

Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer

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We performed a multistage genome-wide association study including 7,683 individuals with pancreatic cancer and 14,397 controls of European descent. Four new loci reached genome-wide significance: rs6971499 at 7q32.3 (*LINC-PINT*, per-allele odds ratio (OR) = 0.79, 95% confidence interval (CI) 0.74–0.84, $P = 3.0 \times 10^{-12}$), rs7190458 at 16q23.1 (*BCAR1/CTRB1/CTRB2*, OR = 1.46, 95% CI 1.30–1.65, $P = 1.1 \times 10^{-10}$), rs9581943 at 13q12.2 (*PDX1*, OR = 1.15, 95% CI 1.10–1.20, $P = 2.4 \times 10^{-9}$) and rs16986825 at 22q12.1 (*ZNRF3*, OR = 1.18, 95% CI 1.12–1.25, $P = 1.2 \times 10^{-8}$). We identified an independent signal in exon 2 of *TERT* at the established region 5p15.33 (rs2736098, OR = 0.80, 95% CI 0.76–0.85, $P = 9.8 \times 10^{-14}$). We also identified a locus at 8q24.21 (rs1561927, $P = 1.3 \times 10^{-7}$) that approached genome-wide significance located 455 kb telomeric of *PVT1*. Our study identified multiple new susceptibility alleles for pancreatic cancer that are worthy of follow-up studies.

Pancreatic cancer is the fourth-leading cause of cancer-related death in the United States and the fifth-leading cause in the European Union^{1,2}. Over 80% of patients have incurable disease at the time of diagnosis, and the majority live for less than 12 months³. Rare, moderately to highly penetrant mutations account for a small fraction of the familial aggregation of pancreatic cancer⁴. In two previous genome-wide association studies (GWAS) called PanScan I⁵ and PanScan II⁶, we identified common variants at four loci that are associated with risk of sporadic pancreatic cancer in European populations. Subsequent GWAS demonstrated five distinct susceptibility loci among individuals of Chinese descent⁷ and three suggestive loci among individuals of Japanese descent⁸.

In the current study (designated PanScan III), we performed a multistage GWAS of 7,683 individuals diagnosed with pancreatic cancer and 14,397 control individuals of European descent (Online Methods, Table 1, Supplementary Table 1 and Supplementary Fig. 1).

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Table 1 Subject numbers and characteristics of pancreatic cancer cases and controls

	Cases <i>n</i> (%)	Controls <i>n</i> (%)
Number of subjects		
Stage 1	1,582	5,203
Stage 2	3,525	3,642
Replication	2,576	5,552
Full study population	7,683	14,397
Geographic region		
United States	4,387 (57.1)	7,962 (55.3)
Central or northern Europe	2,264 (29.5)	3,853 (26.8)
Southern Europe	1,032 (13.4)	2,582 (17.9)
Sex		
Male	4,107 (53.5)	8,841 (61.4)
Female	3,576 (46.5)	5,556 (38.6)
Age, years		
≤60	1,972 (25.7)	4,577 (31.8)
61–70	2,688 (35.0)	5,906 (41.0)
>70	3,023 (39.3)	3,914 (27.2)
Smoking status^a		
Current or past	2,634 (51.6)	4,541 (51.3)
Never	1,642 (32.2)	3,186 (36.0)
Unknown	831 (16.3)	1,118 (12.6)

^aSmoking status information was available for subjects in stages 1 and 2.

In stage 1, we newly genotyped 1,582 cases from 13 prospective cohort studies, 2 case series and 1 case-control study using the Illumina OmniExpress Beadchip array. The control population included 5,203

cancer-free individuals that were genotyped previously using second-generation Illumina SNP microarrays (for example, OmniExpress, Omni 1M or Omni 2.5M) and were drawn from PanScan III prospective cohorts and a Spanish case-control study of bladder cancer. Of the newly genotyped cases, 94% passed quality-control criteria (Online Methods and **Supplementary Tables 2 and 3**), and 712,704 SNPs were included with a minimum call rate of 94%. In stage 2, we used the primary whole genome-scan data from the reported PanScan I⁵ (1,757 cases and 1,801 controls from 12 cohort studies and 1 case-control study typed on the Illumina HumanHap550 array) and PanScan II⁶ (1,768 cases and 1,841 controls from 8 case-control studies typed with the Illumina Human 610-Quad array) studies. To address differences in typed SNPs across the arrays, we used the Division of Cancer Epidemiology and Genetics (DCEG) Imputation Reference Set⁹ to fill in missing genotypes (Online Methods).

In a meta-analysis of stages 1 and 2, we observed robust associations for the four previously identified loci in individuals of European descent: rs687289 at 9q34.2 (*ABO*, OR = 1.27, 95% CI 1.20–1.35, $P = 1.6 \times 10^{-16}$), rs9543325 at 13q22.1 (*KLF5/KLF12*, OR = 1.23, 95% CI 1.18–1.30, $P = 4.3 \times 10^{-14}$), rs10919791 at 1q32.1 (*NR5A2*, OR = 0.79, 95% CI 0.75–0.85, $P = 1.4 \times 10^{-11}$) and rs31490 at 5p15.33 (*CLPTM1L*, OR = 1.20, 95% CI 1.14–1.27, $P = 2.0 \times 10^{-11}$).

