# Genetic variants in the liver kinase B1-AMP-activated protein kinase pathway genes and pancreatic cancer risk

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## Abstract

The liver kinase B1-AMP-activated protein kinase (LKB1-AMPK) pathway has been identified as a new target for cancer therapy, because it controls the glucose and lipid metabolism in response to alterations in nutrients and intracellular energy levels. In the present study, we aimed to identify genetic variants of the LKB1-AMPK pathway genes and their associations with pancreatic cancer (PanC) risk using 15,418 participants of European ancestry from two previously published PanC genome-wide association studies. We found that six novel tagging single-nucleotide polymorphisms (SNPs) (i.e., *MAP2* rs35075084 T>deletion, *PRKAG2* rs2727572 C>T and rs34852782 A>deletion, *TP53* rs9895829 A>G, and *RPTOR* rs62068300 G>A and rs3751936 G>C) were significantly associated with an increased PanC risk. The multivariate logistic regression model incorporating the number of unfavorable genotypes (NUGs) with adjustment for age and sex showed that carriers with 5-6 NUGs had an increased PanC risk (odds ratio=1.24, 95% confidence interval=1.16-1.32 and *P*<0.0001), compared to those with 0-4 NUGs. Subsequent expression quantitative trait loci (eQTL) analysis further revealed that these SNPs were associated with significantly altered mRNA expression levels either in 373 normal lymphoblastoid cell lines (*TP53* SNP rs9895829, *P*<0.05) or in whole blood cells of 369 normal

donors from the genotype-tissue expression project (GTEx) database [*RPTOR* SNP rs60268947 and rs28434589, both in high linkage disequilibrium ($r^2>0.9$) with *RPTOR* rs62068300, *P*<0.001]. Collectively, our findings suggest that these novel SNPs in the LKB1-AMPK pathway genes may modify susceptibility to PanC, possibly by influencing gene expression.

## Keywords

single-nucleotide polymorphism; genome-wide association study; pancreatic cancer risk

## Introduction

Pancreatic cancer (PanC) is one of the most lethal human malignancies, with an overall five-year survival rate lower than 10% and a median survival of six months, and PanC is also the fourth leading cause of cancer-related death and is expected to become the second within the next decade in the United States [1,2]. The exact cause of PanC is not yet well understood, though several risk factors have been identified, including smoking, morbid obesity, having a family history of PanC or pancreatitis, and having certain hereditary conditions [3,4]. More importantly, patients with PanC rarely exhibit symptoms at the early stages, until the disease reaches an advanced stage, which is one of the main reasons for the observed, generally poor survival rates [4,5]. Therefore, prevention and early diagnosis at a curable stage are desperately needed to reduce PanC mortality.

Since Otto Warburg first proposed a connection between cellular metabolism and tumorigenesis nearly 100 years ago, pointing out a new direction for cancer research [6,7], numerous studies have been reported on molecular mechanisms that link the signaling pathways controlling the metabolism to cell growth, in which the metabolism reprogramming was found to be necessary for cancer initiation and progression, a hallmark of cancer [8,9]. PanC is characterized by a severely hypoxic and nutrient-deprived microenvironment, with specific metabolically adaptive mechanisms, such as the Warburg effect, glutamine addiction and autophagy, that all contribute to PanC development and progression, in addition to both oncogenes/tumor suppressors and tumor microenvironment [10,11]. Therefore, targeting any of specific metabolic adaptions becomes an emerging strategy for PanC diagnosis and treatment [12,13].

LKB1 (liver kinase B1, also known as STK11) directly activates the AMPK (AMP-activated protein kinase), which is responsible for nutrient sensing and metabolism reprogramming, and LKB1 is inactivated by mutations found in PanC, and the loss of LKB1 is thought to drive tumorigenesis [14-16].

AMPK is a master regulator of metabolic homeostasis by sensing cellular energy status, the AMP:ATP ratio. When there is an increase in the cellular AMP:ATP ratio, which reflects a decrease in energy supply, AMPK is phosphorylated and activated, promoting catabolic processes and inhibiting anabolic processes to increase the energy level [17].

Recent studies found that meisoindigo can preferentially kill cancer stem cells by interfering cell metabolism via inhibition of LKB1 and activation of AMPK in PanC [18]. Similar results

have also been drawn from metformin, which influences PanC progression by activating the LKB1-AMPK pathway, including inhibition of cell division, promotion of apoptosis and autophagy, down-regulation of circulating insulin, and activation of the immune system [19].

Therefore, we hypothesize that genetic variants of the LKB1-AMPK pathway genes are associated with PanC risk. To test such a hypothesis, we conducted a comprehensive meta-analysis of genetic variants in the LKB1-AMPK pathway genes using two previously published genome-wide association study (GWAS) datasets from the PanScan (i.e., the Pancreatic Cancer Cohort Consortium) and Pancreatic Cancer Case-Control Association Study. We also focused our analysis on the identified single-nucleotide polymorphisms (SNPs) that may change the mRNA expression levels of the genes and thus are likely have functional consequences.

## Methods

### Study Population and GWAS Data

The analysis used two previously published PanC GWASs: Pancreatic Cancer Cohort Consortium (PanScan, phs000206.v5.p3) and the Pancreatic Cancer Case-Control Association Study (dbGaP#:phs000648.v1.p1), which included 15,418 participants (8,474 cases and 6,944 controls). The PanScan GWAS has three phases, including PanScan I, II and III (1,760 cases and 1,780 controls in PanScan I; 1,457 cases and 1,666 controls in PanScan II, 1,538 cases and 0 controls in PanScan III) [20-23]. We then merged the PanScan II and PanScan III into one dataset, PanScan II/III, because PanScan III was a case-only study [24]. Another GWAS dataset was from the Pancreatic Cancer Case-Control Consortium (PanC4) that consisted of 3,719 cases and 3,498 controls from the United States, Europe and Australia (Supplementary Fig. S1). All the participants in these GWASs were of European ancestry, and a written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations for each of the participating institutions, and the present study followed the protocols approved by Duke University Health System Institutional Review Board.

