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Imaging, Diagnosis, Prognosis

A Replication Study and Genome-Wide Scan of Single-Nucleotide Polymorphisms Associated with Pancreatic Cancer Risk and Overall Survival

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Abstract

Purpose: To explore the effects of single-nucleotide polymorphisms (SNP) on pancreatic cancer risk and overall survival (OS).

Experimental Design: The germ line DNA of 531 pancreatic cancer cases and 305 healthy controls from a hospital-based study was genotyped at SNPs previously reported to be associated with pancreatic cancer risk or clinical outcome. We analyzed putative risk SNPs for replication of their reported effects on risk and tested for novel effects on OS. Similarly, we analyzed putative survival-associated SNPs for replication of their reported effects on OS and tested for novel effects on risk. Finally, we conducted a genome-wide association study (GWAS) of OS using a subset of 252 cases, with two subsequent validation sets of 261 and 572 patients, respectively.

Results: Among seven risk SNPs analyzed, two (rs505922 and rs9543325) were associated with risk ($P < 0.05$). Among 24 survival-associated SNPs analyzed, one (rs9350) was associated with OS ($P < 0.05$). No putative risk SNPs or putative survival-associated SNPs were found to be associated with OS or risk, respectively. Furthermore, our GWAS identified a novel SNP [rs1482426, combined stage I and II, $P = 1.7 \times 10^{-6}$, per-allele HR, 1.74; 95% confidence interval (CI), 1.38–2.18] to be putatively associated with OS.

Conclusions: The effects of SNPs on pancreatic cancer risk and OS were replicated in our study, although further work is necessary to understand the functional mechanisms underlying these effects. More importantly, the putative association with OS identified by GWAS suggests that GWAS may be useful in identifying SNPs associated with clinical outcome in pancreatic cancer. *Clin Cancer Res; 18(14); 3942–51. ©2012 AACR.*

Introduction

Pancreatic adenocarcinoma is a rapidly fatal disease with poor long-term prognosis; the 5-year survival rate is estimated to be less than 6%. Despite its relatively low incidence (3% of new cancer cases in the United States) and recent advances in surgical treatment, pancreatic cancer remains the fourth leading cause of cancer mortality in the United States (1, 2). Thus, intense efforts are underway to develop novel strategies for both screening and treatment.

One approach to this challenge has been the identification of inherited genetic variations that are involved with susceptibility to pancreatic cancer. Recently, genome-wide association studies (GWAS) conducted by the PanScan consortium identified 4 loci on chromosomes 9q34 (*ABO*), 13q22, 1q32 (*NR5A2*), and 5p15 (*CLPTM1L-TERT*) associated with increased risk (3, 4). A similar study conducted in the Japanese population identified 3 additional risk loci on chromosomes 6p26, 12p11 (*BICD1*), and 7q36 (*DPP6*; ref. 5). Independent replication of these findings is a critical step toward understanding their functional significance. However, while 3 of the associations reported by PanScan were moderately replicated in the Japan-based study, there have been no reported attempts to independently replicate the risk loci identified in the Japan-based study.

There is also evidence that inherited genetic variations may influence the clinical outcome of pancreatic cancer. In several recent studies, a candidate gene approach was used to identify SNPs within different cellular pathways (e.g., DNA damage repair and mitotic regulation) that are associated with overall survival (OS; refs. 6–17). These findings could potentially identify novel therapeutic targets and/or strategies. Yet because the majority of these associations

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Translational Relevance

The identification of inherited genetic variations that influence susceptibility to and the clinical outcome of pancreatic cancer may yield novel insights about the development and progression of this highly fatal disease. In this study, we provide supporting evidence that genetic variants located on chromosomes 9q34 (*ABO*) and 13q22 are involved in pancreatic cancer susceptibility and that a variant within the *EXO1* gene may be involved in overall survival (OS) from pancreatic cancer. More importantly, using genome-wide methods, we putatively identify a novel locus on chromosome 12q21 associated with OS. Functional analysis of these regions may lead to the development of new strategies for risk prediction and treatment of pancreatic cancer.

have not been replicated in independent patient groups, their generalizability is still unknown. Furthermore, the effects on OS contributed by genetic variations in unexplored regions of the genome are not well-understood.

Here, we addressed these issues by conducting a combined replication and discovery study of genetic variants associated with pancreatic cancer risk and OS within a multi-ethnic, hospital-based case–control group. First, we attempted to replicate the risk associations of SNPs identified by the PanScan or Japan-based pancreatic cancer risk GWAS, and we assessed these SNPs for association with OS. Next, we attempted to replicate the OS associations of SNPs reported by previous candidate gene studies of pancreatic cancer clinical outcome and evaluated these SNPs for association with risk. Finally, we conducted a GWAS to identify novel variants associated with OS.

Materials and Methods

Study population and sample collection

Memorial Sloan-Kettering Cancer Center study. Participants were part of an ongoing hospital-based case–control project conducted in conjunction with the Familial Pancreatic Tumor Registry (FPTR) at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY). Patients were eligible if they were 21 years or older, spoke English, and had pathologically or cytologically confirmed adenocarcinoma of the pancreas. Patients were recruited between June 2003 and July 2009 from the surgical and medical oncology clinics at MSKCC at the time of their initial diagnosis or during follow-up. Controls were spouses of patients or visitors accompanying patients with other diseases, had the same age and language eligibility requirements as the cases, had no personal history of cancer (except for non-melanoma skin cancer), and were not blood relatives of the cases. A total of 531 cases and 305 controls from MSKCC participated in our analyses. The participation rate among approached and eligible individuals was 76% among cases and 56% among controls. The study was approved by the MSKCC Institutional Review Board, and all enrolled participants signed informed consent.

