Received: 31 August 2020 | Revised: 14 December 2020 | Accepted: 4 January 2021

DOI: 10.1002/ijc.33475

CANCER GENETICS AND EPIGENETICS

IJC International Journal of Cancer

Genome-wide scan of long noncoding RNA single nucleotide polymorphisms and pancreatic cancer susceptibility

Chiara Corradi[1] | Manuel Gentiluomo[1] | László Gajdán[2] | Giulia Martina Cavestro[3] | Edita Kreivenaite[4] | Gregorio Di Franco[5] | Cosimo Sperti[6] | Matteo Giaccherini[1] | Maria Chiara Petrone[7] | Francesca Tavano[8] | Domenica Gioffreda[8] | Luca Morelli[5] | Pavel Soucek[9] | Angelo Andriulli[8] | Jakob R. Izbicki[10] | Niccolò Napoli[11] | Ewa Małecka-Panas[12] | Péter Hegyi[13,14] | John P. Neoptolemos[15] | Stefano Landi[1] | Yogesh Vashist[10] | Claudio Pasquali[6] | Ye Lu[16] | Klara Cervena[17,18] | George E. Theodoropoulos[19] | Stefania Moz[6] | Gabriele Capurso[7,20] | Oliver Strobel[15] | Silvia Carrara[21] | Thilo Hackert[15] | Viktor Hlavac[9] | Livia Archibugi[7,20] | Martin Oliverius[22] | Giuseppe Vanella[7,20] | Pavel Vodicka[17,18] | Paolo Giorgio Arcidiacono[7] | Raffaele Pezzilli[23] | Anna Caterina Milanetto[6] | Rita T. Lawlor[24] | Audrius Ivanauskas[4] | Andrea Szentesi[13,14] | Juozas Kupcinskas[4] | Sabrina G. G. Testoni[7] | Martin Lovecek[25] | Michael Nentwich[10] | Maria Gazouli[26] | Claudio Luchini[27] | Raffaella Alessia Zuppardo[3] | Erika Darvasi[14] | Hermann Brenner[28,29,30] | Cristian Gheorghe[31] | Krzysztof Jamroziak[32] | Federico Canzian[16] | Daniele Campa[1]

[1]Department of Biology, University of Pisa, Pisa, Italy

[2]Szent György University Teaching Hospital of Fejér County, Székesfehérvár, Hungary

[3]Division of Experimental Oncology, Gastroenterology and Gastrointestinal Endoscopy Unit, Vita-Salute San Raffaele University, IRCCS Ospedale San Raffaele Scientific Institute, Milan, Italy

[4]Department of Gastroenterology and Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania

[5]General Surgery Unit, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

[6]Department of Surgery, Oncology and Gastroenterology-DiSCOG, University of Padova, Padova, Italy

[7]Pancreato-Biliary Endoscopy and Endosonography Division, Pancreas Translational and Clinical Research Center, Vita-Salute San Raffaele University, IRCCS San Raffaele Scientific Institute, Milan, Italy

[8]Division of Gastroenterology and Research Laboratory, IRCCS Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy

[9]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

[10]Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[11]UO Chirurgia Generale e dei Trapianti, Università di Pisa, Pisa, Italy

Abbreviations: GWAS, genome wide association studies; LD, linkage disequilibrium; lncRNA, long noncoding RNA; lncSNPs, long noncoding RNA single nucleotide polymorphisms; MAF, minor allele frequency; miRNA, micro-RNA; ncRNA, noncoding RNA; PANDoRA, PANcreatic Disease ReseArch Consortium; PCA, principal component analysis; PDAC, pancreatic ductal adenocarcinoma; SNPs, single nucleotide polymorphisms.

Chiara Corradi and Manuel Gentiluomo share the first position.

Federico Canzian and Daniele Campa share the last position.

[Correction added on 03 March 2021, after first online publication: affiliation for Silvia Carrara has been changed.]

