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Genetic variants of the peroxisome proliferator-activated receptor (PPAR) signaling pathway genes and risk of pancreatic cancer

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Abstract

Because the peroxisome proliferator-activated receptor (PPAR) signaling pathway is involved in development and progression of pancreatic cancer, we investigated associations between genetic variants of PPAR pathway genes and pancreatic cancer risk by using three published genome-wide association study datasets including 8,477 cases and 6,946 controls of European ancestry. Expression quantitative trait loci (eQTL) analysis was also performed for correlations between genotypes of the identified genetic variants and mRNA expression levels of their genes by using available databases of the 1000 Genomes, TCGA and GTEx projects. In the single-locus logistic regression analysis, we identified 1,141 out of 17,532 significant single nucleotide polymorphisms (SNPs) in 112 PPAR pathway genes. Further multivariate logistic regression analysis identified three independent, potentially functional loci (rs12947620 in *MED1*, rs11079651 in *PRKCA*, and rs34367566 in *PRKCB*) for pancreatic cancer risk [odds ratio = 1.11, 95% confidence interval = 1.06–1.17, $P = 5.46\times10^{-5}$; 1.10 (1.04–1.15), $P = 1.99\times10^{-4}$; and 1.09 (1.04–1.14), $P = 3.16\times10^{-4}$,

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Conflicts of interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

respectively] among 65 SNPs that passed multiple comparison correction by false discovery rate (FDR <0.2). When risk genotypes of these three SNPs were combined, carriers with 2–3 unfavorable genotypes (NUGs) had a higher risk of pancreatic cancer than those with 0–1 NUGs. The eQTL analysis showed that rs34367566 A>AG was associated with decreased expression levels of *PRKCB* mRNA in 373 lymphoblastoid cell lines. Our findings indicate that genetic variants of PPAR pathway genes, particularly *MED1*, *PRKCA*, and *PRKCB*, may contribute to susceptibility to pancreatic cancer.

Keywords

PPAR; Single nucleotide polymorphism; Genome-wide association study; Pathway analysis; Pancreatic cancer susceptibility

Introduction

Pancreatic cancer is one of the deadliest human cancers, and it is the seventh most common cause of cancer deaths worldwide[1] and the fourth leading cause of cancer-related deaths in the United States[2]. Notably, the incidence has sharply increased up to 15.5/1000000 in the past decades[3]. Many epidemiological studies have consistently reported that some host factors, such as smoking, overweight, a history of diabetes or chronic pancreatitis are risk factors for pancreatic cancer[4–7]. Recently, accumulating evidence suggests that genetic factors also contribute to the development of pancreatic cancer[8,9], because people with a family history of pancreatic cancer are more likely to develop this disease than those without a family history[6]. Among the genetic factors, single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in human genomes and have been identified to be associated with pancreatic cancer risk[9–11]. Therefore, pancreatic cancer as a complex disease is also likely to have a complex interplay between environmental and genetic factors.

Genome-wide association study (GWAS) examines hundreds of thousands of SNPs across the genome at the same time in a hypothesis-free manner, making it a promising approach to studying susceptibility to complex diseases, like caner. GWASs have been used to identify pancreatic cancer susceptibility loci in different ethnic populations[12–18]. To date, a total of 13 loci (9q34.2, 13q22.1, 17q24.3, 7q23.2, 5p15.33, 1q32.1, 16q23.1, 13q12.2, 2p14, 22q12.1, 7p14.1 and 3q28, 8q24.1) have been reported to be associated with pancreatic cancer risk in populations of European ancestry[12–16]. Additionally, some pancreatic cancer susceptibility loci have also been reported in Chinese and Japanese populations[17, 18]. However, based on the stringent genome-wide significance level of $P<5\times10^{-8}$, many potential cancer risk-associated variants may not be detected due to their weak effects at the GWAS level[19]. Recently, the pathway-based analysis of GWAS datasets has been used as a useful hypothesis-driven strategy to search for additional SNPs in multiple genes of a particular biological pathway for susceptibility to complex disease, including cancer[20–22]. Several biological pathways have been suggested as candidate pathways for pancreatic carcinogenesis, such as the platelet-derived growth factor (PDGF) pathway[23], the neuroactive ligand receptor interaction pathway and the olfactory transduction pathways[24] as well as the pancreatic development pathway[25].

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play crucial roles in the regulation of cellular differentiation, development, metabolism, and tumorigenesis. Genetic variants in genes encoding these receptors are thought to contribute to carcinogenesis[26–28]. For example, the Pro12Ala polymorphism in the peroxisome proliferator activated receptor gamma (PPARG) was found to be a risk factor for gastric cancer[26], while PPAR alpha and gamma were found to be associated with breast cancer risk[27]. Another study found that the Pro12Ala polymorphism in PPARG could confer susceptibility to pancreatic cancer[28].