We observed two new SNPs below genome-wide significance ($P < 5 \times 10^{-8}$) in the meta-analysis of stages 1 and 2, plus 11 additional promising SNPs ($P < 5 \times 10^{-5}$) from distinct regions (**Supplementary Table 4**). We carried these 13 SNPs forward for replication (stage 3) in 2,576 cases and 5,552 controls, drawn from (i) cases in stage 1 with DNA quantity insufficient for the full GWAS, (ii) cases and controls

Table 2 Association results for five new pancreatic cancer susceptibility loci and one suggestive locus

Chr.	Nearest gene(s) ^a	SNP	Position ^b	Minor allele ^c	Major allele ^c	Stage	Allelic OR (95% CI)	Minor allele frequency		<i>P</i> ^f
								Controls	Cases	
5p15.33	<i>TERT</i> , <i>MIR4457</i> , <i>CLPTM1L</i>	rs2736098	1,294,086	T	C	Stage 1	0.76 (0.68–0.86)	0.268	0.216	8.22×10^{-6}
						Stage 2	0.82 (0.74–0.90)	0.284	0.259	2.63×10^{-5}
						Replication ^d	0.81 (0.74–0.89)			1.36×10^{-5}
						Combined^e	0.80 (0.76–0.85)			9.78×10^{-14}
7q32.3	<i>LINC-PINT</i>	rs6971499	130,680,521	C	T	Stage 1	0.79 (0.68–0.90)	0.155	0.127	6.58×10^{-4}
						Stage 2	0.81 (0.74–0.90)	0.147	0.124	4.69×10^{-5}
						Replication ^d	0.77 (0.69–0.86)			4.37×10^{-6}
						Combined^e	0.79 (0.74–0.84)			2.98×10^{-12}
16q23.1	<i>BCAR1</i> , <i>CTRB1</i> , <i>CTRB2</i>	rs7190458	75,263,661	A	G	Stage 1	1.61 (1.32–1.96)	0.042	0.065	4.14×10^{-6}
						Stage 2	1.47 (1.20–1.82)	0.039	0.049	2.17×10^{-4}
						Replication ^d	1.33 (1.10–1.61)			3.14×10^{-3}
						Combined^e	1.46 (1.30–1.65)			1.13×10^{-10}
13q12.2	<i>PDX1</i>	rs9581943	28,493,997	A	G	Stage 1	1.23 (1.12–1.35)	0.397	0.441	1.34×10^{-5}
						Stage 2	1.12 (1.05–1.20)	0.406	0.434	5.51×10^{-4}
						Replication ^d	1.11 (1.03–1.20)			4.80×10^{-3}
						Combined^e	1.15 (1.10–1.20)			2.35×10^{-9}
22q12.1	<i>ZNRF3</i>	rs16986825	29,300,306	T	C	Stage 1	1.25 (1.10–1.42)	0.150	0.184	4.96×10^{-4}
						Stage 2	1.15 (1.05–1.26)	0.149	0.168	2.11×10^{-3}
						Replication ^d	1.18 (1.08–1.30)			5.13×10^{-4}
						Combined^e	1.18 (1.12–1.25)			1.18×10^{-8}
8q24.21	<i>MIR1208</i> , <i>PVT1</i>	rs1561927	129,568,078	C	T	Stage 1	0.88 (0.78–0.97)	0.269	0.251	1.59×10^{-2}
						Stage 2	0.86 (0.80–0.93)	0.279	0.250	1.11×10^{-4}
						Replication ^d	0.89 (0.82–0.97)			6.44×10^{-3}
						Combined^e	0.87 (0.83–0.92)			1.30×10^{-7}

Results are shown from an unconditional logistic regression analysis of the genotypes generated in stage 1, stage 2 and the replication (a total of 7,683 individuals diagnosed with pancreatic cancer and 14,397 controls).

^aClosest RefSeq gene(s). Genes located within 25 kb of the given SNP are listed in order of the closest gene to those further away for 5p15.33, 7q32.3, 16q23.1, 13q12.2 and 22q12.1; the closest genes outside this 50 kb window are shown for 8q24.21. ^bPosition of the SNP in NCBI genome build 37 (Hg19). ^cMinor and major alleles. ^dThe replication is a meta-analysis of three groups and, thus, minor allele frequency (MAF) is not listed. ^eNumber of case and control subjects in the joint analysis of stage 1, stage 2 and the replication: rs2736098 (7,199/13,121), rs6971499 (7,435/13,289), rs7190458 (7,412/13,291), rs9581943 (7,415/13,286), rs16986825 (7,413/13,196) and rs1561927 (7,486/13,274). ^f1-degree-of-freedom score test. Chr., chromosome and band; OR, per-allele OR for the minor allele adjusted for age, sex, geographic region and significant principal components for stage 1; per-allele OR adjusted for age, sex, study, arm and significant principal components for stage 2; per-allele OR adjusted for age, sex and study for the replication. Text in bold indicates results from the combined meta-analysis.

from the PANDORA consortium¹⁰ and (iii) cases enrolled in CALGB 80303, a US cooperative group clinical trial¹¹ (**Supplementary Table 5**). We selected additional control subjects from cancer-free individuals genotyped previously using the Illumina HumanHap550 array (Online Methods). Of the 13 SNPs advanced to replication, 9 were associated with pancreatic cancer risk ($P < 0.05$) in the replication stage (**Supplementary Table 6**).