### Gene selection, genotyping, and imputation

The keywords "LKB1-AMPK" was searched in Molecular Signatures Database (MSigDB) (http://www.broadinstitute.org/gsea/msigdb/index.jsp), and all the 58 related genes located on autosomal chromosomes were selected from REACTOME and PID (details presented in Supplementary Table S1). The GWAS genotyping was performed using Illumina HumanHap550v3.0, Human 610-Quadv1_B, HumanOmniExpress-12v1.0 and HumanOmniExpressExome-8v1.0 [25-27]. The SNPs located both in these genes and their ± 500-kb flanking regions were extracted for further imputation by the IMPUTE2 software, using the 1000 Genomes (Phase 1, Release 3) Project as the reference dataset [28,29].

For quality control, the imputed SNPs with an information/accuracy score > 0.4 were qualified for further analysis (with details presented in Table 1 and Supplemental Fig. S2). As a result, there were 14,557 SNPs, 15,866 SNPs and 14,263 SNPs within 5.0 kb up- and down-streams of the 58 LKB1-AMPK pathway genes from populations of the PanScan I,

PanScan II/III and panC4 studies, respectively. The final meta-analysis for all three dataset contained 12,777 SNPs that meets the inclusion criteria: a call rate > 95%, MAF > 1% and Hardy-Weinberg equilibrium (HWE) test $P$ value $> 1 \times 10^{-5}$.

## Association analysis

Unconditional multivariable logistic regression including sex, age and top five principal components was performed using PLINK (version 1.90), assuming an additive genetic model, with assessment of genomic data to control for potential population stratification. The principal components were computed by genome-wide complex trait analysis and the top five principal components with $P$ value less than 0.001 were selected from the logistic regression analysis from all the three studies (Supplementary Table S2), and an odds ratio and its 95% CI were estimated for each SNP with PLINK [30]. A meta-analysis was further employed on the results of a log-additive model of 12,777 SNPs using the fixed-effects inverse-variance method based on β estimates and standard errors with Stata software (v 12, State College, Texas, US). Cochran's Q statistics and $I^2$ were used to assess the heterogeneity [31]. If the Cochran's Q-test $P$ value > 0.100 and the heterogeneity statistic $I^2 <$ 50%, a fixed-effects model was applied. Otherwise, a random-effects model was employed.

False positive report probability (FPRP) is the probability of no true association between a genetic variant and disease, given a statistically significant finding. We chose FPRP to correct for multiple testing, because more than 90% of SNPs (12,379 out of 12,777 under investigation) included in the present study were imputed. The FPRP approach with a prior probability of 0.01 and a hazards ratio (HR) of 2.0 was assigned for an association with genotypes and alleles of each SNP to reduce the probability of false-positive findings. The association between each SNP and PanC risk was evaluated with an additive genetic model in which a cut-off FPRP value 0.02 was considered as a significant association. The multivariable stepwise logistic regression model was also employed to identify independent SNPs. The number of unfavorable genotypes (NUGs) of SNPs with independent effects was calculated to assess the classification performance of the model. All individuals were further dichotomized into low-risk group (0-4 NUGs) and high-risk group (5-6 NUGs) for additional analysis. Besides, Haploview v4.2 [32] was used to produce the Manhattan plot and Linkage disequilibrium (LD) plot, and LocusZoom [33] was employed to construct the regional association plots by using the dataset from the 1000 Genomes Project. Linear regression was used to analyze the correlations between SNPs and corresponding mRNA expression levels. All statistical analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC, USA), if not specified otherwise.

## *In silico* functional prediction and validation

To predict potential functions of the significant SNPs, we used two *in silico* tools: RegulomeDB (http://regulomedb.org/) [34] and HaploReg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) [35]. We performed the expression quantitative trait loci (eQTL) analysis to estimate the associations between SNPs and mRNA expression levels of the corresponding genes by using the mRNA expression data from the lymphoblastoid cell lines of 373 Europeans available in the 1000 Genomes Project [36] and 127 tumor tissues in The Cancer Genome Atlas (TCGA) [37] as well as the eQTL results from the genotype-tissue

expression project (GTEx) database for the whole blood (n=369) and normal pancreatic tissues (n=220) (http://www.gtexportal.org/home/) [38]. In addition, we also compared the mRNA expression levels of targeted gene between tumor and adjacent normal tissues available in the Oncomine™ database (https://www.oncomine.org/) [39].

## Results

### Single locus analysis

The research workflow of the present study design is shown in Fig. 1. We first estimated the associations between selected SNPs [with a minor allele frequency (MAF) 0.01] and PanC risk with the unconditional logistic regression analysis for each of the three populations of European ancestry with 14,557 SNPs, 15,866 SNPs and 14,263 SNPs for PanScan I, PanScan II/III and PanC4, respectively; the single locus analysis revealed that these three study populations had 623, 1713 and 911 SNPs with a nominal $P < 0.05$, respectively (Supplemental Fig. S3). Then, we included a total of 12,777 SNPs in a meta-analysis of the three populations and found that 589 SNPs remained to be associated with PanC risk at $P < 0.05$ in an additive genetic model, of which 16 SNPs on *MAP2*, *PRKAG2*, *TP53* and *RPTOR* passed multiple testing corrections with an FPRP 0.02 (Supplemental Fig. S4a; Table 1).