Although the above-mentioned studies have investigated associations between SNPs in PPAR pathway genes and cancer risk, only a limited number of variants in few candidate genes were included in those studies with limited sample sizes. Thus, there is a need to perform a holistic analysis of SNPs in all genes involved in the PPAR pathway for their contribution to pancreatic cancer susceptibility. In the present study, therefore, we examined associations between 17,532 genetic variants of 112 PPAR pathway genes and pancreatic cancer risk in 8,477 pancreatic cancer cases and 6,946 cancer-free controls of European ancestry.

Materials and Methods

Study populations

The subjects included in the present study has been described in a previous publication[23]. Briefly, there are two published GWASs with available genotyping data to be used for the present study, which include the PanScan study and the Pancreatic Cancer Case Control Association Study. The PanScan study consisted of 5213 cases and 3905 controls[12–15], while the Pancreatic Cancer Case Control Association Study was obtained from the Pancreatic Cancer Case-Control consortium (PanC4), consisting of 4168 cases and 3814 controls from the United States, Europe, and Australia[16]. To focus on the subjects of European ancestry, we included 15,423 individuals (8,477 cases and 6,946 controls) (Table S1) the final analysis. A written informed consent was obtained from each participant for the PanScan study, the present study protocol was approved by Duke University Medical Center Institutional Review Board, and the GWAS data access were granted by National Center for Biotechnology Information (NCBI) for dbGaP accession# phs000206.v5.p3 and dbGaP accession# phs000648.v1.p1.

Gene and SNP selection

The PPAR pathway genes were collected from Molecular Signatures Database (MSigDB) (http://www.broadinstitute.org/gsea/msigdb/index.jsp) by the keyword “peroxisome proliferator-activated receptor” or “PPAR”. In total, 112 genes were selected as candidate genes after removing duplicate genes and genes located in × chromosome (Table S2).

SNP imputation was performed with a 250-kb buffer region on each side of the 112 genes with IMPUTE2 based on the 1000 Genomes Project (Panel 3) (Figure S1). After quality control for SNPs, there were 20,507, 23,324, and 20,230 SNPs within the genes with their ±

2 kb flanking regions for PanScan I, PanScan II/III, and panC4, respectively. The final 17,532 SNPs from each of the three datasets were included in a meta-analysis according to criteria: minor allele frequency (MAF) ≥ 0.01, genotyping rate ≥ 95%, and Hardy-Weinberg equilibrium P value ≥ 1×10^{-5}. All remaining SNPs also passed the quality control of imputation with info ≥ 0.40 in IMPUTE2.

Functional annotation and eQTL analysis

Functional prediction of the identified SNPs was performed by using three online tools: SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm), RegulomeDB (http://www.regulomedb.org/), and HaploReg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php). SNPinfo is a website with a sute of tools for single nucleotide polymorphism detection; RegulomeDB is a website that allows one to identify DNA features and regulatory elements in non-coding regions of the human genome; and HaploReg is a tool for exploring annotations of the noncoding genome at variants on haplotype blocks, such as candidate regulatory SNPs at disease-associated loci.

We performed the expression quantitative trait loci (eQTL) analysis to investigate the correlations between genotypes of the identified SNPs and mRNA expression levels of the corresponding genes. Data from other three sources were also used, including the 1000 Genomes Project, the Cancer Genome Atlas (TCGA) (http://tcga-data.nci.gov/tcga/), and the Genotype-Tissue Expression project (GTEx). In the 1000 Genomes Project, the mRNA expression data were from the lymphoblastoid cell lines of 373 Europeans. In the TCGA, there were genotype and phenotype data for 127 Europeans, while GTEx was an online database of 663 samples with various genotype and gene expression data from different tissues. Additionally, Oncomine™ database was used to compare mRNA expression levels between normal and tumor tissues (https://www.oncomine.org/).

