A pleiotropy scan to discover new susceptibility loci for pancreatic ductal adenocarcinoma

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Abstract

Pleiotropic variants (*i.e.*, genetic polymorphisms influencing more than one phenotype) are often associated with cancer risk. A scan of pleiotropic variants was successfully conducted ten years ago in relation to pancreatic ductal adenocarcinoma susceptibility. However, in the last decade, genetic association studies performed on several human traits have greatly increased the number of known pleiotropic variants. Based on the hypothesis that variants already associated with a least one trait have a higher probability of association with other traits, 61,052 variants reported to be associated by at least one genome wide association study (GWAS) with at least one human trait were tested in the present study consisting of two phases (discovery and validation), comprising a total of 16,055 pancreatic ductal adenocarcinoma (PDAC) cases and 212,149 controls. The meta-analysis of the two phases showed two loci (10q21.1-rs4948550 ($P=6.52\times10^{-5}$) and 7q36.3-rs288762 ($P=3.03\times10^{-5}$) potentially associated with PDAC risk. 10q21.1-rs4948550 shows a high degree of pleiotropy and it is also associated with colorectal cancer risk while 7q36.3-rs288762 is situated 28,558 base pairs upstream of the Sonic Hedgehog (*SHH*) gene, which is involved in the cell differentiation process and PDAC etiopathogenesis. In conclusion, none of the single nucleotide polymorphisms (SNPs) showed a formally statistically significant association after correction for multiple testing. However, given their pleiotropic nature and association with various human traits including colorectal cancer, the two SNPs showing the best associations with PDAC risk merit further investigation through fine mapping and ad hoc functional studies.

Key words: pleiotropy, pancreatic cancer, single nucleotide polymorphism, genetic susceptibility

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a relatively rare disease with a crude incidence rate of 18.7 per 100,000 individuals per year in Europe[1]. Only a small number of potential PDAC risk factors have been identified, such as cigarette smoking, type 2 diabetes mellitus, chronic pancreatitis, overweight and non-O blood groups [2–4]. Recently a study carried out in the context of UK Biobiank (UKBB) has suggested stress as a major contributor for the development of the disease [5]. The genetic susceptibility to PDAC is due to rare high-penetrance mutations and common low-penetrance genetic variants, that alone or in combination are associated with increased risk of developing PDAC [6–24]. However, PDAC is a polygenic and complex multifactorial disease that shares a portion of the genetic background with several human traits [25,26]. For example, the *TERT-CLPTM1L* region is known to be associated with PDAC risk, but it is also associated with risk of melanoma, breast, and cervical cancers [26]. A genetic variant independently associated with more than one trait is defined as pleiotropic, a characteristic shared by many risk loci for a large number of human traits [27]. Pleiotropic polymorphisms could have a crucial role in the genetic architecture of complex diseases due to their influence on different pathways and biological mechanisms [28–30]. Pleiotropic single nucleotide polymorphisms (SNPs) are common in cancer, and there are regions in the genome called "nexus" that are associated with more than one type of cancer [31,32]. In addition, several PDAC risk loci are also associated with non-cancer phenotypes. For example, *ABO* and *TERT* SNPs are associated with a plethora of human traits, such as longevity [33], type II diabetes [34], male infertility [35], mitochondrial DNA copy number [36], and high-density lipoprotein cholesterol level [37]. Therefore, the study of pleiotropic SNPs could be instrumental in unravelling the genetic architecture of human diseases, as SNPs that are already associated with one trait have an increased chance of being associated with other phenotypes. The analysis of the possible association of pleiotropic variants with PDAC was completed in a study comprising 1,087 SNPs in 2,857 PDAC cases and 2,967 controls, in which the authors identified, for the first time, a new PDAC risk locus (rs7310409) in the HNF1 homeobox A *(HNF1A)* gene [38]. In the decade since that study our knowledge of SNP-phenotype associations has greatly increased. With these premises, we aimed to identify novel pleiotropic SNPs associated with PDAC risk in an extensive multi-ethnic study of 16,880 PDAC cases and 219,861 controls.