Although seven SNPs of *TP53* (i.e., rs17884306, rs17879377, rs9891744, rs75732100, rs9895829, rs17883323 and rs8079544) located at 17p13 have been previously reported by the AURORA pathway-based analyses [40], the *TP53* rs35850753 and other eight SNPs (i.e., *MAP2* rs35075084, *PRKAG2* rs2727572, rs12668489, rs2538046 and rs34852782 and *RPTOR* rs62068300 and rs17848685, rs3751936) located at 2q34, 7q36.1 and 17q25.3, respectively, are novel findings, for which we performed additional *in silico* analysis for their functional relevance. The results of these SNPs in each of GWAS datasets and the final meta-analysis are summarized in Table 2. All these SNPs showed a low heterogeneity among the three GWAS datasets (all Q-test $P > 0.100$ and $I^2 < 50.0$).

### LD analysis and stepwise analysis

For the LD analysis, three (i.e., rs2727572, rs12668489 and rs2538046) of the four *PRKAG2* SNPs shared a high LD ($r^2$ 0.80, Supplemental Fig. S4b and Supplemental Fig. S5a); seven (i.e., rs17884306, rs17879377, rs9891744, rs75732100, rs9895829, rs17883323 and rs8079544) of the eight *TP53* SNPs shared a high LD ($r^2$ 0.80, Supplemental Fig. S4d and Supplemental Fig. S5c); and three *RPTOR* SNPs (i.e., rs62068300, rs17848685 and rs3751936) showed a low LD ($r^2$ 0.80, Supplemental Fig. S4c and Supplemental Fig. S5b). Having considered functional prediction and LD, we selected final eight SNPs, including *MAP2* rs35075084, two proxy *PRKAG2* SNPs (i.e., rs2727572 and rs34852782), two proxy *TP53* SNPs (i.e., rs9895829 and rs35850753) and all three SNPs of *RPTOR* (i.e., rs62068300, rs17848685 and rs3751936), for further analysis. Next, we assessed these eight representative SNPs in the presence of age, sex and top five principal components in a multivariate stepwise logistic regression model. As a result, the genotypes of six SNPs remained significantly and independently associated with PanC risk (Supplemental Table S3).

### Genotype effect and the joint-effect of the six significant SNPs

After stepwise analysis, we found that the genotypes of *MAP2* rs35075084 T>deletion, *TP53* rs9895829 A>G, *PRKAG2* rs2727572 C>T and rs34852782 A>deletion, and *RPTOR* rs62068300 G>A and rs3751936 G>C were significantly associated with PanC risk in both additive and dominant genetic models.

In the additive model, the association between each of these six novel SNPs and PanC risk had a linear trend as the frequency of the minor allele increased (trend test: $P = 0.0008$, $P = 0.0004$, $P = 0.0007$, $P = 0.0002$, $P = 0.0009$ and $P = 0.0009$, respectively, Table 3). Consistent with previous results of the single locus analysis, the rs2727572 T allele was associated with an increased PanC risk [odds ratio (OR) = 1.11, 95% confidence interval (CI) = 1.04-1.19 and $P = 0.0034$], the rs34852782 deleted allele was associated with an increased PanC risk (OR = 1.09, 95% CI = 1.02-1.16 and $P = 0.0076$), while the rs35075084 deleted allele, rs9895829 G, rs62068300 A and rs3751936 C alleles were associated with a reduced risk (OR = 0.75, 95% CI = 0.64-0.89, $P = 0.0007$, OR = 0.83, 95% CI = 0.75-0.92, $P = 0.0004$, OR = 0.91, 95% CI = 0.85-0.97, $P = 0.0026$ and OR = 0.91, 95% CI = 0.86-0.97, $P = 0.0051$, respectively), compared with their corresponding wild-type allele (Table 3).

To better estimate the joint association between the six SNPs and PanC risk, we combined risk genotypes of rs35075084 TT, rs2727572 CT/TT, rs34852782 A−/−−, rs9895829 AA, rs62068300 AA and rs3751936 GG into a single genetic score as the NUGs in a dominant model. The significant association between an increased NUGs and an increased PanC risk remained with a significant trend ($P_{trend} < 0.0001$, Table 4). We then dichotomized all individuals into low-risk group (0-4 NUGs) or high-risk group (5-6 NUGs). As shown in Table 4, PanC risk in high-risk group was significantly greater than the low-risk group (OR = 1.24, 95% CI = 1.16-1.32, $P < 0.0001$).

Since the difference in the distribution of age existed in all the dataset, and age is a known risk factor for PanC, we performed subgroup analysis by age group (i.e., < 60y, 60 – 70y and > 70y) and sex to assess any potential interaction. We found that the risk associated with high-risk NUGs was more evident in the >70 year group (OR = 1.27, 95% CI = 1.13-1.41, $P < 0.0001$) and males (OR = 1.25, 95% CI = 1.15-1.37, $P < 0.0001$); however, there was no evidence for an interaction among and between these strata ($P > 0.05$ for all, Supplementary Table S4).

### Genotype and phenotype correlation analysis

To explore the potential function of these six SNPs, we used online prediction tools and performed eQTL analysis. We found that four SNPs are located in the intronic regions, one SNP in 5'-UTR and one SNP in 3'-UTR (Supplemental Table S5). All SNPs are located in the enhancer region of histone H3 mono acetyl K27, which is associated with the higher activation of transcription and defined as an active enhancer marker. Besides, SNP of *TP53* and two SNPs of *RPTOR* also located in DNase I hypersensitive sites where chromatins are sensitive to cleavage by the DNase I and lost its condensed structure, functionally related to transcriptional activity (Supplementary Fig. S6).