[12]Department of Digestive Tract Diseases, Medical University of Lodz, Lodz, Poland

[13]Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary

[14]First Department of Medicine, University of Szeged, Szeged, Hungary

[15]Department of General Surgery, University of Heidelberg, Heidelberg, Germany

[16]Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

[17]Institute of Biology and Medical Genetics, First Medical Faculty, Prague, Czech Republic

[18]Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

[19]Colorectal Unit, First Department of Propaedeutic Surgery, Athens Medical School, National and Kapodistrian University of Athens, Athens, Greece

[20]Sant'Andrea Hospital, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

[21]Department of Gastroenterology, IRCCS Humanitas Research Hospital - Endoscopic Unit, Milan, Italy

[22]Department of Surgery, Faculty Hospital Kralovske Vinohrady and Third Faculty of Medicine, Charles University, Prague, Czech Republic

[23]Gastroenterology, San Carlo Hospital, Potenza, Italy

[24]ARC-NET: Centre for Applied Research on Cancer, University and Hospital Trust of Verona, Verona, Italy

[25]Department of Surgery I, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic

[26]Department of Basic Medical Sciences, Laboratory of Biology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

[27]Department of Diagnostics and Public Health, Section of pathology, University of Verona, Verona, Italy

[28]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany

[29]Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany

[30]German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

[31]Center of Gastroenterology, Fundeni Clinical Institute, Bucharest, Romania

[32]Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Correspondence
Daniele Campa, Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy.
Email: daniele.campa@unipi.it

Funding information
Bundesministerium für Bildung und Forschung, Grant/Award Numbers: 01EY1101, 01GS08114, 01ZX1305C, 01KT1506; Charles University, Grant/Award Number: UNCE/MED/006; Fondazione Arpa; Fondazione Tizzi; Italian Ministry of Health grants, Grant/Award Number: RC1803GA32; "5x1000" voluntary contribution; Ministry of Health of Czech Republic, Grant/Award Numbers: NV 19-03-00097, NV 19-09-00088, NV18/03/00199, NV19-08-00113; PancoBank, Grant/Award Numbers: 159/2002, 301/2001; Biomaterial Bank Heidelberg; Heidelberger Stiftung Chirurgie

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second cancer-related cause of death by 2030. Identifying novel risk factors, including genetic risk loci, could be instrumental in risk stratification and implementation of prevention strategies. Long noncoding RNAs (lncRNAs) are involved in regulation of key biological processes, and the possible role of their genetic variability has been unexplored so far. Combining genome wide association studies and functional data, we investigated the genetic variability in all lncRNAs. We analyzed 9893 PDAC cases and 9969 controls and identified a genome-wide significant association between the rs7046076 SNP and risk of developing PDAC ($P = 9.73 \times 10^{-9}$). This SNP is located in the *NONHSAG053086.2* (*lncSMC2-1*) gene and the risk allele is predicted to disrupt the binding of the lncRNA with the micro-RNA (miRNA) hsa-mir-1256 that regulates several genes involved in cell cycle, such as *CDKN2B*. The *CDKN2B* region is pleiotropic and its genetic variants have been associated with several human diseases, possibly though an imperfect interaction between lncRNA and miRNA. We present a novel PDAC risk locus, supported by a genome-wide statistical significance and a plausible biological mechanism.

KEYWORDS
association study, long noncoding RNA, pancreatic cancer, single nucleotide polymorphism

1 | INTRODUCTION

Pancreatic cancer and particularly pancreatic ductal adenocarcinoma (PDAC) is the fifth cause of cancer-related death in the western world,[1] and it is projected to become the second by 2030.[2] The incidence is almost equal to the mortality, with a survival around 10% at 5 years after diagnosis.[3] One of the reasons for this meager prognosis is the absence of specific symptoms, making early detection and diagnosis a hard challenge.[4] Additionally, surgery remains the only curative treatment, but only a minority of the patients can receive it, because most are diagnosed at advanced stage.[5] A possible strategy to reduce the burden of this disease would be to find biological risk markers that

enable a timely diagnosis, and/or the stratification of the population according to the risk in order to plan preventive strategies. Several epidemiologic PDAC risk factors have been identified, including cigarette smoking, heavy alcohol intake, type two diabetes mellitus and chronic pancreatitis.[6,7] In addition, several studies have identified body mass index as a causative factor for PDAC development using a Mendelian randomization approach.[8-10] The genetic susceptibility to PDAC is the result of the involvement of rare high penetrance mutations and high frequency low penetrance variants discovered through genome wide association studies (GWAS) or large multicentric candidate gene approaches.[11-22]