Participants provided a blood or buccal (mouthwash or saliva) sample to the MSKCC FPTR research study assistant and completed risk factor and family history questionnaires administered by the research study assistant in person or via telephone. Biospecimens were subsequently delivered for genomic DNA extraction and banking to the Molecular Epidemiology Laboratory. DNA was isolated from mouthwash specimens using the Puregene DNA Purification Kit (Qiagen, Inc.), from saliva samples with the Oragene saliva kits (DNA Genotek), and from whole blood using the Gentra Puregene Blood Kit (Qiagen Inc.). DNA samples were hydrated in 1× TE buffer.

Mayo Clinic study. Participants from the Mayo Clinic (Rochester, MN) were identified and recruited as described previously (18). A subset of patients was selected for genotyping on the basis of the following criteria: (i) consented to enroll in the Mayo Clinic SPORE registry; (ii) diagnosed with histopathologically confirmed pancreatic adenocarcinoma (not invasive intraductal papillary mucinous neoplasm or other histology); and (iii) a risk factor questionnaire had been completed. In total, 572 genotyped patients from Mayo Clinic with non-Hispanic Caucasian ethnicity were included in our analyses.

Whole-genome SNP array genotyping and quality control

MSKCC study. Genomic DNA samples from 263 cases were each genotyped on the Illumina CNV370 SNP Bead Array (either the Illumina CNV370-Duo or Illumina CNV370-Quad) at the Genomics Core Laboratory of MSKCC according to the manufacturer's protocol. The array contains probes for 351,496 SNPs. Genotype calls were made in the Illumina BeadStudio software package and exported to PLINK (version 1.07; ref. 19) for processing.

After genotyping, quality control was first applied by removing SNPs with call rates less than 99% or minor allele frequency (MAF) less than 1% in the genotyped cases. In addition, we excluded SNPs that showed extreme deviation from Hardy–Weinberg equilibrium (HWE) in the genotyped cases. Because we intended to conduct a case-only survival analysis and deviation from HWE in cases may be a sign of a true risk SNP, we chose a less stringent approach by excluding SNPs with HWE P value $< 1 \times 10^{-20}$, in the genotyped cases. Identify-by-descent (IBD) analysis was conducted in PLINK to confirm that none of the genotyped cases were blood relatives. Furthermore, none of the genotyped cases were excluded from analysis on the basis of sample-level quality control metrics (overall genotyping rate $\geq$90%; the minimum genotyping rate was found to be 91.5%). Thus, after quality control, 301,250 SNPs (MAF $\geq$ 1%) were available for downstream statistical analysis in 263 cases.

Mayo Clinic study. Whole-genome SNP genotyping of pancreatic cancer cases from the Mayo Clinic study was conducted as previously described (3, 4). In this study, we

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extracted SNP rs1482426 genotypes from the Mayo Clinic data set to conduct stage III analysis in the GWAS.

Mass spectrometry–based SNP genotyping and quality control

We conducted matrix-assisted laser desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry–based SNP genotyping using Sequenom iPlex Gold assays (Sequenom, Inc.). iPlex assays were designed and multiplexed using the MassARRAY Assay Designer software package (Sequenom Inc., version 4.0). In total, we designed 3 multiplexes composed of 64 individual assays targeting risk- and survival-associated SNPs selected for replication study as well as novel survival-associated SNPs identified by GWAS.

PCR amplification primers and single-base extension (SBE) oligonucleotides were synthesized for each SNP assay according to the designed specifications (Integrated DNA Technologies, Inc.). PCR primers within the same multiplex were mixed to a final concentration of 500 nmol/L each. SBE oligonucleotide concentrations were optimized according Sequenom's recommendations. Before use, all DNA samples were whole-genome amplified (WGA) using the Illustra GenomiPhi v2 DNA Amplification Kit (GE Healthcare), following manufacturer's recommendations. The WGA reactions were then diluted by adding 120 μL of reduced TE buffer; an additional 2-fold dilution of the DNA was made as it empirically improved genotyping performance in our hands.

Genotyping reactions were conducted in batches of 384-well plates on the basis of Sequenom recommendations. Briefly, for PCR amplification, template DNA was added to each well along with a master mix containing amplification primers, dNTPs, and HotStar Taq DNA polymerase PCR reagents (Qiagen, Inc.). After PCR cycling, unincorporated dNTPs were neutralized by treatment with shrimp alkaline phosphatase (SAP) supplied by the iPlex Gold reagent kit (Sequenom Inc.). SAP treatment was then followed by SBE reactions using the iPlex Gold Enzyme and Reagent Kit (Sequenom Inc.). Subsequent steps, including reaction desalting, spotting, and SpectroCHIP analysis, were conducted by the MSKCC Sequenom Facility according to protocol. Mass spectra were analyzed with the Typer Software Package (Sequenom Inc., version 4.0) to make genotype calls. Each individual batch of 384-well plates was reviewed for quality control, and the data were exported to PLINK. Batch results were merged together in PLINK. Further quality control was applied by removing 6 SNPs with call rates less than 90% and individuals with overall genotyping rates less than 90%.