We evaluated correlations between SNPs and corresponding mRNA levels in 373 normal lymphoblastoid cell lines from the 1000 Genomes Project. We found rs9895829 G allele was significantly correlated with decreased levels of *TP53* mRNA expression in both additive and dominent model ($P = 0.005$ and $0.039$) by using Student's $t$ test or linear regression analysis of the logarithm transformed expression values ($\log^2$) (Fig. 2a-2b). No other allele was significantly correlated with increased/decreased levels of mRNA expression. However, the rs2727572 T allele of *PRKAG2*, rs62068300 A allele of *RPTOR* and rs3751936 C allele of *RPTOR* were correlated with an observable increased/decreased mRNA expression level in both additive and dominant models, though the differences did not reach the statistical level ($P = 0.363$ and $0.625$, $P = 0.299$ and $0.092$ and $P = 0.163$ and $0.177$, respectively. Supplementary Fig. S7). For SNP rs35075084 and rs34852782, the eQTL results are not available. Next, we used the data from 127 Europeans in the TCGA-PAAD Project to query the eQTL results and assess the correlations. However, we failed to impute the genotype of six SNPs based on the current quality control.

In addition, we used the data from the GTEx database and found that SNP rs60268947 and rs28434589, located in the intron of *RPTOR* and in high LD ($r^2 = 0.91$ and $r^2 = 0.96$, respectively) with SNP rs62068300, had a significant correlation with an increased level of *RPTOR* mRNA expression in whole blood cells of 369 donors from GTEx ($P = 6.5 \times 10^{-12}$ and $P = 1.5 \times 10^{-10}$, respectively, Fig. 2c-2d). For other SNPs, there are no significant correlations from GTEx (Supplemental Table S6).

We also assessed the differences in mRNA expression levels of *MAP2*, *PRKAG2*, *TP53* and *RPTOR* between adjacent normal pancreatic tissues and pancreatic tumor tissues (n=220) from the Oncomine database. We found that *MAP2* and *TP53* mRNA expression levels were statistically significantly higher ($P = 0.002$ and $P = 1.38 \times 10^{-6}$, respectively) in tumor tissues than in normal pancreas, and the mRNA expression levels of *PRKAG2* and *RPTOR* were also higher in tumor tissues than in normal pancreas, though the differences were not statistically significant ($P = 0.061$ and $P = 0.196$) (Supplementary Fig. S8).

## Discussion

In the present study, we evaluated the associations between genetic variants in the 58 LKB1-AMPK pathway genes and PanC risk, using the two existing GWAS datasets: PanScan I, II/III from PanScan study and PanC4 from Pancreatic Cancer Case-Control Association Study. Through the meta-analysis, we identified six novel potential susceptibility loci of *MAP2*, *PRKAG2*, *TP53* and *RPTOR* for PanC risk, located at 2q34, 7q36.1, 17p13.1 and 17q25.3, respectively. We further showed that these variants were independently or jointly associated with an increased PanC risk. Further eQTL analysis revealed that those six novel SNPs might influence the mRNA expression levels of corresponding genes, particularly true for the *MAP2* rs35075084 and *TP53* rs9895829.

LKB1 was initially identified as a tumor suppressor gene responsible for the familial Peutz-Jeghers syndrome and associated with increased risk for gastrointestinal tract cancers, including PanC [41]. The AMPK, which is highly conserved in all eukaryotic cells and exists as a trimeric complex consisting of a catalytic subunit (α subunit) and two regulatory

subunits (β and γ subunits), plays a role in the regulation of cellular energy homeostasis by maintaining cellular energy homeostasis in response to an increased AMP:ATP ratio and restores energy balance by inhibiting anabolic processes that consume ATP, while promoting catabolic processes that generate ATP [12,13,17].

Together, the LKB1-AMPK pathway genes serve as a metabolic checkpoint and a central metabolic switch that governs glucose and lipid metabolisms in response to alterations in nutrients and intracellular energy levels [42,43]. Besides, LKB1-AMPK also controls for cell growth in response to environmental nutrient changes. For example, one central down-stream pathway suppressed by the LKB1-AMPK pathway is the mammalian target of rapamycin (mTOR) pathway, which controls cell growth in all eukaryotes; this signaling pathway is inhibited through the AMPK phosphorylation of tuberous sclerosis complex 2 (TSC2) and regulatory associated protein of mTOR (RPTOR) in conditions of low intracellular ATP levels [44-47].

Microtubule associated protein 2 (MAP2) is localized primarily in dendrites of neurons and involved in microtubule assembly [48,49]. It has been reported that MAP2 participates in the outgrowth of neuronal processes and synaptic plasticity and controls for selective axonal cargo sorting by regulating kinesin activity [50]. In addition, MAP2 is also involved in the protein kinase A-induced decrease in the invasiveness of glioma cells [51]. In the present study, we found that the *MAP2* rs34852782 deleted allele was associated with PanC risk, likely due to the resultant increase in the mRNA expression.

The protein kinase AMP-activated non-catalytic subunit γ 2 (PRKAG2), as a member of AMPK γ subunit family, mutations in which can cause inappropriate AMPK activation under resting conditions and lead to hypertrophic cardiomyopathy associated with the Wolff-Parkinson-White syndrome [52]. Previous studies revealed a nominal association of *PRKAG2* SNPs with diabetes incidence [53] and suggested that *PRKAG2* variants were involved in feed efficiency traits in beef steers [54]. The present study suggests that SNPs rs2727572 and rs34852782 located in the intron of *PRKAG2* were associated with PanC risk, and the variant-associated gene expression may be the mechanism underlying the observed association, but additional studies are needed to validate this speculation.

