ORIGINAL ARTICLE

Genome-wide association study of survival in patients with pancreatic adenocarcinoma

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ABSTRACT

Background and objective Survival of patients with pancreatic adenocarcinoma is limited and few prognostic factors are known. We conducted a two-stage genome-wide association study (GWAS) to identify germline variants associated with survival in patients with pancreatic adenocarcinoma.

Methods We analysed overall survival in relation to single nucleotide polymorphisms (SNPs) among 1005 patients from two large GWAS datasets, PanScan I and ChinaPC. Cox proportional hazards regression was used in an additive genetic model with adjustment for age, sex, clinical stage and the top four principal components of population stratification. The first stage included 642 cases of European ancestry (PanScan), from which the top SNPs (p<10−7) were advanced to a joint analysis with 363 additional patients from China (ChinaPC).

Results In the first stage of cases of European descent, the top-ranked loci were at chromosomes 11p15.4, 18p11.21 and 1p36.13, tagged by rs12362504 (p=1.63×10−7), rs981621 (p=1.65×10−7) and rs16861827 (p=3.75×10−7), respectively. 131 SNPs with p≤10−7 were advanced to a joint analysis with cases from the ChinaPC study. In the joint analysis, the top-ranked SNP was rs10500715 (minor allele frequency, 0.37; p=1.72×10−7) on chromosome 11p15.4, which is intronic to the SET binding factor 2 (SBF2) gene. The HR (95% CI) for death was 0.74 (0.66 to 0.84) in PanScan I, 0.79 (0.65 to 0.97) in ChinaPC and 0.76 (0.68 to 0.84) in the joint analysis.

Conclusions Germline genetic variation in the SBF2 locus was associated with overall survival in patients with pancreatic adenocarcinoma of European and Asian ancestry. This association should be investigated in additional large patient cohorts.

What is already known on this subject?
Five-year overall survival of patients with pancreatic cancer is approximately 5%.
Germline genetic variability can provide important prognostic information for patients with cancer.
Genome-wide association studies have identified several genetic variants associated with the development of pancreatic adenocarcinoma in European and Chinese populations, but few studies have examined variants related to survival.

What are the new findings?
Previously identified genetic loci associated with the development of pancreatic adenocarcinoma were not associated with survival among those with the disease.
In the first stage of the genome-wide association study of patient survival, three regions, 11p15.4, 18p11.21 and 1p36.13, were the top-ranked loci among patients of European ancestry.
In the joint analysis of >1000 pancreatic cancer cases, variants at the SBF2 gene on chromosome 11p15.4 defined the top genetic locus associated with overall survival among patients of European and Chinese descent.
rs10500715 in SBF2 was associated with an HR for death in an additive model of 0.76 (95% CI 0.68 to 0.84; p=1.72×10−7) which was similar in European and Chinese populations and by disease stage.

**Significance of this study**

**How might it impact on clinical practice in the foreseeable future?**

- In patients with pancreatic adenocarcinoma, several germline variants were associated with overall survival.
- If confirmed in further replication and functional studies, these variants may add important information to define patient prognosis, with the potential to impact treatment decisions and clinical trial design.
- Our results highlight a potential role for SBF2 in pancreatic tumourigenesis and further implicate altered membrane trafficking of 3-phosphoinositides in pancreatic cancer growth and progression.

**INTRODUCTION**

Pancreatic cancer is a major cause of cancer-related death across the globe, and 5-year overall survival is approximately 5%. Nevertheless, patient survival times are variable and only partially explained by traditional clinical and pathological features. Accumulating evidence indicates that germline genetic variability can provide important prognostic information for patients with cancer. One mechanism by which germline genetic variability may impact patient survival is through modification of tumour-host interactions. A defining feature of pancreatic adenocarcinoma is the recruitment of host cells, including fibroblasts, immune cells and endothelial cells, which surround the tumour in a dense stromal matrix. This host-derived desmoplastic stroma actively engages with tumour cells and plays a critical role in promoting tumour development and progression. Notably, laboratory studies suggest that treatments which modify the interaction of pancreatic cancer cells with their surrounding stroma can impact survival in genetically engineered mouse models of pancreatic adenocarcinoma.

