Mutations in the pancreatic secretory enzymes *CPA1* and *CPB1* are associated with pancreatic cancer

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To evaluate whether germline variants in genes encoding pancreatic secretory enzymes contribute to pancreatic cancer susceptibility, we sequenced the coding regions of *CPB1* and other genes encoding pancreatic secretory enzymes and known pancreatitis susceptibility genes (*PRSS1*, *CPA1*, *CTRC*, and *SPINK1*) in a hospital series of pancreatic cancer cases and controls. Variants in *CPB1*, *CPA1* (encoding carboxypeptidase B1 and A1), and *CTRC* were evaluated in a second set of cases with familial pancreatic cancer and controls. More deleterious *CPB1* variants, defined as having impaired protein secretion and induction of endoplasmic reticulum (ER) stress in transfected HEK 293T cells, were found in the hospital series of pancreatic cancer cases (5/986, 0.5%) than in controls (0/1,045, $P = 0.027$). Among familial pancreatic cancer cases, ER stress-inducing *CPB1* variants were found in 4 of 593 (0.67%) vs. 0 of 967 additional controls ($P = 0.020$), with a combined prevalence in pancreatic cancer cases of 9/1,579 vs. 0/2,012 controls ($P < 0.01$). More ER stress-inducing *CPA1* variants were also found in the combined set of hospital and familial cases with pancreatic cancer than in controls [7/1,546 vs. 1/2,012; $P = 0.025$; odds ratio, 9.36 (95% CI, 1.15–76.02)]. Overall, 16 (1%) of 1,579 pancreatic cancer cases had an ER stress-inducing *CPA1* or *CPB1* variant, compared with 1 of 2,068 controls ($P < 0.00001$). No other candidate genes had statistically significant differences in variant prevalence between cases and controls. Our study indicates ER stress-inducing variants in *CPB1* and *CPA1* are associated with pancreatic cancer susceptibility and implicate ER stress in pancreatic acinar cells in pancreatic cancer development.

CPB1 | *CPA1* | pancreatic cancer | pancreatitis | ER stress

Pancreatic cancer is the third most common cause of cancer death in the United States with a 5-y survival of only ∼8% (1). Early detection of pancreatic cancer may be the most effective way of reducing the mortality from the disease (2). Identifying those most at risk for developing pancreatic cancer will help improve early-detection efforts. Germline mutations in pancreatic cancer susceptibility genes (*BRCA2*, *ATM*, *PALB2*, *CDKN2A*, *BRCA1*, *STK11*, *PRSS1*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*) as well as numerous low-penetrant loci contribute to pancreatic cancer risk (3–8). Germline mutations in these genes are estimated to account for ∼10% of the familial clustering of

Significance

Much of the inherited susceptibility to pancreatic cancer remains unexplained. Germline variants that cause protein misfolding and impaired secretion of pancreatic enzymes such as *CPA1* (encoding carboxypeptidase A1) can cause pancreatic acinar cell endoplasmic reticulum (ER) stress. We investigated the hypothesis that pancreatic cancer could arise from germline variants in genes encoding pancreatic secretory enzymes that induce pancreatic acinar cell stress. We find ∼1% of 1,579 patients with pancreatic cancer vs. 1 of 2,012 controls have germline variants in the genes encoding *CPA1* and *CPB1* (carboxypeptidase B1) that impair secretion of its protein product and induce ER stress. These findings implicate pancreatic acinar cell stress as a mechanism of pancreatic cancer susceptibility.

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pancreatic cancer (7, 9, 10). Thus, much of the inherited basis for the familial clustering of pancreatic cancer remains unexplained. Mutations in pancreatic cancer susceptibility genes not only contribute to the familial clustering of pancreatic cancer; studies have found that ~5% of patients with apparently sporadic pancreatic cancer carry a deleterious mutation in one of these genes and most of these individuals do not have a family history pointing to an inherited cancer syndrome (11). Chronic pancreatitis, especially hereditary young-onset pancreatitis, is associated with an increased risk of developing pancreatic cancer (12, 13). Most inherited mutations affecting the pancreatitis-susceptibility gene *PRSS1* cause premature trypsin activation (14), but deleterious mutations in *CPA1* and some mutations in *CTRC* and *PRSS1*, genes encoding some of the most abundant proteins secreted by acinar cells, are thought to induce acute and chronic pancreatitis by increasing protein misfolding-induced endoplasmic reticulum (ER) stress (12, 15, 16). Common variants in *SPINK1*, *CTRC*, and *CFTR* that do not induce ER stress and modestly affect the risk of developing pancreatitis have not been implicated in pancreatic cancer susceptibility (17–19). We hypothesized that variants that impair the secretion of pancreatic enzymes from pancreatic acinar cells might induce chronic ER stress and acinar cell injury and thereby predispose to pancreatic cancer development.

