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Genome-Wide Association Study of Survival in Patients with Pancreatic Adenocarcinoma

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Abstract

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.

Design—We analyzed overall survival in relation to single nucleotide polymorphisms (SNPs) among 1,005 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 ($P = 10^{-5}$) 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 ($P=1.63\times 10^{-7}$), rs981621 ($P=1.65\times 10^{-7}$), and rs16861827 ($P=3.75\times 10^{-7}$), respectively. One-hundred thirty-one SNPs with $P = 10^{-5}$ 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; $P=1.72\times 10^{-7}$) on chromosome 11p15.4, which is intronic to the *SET binding factor 2* (*SBF2*) gene. The hazard ratio (95% CI) for death was 0.74 (0.66–0.84) in PanScan I, 0.79 (0.65–0.97) in ChinaPC, and 0.76 (0.68–0.84) in the joint analysis.

Conclusion—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.

Keywords

Pancreatic cancer; GWAS; single nucleotide polymorphism; *SET binding factor 2*

INTRODUCTION

Pancreatic cancer is a major cause of cancer-related death across the globe, and five-year overall survival is approximately five percent.[1–2] Nevertheless, patient survival times are variable and only partially explained by traditional clinical and pathologic 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 tumor-host interactions. A defining feature of pancreatic adenocarcinoma is the recruitment of host cells, including fibroblasts, immune cells, and endothelial cells, which surround the tumor in a dense stromal matrix.[7–9] This host-derived desmoplastic stroma actively engages with tumor cells and plays a critical role in promoting tumor development and progression.[10] Notably, laboratory studies suggest that treatments which modify the interaction of pancreatic cancer cells with its 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 upon our relatively incomplete understanding of tumor and host biology. In contrast, genome-wide approaches are available that allow a more comprehensive evaluation of germline genetic variants that is not reliant upon *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 1,000 cases of pancreatic adenocarcinoma.

MATERIALS AND METHODS

PanScan Population

The Pancreatic Cancer Cohort Consortium (PanScan) genome wide association study (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 United

States and Europe, including Alpha-Tocopherol Beta-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 Professional's 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 Women's Health Study (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 tumors (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.[18, 21] Cohorts obtained consent from participants and approval from their Institutional Review Board (IRB). The Special Studies Institutional Review Board (SSIRB) 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 harmonized 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, (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) 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 tumors as localized, 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 and Supplementary Table 1). Median survival times were slightly shorter among the full population of patients with survival information versus the subset of patients with stage information (Supplemental Table 1). Overall, median survival time (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).

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 Cancer Hospital, Chinese Academy of Medical Sciences (Beijing), and Cancer Hospital, Fudan University (Shanghai), between 2000 and 2011. At recruitment, informed consent was obtained from each subject. The study was approved by the Institutional Review Board 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 World Health Organization 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 tumor 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 harmonized 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, (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. Median survival times were 7.1 months in both those cases with available stage information and those cases without such information.

Genotype and Imputation Analysis

Genotypes of patients in PanScan and ChinaPC were generated using the HumanHap550 chip (Illumina, San Diego, CA) and Affymetrix GeneChip Human Mapping 6.0 set (Affymetrix, Santa Clara, CA), respectively. The procedures of genotyping and quality control in each genome-wide association study have been described previously. [18, 20] In brief, samples with <98% completion and 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 (PCA) 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 CEU and CHB+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 eQTL database.[24] The database includes 405 children of British descent, organized 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 maximize 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 $P < 10^{-5}$ found in PanScan were advanced to a combined analysis in patients independently recruited from the ChinaPC study. To summarize 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 linkage disequilibrium 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 1,005 patients with pancreatic cancer, 19.6% with localized disease, 32.5% with locally advanced disease, and 47.9% with metastatic disease. As expected, stage was strongly associated with survival in both studies ($P < 0.0001$); median survival time (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 were 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 randomized 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 a HR of 3.3 (95% CI, 2.1–5.1) in CALGB 80303,[26] while it resulted in a HR of 0.64 (95% CI, 0.50–0.83) 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, randomized 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 Supplementary Figure 1. 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, 1.23–1.58; $P=1.63\times 10^{-7}$), rs981621 (HR, 1.39; 95% CI, 1.23–1.57; $P=1.65\times 10^{-7}$) and rs16861827 (HR, 1.70; 95% CI, 1.39–2.09; $P=3.75\times 10^{-7}$), respectively (Table 3 and Supplemental Table 2). 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 Supplementary Figure 2. 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 (Supplementary Table 2). 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 a HR for death in an additive model of 0.74 (95% CI, 0.66–0.84) and 0.79 (95% CI, 0.65–0.97), respectively. In the meta-analysis of the two studies, we observed a HR of 0.76 (95% CI, 0.68–0.84) 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 a HR of 0.80 (95% CI, 0.64–1.01) in patients with localized disease, HR of 0.78 (95% CI, 0.65–0.93) in those with locally advanced disease, and HR of 0.81 (95% CI, 0.70–0.92) in those with metastatic disease. 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 expression quantitative trait loci (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.

