ORIGINAL ARTICLE

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

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▶ Additional supplementary files are published online only. To view these files please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2012-303477).

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Revised 26 September 2012
Accepted 20 October 2012
Published Online First 24 November 2012

To cite: Wu C, Kraft P, Stolzenberg-Solomon R, *et al*. *Gut* 2014;**63**: 152–160.

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 ($p \leq 10^{-5}$) 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 ($p=1.63\times10^{-7}$), rs981621 ($p=1.65\times10^{-7}$) and rs16861827 ($p=3.75\times10^{-7}$), respectively. 131 SNPs with $p \leq 10^{-5}$ 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; $p=1.72\times10^{-7}$) 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.

Significance of this study

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; $p=1.72\times10^{-7}$) 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%.[1 2] Nevertheless, patient survival times are variable and only partially explained by traditional clinical and pathological features.[3] Accumulating evidence indicates that germline genetic variability can provide important prognostic information for patients with cancer.[4–6] 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.[7–9] This host-derived desmoplastic stroma actively engages with tumour cells and plays a critical role in promoting tumour development and progression.[10] 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.[8 11 12]

Prior studies of germline variants and pancreatic cancer survival have primarily focused on the evaluation of candidate genes in pathways of suspected importance.[13–17] However, 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.[18–20] 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.[18 21] 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 C250–C259 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 though 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.[18 21] 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 (IUCC) Tumor-Lymph nodes-Metastasis (TNM) staging[22] was converted to the above categories, with AJCC/IUCC 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,[23] 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).

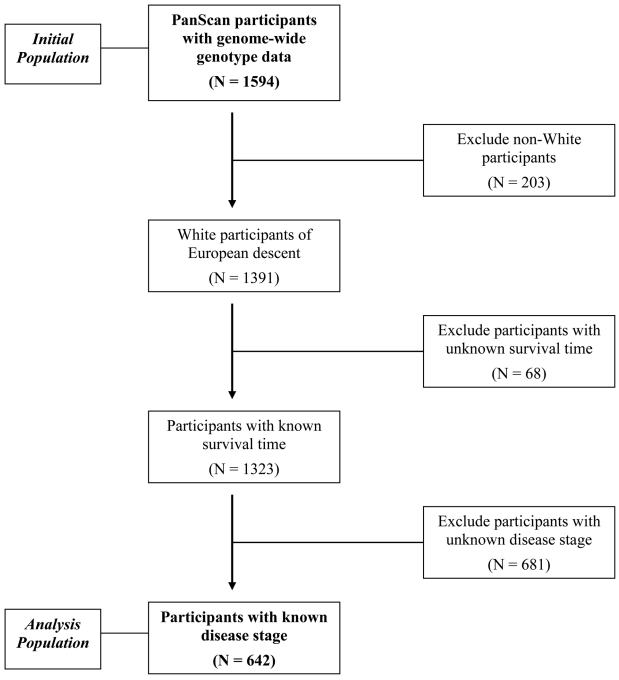

Figure 1 Flow chart of PanScan case eligibility.

ChinaPC population

The ChinaPC case–control GWAS has been described previously in detail.[20] In short, a GWAS was performed among pancreatic cancer cases and controls collected in an ongoing molecular epidemiological study of pancreatic cancer. These case subjects were recruited from the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing), and the Cancer Hospital, Fudan University (Shanghai), between 2000 and 2011. At recruitment, informed consent was obtained from each subject. The study was approved by the IRB of the Chinese Academy of Medical Sciences Cancer Institute. All case subjects had pancreatic ductal adenocarcinoma confirmed histopathologically or cytologically by at least two pathologists according to the WHO classification. Genomic DNA for GWAS analysis was isolated from peripheral blood lymphocytes at the time of diagnosis. Characteristics and clinical information including age, sex, race and tumour stage, were obtained from patients' medical records. As for the PanScan subjects, 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. Those subjects with available genome-wide genotype data, survival time and stage information were included in the analysis. From ChinaPC, 600 cases had genome-wide genotype data and survival times. After exclusion of 237 cases without stage information, 363 cases were available for analysis. MSTs were 7.1 months in both cases with available stage information and cases without such information.