Results

Genes Encoding Pancreatic Secretory Enzymes. We conducted a two-phase study. Phase I had two parts; part 1 was a gene variant discovery phase; genes encoding pancreatic secretory enzymes were sequenced in 986 unselected patients from Johns Hopkins Hospital (JHH) with pancreatic cancer and 1,045 healthy and disease controls. Part 2 of phase I involved functional evaluation of variants; any gene with significantly more rare variants of unknown significance in cases than controls underwent functional analysis of these variants. In phase II, genes identified as having significantly more deleterious (ER stress inducing) variants in the phase I cases vs. controls were similarly evaluated in a second set of cases and controls. The first phase candidate genes included *CPB1*, *PNLIP*, *PNLIPRP2*, *AMY2A*, *CEL*, *CELA2A*, *CELA3A*, *CELA3B*, *CTRB1*, *CLPS*, *PLA2G1B*, and *REG1A*. Among these candidate genes, only *CPB1* had significantly more rare variants in cases than in controls to warrant functional analysis of the variants identified (13/986 vs. 1 of 1,045; $P = 0.0009$) (variants listed in Tables 1 and 2 and *SI Appendix*, Tables S1 and S4–S6). Functional analysis of the phase I *CPB1* variants justified the evaluation of *CPB1* variants in the second independent set of cases and controls. After evaluating *CPB1* as a pancreatic cancer susceptibility gene, we sequenced seven additional genes encoding pancreatic secretory enzymes (*REG1B*, *REG3*, *REG3G*, *REG4*, *CPA2*, *CTRL*, and *PNLIPRP1*) in the phase I cases and controls, but none of these genes had an excess of rare variants in cases to merit functional evaluation. We also evaluated the known pancreatitis susceptibility genes, *PRSS1*, *CPA1*, *CTRC*, *SPINK1*, and *CFTR* (described further below).

All variants of uncertain significance (VUSs) in *CPA1* and *CPB1* in cases and controls were evaluated for loss of secretion, enzyme activity, and ER stress. Some variants have been previously characterized in the literature. The ER stress-inducing or otherwise-defective *CPB1* variants found in cases and controls are listed in Tables 1 and 2; benign variants are listed in *SI Appendix*, Table S1. In phase I, the *CPB1* variants identified included three truncating variants (p.E23*, p.Q130*, and p.Q187*) and one nonstop variant (p.*418W), each found in one patient with pancreatic cancer, as well as an in-frame deletion (c.360_362delCAA; p.N120del) found in two patients with pancreatic cancer, and the missense variant, p.A366P, found in one pancreatic cancer case; no such variants were identified in controls. One rare variant found in the controls (I394M) did not affect protein secretion or enzyme function (Fig. 1); all other variants identified in the phase I controls were previously reported polymorphisms found not to affect protein function (20). Levels of proCpb1 protein secreted from cells transfected with the CPB1 expression plasmid containing the in-frame deletion (c.360_362delCAA; p.N120del) and the missense variant, p.A366P, were markedly reduced compared with wild-type proCpb1 secretion (Fig. 1*A*). Intracellular proCpb1 protein expression in the transfected cells was as expected (*SI Appendix*, Fig. S1*A*). These two variants were also characterized by loss of enzyme activity (Fig. 1*B*). The nonstop variant (p.*418W) also had loss of secretion. One additional variant, p.G383D (c.1148G>A), found in another patient with pancreatic cancer, exhibited reduced enzymatic activity but normal protein secretion (Fig. 1*B*). The secretion and enzyme activity of all other *CPB1* missense variants tested were similar to wild-type *CPB1* (Fig. 1 *A* and *B*).