Material and methods

Study design

The present study was carried out in two phases. First, a discovery phase where pleiotropic SNPs reported to be associated with at least one human trait were tested for association in a case-control study consisting of 8,738 PDAC cases and 7,034 controls. The discovery phase consisted of the PanScan I, II, III [9,11,12], and PanC4 studies [14]that were imputed separately and then merged and analysed together. After the discovery phase two rounds of replication were carried out, the first (replication1) consisting of theEuropean Study into Digestive Illnesses and Genetics (PanGenEU) study [39] and the second (replication2) consisting of the Pancreatic Disease Research (PANDoRA) consortium [24], the

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Japan Pancreatic Cancer Research Consortium (JaPAN) [7,10,40,41] and FinnGen [42]. Replication 2 was carried out in all SNPs that were significant in the discovery and replication 1 phases. **Table 1** shows the number of cases and controls analysed in each dataset, alongside age and sex distribution.

Discovery phase

A list of SNPs associated with at least one human trait at genome-wide significance level ($P<5x10^{-8}$) was obtained from the GWAS Catalog portal. The list contained 126,080 SNP-trait associations, consisting of 73,700 unique SNPs, among which 1,869 did not have reference SNP ID number (rs#) and therefore were not included in the analyses. The list included SNPs associated with any disease and/or any trait and was not restricted by ethnicity. All the selected SNPs were analysed using the genotypes of the PanScan I, II, III and PanC4 GWASs. The genotypes were downloaded from the database of Genotypes and Phenotypes (dbGaP; study accession nos. phs000206.v5.p3 and phs000648.v1.p1; project reference no. 12644). Genotyping and quality control details of these studies have been described in the original publications [9,11,12,14].

The four combined datasets included 9,563 PDAC cases and 8,073 controls. The genotypes were imputed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu), and the Haplotype Reference Consortium (HRC, V.r1.1) as reference panel. The imputation for PanScan I, II, III and PanC4 GWASs was carried out separately for each dataset.

Before imputation, the datasets were filtered applying the following quality controls: removal of individuals with sex mismatches, missing genotypes >2%, relatedness issues (PI_HAT>0.2) and minimal or excessive heterozygosity (>3 standard deviations from the mean). Additionally, the SNPs with a minor allele frequency (MAF) <0.01, call-rate<98%, and evidence for violations of Hardy-Weinberg equilibrium (HWE, $P<1\times10^{-5}$) were discarded. Principal component analysis (PCA) was performed with PLINK 2.0, including the genotypes of phase 3 of the 1000 Genomes Project as reference panel [43]. Individuals not clustering in the PCA with the 1000 Genomes subjects of European descent were excluded from further analysis. After imputation the four datasets were merged using only the SNPs with imputation quality (INFO score r^2) higher than 0.7 (N= 24,735,918 SNPs). The pooled dataset was filtered, removing the variants with call rate<98% (N=11,699,683 SNPs), MAF<1% (N=5,524,684 SNPS) or departure from HWE ($P<1x10^{-5}$, N=2,206 SNPs). The discovery dataset consisted therefore of 8,738 PDAC case and 7,034 controls that were analysed for 7,509,345 SNPs. The "inflation factor" calculated in each dataset did not show evidence of systematic inflation (λ=1.000 for PanScan I, λ=1.015 for PanScan II, λ=1.026 for PanScan III, λ=1.000 for PanC4, and λ=1.000 for the aggregate dataset).

Replication phase

In the replication phase, data obtained from four independent populations were analysed, using the summary statistics of three studies: (I) PanGenEU, (II) FinnGen, and (III) JaPAN. Additionally, the SNPs to be validated were genotyped in the PANDoRA consortium. All populations have been described in detail elsewhere [23,24,41,42,44]. A brief description is also given in **supplementary material 1**.