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.[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.

Cis-eQTL analysis

To examine gene expression differences by genotype at our top locus, we inspected a publically available expression quantitative trait loci (eQTL) database.[24] The database includes 405 children of British descent, organised into 206 sibships, including 297 sib pairs and 11 half-sib pairs. Global gene expression in lymphoblastoid cell lines was measured using Affymetrix HG-U133 Plus 2.0 chips. All 405 children and their parents were genotyped using the Illumina Sentrix Human-1 Genotyping BeadChip. The number of principal components used was chosen to maximise the number of *cis*-eQTLs with genome-wide significance. Association analysis was applied with the FASTASSOC option implemented in MERLIN.

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.[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

Table 1 PanScan and ChinaPC patient characteristics and overall survival

	PanScan (N=642)			ChinaPC (N=363)			Pooled analysis (N=1005)		
	No. (%)	MST*	p Value†	No. (%)	MST*	p Value†	No. (%)	MST*	p Value†
Vital status									
Dead	609 (94.9)	5.9		334 (92.0)	7.1		943 (93.8)	6.1	
Alive	33 (5.1)			29 (8.0)			62 (6.2)		
Sex			0.4522			0.4207			0.9193
Male	239 (37.2)	5.1		218 (60.1)	6.9		457 (45.5)	6.0	
Female	403 (62.8)	6.3		145 (39.9)	7.0		548 (54.5)	6.4	
Age‡			0.0104			0.2627			0.0024
≤68 years	253 (39.4)	7.7		264 (72.7)	6.5		517 (51.4)	7.0	
>68 years	389 (60.6)	5.1		99 (27.3)	5.6		488 (48.6)	5.1	
Stage			<0.0001			<0.0001			<0.0001
Local disease	119 (18.5)	17.6		78 (21.5)	9.3		197 (19.6)	13.5	
Locally advanced disease	170 (26.5)	10.6		157 (43.2)	8.4		327 (32.5)	9.7	
Metastatic disease	353 (55.0)	3.2		128 (35.3)	4.6		481 (47.9)	3.8	

*MST, median survival time, months.
†p Values were calculated using the log-rank test.
‡Median age was 68 years among all cases.

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 time in the PanScan cohort ($p=0.0008$). However, the risk allele identified in CALGB 80303 was protective in the PanScan cohort. Comparing the survival time of patients with the TC genotype versus the TT genotype (referent) resulted in an HR of 3.3 (95% CI 2.1 to 5.1) in CALGB 80303,[26] and an HR of 0.64 (95% CI 0.50 to 0.83) in PanScan. We noted similar results when including only subjects with metastatic disease in the PanScan cohort (data not shown). In CALGB 80303, results were similar after stratification by treatment arm.[25] Although further investigation is required, differences in patient populations between a large, randomised phase III trial and participants from prospective cohorts may have contributed to the discordant results.

Genetic variants associated with patient survival in genome-wide genotyping

The Manhattan plot for the GWAS of pancreatic cancer survival in PanScan is shown in online supplementary figure S1. We identified three independent regions most associated with survival on chromosomes 11p15.4 (four SNPs), 18p11.21 (12 SNPs) and 1p36.13 (one SNP), which were tagged by rs12362504 (HR 1.40; 95% CI 1.23 to 1.58; $p=1.63\times10^{-7}$), rs981621 (HR 1.39; 95% CI 1.23–1.57; $p=1.65\times10^{-7}$) and rs16861827 (HR 1.70; 95% CI 1.39 to 2.09; $p=3.75\times10^{-7}$), 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 $p\leq10^{-5}$ 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. In the meta-analysis of the two studies, we observed an HR of 0.76 (95% CI 0.68 to 0.84) with a p value of 1.72×10^{-7} (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 2 HRs and MSTs by genetic variants previously associated with incident pancreatic cancer in PanScan