For the complete study of 7,683 cases and 14,397 controls, we applied a fixed-effect meta-analysis to the results from the three stages. Overall, six SNPs had P values below genome-wide significance: rs2736098 at 5p15.33 (a second signal in *TERT*, $P = 9.8 \times 10^{-14}$), rs6971499 at 7q32.3 (*LINC-PINT*, $P = 3.0 \times 10^{-12}$), rs7190458 at 16q23.1 (*BCAR1/CTRB1/CTRB2*, $P = 1.1 \times 10^{-10}$), rs9581943 at 13q12.2 (*PDX1*, $P = 2.4 \times 10^{-9}$), rs16986825 at 22q12.1 (*ZNRF3*, $P = 1.2 \times 10^{-8}$) (**Table 2** and **Fig. 1**) and rs4962153 at 9q34.2 (*ADAMTS13*, $P = 1.5 \times 10^{-8}$). In a subsequent conditional analysis described below, rs4962153 in *ADAMTS13* marked the same signal as rs687289 in *ABO* identified in PanScan I and II. An additional locus at 8q24.21 was close to genome-wide significance (rs1561927, $P = 1.3 \times 10^{-7}$) and is located in a region that has been associated previously with multiple cancers (**Table 2** and **Fig. 1**).

The SNP at 7q32.3, rs6971499, maps to an intron in *LINC-PINT*, which is a p53-induced long intergenic non-protein coding RNA located in a 375-kb region between *MKLN1* (encoding muskulin 1) and *KLF14* (**Supplementary Table 7** and **Fig. 1**). Muskulin 1 is an intracellular protein that mediates cell responses to the extracellular matrix, in particular influencing cell adhesion and cytoskeleton organization¹². *KLF14* is a member of the Kruppel-like family of transcription factors, which have been implicated as tumor suppressors, including in mutant *KRAS*-driven tumors¹³. *KLF14* has also been identified as a regulator of several metabolic phenotypes, including type 2 diabetes¹⁴. Notably, the previously established susceptibility locus at 13q22.1 is located in an intergenic region between *KLF5* and *KLF12*, which encode two other members of the Kruppel-like family of transcription factors.

The SNP at 16q23.1, rs7190458, is a synonymous SNP residing in the last exon of *BCAR1* (also known as *P130Cas*) and close to two chymotrypsinogen genes, *CTRB1* (5 kb) and *CTRB2* (23 kb) (**Supplementary Table 7** and **Fig. 1**). Aberrant expression of *BCAR1* has been linked with the transformation and progression of multiple cancer types, and *BCAR1* functions as an adaptor protein that coordinates cell cycle control, cytoskeleton organization and cell migration^{15,16}.