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Summary statistics of PanGenEU were used as first replication (Replication 1), then the variants that showed a statistically significant association (P<0.05) in PanGenEU were genotyped in PANDoRA and looked up in FinnGen and JaPAN (Replication 2) for a total of 7,317 cases and 212,142 controls comprised in the four populations.

PANDoRA sample preparation and genotyping

DNA of cases and controls from PANDoRA was extracted from whole blood, using the QIamp® 96 DNA QIAcube® HT Kit (Qiagen, Hilden, Germany). Genotyping was performed using TaqMan technology (ThermoFisher Applied Biosystems, Waltham MA, USA) in 384-well plates. A similar number of cases and controls was distributed in each plate, and duplicate samples (8%) were added for quality control purposes. Genotypes were determined using the QuantStudio™ 5 Real-Time PCR system (Thermofisher, USA).

Statistical analysis

The association between SNPs and risk of developing PDAC was evaluated through unconditional logistic regression analysis adjusted for sex, age and the top eight principal components for the discovery phase (PanScan I, II, III and PanC4) and for sex, age, and country of origin in the replication phase (PANDoRA). All the statistical analyses were conducted using PLINK 2.0 and R software. The details on the statistical analyses adopted in the PanGenEU, JaPAN and FinnGen GWASs are reported elsewhere [23,41,42]. A meta-analysis was performed for all the variants that showed a statistically significant association in PanGenEU using all the populations (PanScan I, II, III, PanC4, PanGenEU, PANDoRA, JaPAN and FinnGen). Stratified analysis including only European individuals was also performed to avoid confounding bias due to the different ethnic groups. To account for multiple testing, we considered linkage disequilibrium (LDr^2>0.8; 1000 genomes, Europeans) among the SNPs used in the discovery phase to obtain a list of independent variants (N=37,435). The threshold for statistical significance was, therefore, set to $P=0.05/37,435=1.34\times10^{-6}$ using Bonferroni's correction.

Results

Figure 1 shows the flowchart of the study design and replication across the populations. During the discovery phase 73,700 unique SNPs associated with at least one human trait were identified in GWAS Catalog and analysed in the Pancreatic Cancer Cohort Consortium (PanScan I, II and III), and the Pancreatic Cancer Case-Control Consortium (PanC4) datasets, for a total of 8,738 PDAC cases and 7,034 controls; 12,728 variants and their LD proxies (r^2>0.8) were not present in the dataset. Among the 61,052 remaining variants, 428 showed a statistically significant association with PDAC risk (P<0.05) (**Supplementary Table 1**). Among these, 164 SNPs were in LD (r^2>0.8) with known PDAC risk loci, and the remaining 264 variants were pruned (r^2>0.6) to eliminate residual LD and to identify independent SNPs

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to be validated. The final list of SNPs to be further validated consisted of 113 SNPs (**Supplementary Table 1**).

These 113 SNPs were tested in PanGenEU, where seven variants showed an association (P<0.05). One SNP (11p14.2-rs117551578) showed a statistically significant association with the risk of developing PDAC in both the discovery phase and in PanGenEU, but in the opposite direction, therefore it was excluded from the subsequent analyses. **Table 2** shows the seven SNPs associated in PanGenEU, their P-value of association with PDAC risk and the P-value of association with the trait for which they were originally selected.

The remaining six SNPs (6q22.32-rs6919397; 8p23.1-rs2980752; 7q36.3-rs288762; 10q21.1-rs4948550; 12q12-rs12427164; 17q23.2-rs9903801) were analysed in replication 2 (described in the methods), that included genotypes of PANDoRA and summary statistics from FinnGen and JaPAN and then meta-analysed. None of the SNPs showed a statistically significant association in the studies belonging to replication 2. Furthermore, the overall meta-analysis, performed including a total of 16,055 PDAC cases and 212,149 controls, did not show any statistically significant association, considering Bonferroni correction for multiple testing (Table 3). Excluding non-European (Brazilian from PANDoRA and Japanese from JaPAN) individuals from the meta-analysis, lower P-values compared to the discovery phase were observed for 7q36.3-rs288762 (OR=1.08, 95%CI=1.04-1.12, $P=3.03\times10^{-5}$) and for 10q21.1-rs4948550 (OR=0.92, 95%CI=0.89-0.96, $P=6.52\times10^{-5}$).