The recent advances in the knowledge on the regulatory regions of the human genome have highlighted that several GWAS hits associated with a variety of human traits, including PDAC risk, are located in DNA sequences containing noncoding RNA (ncRNA).[23] The vast majority of ncRNAs consists of long noncoding RNA (lncRNA), and recent evidences suggest that around 68% of the human transcriptome consists of lncRNAs.[24] lncRNAs are generally defined as RNA transcripts longer than 200 nucleotides with no protein-coding potential.[25] Although lncRNAs cannot encode any functional protein, they are involved in diverse biological processes, playing essential roles in maintaining cell growth, differentiation and proliferation.[26,27] There is an increasing amount of evidence suggesting their role in cancer development, progression and metastatic spread.[27] The involvement of lncRNAs in cancer could be the results of a plethora of mechanisms such as the regulation of gene expression (epigenetic, transcriptional and post-transcriptional) through the interaction with other regulatory molecules, such as micro-RNAs (miRNAs), and through the direct binding to protein complexes such as transcription factors. All these mechanisms have been reviewed by Slack and Chinnaiyan.[27]

LncRNAs are highly polymorphic and single nucleotide polymorphisms (SNPs) localized in their sequence (lncSNPs) could influence their expression and therefore have an impact on their function as master regulators.[28] At least two PDAC risk loci, identified through GWAS, have been reported to be in lncRNAs (*LINC00673*-rs11655237, *LINC-PINT*-rs6971499), and examples also exist in other cancer types such as colorectal,[29] glioma,[30] lung,[31] hepatocellular carcinoma[32] and ovarian cancer.[33]

Despite these evidences, a systematic search of the effect of lncSNPs in the development of PDAC has never been attempted. With the aim of finding new susceptibility loci, we have scanned the entire human genome for SNPs in lncRNAs and analyzed their possible involvement in PDAC susceptibility by using a two-step study on 9893 cases and 9969 controls.

What's new?

Long non-coding RNAs (lncRNAs) are thought to contribute to cancer development. Here, the authors searched for new lncRNA variants that are associated with risk of pancreatic ductal adenocarcinoma (PDAC). From analysis of 15,000 individuals, they obtained 67 variants associated with PDAC risk. Some of these were located in genes previously associated with PDAC, an outcome which not only validates the method but could shed light on the functional relevance of these genes. The strongest association was to a variant in the *lnc-SMC2-1* gene, and the risk allele is predicted to disrupt cell cycle regulation.

2 | MATERIALS AND METHODS

2.1 | Study populations

For the discovery phase data from the published GWAS PanScan I, PanScan II and PanC4 were downloaded from the database of Genotypes and Phenotypes (dbGaP) website (study accession numbers: phs000206.v5.p3 and phs000648.v1.p1, project reference: #12644). All the individuals were genotyped using either Illumina Infinium HumanHap550v3 (PanScan I), Illumina Infinium Human610-Quad (PanScan II) or HumanOmniExpressExome-8v1 (PanC4) DNA Analysis Genotyping BeadChip. Each participating study (within PanScan I, PanScan II and PanC4) obtained informed consent from study participants, and approval from the responsible institutional review board, as described in the original papers.[34-37] After downloading the genotypes, we performed imputation and quality controls. Briefly, the genotypes were phased using SHAPEIT v2 software. The three GWAS data sets were imputed separately using IMPUTE4 with 1000 Genomes-phase 3 as the reference panel. Prior to imputation, quality control (QC) filters included: removal of individuals with gender mismatches, call rate < 0.98, minimal or excessive heterozygosity (>3 SDs from the mean) or cryptic relatedness (PI_HAT > 0.2). SNPs with minor allele frequency (MAF) < 0.01, call rate < 0.98 or evidence for violations of Hardy-Weinberg Equilibrium ($P < 1 \times 10^{-6}$) were excluded. Postimputation SNPs with low imputation quality (INFO score $r^2 < 0.7$), MAF < 0.01 or call rate < 0.98 were excluded. Principal component analysis (PCA) was carried out with PLINK 2.0 (www.cog-genomics.org/plink/2.0/) including genotypes from all the populations of the phase 3 of the 1000 Genomes Project. Individuals not clustering in the PCA with the 1000 Genomes subjects of European descent were excluded from further analysis. For this phase, genotyping data of 14 269 individuals (7207 cases and 7062 controls) were used. The final data set had genotypes for 7 509 345 SNPs. Additional information regarding SNP filtering for each data set is shown in Table S1. The "inflation factor" did not showed evidence of systematic inflation (λ = 1.000 for PanScan I, λ = 1.015 for PanScan II, λ = 1.000 for PanC4, and λ = 1.000 for the aggregate data set). For the replication phase, the genotyping was conducted in 5593 individuals (2686 PDAC patients and 2907 controls) belonging to the PANcreatic Disease ReseArch Consortium (PANDoRA). PANDoRA has been described in detail elsewhere.[38] It is a multicentric study consisting of 11 European countries (Greece, Italy, Germany, Netherland, Denmark, Czech Republic, Hungary, Poland, Ukraine, Lithuania and