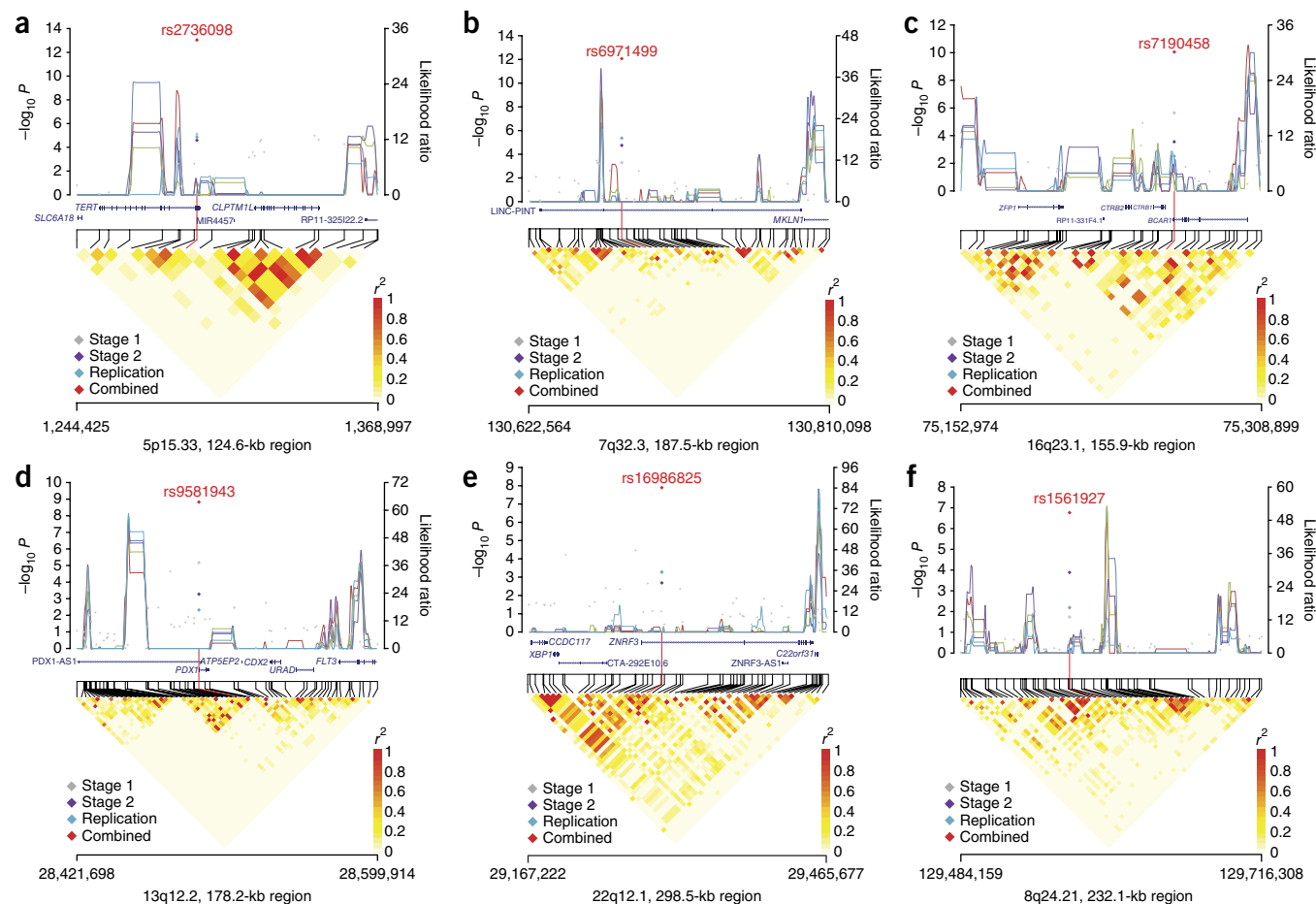


Figure 1 Association results, recombination hotspots and LD plots for new pancreatic cancer susceptibility regions and one suggestive region. (a–f) Results in the newly identified susceptibility regions (a–e) and the suggestive region (f). Top, association results of GWAS data from stage 1 (gray diamonds), stage 2 (purple diamonds) and the replication (blue diamonds), as well as the combined data from stages 1–3 (red diamonds), plotted against $-\log_{10} P$ values (left y axis). Overlaid are likelihood ratio statistics (right y axis) estimating putative recombination hotspots across the region on the basis of five unique sets of 100 randomly selected control samples. Bottom, LD heat maps based on r^2 values from the total control populations for all SNPs included in the GWAS. The data are based on a total of 7,683 individuals with pancreatic cancer and 14,397 controls of European descent. Shown are results for 5p15.33 (a), 7q32.3 (b), 16q23.1 (c), 13q12.2 (d), 22q12.1 (e) and 8q24.21 (f).

Table 3 Conditional analyses of SNPs at chromosomes 5p15.33 and 9q34.2

New SNP	Chr.	Position ^a	Gene	OR ^b (95% CI)	P ^b	Conditional OR ^c (95% CI)	Conditional P ^c	Established SNP	r ^{2,d}	OR ^e (95% CI)	P ^e	Conditional OR ^f (95% CI)	Conditional P ^f
rs2736098	5p15.33	1,294,086	<i>TERT</i> , <i>CLPTMIL</i>	0.80 (0.74–0.86)	1.42 × 10 ^{−9}	0.88 (0.80–0.95)	2.44 × 10 ^{−3}	rs401681/rs31490	0.22	0.83 (0.79–0.88)	1.97 × 10 ^{−11}	0.86 (0.81–0.92)	7.55 × 10 ^{−6}
rs4962153	9q34.2	136,323,754	<i>ADAMTS13</i> , <i>ABO</i>	1.20 (1.10–1.30)	1.97 × 10 ^{−5}	1.05 (0.96–1.16)	0.28	rs505902/rs687289	0.17	1.27 (1.20–1.35)	1.64 × 10 ^{−16}	1.25 (1.18–1.33)	6.28 × 10 ^{−12}

^aPosition of the SNP in NCBI genome build 37 (Hg19). ^bPer-allele ORs for the minor allele and P values for the new SNP from the unconditional meta-analysis of stage 1 and stage 2. ^cPer-allele ORs for the minor allele and P values for the new SNP from the conditional meta-analysis of stage 1 and stage 2. ^dr² LD values between the new SNP and the established SNP at the locus based on 1000G CEU data. ^ePer-allele ORs for the minor allele and P values for the established SNP from the unconditional meta-analysis of stage 1 and stage 2. ^fPer-allele ORs for the minor allele and P values for the established SNP from the conditional meta-analysis. Chr., chromosome.

The chymotrypsinogens are members of a family of serine proteases that are secreted by the pancreas into the gastrointestinal tract¹⁷. Mutations in the related genes *PRSS1* (encoding trypsin 1) and *CTRC* have been associated with hereditary pancreatitis¹⁸, a known risk factor for pancreatic cancer¹⁹. In addition, a susceptibility locus for types 1 and 2 diabetes^{20,21} is located 16 kb centromeric to rs7190458 (rs7202877, $r^2 = 0.32$ in the 1000 Genomes Project (1000G) CEU data). Functional analyses have indicated that this variant (rs7202877) leads to impaired pancreatic beta-cell function²² and influences the expression of *CTRB1* and *CTRB2* in pancreatic tissue²³.

At chromosome 13q12.2, the newly identified SNP, rs9581943, is approximately 200 bp upstream of *PDX1* (encoding pancreatic and duodenal homeobox1 protein 1) and is intronic to *PDX1-AS1* (encoding *PDX1* antisense RNA 1), a recently identified noncoding RNA (**Supplementary Table 7** and **Fig. 1**). *PDX1* is critical for early pancreatic development, has a role in differentiation of the exocrine pancreas and regulates beta-cell function in the mature pancreas^{24,25}. Mutations in *PDX1* have been linked to agenesis of the pancreas²⁴ and maturity onset diabetes of the young²⁶, a dominantly inherited disorder of nonautoimmune diabetes. Furthermore, *PDX1* has been implicated in glucose-dependent regulation of insulin gene transcription²⁷, and GWAS have identified a SNP (rs2293941, $r^2 = 0.20$ in the 1000G CEU data) at the *PDX1* locus that is associated with fasting glucose levels²⁸.