Discussion

Among the six SNPs selected for the replication phase in PANDoRA, JaPAN, and FinnGen, none showed significant associations (P<0.05) in the studies taken individually; however, 10q21.1-rs4948550 and 7q36.3-rs288762 showed a lower P-value in the meta-analysis compared with the discovery phase. In addition, removing individuals of non-European or of admixed ancestry from the analysis, the significance level of the results improved further for 7q36.3-rs288762 ($P_{value}=3.03\times10^{-5}$) and for 10q21.1-rs4948550 ($P_{value}=6.52\times10^{-5}$). This improvement could be explained by the fact that the two SNPs are just risk markers, not directly responsible for the disease and, therefore, the presence of a different LD architecture across populations could dilute the results when considering different ethnicities together. The causative SNP could be linked to the markers in central Europeans but not in Asians, or Brazilians, or in LD with a variant that was not genotyped and that is in different LD blocks in the various populations. However, none of the variants reached the P-value threshold set for this study, considering the correction for multiple testing ($P=1.34\times10^{-6}$).

The best association was observed for 10q21.1-rs4948550 which is located in the BicC Family RNA Binding Protein 1 (*BICC1*) gene, that encodes an RNA-binding protein that regulates cell proliferation and apoptosis [45]. This SNP is a missense variant that leads to the aminoacidic variation Ser943Pro, for which a benign clinical significance is reported on NCBI dbSNP portal (www.ncbi.nlm.nih.gov). The 10q21.1-rs4948550 region shows a high degree of pleiotropy since the SNP (or the SNPs in LD with it) is associated with cardiovascular disease, bilateral cleft lip, morningness (*i.e.,* the individual preference of

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waking up early) and colorectal cancer [46–48]. In particular, 10q21.1-rs4948550 is in strong LD with rs4948317 (r^2=0.82, D'=0.98 in Europeans) which was identified to be associated with risk of colorectal cancer (CRC, P=7x10^{-8}) in a GWAS study carried out in Est Asian individuals. The connection between CRC and PDAC is intriguing because the two tumours share several risk loci, for example 5p15.33-*TERT*, 16q24.1-*LINC01081/LINC00917*, 7p12.3-*TNS3* and the region of *ABO* on 9q34 [9,12,49–52]. Therefore, the results of our study may suggest another potential pleiotropic locus shared by PDAC and CRC, highlighting the importance of pleiotropy in human neoplastic diseases and a possible overlap in pathways and mechanisms that lead to the development of the two diseases.

The other potentially interesting SNP, 7q36.3-rs288762, is situated 28,558 base pairs upstream of the sonic hedgehog (*SHH*) gene, that is involved in cell differentiation. *SHH* has been observed to be overexpressed in cancer patients, thus creating a favourable environment for metastasis, proliferation, and drug resistance [11,53,54]. Three SNPs located in the *SHH* gene (rs167020, rs172310, rs288746) are already known to be associated with the risk of developing PDAC [12], highlighting the importance of the genetic variability of this region in PDAC. These three SNPs are in weak LD withrs288762, (r^2<0.35 in the European population). This region is particularly interesting due to the different results obtained with cohort and case-control studies. Amundadottir and colleagues observed a strong association for rs167020 (P=1.76x10^{-7}), rs172310 (P=2.01x10^{-7}) and rs288746 (P=1.35x10^{-4}) in prospective cohorts included in PanScan-I, but the associations were not confirmed in the retrospective studies used for replication (P=0.122, P=0.095 and P=0.108, respectively) [12]. Similarly, in our study, 7q36.3-rs288762 was observed to be associated with PDAC risk in the discovery phase (P=1.09×10^{-4}), that included mainly prospective studies, among which those analysed in 2009 by Amundadottir and colleagues, but not in the European retrospective populations used in the replication phase (PanGenEU P=0.03, PANDoRA P=0.66 and FinnGen P=0.37). Interestingly, Regan et al. observed that non canonical expression of SHH pathways positively regulates WNT signalling and may be crucial for colon cancer stem cell survival once again highlighting a connection between PDAC and CRC. [55]. Moreover, the T allele of rs288762, associated with an increased risk of developing PDAC, is also significantly associated with a low estimated glomerular filtration rate. This trait was already found to be associated with one (rs9903801) of the six SNPs analysed in Replication 2, suggesting a possible correlation between the risk of developing PDAC and a low estimated glomerular filtration rate. Additionally, it is interesting to note that C-reactive protein/albumin ratio is a predictor of pancreatic cancer survival, however further studies are warranted to characterise the mechanism linking these two traits.

