Potential Functional Variants in *SMC2* and *TP53* in the AURORA Pathway Genes and Risk of Pancreatic Cancer

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Abbreviations: SNP, single nucleotide polymorphisms; GWAS, genome-wide association studies; MAF, minor allele frequency; HWE, Hardy-Weinberg Equilibrium; eQTL, expression quantitative trait loci; OR, odds ratio; CI, confidence interval; FDR, false discovery rate; LD, linkage disequilibrium; NPG, number of protective genotypes;

Key Words: single nucleotide polymorphism; AURORA; pancreatic cancer susceptibility; pathway analysis.

Abstract

The AURORA pathway participates in mitosis and cell division, and alterations in mitosis and cell division can lead to carcinogenesis. Therefore, genetic variants in the AURORA pathway genes may be associated with susceptibility to pancreatic cancer. To test this hypothesis, we used three large, publically available pancreatic cancer genome-wide association studies (GWASs) datasets (PanScan I, II/III and PanC4) to assess the associations of 7,168 single nucleotide polymorphisms (SNPs) in a set of 62 genes of this pathway with pancreatic cancer risk (8,477 cases and 6,946 controls of European ancestry). We identify 15 significant pancreatic cancer risk-associated SNPs in three genes (*SMC2, ARHGEF7* and *TP53*) after correction for multiple comparisons by a false discovery rate (FDR) < 0.20. Through further linkage disequilibrium analysis, SNP functional prediction and stepwise logistic regression analysis, we focused on three SNPs: rs3818626 in *SMC2*, rs79447092 in *ARHGEF7* and rs9895829 in *TP53*. We found that these three SNPs were associated with pancreatic cancer risk [odds ratio (OR) = 1.12, 95% confidence interval (CI) = 1.07-1.17 and *P* = 2.20E-06 for the rs3818626 C allele; OR = 0.76, CI = 0.66-0.88 and *P* = 1.46E-04 for the rs79447092 A allele; and OR = 0.82, CI = 0.74-0.91 and *P* = 1.51E-04 for the rs9895829 G allele]. Their joint effect as the number of protective genotypes (NPGs) also showed a significant association with pancreatic cancer risk (trend test $P \leq 0.001$). Finally, we performed an eQTL analysis and found that rs3818626 and rs9895829 were significantly associated with *SMC2* and *TP53* mRNA expression levels in 373 lymphoblastoid cell lines, respectively. In conclusion, these three representative SNPs may be potentially susceptibility loci for pancreatic cancer and warrant additional validation.

Introduction

Pancreatic cancer is a highly lethal malignancy and estimated to cause approximately 43,090 cancer-related deaths in the United States in 2017 (1). Some environmental factors, such as cigarette smoking, alcohol intake, diabetes, obesity and chronic pancreatitis have been identified as risk factors for pancreatic cancer (2,3). Genetic factors are also known to play an important role in pancreatic cancer etiology. For example, germline mutations in *BRCA2, PALB2, CDKN2A, ATM, STK11, PRSS1, SPINK1* and DNA mismatch repair genes have been reported to be involved in pancreatic carcinogenesis (4-9).

Other genetic factors, such as common single nucleotide polymorphisms (SNPs), are also reported to be associated with pancreatic cancer risk, in several prior genome-wide association studies (GWASs) (10-13). Many pancreatic cancer susceptibility loci have been identified, such as 1q32.1(*NR5A2*), 2p13.3 (*ETAA1*), 3q29 (*TP63*), 5p15.33 (*TERT, CLPTM1*), 7p13 (*SUGCT*), 7q32.3(*LINC-PINT*), 8q24.21(*MYC*), 9q34.2(*ABO*), 13q12.2 (*PDX1*), 13q22.1(*KLF5*), 16q23.1(*BCAR1*), 17q25.1 (*LINC00673)* and 22q12.1 (*ZNRF3*), particularly in European populations (10-13). However, many of the SNPs identified by GWAS are not functionally related to possible mechanisms associated with the disease, and identification of the causal alleles that provide a clue to biologically-plausible genes and pathways remains difficult. Therefore, we sought to perform a pathway-based analysis as a hypothesis-driven approach with fewer SNPs selected from available GWAS datasets to reduce multiple tests and also to identify possible functional SNPs associated with pancreatic cancer risk. We have applied this approach in lung cancer research, having identifed previously unreported susceptibility loci in genes involved in the pathways of centrosome (14), DNA repair (15), LncRNA (16) and RNA degradation (17). In the present study, we applied this pathway-based approach to investigate the associations between genetic variants of the gene-set involved in the AURORA pathway and pancreatic cancer risk.

