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Genome-wide association study identifies an early onset pancreatic cancer risk locus

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Abbreviations: EOPC, early onset pancreatic cancer; eQTLs, expression quantitative trait loci; GWAS, genome-wide association study; LD, linkage disequilibrium; MAF, minor allele frequency; NEOPC, Non-EOPC; OR, odds ratio; P, *P*-value or probability value; PanC4, Pancreatic Cancer Case-Control Consortium; PANDoRA, PANcreatic Disease ReseArch; PDAC, pancreatic ductal adenocarcinoma; VEOPC, very early onset pancreatic cancer.

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Abstract

Early onset pancreatic cancer (EOPC) is a rare disease with a very high mortality rate. Almost nothing is known on the genetic susceptibility of EOPC, therefore, we performed a genome-wide association study (GWAS) to identify novel genetic variants specific for patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) at younger ages. In the first phase, conducted on 821 cases with age of onset ≤60 years, of whom 198 with age of onset ≤50, and 3227 controls from PanScan I-II, we observed four SNPs (rs7155613, rs2328991, rs4891017 and rs12610094) showing an association with EOPC risk ($P < 1 \times 10^{-4}$). We replicated these SNPs in the PANcreatic Disease ReseArch (PANDoRA) consortium and used additional in silico data from PanScan III and PanC4. Among these four variants rs2328991 was significant in an independent set of 855 cases with age of onset ≤60 years, of whom 265 with age of onset ≤50, and 4142 controls from the PANDoRA consortium while in the in silico data, we observed no statistically significant association. However, the resulting meta-analysis supported the association ($P = 1.15 \times 10^{-4}$). In conclusion, we propose a novel variant rs2328991 to be involved in EOPC risk. Even though it was not possible to find a mechanistic link between the variant and the function, the association is supported by a solid statistical significance obtained in the largest study on EOPC genetics present so far in the literature.

KEYWORDS

early onset, genome-wide association study, pancreatic cancer, single nucleotide polymorphisms, very early onset pancreatic cancer

1 | INTRODUCTION

Pancreatic cancer is the fifth most common cause of cancer death worldwide[1] and it is projected to become the second by 2030.[2] Several epidemiologic factors show a strong effect on pancreatic cancer susceptibility including smoking, alcohol consumption and obesity.[3] In the last 10 years, almost 30 sporadic pancreatic cancer risk loci have been identified through genome wide association studies (GWAS) and large scale candidate gene studies.[4-15]

The median age of onset of pancreatic cancer is 71 years and around 20% of subjects develop it before 60 years of age, defined as early onset pancreatic cancer (EOPC).[16-19] Only around 3% of cases are diagnosed before 45 years of age (very early onset pancreatic cancer, VEOPC).[17] EOPC and VEOPC share the majority of epidemiologic risk factors with non-EOPC (NEOPC), with smoking being the strongest for both ages of onset.[16,17] Not much is known on the genetic background of EOPC, however in a recent report Ben-Aharon and colleagues comparing the somatic mutation landscape of NEOPC and EOPC, found several differences in the pathways involved.[20] In addition, only one study has been performed to identify germline single nucleotide polymorphisms (SNPs) specifically associated with EOPC.[21] In that manuscript, Chen et al identified eight SNPs associated with an earlier diagnosis. A better understanding of the genetic background could be extremely helpful in identifying molecular pathways that could lead to early carcinogenesis and therefore expand our understanding on the disease. With these premises, we performed a GWAS on EOPC with the aim of identifying novel variants specific for younger pancreatic ductal adenocarcinoma (PDAC) patients.

What's new?

Early-onset pancreatic cancer (EOPC), diagnosed between ages 45 and 60, accounts for about one-fifth of all pancreatic cancers. Nonetheless, while multiple epidemiological risk factors for EOPC have been identified, very little is known about genetic susceptibility. The present genome-wide association study identifies four novel single nucleotide polymorphisms specific for patients diagnosed with pancreatic cancer at younger ages. Among the variants, 13q22.3_rs2328991 was associated with elevated risk in an independent set of pancreatic cancer patients, some of whom experienced disease onset at age 50 or younger. The findings highlight a need for further research on the genetics of EOPC.