A clear strength of this study is the large sample size, with 16,055 cases of PDAC and 212,149 controls, and a rigorous multi-phase process to eliminate spurious findings.

A possible limitation is the lack of a prospective cohort to replicate the finding on 7q36.2 to shed more light on the association of rs288762, located at this locus, since it appears to have different effect in prospective and retrospective studies. Another possible limitation is the fact that in the FinnGen study exocrine and endocrine pancreatic cancers are not divided. However, considering the very low prevalence of the latter (less than 2% of all pancreatic cancers), it is unlikely that this would have changed our results.

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In conclusion, none of the SNPs showed a formally statistically significant association after correction for multiple testing. However, due to their pleiotropic nature and their connection with CRC, the two SNPs showing the best associations with PDAC risk merit further investigation through fine mapping and ad hoc functional studies.

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Figure 1: Flowchart of SNP selection.

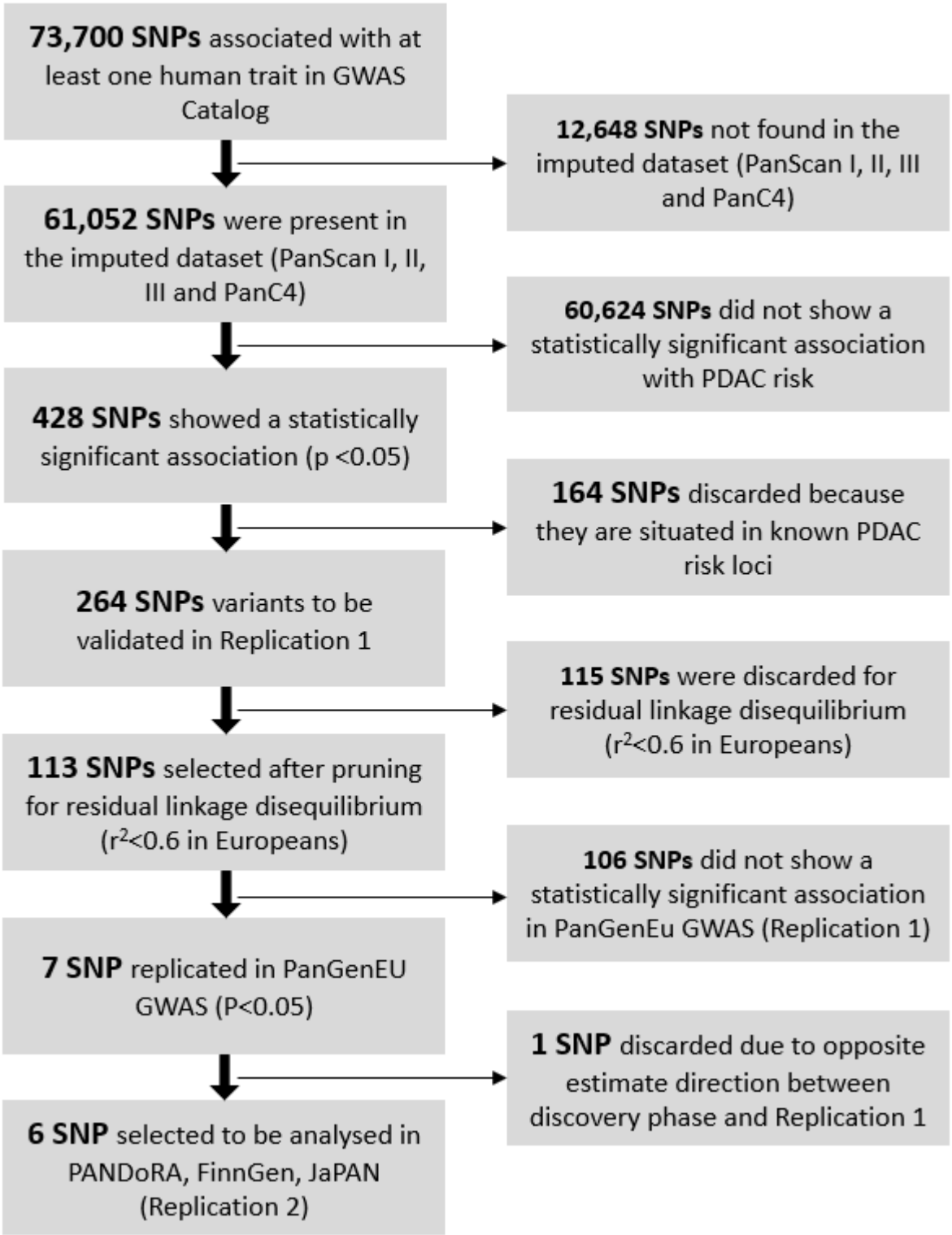

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Table 1. Description of the study populations.

	PanScan I II III & PanC4	***PanGenEU***	***PANDoRA***	***JaPAN[1]***	***FinnGen[2]***	***Total***
Study phase	Discovery	Replication 1	Replication 2	Replication 2	Replication 2	
Number of subjects						
Cases	8,738	1,317	3,442	2,039	519	16,055
Controls	7,034	1,616	3,928	32,592	174,006	212,149
Total	15,772	2,933	7,370	34,631	174,525	235,231
Median age (25%-75% percentiles)						