Genomic instability is one of the known cancer hallmarks that provide a driving power for cancer initiation and development. Aneuploidy and chromosome instability are two forms of genomic instability, regulated by a number of cell-cycle dependent kinases (18-20), of which mitotic kinases play a key role in mitosis checkpoints and the maintenance of chromosome integrity and segregation. The Aurora kinases are a family of mitotic serine threonine/kinases including three members: Aurora A, B and C that participate in mitosis and cell division, including centrosome duplication, spindle formation, chromosome alignment, checkpoint activation and cytokinesis (21,22). Studies showed that overexpression of one mitotic kinase, Aurora A, can lead to centrosome amplification, inducing chromosomal instability (23,24). The Aurora kinases have been reported to be overexpressed in a wide range of human cancers, including pancreatic cancer (25,26), and thus targeted for the treatment of pancreatic cancer (27). Previous studies also revealed that a genetic variant in Aurora A was associated with risks of multiple cancers (28).

Some studies suggested that *TP53* (29,30) and *BIRC5* (31) play a role in the AURORA signaling pathway and thus are likely to be involved in pancreatic carcinogenesis. Other studies showed that *MDM2* (32) and *AKT1* (33) in this pathway were associated with tumor progression of pancreatic cancer. However, these studies did not include other related genes or SNPs of genes involved in the AURORA pathway. In the present study, we comprehensively investigated associations between common genetic variants of all possible genes likely to be involved in the AURORA pathway and pancreatic cancer risk.

Methods and Materials

Study subjects

We used the genotyping data of participants of European ancestry from two published GWASs, which were downloaded from the dbGaP website: the PanScan study (dbGap#: phs000206.v5. p3) and the Pancreatic Cancer Case Control Association Study (dbGaP #: phs000648. v1. p1) (34,35). The ancestry information was imputed based on principal component analysis and self-reported in former and latter studies, respectively. The PanScan GWAS was previously performed in three phases: PanScan I, II and III (1,921 cases and 2,016 controls in PanScan I; 1754 cases, 1889 controls in PanScan II; 1538 cases, 0 controls in PanScan III) (10-12). We merged the PanScan II and PanScan III into one dataset "PanScan II/III", because the control data in PanScan III were not found in dbGaP. The other Pancreatic Cancer Case Control Association Study from the Pancreatic Cancer Case-Control consortium (PanC4) includes 4168 cases and 3814 controls (13,36,37). Therefore, these three datasets (PanScan I, PanScan II/III and PanC4) from dbGAP included a total of 15,423 individuals (8,477 cases and 6,946 controls) for the final analysis. All the cases were diagnosed with a primary adenocarcinoma of the exocrine pancreas. A written informed consent was obtained from all participants in the original GWASs. All the original studies were performed in accordance with the relevant guidelines and regulations for each of the participating institutions, and the present study followed the study protocols approved by the Duke University Health System Institutional Review Board. **Supplementary Table S1** showed the distributions of demographic characteristics of the three GWAS datasets.

Selection of SNPs in the gene-set of the AURORA pathway

Genes in the AURORA pathway were selected from the Molecular Signatures Database (C2) (38). Overall, a set of 62 genes involved in the AURORA pathway from the PID??? dataset were selected (details presented in **Supplementary Table S2**). SNPs located in these genes and their ± 5-kb flanking regions were extracted from the original GWAS datasets based on the following selection criteria: (1) minor allele frequency (MAF) ≥ 1%, (2) genotyping rate ≥ 95%, and (3) Hardy-Weinberg Equilibrium (HWE) exact *P* value ≥ 10^{-5}. We used IMPUTE2 v2.1.1 software to impute untyped SNPs in our target regions, using a 500-kb buffer in our case-control data and the 1000 Genomes Project data (phase 3, released October 2015) as the imputation reference panel. After imputation, we extracted 7,757, 7,611 and 7,665 SNPs within 5-kb up- and down-streams of genes in the AURORA pathway from populations of the PanScan I, PanScan II/III and panC4 studies, respectively. The final meta-analysis contained 7,168 SNPs for each of the datasets with imputation quality (info) > 0.4. The detailed workflow is shown in **Figure 1**.

Functional prediction and validation

SNPinfo (39), RegulomeDB (40) and HaploReg (41) were used to predict SNP-associated potential functions. The expression quantitative trait loci (eQTL) analysis was performed by using the genotyping and expression data from the lymphoblastoid cell lines of 373 European individuals from Genetic European Variation in Health and Disease Consortium (GEUVADIS) and the 1000 Genomes Project (phase I integrated release 3, March 2012) (42). We also tested the correlations between the identified SNPs and the corresponding genes' expression levels in normal pancreatic tissues using the online GTEx database (https://gtexportal.org)

