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Genetic and non-genetic risk factors for early-onset pancreatic cancer

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ABSTRACT

Background: Early-onset pancreatic cancer (EOPC) represents 5–10% of all pancreatic ductal adenocarcinoma (PDAC) cases, and the etiology of this form is poorly understood. It is not clear if established PDAC risk factors have the same relevance for younger patients. This study aims to identify genetic and non-genetic risk factors specific to EOPC.
Methods: A genome-wide association study was performed, analysing 912 EOPC cases and 10 222 controls, divided into discovery and replication phases. Furthermore, the associations between a polygenic risk score (PRS), smoking, alcohol consumption, type 2 diabetes and PDAC risk were also assessed.
Results: Six novel SNPs were associated with EOPC risk in the discovery phase, but not in the replication phase. The PRS, smoking, and diabetes affected EOPC risk. The OR comparing current smokers to never-smokers was 2.92 (95% CI 1.69–5.04, $P = 1.44 \times 10^{-4}$). For diabetes, the corresponding OR was 14.95 (95% CI 3.41–65.50, $P = 3.58 \times 10^{-4}$).
Conclusion: In conclusion, we did not identify novel genetic variants associated specifically with EOPC, and we found that established PDAC risk variants do not have a strong age-dependent effect. Furthermore, we add to the evidence pointing to the role of smoking and diabetes in EOPC.
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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) represents the seventh leading cause of cancer death worldwide [1] and it is projected to become the third by 2025 [1,2]. PDAC is a complex disease with a multifactorial etiology for which several epidemiologic risk factors have been identified, including age, type 2 diabetes mellitus, smoking, alcohol consumption and chronic pancreatitis and the presence of non-invasive cyst, such as intraductal papillary mucinous neoplasm (IPMN) [3–9]. Moreover, genetic factors play an important role in the development of PDAC, as highlighted by the results reported by several genome-wide association studies (GWAS) and more focused reports on specific genomic regions [10–23]. PDAC typically affects people in their late adult life, is most frequently diagnosed among people aged 65–74 with a median age of onset of 70 years (accessed on August 25, 2022, https://seer.cancer.gov/statfacts/html/pancreas.html). Subjects diagnosed at younger ages are defined as early-onset (EOPC) if they are diagnosed before 50 years of age. EOPC accounts for 5–10% of PDAC cases [24]. In recent years the incidence in the younger population has been increasing [25]. Even though EOPC represents only a small fraction of PDAC cases, it largely contributes to the societal burden of the disease, with a high number of potential years of life lost (PYLL) [26]. In several European countries around 40% of PYLL due to PDAC has been attributed to EOPC [26].

Studies focused on younger patients are limited and the causes triggering early onset are still largely unknown, although most of the epidemiologic risk factors identified for PDAC seem to play a role also in the earlier onset of disease [24,27,28]. Little is known on the genetic background of EOPC since only two studies were performed to identify germline variants specifically associated with EOPC, suggesting nine risk loci [29,30]. To date, only one GWAS has been conducted to identify genetic variants associated with the risk of developing EOPC, scanning 630 600 genetic variants in 198 EOPC cases and 3227 controls genotyped in the PanScan I and II studies [30]. In addition, a recent study has examined the established modifiable and nonmodifiable PDAC risk factors to estimate their association and attributable risk across different age-groups, showing that both inherited and lifestyle factors were slightly more strongly associated with pancreatic cancer risk at younger ages [28].

Since the factors that influence the development of PDAC in younger patients remain to be determined, this study aimed at identifying novel EOPC-specific SNPs and to validate the known PDAC risk loci in younger patients. In this report the variants were investigated individually and with a polygenic risk score (PRS) in 912 EOPC cases and 10 222 controls, using a standard two-phase approach. Additionally, three non-genetic risk PDAC factors (cigarette smoking, alcohol consumption and diabetes) were also investigated.

2. Materials and methods

2.1. Study design

First, a GWAS on EOPC risk was performed using a two-phase approach (discovery/replication). The discovery phase was con-

Table 1
Description of the study population.