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 $<5\times 10^{-7}$, this association is likely to be replicated in follow-up studies,[26] although it did not reach $P<5\times 10^{-8}$, 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 $P < 5 \times 10^{-7}$ 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. [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 tumor suppressor gene *phosphatase and tensin homolog (PTEN)*, [29] MTMR proteins function as phosphoinositide- 3-phosphatases and antagonize 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 localization 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 characterized 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 (EGFR), with resultant alterations in downstream signaling. [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 genome wide association studies 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 HDL and rs10734652 with stature, also located within introns of *SBF2*. The most strongly associated SNP in our combined analysis, rs10500715, is in moderate linkage disequilibrium 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 *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-controls 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 programs 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 programs 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, linkage disequilibrium 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 versus 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 utilized 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 five percent, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance of this study

What is already known about the subject?

- ▶ Five-year overall survival of patients with pancreatic cancer is approximately five percent.
- ▶ Germline genetic variability can provide important prognostic information for patients with cancer.
- ▶ Genome-wide association studies (GWAS) 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 GWAS 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 a HR for death in an additive model of 0.76 (95% CI, 0.68–0.84; *P*-value, 1.72×10^{-7}) which was similar in European and Chinese populations and by disease stage.

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 tumorigenesis and further implicate altered membrane trafficking of 3-phosphoinositides in pancreatic cancer growth and progression.