TABLE 1 Description of the study population

	PanScan	PanC4	PANDoRA	Total
Diagnosis				
Controls	3320	3742	2907	9893
PDAC cases	3274	3933	2686	9969
Total	6594	7675	5593	19 862
Median age (Q1-Q3)				
Controls	65 (55-75)	65 (55-75)	59 (47-67)	65 (55-75)
PDAC cases	65 (65-75)	65 (55-75)	66 (58-73)	65 (55-75)
Sex				
Female	52%	57%	45%	52%
Male	48%	43%	55%	48%

Note: The age values in the PanScan and PanC4 data sets obtained from dbGaP were reported in 10-year categories. We used the value of 35 for the age of all subjects belonging to the 30 to 39 category. Likewise, for the other age categories we used 45, 55, 65, 75, and 85 years.
Abbreviations: PANDoRA, PANcreatic Disease ReseArch Consortium; PDAC, pancreatic ductal adenocarcinoma.

United Kingdom), Brazil and Japan. PDAC cases were defined by an established diagnosis of PDAC and controls were individuals of the general population without a pancreatic disease at recruitment, individuals that were hospitalized for nontumor-related causes, or blood donors. For each subject, information on sex, age (age at diagnosis for cases and age at recruitment for controls) and country of origin was collected. 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. A description of the populations used is shown in Table 1.

2.2 | Identification of lncRNA and lncSNPs

To obtain the list of all known human lncRNAs, the publicly available database NONCODE (http://www.noncode.org) was used. The database uses a Coding Noncoding Index algorithm to discriminate between ncRNA and protein-coding RNA through the coding potential of each transcript.[39] The database uses a unique nomenclature for the lncRNA, described in detail by Xie et al.[40] The NONCODE database was consulted on March 22, 2019 and the list consisted of 11 857 human lncRNAs. To identify all the lncSNPs in each of the sequences identified through NONCODE, we used LncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP#!/), a database of functional SNPs and mutations in human and mouse lncRNAs, obtaining a list of 10 205 295 lncSNPs.[41]

2.3 | Sample preparation and genotyping

DNA of PANDoRA cases and controls was extracted from whole blood, using the Qlamp 96 DNA QIAcube HT Kit (Qiagen, Hilden, Germany). Genotyping was done using TaqMan technology (ThermoFisher Applied Biosystems, Waltham, Massachusetts) in 384-well plates according to manufacturer's recommendations. In each plate, an approximately equal number of cases and controls were used, and duplicate samples (8%) and no template controls were added for QC purposes. Genotyping calls were made using QuantStudio 5 Real-Time PCR system (Thermofisher) and QuantStudio software.

2.4 | Data filtering and statistical analysis

Out of the 10 205 295 lncSNPs identified through LncRNASNP2, 9 787 663 had MAF < 0.01 and were then discarded. Out of the remaining 417 632 lncSNPs, 121 555 were not present in the imputed PanScan + PanC4 data sets. The final list of variants used in the analysis consisted of 296 077 lncSNPs. The logistic analysis was carried out with PLINK 2.0 (www.cog-genomics.org/plink/2.0/).

The five resulting independent SNPs with the lowest *P* values of association with PDAC risk were then genotyped in a population consisting of 2686 PDAC patients and 2907 controls from PANDoRA. We observed no deviation from Hardy-Weinberg equilibrium in any of the genotyped SNPs. The average call rate was 98%. This replication analysis was adjusted for sex, age and country of origin.

In addition, a gene-based analysis was performed through the MAGMA v1.08 software to test the association between all long noncoding genes and PDAC risk.[42]

Finally, a fixed effect meta-analysis between the results of the two phases was conducted in the 19 862 individuals included in the two study phases using R software package (https://cran.r-project.org/web/packages/rmeta). The Bonferroni-corrected threshold for statistical significance was $0.05/296077 = 1.69 \times 10^{-7}$.

2.5 | Bioinformatic tools

We used several databases to link the SNPs with the best associations with a potential functional explanation. To identify the possible effect of the SNPs on gene expression, we used the data available in the Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org). We used LncRNASNP2, miRbase (http://www.mirbase.org) (release 22) and miRDB 6.0 tool (http://mirdb.org) to identify potential interactions between lncRNAs and miRNAs to assess the potential effect on gene expression. We used HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and RegulomedB (https://www.regulomedb.org) to test the regulatory potential (ie, possible change in transcription factors affinity, regulation of chromatin state). Finally, we used LDlink (https://ldlink.nci.nih.gov) to explore the linkage disequilibrium (LD) between the variants we have identified and polymorphisms reported in the literature. We have also analyzed the regions nearby the significant SNPs to look for regulatory regions using the ensemble website (https://www.ensembl.org/).