Discovery phase					
Study	EOPC	non EOPC	All cases	All controls	Total
PanScan I-III	272	4585	4857	3418	8275
PanC4	331	3322	3653	3479	7132
Total	603	7907	8510	6897	15 407
Sex					
Male	324	4285	4609	3735	8344
Female	279	3622	3901	3162	7063
Replication phase					
Consortium	EOPC	non EOPC	All cases	All controls	Total
PANDoRA	309	2971	3280	3325	6605
Sex					
Male	181	1593	1774	1810	3584
Female	128	1378	1506	1515	3021

ducted using genotyping data from four GWAS studies on PDAC risk, the Pancreatic Cancer Cohort Consortium (PanScan I, II and III studies, from here on will indicate with the generic abbreviation "PanScan") and the Pancreatic Cancer Case-Control Consortium (PanC4). In the replication phase, genotyping of the most significant SNPs was performed using additional cases and controls from the PANcreatic Disease ReseArch (PANDoRA) consortium [31].

2.2. Data filtering, sample preparation and genotyping

In the discovery phase, the genotyping data of PanScan and PanC4 were downloaded from the database of Genotypes and Phenotypes (dbGaP; study accession number phs000206.v5.p3 and phs000648.v1.p1; project reference no. 12644). For all datasets obtained from dbGaP, genotyping and genotyping quality control procedures and data collection were thoroughly described in the original publications [10–14]. GWAS datasets were imputed using the Michigan imputation Server (https://imputationserver.sph.umich.edu) and the Haplotype Reference Consortium (HRC, V.r1.1) as reference panel. Before the imputation process, individuals with sex mismatches, call rate <0.98, minimal or excessive heterozygosity (>3 standard deviations for the mean) or cryptic relatedness (PI_HAT >0.2) were removed from the datasets. Variants with minor allele frequency (MAF) <0.01 or evidence of violation of Hardy-Weinberg equilibrium ($P < 10^{-6}$) were excluded. All datasets were imputed separately and then merged. After imputation, variants with MAF <0.01 and low-quality imputation score (INFO score <0.7) were removed. The final data set consisted of 6 993 629 SNPs and 603 EOPC cases and 6897 controls.

The replication phase consisted of an independent set of 309 EOPC cases and 3325 controls from the PANDoRA consortium [31]. The PANDoRA consortium is constituted by several research groups across 11 European countries (Greece, Italy, Germany, the Netherlands, Denmark, Czech Republic, Hungary, Poland, Ukraine, Lithuania, United Kingdom), Japan and Brazil. Cases were defined by a confirmed diagnosis of pancreatic adenocarcinoma, whereas the controls were individuals from the general population without a pancreatic disease at recruitment, individuals who were hospitalised for non-tumor related causes, or blood donors. For each subject data on age (diagnosis for the cases/recruitment for controls) and sex were retrospectively acquired. For a subset of subjects from PANDoRA data on smoking habits, alcohol consumption and diagnosis of type 2 diabetes were also collected. Table 1 summarises the subjects used for this study.

DNA of PANDoRA samples was extracted from circulating blood using the Qiagen mini kit (Qiagen, Hilden, Germany), transferred in 384-well plates and dried. The genotyping was carried out with TaqMan™ and KASP™ technologies. Each plate included No Template Controls (NTCs) and 8% of duplicated samples for quality control purposes. After the PCR reaction, the fluorescence emission of each sample was measured to determine genotypes using the QuantStudio™ 5 Real-Time PCR system (Thermofisher Applied Biosystems, USA) and QuantStudio software.

2.3. Polygenic risk score: SNPs selection and score computation

In addition, previously reported PDAC risk loci were also analysed in EOPC patients individually and combined in a polygenic risk score (PRS). To build the PRS, 28 SNPs previously identified by PDAC GWAS, with a genome-wide significance level ($P < 5 \times 10^{-8}$) of association or close to that threshold ($P < 10^{-7}$), were combined. To infer the ABO blood group, two SNPs were genotyped (rs505922 and rs8176746). In detail, rs505922 discriminates O from non-O and rs8176746 distinguishes between ABO A and B alleles [32–34]. The final selection consisted of 30 SNPs, as described in supplementary table 1. More detail on the procedure to calculate the PDAC risk PRS is reported by Galeotti et al. [35].

A weighted score was generated by summing the number of risk alleles each multiplied by the effect size ($\beta = \ln(OR)$) of each allele and ABO blood group, using the β reported in the literature. The PRS was computed separately for the subjects of PanScan-PanC4 and PANDoRA, including only the subjects with a call rate of 100%. To reduce the effect of distribution outliers, the scores were divided in quintiles based on their distribution in the control population.