Statistical analysis

There were 20 principal components (PCs) in the pancreatic cancer GWAS datasets, and these 20 PCs were first analyzed for their associations with pancreatic cancer risk by univariate logistic regression analysis (Table S3). As a result, the top five significant PCs with P value < 0.001 were included as covariates for the adjustment in further multivariate analyses. For each SNP, odd ratios (ORs) and 95% confidence intervals (CIs) were estimated in a single-locus analysis by unconditional logistic regression with adjustment for age, sex and significant PCs by using PLINK 1.9. Cochran's Q statistics and I^2 were performed to estimate heterogeneity. If there was no heterogeneity (I^2 <50% and Q-test P >0.1), a meta-analysis in fixed-effects models was used to calculate associations between SNPs and pancreatic cancer risk in the combined dataset of all three studies. The false discovery rate (FDR) with a cut-off 0.2 for multiple comparison correction was used to reduce the probability of false-positive findings. The independent SNPs were further determined by the stepwise multivariate logistic regression analysis with adjustment for other available covariates and previously published risk-associated SNPs from the same study populations. The risk associated with genotypes of the identified significant and independent SNPs was further assessed in a genetic score (the combined genotypes) and in stratified analyses by the available covariates. In the eQTL analysis, associations between genotypes of the SNPs and

mRNA expression levels were calculated by using a general linear regression model. Manhattan plots and linkage disequilibrium (LD) plots were made by Haploview v4.2. Regional association plots were generated by LocusZoom (http://locuszoom.sph.umich.edu/locuszoom/). Unless specified otherwise, all statistical analyses were performed using R (version 3.2.2), SAS (version 9.4; SAS Institute, Cary, NC, USA), and PLINK (version 1.07).

Results

Subject characteristics

The final analysis included 8,477 cases and 6,946 controls whose demographic characteristics have been described previously (Table S1). In brief, sex was similarly distributed between the case and control groups in all three studies, but the cases were younger than the controls in all three studies.

Associations between SNPs and pancreatic cancer risk

The study workflow is outlined in Figure 1. There were 20,507, 23,324, and 20,230 SNPs in PanScan I, PanScan II/III, and panC4, respectively. The single-locus analysis showed that there were 1338, 1171 and 1036 SNPs with a nominal $P < 0.05$ in PanScan I, PanScan II/III, and panC4, respectively (Figure S2). Then, a meta-analysis using the summary genotyping data from these three GWASs was performed. An overview of overall associations is shown in the Manhattan plot (Figure 2A). A total of 1141 SNPs of 112 PPAR pathway genes were identified with a nominal $P < 0.05$ in the meta-analysis. Of these, 65 SNPs in *MED1, PRKCA* and *PRKCB* remained statistically significant after multiple test correction by FDR < 0.2. Of these 65 SNPs, there were 46 SNPs in *MED1*, one SNP in *PRKCA*, and 18 SNPs in *PRKCB*. More detailed information for 65 SNPs (including position, effect allele, relative minor allelic frequency, effect sizes, unadjusted and FDR, and adjusted *P* values) are summarized in Table 1 and Table S4.

LD analysis and functional prediction

Because there was only one SNP in *PRKCA*, we performed the LD analysis for *MED1* and *PRKCB*. The results showed that two blocks (of seven and 35 SNPs) shared a high LD in *MED1*, respectively ($r^2 \geq 0.80$, Figure 2B and 2C), and another 17 SNPs shared a high LD in *PRKCB* ($r^2 \geq 0.80$, Figure 2D and 2E). Functional prediction was performed for these 65 SNPs by using SNPinfo, RegulomeDB, and HaploReg. According to the LD map and online functional prediction (Table S5), five SNPs (rs12947620, rs8076041 and rs12945015 in *MED1*, rs34367566 in *PRKCB,* and rs11079651 in *PRKCA*) were selected as the tagSNPs for further analysis.

Independent associations between SNPs and pancreatic cancer risk

Then, we included the five potentially functional SNPs (i.e. rs12947620, rs8076041, rs12945015, rs34367566 and rs11079651) in a multivariate stepwise logistic regression model. After adjustment for sex, age, and the top five PCs ($P < 0.001$), three SNPs (i.e., rs12947620, rs11079651 and rs34367566) were found to be independently associated with pancreatic cancer risk (Table 2). Furthermore, we put these three SNPs and all previously

published risk-associated SNPs from the same study populations of pancreatic cancer studies into a multivariate model and found that all of these three SNPs remained significantly associated with pancreatic cancer risk (Table S6).

Specifically, the SNP rs12947620 C>T was significantly associated with risk in all genetic models. In an additive model, there was a linear trend between the number of rs12947620 T allele and pancreatic cancer risk (P_{trend} <0.001), while the SNP rs11079651 C>T was also significantly associated with risk in all genetic models with a linear trend (P_{trend} =0.0001). Similarly, the SNP rs34367566 A>AG was significantly associated with pancreatic cancer risk only in additive and dominant models. In the additive model, carriers with the rs34367566 AG allele had a high risk of pancreatic cancer, and the risk increased as the number of minor alleles increased (P_{trend} =0.0004) (Table 3). Compared with the reference genotype in a dominant genetic model, carriers with *MED1* rs12947620 CT+TT, *PRKCA* rs11079651 CT+TT, and *PRKCB* rs34367566 A/AG+AG/AG genotypes had a higher risk of pancreatic cancer (OR=1.12, 95% CI=1.05–1.19, *P*=0.0008; OR=1.12, 1.05–1.19, 0.0008; and 1.14, 1.06–1.22, 0.0002; respectively).