A subset of individuals ($n = 263$ cases and 203 controls) who were genotyped by iPlex assays was also genotyped by array-based methods (described above). Thus, to assess the overall quality of our iPlex genotyping experiment, we analyzed the results for concordance with array-based calls. In the analysis of 39 SNPs that were present on the array and in the iPlex experiment, we found the overall concordance rate to be more than 99.7%.

Statistical methods

Risk association. SNPs were analyzed for association with pancreatic cancer risk by use of a logistic regression model whereby genotypes were coded as either 0, 1, or 2 depending on the number of minor alleles carried by an individual; race, age (years), and gender (male or female) were included as covariates. This model estimated a log-additive effect of each SNP on disease risk per additional minor allele. Statistical significance was determined using a 1 degree of freedom (*df*) Wald test. For the replication study of previously reported risk SNPs, power analysis was conducted using the CaTS tool (20) on the basis of a disease prevalence of 0.01%, the reported effect size of each SNP, the MAF of each SNP observed in controls, our sample size, and a significance level of 0.05.

OS association. Survival analyses were conducted separately in the MSKCC and Mayo Clinic studies.

MSKCC study. SNPs and clinical variables were analyzed for association with OS by use of a Cox proportional hazards model as implemented in the *survival* package for R. We measured OS from the date of diagnosis until the date of death (any cause) or last follow-up. Notably, the cases in our study were recruited at variable times following diagnosis—a setting which potentially may lead to biased HR estimates (21). Therefore, we modified the analysis to allow for left truncation, whereby cases were considered to be at risk of death only after the time of study recruitment. Of the initial 531 cases included in our study, 18 cases were either missing the date of diagnosis ($n = 1$) or were lost to follow-up ($n = 17$) after their initial recruitment. Therefore, follow-up data were available for a total of 513 cases.

To analyze a given SNP for association with OS, we considered one or more of the following Cox regression models. Model 1: a univariate Cox model with SNP genotypes coded as either 0, 1, or 2 depending on the number of minor alleles carried by an individual; model 2: a multivariate Cox model including the SNP (coded as in model 1) and race/ethnicity as a covariate; model 3: a multivariate Cox model including the SNP (coded as in model 1), race/ethnicity, Eastern Cooperative Oncology Group (ECOG) performance status (coded as integer values between 0 and 5); clinical stage at diagnosis (which was abstracted from the medical record and categorized as either localized, locally advanced, or metastatic), and radiation treatment (yes/no) as covariates. The clinical variables included in model 3 were each chosen on the basis of having significant ($P \leq 0.05$) HR estimates when individually tested in a univariate Cox model (Supplementary Table S1). Using the "strata()" function in the R *survival* package, model 3 was further stratified by surgical resection status (yes/no), as this variable was found to violate the Cox proportional hazards assumption (data not shown).

In all of the above models, a log-additive effect (HR per additional minor allele) was estimated for each SNP; statistical significance was determined using a 1-*df* Wald test. Cumulative survival probabilities within different SNP genotype strata were estimated by Kaplan–Meier analysis methods.

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Table 1. Summary of the overall study design and results

		Findings	
SNPs analyzed	**Study samples**	**Risk analysis**	**Survival analysis**
Seven SNPs identified by previous GWAS of pancreatic cancer risk (refs. 3–5)	531 cases, 305 controls (MSKCC)	Independent replication of the risk associations of SNPs: rs505922 (chr 9q34, *ABO*) and rs9543325 (chr 13q22)	No significant findings
Twenty-four SNPs identified by previous candidate gene/pathway studies of pancreatic cancer clinical outcome (refs. 6–17)	513 cases (MSKCC)	No significant findings	Independent replication of the OS association of SNP: rs9350 (chr 1q43, *EXO1*)
GWAS of OS from pancreatic cancer (this study)		Not conducted	Putative identification of a novel SNP associated with OS: rs1482426 (chr 12q21)
Stage I (discovery): 301,250 SNPs	252 cases (MSKCC)		
Stage II (validation): 22 SNPs	261 cases (MSKCC)		
Stage III (Replication): 1 SNP	572 cases (Mayo Clinic)		

The first stage of the survival GWAS used whole-genome SNP array data for 252 of the original subset of 263 cases genotyped on the Illumina CNV370 chip that had follow-up information. SNPs from the array with MAF $\geq 1\%$ were each tested for association with OS using model 1 (described above) in the full ($n = 252$) discovery case set. SNPs were carried forward to a validation stage on the basis of the following criteria: (i) the proportional hazards assumption was not violated, as determined by evaluation of the Schoenfeld residuals and (ii) association test, $P < 1 \times 10^{-4}$. In the validation stage, an independent set of 261 cases was used for analysis. Each SNP carried forward from the first stage of the GWAS was analyzed using the validation case set under model 2 (to adjust for the multi-ethnic composition of the validation case set).