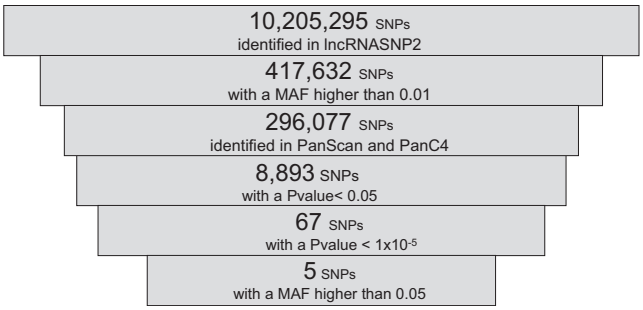

FIGURE 1 Selection/elimination process of the lncSNPs. lncSNPs, long noncoding RNA single nucleotide polymorphisms

3 | RESULTS

This study was performed using a two-phase approach, a discovery phase consisting of data on 7207 cases and 7062 controls from four GWAS conducted on PDAC risk (PanScan I, PanScan II and PanC4), and a validation phase, in which we performed de novo genotyping of the most significant SNPs, comprising 2686 PDAC patients and 2907 controls from the PANcreatic Disease ReseArch (PANDoRA) consortium. A description of the populations studied is shown in Table 1. We used the NONCODE and the LncRNASNP2 databases to establish a comprehensive list of 296 077 lncSNPs. By testing their associations in the first phase, we observed 8893 lncSNPs with a statistically significant association with PDAC risk considering a threshold of $P < .05$ (P values ranging from 5.06×10^{-19} to .049). We applied a filter of $P < 1 \times 10^{-4}$ in order to maximize the chances of reaching genome-wide significance in the combined PanScan + PanC4 + PANDoRA data set and observed 67 SNPs below that threshold (Table S2). We then pruned for residual LD among the lncSNPs and between the lncSNPs and variants already reported in the literature to be associated with risk of developing PDAC. Details of the associations with established and putative loci, as well as localizations of SNPs in lncRNAs and predictions of impact on lncRNA-miRNA binding, are reported in Table S3. We obtained five candidates (rs6931760, rs6489786, rs7046076, rs7663891, rs73335863) that were associated with PDAC risk with a $P < 1 \times 10^{-4}$, had MAF > 0.05 and were independent from known risk loci. Figure 1 shows a scheme of the selection/elimination process of the lncSNPs and Figure S1 shows regional plots for all the loci in the PanScan + PanC4 data set. The gene-based analysis using MAGMA revealed that 3108 long noncoding genes were associated to PDAC risk ($P < .05$) and 11 considering a Bonferroni-corrected threshold ($0.05/11857 = 4.22 \times 10^{-6}$). The top six genes overlap or have SNPs in LD with known PDAC risk loci. Our five candidates SNPs map to genes that have a range of P values (multitest of MAGMA) from 6.06×10^{-7} to 3.25×10^{-4}. In particular, the NONHSAG053086.2 gene, where rs7046076 maps, showed an association with a very strong statistical significance $P = 6.06 \times 10^{-7}$. The results for all the genes showing statistically significant associations are listed in Table S4.

In the replication phase, the five SNPs were tested in the PANDoRA population. We observed that the C allele of rs7046076 was associated with an increase in PDAC risk in the additive model ($OR_{add} = 1.14$, 95% CI = 1.04-1.24, $P = .004$) and in the codominant model ($OR_{[C/C \text{ vs } T/T]} = 1.33$, 95% CI = 1.09-1.62, $P = .005$).

The results were meta-analyzed with the data from the discovery phase and we found a genome-wide significant risk associated with the C allele of the rs7046076 SNP ($OR_{add} = 1.13$, 95% CI = 1.09-1.18, $P = 9.73 \times 10^{-9}$). Table 2 shows the results of the discovery phase, of the validation phase and of the meta-analysis for the five SNPs that were genotyped in PANDoRA. Figure 2 shows the meta-analysis for rs7046076. In addition, we have also checked the results of the five SNPs in a pancreatic cancer GWAS conducted in the Japanese population and we observed that rs7663891 showed an association with the risk of developing the disease, with the estimate that goes in the same direction of our replication phase (OR = 1.08, 95% CI = 1.07-1.09 and $P = .0499$)[14] but not of the discovery phase.