Statistical analysis

We performed an unconditional logistic regression analysis with the PLINK (v1.90) software to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) by using the genotyping data and the best-guess genotypes from imputation (43,44). Age, sex and top significant principal components were adjusted for in logistic regression models, including the top five and seven significant principal components in the analysis of Panscan I/II/III data and PanC4 data, respectively. A meta-analysis was performed for the selected 7,168 SNPs with Stata software (v12, State College, Texas, US). We tested for the heterogeneity among the datasets by using the Cochran's Q statistic and investigated the proportion of the total variation by the I^2 statistic. When there was no heterogeneity among the GWAS datasets (Q-test $P > 0.100$ and $I^2 < 50\%$), we used the fixed-effects model; otherwise, we used the random-effects model. We also performed the gene-based test by using the VEGAS (versatile gene-based association study) approach integrated in the VEGAS2 program (45,46). Briefly, for a given gene with n low linkage disequilibrium (LD) SNPs, the association *P* values were first converted to one Chi-squared statistics with one degree of freedom. The gene-based test statistic was then calculated by adding up all of the Chi-squared statistics within that gene. A large number of simulations were performed by using the multivariate normal distribution, and the empirical gene-based *P* value is the proportion of the simulated test statistics that exceeded the observed gene-based test statistic. We controlled for multiple testing with a threshold of a false discovery rate (FDR) of < 0.20. LocusZoom (http://locuszoom.sph.umich.edu/locuszoom/) (reference version: 1000 Genomes, Nov 24, 2014; EUR) was applied to generate the regional association plots (47). Manhattan plot and LD plot were generated by Haploview v4.2 (48). Finally, the joint effect analysis, stratified analysis and stepwise analysis were conducted with SAS (Version 9.3; SAS Institute, Cary, NC, USA).

Results

Association Analysis using three GWAS datasets

We first performed logistic regression analysis to estimate the associations between common SNPs (MAF>0.01) and pancreatic cancer risk in the three available pancreatic cancer GWAS datasets. There were 7,757, 7,611 and 7,665 SNPs in PanScan I, PanScan II/III and PanC4 datasets, respectively. As a result, 7,168 SNPs from a set of 62 genes were included in a meta-analysis. All the associations between SNPs of these genes and pancreatic cancer risk as identified by the single-locus analysis are presented in a Manhattan plot (**Supplementary Figure S1**). Overall, 15 SNPs in three genes (*SMC2, ARHGEF7* and *TP53*) passed the multiple-testing correction by FDR< 0.20. It should be mentioned that only the six SNPs in *SMC2* had passed the Bonferroni correction, which is a more stringent test assuming that all the tested SNPs are independent. The SNPs' locations and their associations with pancreatic cancer risk are presented in **Table 1**. The results of the meta-analysis with a random-effects model and the imputation qualities of these SNPs are shown in **Supplementary Table S3**. The chromosome regions of the three genes are novel findings, because they were not previously reported by any of these three pancreatic cancer GWASs, and therefore we performed further functional analysis. By using the VEGAS method, we performed the gene-based test and found seven genes (*SMC2, KIF20A, RHOA,* *TP53, EVI5, AURKC* and *NCAPD2*) with an empirical *P*-value < 0.05 (**Supplementary Table S4**), four of which passed the multiple testing correction with an FDR < 0.2. However, no significance was found for *ARHGEF7*.

LD and functional prediction

Based on the LD analysis ($r^2 > 0.80$) (**Supplementary Figure S2 d** and **e**) and *in silico* SNP functional prediction (SNPinfo, RegulomeDB and HaploReg) **(Supplementary Table S5)**, we chose four representative SNPs (i.e., rs3818626 and rs4742901 in *SMC2*, rs79447092 in *ARHGEF7*, and rs9895829 in *TP53*) for further analyses. Then, we employed the multivariate stepwise logistic regression analysis with adjustment for age, sex, and data source (components). As a result, three representative SNPs (i.e., rs3818626 in *SMC2*, rs79447092 in *ARHGEF7* and rs9895829 in *TP53*) remained statistically significantly associated with pancreatic cancer risk (**Supplementary Table S6**). Regional association plots of the three SNPs in the 200-kb up- and down-stream regions are shown in **Supplementary Figure S2 a, b** and **c.** The final meta-analysis results of three representative SNPs are summarized in **Figure 2.** We also presented individual association results for each of the three identified SNPs in the three GWAS datasets, which shows that our results are consistent across the three datasets (**Supplementary Table S7**) and thus reliable.

Potentially functional SNPs and pancreatic cancer risk

We performed risk analysis with different genetic models for each of representative SNPs by using logistic regression analysis (**Table 2**). We found that rs3818626 in *SMC2* was associated with an increased pancreatic cancer risk, whereas rs79447092 in *ARHGEF7* and

TP53 rs9895829 were associated with a decreased pancreatic cancer risk in both additive and dominant models.

To evaluate the joint effect of these three representative SNPs on pancreatic cancer risk, we combined the number of protective genotypes (NPGs) of rs3818626 TT, rs79447092 TA+AA, and rs9895829 AG+GG into a genetic score and divided all the patients into four groups: 0-3 risk genotypes, and we found that there was a significant association between an increased NPGs and pancreatic cancer risk in a dominant model (**Table 3**). Then, all participants were divided into a low-protection group (0 NPGs) and a high-protection group (1-3 NPGs). We found that the high-protection group had a significantly decreased cancer risk (**Table 3**), compared with the low-protection group.