For the replication study of SNPs previously reported for association with OS, power analysis was conducted using methods described by Schoenfeld (22) on the basis of the MAF of each SNP in the CEU HapMap sample and its reported effect size

Mayo Clinic study. OS was measured from the date of diagnosis until the date of death (from all causes) or last follow-up for those not known to be deceased at the time of analysis. SNPs were coded as the number of minor alleles carried (0, 1, or 2) by an individual. Under an additive genetic model, associations between SNPs and OS were analyzed using Cox proportional hazards regression as implemented in the R *survival* package. Significance was determined using a χ^2 test comparing the association model with and without the SNP using the *anova.coxph* function. In addition to univariate SNP analyses, multivariable models were also considered which adjusted for age at diagnosis, sex, Karnofsky performance score, body mass index (BMI), and pancreatic cancer stage.

Results

Study design and characteristics of the study participants

The overall design and principal findings of our combined replication/discovery study are summarized in Table 1. A total of 836 individuals (531 pancreatic cancer cases and 305 healthy, unrelated controls) from a case–control study group based at the MSKCC were included in the analyses of previously reported risk SNPs, previously reported survival SNPs, or stages I and II of the GWAS for novel survival SNPs described below. Table 2 describes the

Table 2. Demographics of the MSKCC case–control study population

	Cases ($N = 531$)	**Controls ($N = 305$)**
	***n* (%)**	***n* (%)**
Gender		
Male	290 (55)	127 (42)
Female	241 (45)	178 (58)
Age, y		
≤50	83 (16)	80 (26)
51–60	129 (24)	86 (28)
61–70	184 (35)	100 (33)
>70	135 (25)	39 (13)
Race/ethnicity		
White/Caucasian	495 (93)	283 (93)
Black/African-American	21 (4)	10 (3)
Asian (East)	10 (2)	11 (4)
Asian (Indian)	5 (1)	1 (0)

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Table 3. Clinical characteristics of MSKCC cases used in survival analysis

	Patient subset	
	Stage I: GWAS (*N* = 252)	**Stage II: validation (*N* = 261)**
	***n* (%)**	***n* (%)**
Gender		
Male	141 (56)	141 (54)
Female	111 (44)	120 (46)
Race/ethnicity		
White/Caucasian	252 (100)	226 (87)
Black/African-American	0 (0)	21 (8)
Asian (East)	0 (0)	10 (4)
Asian (Indian)	0 (0)	4 (2)
Surgical resection		
Yes	102 (40)	109 (42)
No	150 (60)	152 (58)
ECOG performance status		
≤1	203 (81)	208 (80)
>1	11 (4)	15 (6)
Missing	38 (15)	38 (15)
Clinical stage		
Localized	89 (35)	100 (38)
Locally advanced	65 (26)	56 (21)
Metastatic	87 (35)	82 (31)
Missing	11 (4)	23 (9)
Chemotherapy		
Yes	232 (92)	234 (90)
No	20 (8)	27 (10)
Radiation		
Yes	90 (36)	89 (34)
No	162 (64)	172 (66)

overall demographics of MSKCC cases and controls used in our study. Table 3 describes the clinical characteristics of MSKCC cases pertaining to survival analysis. An additional 572 pancreatic cancer cases from the Mayo Clinic (described in ref. 23) were included as part of the stage III analysis of a putative survival-associated SNP identified by GWAS.

Replication analysis of SNPs associated with pancreatic cancer risk

We first determined whether pancreatic cancer risk associations identified by GWAS could be replicated in the MSKCC case–control group. From the PanScan studies, we selected SNPs, rs3790844, rs401681, rs505922, and rs9543325, which were reported to have the strongest association signal (lowest *P* value) at-risk loci on chromosomes 1q32, 5p15, 9q34, and 13q22, respectively. From the Japan-based study, we selected SNPs rs9502893 (6p26), rs6464375 (7q36), and rs708224 (12q11) on the basis of having the 3 smallest reported *P* values in that study ($P = 3.3 \times 10^{-7}$, 4.4×10^{-7}, and 3.3×10^{-7}, respectively).

After genotyping cases and controls, each SNP was analyzed for association with risk by use of a logistic regression model adjusted for race, age, and gender (Table 4). Notably, a subset ($n = 283$) of individuals in the MSKCC case-control group also participated in the PanScan study. Thus, for the purpose of independent replication, we excluded these individuals from the analysis of risk SNPs identified by PanScan. Overall, at a nominal *P* value of 0.05, we replicated the risk associations of SNPs rs505922 [$P = 0.002$; per-allele OR, 1.65; 95% confidence interval (CI), 1.20–2.26] located in the *ABO* gene and rs9543325 ($P = 0.02$; per-allele OR, 1.42; 95% CI, 1.06–1.90) located in a nongenic region of chromosome 13q22.

Survival analysis of SNPs associated with pancreatic cancer risk

We next determined whether each of the 7 SNPs selected for risk replication was also associated with OS of pancreatic cancer in the MSKCC case group. The SNPs were analyzed by use of Cox proportional hazard models adjusted for race (Supplementary Table S2). However, no statistically significant ($P < 0.05$) associations were observed.