Combined and stratified analyses

In a dominant model, we combined risk genotypes (i.e., rs12947620 CT+TT, rs11079651 CT+TT, and rs34367566 A/AG+AG/AG) into a single variable as the number of unfavorable genotypes (NUGs) that were further divided into: 0, 1, 2, and 3. The trend test showed that there was a significant trend in association of an increased NUGs with an increased pancreatic cancer risk (*P* <0.0001, Table 3). We further dichotomized all the subjects by NUG into two groups: low-score group (0–1) and high score group (2–3). Compared with the low-score group, individuals of the high-score group had a significantly higher risk of developing pancreatic cancer (OR=1.20, 95% CI=1.12–1.28, *P* <0.0001). Using this dichotomized variable, we further performed stratified analysis by age and sex to investigate their interactions with the combined risk genotypes on pancreatic cancer risk. We found that those with a high NUG score had a higher risk of pancreatic cancer than those of the low-score group in both males and females, but the results were similar among different age groups (< 60, 60–70, and > 70); thus, there was no statistical evidence for an interaction. (Table S7).

eQTL analysis

We also performed eQTL to investigate the correlations between variant genotypes and their gene expression levels. By using the available data on genotypes and gene expression in the 1000 Genomes Project, we found that the rs34367566 genotypes were significantly associated with a decreased mRNA expression level of *PRKCB* in both additive and recessive models (*P* = 0.037 and *P* = 0.046, respectively; Figure 3), but not for other two SNPs (*MED1* rs12947620 and *PRKCA* rs11079651); however, none of positive correlations were observed in the TCGA database, and no data for three SNPs available in the online GTEx database. Finally, we found that expression levels of all three genes, *MED1*, *PRKCA*, and *PRKCB*, were higher in tumor tissues than in normal tissues in the Oncomine™ database (Figure S3).

Discussion

Prior studies have reported associations between genetic variants in PPAR pathway genes and risk of cancers of the stomach, breasts, and pancreas[26–28] for a limited number of variants in a small number of candidate genes. These candidate gene studies typically investigated the PPAR alpha and gamma genes in populations with limited sample sizes. In the present study, we investigated the associations between 17,532 genetic variants of 112 PPAR pathway genes and pancreatic cancer risk in 8,477 cases with pancreatic cancer and 6,946 controls. We found that *MED1* rs12947620, *PRKCA* rs11079651, and *PRKCB* rs34367566 were significantly associated with an increased risk of pancreatic cancer in populations of European ancestry. Furthermore, functional prediction analyses showed that the rs34367566 variant AG allele was associated with a decreased mRNA expression level of *PRKCB*. Interestingly, we also found that the expression levels of all three genes, *MED1*, *PRKCA*, and *PRKCB*, were higher in tumor tissues than in normal tissues, suggesting an oncogenic role of the PPAR pathway genes in pancreatic cancer. To the best of our knowledge, the present study is the first to comprehensively evaluate the roles of common genetic variants in associations between all the PPAR pathway genes and pancreatic cancer risk.

The *MED1* gene, also known as *MBD4* harboring the most significantly risk-associated SNP rs12947620, encodes a protein called the mediator of RNA polymerase II transcription subunit 1. This protein has been reported to be involved in several cellular processes, including apoptotic response to DNA damage[29], transcriptional repression[30], and chromosomal stability[31]. Additionally, MED1 can bind to the mismatch repair (MMR) protein MLH1 and modulate expression levels of the core MMR proteins[32]. Studies have shown that *MED1* mutations frequently occurred in cancers with microsatellite instability (MSI), including pancreatic cancer[33–35], while other studies indicated that MED1 suppressed mutations at CpG sites in mammalian genomes and that its loss could promote tumor formation[36,37]. However, the exact molecular mechanism how *MED1* rs12947620 may increase pancreatic cancer risk is not fully understood yet, which requires additional mechanistic and functional studies.