2.4. Non-genetic risk factors analysis

Finally, as an exploratory analysis, the association of cigarette smoking and alcohol consumption with the risk of developing PDAC was tested, expressed as never, current, and ever smoker or drinker, respectively. In addition, the association between type 2 diabetes and risk of developing PDAC was tested by dividing patients into subjects who had been diagnosed with diabetes more than two years before PDAC diagnosis and those who had been diagnosed within two years before PDAC diagnosis. All the analyses were performed on a subset of subjects from PANDoRA, for which these data were available, considering two different age cut-offs: subjects younger than 50, and older than 50 years. The analysis with all cases and controls without age division was also performed.

2.5. Statistical analyses

In the discovery phase, the association of 6 993, 29 SNPs with EOPC risk was analysed in the aggregated PanScan and PanC4 dataset, using an unconditional logistic regression (using controls of all ages) carried out with PLINK 2.0 (www.cog-genomics.org/plink/2.0/). SNPs that showed a *p*-value of association lower than an arbitrary threshold of 1×10^{-4} in the discovery phase and that were independent ($r^2 < 0.7$ in the European population) from any known PDAC locus and/or with each other, were selected for the replication phase performed using the PANDoRA subjects. In PANDoRA, in addition to the analysis using EOPC cases vs all controls, the SNPs identified in the discovery phase were tested also considering EOPC cases vs non-EOPC cases. Finally, a meta-analysis considering PanScan, PanC4 and PANDoRA was carried out, with a final sample size of 912 EOPC cases and 10 222 controls. A fixed or random effect was used depending on the heterogeneity observed for each variant. The meta-analysis was carried out using "rmeta" package for R (https://cran.r-project.org/web/packages/rmeta).

The association between the 28 known PDAC risk SNPs was assessed, considering different age thresholds for the onset of the disease (≤50 years, and >50 years) in PanScan-PanC4 (including

Table 2
Case-control and case-case analyses in all phases and meta-analysis.

SNP (M/m)[a]	Study	EOPC vs all controls						non-EOPC vs all controls					EOPC vs non-EOPC cases				
		Cases/Controls			Allelic Model m vs M			Cases/Controls			Allelic Model m vs M		Cases/Cases			Allelic Model m vs M	
		MM	Mm	mm	OR (95% CI)	*p*-value	P_{het}	MM	Mm	mm	OR (95% CI)	*p*-value	MM	Mm	mm	OR (95% CI)	*p*-value
rs1381553 (T/A)	PanScan+ PanC4	470/5822	113/963	13/43	1.60 (1.32–1.92)	**9.88×10^{-7}**	-	6646/5822	1124/963	43/43	1.01 (0.93–1.10)	0.804	470/6646	113/1124	13/43	1.58 (1.31–1.91)	**2.42×10^{-6}**
	PANDoRA	270/2846	38/461	1/18	0.94 (0.67–1.34)	0.75	-	2517/2846	436/461	18/18	1.08 (0.94–1.24)	0.255	270/2517	38/436	1/18	0.87 (0.62–1.23)	0.44
	Meta-analysis	-	-	-	1.25 (0.75–2.10)	0.397	**0.008**	-	-	-	-	-	-	-	-	-	-
rs3984967 (T/A)	PanScan+ PanC4	362/4629	207/2045	34/212	1.38 (1.20–1.59)	**9.88×10^{-6}**	-	5253/4629	2374/2045	267/212	1.03 (0.97–1.09)	0.410	362/5253	207/2374	34/267	1.33 (1.15–1.53)	**9.30×10^{-5}**
	PANDoRA	201/2225	97/976	11/124	1.04 (0.83–1.29)	0.751	-	2050/2225	825/976	96/124	0.91 (0.83–1.00)	0.059	201/2050	97/825	11/96	1.11 (0.89–1.38)	0.344
	Meta-analysis	-	-	-	1.21 (0.92–1.60)	0.169	**0.035**	-	-	-	-	-	-	-	-	-	-
rs11257929 (T/G)	PanScan+ PanC4	482/5945	115/908	6/29	1.57 (1.29–1.91)	**9.28×10^{-6}**	-	6757/5945	1094/908	41/29	1.06 (0.96–1.15)	0.239	482/6757	115/1094	6/41	1.48 (1.22–1.81)	**8.94×10^{-5}**
	PANDoRA	266/2865	43/437	0/2	0.94 (0.67–1.32)	0.712	-	2534/2865	411/437	26/23	1.06 (0.92–1.21)	0.425	266/2534	43,411	0/26	0.89 (0.64–1.24)	0.497
	Meta-analysis	-	-	-	1.24 (0.75–2.04)	0.404	**0.011**	-	-	-	-	-	-	-	-	-	-
rs12671911 (C/T)	PanScan+ PanC4	361/4689	202/1862	31/217	1.40 (1.21–1.62)	**4.38×10^{-6}**	-	5355/4689	2157/1862	202/217	0.99 (0.93–1.05)	0.725	361/5355	202/2157	31/202	1.44 (1.25–1.67)	**1.18×10^{-6}**
	PANDoRA	225/2409	81/827	3/89	0.95 (0.74–1.22)	0.668	-	2129/2409	761/827	81/89	1.04 (0.94–1.15)	0.454	225/2129	81/761	3/81	0.9 (0.71–1.15)	0.406
	Meta-analysis	-	-	-	1.17 (0.80–1.71)	0.418	**0.009**	-	-	-	-	-	-	-	-	-	-
rs72718310 (T/A)	PanScan+ PanC4	547/6511	45/238	0/1	2.23 (1.60–3.10)	**2.22×10^{-6}**	-	7437/6511	287/238	2/1	1.06 (0.89–1.26)	0.517	547/7437	45/287	0/2	2.02 (1.46–2.80)	**2.48×10^{-5}**
	PANDoRA	296/3186	13/139	0/0	1.07 (0.57–2.00)	0.833	-	2833/3186	132/139	6/0	1.18 (0.92–1.50)	0.186	296/2833	13/132	0/6	0.75 (0.41–1.36)	0.345
	Meta-analysis	-	-	-	1.62 (0.80–3.31)	0.182	**0.043**	-	-	-	-	-	-	-	-	-	-
rs111703463 (G/A)	PanScan+ PanC4	541/6468	55/344	1/4	1.93 (1.44–2.58)	**8.96×10^{-6}**	-	7401/6468	404/344	8/4	1.02 (0.88–1.17)	0.821	541/7401	55/404	1/8	1.86 (1.40–2.47)	**2.38×10^{-5}**
	PANDoRA	287/3124	21/200	1/1	1.11 (0.68–1.81)	0.668	-	2768/3124	201/200	2/1	1.09 (0.89–1.34)	0.397	287/2768	21/201	1/2	1.17 (0.74–1.85)	0.494
	Meta-analysis	-	-	-	1.52 (0.89–2.60)	0.128	0.057	-	-	-	-	-	-	-	-	-	-