Since variants that impair normal pancreatic enzyme secretion can cause ER stress as a result of protein misfolding (21), we investigated whether *CPB1* variants lacking normal secretion induced ER stress as determined by elevated expression of BiP protein (also known as GRP78 or HSPA5) (22). Transfection of the secretion-impaired variants resulted in ER stress (defined as a significant induction of BiP protein expression relative to wild-type *CPB1* and functional *CPB1* missense variants, Fig. 1 *C* and *E*). We also evaluated the secretion, enzymatic activity, and ER stress effects of the three premature truncating variants recognizing that most premature truncating transcripts undergo nonsense-mediated decay (NMD) that would limit their pathogenicity. Transfection of these three premature truncating variants did not yield detectable protein secretion or enzymatic activity (*SI Appendix*, Fig. S1*B*). Although transfection of these premature truncating variants induced ER stress in vitro (Fig. 1*C*), only the c.67G>T, p.E23* variant is expected to cause pancreatic acinar cell ER stress in vivo. This is because the truncating variant is very close to the start codon and not predicted to

Table 1. Deleterious *CPB1* variants: First-phase study (PC-JHH vs. controls)

Chr/position	rsID	AA change	Nucleotide change[†]	Function	Loss of secretion	Loss of activity	ER stress	Classification	PC, $n = 986$	Controls, $n = 1,045$
3/148545677	rs780957048	p.E23*	c.67G>T	Nonsense	Yes	Yes	Yes	Deleterious[‡]	1	0
3/148558556		p.N120del	c.360_362delCAA	In-frame	Yes	Yes	Yes	Deleterious[‡]	2	0
3/148558676		p.Q130*	c.388C>T	Nonsense	Yes	Yes	No	Defective[§]	1	0
3/148559694	rs141911824	p.Q187*	c.559C>T	Nonsense	Yes	Yes	Yes[¶]	Defective[¶]	1	0
3/148577631		p.A366P	c.1096G>C	Missense	Yes	Yes	Yes	Deleterious[‡]	1	0
3/148577683	rs762454832	p.G383D	c.1148G>A	Missense	No	Yes	No	Defective[#]	1	0
3/148577788	rs201519774	p.X418W	c.1253A>G	Nonstop	Yes	Yes	Yes	Deleterious[‡]	1	0

CPB1 transcript NM_001871.2. AA, amino acid; JHH, Johns Hopkins Hospital; PC, pancreatic cancer.
[†]All heterozygous.
[‡]Induces ER stress.
[§]Loss of secretion but no ER stress.
[¶]Transcript predicted to undergo NMD so predicted to not induce ER stress in vivo.
[#]Loss of enzyme activity but no ER stress.

Table 2. Deleterious *CPB1* variants: Second-phase study (FPC vs. controls)

Chr/position	rsID	AA change	Nucleotide change[†]	Function	Loss of secretion	Loss of activity	ER stress	Classification	FPC, $n = 593$	BCCS, $n = 1,934$
3/148558724	rs143479075	p.G146R	c.436G>A	Missense	Yes	Yes	Yes	Deleterious[‡]	1	0
3/148559694	rs141911824	p.Q187*	c.559C>T	Nonsense	Yes	Yes	Yes[§]	Defective[§]	1	0
3/148577625	rs200456954	p.D364Y	c.1090G>T	Missense	No[¶]	Yes[¶]	No	Defective[#]	1	0
3/148577788	rs201519774	p.X418W	c.1253A>G	Nonstop	Yes	Yes	Yes	Deleterious[‡]	3	0
3/148558659	rs201950041	p.Sp	c.373–2A>G	Splice-site	NA	NA	NA	Defective[§]	0	1

CPB1 transcript NM_001871.2. AA, amino acid; BCCS, bipolar case control study; FPC, familial pancreatic cancer.
[†]All heterozygous.
[‡]Induces ER stress.
[§]Transcript predicted to undergo NMD so predicted to not induce ER stress in vivo.
[¶]Published data.
[#]Loss of enzyme activity but no ER stress.

undergo NMD (23–26), whereas the transcripts of the other premature truncating variants (c.559C>T, p.Q187*, and c.388C>T, p.Q130*) are predicted to undergo NMD and not expected to translate enough protein to cause ER stress in vivo.

Thus, 5 of the 986 patients with pancreatic cancer but none of the 1,045 controls in the first-phase study had *CPB1* variants predicted to induce ER stress in vivo ($P = 0.027$) (Tables 1 and 2). Two of these five *CPB1* mutation carriers had a family history of pancreatic cancer; none had a history of acute or chronic pancreatitis or pancreatic insufficiency (*SI Appendix*, Table S2).