Stratified analysis of combined protective genotypes and pancreatic cancer

To further analyze the interactive effect in associations between genotypes and pancreatic cancer risk, we performed stratified analysis by age and sex. In subgroup analysis by age (**Supplementary Table S8**), we found that the high-protection group had a significantly decreased cancer risk in all age subgroups (<60, 60-70 and >70), and both sex groups (male: OR = 0.86, 95% CI = 0.78-0.94 and P < 0.001; female: OR = 0.80, 95% CI = 0.72-0.88 and P < 0.001. **Supplementary Table S8**), compared with the low-protection group. There were no differences in the risk among these subgroups.

Functional validation by the eQTL analysis

We further performed the eQTL analysis to assess the associations between the representative SNPs and their mRNA expression levels, and we found that *SMC2* mRNA expression levels significantly decreased as the number of the rs3818626 risk alleles (C) increased in an additive model (P = 0.0007) (**Figure 3a**). The eQTL analysis result of rs9895829 in *TP53* was also significant in an additive model, demonstrating that the protective (A) allele was associated with higher TP53 expression levels (*P* = 0.005) (**Figure 3c**). However, we did not find such a correlation for rs79447092 in an additive model **(Figure 3b).** We have also tested the correlations between genotypes of the three identified SNPs and the corresponding genes' expression levels in the GTEx database (https://gtexportal.org), but we did not find significant results (**Supplementary Table S9**).

Discussion

In the present study, we investigated the associations between genetic variants in the AURORA pathway and pancreatic cancer risk using two published GWASs (PanScan study and PanC4 study). We found that three novel SNPs associations (i.e., rs3818626 in *SMC2*, rs79447092 in *ARHGEF7* and rs9895829 in *TP53*) were independently and jointly associated with pancreatic cancer risk. Further functional analyses showed that rs3818626 and rs9895829 were significantly associated with decreased mRNA expression levels of *SMC2* and *TP53*, respectively.

The structural maintenance of chromosome 2 (*SMC2)* protein product belongs to the condensin complex and plays an important role in packaging of chromatin before cell division and DNA damage response, which is required for proper chromosome segregation and maintenance of chromosomal stability (49). SMC2 plays a dual role in development and progression of cancer. For example, emerging evidence has showed that SMC2 may have a pro-oncogenic function and that SMC2 is involved in the mitotic cell division and also a direct transcriptional target of the oncogenic WNT signaling (50). Experimental studies suggested that *SMC2* knockdown would suppress tumor growth in colorectal cancer (50) and increase apoptosis in neuroblastoma cells (49). Many studies also showed significantly higher *SMC2* mRNA expression levels in human pancreatic cancer tissues than in adjacent non-neoplastic pancreas tissues (25,51). On the other hand, tumor suppressor p53-binding protein 1 known as p53-binding protein 1 or 53BP1, is a tumor suppressor, and 53BP1 nuclear bodies are partially suppressed by knocking down *SMC2* (52). The PanC4 GWAS previously reported that rs10991043 near *SMC2* reached 7.00×10^{-8} in association with pancreatic cancer risk, but this association was not observed in other pancreatic GWASs (13). In the present study, we found that the representative *SMC2* SNP rs3818626 was associated with pancreatic cancer risk in both PanC4 and PanScan GWAS datasets, but in moderate LD with the previously reported rs10991043 ($r^2 = 0.53$). More importantly, the SNP rs3818626 was predicted to be involved in TFBS/Splicing with a Regulome DB Score 2b and also associated with *SMC2* mRNA expression levels in 373 lymphoblastoid cell lines by the eQTL analysis. A similar trend was found between rs3818626 and the mRNA expression levels of *SMC2* in normal pancreatic tissues in the GTEx data, but the correlation was not significant, which might be due to small sample size or transcription specificity between tissues. Therefore, the finding of the association between *SMC2* rs3818626 and pancreatic cancer risk is biologically plausible.

P53 protein (encoded by *TP53* gene) is responsive to DNA damage, hypoxia, metabolic stress and oncogenic activation. The P53 protein suppresses cancer formation through its role in regulating cell cycle and apoptosis. This tumor suppressor gene is frequently mutated in various solid tumors, including pancreatic cancer (53,54). Although most of the mutations lead to loss of p53 function in inducing apoptosis and senescence, recent evidence shows that p53 inactivation/dysfunction would also directly or indirectly leads to promote tumorigenesis (55-57). In the present study, seven SNPs were found to be significantly associated with pancreatic cancer risk after multiple-testing correction by an FDR < 0.20. The representative SNP rs9895829 (with a Regulome DB Score 1f) was associated with *TP53* mRNA expression levels in 373 lymphoblastoid cell lines by the eQTL analysis, potentially affecting p53 activation and function. Similarly, a non-significant trend was found in normal pancreatic tissues in the GTEx data. These observations suggest that the association between rs9895829 and pancreatic cancer risk is also biologically plausible.