Statistically significant results (*p*-value <0.05) are in bold
[a] M/m: major and minor allele; OR(CI): odds ratio (confidence interval); P_{het}: *p*-value of heterogeneity test. All analyses were adjusted for sex and the eight principal components (PanScan + PanC4) or sex and country of origin (PANDoRA).

Table 3
Association between PRS and PDAC risk.

Cases vs controls ≤50 years								
	PanScan I-III + PanC4				PANDoRA			
Quintile	Cas	Con	OR (95% CI)	*p*-value	Cas	Con	OR (95% CI)	*p*-value
1st	29	79	1.00 (reference)	-	10	127	1.00 (reference)	-
2nd vs 1st	39	73	1.41 (0.79–2.53)	0.245	3	129	0.26 (0.05–1.26)	0.095
3rd vs 1st	87	73	3.30 (1.94–5.62)	1.42×10^{-5}	14	125	1.71 (0.67–4.38)	0.262
4th vs 1st	109	75	4.04 (2.39–6.80)	2.56×10^{-7}	20	122	2.35 (0.95–5.79)	0.063
5th vs 1st	135	75	4.93 (2.94–8.26)	3.01×10^{-9}	23	125	2.90 (1.17–7.17)	2.14×10^{-2}
Total	399	375			70	628		
Cases vs controls >50 years								
	PanScan I-III + PanC4				PANDoRA			
Quintile	Cas	Con	OR (95% CI)	*p*-value	Cas	Con	OR (95% CI)	*p*-value
1st	501	685	1.00 (reference)	-	103	280	1.00 (reference)	-
2nd vs 1st	759	703	1.48 (1.27–1.73)	9.77×10^{-7}	147	274	1.38 (0.97–0.95)	0.071
3rd vs 1st	945	659	1.96 (1.69–2.29)	6.15×10^{-17}	174	275	1.76 (1.26–2.48)	1.03×10^{-3}
4th vs 1st	1168	691	2.28 (1.96–2.65)	3.33×10^{-25}	189	271	2.02 (1.44–2.82)	4.12×10^{-5}
5th vs 1st	1632	670	3.29 (2.84–3.81)	2.37×10^{-51}	252	270	2.44 (1.76–3.38)	9.16×10^{-8}
Total	5005	3408			865	1370		

Cas: number of cases; Con: number of controls. The quintiles were defined using the distribution of the controls. All analyses were adjusted for sex and the eight principal components (PanScan + PanC4) or sex and country of origin (PANDoRA).