2 | MATERIALS AND METHODS

2.1 | Populations used in the study

We used a two-phase approach: in the discovery phase, we have used the Pancreatic Cancer Cohort Consortium (PanScan) study that has been fully described elsewhere.[4,8] Briefly, case and control data and DNA samples were collected from 12 cohort studies and 8 case-control studies. Cases were defined as those individuals having primary adenocarcinoma of the exocrine pancreas. Controls were frequency matched to cases and were free of pancreatic cancer at the time of enrolment. Matching criteria varied according to the studies within PanScan I-II. We analyzed 3133 PDAC patients among whom there were 821 cases with the age of onset ≤60 years and 198 with the age of onset ≤50, and 3227 controls. For replication, we used three populations: phase three of the PanScan (PanScan III) study,[10] the Pancreatic Cancer Case-Control Consortium (PanC4)[12] and the PANcreatic Disease ReseArch (PANDoRA) consortium.[22] For PanScan III and PanC4, the replication was done "in silico" using data from PANDoRA includes studies from nine European countries in which cases were defined by an established diagnosis of PDAC and controls were individuals from the general population without a pancreatic disease at recruitment, individuals who were hospitalized for nontumor related causes, or blood donors. Table 1 summarizes the subjects used for our study. We validated the results with a total of 8096 cases (695 younger than 50 and 2385 younger than 60) and 7741 controls.

2.2 | Data filtering, sample preparation and genotyping

For the first phase (PanScan I-II), we downloaded the genotyping data from the database of Genotypes and Phenotypes (dbGaP, study accession number phs000206.v5.p3, project reference #12644). We used 60 and 50 years of age as thresholds to define groups of EOPC cases. Ages are coded in 10-year intervals in data downloaded from dbGaP (eg, 40-50, 50-60, etc.), therefore, we were unable to analyze only cases younger than 45, which correspond to the exact definition of VEOPC. The validation in PanScan III and PanC4 was done in silico using data from dbGaP. For all datasets obtained from dbGaP, genotyping procedures, genotyping quality control checks, data collection were thoroughly reported in the original publications.[4,6,8,10] We removed individuals with gender mismatches, call rate <0.9, minimal or excessive heterozygosity (>3 SD from the mean) or cryptic relatedness (PI_HAT>0.2). We discarded SNPs with a minor allele frequency <0.5%, completion rate <90%, evidence for violations of Hardy-Weinberg Equilibrium ($P < 10^{-6}$). Principal component analysis was carried out including genotypes from all the populations of the phase 3 of the 1000 Genomes Project (http://www.internationalgenome.org/). Individuals not clustering with the 1000 Genomes subjects of European descent were excluded from further analysis.

We performed de novo genotyping for PANDoRA. DNAs were extracted from blood samples using the Qiagen mini kit (Qiagen, Hilden, Germany) at the German Cancer Research Center (DKFZ) in Heidelberg, according to the manufacturer's protocol. DNA concentration was measured using spectrophotometer and samples were

TABLE 1 Description of the study population

Study phase	Country/study	Cases	Controls	Total
Phase 1 (PanScan)	PanScan I	2040	2048	4088
	PanScan II	1093	1179	2272
	Total	3133	3227	6360
	Sex			
	Male	52.9%	53.2%	53.0%
	Female	47.1%	46.8%	47.0%
	Age			
	Median, years	65	65	65
	≤50, N	198	217	415
	≤60, N	821	800	1621
Phase 2 (PANDoRA)	Czech Republic	347	431	778
	Germany	839	1708	2547
	Greece	114	16	130
	Hungary	259	332	591
	Italy	792	1282	2074
	Lithuania	57	189	246
	Netherlands	117	0	117
	Poland	99	184	283
	United Kingdom	87	0	87
	Total	2711	4142	6853
	Sex			
	Male	56%	54%	55%
	Female	44%	46%	45%
	Age			
	Median, years	64	56	59
	≤50, N	265	1256	1521
	≤60, N	855	2505	3360
Phase 2 (PanScan PanC4)	*PanScan III*	1522	—	1522
	Sex			
	Male	49%	—	49%
	Female	51%	—	51%
	Age			
	Median, years	69	—	69
	≤50, N	76	—	76
	≤60, N	354	—	354
	PanC4	3863	3599	7462
	Sex			
	Male	58%	56%	57%
	Female	42%	44%	43%
	Age			
	Median, years	65	64	65
	≤50, N	354	375	729
	≤60, N	1176	1277	1176

kept frozen till genotyping. Genotyping was performed using TaqMan technology assays as recommended by the manufacturer in 384 well plates using 10% of samples as duplicates for quality control purposes. We observed no deviation from Hardy-Weinberg equilibrium in the controls, an average call rate >99% and a concordance with the duplicated samples of 99.2%.