Replication analysis of SNPs associated with OS of pancreatic cancer

Using a candidate gene approach, several recent studies have reported SNPs that are associated with OS of pancreatic cancer. For replication analysis, we selected a set of 24 previously reported SNPs on the basis of 3 criteria: (i) we had adequate (>80%) study power to detect the reported HRs under a dominant model, assuming $\alpha = 0.05$; (ii) a genotyping assay could be designed; and (iii) genotyping met quality control criteria (see Materials and Methods). After genotyping, we tested each SNP for association with OS in the MSKCC case group using a Cox proportional hazards model adjusted for race (Table 5).

We observed one nominally significant association ($P < 0.05$) at SNP rs9350 ($P = 0.007$; per-allele HR, 1.26; 95% CI, 1.07–1.50), located in the *EXO1* gene, with an estimated HR that was consistent with previous reports. Patients who were heterozygous or homozygous minor allele carriers of rs9350 had an estimated median survival of 0.99 years (95% CI, 0.80–1.21), versus 1.28 years (95% CI, 1.12–1.49) for homozygous major allele carriers (Supplementary Fig. S1a). In multivariate Cox analysis—stratified by surgical resection (yes/no) and adjusted for race, ECOG status, clinical stage, and radiation treatment—rs9350 ($P = 0.002$; adjusted per-allele HR, 1.33; 95% CI, 1.11–1.60) remained statistically significantly associated with OS (Supplementary Table S4).

Notably, we observed one additional SNP—rs8191754 ($P = 0.02$; per-allele HR, 0.77; 95% CI, 0.62–0.95), located in the *IGF2R* gene—to be associated with OS at $P < 0.05$ in our study. However, the estimated HR for this SNP was in the opposite direction of previous reports (9).

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Table 4. Replication of pancreatic cancer risk associations for SNPs identified by previous GWAS

		Subjects		MAF					
SNP, minor allele, Chr, genes	Ref.	Controls	Cases	Controls	Cases	Reported OR (95% CI)	Estimated OR[a] (95% CI)	P^b	Power[c]
rs505922[d], C, 9q34, *ABO*	3	149	385	0.34	0.43	1.20 (1.12–1.28)	1.65 (1.20–2.26)	0.002	26%
rs9543325[d], G, 13q22.1, none	4	148	386	0.40	0.49	1.26 (1.18–1.35)	1.42 (1.06–1.90)	0.02	39%
rs708224, A, 12p11.21, *BICD1*	5	303	525	0.38	0.42	1.32 (1.19–1.47)	1.21 (0.98–1.50)	0.08	77%
rs9502893, G, 6p25.3, *FOXQ1*	5	303	528	0.42	0.46	1.29 (1.17–1.43)	1.20 (0.97–1.48)	0.09	70%
rs401681[d], A, 5p15.33, *CLPTM1L*	4	149	390	0.43	0.48	1.19 (1.11–1.27)	1.22 (0.91–1.62)	0.18	25%
rs6464375, A, 7q36.2, *DPP6*	5	305	531	0.05	0.05	3.73 (2.24–6.21)[e]	0.44 (0.04–5.39)[e]	0.52	9%
rs3790844[d], G, 1q32.1, *NR5A2*	4	152	392	0.24	0.21	0.77 (0.71–0.84)	0.93 (0.66–1.30)	0.66	33%

Abbreviations: Chr, chromosome; Ref., reference.

[a]Multivariable logistic regression model adjusted for race, gender, and age.

[b]One-df Wald test for significance.

[c]Power calculation based on a disease prevalence of 0.01% in the population, the reported effect size, the MAF observed in controls, the indicated sample size, and significance level of 0.05.

[d]SNP was analyzed after excluding cases and controls who participated in the PanScan study.

[e]SNP was analyzed under a recessive model consistent with its original report in the work of Low and colleagues (5).

Risk analysis of SNPs associated with OS of pancreatic cancer

We next tested whether each of the 24 SNPs chosen for OS analysis was also associated with pancreatic cancer risk in the MSKCC case–control group. The SNPs were analyzed using a log-additive logistic regression model adjusted for race, age, and gender (Supplementary Table S3). However, no statistically significant ($P < 0.05$) associations were observed.

Table 5. Replication of OS associations for SNPs identified by previous candidate gene studies of pancreatic cancer clinical outcome

SNP	Ref.	Gene	Minor allele	Estimated HR[a] (95% CI)	P^b
rs9350	10	*EXO1*	T	1.26 (1.07–1.50)	0.007
rs8191754	9	*IGF2R*	G	0.77 (0.62–0.95)	0.02
rs8041224	9	*IGF1R*	T	1.15 (0.99–1.33)	0.06
rs12090453	8	*GPSM2*	C	1.15 (0.99–1.33)	0.07
rs2854744	9	*IGFBP3*	A	1.14 (0.99–1.31)	0.07
rs735943	10	*EXO1*	A	0.88 (0.76–1.02)	0.08
rs2272615	14	*POLB*	G	1.19 (0.94–1.51)	0.14
rs2946834	9	*IGF1*	T	0.91 (0.77–1.08)	0.28
rs3218536	15	*XRCC2*	T	0.88 (0.69–1.13)	0.31
rs2953993	14	*POLB*	A	1.16 (0.86–1.55)	0.33
rs2134808	8	*TUBG1*	C	0.93 (0.80–1.08)	0.34
rs302864	8	*TEX14*	T	1.14 (0.86–1.50)	0.36
rs2431238	8	*APC*	T	1.05 (0.91–1.22)	0.49
rs293794	14	*hOGG1*	C	1.07 (0.87–1.31)	0.52
rs664143	17	*ATM*	T	0.95 (0.81–1.11)	0.53
rs1805355	10	*MSH3*	A	0.94 (0.73–1.21)	0.61
rs3743262	9	*IGF1R*	T	0.92 (0.67–1.28)	0.63
rs12437963	9	*IGF1R*	G	0.96 (0.78–1.17)	0.67
rs664677	13	*ATM*	C	0.97 (0.83–1.14)	0.74
rs2066827	6	*p27*	C	0.98 (0.82–1.16)	0.78
rs7928320	8	*KIAA0999*	T	0.98 (0.81–1.18)	0.85
rs11079571	8	*AXIN2*	A	1.02 (0.84–1.23)	0.88
rs521102	17	*CHEK1*	T	1.00 (0.86–1.17)	0.95
rs5742933	10	*PMS1*	C	1.00 (0.84–1.18)	0.96