To confirm the association between defective *CPB1* variants and pancreatic cancer, we evaluated *CPB1* variants in the second set of cases and controls that had undergone whole-genome sequencing [593 patients with familial pancreatic cancer and 967 controls from the bipolar case control consortium (BCCS) (7)]. All *CPB1* variants of unknown significance found in cases or controls were subjected to functional analysis. Among the patients with familial pancreatic cancer, one had the *CPB1* p.Q187* nonsense variant (predicted to be benign). In addition, three patients with familial pancreatic cancer from unrelated families and one sibling also with pancreatic cancer had the ER stress-inducing p.*418W variant; one pancreatic cancer case had a missense variant (p.D364Y) that impaired enzyme function but did not impair protein secretion (20) (Tables 1 and 2 and *SI Appendix*, Table S1). One pancreatic cancer case had the missense variant, p.G146R, characterized by markedly reduced proCpb1 secretion compared with wild-type *CPB1* (Fig. 1*D*), despite similar intracellular protein expression by Western blot (*SI Appendix*, Fig. S1*C*). This variant also had loss of enzyme activity (Fig. 1*B*) and induced ER stress as measured by a significant induction of BiP in transfected cells (Fig. 1 *C* and *E*). One BCCS control had a splice-site variant (c.373-2A>G) judged to be benign as its transcript would be predicted to undergo NMD. All other *CPB1* variants found in the controls were known polymorphisms found not to affect protein function (20) (*SI Appendix*, Table S1). Overall, ER stress-inducing *CPB1* variants were identified significantly more often in patients with familial pancreatic cancer (4 of the 593 kindred, as well as 1 sibling also with pancreatic cancer) than in BCCS controls (0/967, $P = 0.020$) (Tables 1 and 2). In the combined set of cases and controls from both phases, there were ER stress-inducing *CPB1* variants found in 9 pancreatic cancer cases compared with 0 controls (9/1,579 vs. 0/2,012, $P < 0.01$). In contrast, there was no significant difference in the prevalence of rare variants classified as benign between cases and controls (23/1,579 vs. 22/2,012, $P = 0.33$; *SI Appendix*, Table S1).

We also compared the prevalence of the ER stress-inducing *CPB1* variants identified in our cases to their prevalence in the ExAC database (exac.broadinstitute.org/): Only 4 of 60,649 ExAC controls had one of these defective *CPB1* variants ($P < 0.0001$ compared with the 9/1,579 pancreatic cancer cases) (*SI Appendix*, Table S3).

Pancreatitis Susceptibility Genes. Among the pancreatitis susceptibility genes (*CPA1*, *CTRC*, *PRSS1*, *SPINK1*, and *CFTR*), multiple rare VUSs in *CPA1* were found in both cases and controls. Since rare variants in *CPA1* have been found to induce ER stress and are associated with pancreatitis susceptibility, we evaluated the secretion/ER stress-inducing effect of *CPA1* variants. In the first phase, there were three rare *CPA1* missense VUSs (one p.R386C and two p.R237C variants in cases, and one p.G55W variant in a control) and three truncating variants identified in the pancreatic cancer cases. Cells transfected with the p.R237C and p.R386C variants, but not the p.G55W variant, had reduced protein secretion despite similar intracellular Cpa1 expression by Western blot (*SI Appendix*, Figs. S2*A* and S3*B*). The two missense variants in cases with loss of secretion (p.R237C and R386C) induced ER stress (significant induction of BiP expression; *SI Appendix*, Fig. S2*B*); they also exhibited reduced enzymatic activity (*SI Appendix*, Fig. S2*C*). The three premature truncating *CPA1* variants (two p.R27* and one p.Y318fs) found in cases (*SI Appendix*, Table S4) were characterized by loss of secretion and loss of enzyme activity (*SI Appendix*, Fig. S3 *A* and *C*) but were all considered not deleterious: the Y318fs variant induced ER stress in vitro, but its transcript is predicted to undergo NMD and so the variant is likely to be benign, and while the p.R27* is not predicted to undergo NMD, it did not induce ER stress in vitro so it was considered benign. Thus, 3 of 986 phase I cases and 0 of 1,045 phase I controls ($P = 0.115$) had an ER stress-inducing *CPA1* variant. To increase our sample size, we evaluated *CPA1* in the phase II cases/controls. In phase II, five *CPA1* missense VUSs (p.E99K, p.R110Q, p.T164M, p.R240Q, and p.W367C) and one in-frame variant (p.T409ins, adding 8 aa to the protein) were identified in the cases, and one missense variant, p.R234H in the controls. Functional analysis of these variants found four variants in the phase II cases (p.T164M, p.R240Q, and p.W367C and the p.T409ins in-frame variant), as having reduced or absent secretion and enzyme activity and also induced ER stress (*SI Appendix*, Fig. S2 *C–E*). The p.R240Q variant was recently reported as similarly dysfunctional in a case with pancreatitis (27). One variant in the phase II controls (p.R234H) was also characterized by loss of protein secretion and ER stress. Of the truncating *CPA1* variants in the phase II set, three cases had the benign *CPA1* p.R27* nonsense variant, one had a p.Y119* nonsense variant, and one BCCS control had a p.L134 frameshift variant; the transcripts of these variants were predicted to undergo NMD in vivo and were therefore considered benign (the p.L314fs variant exhibited elevated expression of BiP protein, whereas p.Y119* did not) (Tables 3 and 4). None of the cases with an ER stress-inducing *CPA1* variant had a personal history of pancreatitis or pancreatic insufficiency. All other *CPA1* variants found in phase II cases or controls were known polymorphisms not found to affect protein function (*SI Appendix*, Table S4). Thus, in phase II, 4 of 593 familial cases and 1 of 967 controls ($P = 0.07$) had an ER stress-inducing *CPA1* variant. Combining both phase I and phase II results, more ER stress-inducing *CPA1* variants were found in hospital/familial pancreatic cancer cases than in controls [7/1,546 vs. 1/2,068; $P = 0.025$; odds ratio, 9.36 (95% CI, 1.15–76.02)]. In contrast, there was no significant difference in the prevalence of rare variants