2.3 | Statistical analysis

In phase one, we performed a GWAS on EOPC risk, analyzing 630 600 SNPs with unconditional logistic regression, adjusted for study, sex, two main principal components and study. We performed four analyses: (a) considering individuals ≤50 years when diagnosed with PDAC vs all controls, (b) individuals ≤60 years when diagnosed with PDAC vs all controls, (c) a case-case analysis considering cases ≤50 vs older cases and (d) cases ≤60 vs older cases. For all analyses, we used an allelic, a dominant and a recessive inheritance model, using the more common allele in the controls as reference. All non-Caucasian individuals were excluded from the analyses.

We replicated in the second phase all SNPs that reached a *P*-value of at least 1×10^{-4} in at least one of the analyses done. Data from PanScan III was used for replication of the case-case analysis (PanScan III consists of only cases), while data from PanC4 and PANDoRA was used for all the analyses. Hardy-Weinberg equilibrium was checked for the SNPs that were genotyped in phase two of the study using Pearson exact chi square. In the second phase, analyses were also performed with unconditional logistic regression adjusting for sex and the two principal components (PanScan III and PanC4) or sex and country of origin (PANDoRA).

We performed two meta-analyses calculating heterogeneity between the studies (one for the case-control and one for the case-case analyses) using both fixed effect model and random effect model (depending on the heterogeneity) between phase one and phase two with a final sample size of 3206 EOPC, 893 cases with an onset before 50 years, 11 229 total PDAC cases and 10 908 controls.

In addition, to compare our data with the paper by Chen et al, we also checked the PanScan data for the SNP significant in their analysis. For none of the 8 SNPs reported by Chen et al, there were genotyping data in PanScan. However, for three of them (chr20_rs2766669, chr11_rs12803915, chr6_rs1559849), we found a SNP in LD ($r^2 > 0.80$) that we used as surrogate in the analysis.

2.4 | Bioinformatic analysis

To understand the functional relevance of the SNPs significant after the second phase, we used RegulomeDB (http://regulome.stanford.edu/)[23] and HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php)[24] to identify the regulatory potential of the region nearby the SNPs. The Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org/)[25] was used to identify potential eQTLs. Finally, we used SNP Nexus (https://snp-nexus.org)[26] as a database for functional annotation of SNPs.