Abbreviation: Ref, reference.

[a]Cox proportional hazards model adjusted for race.

[b]One-df Wald test for significance.

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Genome-wide scan for novel SNPs associated with OS

To identify novel SNPs associated with OS, we conducted a 3-stage GWAS. The first stage of analysis was conducted within a set of 252 MSKCC cases for which we had follow-up information and whole-genome SNP array data. We tested each of 301,250 SNPs (with MAF $\geq$ 1% in cases) for association with OS under a univariate Cox proportional hazards model. None of SNPs tested in this analysis reached genome-wide significance ($P < 5 \times 10^{-8}$; Supplementary Figs. S2 and S3). However, 22 candidate SNPs with $P < 1 \times 10^{-4}$ were carried forward to a second (validation) stage. Genotyping was conducted in the validation case set ($n =$ 261 MSKCC cases), and each SNP was tested for association with OS using a Cox proportional hazards model adjusted for race (Table 6).

Simultaneously, under the hypothesis that different genetic variants play a role in the survival of pancreatic cancer cases that undergo surgical tumor resection compared with cases that do not, we conducted similar analyses (up to stage I and II) in those respective case subgroups (data not shown). However, as no SNPs tested in stage II were significant in those analyses, we focused the remainder of our study on candidate SNPs identified in full-group analysis.

Notably, of the 22 candidate SNPs tested in stage II, we successfully validated the association of SNP rs1482426 ($P = 0.001$; per-allele HR, 1.73; 95% CI, 1.25–2.40), which is located in a nongenic region of chromosome 12q21 (Supplementary Fig. S4). In a combined analysis using both the GWAS (discovery) and validation case sets, rs1482426 was found to improve in overall rank and P value ($P = 1.7 \times 10^{-6}$; per-allele HR, 1.74; 95% CI, 1.38–2.18). Patients who were heterozygous or homozygous minor allele carriers of rs1482426 had an estimated median survival of 0.78 years (95% CI, 0.53–0.95) versus 1.28 years (95% CI, 1.19–1.49) for homozygous major allele carriers (Supplementary Fig. S1b). Furthermore, multivariate Cox analysis of the combined case sets—stratified by surgical resection (yes/no) and adjusted for race, ECOG status, clinical stage, and radiation treatment—showed that rs1482426 was independently associated with OS ($P = 9.0 \times 10^{-6}$; per-allele HR, 1.70; 95% CI, 1.34–2.14; Supplementary Table S4).

To gain further statistical evidence of its association with OS, we selected SNP rs1482426 for a third (replication) stage of analysis involving an independent case group ($n =$ 572) based at the Mayo Clinic. In univariate Cox regression analysis of rs1482426, we observed suggestive evidence of

Table 6. Summarized results from stages I and II of the GWAS of OS from pancreatic cancer