Cases	65 (55-75)	66 (57-73)	66(58-73)	62.7| 66.3	-	-
Controls	65 (55-75)	65 (55-75)	59(50-66)	43.6| 56.3	-	-
Sex						
Female	46%	42%	47%	-	-	-
Male	54%	58%	53%	-	-	-

[1]The information about sex and age are reported as minimum | maximum value.

[2] We used the FinnGen documentation of R4 Release.

"-": Information not available in the original database.

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Table 2: SNPs associated with PDAC risk in discovery phase (PanScan and PanC4) that were found associated also in replication 1 (PanGenEU GWAS).

SNP	Locus	M/m	MAF in CEU	Results of replication 1		Associated traits in GWAS Catalog		
				OR (95%CI)	Pvalue	Traits	OR (95%CI)	Pvalue
rs6919397	6q22.32	T/G	0.409	0.80 (0.69-0.92)	0.002	Neuroticism	NR (NR)	$4x10^{-8}$
						Type 1 diabetes (rs9388489, r^2=0.99, D'=1.00) #	1.17 (1.10-1.24)	$4x10^{-13}$
						Type 2 diabetes (rs4897182, r^2=0.97, D'=0.99) #	1.05 (1.02-1.11)	$3x10^{-8}$
rs288762	7q36.3	C/T	0.384	1.17 (1.01-1.36)	0.034	Estimated glomerular filtration rate in non-diabetics	1.44 (1.11-2.30)	$2x10^{-11}$
rs2980752	8p23.1	C/A	0.294	0.84 (0.72-0.99)	0.034	Heel bone mineral density	1.03 (1.00-1.07)	$3x10^{-50}$
						Triglyceride levels (rs2980755, r^2=0.41, D'=0.91) #	0.98 (0.95-0.99)	$1x10^{-10}$
rs4948550	10q21.1	C/T	0.328	0.85 (0.73-1.00)	0.049	Cardiovascular disease	NR (NR)	$7x10^{-8}$
						Colorectal cancer (rs4948317, r^2=0.82, D'=0.97) #	1.10 (1.06-1.13)	$7x10^{-8}$
						Morning person (rs2893787, r^2=0.91, D'=0.99) #	NR (NR)	$2x10^{-8}$
rs35138700*	12q12	C/T	0.394	1.18 (1.02-1.37)	0.029	Morning person	1.05 (NR)	$4x10^{-43}$
						Type 2 diabetes (rs7315028, r^2=0.57, D'=0.96) #	1.13 (1.04-1.33)	$2x10^{-8}$