TABLE 2 Case-control analysis in all study phases and meta-analysis

	MM	Mm	mm	m vs M			Mm + mm vs MM			mm vs MM + Mm		
SNP (M/m)	Cases/controls			OR (95% CI)	P_{all}	$P_{het.}$	OR (95% CI)	P_{dom}	*P* dom.	OR (95% CI)	P_{rec}	$P_{het.}$
rs7155613 (A/C)												
PanScan I & II ≤ 50	41/888	95/1587	62/754	1.34 (1.09-1.65)	**4.31E−03**		1.46 (1.02-2.08)	**.035**		1.51 (1.11-2.07)	**8.55E−03**	
PanC4 ≤ 50	106/966	155/1733	86/802	0.96 (0.82-1.12)	.580		0.84 (0.66-1.07)	.167		1.07 (0.83-1.39)	.595	
PANDoRA ≤ 50	68/1109	113/1954	42/1036	0.87 (0.71-1.06)	.153		0.91 (0.66-1.24)	.537		0.73 (0.51-1.04)	.083	
Meta-analysis				1.03 (0.82-1.31)[a]	.781	**8.00E−03**	1.02 (0.74-1.4)[a]	.917	**.037**	1.07 (0.73-1.55)[a]	.742	**.011**
PanScan I & II ≤ 60	178/888	395/1587	248/745	1.29 (1.15-1.43)	**4.83E−06**		1.36 (1.13-1.64)	**6.**21E−04		1.44 (1.22-1.71)	**2.66E−05**	
PanC4 ≤ 60	308/966	550/1733	292/802	1.05 (0.95-1.15)	.341		1.02 (0.87-1.18)	.842		1.12 (0.96-1.31)	.167	
PANDoRA ≤ 60	213/1109	360/1954	192/1036	0.97 (0.87-1.09)	.597		0.96 (0.80-1.15)	.668		0.96 (0.80-1.16)	.662	
Meta-analysis				1.10 (0.93-1.29)[a]	.267	**9.72E−04**	1.1 (0.90-1.34)[a]	.365	**.018**	1.16 (0.93-1.45)[a]	.196	**5.33E−03**
rs2328991 (G/C)												
PanScan I & II ≤ 50	136/2519	59/659	3/46	1.47 (1.12-1.95)	**6.43E−03**		1.61 (1.18-2.20)	**4.04E−04**		1.04 (0.32-3.40)	.855	
PanC4 ≤ 50	271/2793	77/734	5/62	1.04 (0.82–1.31)	.759		1.07 (0.82-1.39)	.615		0.79 (0.31-1.99)	.616	
PANDoRA ≤ 50	207/3251	54/792	4/61	1.10 (0.83-1.47)	.509		1.10 (0.80-1.52)	.554		1.28 (0.46-3.58)	.636	
Meta-analysis				1.17 (1.01-1.36)	**.040**	.153	1.22 (1.03-1.45)	**.023**	.111	1 (0.55-1.81)	.991	.789
PanScan I & II ≤ 60	590/2519	210/659	19/46	1.35 (1.16-1.58)	**1.52E−04**		1.39 (1.16-1.65)	**2.20E−04**		1.64 (0.95-2.83)	.070	
PanC4 ≤ 60	899/2793	255/734	20/62	1.06 (0.92-1.22)	.393		1.09 (0.93-1.27)	.308		0.95 (0.57-1.58)	.841	
PANDoRA ≤60	652/3251	183/792	16/61	1.20 (1.02–1.41)	**.030**		1.21 (1.01-1.45)	**.044**		1.46 (0.84-2.57)	.183	
Meta-analysis				1.19 (1.09–1.30)	**1.15E−04**	.076	1.21 (1.10-1.34)	**1.38E−04**	.128	1.29 (0.95-1.76)	.104	.314
rs4891017 (G/A)												
PanScan I & II ≤ 50	105/1277	77/1510	16/440	0.65 (0.52-0.81)	**1.77E−04**		0.58 (0.44-0.78)	**1.79E−04**		0.56 (0.33-0.94)	**.026**	
PanC4 ≤ 50	148/1448	155/1596	41/418	0.97 (0.82-1.15)	.727		0.96 (0.76-1.20)	.700		0.98 (0.69-1.38)	.891	
PANDoRA ≤ 50	107/1456	103/1526	34/438	0.93 (0.75-1.14)	.479		0.91 (0.68-1.21)	.499		0.91 (0.58-1.41)	.660	
Meta-analysis				0.84 (0.66–1.07)[a]	.160	**.014**	0.8 (0.59-1.09)[a]	.160	**.019**	0.85 (0.67-1.08)	.190	.204
PanScan I & II ≤ 60	373/1277	345/1510	103/440	0.86 (0.77-0.97)	**.010**		0.79 (0.67-0.92)	**2.28E−03**		0.92 (0.73-1.15)	.414	
PanC4 ≤ 60	486/1448	491/1596	148/418	1.00 (0.90-1.10)	.934		0.95 (0.83-1.09)	.471		1.10 (0.90-1.34)	.362	
PANDoRA ≤ 60	352/1456	322/1526	117/438	0.98 (0.87-1.11)	.766		0.92 (0.78-1.08)	.316		1.11 (0.88-1.42)	.383	
Meta-analysis				0.95 (0.89-1.01)	.110	.128	0.89 (0.82-0.97)	**8.95E−03**	.201	1.04 (0.92-1.18)	0.515	.426

(Continues)

TABLE 2 (Continued)

SNP (M/m)	MM	Mm	mm	m vs M			Mm + mm vs MM			mm vs MM + Mm		
	Cases/controls			OR (95% CI)	P_{all}	$P_{het.}$	OR (95% CI)	P_{dom}	*P* dom.	OR (95% CI)	P_{rec}	$P_{het.}$
rs12610094 (A/G)												
PanScan I & II ≤ 50	45/1171	107/1553	46/503	1.53 (1.25-1.88)	**4.89E−05**		1.93 (1.37-2.71)	**1.09E−04**		1.61 (1.14-2.27)	**4.43E−03**	
PanC4 ≤ 50	116/1281	175/1705	63/613	1.08 (0.93-1.26)	.328		1.16 (0.92-1.46)	.221		1.04 (0.78-1.39)	.789	
PANDoRA ≤ 50	87/1222	132/1704	46/593	0.99 (0.82-1.21)	0.956		1.06 (0.79-1.42)	.688		0.89 (0.62-1.29)	.549	
Meta-analysis				1.17 (0.92-1.49)[a]	.194	**5.37E−03**	1.31 (0.95-1.82)[a]	.102	**.020**	1.14 (0.82-1.59)[a]	.432	**.050**
PanScan I & II ≤ 60	246/1171	414/1553	161/503	1.23 (1.1-1.38)	**2.21E−04**		1.33 (1.12-1.57)	**6.94E−04**		1.30 (1.07-1.59)	**5.45E−03**	
PanC4 ≤ 60	404/1281	583/1705	189/613	1.00 (0.91-1.1)	.972		1.06 (0.92-1.22)	.428		0.92 (0.77-1.1)	.344	
PANDoRA ≤60	320/1222	395/1704	133/593	0.90 (0.80-1.01)	.080		0.87 (0.74-1.03)	.100		0.88 (0.7-1.09)	.244	
Meta-analysis				1.03 (0.87-1.23)[a]	.698	**5.74E−04**	1.07 (0.85-1.34)[a]	.561	**2.03E−03**	1.02 (0.8-1.3)[a]	.882	**.013**