While a large proportion of cancer genomics research has been focusing on somatic mutations in *TP53*, a well-studied tumor suppresser, this gene does have a number of germline variants. Significantly, the majority of somatic mutations in *TP53* occur in the codons for amino acid positions 175, 245, 248, 273 and 282 of the exons (58). In the present study, however, the SNP rs9895829, although located in an intron, was found to be associated with a decreased pancreatic cancer risk, as a result of an effect of the G allele that was associated with higher *TP53* mRNA expression levels.

ARHGEF7 has many aliases, such as Rho Guanine Nucleotide Exchange Factor *(GEF) 7*, *PAK-Interacting Exchange Factor Beta, BETA-PIX, COOL-1,* and *P85SPR* (http://www.genecards.org/cgi-bin/carddisp.pl?gene=ARHGEF7). ARHGEF7 participates in the Hippo pathway to promote the tumorigenesis (59). Although we also found that *ARHGEF7* SNP rs79447092 was associated with pancreatic cancer risk, we did not find an association between the representative SNP rs79447092 and *ARHGEF7* mRNA expression levels.

The present study has some limitations. First of all, although we found two AURORA pathways from the Molecular Signatures Database, there may be other relevant genes that we failed to include. Secondly, we cannot get detailed clinical data for the study populations, such as family history, smoking status, alcohol intake, diabetes, obesity, chronic pancreatitis in the publically available GWAS datasets, to perform either further adjustment or stratified analysis. Finally, although we chose the representative SNPs by *in silico* SNP functional prediction tools and assessment by the eQTL analysis, more direct functional validations are needed to support our findings.

In conclusion, the present study revealed three potentially susceptibility loci in *SMC2, ARHGEF7* and *TP53*, which were associated with pancreatic cancer risk in 8,463 cases and 6,970 controls of European descent. The joint effect analysis demonstrated a significant association between an increased NPGs and pancreatic cancer risk. Further validations and functional evaluations of these genetic variants are warranted to support these findings.

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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.

Conflict of interest

The authors disclose no potential conflicts of interest.

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Table 1. Associations between SNPs in the AURORA Pathway and Pancreatic Cancer Risk with FDR < 0.20

SNP	**Gene**	**Chr.**	**Allele**[a]	**Position (hg19)**	***I^2***	**EAF**[b]	**OR (95% CI)**[c]	***P***[d]	**FDR**
rs10820603	*SMC2*	9	A/G	106877939	0	0.44	1.12 (1.07-1.18)	8.39E-07	0.003
rs7872034	*SMC2*	9	A/G	106896809	0	0.44	1.12 (1.07-1.17)	9.97E-07	0.003
rs3818626	*SMC2*	9	T/C	106856633	0	0.44	1.12 (1.07-1.17)	2.20E-06	0.003
rs4743687	*SMC2*	9	T/C	106856910	0	0.44	1.12 (1.07-1.17)	1.97E-06	0.003
rs4742906	*SMC2*	9	G/A	106857078	0	0.44	1.12 (1.07-1.17)	1.33E-06	0.003
rs7028408	*SMC2*	9	A/G	106859811	0	0.44	1.12 (1.07-1.17)	2.12E-06	0.003
rs4742901	*SMC2*	9	T/C	106856043	8.87	0.29	1.10 (1.04-1.15)	2.92E-04	0.149
rs79447092	*ARHGEF7*	13	T/A	111809308	0	0.03	0.76 (0.66-0.88)	1.46E-04	0.108
rs17884306	*TP53*	17	C/T	7572101	0	0.06	0.82 (0.74-0.91)	1.45E-04	0.108
rs9891744	*TP53*	17	C/T	7574864	0	0.06	0.81 (0.73-0.90)	1.26E-04	0.108
rs9895829	*TP53*	17	A/G	7578679	0	0.06	0.82 (0.74-0.91)	1.51E-04	0.108
rs17883323	*TP53*	17	G/T	7579619	0	0.06	0.82 (0.74-0.91)	1.77E-04	0.111
rs8079544	*TP53*	17	C/T	7580052	0	0.06	0.82 (0.74-0.91)	1.86E-04	0.111
rs75732100	*TP53*	17	C/T	7576348	0	0.06	0.82 (0.74-0.91)	2.29E-04	0.126
rs17879377	*TP53*	17	C/T	7574721	0	0.05	0.82 (0.73-0.91)	3.28E-04	0.157

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

[a] Reference allele/effect allele.

[b] EAF was EAF in PanScan I + PanScan II/III + PanC4 controls;

[c] Fixed effect models were used when no heterogeneity was found between studies (Q test P > 0.10 and I2 < 50.0%); otherwise, random effect models were used.

[d] Meta-analysis of the three studies.