8510 PDAC cases and 6897 controls) and in PANDoRA (3280 PDAC cases and 3325 controls). Stratified analyses that categorized cases and controls by age (≤50 years, and >50 years) were used to assess the associations of the PRS and the non-genetic risk factors with the risk of developing PDAC. For the non-genetic risk factors (cigarette smoking, alcohol consumption, and diabetes) the same age thresholds were used, but their effect was analysed only in PANDoRA, since the datasets downloaded from dbGaP lack this information. For all the genetic analyses, the more common allele among the controls was assigned as the reference category and the allelic model was used. All the genetic analyses in PanScan-PanC4 were performed using unconditional logistic regression adjusted for sex and the top eight principal components, computed from the GWAS data. Since PANDoRA lacks GWAS data, therefore no principal components are available, PANDoRA analyses were adjusted for sex and country of origin.

3. Results

3.1. Early-onset PDAC risk

The results of the case-control analysis conducted in PanScan-PanC4 showed six SNPs (*SLC14A2*-rs1381553, *SEMA6A*-rs3984967, *CAMK1D*-rs11257929, *CNTNAP2*-rs12671911, 1p22.2-rs72718310 and 18q12.3-rs111703463) that were associated with EOPC risk (*p*-value $<10^{-4}$). These variants were genotyped in PANDoRA using 309 EOPC cases and 3325 controls, but no statistically significant associations were observed. The analysis performed in PANDoRA including EOPC vs non-EOPC cases also did not show any statistically significant associations. The results of all the analyses, including a meta-analysis between PanScan-PanC4 and PANDoRA are shown in Table 2. We observed heterogeneity in all the metanalyses conducted and therefore a random model was also applied, with the all the results showing non statistically significant associations. The forest plots showing the results of the meta-analyses are reported in Fig. 1.

The analyses performed to validate the association with the 28 established PDAC risk SNPs showed that 19 of them replicated the association (P <0.05) in EOPC, in at least one among the discovery and replication datasets, as reported in supplementary table 1.

3.2. Polygenic risk score

The association analyses between the PRS and PDAC risk stratified by age showed a higher risk of developing PDAC for subjects in the fifth quintile of the score compared with subjects in the first quintile, with similar results in all age groups, both in PanScan-PanC4 and PANDoRA. Therefore, no difference was observed in the cumulative effect of the known PDAC-susceptibility SNPs between young and older subjects. The results of PRS analyses are reported in Table 3.

3.3. Association between non-genetic risk factors and EOPC

The association of cigarette smoking, alcohol consumption and diabetes with PDAC risk was tested across two age categories in PANDoRA. The results showed that being a current smoker is associated with increased risk of developing PDAC across both age categories. We observed that for EOPC, the risk increased nearly threefold (OR=2.92, 95% CI 1.69–5.04, $P = 1.44 \times 10^{-4}$), while for non EOPC the risk is lower (OR=1.51, 95% CI 1.12–2.03, $P = 0.006$), when comparing current smokers to never smokers. The risk calculated analysing all study subjects was (OR=1.81, 95% CI 1.40–2.34, $P = 7.97 \times 10^{-6}$). Diabetes was also associated with PDAC