SNP, chromosome, gene	No. (%)	MST*	HR (95% CI)†	p Value†
rs9543325, Chr13, none			1.09 (0.96 to 1.24)	0.1769
TT	207 (32.2)	6.1		
TC	331 (51.6)	6.4		
CC	104 (16.2)	5.1		
rs3790844, Chr1, *NR5A2*			1.04 (0.90 to 1.21)	0.5699
AA	414 (64.5)	5.4		
AG	203 (31.6)	6.6		
GG	25 (3.9)	6.9		
rs401681, Chr5, *CLPTM1L-TERT*			1.06 (0.95 to 1.19)	0.2916
CC	189 (29.4)	6.5		
CT	316 (49.2)	5.2		
TT	137 (21.4)	7.7		
rs505922, Chr9, *ABO*			1.05 (0.93 to 1.18)	0.4590
TT	224 (34.9)	5.1		
TC	312 (48.6)	5.3		
CC	106 (16.5)	8.0		
rs167020, Chr7, *SHH*			0.99 (0.87 to 1.12)	0.8724
GG	314 (48.9)	5.7		
GA	264 (41.1)	6.1		
AA	64 (10.0)	5.0		

*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.

rs10500715 genotype was not statistically significantly 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 8.64×10^{-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 3 HRs and MSTs by genotype for significant tagSNPs ($p<5\times10^{-7}$) in PanScan survival GWAS

SNP, chromosome, gene	No. (%)	MST*	HR (95% CI)†	p Value†
rs12362504, Chr11p15.4, *SBF2*			1.40 (1.23 to 1.58)	1.63×10^{-7}
TT	319 (49.7)	6.9		
TC	266 (41.4)	4.6		
CC	57 (8.9)	5.1		
rs981621, Chr18p11.21, *C18orf1*			1.39 (1.23 to 1.57)	1.65×10^{-7}
AA	266 (41.4)	8.1		
AG	298 (46.4)	5.0		
GG	78 (12.2)	3.7		
rs16861827, Chr1p36.13, *IGSF21*			1.70 (1.39 to 2.09)	3.75×10^{-7}
CC	513 (79.9)	6.5		
CT	125 (19.5)	3.8		
TT	4 (0.6)	2.5		

*MST, median survival time, months.
†HR (95% CI). HR and p value 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.
GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

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

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

*MST, median survival time, months.
†HR (95% CI). HR and p value 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.