Prior studies of germline variants and pancreatic cancer survival have primarily focused on the evaluation of candidate genes in pathways of suspected importance. This approach relies on our relatively incomplete understanding of tumour and host biology. In contrast, genome-wide approaches are available that allow a more comprehensive evaluation of germline genetic variants that is not reliant on a priori hypotheses. Recently, genome-wide association studies (GWAS) have identified several genetic variants associated with the development of pancreatic adenocarcinoma in European and Chinese populations. In a two-stage genome-wide study of survival, we used these data to evaluate the association of germline variants with overall survival in over 1000 cases of pancreatic adenocarcinoma.

**MATERIALS AND METHODS**

**PanScan population**

The Pancreatic Cancer Cohort Consortium (PanScan) GWAS has been described previously, in detail. In short, PanScan I included cases and controls from 11 prospective cohort studies from European populations in the USA and Europe: α-Tocopherol β-Carotene Cancer Prevention Study (ATBC); CLUE II; American Cancer Society Cancer Prevention Study-II (CPS II); European Prospective Investigation into Cancer and Nutrition Study (EPIC); Health Professionals Follow-up Study (HPFS); New York University Women’s Health Study (NYU-WHS); Nurses’ Health Study (NHS); Physicians’ Health Study I (PHS I); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO); Women’s Health Initiative (WHI); and WHS. In each cohort, a defined population of subjects was followed prospectively with assessments of lifestyle factors and ascertainment of cancer diagnoses. Cases included subjects with incident primary pancreatic adenocarcinoma (ICD-O-3 code C25.0–C25.9 or C25.0–C25.3, C25.7–C25.9). All subjects with non-exocrine pancreatic tumours (C25.4, histology type, 8150, 8151, 8153, 8155, 8240, 8246) were excluded.

Each cohort study selected participants with blood or buccal cells collected prior to cancer diagnosis. Incident pancreatic cancer cases identified by self-report, report of next-of-kin, linkage with local or national cancer registries, or through national death indices were confirmed by subsequent medical record review, cancer registry report, and/or death registry report, without prior knowledge of genetic data. In the 11 participating cohorts, covariate data were collected through written questionnaires or in-person interviews. Data were requested from each cohort on participants’ age, gender and race/ethnicity (European, Asian, African, other). Detailed descriptions of data collection methods have been published previously. Cohorts obtained consent from participants and approval from their Institutional Review Board (IRB). The Special Studies IRB of the National Cancer Institute approved the pooled PanScan study.

For the current survival analysis, each cohort also provided survival time and stage information for pancreatic cancer cases included in PanScan. Survival time was defined as the number of days between the date of diagnosis and the date of death or date of last known contact. Stage data were harmonised into three categories: (1) local disease amenable to surgical resection; (2) locally advanced disease with extra-pancreatic extension rendering it unresectable, but without distant metastases; and (3) distant metastatic disease. For eight cohorts (CLUE II, EPIC, HPFS, NHS, NYU-WHS, PHS, PLCO and WHS), American Joint Committee on Cancer (AJCC)/International Union for Cancer Control (I.U.C.C) Tumor-Lymph nodes-Metastasis (TNM) staging was converted to the above categories, with AJCC/I.U.C.C stages I and II indicating local disease, stage III indicating locally advanced disease, and stage IV indicating metastatic disease. Two cohorts (CPS and WHI) provided data using Surveillance Epidemiology End Results summary staging, which classifies tumours as localised, regional or distant. These stages were included as local, locally advanced and metastatic disease, respectively. Stage data were not available for one study (ATBC). Given the known strong association between stage and survival, subjects of European descent were included in the final analysis if they had available survival time and stage information, in addition to genotype data. From PanScan I, 1323 cases of European descent were available with genome-wide genotype data and survival time. Among these cases, 642 cases had stage information and were included in the analysis (figure 1; see also online supplementary table S1). Median survival times (MSTs) were slightly shorter among the full population of patients with survival information versus the subset of patients with stage information (see online supplementary table S1). Overall, MST was 5.0 months for all cases and 5.9 months for the subset of cases with available stage data. Age and gender were similar in the full PanScan population of European descent (median age, 68 years; 48% male) and the population with survival and stage information (median age, 71 years; 37% male).
have been described previously.\textsuperscript{18–20} In brief, samples with <98% completion and single nucleotide polymorphism (SNP) assays with call rates <90% were excluded. Only SNPs with minor allele frequency >0.01 and mapped on autosomal chromosomes were included for analysis. A principal component analysis of DNA samples in this study was performed with EIGENSTRAT. Four principal components were effective for distinguishing significant population groups and were included as quantitative covariates to correct for genetic admixture. To increase the spectrum of variants tested in the current study of overall survival, we used MACH software to impute untyped markers using linkage disequilibrium (LD) and haplotype information from HapMap phase II CEPH, Utah residents with ancestry from northern and western Europe (CEU) and Han Chinese in Beijing, China (CHB)+Japanese in Tokyo, Japan (JPT) as the reference sets for PanScan and ChinaPC participants, respectively. After quality control of imputation data, 2,731,086 SNPs in PanScan and 2,307,550 SNPs in ChinaPC were available for analysis.