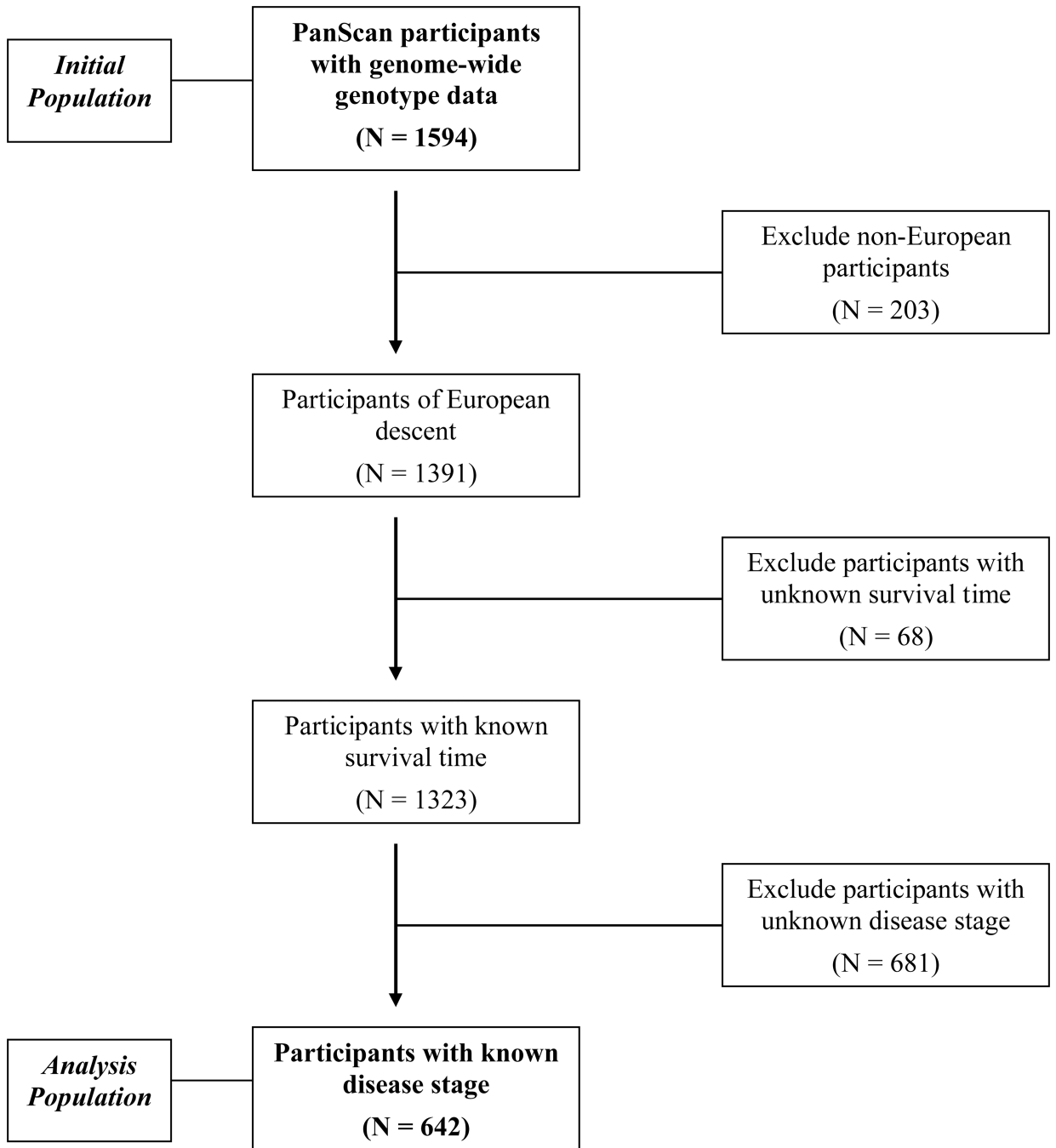
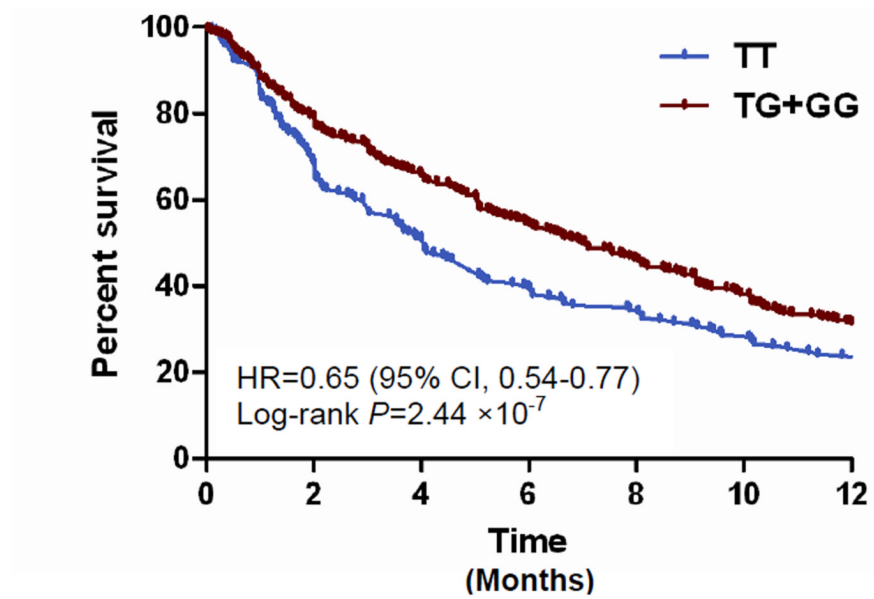