The *PKC* gene, tagged by two other risk-associated SNPs rs11079651 and rs34367566 identified in the present study, encodes a protein called protein kinase C (PKC), a family of homologous serine/threonine protein kinases. It is known that PKC participates in diverse cellular process by transducing signals, including proliferation, differentiation, apoptosis, and angiogenesis[38]. The PKC family is subdivided into three groups, the classical PKC (cPKC), novel PKC (nPKC) and atypical PKC (aPKC), of which the classical PKCs include PKCα, PKCβI, PKCβII and PKCγ[39,40]. The two SNPs identified in the present study belong to cPKC, a protein that regulates many aspects of tumorigenesis, including initiation, progression and metastasis[41]. Although different isoforms have different roles, they all act to influence similar signaling pathway downstream genes. For example, cPKCs are mainly activated by growth factors such as epidermal growth factor and oxidative stress, leading to the activation of transcription factors, such as nuclear factor-κB, signal transducer and activator of transcription. Both proteins have been demonstrated to play a role in metastasis and survival in patients with pancreatic cancer[42–44]. In the present study, we found that

rs34367566 AG variant genotypes were significantly associated with a decreased mRNA expression level of *PRKCB* in both additive and recessive models. However, the mRNA expression levers of *PRKCB* were higher in tumor tissues than in normal tissues in the OncomineTM database. It is possible that the expression levels of *PRKCB* may be affected by other genes, such as an imbalanced activation of the PPAP signaling pathway caused by mutations in the genes in tumor tissues, which may lead to abnormal expression of *PRKCB*. Future functional studies are necessary to elucidate the exact mechanism how *PRKCB* rs34367566 affects pancreatic cancer risk. Finally, the PPARs have been reported in numerous pathophysiological conditions, and many ligands have been designed to target PPARs receptors. However, to classify the therapeutic targets for drug development, more detailed mechanistic studies are needed[45].

The present study has several limitations. First of all, both of the two available pancreatic cancer GWAS datasets were from populations of European ancestry; therefore, the findings may not be generalized to other ethnic populations. Second, the access to clinical information, such as smoking, history of pancreatitis, diabetes, obesity and other data relevant to risk, was limited and therefore could not be integrated into the model for further adjustment in the risk analysis. Finally, the biological mechanisms by which the SNPs of the identified genes may influence pancreatic cancer risk remain unclear. Therefore, additional comprehensive mechanistic studies are needed to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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PanScan

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PanC4

The patients and controls for this study were derived from the following PANC4 studies: Johns Hopkins National Familial Pancreas Tumor Registry, Mayo Clinic Biospecimen Resource for Pancreas Research, Ontario Pancreas Cancer Study (OPCS), Yale University, MD Anderson Case Control Study, Queensland Pancreatic Cancer Study, University of California San Francisco Molecular Epidemiology of Pancreatic Cancer Study, International Agency of Cancer Research and Memorial Sloan Kettering Cancer Center. This work is supported by NCI R01CA154823 Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN2682011000111. The dbGaP accession number for this study used in this manuscript is phs000648.v1.p1.

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Abbreviations:

SNPs	Single nucleotide polymorphisms
GWAS	Genome-wide association study
PDGF	platelet-derived growth factor
PPARs	Peroxisome proliferator-activated receptors
PPARG	peroxisome proliferator activated receptor gamma
MAF	minor allele frequency
eQTL	expression quantitative trait loci
TCGA	the Cancer Genome Atlas
GTEx	genotype-tissue expression project
PC	principal components
ORs	odd ratios
CIs	confidence intervals
FDR	false discovery rate
LD	linkage disequilibrium
NUGs	number of unfavorable genotypes
MMR	mismatch repair
MSI	microsatellite instability
PKC	protein kinase C