Tumor protein p53 (TP53) has many mechanisms of anticancer function and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis [55]. *TP53* somatically mutated in 50-80% of PanC [2,56-58]. As we mentioned before, SNPs rs9895829 of *TP53* has been previously reported by the AURORA pathway-based analyses. In our present study we found that SNPs rs9895829 had a significant correlation with a decreased *TP53* mRNA expression and was associated with PanC risk.

RPTOR is a crucial component of MTORC1 and negatively regulates mTOR [59]. When the intracellular energy level is low, AMPK directly phosphorylates RPTOR at Ser722 and Ser792, reducing mTOR kinase activities [60,61]. One study suggested that SNP rs11868112 of *RPTOR* exhibits a strong association with temperature variables that contribute to climate adaptations [62]. The present study found that SNPs rs62068300 and rs3751936 of *RPTOR* were associated with PanC risk. Because two SNPs (rs60268947 and rs28434589) in high

LD ($r^2 > 0.90$) with rs62068300 had a significant correlation with an increased *RPTOR* mRNA expression in whole blood cells from GTEx, and genetic variants in *RPTOR* are likely to play a role in carcinogenesis of PanC.

Although the present study observed associations between six novel genetic variants in the LKB1-AMPK pathway genes and PanC risk, it has also several limitations. First, we had no access to family history in the publically available datasets, which might have an impact on PanC risk. Second, since we only used the available online tools and eQTL analysis to evaluate function of a particular SNP, further functional investigations are required. Third, we are still not sure which SNP in the LKB1-AMPK pathway genes may have played a major role in or how jointly they may have an impact on PanC risk. Finally, because all selected subjects in two GWAS studies were from Caucasian populations, the results may not be generalizable to the general populations.

In summary, we report some significant associations between genetic variants in 58 LKB1-AMPK pathway genes and PanC risk in European populations. Six SNPs (i.e., *MAP2* rs35075084 T > deletion, *PRKAG2* rs2727572 C > T and rs34852782 A > deletion, *TP53* rs9895829, *RPTOR* rs62068300 G > A and rs3751936 G > C) were found to be significantly associated with an increased PanC risk, possibly by influencing their gene expression. More population validations and additional functional studies are needed to explore possible molecular mechanisms in the etiology of PanC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

## Abbreviations:

**PanC** pancreatic cancer

**SNP** single nucleotide polymorphism

**NUGs** number of unfavorable genotypes

**OR** odds ratio

**CI** confidence interval

**GWAS** genome-wide association studies

**FPRP** false positive report probability

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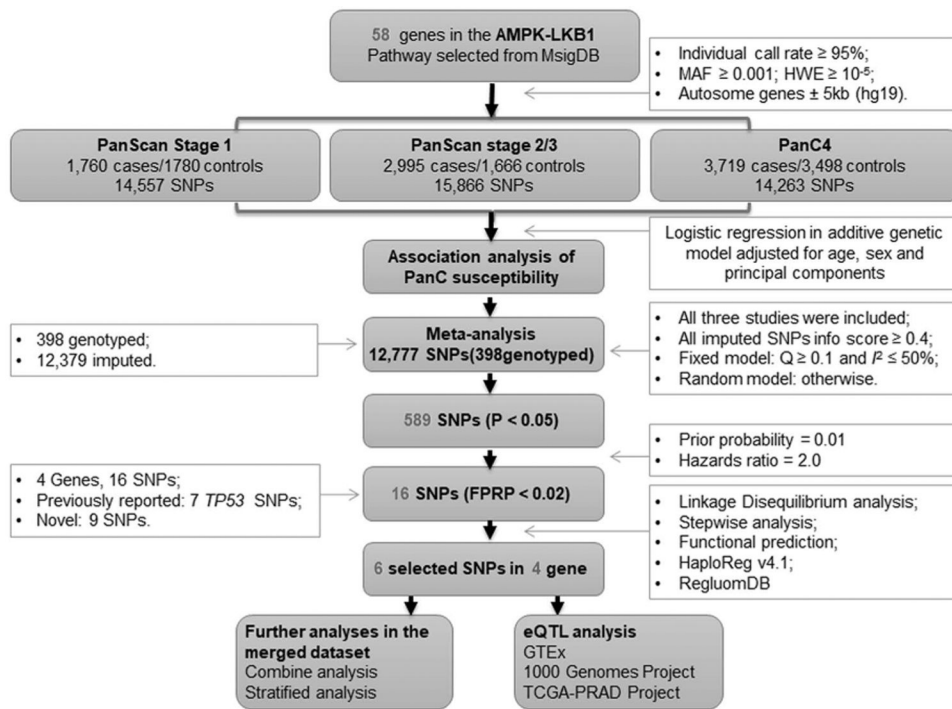
**Fig. 1. The workflow of the analysis.**

MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; SNP: single nucleotide polymorphism; PanC: pancreatic cancer; FPRP: false positive report probability; eQTL: expression quantitative trait loci.
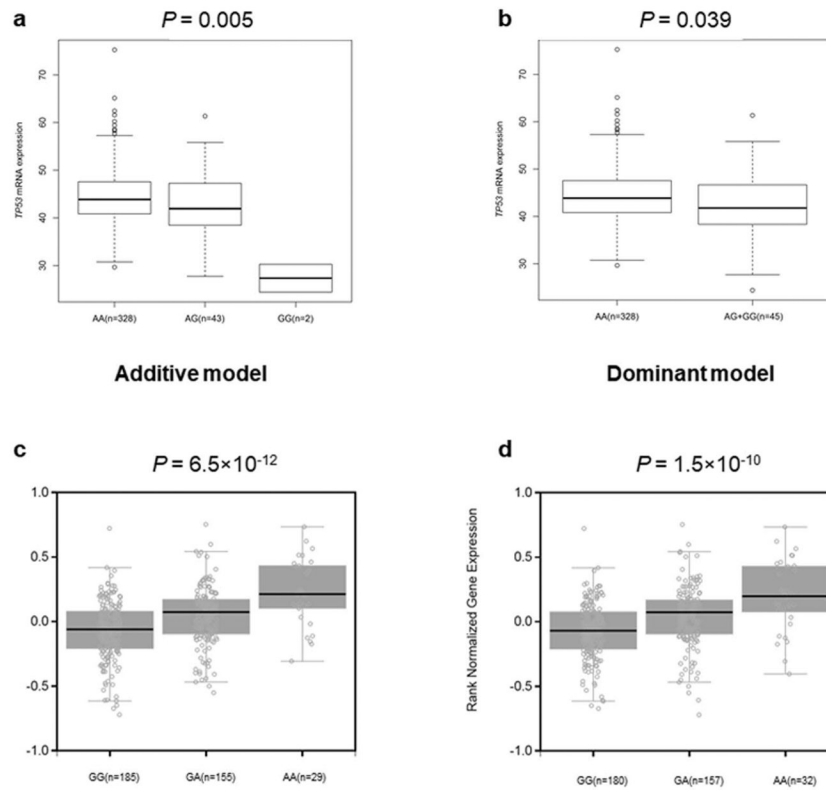
**Fig. 2. The eQTL analyses of the functional SNPs.**
The eQTL results of the SNP rs9895829 of *TP53* in 373 normal lymphoblastoid cell lines from the 1000 Genomes Project in (**a**) additive and (**b**) dominant model and (**c**-**d**) GTEx results of the two SNPs in high LD with SNP rs62068300 of *RPTOR* in whole blood cells of 369 normal participants. eQTL: expression quantitative trait loci; SNP: single nucleotide polymorphism; GTEx: genotype-tissue expression; LD: Linkage disequilibrium.

**Table 1.**

Associations between 16 SNPs in the AMPK-LKB1 pathway genes and pancreatic cancer risk with an FPRP < 0.02.

| SNP rs ID# | Locus | Gene | Allele[a] | IS1[b] | IS2[b] | IS3[b] | EAF1[c] | EAF2[c] | EAF3[c] | OR (95% CI)[d] | P[e] | FPRP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| rs35075084 | 2q34 | MAP2 | CT>C | 0.540 | 0.602 | 0.545 | 0.019 | 0.023 | 0.023 | 0.76 (0.64-0.90) | 0.0010 | 0.020 |
| rs2727572 | 7q36.1 | PRKAG2 | C>T | 0.982 | 0.979 | 0.982 | 0.473 | 0.454 | 0.445 | 1.08 (1.03-1.13) | 0.0011 | 0.019 |
| rs12668489 | 7q36.1 | PRKAG2 | C>T | 0.985 | 0.982 | 0.986 | 0.473 | 0.456 | 0.448 | 1.08 (1.03-1.13) | 0.0010 | 0.018 |
| rs2538046 | 7q36.1 | PRKAG2 | G>A | 0.995 | 0.993 | 0.991 | 0.505 | 0.486 | 0.474 | 1.09 (1.04-1.14) | 0.0003 | 0.005 |
| rs34852782 | 7q36.1 | PRKAG2 | TA>T | 0.886 | 0.860 | 0.791 | 0.283 | 0.272 | 0.275 | 1.10 (1.05-1.16) | 0.0002 | 0.003 |
| rs17884306 | 17p13.1 | TP53 | C>T | 0.979 | 0.973 | 0.971 | 0.060 | 0.058 | 0.057 | 0.83 (0.75-0.93) | 0.0004 | 0.007 |
| rs17879377 | 17p13.1 | TP53 | C>T | 0.972 | 0.969 | 0.966 | 0.051 | 0.053 | 0.050 | 0.84 (0.74-0.94) | 0.0011 | 0.020 |
| rs9891744 | 17p13.1 | TP53 | C>T | 0.980 | 0.975 | 0.976 | 0.060 | 0.059 | 0.058 | 0.82 (0.73-0.91) | 0.0001 | 0.002 |
| rs75732100 | 17p13.1 | TP53 | C>T | 0.985 | 0.980 | 0.981 | 0.060 | 0.058 | 0.058 | 0.82 (0.74-0.92) | 0.0002 | 0.004 |
| rs35850753 | 17p13.1 | TP53 | C>T | 0.963 | 0.948 | 0.952 | 0.021 | 0.022 | 0.023 | 0.74 (0.62-0.88) | 0.0004 | 0.008 |
| rs9895829 | 17p13.1 | TP53 | A>G | 0.991 | 0.987 | 0.991 | 0.061 | 0.058 | 0.058 | 0.82 (0.74-0.92) | 0.0002 | 0.003 |
| rs17883323 | 17p13.1 | TP53 | G>T | 0.989 | 0.987 | 0.991 | 0.061 | 0.058 | 0.057 | 0.83 (0.74-0.92) | 0.0002 | 0.004 |
| rs8079544 | 17p13.1 | TP53 | G>T | 1.000 | 1.000 | 1.000 | 0.060 | 0.058 | 0.058 | 0.82 (0.74-0.92) | 0.0002 | 0.003 |
| rs62068300 | 17q25.3 | RPTOR | G>A | 0.973 | 0.974 | 0.978 | 0.308 | 0.320 | 0.330 | 0.92 (0.87-0.97) | 0.0008 | 0.015 |
| rs17848685 | 17q25.3 | RPTOR | C>G | 0.729 | 0.701 | 0.822 | 0.213 | 0.228 | 0.234 | 0.91 (0.86-0.97) | 0.0011 | 0.019 |
| rs3751936 | 17q25.3 | RPTOR | G>C | 0.897 | 0.883 | 0.906 | 0.264 | 0.268 | 0.258 | 0.91 (0.86-0.97) | 0.0008 | 0.014 |

Abbreviations: SNP: single nucleotide polymorphism; FPRP: false positive report probability; IS: information score; EAF: effect allele frequency; OR: odds ratio; CI: confidence interval.