The signal at 22q12.1, rs16986825, maps to an intron in *ZNRF3* (zinc and ring finger 3) (**Supplementary Table 7** and **Fig. 1**), encoding a cell-surface transmembrane E3 ubiquitin protein ligase that is a negative regulator of the Wnt signaling pathway²⁹. Additionally, *CHEK2* is located 162 kb centromeric to the marker SNP and encodes a cell cycle-checkpoint kinase that cooperates with P53, BRCA1 and ATM in response to DNA damage³⁰. Alterations in *CHEK2* have been implicated in susceptibility to several cancer types³¹.

We performed conditional analyses to assess whether the newly identified SNPs at 5p15.33 (*CLPTMIL* and *TERT*) and 9q34.2 (*ABO* and *ADAMTS13*) were independent from those identified previously. After conditioning on the reported SNP within intron 13 of *CLPTMIL*, the newly identified synonymous SNP within the second exon of *TERT* (rs2736098) remained statistically significant ($P = 2.4 \times 10^{-3}$) (**Table 3**). Two strong recombination hotspots lie between the established and new SNPs in the 1000G CEU data (likelihood ratios of 27.1 and 261.0)³², and the two SNPs are in modest linkage disequilibrium (LD; $r^2 = 0.22$ in the 1000G CEU data) (**Fig. 1**).

TERT encodes the catalytic subunit of telomerase reverse transcriptase, a component of the ribonucleoprotein complex that maintains the integrity of chromosome ends. Inherited mutations affecting *TERT* underlie cases of dyskeratosis congenita, aplastic anemia, acute myeloid leukemia, familial melanoma and pulmonary fibrosis^{33,34}. *CLPTMIL* encodes the cleft lip and palate associated transmembrane 1-like protein that is involved in mediating apoptosis, aneuploidy, cisplatin resistance and RAS-mediated malignant transformation^{35,36}. Variants across the *TERT* and *CLPTMIL* region have been associated previously with risk of multiple cancers. Furthermore, independent signals within *TERT* and *CLPTMIL* have been identified for bladder cancer³⁷, chronic lymphocytic leukemia³⁸ and lung cancer^{37,39}, and fine-mapping studies have identified at least four independent signals across the *TERT* and *CLPTMIL* region that are associated with cancer^{40,41}. The new SNP identified in PanScan III (rs2736098) is located in a region of LD spanning ~4 kb from the promoter region to exon 2 of *TERT*. This SNP and several correlated SNPs have been associated with telomere length in white blood cells, as well as *TERT* promoter activity^{37,40,41}. The minor allele of rs2736098 that is associated with

a lower risk of pancreatic cancer in PanScan is also associated with longer telomeres and lower risk of breast cancer⁴⁰. Although further characterization of this region will be necessary, the new SNP in exon 2 of *TERT* appears to mark an independent risk locus for pancreatic cancer.

After conditioning on the established SNP at 9q34.2 in the first intron of *ABO*, the SNP rs4962153 in *ADAMTS13* identified in PanScan III was not statistically significant ($P = 0.28$), indicating that these two SNPs point to the same susceptibility haplotype (Table 3).

We identified a promising risk locus at 8q24.21 (rs1561927, $P = 1.3 \times 10^{-7}$) in a nongenic region between *PVT1* and *LINC00977* (Supplementary Table 7 and Fig. 1). 8q24.21 is known to contain multiple cancer susceptibility loci that span over 2 Mb^{42,43}. The promising pancreatic cancer SNP is in LD with a SNP that is associated with ovarian cancer risk (rs10088218, $r^2 = 0.37$ in the 1000G CEU data, 24 kb upstream)⁴⁴, and the closest genes are centromeric to rs1561927: *MIR1208* (406 kb), *PVT1* (455 kb) and *MYC* (814 kb). Several 8q24.21 risk loci have been shown to interact with *MYC* or *PVT1* promoters through long-range chromosomal interaction, and allele-specific effects on the expression of both genes have been reported^{42,45,46}. However, these loci are located more than 1 Mb upstream of rs1561927 on 8q24.21 ($r^2 < 0.03$ in the 1000G CEU data).

In stratified analyses, we noted no statistically significant heterogeneity by geographic region or smoking status (Supplementary Tables 8 and 9). In a preliminary analysis that included 173 cases and 430 controls of Asian ancestry (Supplementary Table 10), we examined the susceptibility loci identified in individuals of European descent^{5,6} (Table 2). We also assessed previously published pancreatic cancer risk loci from individuals of Chinese⁷ and Japanese⁸ ancestry, noting no loci and one locus, respectively, as being nominally statistically significant in PanScan (Supplementary Table 11).

To pursue the first steps toward understanding the functional underpinnings of the newly identified risk alleles, we conducted bioinformatic analyses using HaploReg⁴⁷ (Supplementary Table 12). We also evaluated expression quantitative trait locus (eQTL) effects^{48–50} (Supplementary Table 12). We noted *cis*-eQTLs on chromosome 16q23.1 in peripheral blood (*CFDP1*), chromosome 13q12.2 in skin and liver (*POMP*), chromosome 22q12.1 in liver and peripheral blood (*CCDC117*) and peripheral blood (*XBPI*) and chromosome 8q24.21 in adipose tissue (*PVT1*). *XBPI* at chromosome 22q12.1 regulates pancreatic beta-cell function with effects on systemic glucose control⁵¹ and modulates acinar cell homeostasis²⁵. In a gene set enrichment analysis⁵² of genes within 100 kb of the ten index SNPs identified in PanScan, the only statistically significant pathway was maturity onset diabetes of the young ($P = 3.3 \times 10^{-4}$). Understanding the functional consequences of pancreatic cancer susceptibility variants will require further laboratory investigation.