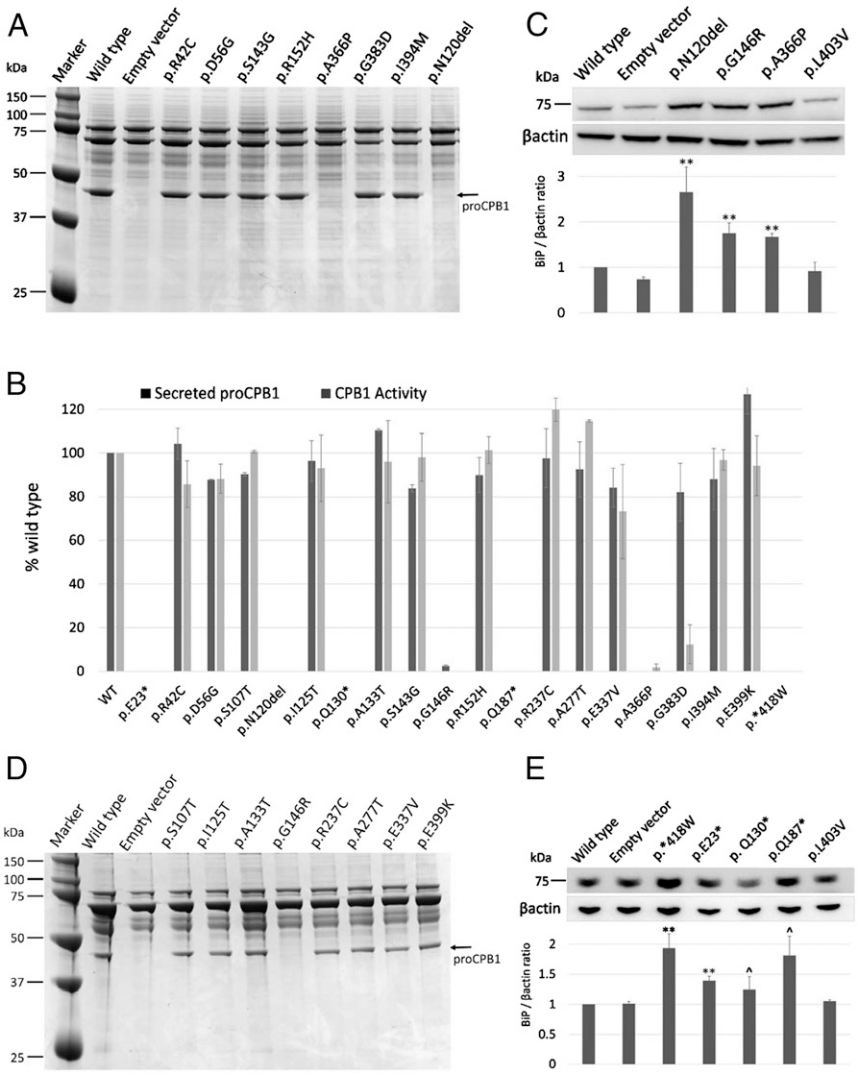

Fig. 1. Secretion, enzymatic activity, and ER stress of *CPB1* variants. (*A* and *D*) Secreted proCpb1 protein by SDS/PAGE and Coomassie Blue staining. (*B*) Cpb1 secretion (black bars) and enzymatic activity (gray bars) relative to wild-type *CPB1* levels (average ± SE, $n = 3$). (*C*, *Top*) Expression of BiP in cells transfected with *CPB1* wild type, and truncating and nonstop deleterious variants lacking secretion (p.*418W, p.E23*, p.Q130*, p.Q187*). (*C* and *E*, *Bottom*) Relative BiP/actin protein expression quantified by densitometry (average ± SE, $n =$ 3 independent transfections). (*C*, *Top*) Expression of the ER stress protein BiP (Grp78) in cells transfected with wild-type *CPB1*, deleterious variants lacking secretion (p.N120del, p.G146R, p.A366P), and a benign variant (p.L403V). **$P < 0.01$–0.02; ^variant transcript predicted to undergo NMD in vivo.

classified as benign between cases and controls (30/1,546 vs. 40/2,012; $P = 0.85$; *SI Appendix*, Table S4).

In the ExAC database, 24 of 60,677 ExAC controls had one of the *CPA1* ER stress-inducing *CPA1* variants, significantly fewer than in our pancreatic cancer series ($P < 0.0001$), as shown in *SI Appendix*, Table S3.

Overall, 16 of 1,546 patients with pancreatic cancer were identified as having an ER stress-inducing *CPA1* or *CPB1* variant, compared with 1 of 2,068 disease controls ($P < 0.00001$). None of the patients with an ER stress-inducing *CPA1* or *CPB1* variant carried a deleterious variant in a known pancreatic cancer susceptibility gene and other relevant risk factors such as ethanol abuse. In addition to the ER stress-inducing variants, more pancreatic cancer cases (12 of 1,546) than controls (3 of 2,068) had dysfunctional *CPA1*/*CPB1* variants (lacking secretion and/or enzymatic activity but not ER stress-inducing) ($P = 0.0065$), but this difference could be explained by the p.R27* variant found in five cases but no controls. There was no significant difference in the prevalence of known loss-of-function variants or VUSs in *PRSS1*, *SPINK1*, and *CFTR* between cases and controls.