Figure 1.
Flow chart of PanScan case eligibility

(A) PanScan I



(B) Combined Analysis

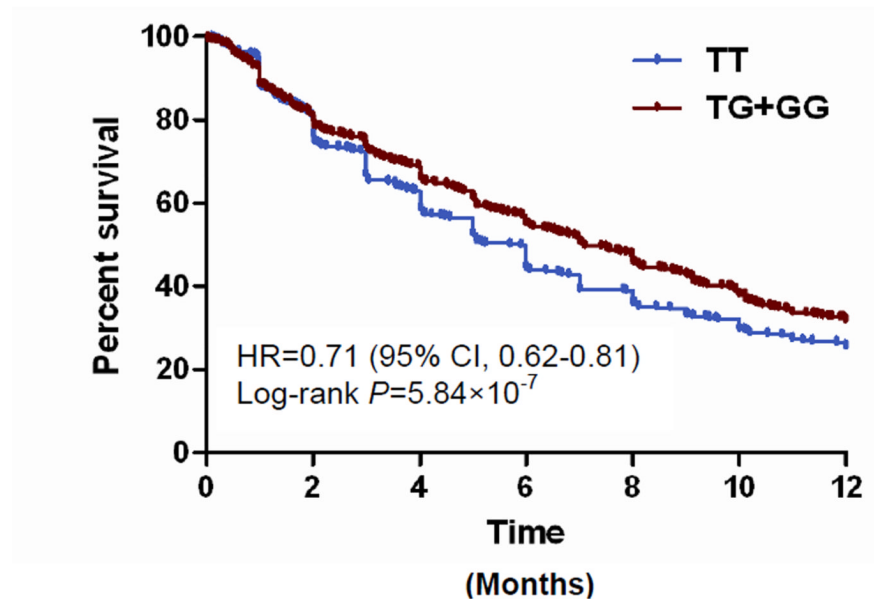


Figure 2.
Kaplan-Meier curves by rs10500715 T>G genotypes using a dominant model in the PanScan and Combined Analyses
Abbreviations: CI, confidence interval; HR, hazard ratio