Note: Statistically significant results ($P < .05$) are in bold; M, major allele; m, minor allele; m vs M, allelic model; P_{all}, *P* value of logistic regression using allelic model; $P_{het.}$, *P* value heterozygosity of meta-analysis; Mm + mm vs MM, dominant model; mm vs MM + Mm, recessive model. All analyses were adjusted for sex and the two principal components (PanScan and PanC4) or sex and country of origin (PANDoRA).
[a]Meta-analysis performed using a random-effects meta-analysis model.

3 | RESULTS

In the first phase, which was conducted at a genome-wide scale on 3133 PDAC patients (among which 821 cases with the age of onset ≤60 years and 198 with the age of onset ≤50) and 3227 controls we observed four SNPs (14q24.3_rs7155613, 13q22.3_rs2328991, *ZNF516*_rs4891017 and *OR7G2*_rs12610094) that showed an association with EOPC risk with $P < 1 \times 10^{-4}$ in the case-control analyses and/or in the case-case analyses.

We genotyped these SNPs in the PANDoRA consortium and used additional in silico data from PanScan III and PanC4. In the *in silico* data, we observed no statistically significant association in the selected SNPs. In PANDoRA, instead, using cases younger than 60 and all controls, we observed that carriers of the rare allele (C) of the 13q22.3_rs2328991 SNP showed a nominally statistically significant association with increased risk of developing EOPC (OR = 1.20, 95% CI 1.02-1.41, $P = 3.00 \times 10^{-2}$) even though if considering multiple testing correction ($P < .05/4 = 0.0125$) this association was not significant. In the meta-analysis between phase one and phase two of the study (PanScan I-II, PanC4, PANDoRA), we observed an association between the 13q22.3_rs2328991 C allele and an increased risk of developing EOPC (OR = 1.19, 95% CI 1.09-1.30, $P = 1.15 \times 10^{-4}$) with no heterogeneity ($P > .05$). The results of the case-control analyses are shown in Table 2. In the case-case analysis of the second phase alone, using 60 years as age cutoff, in PANDoRA, we observed that the minor allele (C) of the 13q22.3_rs2328991 showed a tendency of being associated with younger age of onset of PDAC reaching a statistically significant association (considering a threshold of $P < .05$) in the recessive model of inheritance (OR = 2.06, 95% CI 1.05-4.05, $P = 3.50 \times 10^{-2}$). The case-case analyses also showed an association between 14q24.3_rs7155613 and age of onset of the disease (OR = 0.81, 95% CI 0.67-0.98, $P = 3.30 \times 10^{-2}$). PanScan III and PanC4 analysis did not show any statistically significant associations. The case-case meta-analysis showed significant results for all the four SNPs with the exception of 14q24.3_rs7155613. The results of the case-case analyses are shown in Table 3.

In addition, we also checked in PanScan the possible associations with EOPC risk of the eight variants identified by Chen and colleagues.[21] We could find only three of the eight variants in the PanScan database and we observed no statistically significant association with EOPC risk.

3.1 | Bioinformatic analysis

Using HaploReg we found that rs2328991 is in strong linkage disequilibrium (LD) with 6 SNPs (rs17355129, rs7322104, rs76406862, rs80009395, rs79737810, rs76655255) in the Caucasian population and that is in a DNAse sensitive region. The bioinformatic tool does not show report any eQTLs for any of the SNPs. Regulome DB shows a score of 4 for rs2328991, a score of 5 for rs17355129 and rs76655255 a score of 6 for rs79737810 and no data for all other