### Genotype and imputation analysis

Genotypes of patients in PanScan and ChinaPC were generated using the HumanHap550 chip (Illumina, San Diego, California, USA) and Affymetrix GeneChip Human Mapping 6.0 set (Affymetrix, Santa Clara, California, USA), respectively. The procedures of genotyping and quality control in each GWAS have been described previously.\textsuperscript{18–20} In brief, samples with <98% completion and single nucleotide polymorphism (SNP) assays with call rates <90% were excluded. Only SNPs with minor allele frequency >0.01 and mapped on autosomal chromosomes were included for analysis. A principal component analysis of DNA samples in this study was performed with EIGENSTRAT. Four principal components were effective for distinguishing significant population groups and were included as quantitative covariates to correct for genetic admixture. To increase the spectrum of variants tested in the current study of overall survival, we used MACH software to impute untyped markers using linkage disequilibrium (LD) and haplotype information from HapMap phase II CEPH, Utah residents with ancestry from northern and western Europe (CEU) and Han Chinese in Beijing, China (CHB)+Japanese in Tokyo, Japan (JPT) as the reference sets for PanScan and ChinaPC participants, respectively. After quality control of imputation data, 2,731,086 SNPs in PanScan and 2,307,550 SNPs in ChinaPC were available for analysis.

### Statistical analysis

For each of the selected SNPs, we performed Cox proportional hazards regression under a log-additive genetic model with adjustment for covariates that might influence patient survival, including age (continuous), sex (male or female), stage of disease (local, locally advanced and metastatic as ordinal categories) and the top four principal components of population stratification in both PanScan I and ChinaPC studies.\textsuperscript{18–20} The overall survival time was defined as the time from pancreatic cancer diagnosis to either death or the last known date alive. Patients known to be alive were censored at the time of last contact. The top SNPs with \( p \leq 10^{-5} \) found in PanScan were advanced to a combined analysis in patients independently recruited from the ChinaPC study. To summarise results for the two datasets, we performed a meta-analysis to obtain the summary HR and 95% CI using METAL software (http://www.sph.umich.edu/csg/abecasis/metal). Haploview software was used to determine pair-wise LD structure across the studied genomic regions. Kaplan–Meier survival estimates were plotted and \( p \) values were assessed using the log-rank test. Survival analyses were performed with SAS software. All statistical tests were two-sided.

### RESULTS

#### Patient characteristics

The characteristics of the 642 pancreatic cancer cases from the PanScan cohort and the 363 cases from the ChinaPC study are shown in table 1. Median follow-up time for cases still alive was 64 months in PanScan and 17 months in ChinaPC. In PanScan, 609 (94.9%) patients had died, while 334 (92.0%) had died in...
the ChinaPC study. In the combined analysis, we included 1005 patients with pancreatic cancer, 19.6% with localised disease, 32.5% with locally advanced disease and 47.9% with metastatic disease. As expected, stage was strongly associated with survival in both studies (p<0.0001); MST was 13.5, 9.7 and 3.8 months for patients with local, locally advanced and metastatic disease, respectively, in the combined study. The MST for patients in PanScan and ChinaPC studies was 5.9 months and 7.1 months, respectively.

Germline variants associated with incident pancreatic cancer or patient survival in previous GWAS

Four susceptibility loci, 13q22.1, 1q32.1, 5p15.33 and 9q34, have been associated with pancreatic cancer risk in two pancreatic cancer GWAS of European ancestry (PanScan I and II).18–19 To determine if the top SNPs at these loci might also be associated with patient survival, we investigated the associations between these SNPs and survival time in the PanScan cohort. We also evaluated rs167020 on chromosome 7q36, which was associated with incident pancreatic cancer in the prospective cohorts participating in PanScan I.18 None of the top SNPs at these loci were significantly associated with survival (p>0.05) (table 2).