[a] Referring to "common allele/effect allele."

[b] IS1 was EAF in PanScan I controls; IS2 was EAF in PanScan II/III controls; IS3 was EAF in PanC4 controls.

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

[d] 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.

[e] Obtained from the meta-analysis of the three studies.

**Table 2.**

The results of these 16 SNPs in each of GWAS datasets and the final meta-analysis.

| SNP rs ID# | Allele | Position | Gene | PanScan_stage1 | | PanScan_stage2 | | PanC4 | | All GWAS combined | | Heterogeneity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P | Q | $I^2$ |
| rs35075084 | CT>C | 210473015 | MAP2 | 0.78 (0.54-1.13) | 0.188 | 0.88 (0.66-1.18) | 0.400 | 0.68 (0.54-0.86) | 0.001 | 0.76 (0.64-0.90) | 0.0010 | 0.398 | 0 |
| rs2727572 | C>T | 151298246 | PRKAG2 | 1.03 (0.94-1.13) | 0.509 | 1.08 (1.00-1.18) | 0.064 | 1.10 (1.03-1.18) | 0.005 | 1.08 (1.03-1.13) | 0.0011 | 0.541 | 0 |
| rs34852782 | TA>T | 151566171 | PRKAG2 | 1.08 (0.97-1.20) | 0.144 | 1.11 (1.01-1.22) | 0.036 | 1.11 (1.03-1.20) | 0.005 | 1.10 (1.05-1.16) | 0.0002 | 0.902 | 0 |
| rs12668489 | C>T | 151301725 | PRKAG2 | 1.04 (0.95-1.15) | 0.362 | 1.08 (0.99-1.17) | 0.087 | 1.10 (1.03-1.17) | 0.005 | 1.08 (1.03-1.13) | 0.0010 | 0.675 | 0 |
| rs2538046 | G>A | 151314162 | PRKAG2 | 0.94 (0.85-1.03) | 0.161 | 1.07 (0.98-1.16) | 0.132 | 1.11 (1.04-1.19) | 0.002 | 1.09 (1.04-1.14) | 0.0003 | 0.715 | 0 |
| rs17884306 | C>T | 7572101 | TP53 | 0.81 (0.65-0.99) | 0.044 | 0.82 (0.68-0.99) | 0.039 | 0.85 (0.73-0.99) | 0.032 | 0.83 (0.75-0.93) | 0.0004 | 0.912 | 0 |
| rs17879377 | C>T | 7574721 | TP53 | 0.88 (0.70-1.10) | 0.256 | 0.78 (0.64-0.95) | 0.015 | 0.85 (0.73-1.00) | 0.044 | 0.84 (0.74-0.94) | 0.0011 | 0.706 | 0 |
| rs9891744 | C>T | 7574864 | TP53 | 0.79 (0.64-0.98) | 0.032 | 0.81 (0.67-0.98) | 0.028 | 0.83 (0.72-0.97) | 0.016 | 0.82 (0.73-0.91) | 0.0001 | 0.817 | 0 |
| rs75732100 | C>T | 7576348 | TP53 | 0.80 (0.65-0.99) | 0.037 | 0.83 (0.69-1.00) | 0.046 | 0.84 (0.72-0.97) | 0.018 | 0.82 (0.74-0.92) | 0.0002 | 0.947 | 0 |
| rs35850753 | C>T | 7578671 | TP53 | 0.94 (0.67-1.31) | 0.712 | 0.60 (0.43-0.83) | 0.002 | 0.73 (0.58-0.93) | 0.010 | 0.74 (0.62-0.88) | 0.0004 | 0.168 | 43.97 |
| rs9895829 | A>G | 7578679 | TP53 | 0.79 (0.64-0.98) | 0.029 | 0.83 (0.69-1.00) | 0.046 | 0.84 (0.72-0.97) | 0.020 | 0.82 (0.74-0.92) | 0.0002 | 0.913 | 0 |
| rs17883323 | G>T | 7579619 | TP53 | 0.79 (0.64-0.98) | 0.029 | 0.82 (0.68-0.99) | 0.042 | 0.84 (0.73-0.98) | 0.024 | 0.83 (0.74-0.92) | 0.0002 | 0.896 | 0 |
| rs8079544 | G>T | 7580052 | TP53 | 0.80 (0.65-0.99) | 0.036 | 0.82 (0.68-0.99) | 0.039 | 0.83 (0.72-0.97) | 0.017 | 0.82 (0.74-0.92) | 0.0002 | 0.948 | 0 |
| rs62068300 | G>A | 78574727 | RPTOR | 0.96 (0.87-1.07) | 0.479 | 0.92 (0.84-1.01) | 0.083 | 0.90 (0.84-0.96) | 0.003 | 0.92 (0.87-0.97) | 0.0008 | 0.537 | 0 |
| rs17848685 | C>G | 78599562 | RPTOR | 1.00 (0.89-1.12) | 0.998 | 0.92 (0.83-1.02) | 0.099 | 0.87 (0.80-0.94) | 0.001 | 0.91 (0.86-0.97) | 0.0011 | 0.150 | 47.36 |
| rs3751936 | G>C | 78938204 | RPTOR | 1.00 (0.90-1.11) | 0.960 | 0.89 (0.81-0.98) | 0.017 | 0.89 (0.82-0.96) | 0.003 | 0.91 (0.86-0.97) | 0.0008 | 0.178 | 41.98 |

Abbreviations: SNP: single nucleotide polymorphism; GWAS: genome-wide association study; OR: odds ratio; CI: confidence interval.