For *CTRC*, we identified one patient with pancreatic cancer without a history of pancreatitis who had a known deleterious missense *CTRC* variant associated with pancreatitis susceptibility (p.Q48R: which has been reported to lack normal secretion and to induce ER stress) and one patient with a p.F68I variant (not reported previously). The p.F68I variant was associated with reduced protein secretion (~60%), and expression of this variant induced elevated BiP protein expression consistent with inducing ER stress. We also sequenced *CTRC* and the other pancreatitis susceptibility genes in 33 patients who had undergone pancreatic resection for chronic pancreatitis, and one of these patients carried a p.T54M variant of unknown significance. As shown in *SI Appendix*, Fig. S3*C*, the p.T54M variant was characterized by loss of protein secretion and elevated expression of BiP protein, consistent with ER stress (*SI Appendix*, Fig. S3*E*). Three patients with pancreatic cancer also carried the p.R254W variant (reported to be associated with a moderate increased risk of developing chronic pancreatitis) (28). None of these patients gave a history of pancreatitis. The p.R254W variant had somewhat reduced protein secretion compared with wild-type protein, but it did not induce ER stress (*SI Appendix*, Fig. S3 *C* and *D*). These results for the p.R254W variant have been reported previously (15, 28). We also compared the prevalence of the R254W variant in familial pancreatic cancer cases vs. the BCCS controls but found no significant difference (5 of 593 vs. 9 of 967). All other *CTRC* variants identified in cases and controls were benign (*SI Appendix*, Table S5). Overall, two patients with pancreatic cancer, one patient with chronic pancreatitis, and none of the controls had an ER-stress-inducing *CTRC* variant.