We then investigated a genetic locus on chromosome 6, which was associated with survival in patients with advanced pancreatic adenocarcinoma participating in a randomised clinical trial of gemcitabine plus placebo versus gemcitabine plus bevacizumab (CALGB 80303).25 The top SNP from the analysis in CALGB 80303 (rs763780) was also associated with survival in patients with advanced pancreatic cancer participating in a randomised clinical trial (table 2). These two SNPs were also located in the top two SNPs, rs10500715 and rs7106914, selected as tag SNPs in this region for further evaluation in a joint analysis with cases from the ChinaPC study. In CALGB 80303 was protective in the PanScan analysis, respectively (table 3 and online supplementary table S2). These SNPs were clustered in SBF2 on Chr11p15.4, C18orf1 on Chr18p11.21 and IGSF21 on Chr1p36.13. LD plots of the SNPs on chromosomes 11p15.4 and 18p11.21 are shown in online supplementary figure S2. In 200 kb regions flanking rs16861827, there were 56 nominally significant SNPs with p values ranging from 4.96×10⁻⁵ to 0.048.

We selected the SNPs with p<10⁻⁵ from PanScan (131 SNPs) to evaluate in a joint analysis with cases from the ChinaPC study (see online supplementary table S2). In the joint analysis, the top two SNPs, rs10500715 and rs7106914, were identified on chromosome 11p15.4. These two SNPs were also located in the SBF2 gene, 43 520 and 48 429 base pairs from the top SNP identified in the PanScan analysis, respectively. These two SNPs were in perfect LD with each other in both populations; we selected rs10500715 as the tag SNP in this region for further analysis. In PanScan and ChinaPC participants, rs10500715 was associated with an HR for death in an additive model of 0.74 (95% CI 0.66 to 0.84) and 0.79 (95% CI 0.65 to 0.97), respectively (table 4). The p value for heterogeneity was 0.30 across the two studies. The association of rs10500715 with patient survival was similar by disease stage, with an HR of 0.80 (95% CI 0.64 to 1.01) in patients with localised disease, HR of 0.78 (95% CI 0.65 to 0.93) in those with locally advanced disease and HR of 0.81 (95% CI 0.70 to 0.92) in those with metastatic disease.

### Table 1 PanScan and ChinaPC patient characteristics and overall survival

<table>
<thead>
<tr>
<th></th>
<th>PanScan (N=642)</th>
<th>ChinaPC (N=363)</th>
<th>Pooled analysis (N=1005)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>609 (94.9)</td>
<td>334 (92.0)</td>
<td>943 (93.8)</td>
</tr>
<tr>
<td>Alive</td>
<td>33 (5.1)</td>
<td>29 (8.0)</td>
<td>62 (6.2)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>239 (37.2)</td>
<td>218 (60.1)</td>
<td>457 (45.5)</td>
</tr>
<tr>
<td>Female</td>
<td>403 (62.8)</td>
<td>145 (39.9)</td>
<td>548 (54.5)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤68 years</td>
<td>253 (39.4)</td>
<td>264 (72.7)</td>
<td>517 (51.4)</td>
</tr>
<tr>
<td>&gt;68 years</td>
<td>389 (60.6)</td>
<td>99 (27.3)</td>
<td>488 (48.6)</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local disease</td>
<td>119 (18.5)</td>
<td>78 (21.5)</td>
<td>197 (19.6)</td>
</tr>
<tr>
<td>Locally advanced disease</td>
<td>170 (26.5)</td>
<td>157 (43.2)</td>
<td>327 (32.5)</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>353 (55.0)</td>
<td>128 (35.3)</td>
<td>481 (47.9)</td>
</tr>
</tbody>
</table>

*ST, median survival time, months.
†p Values were calculated using the log-rank test.
‡Median age was 68 years among all cases.
rs10500715 genotype was not statistically associated with clinical stage in a joint analysis (p=0.10).

In a dominant model for rs10500715, the median overall survival was 4.1 months for cases with the TT genotype and 7.0 months for those with TG or GG genotype in PanScan (figure 2). Multiple additional SNPs at the SBF2 gene locus were of marginal statistical significance in the PanScan cohort and combined analysis (figure 3); seven SNPs in high LD \((r^2=0.76–1.00)\) in PanScan were associated with overall survival with \(p\) values of \(8.64\times10^{-7}\) to 0.0002. We examined whether the top SNPs in SBF2 map to reported eQTLs for nearby genes using data from a published eQTL dataset.\(^{24}\) However, we did not find that the SNPs were associated with known eQTLs in this dataset.