Table 2. Associations between the top three representative SNPs and pancreatic cancer risk in the combined dataset of PanScan and PanC4 studies

Genotype		**Group**		**OR (95% CI)**[1]	***P***[1]
		Case (%)	**Control (%)**		
rs3818626					
	TT	2402 (28.4)	2182 (31.5)	1.00	
	TC	4247 (50.2)	3427 (49.4)	1.13 (1.05-1.21)	0.001
	CC	1808 (21.4)	1323 (19.1)	1.24 (1.13-1.36)	< 0.001
	Trend test				< 0.001
Dominant model					
	TT	2402 (52.4)	2182 (47.6)	1.00	
	TC+CC	6055 (56.0)	4750 (44.0)	1.16 (1.08-1.24)	< 0.001
rs79447092					
	TT	7944 (95.2)	6421 (94.0)	1.00	
	TA	394 (4.72)	395 (5.79)	0.80 (0.69-0.93)	0.003
	AA	4 (0.05)	12 (0.18)	0.29 (0.09-0.90)	0.032
	Trend test				< 0.001
Dominant model					
	TT	7944 (55.3)	6421 (44.7)	1.00	
	TA+AA	398 (49.4)	407 (50.6)	0.79 (0.68-0.91)	0.001
rs9895829					
	AA	7675 (90.7)	6153 (88.6)	1.00	
	AG	775 (9.15)	769 (11.1)	0.81 (0.73-0.90)	< 0.001
	GG	17 (0.20)	20 (0.29)	0.62 (0.32-1.19)	0.149
	Trend test				< 0.001
Dominant model					
	AA	7675 (55.5)	6153 (44.5)	1.00	
	AG+GG	792 (50.1)	789 (49.9)	0.81 (0.73-0.90)	< 0.001

SNP, single nucleotide polymorphism; OR, odds ratio; CI. Confidence interval;

[1] Adjusted for age, sex and data source;

Table 3. Associations between NPGs and risk of pancreatic cancer[1]

NPG[2]	**Group**		**OR (95% CI)**[3]	***P***[3]
	case (%)	**control (%)**		
0	5137 (61.8)	3900 (57.3)	1.00	
1	2827 (34.0)	2505 (36.8)	0.86 (0.80-0.92)	<0.001
2	342 (4.1)	397 (5.83)	0.66 (0.56-0.76)	<0.001
3	7 (0.1)	8 (0.1)	0.78 (0.28-2.16)	0.634
Trend test				<0.001
0	5137 (61.8)	3900 (57.3)	1.00	
1-3	3176 (38.2)	2910 (42.7)	0.83 (0.78-0.88)	<0.001

NPG, number of protective genotypes; OR, odds ratio; CI. Confidence.

[1] The logistic regression analysis was performed in the combined dataset of PanScan and PanC4 studies;

[2] Protective genotypes were rs3818626 TT, rs79447092 TA+AA, and rs9895829 AG+GG;

[3] Logistic regression analyses with adjustment for age, sex and data source.