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rs7214227*	17q23.2	C/T	0.146	0.76 (0.63-0.93)	0.014	Estimated glomerular filtration rate	1.01 (1.00-1.01)	$2x10^{-36}$
						Renal function-related traits (rs11868441, r^2=0.61, D'=0.89) #	1.01 (1.00-1.01)	$2x10^{-9}$

*SNPs selected as proxy (r^2=1, D'≥ 0.94).

Traits reported in GWAS Catalog for variants in LD (r^2>0.4) with the SNPs analysed in replication 1.

M/m: major/minor allele; OR (95%CI): odds ratio and its 95% confidence interval; NR: data nor reported in GWAS Catalog, the Pvalue refers to the association with the reported trait.

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Table 3: Associations between selected SNPs and PDAC risk in the individual studies and in the meta-analysis.

SNP, locus, M/m		*PanScan/PanC 4*	*PanGenEU*	*PANDoRA*[1]	*PANDoRA*[2]	*JaPAN*	*FinnGen*	*Meta-analysis*[1]	*Meta-analysis*[2]
rs6919397	OR (95%CI)	0.92 (0.88-0.96)	0.80 (0.69-0.92)	098 (0.92-1.05)	0.98 (0.91-1.05)	-	1.06 (0.93-1.2)	0.94 (0.87-1.02)	0.95 (0.88-1.03)
6q22.32	P_{value}	1.31×10^{-4}	2.44×10^{-3}	6.17×10^{-1}	5.40×10^{-1}	-	3.84×10^{-1}	1.35×10^{-1}	2.17×10^{-1}
T/G	P_{value} Het.							1.18×10^{-2}	9.70×10^{-3}
rs288762	OR (95%CI)	1.10 (1.05-1.15)	1.17 (1.01-1.36)	1.02 (0.95-1.10)	1.02 (0.95-1.09)	1.02 (0.94-1.11)	1.09 (0.96-1.24)	1.07 (1.04-1.11)	1.08 (1.04-1.12)
7q36.3	P_{value}	1.09×10^{-4}	3.38×10^{-2}	5.04×10^{-1}	6.60×10^{-1}	6.13×10^{-1}	1.79×10-[1]	3.98×10^{-5}	3.03×10^{-5}
C/T	P_{value} Het.							2.52×10^{-1}	2.13×10^{-1}
rs2980752	OR (95%CI)	0.90 (0.85-0.95)	0.84 (0.72-0.99)	0.98 (0.91-1.05)	0.98 (0.91-1.06)	1.02 (0.95-1.10)	0.97 (0.85-1.10)	0.95 (0.89-1.01)	0.92 (0.89-0.96)
8p23.1	P_{value}	2.92×10^{-4}	3.38×10^{-2}	5.29×10^{-1}	5.40×10^{-1}	5.33×10^{-1}	6.28×10^{-1}	7.98×10^{-2}	1.38×10^{-4}
C/A	P_{value} Het.							2.55×10^{-2}	1.71×10^{-1}
rs4948550	OR (95%CI)	0.91 (0.87-0.96)	0.85 (0.73-1.00)	0.97 (0.89-1.04)	0.95 (0.88-1.03)	0.99 (0.91-1.07)	0.99 (0.86-1.14)	0.94 (0.91-0.97)	0.92 (0.89-0.96)
10q21.1	P_{value}	2.92×10^{-4}	4.99×10^{-2}	3.64×10^{-1}	2.40×10^{-1}	7.74×10^{-1}	8.84×10^{-1}	3.91×10^{-4}	6.52×10^{-5}
C/T	P_{value} Het.							2.58×10^{-1}	4.47×10^{-1}
*rs35138700**	OR (95%CI)	1.11 (1.04-1.18)	1.18 (1.02-1.37)	1.01 (0.94-1.09)	1.02 (0.93-1.12)	1.03 (0.93-1.14)	0.91 (0.93-1.20)	1.05 (0.97-1.12)	1.05 (0.96-1.16)
12q12	P_{value}	9.32×10^{-5}	2.86×10^{-2}	7.11×10^{-1}	6.40×10^{-1}	5.29×10^{-1}	1.48×10^{-1}	2.19×10^{-1}	2.92×10^{-1}
C/T	P_{value} Het.							1.24×10^{-2}	1.06×10^{-2}
*rs7214227**	OR (95%CI)	0.9 (0.85-0.96)	0.76 (0.63-0.93)	1.03 (0.94-1.14)	1.01 (0.92-1.11)	1.03 (0.93-1.14)	0.92 (0.77-1.10)	0.94 (0.86-1.03)	0.92 (0.88-0.97)
17q23.2	P_{value}	1.14×10^{-3}	6.68×10^{-3}	4.74×10^{-1}	8.60×10^{-1}	5.87×10^{-1}	3.72×10^{-1}	1.93×10^{-1}	8.89×10^{-4}
C/T	P_{value} Het.							1.06×10^{-2}	5.18×10^{-2}