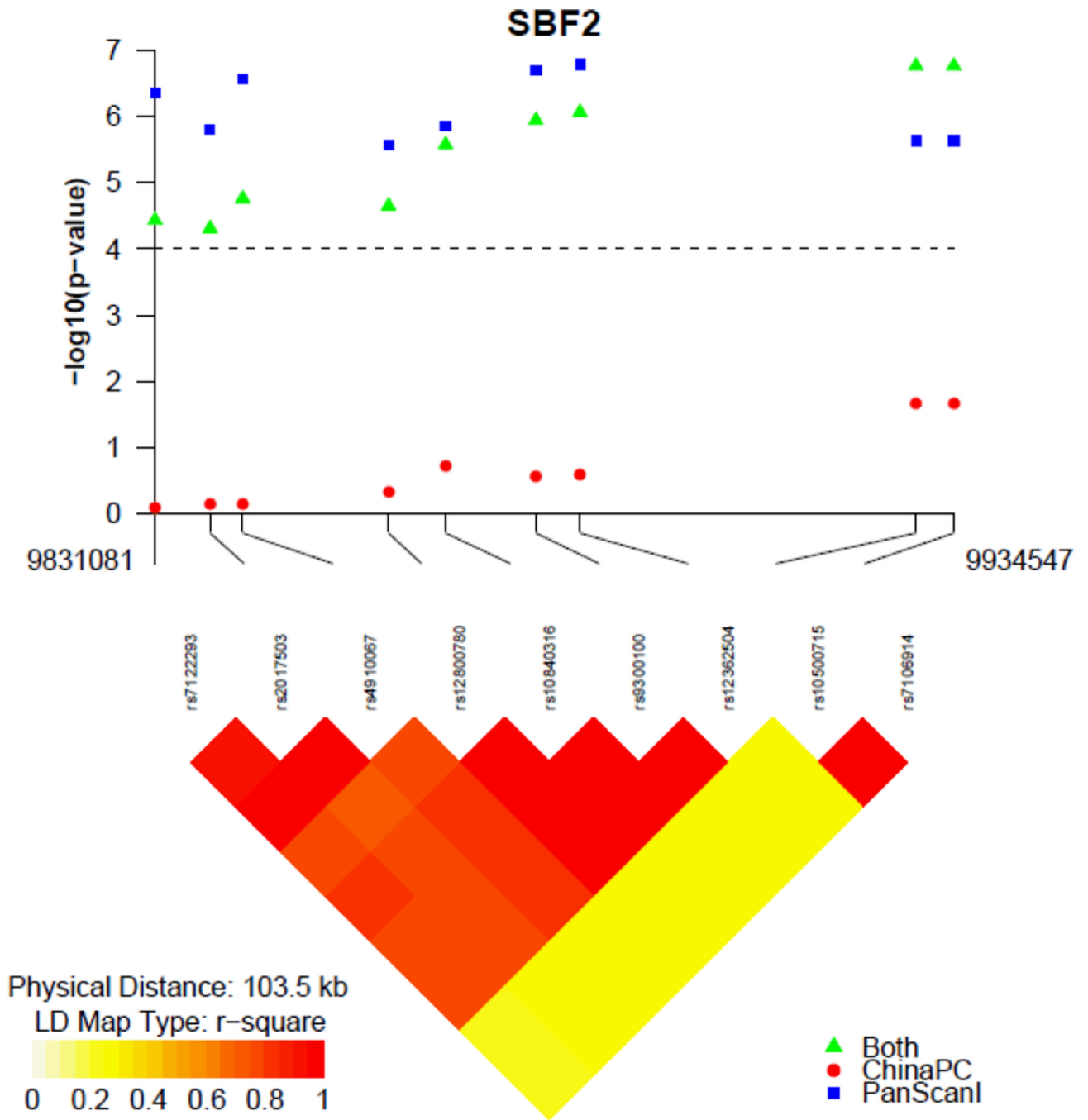


Figure 3. Association with survival and linkage disequilibrium of single nucleotide polymorphisms at the *SBF2* gene locus
 Association results are shown in the top panel for the joint analysis (green triangles), PanScan I (blue squares) and ChinaPC (red circle). The linkage disequilibrium plot was based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

Table 1

PanScan and ChinaPC patient characteristics and overall survival

	PanScan (N=642)			ChinaPC (N=363)			Pooled Analysis (N=1005)		
	No (%)	MST*	P [†]	No (%)	MST*	P [†]	No (%)	MST*	P [†]
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

Table 2

Hazard ratios and median survival times by genetic variants previously associated with incident pancreatic cancer in PanScan

SNP, Chromosome, Gene	No. (%)	MST*	HR (95% CI) [†]	P [†]
rs9543325 , Chr13, None			1.09 (0.96–1.24)	0.1769
TT	207 (32.2)	6.1		
TC	331 (51.6)	6.4		
CC	104 (16.2)	5.1		
rs3790844 , Chr1, <i>NR5A2</i>			1.04 (0.90–1.21)	0.5699
AA	414 (64.5)	5.4		
AG	203 (31.6)	6.6		
GG	25 (3.9)	6.9		
rs401681 , Chr5, <i>CLPTMIL-TERT</i>			1.06 (0.95–1.19)	0.2916
CC	189 (29.4)	6.5		
CT	316 (49.2)	5.2		
TT	137 (21.4)	7.7		
rs505922 , Chr9, <i>ABO</i>			1.05 (0.93–1.18)	0.4590
TT	224 (34.9)	5.1		
TC	312 (48.6)	5.3		
CC	106 (16.5)	8.0		
rs167020 , Chr7, <i>SHH</i>			0.99 (0.87–1.12)	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) = hazard ratio (95% confidence interval). 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

Table 3

Hazard ratios and median survival times by genotype for significant tagSNPs ($P < 5 \times 10^{-7}$) in PanScan survival GWAS.

SNP, Chromosome, Gene	No. (%)	MST*	HR (95% CI) [†]	P [†]
rs12362504, Chr11p15.4, <i>SBF2</i>			1.40 (1.23–1.58)	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, <i>CI8orf1</i>			1.39 (1.23–1.57)	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, <i>IGSF21</i>			1.70 (1.39–2.09)	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) = hazard ratio (95% confidence interval). 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

Table 4

Hazard ratios and median survival times by rs10500715 T>G genotypes in the PanScan and ChinaPC studies

	rs10500715		
	No. (%)	MST*	HR (95% CI) [†]
PanScan Study			0.74 (0.66–0.84)
TT	204 (31.8)	4.1	
GT	313 (48.8)	6.6	
GG	125 (19.4)	7.5	
ChinaPC Study			0.79 (0.65–0.97)
TT	235 (64.7)	6.1	
GT	113 (31.1)	7.9	
GG	15 (4.2)	5.4	
Meta-Analysis			0.76 (0.68–0.84)
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) = hazard ratio (95% confidence interval). 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