Figure 1

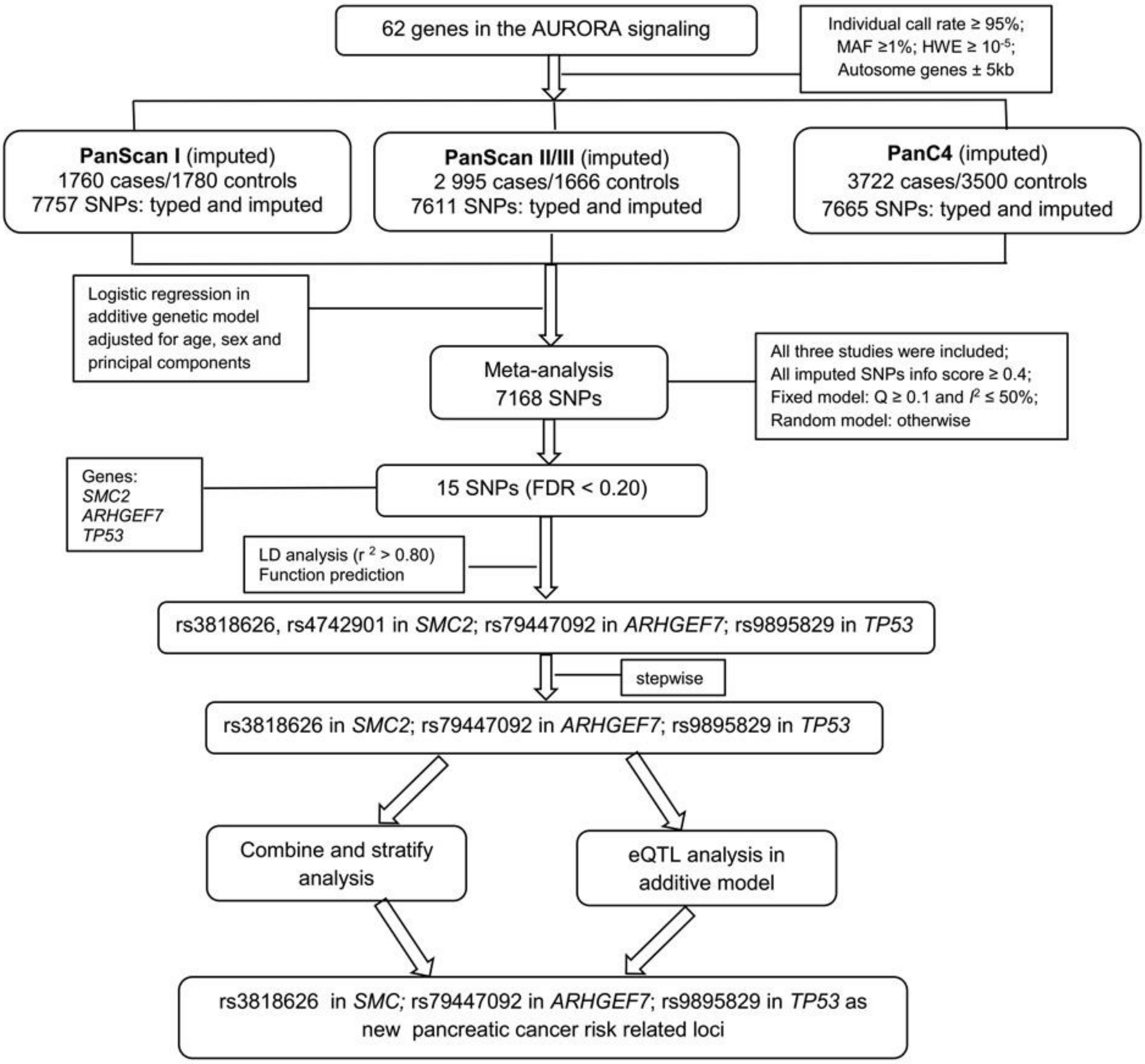

62 genes in the AURORA signaling
Individual call rate ≥ 95%; MAF ≥1%; HWE ≥ 10^{-5}; Autosome genes ± 5kb
PanScan I (imputed) 1760 cases/1780 controls 7757 SNPs: typed and imputed
PanScan II/III (imputed) 2 995 cases/1666 controls 7611 SNPs: typed and imputed
PanC4 (imputed) 3722 cases/3500 controls 7665 SNPs: typed and imputed
Logistic regression in additive genetic model adjusted for age, sex and principal components
Meta-analysis 7168 SNPs
All three studies were included; All imputed SNPs info score ≥ 0.4; Fixed model: Q ≥ 0.1 and I^2 ≤ 50%; Random model: otherwise
Genes: *SMC2* *ARHGEF7* *TP53*
15 SNPs (FDR < 0.20)
LD analysis (r^2 > 0.80) Function prediction
rs3818626, rs4742901 in *SMC2*; rs79447092 in *ARHGEF7*; rs9895829 in *TP53*
stepwise
rs3818626 in *SMC2*; rs79447092 in *ARHGEF7*; rs9895829 in *TP53*
Combine and stratify analysis
eQTL analysis in additive model
rs3818626 in *SMC;* rs79447092 in *ARHGEF7*; rs9895829 in *TP53* as new pancreatic cancer risk related loci