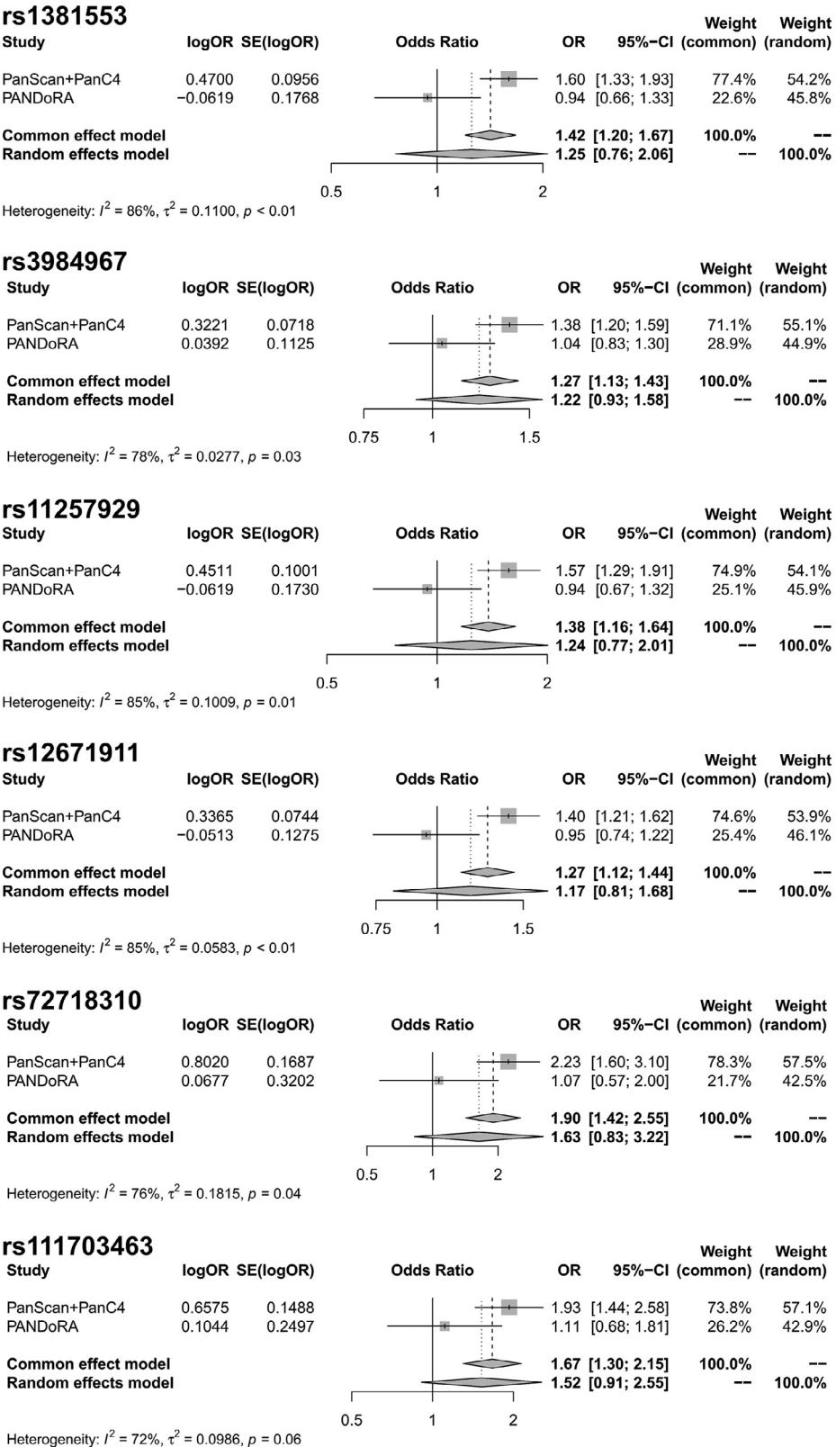

Fig. 1. Forest plots of the meta-analyses between PanScan-PanC4 and PANDoRA results.

risk in all age categories, showing a very strong effect for EOPC (OR=14.95, 95% CI 3.41–65.50, $P = 3.58 \times 10^{-4}$). We also observed in all the age groups a very strong effect of diabetes diagnosed within two years of PDAC diagnosis, with OR values between 11 (in subjects older than 50) to 16 (in EOPC compared with all the controls). Instead, this effect is much less evident if not entirely absent in some age groups when considering the subjects with diabetes diagnosed at least two years before PDAC diagnosis. The results of the diabetes analyses are reported in Table 4. Conversely, alcohol consumption was not associated with increased PDAC risk in

Table 4
Association between non-genetic risk factors and PDAC risk.