Table 2  HRs and MSTs by genotype variants previously associated with incident pancreatic cancer in PanScan

<table>
<thead>
<tr>
<th>SNP, chromosome, gene</th>
<th>No. (%)</th>
<th>MST*</th>
<th>HR (95% CI)†</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9543325, Chr13, none</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>207 (32.2)</td>
<td>6.1</td>
<td>1.09 (0.96 to 1.24)</td>
<td>0.1769</td>
</tr>
<tr>
<td>TC</td>
<td>331 (51.6)</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>104 (16.2)</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3790844, Chr1, NRSa2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>414 (64.5)</td>
<td>5.4</td>
<td>1.04 (0.90 to 1.21)</td>
<td>0.5699</td>
</tr>
<tr>
<td>AG</td>
<td>203 (31.6)</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>25 (3.9)</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs401681, Chr5, CLPTM1-TERT</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>CC</td>
<td>189 (29.4)</td>
<td>6.5</td>
<td>1.06 (0.95 to 1.19)</td>
<td>0.2916</td>
</tr>
<tr>
<td>CT</td>
<td>316 (49.2)</td>
<td>5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>137 (21.4)</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs505922, Chr9, ABO</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TT</td>
<td>224 (34.9)</td>
<td>5.1</td>
<td>1.05 (0.93 to 1.18)</td>
<td>0.4590</td>
</tr>
<tr>
<td>TC</td>
<td>312 (48.6)</td>
<td>5.3</td>
<td></td>
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</tr>
<tr>
<td>CC</td>
<td>106 (16.5)</td>
<td>8.0</td>
<td></td>
<td></td>
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<tr>
<td>rs167020, Chr7, SHH</td>
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<td></td>
<td></td>
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<tr>
<td>GG</td>
<td>314 (48.9)</td>
<td>5.7</td>
<td>0.99 (0.87 to 1.12)</td>
<td>0.8724</td>
</tr>
<tr>
<td>GA</td>
<td>264 (41.1)</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>64 (10.0)</td>
<td>5.0</td>
<td></td>
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</tbody>
</table>

*MST, median survival time, months.
†HR (95% CI). HR and p values calculated using multivariable-adjusted Cox regression under a log-additive genetic model, adjusting for age, sex, stage of disease, and the top four principal components of population stratification.
SNP, single nucleotide polymorphism.

Table 3  HRs and MSTs by genotype for significant tagSNPs \((p<5\times10^{-7})\) in PanScan survival GWAS

<table>
<thead>
<tr>
<th>SNP, chromosome, gene</th>
<th>No. (%)</th>
<th>MST*</th>
<th>HR (95% CI)†</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12362504, Chr11p15.4, SBF2</td>
<td></td>
<td></td>
<td>1.40 (1.23 to 1.58)</td>
<td>1.63×10^{-7}</td>
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<tr>
<td>TT</td>
<td>319 (49.7)</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>266 (41.4)</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>57 (8.9)</td>
<td>5.1</td>
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<td></td>
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<tr>
<td>rs981621, Chr18p11.21, C18orf11</td>
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<td></td>
<td>1.39 (1.23 to 1.57)</td>
<td>1.65×10^{-7}</td>
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<tr>
<td>AA</td>
<td>266 (41.4)</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>298 (46.4)</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>78 (12.2)</td>
<td>3.7</td>
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</tr>
<tr>
<td>rs16861827, Chr1p36.13, IGSF21</td>
<td></td>
<td></td>
<td>1.70 (1.39 to 2.09)</td>
<td>3.75×10^{-7}</td>
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<tr>
<td>CC</td>
<td>513 (79.9)</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>125 (19.5)</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4 (0.6)</td>
<td>2.5</td>
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</table>

*MST, median survival time, months.
†HR (95% CI). HR and p values calculated using multivariable-adjusted Cox regression under a log-additive genetic model, adjusting for age, sex, stage of disease, and the top four principal components of population stratification.
SNP, single nucleotide polymorphism.