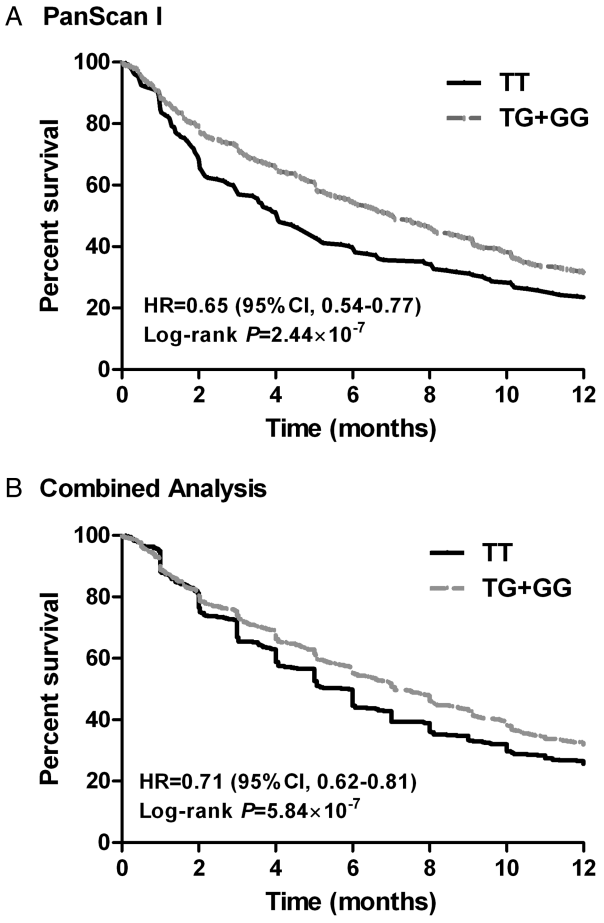

Figure 2 Kaplan–Meier curves by rs10500715 T>G genotypes using a dominant model in the PanScan and combined analyses.

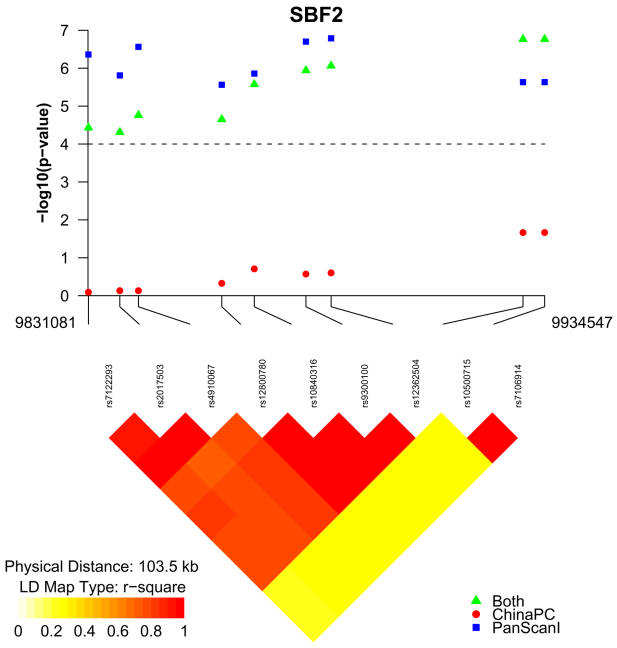

Figure 3 Association with survival and linkage disequilibrium (LD) of single nucleotide polymorphisms at the *SBF2* gene locus. Association results are shown in the top panel for the joint analysis (green triangles), PanScan I (blue squares) and ChinaPC (red circle). The LD plot was based on genotypes in the PanScan I cohort. Locations are from NCBI Genome Build 36.

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 $<5\times10^{-7}$, this association is likely to be replicated in follow-up studies,[26] although it did not reach $p<5\times10^{-8}$, 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 $p<5\times10^{-7}$ 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.[27 28] 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*),[29] MTMR proteins function as phosphoinositide-3-phosphatases and antagonise the activity of specific classes of phosphatidylinositol-3-kinases.[30] 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.[31]

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.[32 33] The known mutations in human *SBF2* lead to a shortened or truncated protein,[33 34] and mice genetically engineered for loss of murine SBF2 develop peripheral neuropathy similar to that seen in humans with CMT4B.[35 36] 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.[37] 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.[30] 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[28] and human stature in European and Chinese populations.[38] 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* (*C18orf1*) and *immunoglobin 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 43 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 $p<5\times10^{-7}$; 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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Acknowledgements The authors would like to thank the participants and staff of all of the cohorts studies involved in this analysis. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators is available. We are grateful for the contributions of Drs. Yu-Tang Gao and Yong-Bing Xiang in these two cohort studies. The studies would not be possible without the continuing support and devotion from the study participants and staff of the SMHS and SWHS. The authors thank Drs. Christine Berg and Philip Prorok, Division of Cancer Prevention, National Cancer Institute, the Screening Center investigators and staff of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, Susan Yurgalevitch and staff at Westat, Inc for their assistance with survival data abstraction, and Craig Williams, Michael Furr and staff at Information Management Services, Inc. Most importantly, we acknowledge the study participants for their contributions to making this study possible. For the EPIC cohorts, all coauthors coordinated the initial recruitment and management of the studies. All authors contributed to the final paper. The authors thank all of the participants who took part in this research and the funders and support and technical staff who made this study possible. The authors express their appreciation to the participants of the CLUE II cohort, and thank the staff at the George W. Comstock Center for Public Health Research and Prevention for their dedication and contributions to the study: Judy Hoffman-Bolton, Clara Krumpe, Kitty Spoonire and Betty Miner. Cancer incidence data have been provided by the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Department of Health and Mental Hygiene, 201 W. Preston Street, Room 400, Baltimore, MD 21201, www.fha.state.md.us/cancer/ registry/, 410-767-4055. The authors thank all of the men and women in the Cancer Prevention Study II Nutrition Cohort for their many years of dedicated participation in the study.