We investigated the functional potential of rs7046076 with all the tools described in the methods section. GTEx and Haploreg showed that rs7046076 is a multitissue eQTL, with no statistically significant association in the pancreatic tissue. According to the LncRNASNP2 website, rs7046076 lies in the *NONHSAG053086.2* lncRNA that binds to the hsa-mir-1256, which according to miRDB regulates 381 genes. The C allele of rs7046076 disrupts the binding between *NONHSAG053086.2* and hsa-mir-1256, with $\Delta\Delta G = -17.46$ kCal/mol (predicted by LncRNASNP2). LDlink showed that rs4742902, that is in LD with rs7046076 ($r^2 = 0.83$ in the European populations of 1000 Genomes), has a RegulomeBD rank of 2b and a score of 0.84, indicating that the SNP probably binds to a transcription factor and is situated in a region sensible to DNases. Using Ensembl, we found that nearby the five SNPs there are several regulatory regions (Table S5).

4 | DISCUSSION

In the last decade, GWAS have been an invaluable tool to identify thousands of susceptibility loci in many human complex traits; however, they suffer from two inherent problems: (a) the majority of the identified SNPs are situated in noncoding region that make their functional interpretation difficult, and (b) considering the stringent statistical threshold imposed by multiple comparisons (usually $P < 5 \times 10^{-8}$), only the top results are usually reported, possibly leaving out a large number of false negatives. To overcome these limitations, in our study we combined GWAS and functional data in order to identify novel variants involved in PDAC susceptibility. We focused on lncRNAs because they are an emergent biomarker in a plethora of human diseases.

LncRNA deregulation plays a key role in different human cancers, including PDAC.[26,27,43,44] The molecular mechanisms through which lncRNA could affect cancer development, progression and metastatic spread are multiple and include regulatory interactions with DNA, RNA (mRNAs and miRNAs) and proteins (eg, transcription factors and chromatin modifying complexes).[3,27]

In the last years, several in vitro studies have shown that deregulation of lncRNA expression patterns plays an essential role in growth,

TABLE 2 Case-control analysis of the five candidate SNPs selected after the discovery phase of the study