	Subjects younger than (≤)50 years			Subjects older than 50 years			EOPC vs all controls			All study subjects		
Cigarette smoking	Cas/Con	OR (95% CI)	*p*-value	Cas/Con	OR (95% CI)	*p*-value	Cas/Con	OR (95% CI)	*p*-value	Cas/Con	OR (95% CI)	*p*-value
Non-smokers (never)	81/113	*Ref*		881/250	*Ref*		81/363	Ref		962/363	*Ref*	
Smokers	78/52	2.08 (1.31–3.32)	0.002	815/185	1.23 (0.99–1.54)	0.065	78/237	2.05 (1.32–3.19)	1.52×10^{-3}	893/237	1.40 (1.15–1.70)	9.77×10^{-4}
Current smokers	58/27	2.92 (1.69–5.04)	1.44×10^{-4}	395/77	1.51 (1.12–2.03)	0.006	58/104	2.59 (1.58–4.23)	1.68×10^{-4}	453/104	1.81 (1.40–2.34)	7.97×10^{-6}
Former smokers	20/25	1.17 (0.59–2.30)	0.666	420/108	1.00 (0.77–1.31)	0.986	20/363	1.20 (0.64–2.25)	0.564	440/133	1.07 (0.84–1.37)	0.574
Alcohol drinking	**Cas/Con**	**OR (95% CI)**	*p*-value	**Cas/Con**	**OR (95% CI)**	*p*-value				**Cas/Con**	**OR (95% CI)**	*p*-value
Non-drinker	60/52	*Ref*		844/167	*Ref*		60/219	*Ref*		904/219	*Ref*	
Drinker	63/38	1.10 (0.61–1.98)	0.762	772/165	0.82 (0.64–1.05)	1.120	63/203	0.94 (0.55–1.61)	0.833	835/203	0.87 (0.69–1.1)	1.31
Current drinker	59/38	1.05 (0.58–1.89)	0.882	704/165	0.75 (0.58–0.96)	0.611	59/203	0.89 (0.52–1.53)	0.679	763/203	0.80 (0.63–1.01)	0.87
Diabetes	**Cas/Con**	**OR (95% CI)**	*p*-value	**Cas/Con**	**OR (95% CI)**	*p*-value				**Cas/Con**	**OR (95% CI)**	*p*-value
Not diabetic	85/112			863/327			85/439	*Ref*		948/439		
Diabetics	24/2	14.95 (3.41–65.50)	3.58×10^{-4}	526/70	2.77 (2.09–3.67)	5.49×10^{-12}	24/72	4.72 (2.29–9.74)	3.20×10^{-5}	550/72	3.13 (2.38–4.13)	2.90×10^{-15}
Diabetes diagnosed a least 2 years before PDAC diagnosis	2/1	1.82 (0.16–21.09)	0.633	123/30	1.53 (1.00–2.33)	0.049	2/31	0.98 (0.21–4.62)	0.98	125/31	1.60 (1.06–2.43)	0.03
Diagnosis of diabetes within 2 years of PDAC diagnosis	7/0	-	-	91/3	11.46 (3.59–36.51)	4.29×10^{-5}	7/3	16.64 (2.93–94.36)	1.52×10^{-3}	98/3	13.99 (4.39–44.51)	9.96×10^{-6}

OR (95% CI): odds ratio (95% confidence interval); **Cas/Con**: Cases/Controls. All analyses were adjusted for sex, and age.

EOPC and non-EOPC. When subjects (cases and controls) of all ages were considered, the results showed that both smoking and diabetes were associated with an increased risk of developing PDAC, while for alcohol consumption, no statistically significant associations were observed. The results of all the analyses are reported in Table 4.

4. Discussion

PDAC is rare before 50 years and the causes triggering the early disease are still unknown and currently only few SNPs have been specifically associated with EOPC [29,30].

In this study, we aimed to identify novel specific SNPs for EOPC performing a genome-wide analysis. Using the data from PanScan and PanC4, we identified six novel variants statistically associated with the risk of developing EOPC ($P < 10^{-4}$), but none of these replicated in PANDoRA. Additionally, the results of the metanalysis show statistically significant heterogeneity values for all SNPs except for rs111703463. The heterogeneity is explained by the fact that the results of the discovery and validation phases go in different directions (i.e., the risk allele is inverted). This discrepancy could be explained by the low frequencies of the SNPs (MAF <10%) which probably increased the chance finding observed in the discovery phase, and highlights the importance of replication in epidemiologic studies, to avoid reporting false positives. This study represents the largest GWAS on EOPC risk performed so far, with more than 900 EOPC cases analysed in total. We observed that the majority (19 out of 28) of the known PDAC risk loci replicated their associations considering younger ages of onset, both in PanScan-PanC4 and PANDoRA.