Discussion

Pancreatic acinar cells secrete large amounts of protein, so they have a robust unfolded protein response to help protect them from ER stress due to protein misfolding, but this response can

Table 3. Deleterious CPA1 variants: First-phase study (PC-JHH vs. controls)

Chr/position	rsID	AA_change	Nucleotide change[†]	Function	Loss of secretion	Loss of activity	ER stress	Classification	PC, $n = 953$	Controls, $n = 1,045$
7/130020952	rs141209213	p.R27*	c.79C>T	Nonsense	Yes	Yes	No	Defective[‡]	2	0
7/130024389	rs184981267	p.R237C	c.709C>T	Missense	Yes	Yes	Yes	Deleterious[§]	2	0
7/130025152		p.Y318fs	c.954_955delCA	Frameshift	Yes	Yes	Yes[¶]	Defective[¶]	1	0
7/130027748		p.R386C	c.1156C>T	Missense	Yes	Yes	Yes	Deleterious[§]	1	0

CPA1 transcript NM_001868.2. AA, amino acid; JHH, Johns Hopkins Hospital; PC, pancreatic cancer.
[†]All heterozygous.
[‡]Loss of secretion but no ER stress.
[§]Induces ER stress.
[¶]Transcript predicted to undergo NMD so predicted to not induce ER stress in vivo.

be overwhelmed. Pancreatic acinar cell ER stress is hypothesized to be the mechanism by which *CPA1* variants lead to hereditary pancreatitis, but ER stress of pancreatic acinar cells has not been identified as a mechanism by which patients can become susceptible to pancreatic cancer. CPA1 and CPB1 have a restricted pattern of tissue expression and are among the most highly expressed genes in pancreatic acinar cells; Cpb1 constitutes ∼2% of total pancreatic cytosol protein (29, 30); it is not expressed in pancreatic duct cells (www.proteinatlas.org) and, like other pancreatic secretory enzymes, is stored in an inactive form in zymogen granules. It is notable that none of the patients with ER stress-inducing *CPA1* or *CPB1* variants in this study had a clinical history of recurrent acute pancreatitis or chronic pancreatitis. *CPB1* has not been identified as a pancreatitis susceptibility gene; one report of a study of cases with pancreatitis and controls did not identify any *CPB1* variants in cases or controls that significantly reduced Cpb1 secretion (20). The first report describing *CPA1* as a cause of hereditary pancreatitis found that variants predicted to be deleterious conferred a high odds for pancreatitis susceptibility (12), but more recent studies in other populations have found such variants were associated with more modest odd ratios of having pancreatitis (27, 31), indicating that carriers of these variants often do not develop clinical pancreatitis (27, 31). Since ER stress can result in apoptosis, it is likely that carriers of ER stress-inducing *CPA1* and *CPB1* variants are prone to pancreatic acinar cell apoptosis, loss of acinar glands, and fatty replacement. Only when ER stress causes excessive apoptosis that results in necrosis and a local inflammatory response is there the potential for the inflammation to progress to a clinical attack of pancreatitis (21). A similar phenotype has been identified in rare ER stress-inducing mutations in the gene *CEL* (which encodes carboxyl ester lipase) that also cause maturity onset diabetes of the young (MODY) (32–34); some of these patients will develop sufficient loss of pancreas tissue to cause pancreatic insufficiency (34). In addition to rare variants in *CEL* that can cause MODY, a fusion allele involving CEL and its pseudogene (CEL-HYB) has been implicated as a cause of chronic pancreatitis in one study (35, 36), but has not been associated with having pancreatic cancer (37, 38). Many patients with pancreatic cancer develop pancreatic atrophy that can be attributed to obstructive atrophy from the tumor, precluding the opportunity to determine whether the pancreatic cancer cases with ER stress-inducing *CPA1* or *CPB1* mutations identified in this study developed pancreatic atrophy before tumor development. Focal pancreatic atrophy and fatty replacement have been described in some patients from pancreatic cancer families who have undergone pancreatic resection, but this has been attributed to secondary effects of PanIN obstructing small pancreatic ductules (39). The increased prevalence of ER stress-inducing *CPA1* and *CPB1* variants in patients with pancreatic cancer is consistent with the hypothesis that these variants can contribute to pancreatic cancer susceptibility probably as a consequence of chronic low-grade inflammation arising from acinar cell injury.

Patients with ER stress-inducing variants in *CPA1* and *CPB1* could potentially benefit from pancreatic screening and surveillance (40), but further investigation is needed to determine their lifetime risk of pancreatic cancer. Other factors that affect the unfolded protein response and cause ER stress such as excess alcohol consumption could be important in determining pancreatic cancer susceptibility in patients with ER stress-inducing variants (41), but this requires more investigation.