SNP, minor allele, chromosome, gene[a]	Discovery		Validation		Combined	
	HR[b] (95% CI)	P[c]	HR[d] (95% CI)	P[c]	HR[d] (95% CI)	P[c]
rs1482426, G, 12q21, —	1.95 (1.42–2.67)	3.6×10^{-5}	1.73 (1.25–2.40)	0.001	1.74 (1.38–2.18)	1.7×10^{-6}
rs4285214, G, 5q23, *ZNF608*	1.65 (1.35–2.02)	7.9×10^{-7}	1.09 (0.90–1.31)	0.39	1.33 (1.16–1.53)	4.1×10^{-5}
rs7849571, G, 9p21, —	1.63 (1.28–2.07)	7.2×10^{-5}	1.14 (0.90–1.43)	0.27	1.35 (1.14–1.59)	3.7×10^{-4}
rs11151040, T, 18q23, —	2.57 (1.61–4.10)	7.6×10^{-5}	1.27 (0.76–2.15)	0.36	1.84 (1.30–2.61)	5.6×10^{-4}
rs3747572, C, 16p13, *GLIS2*	1.83 (1.39–2.40)	1.3×10^{-5}	1.10 (0.86–1.42)	0.44	1.38 (1.15–1.66)	6.2×10^{-4}
rs10189511, A, 2q34, —	2.11 (1.51–2.95)	1.2×10^{-5}	1.07 (0.77–1.48)	0.70	1.50 (1.19–1.89)	6.7×10^{-4}
rs1344963, T, 12q21, —	2.59 (1.69–3.97)	1.4×10^{-5}	1.18 (0.85–1.66)	0.32	1.56 (1.20–2.03)	8.5×10^{-4}
rs4903736, A, 14q24, —	1.59 (1.27–1.98)	4.5×10^{-5}	1.02 (0.78–1.32)	0.90	1.33 (1.12–1.58)	9.8×10^{-4}
rs11024097, T, 11p15, *PLEKHA7*	1.54 (1.25–1.90)	4.2×10^{-5}	1.01 (0.81–1.26)	0.91	1.28 (1.10–1.49)	0.001
rs4903741, C, 14q24, —	1.57 (1.25–1.96)	8.7×10^{-5}	1.04 (0.83–1.31)	0.72	1.29 (1.10–1.52)	0.002
rs12835268, C, Xp21, —	1.50 (1.24–1.82)	3.4×10^{-5}	1.05 (0.86–1.28)	0.64	1.23 (1.08–1.41)	0.003
rs16867625, T, 8q22, —	2.33 (1.55–3.51)	5.0×10^{-5}	1.15 (0.75–1.75)	0.52	1.55 (1.16–2.08)	0.003
rs7016046, A, 8q24, —	1.83 (1.38–2.43)	3.1×10^{-5}	1.02 (0.76–1.38)	0.89	1.36 (1.11–1.67)	0.004
rs2056096, T, 18q12, —	1.55 (1.25–1.93)	7.3×10^{-5}	0.97 (0.78–1.20)	0.76	1.24 (1.07–1.44)	0.005
rs8034546, T, 15q14, —	1.62 (1.29–2.04)	4.0×10^{-5}	0.97 (0.75–1.26)	0.85	1.27 (1.07–1.51)	0.005
rs1867348, T, 6q25, *IGF2R*	2.02 (1.43–2.85)	6.3×10^{-5}	0.98 (0.70–1.37)	0.90	1.38 (1.08–1.75)	0.009
rs956518, G, 4p15, —	0.62 (0.50–0.79)	5.7×10^{-5}	1.13 (0.92–1.40)	0.24	0.82 (0.70–0.95)	0.01
rs10167103, C, 2q14, —	1.53 (1.25–1.88)	5.0×10^{-5}	0.92 (0.76–1.13)	0.43	1.20 (1.04–1.38)	0.01
rs38402, G, 7p15, *GGCT*	4.75 (2.19–10.31)	8.2×10^{-5}	1.25 (0.65–2.41)	0.50	1.86 (1.13–3.05)	0.01
rs3795244, T, 17q11, *ZNF207*	2.46 (1.69–3.58)	3.0×10^{-6}	0.89 (0.61–1.29)	0.53	1.34 (1.03–1.74)	0.03
rs1944395, C, 18q12, —	1.50 (1.23–1.84)	8.3×10^{-5}	0.86 (0.70–1.06)	0.16	1.16 (1.01–1.35)	0.04
rs1388193, T, 13q31, —	1.77 (1.34–2.34)	6.5×10^{-5}	0.72 (0.53–0.99)	0.04	1.13 (0.92–1.38)	0.25

[a]Closest RefSeq annotated gene within ±20 kb.
[b]Univariate Cox proportional hazards model.
[c]One-df Wald test for significance.
[d]Cox proportional hazards model adjusted for race.

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association ($P = 0.0755$; per-allele HR, 1.19) in the Mayo case group that, although weaker, was consistent with the direction of effect estimated in stages I and II of the GWAS.

Discussion

In this study, we have replicated previously reported pancreatic cancer risk and survival SNPs, and we have identified a novel putative pancreatic cancer survival SNP. We first focused on a replication analysis of SNPs reported by recent pancreatic cancer risk GWAS. Both the *ABO* locus (marked by SNP rs505922) and the 13q22 locus (marked by SNP rs9543325) were found to be associated with risk in our case–control group. Notably, our estimate of the per-allele OR for SNP rs505922 (per-allele OR, 1.65; 95% CI, 1.20–2.26) is larger than those reported by PanScan or the Japan-based GWAS. Indeed, it is also larger than most ORs estimated for other common cancer risk SNPs. However, as the 95% CI of our estimate overlaps with previous reports, we cannot draw specific conclusions about this observation.

In addition, we observed that 3 other SNPs in our risk replication analysis—rs708224 (*BICD1*), rs9502893 (*FOXQ1*), and rs401681 (*CLPTM1L*)—had OR estimates that were trending toward significance ($P < 0.2$) and were consistent with previous GWAS reports. Indeed, on the basis of our power calculations, we had only weak-to-moderate study power to replicate the associations of previously reported risk SNPs chosen for analysis (Table 4). Thus, we emphasize that a major limitation of our risk replication analysis was its relatively small sample size. Nonetheless, for several of the SNPs, our results contribute suggestive evidence of their roles in pancreatic cancer.