Table 4  HRs and MSTs by rs10500715 T>G genotypes in the PanScan and ChinaPC studies

<table>
<thead>
<tr>
<th>rs10500715</th>
<th>No. (%)</th>
<th>MST*</th>
<th>HR (95% CI)†</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PanScan study</strong></td>
<td></td>
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<tr>
<td>TT</td>
<td>204 (31.8)</td>
<td>4.1</td>
<td>0.74 (0.66 to 0.84)</td>
<td>2.33×10^{-6}</td>
</tr>
<tr>
<td>GT</td>
<td>313 (48.8)</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>125 (19.4)</td>
<td>7.5</td>
<td></td>
<td></td>
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<tr>
<td><strong>ChinaPC study</strong></td>
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</tr>
<tr>
<td>TT</td>
<td>235 (64.7)</td>
<td>6.1</td>
<td>0.79 (0.65 to 0.97)</td>
<td>0.0216</td>
</tr>
<tr>
<td>GT</td>
<td>113 (31.1)</td>
<td>7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>15 (4.2)</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Meta-analysis</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TT</td>
<td>439 (43.7)</td>
<td>5.7</td>
<td>0.76 (0.68 to 0.84)</td>
<td>1.72×10^{-7}</td>
</tr>
<tr>
<td>GT</td>
<td>426 (42.4)</td>
<td>7.1</td>
<td></td>
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</tr>
<tr>
<td>GG</td>
<td>140 (13.9)</td>
<td>7.3</td>
<td></td>
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</tbody>
</table>

*MST, median survival time, months.
†HR (95% CI). HR and p values calculated using multivariable-adjusted Cox regression under a log-additive genetic model, adjusting for age, sex, stage of disease, and the top four principal components of population stratification.
SNP, single nucleotide polymorphism.
DISCUSSION

In this genome-wide interrogation of germline genetic variants associated with pancreatic cancer survival, we used a two-stage analytical approach that took advantage of two large GWAS of pancreatic cancer in two independent populations. In both the PanScan population that included cases of European descent and in a combined analysis with cases from China, SNPs in the SET binding factor 2 (SBF2) gene were associated with survival time among patients with pancreatic adenocarcinoma. With a p value <5×10^{-7}, this association is likely to be replicated in follow-up studies, although it did not reach p<5×10^{-8}, which is often cited as a threshold for genome-wide significance in GWAS of incident disease. We also identified two additional genetic loci associated with pancreatic cancer survival in the PanScan cohort, which were not significant at p<5×10^{-7} in the combined analysis. Several loci associated with incident pancreatic cancer in two prior PanScan studies were not associated with patient survival.

The SNPs most highly associated with pancreatic cancer survival in the PanScan population (rs12362504) and in the joint analysis (rs10500715) are located intronic to the SBF2 gene and are in moderate linkage disequilibrium (r^2=0.33) in the CEU population. SBF2 spans >500 kb and 40 exons on chromosome 11p15.4 and is highly conserved across eukaryotes. This gene encodes for a protein in the myotubularin family of lipid phosphatases and is also known as myotubularin-related protein-13 (MTMR13). Similar to the known tumour suppressor gene phosphatase and tensin homolog (PTEN), MTMR proteins function as phosphoinositide-3-phosphatases and antagonise the activity of specific classes of phosphatidylinositol-3-kinases. Although it contains an inactive phosphatase domain, SBF2 enhances the catalytic activity of and may alter the cellular localisation of MTMR2, a phosphatase-competent member of the MTMR family.

Notably, mutations in either SBF2 or MTMR2 lead to the development of Charcot-Marie-Tooth disease type 4B (CMT4B), an autosomal recessive disorder characterised by severe demyelinating peripheral neuropathy. The known mutations in human SBF2 lead to a shortened or truncated protein, and mice genetically engineered for loss of murine SBF2 develop peripheral neuropathy similar to that seen in humans with CMT4B. The pathogenic mechanisms appear related to altered membrane trafficking of 3-phosphoinositides within nerve-supporting Schwann cells, due to malfunctioning of the SBF2–MTMR2 complex. Importantly, in other cell types, SBF2 and MTMR2 appear to influence the sorting and degradation of cell surface receptors, such as the epidermal growth factor receptor, with resultant alterations in downstream signalling. For the SNPs identified in SBF2 in the current study, we did not identify alterations in expression of SBF2 or nearby genes using a publically available eQTL database. Further investigation will be necessary to determine the causative one or more SNPs marked by the single nucleotide changes identified in the current study, and the functional impact of such changes.