								Additive model		M/m vs M/M		m/m vs M/M	
SNP (M/m)	**Phase**	**N. Co/Ca**	**MAF Co**	**MAF Ca**	**Hom Co/Ca**	**Het Co/Ca**	**Hom Co/Ca**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
rs7663891 (T/C)	Dis.	6936/7081	30.05%	28.12%	3402/3668	2899/2843	635/570	0.90 (0.86-0.95)	8.73×10^{-5}	0.90 (0.84-0.97)	.003	0.82 (0.72-0.92)	.001
	Rep.	2885/2670	30.99%	30.97%	1392/1262	1198/1162	295/246	1.04 (0.95-1.13)	.418	1.09 (0.97-1.23)	.149	1.01 (0.82-1.23)	.931
	Met.	9821/9751	30.33%	28.90%	4794/4930	4097/4005	930/816	0.96 (0.84-1.11)	.602	0.98 (0.82-1.19)	.870	0.90 (0.73-1.10)	.283
Imputation score	PanScan-I: 0.989, PanScan II: 0.988, PanC4: 0.998												
rs6931760 (G/C)	Dis.	6998/7147	31.74%	29.66%	3287/3559	2980/2937	731/651	0.90 (0.85-0.95)	3.43×10^{-5}	0.90 (0.84-0.96)	.003	0.81 (0.72-0.91)	3.33×10^{-4}
	Rep.	2663/2562	35.43%	33.65%	1141/1140	1157/1120	365/302	0.93 (0.85-1.01)	.093	0.96 (0.85-1.09)	.568	0.84 (0.69-1.01)	.064
	Met.	9661/9709	32.76%	30.71%	4428/4699	4137/4057	1096/953	0.91 (0.87-0.95)	5.96×10^{-5}	0.91 (0.86-0.97)	.002	0.82 (0.74-0.90)	8.01×10^{-5}
Imputation score	PanScan-I: 0.996, PanScan-II: 0.995, PanC4: 0.997												
rs7046076 (T/C)	Dis.	7038/7187	31.00%	33.78%	3346/3172	3020/3175	672/840	1.13 (1.08-1.19)	9.73×10^{-7}	1.11 (1.03-1.19)	.004	1.31 (1.17-1.47)	2.39×10^{-6}
	Rep.	2839/2648	29.99%	32.91%	1394/1198	1187/1157	258/293	1.14 (1.04-1.24)	.004	1.11 (0.98-1.25)	.102	1.33 (1.09-1.62)	.005
	Met.	9877/9835	30.71%	33.54%	4740/4370	4207/4332	930/1133	1.13 (1.09-1.18)	9.73×10^{-9}	1.11 (1.04-1.18)	9.87×10^{-4}	1.32 (1.19-1.45)	5.75×10^{-8}
Imputation score	PanScan-I: 1.000, PanScan-II: 1.000, PanC4: 1.000												
rs6489786 (G/A)	Dis.	7014/7162	34.82%	37.32%	2976/2831	3191/3316	847/1015	1.11 (1.05-1.16)	5.84×10^{-5}	1.08 (1.01-1.16)	.024	1.24 (1.11-1.38)	6.81×10^{-5}
	Rep.	2836/2639	36.05%	37.27%	1171/1058	1285/1195	380/386	1.04 (0.92-1.18)	.197	1.04 (0.92-1.18)	.524	1.13 (0.94-1.35)	.188
	Met.	9850/9801	35.18%	37.31%	4147/3889	4476/4511	1227/1401	1.10 (1.05-1.15)	5.31×10^{-5}	1.07 (1.01-1.14)	.028	1.21 (1.10-1.33)	6.31×10^{-5}
Imputation score	PanScan-I: 0.999, PanScan-II: 0.998, PanC4: 0.999												
rs73335863 (T/C)	Dis.	6514/6628	4.54%	5.71%	5937/5897	563/705	14/26	1.27 (1.13-1.41)	2.64×10^{-5}	1.26 (1.12-1.41)	1.18×10^{-4}	1.8 (0.94-3.46)	.076
	Rep.	2848/2636	6.62%	6.87%	2496/2290	327/330	25/16	1.03 (0.88-1.21)	.686	1.06 (0.89-1.26)	.537	0.90 (0.46-1.76)	.755
	Met.	9362/9264	5.17%	6.04%	8433/8187	890/1035	39/42	1.15 (0.94-1.42)	.173	1.17 (0.99-1.38)	.065	1.28 (0.65-2.52)	.478
Imputation score	PanScan-I: 0.950, PanScan-II: 0.946, PanC4: 0.946												

Note: All analyses were adjusted by age, sex, and the first eight principal components. Meta-analysis was performed applying the fixed-effects model (rs6931760, rs7046076 and rs6489786) or random-effects model for SNPs showing heterogeneity (rs7663891 and rs73335863).

Abbreviations: Ca, cases; Co, controls; Dis., discovery phase; M, major allele; m, minor allele; M/m vs M/M and m/m vs M/M, codominant model; MAF, minor allele frequency; Met., meta-analysis; OR, odds ratio; Rep., replication phase; SNPs, single nucleotide polymorphisms.