TABLE 3 Case-case analysis in all study phases and meta-analysis

	MM	Mm	mm	m vs M			Mm + mm vs MM			mm vs MM + Mm		
SNP (M/m)		≤50/>50		OR	P_{all}	$P_{het.}$	OR	P_{dom}	$P_{het.}$	OR	P_{rec}	$P_{het.}$
rs7155613 (A/C)												
PanScan I & II [cases ≤50 vs cases >50]	41/797	95/1435	62/697	1.30 (1.06-1.59)	**4.31E−03**		1.39 (0.98-1.99)	**.035**		1.45 (1.06-1.98)	**8.55E−03**	
PANDoRA [cases ≤50 vs cases >50]	68/613	113/1080	42/567	0.81 (0.67–0.98)	**.033**		0.83 (0.61-1.12)	.225		0.66 (0.46-0.94)	**.022**	
PanScan III [cases ≤50 vs cases >50]	14/384	48/713	13/359	0.96 (0.68-1.34)	.789		1.42 (0.78-2.59)	.252		0.61 (0.33-1.13)	.120	
PanC4 [cases ≤50 Vs cases >50]	106/922	155/1675	86/829	0.94 (0.80-1.10)	.432		0.83 (0.65-1.06)	.141		1.03 (0.79-1.33)	.828	
Meta-analysis				0.99 (0.80-1.21)[a]	.898	**8.91E−03**	1.02 (0.77-1.36)[a]	.876	**.045**	0.92 (0.63-1.34)[a]	.658	**4.33E−03**
PanScan I & II [cases ≤60 vs cases >60]	178/660	395/1135	248/511	1.33 (1.19-1.49)	**1.82E−07**		1.42 (1.18-1.72)	**1.16E−04**		1.52 (1.27-1.82)	**3.86E−06**	
PANDoRA [cases ≤60 vs cases >60]	213/468	360/833	190/418	0.98 (0.87–1.11)	.770		0.95 (0.78-1.15)	.567		1.01 (0.83-1.23)	.917	
PanScan III [cases ≤60 vs cases >60]	61/301	146/615	72/300	1.05 (0.87-1.27)	.609		1.11 (0.80–1.52)	.535		1.03 (0.76-1.40)	.831	
PanC4 [cases ≤60 vs cases >60]	308/720	550/1280	292/623	1.05 (0.95-1.16)	.333		1.04 (0.88-1.21)	.664		1.10 (0.94-1.29)	.244	
Meta-analysis				1.10 (0.95-1.27)[a]	.196	**1.46E−03**	1.12 (0.93-1.34)[a]	.240	**.022**	1.16 (0.95-1.41)[a]	.141	**9.22E−03**
rs2328991 (G/C)												
PanScan I & II [cases ≤50 vs cases >50]	136/2285	59/598	3/48	1.46 (1.10-1.92)	**8.00E−03**		1.60 (1.17-2.20)	**4.31E−04**		0.98 (0.30-3.17)	.855	
PANDoRA [cases ≤50 vs cases >50]	207/1858	54/549	4/31	0.95 (0.71-1.26)	.712		0.91 (0.67-1.25)	.572		1.43 (0.50-4.11)	.504	
PanScan III [cases ≤50 vs cases >50]	53/1095	20/320	2/26	1.28 (0.82-2.01)	.282		1.31 (0.78-2.19)	.305		1.53 (0.35-6.66)	.573	
PanC4 [cases ≤50 vs cases >50]	271/2701	77/746	5/56	1.01 (0.80-1.28)	.939		1.02 (0.79–1.33)	.872		0.89 (0.35-2.23)	.796	
Meta-analysis				1.13 (0.97-1.30)	.110	.120	1.14 (0.97-1.34)	.112	.060	1.12 (0.64-1.95)	.689	.881
PanScan I & II [cases ≤60 vs cases >60]	590/1831	210/447	19/32	1.43 (1.21-1.69)	**1.39E−05**		1.48 (1.23-1.78)	**2.18E−05**		1.73 (0.97-3.10)	.070	
PANDoRA [cases ≤60 vs cases >60]	652/1413	183/420	5/56	1.04 (0.87-1.24)	.684		0.99 (0.82-1.20)	.904		2.06 (1.05–4.05)	**.035**	
PanScan III [cases ≤60 vs cases >60]	202/946	72/268	8/20	1.29 (1.00-1.67)	.054		1.29 (0.96-1.73)	.091		1.89 (0.81-4.40)	.140	
PanC4 [cases ≤60 vs cases >60]	899/2073	255/568	20/41	1.04 (0.90-1.20)	.622		1.04 (0.88-1.22)	.660		1.10 (0.64-1.90)	.719	
Meta-analysis				1.18 (1.01-1.40)[a]	.053	**.016**	1.18 (0.97-1.43)[a]	.108	**9.08E−03**	1.56 (1.14-2.14)	**5.97E−03**	.465
rs4891017 (G/A)												
PanScan I & II [cases ≤50 vs cases >50]	105/1130	77/1384	16/421	0.62 (0.49-0.78)	**3.80E−05**		0.55 (0.41-0.74)	**5.14E−05**	–	0.52 (0.31-0.87)	**.014**	
PANDoRA [cases ≤50 vs cases >50]	107/999	103/1015	34/313	0.97 (0.80-1.17)	.729		0.95 (0.72-1.24)	.692	–	0.97 (0.66-1.44)	.896	
PanScan III [cases ≤50 vs cases >50]	33/545	32/655	8/190	0.81 (0.57-1.15)	.242		0.77 (0.48-1.23)	.271		0.75 (0.35-1.59)	.451	
PanC4 [cases ≤50 vs cases >50]	148/1387	155/1567	41/426	0.94 (0.80-1.11)	.471		0.92 (0.74-1.16)	.489		0.93 (0.66-1.30)	.659	
Meta-analysis				0.83 (0.68-1.02)[a]	.081	**.015**	0.79 (0.61-1.03)[a]	.075	**.027**	0.83 (0.67-1.04)	.103	.234
PanScan I & II [cases ≤60 vs cases >60]	373/862	345/1116	103/334	0.80 (0.71-0.90)	**2.98E-04**		0.71 (0.6-0.83)	**4.05E−05**	–	0.85 (0.67-1.07)	.177	
PANDoRA [cases ≤60 vs cases >60]	352/754	322/796	117/230	0.98 (0.87–1.11)	.726		0.90 (0.76-1.07)	.247	–	1.13 (0.88-1.44)	.333	
PanScan III [cases ≤60 vs cases >60]	109/469	134/553	32/166	0.91 (0.74-1.10)	.325		0.95 (0.72–1.24)	.685		0.75 (0.50-1.13)	.173	