In a linear-mixed model analysis⁵³ (Online Methods), we estimated that the heritability for pancreatic cancer due to common SNPs present on GWAS arrays was 13% (95% CI 4–22%). Furthermore, we estimated that the nine loci identified in individuals of European ancestry account for approximately 9% of the total heritability tagged by common SNPs. We also evaluated the cumulative association with pancreatic cancer of risk alleles at susceptibility loci identified in individuals of European descent. Compared to individuals with the most prevalent number of risk alleles in controls ($n = 10$), those with ≤ 6 risk alleles had an OR of 0.55 (95% CI 0.44–0.68) and those with ≥ 14 risk alleles had an OR of 2.24 (95% CI 1.80–2.80) for pancreatic cancer (Supplementary Fig. 2).

In conclusion, our multistage GWAS revealed new loci that are associated with pancreatic cancer risk, as well as promising loci that

merit follow-up study. Several of the new loci harbor plausible candidate genes that have been implicated in pancreas development, pancreatic beta-cell function and predisposition to diabetes. Further investigation is warranted to understand the biological underpinnings of these common pancreatic cancer susceptibility alleles.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

B.M.W., C.R., P.K., C.K., G.M.P., P.H., C.F., S.J.C., R.S.S.-S. and L.T.A. organized and designed the study. B.M.W., C.R., F.C., L.B., R.S.S.-S. and L.T.A. conducted and supervised the genotyping of samples. B.M.W., C.R., P.K., C.K., Z.W., R.B., R.S.S.-S. and L.T.A. contributed to the design and execution of statistical analyses. B.M.W., R.S.S.-S. and L.T.A. wrote the first draft of the manuscript. B.M.W., C.R., P.K., C.K., G.M.P., A.A.A., L.B.-F., P.M.B., J.B., F.C., E.J.D., S.G., G.G.G., G.E.G., P.J.G., E.J.J., A.K., A.P.K., L.N.K., M.H.K., D.L., N.M., S.H.O., H.A.R., H.D.S., K.V., E.W., W.Z., C.C.A., D.A., G.A., M.A.A., D.B., S.I.B., M.-C.B.-R., M.B., M.W.B., H.B.B.-d.-M., P.B., D.C., N.E.C., G.C., C.C., M.C., E.C., J.E., N.F., J.M.G., N.A.G., E.L.G., M. Goggins, M.J.G., M. Gross, C.A.H., M.H., K.J.H., B.E.H., E.A.H., N.H., D.J.H., F.I., M.J., R.K., T.J.K., K.-T.K., E.A.K., M.K., V.K., J.K., R.C.K., A.L., M.T.L., S.L., L.L.M., A.M., S.M., R.L.M., Y.N., A.L.O., K.O., A.V.P., P.H.M.P., U.P., R.P., A.P., M.P., F.X.R., E.R., N.R., A.S., X.-O.S., D.T.S., P.S., M.S., R.T.-W., P.R.T., G.E.T., M.T., A.T., G.S.T., D.T., P.V., J.W.-W., N.W., C.W., H.Y., K.Y., A.Z.-J., R.H., P.H., C.F., S.J.C., R.S.S.-S. and L.T.A. conducted the epidemiological studies and contributed samples to the GWAS and/or follow-up genotyping. All authors contributed to the writing of the manuscript.

COMPETING FINANCIAL INTERESTS

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ONLINE METHODS

Stage 1: GWAS for PanScan III. We conducted a GWAS of pancreatic cancer using case and control subjects from 17 studies (**Supplementary Table 1**). Cases with pancreatic cancer included individuals newly identified from nine cohort studies that participated in PanScan I⁵, as well as those from five new cohort studies, two new case series and one new case-control study. The new cohort studies included the Agricultural Health Study (AHS)⁵⁴, the Melbourne Collaborative Cohort Study (MCCS)⁵⁵, the Multiethnic Cohort Study (MEC)⁵⁶, the Selenium and Vitamin E Cancer Prevention Trial (SELECT)⁵⁷ and the Vitamins and Lifestyle Study (VITAL)⁵⁸. The new case-based studies were the Gastrointestinal Cancer Clinic of Dana-Farber Cancer Institute (DFCI-GCC) study, the Spanish Pancreatic Cancer Study PANKRAS-II⁵⁹ and the PANDORA-Heidelberg pancreatic cancer case-control study¹⁰. Cases were defined as those individuals having primary adenocarcinoma of the exocrine pancreas (ICD-O-3 code C250-C259). Those with non-exocrine pancreatic tumors (histology types 8150, 8151, 8153, 8155 and 8240) were excluded. Each participating study obtained informed consent from study participants, approval from its institutional review board (IRB) for this study and IRB certification permitting data sharing in accordance with the NIH Policy for Sharing of Data Obtained in NIH-Supported or NIH-Conducted Genome-Wide Association Studies.