In addition to the ER stress-inducing variants in *CPA1* and *CPB1*, there were several loss-of-function *CPA1* and *CPB1* variants that either did not induce ER stress in vitro or were not predicted to cause ER stress in vivo because the variant transcript

Table 4. Deleterious CPA1 variants: Second-phase study (FPC vs. controls)

Chr/position	rsID	AA_change	Nucleotide change[†]	Function	Loss of secretion	Loss of activity	ER stress	Classification	FPC, $n = 593$	BCCS, $n = 967$
7/130020952	rs141209213	p.R27*	c.79C>T	Nonsense	Yes	Yes	No	Defective[‡]	3	0
7/130021652		p.R110Q	c.329G>A	Missense	No	Yes	ND	Defective[§]	0	1
7/130021680		p.Y119*	c.357C>A	Nonsense	Yes	Yes	No	Defective[‡]	1	0
7/130021967		p.L134fs	c.401delT	Frameshift	Yes	Yes	Yes	Defective[¶]	0	1
7/130023239		p.T164M	c.491C>T	Missense	Yes	Yes	Yes	Deleterious[#]	1	0
7/130024381		p.R234H	c.701G>A	Missense	Yes	Yes	Yes	Deleterious[#]	0	1
7/130024399		p.R240Q	c.719G>A	Missense	Yes	Yes	Yes	Deleterious[#]	1	0
7/130027693		p.W367C	c.1101G>T	Missense	Yes	Yes	Yes	Deleterious[#]	1	0
7/130027818		p.T409ins	c.1226C>ccatcatggag-cacaccctgaatca	In-frame	Yes	Yes	Yes	Deleterious[#]	1	0

CPA1 transcript NM_001868.2. AA, amino acid; BCCS, bipolar case control study; FPC, familial pancreatic cancer.
[†]All heterozygous.
[‡]Loss of secretion but no ER stress.
[§]Loss of enzyme activity only.
[¶]Transcript predicted to undergo NMD so predicted to not induce ER stress in vivo.
[#]Induces ER stress.

was predicted to undergo NMD. Predicting the pathogenicity of premature truncating variants can be difficult because even if a transcript undergoes NMD, the extent of transcript degradation by NMD can vary and NMD may not always sufficiently degrade transcript expression to prevent pathogenicity. Whether a premature truncating variant-containing transcript undergoes NMD depends primarily on the location of the premature truncating variant relative to the 3′ exon junction, but other factors influence whether NMD of a given transcript occurs and the extent of transcript degradation by NMD (23–25).

Two ER stress-inducing *CTRC* variants were also identified in pancreatic cancer cases; further investigation is needed to determine whether ER stress-inducing *CTRC* variants are more common in pancreatic cancer cases than in controls. One potential reason why variants in *CPB1* and *CPA1* but not most other genes encoding pancreatic enzymes would predispose to pancreatic cancer might be their level of expression; *CPB1* and *CPA1* are among the highest expressed genes in acinar cells and so variant Cpb1 and Cpa1 proteins with impaired secretion would be expected to cause more ER stress than pancreatic enzymes with lower levels of expression.

Our results support the hypothesis that germline mutations that cause defective protein secretion and ER stress in pancreatic acinar cells can promote the development of pancreatic ductal adenocarcinoma. In particular, we find that defective variants in *CPA1* and *CPB1* are more common in patients with pancreatic cancer than in controls, implicating ER stress of pancreatic acinar cells due to impaired protein secretion in pancreatic cancer susceptibility.

Materials and Methods

Study Population. The phase I study included 986 patients with pancreatic cancer who were evaluated and treated at JHH between 1993 and 2015 (*SI Appendix*, Table S7). Patient information including family history was obtained from the JHH medical record and from the National Familial Pancreas Tumor Registry (NFPTR). The control group is described in ref. 38. For the phase II study, the 593 familial pancreatic cancer kindred from 10 North American pancreatic cancer family registries and the 967 controls from the BCCS are further described in refs. 7 and 42, respectively. This study was approved by the Johns Hopkins and all other participating Institutional Review Boards (Mayo Clinic, Rochester, Dana Farber Cancer Institute, University of Pittsburgh, University of Pennsylvania, Memorial Sloan Kettering Cancer Center, University of Toronto, University of Michigan, Karmonos Cancer Institute, Wayne State University, and McGill University), and written informed consent was provided from all enrolled patients.

Next-Generation Sequencing. Next-generation sequencing was performed as previously described (43). See *SI Appendix, SI Materials and Materials*, for additional description of methods.

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