(Continues)

TABLE 3 (Continued)

SNP (M/m)	MM	Mm	mm	m vs M			Mm + mm vs MM			mm vs MM + Mm		
		≤50/>50		OR	P_{all}	$P_{het.}$	OR	P_{dom}	$P_{het.}$	OR	P_{rec}	$P_{het.}$
PanC4 [cases ≤60 vs cases >60]	486/1049	491/1231	148/319	0.95 (0.86-1.06)	.347		0.88 (0.77-1.02)	.090		1.07 (0.86-1.31)	.552	
Meta-analysis				0.91 (0.85-0.97)	**2.85E−03**	.086	0.84 (0.77-0.92)	**6.57E−05**	.114	0.98 (0.87–1.11)	.776	.170
rs12610094 (A/G)												
PanScan I & II [cases ≤50 vs cases >50]	45/1026	107/1412	46/497	1.43 (1.16-1.75)	**6.60E−04**		1.80 (1.28-2.53)	**4.45E−04**	—	1.44 (1.02-2.03)	**.023**	
PANDoRA [cases ≤50 vs cases >50]	87/856	132/1157	46/407	1.06 (0.88-1.28)	.514		1.12 (0.85-1.47)	.408	—	1.03 (0.73-1.45)	.872	
PanScan III [cases ≤50 vs cases >50]	28/509	30/722	18/215	1.18 (0.85-1.66)	.326		0.95 (0.59-1.54)	.832		1.83 (1.05-3.18)	**.033**	
PanC4 [cases ≤50 vs cases >50]	116/1229	175/1683	63/597	1.08 (0.92-1.26)	.357		1.12 (0.89-1.42)	.328		1.07 (0.80-1.43)	.640	
Meta-analysis				1.16 (1.05-1.28)	**4.21E−03**	.129	1.21 (1.04-1.40)	**.013**	.074	1.21 (1.01-1.44)	**.035**	.199
PanScan I & II [cases ≤60 vs cases >60]	246/825	414/1105	161/382	1.18 (1.05-1.32)	**1.87E−03**		1.28 (1.08-1.52)	**3.00E−03**		1.20 (0.97-1.47)	**.045**	
PANDoRA [cases ≤60 vs cases >60]	320/623	395/894	133/320	0.90 (0.80–1.01)	.073		0.86 (0.72-1.01)	.071		0.89 (0.71-1.11)	.302	
PanScan III [cases ≤60 vs cases >60]	99/438	139/613	47/186	1.07 (0.88-1.30)	.490		1.05 (0.80-1.38)	.748		1.17 (0.82-1.67)	.377	
PanC4 [cases ≤60 vs cases >60]	404/941	583/1275	189/471	0.99 (0.89-1.09)	.789		1.04 (0.90–1.20)	.611		0.90 (0.75-1.08)	.255	
Meta-analysis				1.03 (0.91-1.16)[a]	.675	**.011**	1.05 (0.88-1.24)[a]	.601	**.015**	1.00 (0.89-1.12)	.993	.110

Note: Statistically significant results ($P < .05$) are in bold; M, major allele; m, minor allele; m vs M, allelic model; P_{all}, P value of logistic regression using allelic model; $P_{het.}$, P value heterozygosity of meta-analysis; Mm + mm vs MM, dominant model; mm vs MM + Mm, recessive model. All analyses were adjusted for sex and the two principal components (PanScan and PanC4) or sex and country of origin (PANDoRA).