Contributors Study concept and design: CW, PK, CSF, DL, BMW. Acquisition of data: all coauthors. Analysis and interpretation of data: CW, PK, CSF, DL, BMW. Drafting of the manuscript: CW, PK, CCF, DL, BMW. Critical revision of the manuscript for important intellectual content: all coauthors. Statistical analysis: CW, ES, PM, MX, PK. Obtained funding: PK, RS-S, PH, CCF, DL, BMW. Administrative, technical or material support: CW, PK, RS-S, MB, GST, CCF, DL, BMW. Study supervision: PK, CSF, DL, BMW.

Funding The NYU Women's Health Study is supported by research grants R01CA034588, R01CA098661, center grant P30CA016087 from the NCI and center grant ES000260 from the National Institute of Environmental Health Sciences. The WHI programme is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services through contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221. PHS was supported by grants CA 97193, CA 34944, CA 40360, HL 26490, and HL 34595 from the National Institutes of Health (Bethesda, MD). The NHS, HPFS and WHS at Harvard were supported by the National Cancer Institute, National Institutes of Health (grants P01 CA87969, P01 CA55075, P50 CA127003, R01 CA124908). BMW was supported by NCI K07 CA140790, an American Society of Clinical Oncology Career Development Award, Howard Hughes Medical Institute Early Career Physician-Scientist Award, and the Lustgarten Foundation for Pancreatic Cancer Research. The Shanghai Men's Health Study was supported by the National Cancer Institute extramural research grant (R01 CA82729). The Shanghai Women's Health Study was supported by the National Cancer Institute extramural research grant (R37 CA70867) and, partially for biological sample collection, by the Intramural Research Program of National Cancer Institute (Division of Cancer Epidemiology and Genetics). PLCO was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, DHHS. The ATBC Study was supported by US Public Health Service contracts N01-CN-45165, N01-RC-45035, N01-RC-37004, and HHSN261201000006C from the National Cancer Institute, Department of Health and Human Services, and by funding from the Intramural Research Program of the National Cancer Institute. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue contre le Cancer, Société 3M, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Medicale (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); Ministry of Health and Social Solidarity, Stavros Niarchos Foundation and Hellenic Health Foundation (Greece); Italian Association for Research on Cancer (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council (UK). CLUE II was supported by National Institute of Aging grant (5U01AG018033) and National Cancer Institute grants (CA105069, CA73790). We acknowledge the State of Maryland, the Maryland Cigarette Restitution Fund, and the National Program of Cancer Registries (NPCR) of the Centers for Disease Control and Prevention (CDC) for the funds that helped support the availability of the cancer registry data. The Cancer Prevention Study II Nutrition Cohort is supported by the American Cancer Society. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organisations imply endorsement by the US Government.

Competing interests None.

Patient consent Obtained.

Ethics approval The Special Studies Institutional Review Board (SSIRB) of the National Cancer Institute approved the pooled PanScan study.

Provenance and peer review Not commissioned; externally peer reviewed.

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

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Gut 2014 63: 152-160 originally published online November 24, 2012
doi: 10.1136/gutjnl-2012-303477