We next turned toward the analysis of 24 SNPs that have been previously implicated in modulating OS from pancreatic cancer. We focused this analysis on SNPs for which we had more than 80% to replicate their effects on OS. Using a Cox regression model adjusted for race, we observed only one SNP association having a P value ≤ 0.05 and an estimated HR that was consistent with the direction and magnitude of previous reports: rs9350, a nonsynonymous coding SNP in the DNA damage repair gene exonuclease 1 (*EXO1*). Notably, whereas we primarily considered the log-additive effects of minor alleles on OS, previous studies of rs9350 considered a dominant model of effect. Specifically, rs9350 was first analyzed by Dong and colleagues (10) by comparing the survival of CT/TT versus CC genotype groups (HR, 1.89; 95% CI, 1.25–2.87). To compare our results more directly with previous studies, we examined differences in estimated survival probability according to rs9350 genotypes after applying a dominant model grouping scheme (Supplementary Fig. S1a). In this setting, we once again observed a significant and consistent effect of SNP rs9350 (CT/TT vs. CC: HR, 1.41; 95% CI, 1.14–1.74).

Furthermore, in multivariate analysis adjusting for other clinical factors related to OS, SNP rs9350 (*EXO1*) remained independently associated. *EXO1* encodes a 5′-3′ exonuclease that interacts with several components of the DNA mismatch repair pathway (24). It is currently unknown whether rs9350 might itself be a functional SNP modulating *EXO1* activity or whether it lies in linkage disequilibrium with the true functional SNP. However, in conjunction with recent follow-up studies by Dong and colleagues that found additional SNPs in *EXO1* associated with OS (11), our results provide a third line of evidence suggesting the importance of *EXO1* in pancreatic tumor biology. Additional studies are needed to understand the functional mechanisms by which this gene (and the associated SNPs) is modulating clinical outcome in patients with pancreatic cancer.

In contrast, the direction of our results for SNP rs8191754 (per-allele HR, 0.77; 95% CI, 0.62–0.95) was nominally significant ($P < 0.05$) but not consistent with previous reports. Overall, we were unable to replicate the survival associations of most previously reported SNPs analyzed in this study. Importantly, however, we emphasize that such discordant findings do not directly imply that previous reports were false-positives. Rather, given the complex spectrum of factors that influence survival from pancreatic cancer, we would first speculate that clinical and/or genetic ancestral differences between our patient population and previously studied populations might explain the discordant results.

In this study, we also assessed whether SNPs involved in pancreatic cancer risk might also be involved in OS (and vice versa). This hypothesis was suggested by previous studies that found the same factors could influence both pancreatic cancer risk and survival (e.g., self-reported allergies or high BMI; refs. 23, 25–30). We tested SNPs identified by pancreatic cancer risk GWAS for association with OS. Conversely, we tested SNPs identified by candidate gene studies of pancreatic cancer survival for risk association. Our results, however, do not provide strong evidence that either phenomenon is occurring for these sets of SNPs.

Finally, using an unbiased genome-wide approach, we sought to identify preliminary evidence of novel SNPs associated with the clinical outcome of pancreatic cancer. Putative associations were identified by GWAS of OS and then validated using independent case groups. This approach yielded at least one novel putative association at SNP rs1482426, which lies in a nongenic region of chromosome 12q21. Interestingly, the gene *SLC6A15* (solute carrier family 6, neutral amino acid transporter member 15) is located approximately 730 kb downstream of rs1482426, adjacent to the linkage disequilibrium region containing rs1482426, and was recently found to be somatically mutated in 2 sequenced pancreatic cancer exomes (31).

However, we emphasize that our GWAS results should be interpreted as preliminary. Additional studies are needed to confirm this association in larger patient samples and to understand the functional significance of this region in pancreatic cancer survival. Importantly, although the direction of its effect on OS was consistent throughout all 3 stages of our GWAS, rs1482426 was estimated to have a much

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weaker effect (HR ≈ 1.2) in stage III than in stages I and II (per-allele HR ≈ 1.7). Several factors could have led to this disparity. For example, it is possible that the effect observed in stages I and II was an overestimate of the true effect size of rs1482426 in the population—that is, the winner's curse phenomenon (32). In addition, the patients analyzed in stages I and II were based at MSKCC, whereas patients analyzed in stage II were based at the Mayo Clinic. Thus, we also speculate that differences in clinical and/or demographic characteristics between these 2 patient samples may partially explain the discordant effect sizes. Finally, we note that the overall results of our GWAS were not strictly significant at the genome-wide level. Therefore, we cannot rule out the possibility that the observed effect of rs1482426 on survival was a false-positive finding.

Nonetheless, toward the goal of identifying novel loci that play a role in pancreatic cancer outcome, our study shows both the potential use of GWAS and some of the challenges faced in its design. Ideally, a large multicenter consortium study such as PanScan would be well-powered to identify robust and novel associations of SNPs with OS. However, that approach would be faced with the complex challenge of merging clinical data across the various centers. Here, at the cost of overall study power, we chose to focus the discovery and validation stages of our GWAS on patients treated at a single center (MSKCC), followed by replication in patients from an independent center (Mayo Clinic).

In conclusion, we have provided evidence of independent replication for several previously reported SNPs associated with pancreatic cancer risk and OS. Furthermore, we used an unbiased, genome-wide approach to identify a novel locus putatively associated with OS. Our study adds further supporting evidence that inherited genetic variations may play important biologic roles in pancreatic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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