[a]Meta-analysis performed using a random-effects meta-analysis model.

SNPs. Scores ranging from 6 to 4 represent a minimal binding evidence. The GTEx project does not show any statistically significant eQTLs for any of the selected SNPs.

4 | DISCUSSION

EOPC is a rare disease with a very high mortality rate, for which very few specific risk factors have been identified.[17] Only a small number of genetic variants have been suggested in this regard.[21] In this report, we aimed at uncovering novel polymorphisms associated with the disease. Our results suggest a potential involvement of 13q22.3_rs2328991 that is associated independently in PanScan I-II, PANDoRA and also in the meta-analysis with the same direction of the association. The minor allele of the SNP is associated with an increased chance of developing EOPC in the case-control analysis and also it is also associated with an increased chance of developing EOPC in comparison with NEOPC. The SNP however is associated with the disease in only one of the populations used for replication.

None of our results reached genome-wide statistical significance ($P < 5 \times 10^{-8}$) in either phase or in the meta-analysis. The lowest *P*-value we observed for the meta-analysis of PanScan, PanC4 and PANDoRA data is 1.15×10^{-4} for the association of 13q22.3_rs2328991 with risk of pancreatic cancer under 60 years. This observation therefore has to be considered suggestive. However, the concordance of the results between two independent phases of our study is encouraging. No association, including that of 13q22.3_rs2328991, was consistently observed when considering cases diagnosed under 50 years of age. This is likely a reflection of the small numbers of cases in this category.

13q22.3_rs2328991 is situated 57 kb at the 3′ end of the potassium channel tetramerization domain containing 12 (KCTD12, OMIM no. 610521). In the last years, this gene has been the focus of several studies linking it to carcinogenesis. For example, Hasegawa and collaborators have found that KCTD12 expression is associated with diagnosis and prognosis of gastrointestinal stromal tumors (GISTs).[27] The possible mechanism by which the KCTD12 protein may exert an oncogenic push is facilitating the entrance of the cell in the M phase and therefore promoting cell proliferation through the dephosphorylation of Cyclin-Dependent Kinase 1 (CDK1, OMIM no. 116940).[28] CDK1 is the catalytic subunit of a protein kinase complex essential for G2/M transition the aberrant expression of which is associated with PDAC.[29]

Considering the lack of bioinformatic data a mechanistic link between the SNP function and the gene expression is difficult to establish, a possible explanation for the association of 13q22.3_rs2328991 is that it could be associated with yet an unknown polymorphism possibly with a lower minor allele frequency (MAF) that could be the real culprit of the association. Fine mapping approaches have indeed successfully been used to identify rare variants close to GWAS findings.[30]

The increasing evidence of the involvement of pleiotropic regions in cancer etiology and the vicinity of a gene involved in cell cycle regulation make this finding potentially interesting.

For the three SNPs reported by Chen et al for which we had data we did not observe any statistically significant association. Possible reasons for these differences include the specific selection of candidate regions, the different analytical design of the studies.

An obvious strength of this report is its large sample size because with 3206 EOPC cases this is by far the largest study on biological determinants of this disease. A potential limitation of the study is the lack of data on epidemiologic risk factors since it is not possible to download covariate data from dbGaP. Considering that smoking behavior and family history of pancreatic cancer are strong risk factors for EOPC[17] this could have led us to miss some associations or to not estimate correctly the associations we found. It is possible that the lack of adjustment for smoking and family history may cover the discovery of other potential SNPs. In addition, in the manuscript by Raimondi and colleagues the authors did not find markedly different percentage of familial cases in EOPC and VEOPC compared to NEOPC cases and a comparable effect of smoking in younger vs older cases.[17] Therefore, it is unlikely that the patients in our study are enriched by familial cases or by heavy smoking.

Our result suggests the possible involvement of 13q22.3_rs2328991 in EOPC development in the largest study performed so far. However, it was not possible to find a mechanistic link between the variant and the function. These results need to be validated in larger datasets.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

DATA ACCESSIBILITY

The PanScan genotyping data are available from the database of Genotypes and Phenotypes (dbGaP, study accession number phs000206.v5.p3). 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.

ETHICS APPROVAL

Written informed consent was obtained from each participant. The PANDoRA study protocol was approved by the Ethics Commission of the Medical Faculty of the University of Heidelberg.

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