All samples from cases with pancreatic cancer that had sufficient DNA ($n = 1,894$) were genotyped on the Illumina OmniExpress chip at the NCI Cancer Genomic Research Laboratory (CGR) (**Supplementary Table 2**). Genotypes were called using the Illumina GenomeStudio software. Genotype clusters for new cases were estimated using samples with a completion rate of 98% to optimize accuracy. Genotypes for all samples, including those initially excluded, were subsequently called on the basis of the optimized cluster file. Extensive quality-control metrics were applied to the data: SNPs with a call rate <94% or Hardy-Weinberg proportion $P < 1 \times 10^{-7}$ were excluded ($n = 18,765$); samples with a call rate <94% ($n = 78$) and those with mean heterozygosity <26% or >33% ($n = 2$) based on autosomal SNPs or gender discordance (>5% heterozygosity based on the X chromosome SNPs for males or <20% heterozygosity based on the X chromosome SNPs for females, $n = 5$) were excluded. Unexpected duplicates (>99.9% concordance, $n = 3$) and first-degree relatives ($n = 2$, on the basis of identity-by-descent sharing with $\text{Pi-hat} > 0.40$) were removed. Quality-control duplicate samples in PanScan III ($n = 38$ pairs) showed >99.9% genotype concordance. Duplicates with PanScan I or II were removed (>99.9% concordance, $n = 21$). Ancestry was assessed using the Genotyping Library and Utilities (GLU) struct.admix module. Participants with <80% European ancestry ($n = 199$) were excluded for the primary analysis of individuals of European ancestry (**Supplementary Fig. 3**). After exclusions, 1,582 cases of European ancestry were available for analysis (**Supplementary Tables 2 and 3**).

Controls of ≥80% European ancestry were drawn from ten of the studies included in PanScan III (Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study (ATBC), American Cancer Society Cancer Prevention Study-II Cohort (CPS-II), European Prospective Investigation into Cancer and Nutrition (EPIC), Health Professionals Follow-Up Study (HPFS), Melbourne Collaborative Cohort Study (MCCS), Multiethnic Cohort Study (MEC), Nurses' Health Study (NHS), Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), Spanish Pancreatic Cancer Study (SPCS, PANKRAS-II and Spanish Bladder Cancer SBC/EPICURO studies)⁶⁰ and Women's Health Initiative (WHI)). These controls had no history of cancer, were not included in PanScan I or PanScan II and had been genotyped previously at CGR on the Illumina OmniExpress, Omni1M or Omni2.5M arrays with extensive quality control, as previously described^{9,61–64}. In total, 5,203 controls were included in the analysis (**Supplementary Tables 2 and 3**). A total of 608,202 SNPs with overall completion rate >80% in both cases and controls were advanced to the association analysis. To evaluate population substructure, a principal components analysis was performed using the struct.pca module of GLU, version 1.0, which is similar to EIGENSTRAT⁶⁵. Plots of the first six principal components are shown in **Supplementary Figure 4**. The estimated inflation of the test statistic, λ , was 1.02 (ref. 66); a quantile-quantile (QQ) plot is shown in **Supplementary Figure 5**.

Association analysis was performed assuming a log-additive genetic model and adjusting for age, sex, geographic region and six significant eigenvectors

(i.e., eigenvectors that were nominally significant in a baseline risk model adjusting for age, sex and geographic region). Geographic region was defined as REGION_US (United States): AgHealth, CPS-II, DFCI, HPFS, MEC, NHS, NYU-WHS, PHS, PLCO, SELECT, VITAL, WHI; REGION_CNE (central and northern Europe): ATBC, EPIC, PANDORA-Heidelberg, MCCS (Melbourne); and REGION_SE (southern Europe): SBCS (Spain controls), PANKRAS-II (cases). All data analyses and management were conducted using GLU.

Stage 2: PanScan I and II data. The second stage involved the primary whole genome-scan data from the previously reported PanScan I⁵ and PanScan II⁶ studies. PanScan I and PanScan II were genotyped on the Illumina HumanHap550 Infinium II and the Human 610-Quad chips, respectively, whereas PanScan III was genotyped on the OmniExpress chip. As the number of overlapping SNPs between the three chips is moderate (~300,000), imputation of missing genotypes was performed using phased haplotypes from the DCEG reference set and IMPUTE2 (refs. 9,67). The DCEG reference set is well designed for 'filling in' missing genotypes across chip designs in PanScan, as it is based on several of the same studies included in PanScan, and the imputation accuracy is improved over 1,000G and HapMap data⁹. Imputed SNPs with low MAF (<0.01) or low-quality scores (IMPUTE2 information score <0.3) were removed before the association analysis. The same quality thresholds as described above for stage 1 were applied for stage 2. The final numbers of cases and controls included in stage 2 were 1,757 cases and 1,801 controls from PanScan I and 1,768 cases and 1,841 controls from PanScan II.

To combine the data from PanScan I, II and III, meta-analyses were performed using the fixed-effects inverse-variance method based on the β estimates and standard errors. No heterogeneity was observed across stages 1 and 2 for the SNPs identified as GWAS significant or suggestive in the full study (P heterogeneity ≥ 0.11 ; **Supplementary Table 4**). A Manhattan plot for the results of the meta-analysis of stage 1 and stage 2 is shown in **Supplementary Figure 6**.

Association analysis was also performed in 173 cases and 430 controls of Asian ancestry from the Shanghai Men's and Women's Health Study (SMWHS) (**Supplementary Table 10**). This analysis included case and control subjects from stages 1 and 2 of PanScan III and previously genotyped control subjects from SMWHS⁶⁸. The quality-control methods and association analysis were performed as described above for the European ancestry subjects.