Figure 2

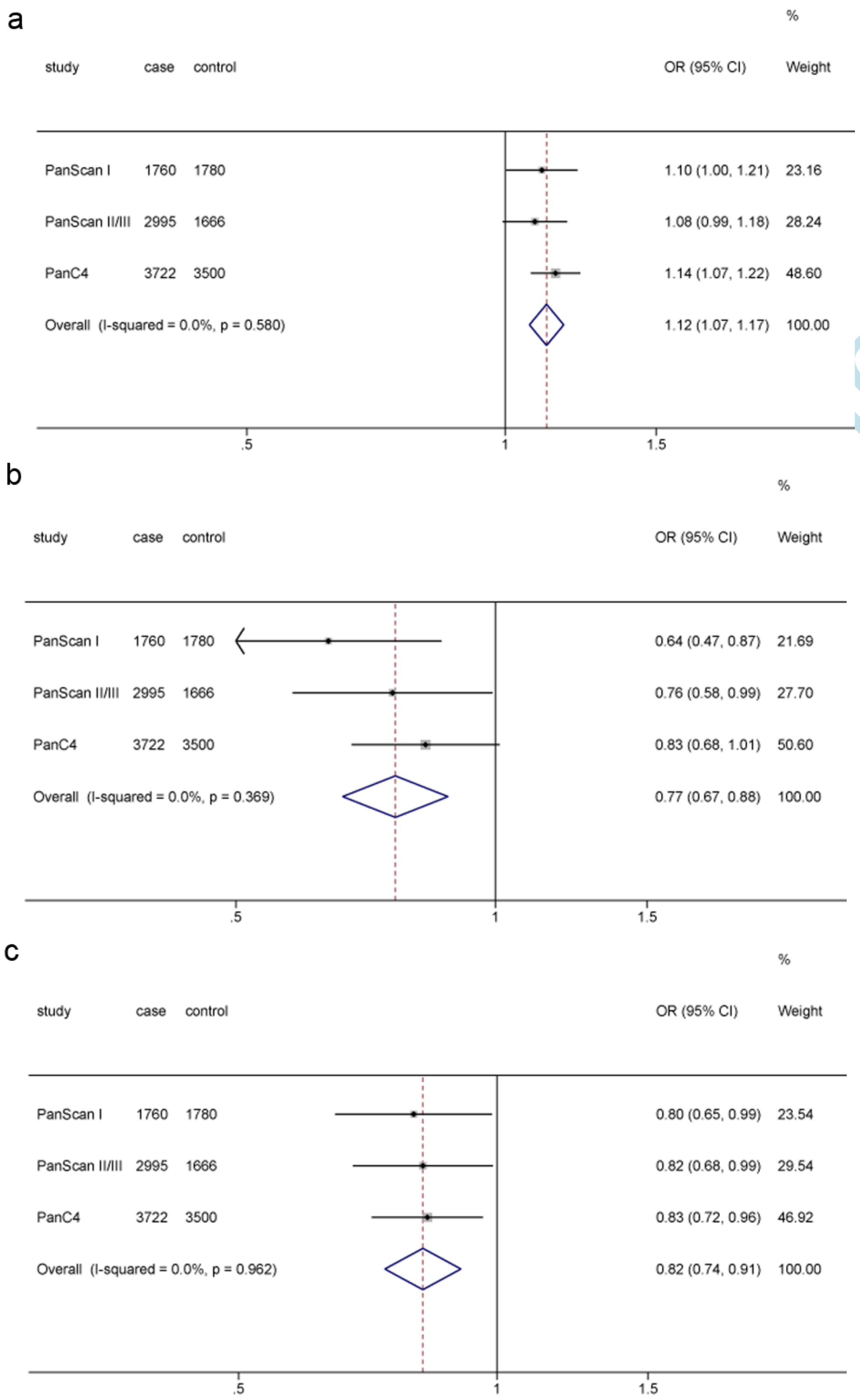
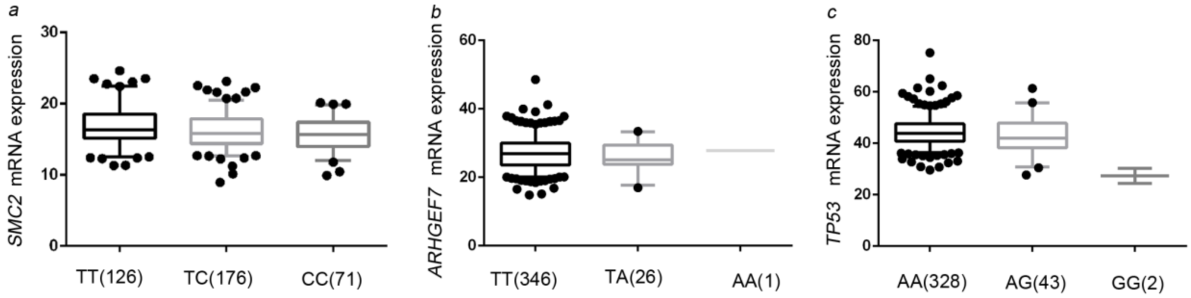

a
%
study case control OR (95% CI) Weight
PanScan I 1760 1780 1.10 (1.00, 1.21) 23.16
PanScan II/III 2995 1666 1.08 (0.99, 1.18) 28.24
PanC4 3722 3500 1.14 (1.07, 1.22) 48.60
Overall (I-squared = 0.0%, p = 0.580) 1.12 (1.07, 1.17) 100.00
.5
1
1.5
b
%
study case control OR (95% CI) Weight
PanScan I 1760 1780 0.64 (0.47, 0.87) 21.69
PanScan II/III 2995 1666 0.76 (0.58, 0.99) 27.70
PanC4 3722 3500 0.83 (0.68, 1.01) 50.60
Overall (I-squared = 0.0%, p = 0.369) 0.77 (0.67, 0.88) 100.00
.5
1
1.5
c
%
study case control OR (95% CI) Weight
PanScan I 1760 1780 0.80 (0.65, 0.99) 23.54
PanScan II/III 2995 1666 0.82 (0.68, 0.99) 29.54
PanC4 3722 3500 0.83 (0.72, 0.96) 46.92
Overall (I-squared = 0.0%, p = 0.962) 0.82 (0.74, 0.91) 100.00
.5
1
1.5

Figure 3


a
SMC2 mRNA expression
0
10
20
30
TT(126)
TC(176)
CC(71)
b
ARHGEF7 mRNA expression
0
20
40
60
TT(346)
TA(26)
AA(1)
c
TP53 mRNA expression
0
20
40
60
80
AA(328)
AG(43)
GG(2)