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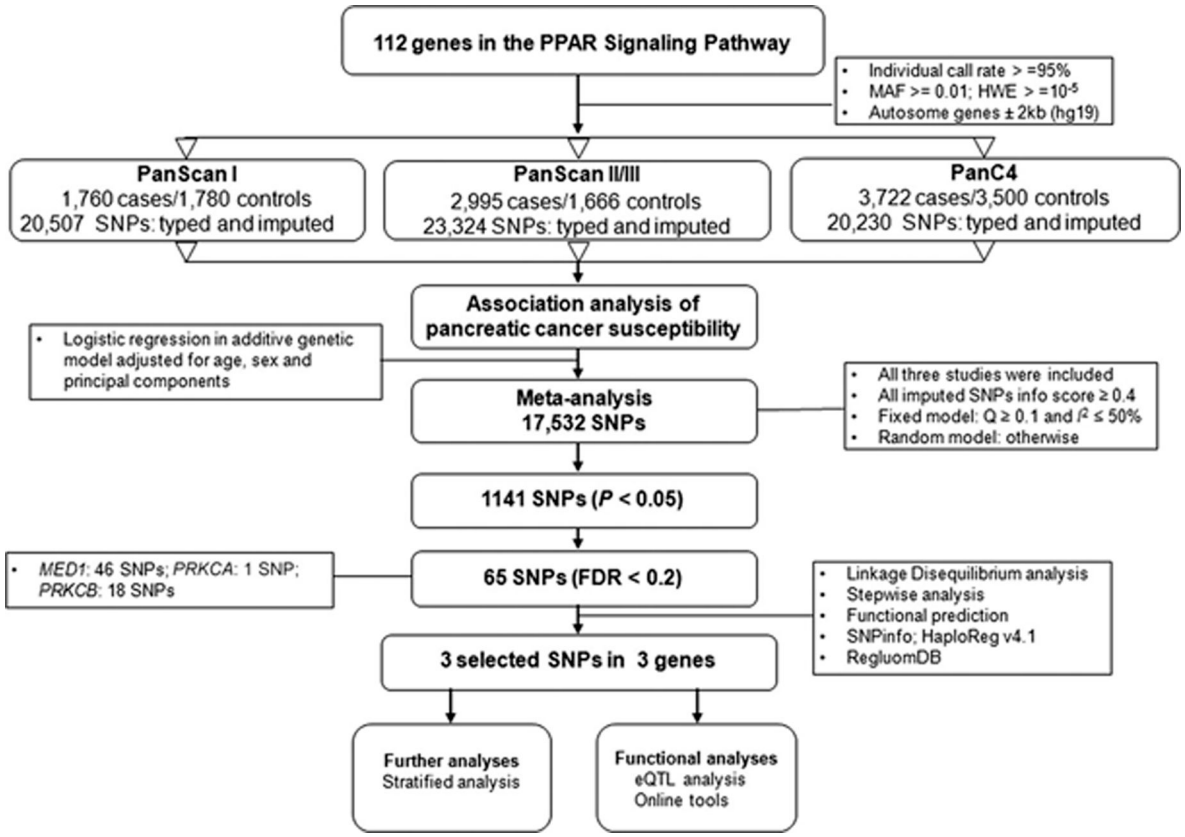

Figure 1. The workflow of the analysis.

(Abbreviations: PPAR: Peroxisome proliferator-activated receptor; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; PanC: pancreatic cancer; SNP: single-nucleotide polymorphism; FDR: false discovery rate; eQTL, expression quantitative trait loci)