Recently, two GWAS have implicated SBF2 as a susceptibility locus for circulating lipoproteins in European populations and human stature in European and Chinese populations. The most strongly associated SNPs from these studies were rs7938647 with circulating high density lipoprotein (HDL) and rs10734652 with stature, also located within introns of SBF2.
The most strongly associated SNP in our combined analysis, rs10500715, is in moderate LD with rs7938647 and rs10734652, with $r^2$ values of 0.40 and 0.56, respectively in the PanScan cohort. Interestingly, pancreatic cancer incidence or mortality has been associated with height, obesity and metabolic derangements related to insulin resistance.39–42

In the PanScan population, variants at chromosomes 18p11.21 and 1p36.13 were also associated with survival. However, these associations were no longer significant in the combined analysis with cases from the ChinaPC study. Furthermore, the genes at these loci, chromosome 18 open reading frame 1 (C1orf81) and immunoglobulin superfamily member 21 (IGSF21), respectively, encode for proteins with unclear function. Additional studies of these variants in populations of European and Asian ancestry will be necessary to determine whether these loci are truly associated with survival of patients with pancreatic adenocarcinoma.

The current study has a number of important strengths. In the PanScan population, large numbers of cohort participants provided germline DNA at a baseline time point and were then followed prospectively for development of disease. Pancreatic cancer diagnoses were subsequently determined from notification near the time of diagnosis or review of cancer and death registries. Therefore, the full spectrum of cases were ascertained, in terms of disease aggressiveness and stage of disease, rather than only those patients well enough to be captured in case-control studies. This is of particular importance to studies of rapidly fatal diseases, such as pancreatic cancer, in which a better-prognosis population can result when subjects donate biologic samples after diagnosis. Furthermore, our study included a large number of pancreatic cancer cases with genome-wide SNP data, and these data originated from two well established GWAS with strict quality control procedures.18–20 We also pursued a two-stage design, with an initial analysis in PanScan participants and a subsequent combined analysis with ChinaPC participants, in an attempt to reduce the likelihood of false positive results.

Our study also has limitations. Among our participants, treatment programmes likely varied, and we could not control for differences in treatment as the PanScan cohorts generally did not collect this information. Nevertheless, chemotherapy and radiation have had only a modest impact on patient survival,3 and it is highly unlikely that treatment programmes varied systematically by germline genotype. As described, we also performed combined analyses with a second group of pancreatic cancer cases, drawn from a case-control study in China, to reduce the chance of false positive results. However, LD differs between Europeans and Asians, which can lead to false negative results in analyses that combine subjects of different race/ethnicity. Specifically, some loci may impact survival only in a particular race/ethnicity or the index signal may be best tagged by different polymorphisms in subjects of different race/ethnicity, phenomena demonstrated in studies of cancer risk.19 20 41 44 The difference in study design (nested prospective cohort study vs hospital-based case-control study) can also lead to false negative results in combined analyses due to recruitment of patients with dissimilar disease characteristics. However, all patients were known to have pancreatic adenocarcinoma, and all had available information on disease stage. Our top SNPs had significance levels of $p<5\times 10^{-7}$; further studies are necessary to replicate these findings in additional large patient cohorts. As is inherent in the GWAS design, we have identified loci associated with pancreatic cancer survival, but further work is necessary to investigate the biologic mechanisms by which polymorphisms at these loci impact survival. Although we examined published eQTL datasets, we did not identify known gene expression changes related to the most significant SNPs in SBF2. Finally, we utilised overall mortality data in our analyses, as opposed to pancreatic cancer-specific mortality. However, pancreatic cancer is a highly lethal malignancy with cure rate less than 5%, such that overall mortality is a good surrogate for cancer-specific mortality in patients with this disease.

In summary, we performed a genome-wide analysis of germline genetic variants and survival of patients with pancreatic adenocarcinoma. Our large study implicates the SBF2 locus on chromosome 11p15.4 as a genetic region associated with overall survival among these patients. These results further implicate altered membrane trafficking of 3-phosphoinositides in pancreatic cancer growth and progression. Additional large datasets are needed to evaluate germline genetic variants and survival in patients with this highly lethal malignancy.

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Pancreas


Genome-wide association study of survival in patients with pancreatic adenocarcinoma

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