[1] result with all subjects; [2] results excluding non-European subjects.

* SNPs selected as proxy (r^2=1, D'≥ 0.94).

m= minor allele; M= major allele; "P_{value} Het" = P_{value} of heterogeneity. When the studies were heterogeneous, we performed the meta-analysis with the random effect model, while when studies were not heterogenous, we used the fixed-effect model.

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Competing interests:

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Ethics statement: each participating study obtained approval from the responsible institutional review board (IRB) and IRB certification permitting data sharing in accordance with the NIH Policy for sharing of Data Obtained in NIH-Supported or NIH-Conducted Genome Wide Association Studies. The PANDoRA study protocol was approved by the Ethics Commission of the Medical Faculty of the University of Heidelberg. In accordance with the Declaration of Helsinki, written informed consent was obtained from each participant. IRB ethical approval and written informed consent was obtained by all participating centres contributing to PanGenEU Study. The FinnGen study was approved by the ethical Review Board of the Hospital District of Helsinki and Uusimaa. FinnGen participants provided written, informed consent. For JaPAN, written informed consent was obtained from all study participants, and the study protocol was approved by the Ethical Review Board of Aichi Medical University, the Institutional Ethics Committee of Aichi Cancer Center, the Human Genome and Gene Analysis Research Ethics Committee of Nagoya University, and the ethics committees of all participating hospitals.

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Data availability: the PanScan and PanC4 genotyping data are available from the database of Genotypes and Phenotypes (dbGaP, study accession numbers phs000206.v5.p3 and phs000648.v1.p1). JaPAN data are available from the JaPAN consortium website (www.aichi-med-u.ac.jp/JaPAN). FinnGen summary statistics are available from the FinnGen study website (www.finngen.fi). The PANDoRA primary data for this work will be made available to researchers who submit a reasonable request to the corresponding author, conditional to approval by the PANDoRA Steering Committee and Ethics Commission of the Medical Faculty of the University of Heidelberg. Data will be stripped from all information allowing identification of study participants. PanGenEU GWAS summary statistics are available in GWAS catalog repository.

Author contribution: D.C. conceived and designed the study. M.Gi., M.R., performed the lab work, M.Ge., M.R. analysed the data. All the authors contributed with the interpretation of the data. M.Gi., M.Ge., D.C. wrote the first draft of the manuscript and all authors contributed to the writing and approve of the final version of the manuscript.

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