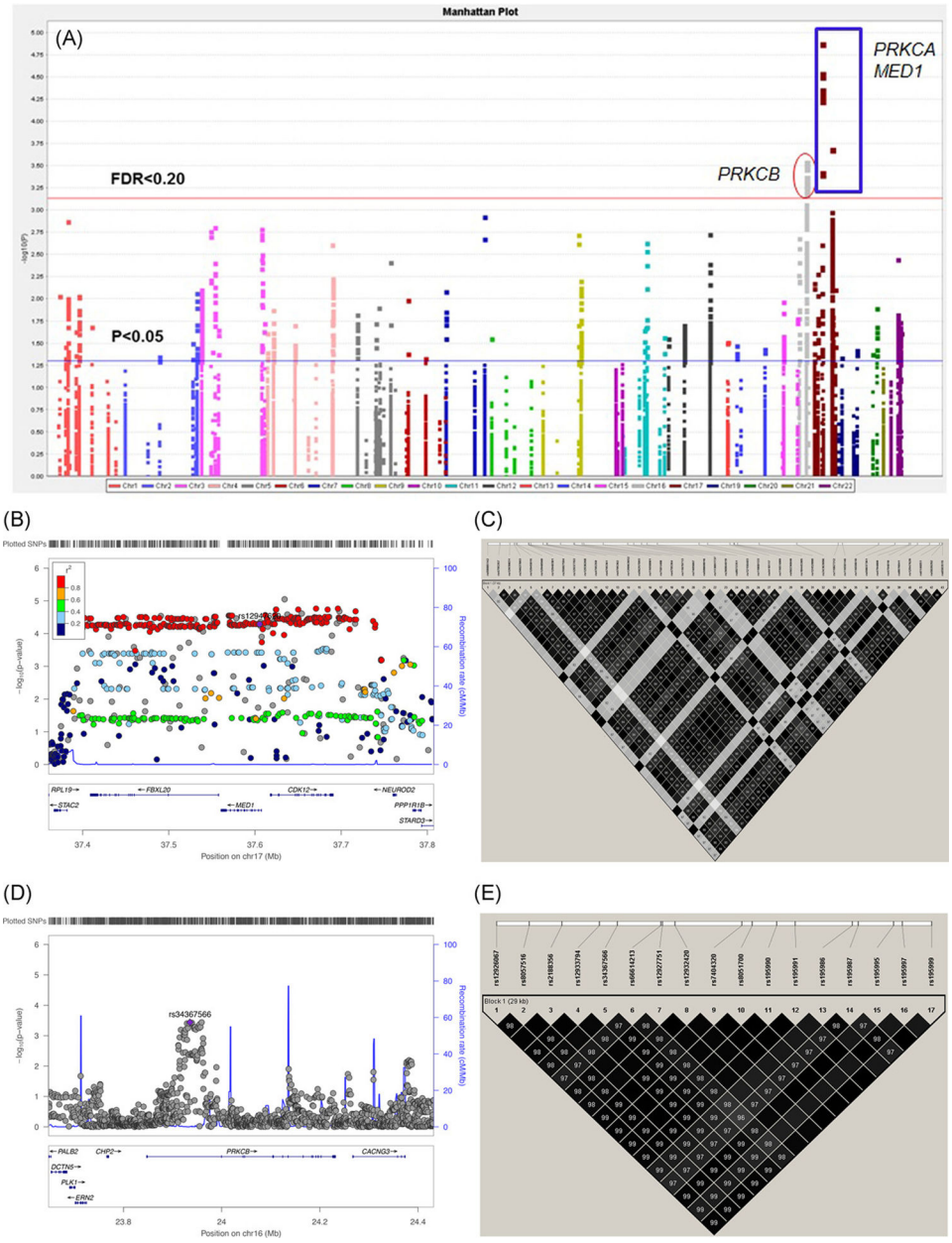

Figure 2. Screening for pancreatic cancer (PanC) risk-associated single nucleotide polymorphisms (SNPs).

(A) Manhattan plot of the association results of 17,532 SNPs in 112 peroxisome proliferator-activated receptor (PPAR) signaling pathway genes and PanC risk in the meta-analysis of three genome-wide association study (GWAS) datasets. Blue horizontal line indicates $P = 0.05$ and red horizontal line indicates false discovery rate (FDR) = 0.2. (B) Regional association plots and (C) Linkage disequilibrium plot of significant SNPs of the *MED1* gene. (D) Regional association plots and (E) Linkage disequilibrium plot of significant SNPs of the *PRKCB* gene

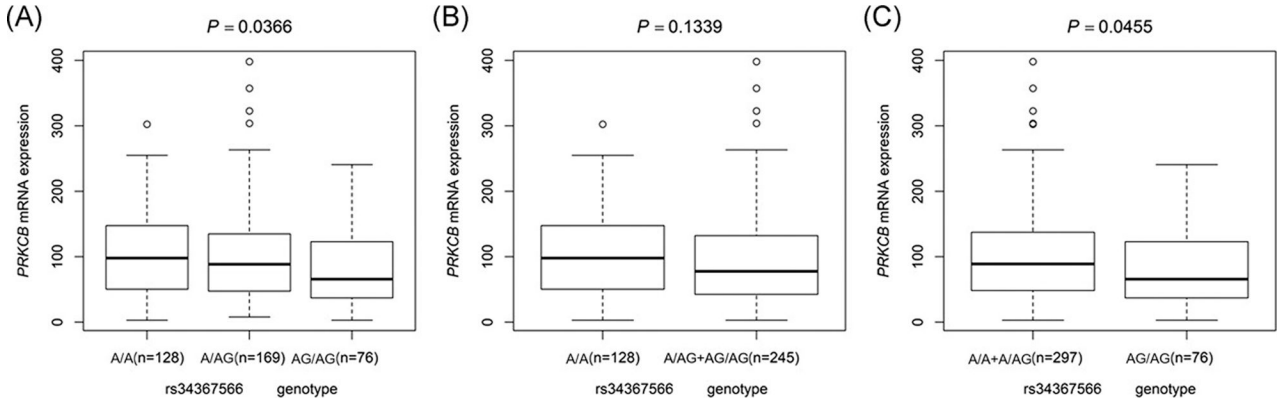

Figure 3. The expression quantitative trait loci (eQTLs) analysis from the 1000 Genomes Project.
Correlation between *PRKCB* mRNA expression and rs34367566 genotypes in blood cells in the (A) additive model, (B) dominant model, (C) recessive model.

Table 1.