**Table 3.**

Analysis of associations between PanC risk and the six SNPs in the dataset of PanScan and PanC4 studies.

| SNP rs ID# & genetic model | Genotype | Group Case (%) | Control (%) | OR (95% CI)[a] | P[b] |
|---|---|---|---|---|---|
| *MAP2* rs35075084 CT>C[b] | | | | | |
| Additive | TT | 8221 (96.49) | 6798 (95.60) | 1.00 | -- |
| | T- | 295 (3.46) | 310 (4.36) | 0.75 (0.64-0.89) | 0.0006 |
| | -- | 4 (0.05) | 3 (0.04) | 0.99 (0.22-4.44) | 0.9871 |
| | Trend test | | | | 0.0008 |
| Dominant | T-+-- | 299 (3.51) | 313 (4.40) | 0.75 (0.64-0.89) | 0.0007 |
| *TP53* rs9895829 A>G | | | | | |
| Additive | AA | 7729 (90.56) | 6315 (88.62) | 1.00 | -- |
| | AG | 788 (9.23) | 790 (11.09) | 0.83 (0.75-0.93) | 0.0006 |
| | GG | 18 (0.21) | 21 (0.29) | 0.73 (0.39-1.37) | 0.3285 |
| | Trend test | | | | 0.0004 |
| Dominant | AG+GG | 806 (9.44) | 811 (11.38) | 0.83 (0.75-0.92) | 0.0004 |
| *PRKAG2* rs2727572 C>T | | | | | |
| Additive | CC | 2432 (28.50) | 2146 (30.00) | 1.00 | -- |
| | CT | 4170 (48.87) | 3485 (48.72) | 1.09 (1.01-1.17) | 0.0283 |
| | TT | 1930 (22.62) | 1522 (21.28) | 1.17 (1.07-1.28) | 0.0008 |
| | Trend test | | | | 0.0007 |
| Dominant | CT+TT | 6100 (71.50) | 4980 (69.62) | 1.11 (1.04-1.19) | 0.0034 |
| *PRKAG2* s34852782 TA>T[b] | | | | | |
| Additive | AA | 4237 (49.98) | 3687 (51.98) | | |
| | A- | 3492 (41.49) | 2893 (40.79) | 1.06 (0.99-1.13) | 0.1161 |
| | -- | 749 (8.83) | 513 (7.23) | 1.29 (1.15-1.46) | <.0001 |
| | Trend test | | | | 0.0002 |
| Dominant | A-+-- | 4241 (50.02) | 3406 (48.02) | 1.09 (1.02-1.16) | 0.0076 |
| *RPTOR* rs62068300 G>A | | | | | |
| Additive | GG | 4093 (47.97) | 3278 (46.00) | | |
| | GA | 3639 (42.65) | 3116 (43.73) | 0.92 (0.86-0.98) | 0.0149 |
| | AA | 801 (9.39) | 732 (10.27) | 0.85 (0.76-0.95) | 0.0043 |
| | Trend test | | | | 0.0009 |
| Dominant | GA+AA | 4440 (52.03) | 3848 (54.00) | 0.91 (0.85-0.97) | 0.0026 |
| *RPTOR* rs3751936 G>C | | | | | |
| Additive | GG | 4887 (25.28) | 3886 (27.01) | 1.00 | -- |
| | GC | 3119 (49.55) | 2710 (49.40) | 0.93 (0.87-1.00) | 0.0347 |
| | CC | 513 (25.17) | 512 (23.59) | 0.82 (0.72-0.93) | 0.0025 |
| | Trend test | | | | 0.0009 |
| Dominant | GC+CC | 3632 (74.72) | 3222 (72.99) | 0.91 (0.86-0.97) | 0.0051 |

Abbreviations: PanC: pancreatic cancer; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

[a]Obtained from Logistic regression models with adjustment for age, sex, and top five significant principal components.

[b]Base deletion.

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**Table 4.**

Combined risk genotypes of the six SNPs and the risk of pancreatic cancer.

| NUG | Group | | OR (95% CI)[a] | P[b] |
| --- | --- | --- | --- | --- |
| | Case (%) | Control (%) | | |
| 0 | 1 (0.01) | 1 (0.01) | 1.00 | -- |
| 1 | 34 (0.40) | 50 (0.71) | 1.75 (0.11-29.08) | 0.6963 |
| 2 | 459 (5.44) | 467 (6.61) | 1.48 (0.94-2.34) | 0.0911 |
| 3 | 1738 (20.59) | 1571 (22.25) | 1.66 (1.07-2.59) | 0.0253 |
| 4 | 3014 (35.70) | 2626 (37.20) | 1.75 (1.12-2.72) | 0.0133 |
| 5 | 2450 (29.02) | 1836 (26.01) | 2.04 (1.31-3.18) | 0.0016 |
| 6 | 746 (8.84) | 509 (7.21) | 2.23 (1.42-3.51) | 0.0005 |
| Trend test | | | | <.0001 |
| 0-4 | 5246 (62.14) | 4715 (66.78) | 1.00 | -- |
| 5-6 | 3196 (37.86) | 2345 (33.22) | 1.24 (1.16-1.32) | <.0001 |

Abbreviations: SNP: single nucleotide polymorphism; PanC: pancreatic cancer; NUG: number of unfavorable genotype; OR: odds ratio; CI: confidence interval.

[a] Risk genotypes were rs35075084 TT, rs2727572 CT+TT, rs34852782 A-+--, rs9895829 AA, rs62068300 GG and rs3751936 GG.

[b] Logistic regression analyses were adjusted for age, sex and the top five principal components.