Stage 3: replication studies. Thirteen SNPs (P value threshold of $<5 \times 10^{-5}$) were taken forward for *de novo* replication in an additional 2,576 cases and 5,552 controls. The replication samples were analyzed individually as three groups: (i) CGR: pancreatic cancer case and control subjects from CARET⁶⁹ plus samples from cases that did not have sufficient DNA for the full GWAS and control subjects previously genotyped at CGR; (ii) PANDORA: case and control subjects from the PANDORA pancreatic cancer case-control consortium¹⁰ (no overlap with the PANDORA-Heidelberg cases genotyped in stage 1); and (iii) CALBG/Alliance 80303: cases from a randomized clinical trial of gemcitabine plus placebo versus gemcitabine plus bevacizumab¹¹ and control subjects previously genotyped at CGR (**Supplementary Table 5**).

Genotyping for cases in group (i) was performed using custom TaqMan genotyping assays (Applied Biosystems) at CGR. Genotyping for cases and controls from PANDORA (group ii) was performed in the same manner but at the German Cancer Research Center (DKFZ) in Heidelberg, Germany. Quality-control duplicate samples in the replication (CGR, $n = 20$ pairs; PANDORA, $n = 512$ pairs) showed >99.9% genotype concordance. Patients enrolled in CALBG/Alliance 80303 (group iii) were previously genotyped using the Illumina HumanHap550v3 Genotyping BeadChip array¹¹. Control subjects from PLCO previously genotyped at CGR using the Illumina HumanHap550v3 Genotyping BeadChip array⁷⁰ were used for groups (i) and (iii) (**Supplementary Table 5**) and did not overlap with control subjects included in PanScan I, II or III. CALBG/Alliance 80303 and control genotypes were imputed to OmniExpress SNP content in the same manner as described above for stage 1. Quality-control thresholds and exclusions for samples and loci in the replication are listed in **Supplementary Table 5**. Association results for the replication studies were adjusted for age, sex and study, and a meta-analysis of the three replication groups was performed using the fixed-effects inverse-variance method based on the β estimates and standard errors

(Supplementary Table 6). This was followed by a meta-analysis of stages 1 and 2 and the replication for the 13 SNPs using the same fixed-effects inverse-variance method.

Technical validation. A comparison of the genotyping calls from the imputation of PanScan I and II into the OmniExpress array contents and confirmatory TaqMan assays ($n = 511$ samples from PanScan I and II) yielded r^2 values of 0.74, 0.96, 0.56, 0.99, 0.98 and 1.00 for rs2736098, rs6971499, rs7190458, rs9581943, rs16986825 and rs1561927, respectively.

Estimate of recombination hotspots. To identify recombination hotspots, we used SequenceLDhot³², a program that uses the approximate marginal likelihood method⁷¹ and calculates likelihood ratio statistics at a set of possible hotspots. We tested five unique sets of 100 control samples. The PHASE v2.1 program was used to calculate background recombination rates^{72,73}, and LD heat maps were visualized using the snp.plotter program⁷⁴. For an estimation of recombination hotspots between loci in *TERT* and *CLPTM1L* on chromosome 5p15.33, we used the 1000G (version 3) CEU data.

Heritability analysis. To estimate the heritability explained by common SNPs present on GWAS arrays on the liability scale (lifetime disease risk of 0.015), we used GCTA^{53,75} on a set of LD-pruned SNPs ($r^2 < 0.5$) that passed the following stringent quality-control thresholds: MAF $> 1\%$, SNP missing rates $< 5\%$, subject missing rate $< 1\%$ and Hardy-Weinberg equilibrium P values $> 10^{-4}$. Nonautosomal SNPs and pairs of subjects with genetic relatedness $> 5\%$ were removed. These analyses were run separately in PanScan I, II and III adjusting for age, sex, study (or geographic region in PanScan III) and the significant principal components in each study. PanScan III analyses were restricted to participating studies that contributed both cases and controls. PanScan I, II and III results were combined in a meta-analysis. We repeated the analyses restricted to the genome wide-significant SNPs in individuals of European ancestry to estimate the proportion of heritability tagged by these nine SNPs.

Further follow-up analyses. We constructed a genetic risk score for pancreatic cancer incorporating the susceptibility loci identified in PanScan I, II and III. For this analysis, subjects could possess 0–20 risk alleles based on their genotypes at the 10 identified loci. ORs were calculated using multivariable-adjusted unconditional logistic regression with meta-analysis to combine data from stages 1 and 2, as was done in the analyses of individual SNPs. Replication samples were not genotyped for the four susceptibility loci identified in PanScan I and II, and therefore these subjects could not be included in the risk score analysis. Subjects with missing genotypes for one or more of the ten SNPs ($n = 898$) were assigned the most common genotype at that SNP among cases or controls. In sensitivity analyses, results were unchanged if these subjects were excluded. Using 1000G CEU data, we identified SNPs with $r^2 > 0.7$ with our lead SNP. We used HaploReg v2 (ref. 47), a tool for exploring noncoding functional annotation using ENCODE data, to evaluate the genome surrounding our SNPs (Supplementary Table 12). In addition, we evaluated *cis* associations between all new and promising SNPs discovered in this study and the expression of nearby genes in skin biopsies, adipose biopsies and nontransformed peripheral blood samples from subjects of European descent

from publically available data sets^{48,50} (Supplementary Table 12). Gene set enrichment analysis was also performed for genes in pancreatic cancer risk loci identified in subjects of European descent (in a window of 100 kb centered on the most significant SNP in each locus) based on KEGG (Kyoto Encyclopedia of Genes and Genomes) annotations using GeneCodis3 with reporting of the corrected hypergeometric P value⁵².

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