Associations between three independently pancreatic cancer risk-associated SNPs in the PPAR signaling pathway

SNP	Gene	Chr.	Position	Allele[a]	EAF1[b]	EAF2[b]	EAF3[b]	OR (95% CI)	*P*[d]	FDR
rs12947620	*MED1*	17	37605364	T/C	0.2531	0.2512	0.2521	1.11 (1.06–1.17)	5.46×10^{-5}	0.026
rs11079651	*PRKCA*	17	64346169	T/C	0.3177	0.3045	0.2991	1.10 (1.04–1.15)	1.99×10^{-4}	0.090
rs34367566	*PRKCB*	16	23935503	AG/A	0.4135	0.4217	0.4249	1.09 (1.04–1.14)	3.16×10^{-4}	0.129

Chr, chromosome; EAF, effect allele frequency; FDR, false discovery rate; OR, odds ratio; SNP, single nucleotide polymorphism

[a] Effect allele/reference allele

[b] EAF1 was EAF in PanScan I controls; EAF2 was EAF in PanScan II/III controls; EAF3 was EAF in PanC4 controls

[c] Fixed effects models were used when no heterogeneity was found between studies (Q_{test} $P > 0.10$ and $I^2 < 50.0\%$); otherwise, random effects models were used

[d] Meta-analysis of the three studies

Table 2.

SNPs as independent predictors for pancreatic cancer risk evaluated by stepwise logistic regression analysis*

Parameter	Sub group	Beta	SE	Chi-Square	OR (95% CI)	P^1
Sex	male vs.female	−0.004	0.03	0.02	1.00 (0.93–1.06)	0.8946
Age	60–70 vs. <60	0.132	0.04	10.22	1.14 (1.05–1.24)	0.0014
Age	>70 vs. <60	0.191	0.04	21.75	1.21 (1.12–1.31)	<0.0001
rs34367566	AG/A	0.085	0.02	13.04	1.09 (1.04–1.14)	0.0003
rs12947620	T/C	0.110	0.03	17.75	1.12 (1.06–1.18)	<0.0001
rs11079651	T/C	0.096	0.02	15.09	1.10 (1.05–1.16)	0.0001

SE, standard error; OR, odds ratio; CI, Confidence interval

*15 subjects with missing date were excluded

1Stepwise analysis included age, sex, top 5 PC (Principal Components) and 5 SNPs (rs12947620, rs12945015, rs8076041, rs11079651 and rs34367566)

Table 3.

Associations between genotypes of three SNPs and pancreatic cancer risk

Genotype	Group		OR (95% CI)[1]	*P*[1]
	Case (%)	Control (%)		
rs34367566 A>AG				
A/A	2578 (30.41)	2347 (33.79)	1.00	--
A/AG	4231 (49.91)	3347 (48.19)	1.13 (1.05–1.22)	0.0009
AG/AG	1668 (19.68)	1252 (18.02)	1.16 (1.06–1.28)	0.0014
Trend test				0.0004
A/AG+AG/AG	5899 (69.59)	4599 (66.21)	1.14 (1.06–1.22)	0.0002
AG/AG	1668 (19.68)	1252 (18.02)	1.08 (1.00–1.17)	0.0668
rs12947620 C>T				
C/C	4477 (52.81)	3877 (55.82)	1.00	--
C/T	3335 (39.34)	2635 (37.93)	1.09 (1.02–1.16)	0.0170
T/T	665 (7.85)	434 (6.25)	1.30 (1.14–1.48)	<0.0001
Trend test				<0.0001
C/T+T/T	4000 (47.19)	3069 (44.18)	1.12 (1.05–1.19)	0.0008
T/T	665 (7.85)	434 (6.25)	1.26 (1.11–1.43)	0.0004
rs11079651 C>T[2]				
C/C	3890 (45.92)	3366 (48.49)	1.00	--
C/T	3663 (43.23)	2915 (41.99)	1.09 (1.02–1.17)	0.0096
T/T	919 (10.85)	661 (9.52)	1.21 (1.09–1.36)	0.0006
Trend test				0.0001
C/T+T/T	4582 (54.08)	3576 (51.51)	1.12 (1.05–1.19)	0.0008
T/T	919 (10.85)	661 (9.52)	1.16 (1.05–1.29)	0.0051
Number of unfavorable genotype[3]				
0	598 (7.06)	596 (8.58)	1.00	--
1	2767 (32.66)	2485 (35.80)	1.09 (0.96–1.24)	0.1733
2	3614 (42.66)	2830 (40.77)	1.24 (1.10–1.41)	0.0006
3	1493 (17.62)	1031 (14.85)	1.40 (1.22–1.61)	<.0001
Trend test				<.0001
0–1	3365 (39.72)	3081 (44.38)	1.00	--
2–3	5107 (60.28)	3861 (55.62)	1.20 (1.12–1.28)	<.0001

OR, odds ratio; CI. Confidence interval

[1]Adjusted for age, sex and top five principle components;

[2]Nine subjects with missing date were excluded;

[3]Risk genotypes were rs12947620 C/T+T/T, rs11079651 C/T+T/T and rs34367566 A/AG+AG/AG