The subjects in the highest quintile of the PRS generated using the currently known PDAC risk loci had an increased risk of developing PDAC when compared with the group of subjects in the lowest quintile, with very similar results in regards of the direction of effects and the level of statistical significance, independently from the age of onset of the disease. A recent study, conducted in the context of PanScan-PanC4, examining the association of a PRS consisting of 22 SNPs, showed a slightly increased risk for EOPC (OR=6.91, 95% CI 4.60–10.40) compared to late-onset PDAC (OR=4.12, 95% CI 3.08–5.52) [28]. These ORs were observed when subjects in the top vs bottom 10% of the allelic distributions were compared, substantially confirming what we observed. These results taken together suggest that there is not a strong correlation between the known genetic loci and the age of onset of PDAC. Studies on prostate and breast cancer have found similar results, showing that several previously established risk loci are also associated with the risk in younger cases [36–39]. Therefore, the only possible way to better understand the specific genetic factors for EOPC will be to conduct larger studies, to increase the statistical power of the study and improve the chances of detecting even the associations with a minor effect that in current studies cannot be observed, although this is extremely difficult considering the rarity of PDAC at earlier stages.

We also examined the association of non-genetic risk factors with PDAC risk across different ages and observed that smoking and diabetes increase the risk of developing PDAC across all age categories analysed in our study. Smoking is one of the strongest PDAC risk factors and it increases the risk at all ages, although we observed a weak tendency of smoking having a stronger effect in EOPC (OR=2.92, 95% CI 1.69–5.04) compared to non-EOPC individuals (OR=1.51, 95% CI 1.12–2.03). These results are in agreement with the literature, suggesting the role of smoking as PDAC risk factors with a weak age-dependent effect [28,40].

To date, the association between diabetes and age of onset of PDAC has been poorly investigated, with a limited number of studies on the topic. We observed a substantial increase in

risk for EOPC patients, but only when diabetes was diagnosed within two years of PDAC diagnosis, probably reflecting type 3c diabetes.

In contrast to several studies that reported that alcohol was associated with an increased risk for younger PDAC onset and proposed a dose-dependent effect [41–43] we did not observe an association between alcohol and EOPC risk. However, in PANDoRA we do not have data on the dose of alcohol consumed and only recorded the data as a dichotomous variable, clearly limiting the possible generalization of our findings. The analyses conducted on non-genetic risk factors provide some valuable indications for setting up future studies. However, the results should not be considered conclusive given the limited number of subjects for whom these data were available for patients under 50 years. The low number of EOPC cases with data on non-genetic risk factors is due to the low incidence of PDAC in young subjects and partly to the nature of retrospective multicentric studies (e.g., PANDoRA) where it is difficult to collect and harmonize environmental and lifestyle variables. As an example, in PANDoRA the variable alcohol consumption has been recorded differently across the centers that comprise the consortium and therefore the only possibility in the aggregated data was to code it as a dichotomous variable (i.e., drinkers vs non-drinker) limiting the power to detect associations. However, the effect that we observed, even though in a limited number of subjects is in line with what has been already reported in the literature for smoking and diabetes. An additional limitation is also represented by the fact that we could not test other risk factors for PDAC such as BMI and pancreatitis since these data were not available in PANDoRA." In conclusion, we did not identify novel specific genetic variants associated with EOPC, we substantially validated the associations reported for non-EOPC disease either analysing them individually or in a PRS, suggesting that these variants do not have a strong age dependent effect. Furthermore, we add to the evidence pointing to the role of cigarette smoking and diabetes in EOPC.

Conflict of interest

M.F.B. has received research funding from Celgene and Lead Pharma and acted as a consultant to Servier. **H.W.M.L.** reports research funding and/or medication supply from Bristol-Myers Squibb, Bayer Schering Pharma, Celgene, Janssen-Cilag, Lilly, Nordic Pharma, Philips Healthcare, Roche, Merck Sharp and Dohme, Servier, Incyte; and consultant/advisory board member for Lilly, Nordic Pharma, Bristol-Myers Squibb, Dragonfly, Merck Sharp and Dohme, Servier, all outside the submitted work. The other authors do not have any conflict of interest to declare.

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Author contributions

D.C. conceived and designed the study. **Y.N.** performed the lab work. **Y.N. an M.Ge.** performed data curation and analysis. **M.Ge.** drafted the manuscript. **D.C., M.Ge., Y.N.** reviewed and edited the manuscript. All other authors provided samples and data.

Ethics approval and consent to participate

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.

Consent for publication

Not applicable.

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). 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, Germany. Data will be stripped from all information allowing identification of study participants.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.02.023.

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