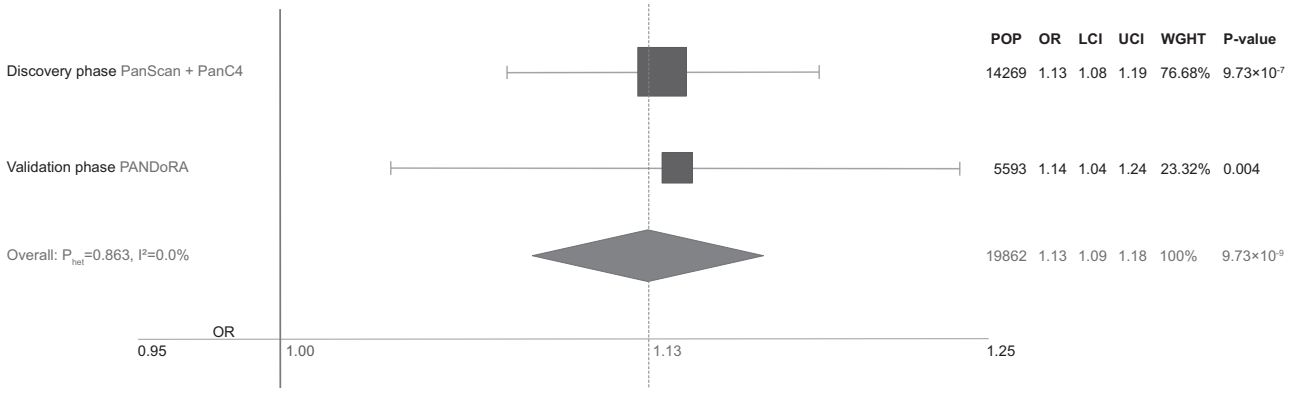

FIGURE 2 Meta-analysis for rs7046076. *Discovery phase*: PanScan + PanC4; *Validation phase*: PANDoRA; *POP*: number of cases + controls; *WGHT*: relative weight of the meta-analysis components; *P value*: association with PDAC risk; *Overall $P_{het}$and I^2*: measures of heterogeneity between the meta-analysis components. LCI, lower bound of the 95% confidence interval; OR, odds ratio; PDAC, pancreatic ductal adenocarcinoma; UCI, upper bound of the 95% confidence interval

invasive and metastatic potential of PDAC cells, as reviewed by Huang et al.[26] In particular, lncRNA-miRNA interactions seem to be of primary importance in PDAC aggressiveness.[26] Recent evidences suggest that functional polymorphisms, regulating lncRNA expression, could play a decisive role in modulating the risk of developing several cancer types.[29-33,45] With these premises, we investigated the genetic variability of lncRNAs across the genome in relation to PDAC risk.

We used a two-phase approach and analyzing almost 15 000 individuals in the discovery phase we identified 67 lncSNPs that were associated with PDAC risk ($P < 1 \times 10^{-4}$); filtering for residual LD and possible association with known loci we ended up with a list of five variants to be tested in a validation phase.

The strongest association we observed, from a statistical point of view, is the increase in risk of developing PDAC associated with the C allele of the rs7046076 variant. This SNP was significant in both study phases and reaches genome-wide significance ($P = 9.73 \times 10^{-9}$) in the meta-analysis of the two phases. rs7046076 is in the region of *SMC2*, a locus previously reported as suggestively associated with PDAC risk,[11] and is in moderate LD ($r^2 = 0.79$, $D' = 1$) with rs10991043, the top SNP reported in that locus. This SNP maps to the *NONHSAG053086.2* lncRNA (also known as *lnc-SMC2-1*) that lies on chromosome 9q31.2 (at position 106 786 881, hg38), more than 2.6 megabases away from the well-known *ABO*-rs505922 locus at 9q34 ($r^2 = 0$ in the populations of European descent of 1000 Genomes). *NONHSAG053086.2* binds to a miRNA (hsa-mir-1256), which is expressed in several tissues, including pancreatic tissue, and regulates 381 genes. Among the genes with the highest score for binding with hsa-mir-1256, there are *CDKN2B* and *DAAM1*. *CDKN2B* lies on 9p21.3, a pleiotropic stretch of DNA that includes in addition to *CDKN2B* also *CDKN2A* and *CDKN2B-AS1*, and has a very complex genomic context and regulation. There are convincing epidemiologic and molecular evidences pointing to a key role for the region in cancer etiology. For example, the *CDKN2A* gene is frequently mutated in PDAC,[46] while *CDKN2B* genetic germline variability has been consistently shown by us and others to be associated with risk of PDAC and pancreatic neuroendocrine tumors.[12,47,48] The presence of the rs7046076 C allele causes the loss of miRNA-lncRNA binding (as shown in the LncRNASNP2 database), and therefore could be involved in the deregulation of the genes on 9p21.3 increasing PDAC risk. In addition, *NONHSAG053086.2* regulates *DAAM1* (14q23.1) through the binding with hsa-mir-1256. The protein encoded by this gene promotes directional vesicular transport and facilitates cell movement. Ang et al have shown that the loss of this protein could lead to random migration of the cell,[49] and a perturbation of DAAM1 regulation has been reported to facilitate cancer progression and metastasis through random cell migration.[50] Moreover, using the MAGMA software, an association with *NONHSAG053086.2* gene, where rs7046076 maps, and PDAC risk was highlighted with a p multi of 6.06×10^{-7}. All these evidences confirm that this locus is associated with the PDAC risk. Using Ensembl, we observed the presence of are several putatively regulatory regions with variants in LD with the top five SNPs. These regions seem to have a role in the pancreatic tissues, it is however, difficult to directly link their effect with the variation determined by the SNPs. Follow-up studies aimed of uncovering the effect of the polymorphisms, especially rs7046076 are therefore warranted.

It is noteworthy that our approach selected a number of SNPs belonging to loci that were previously reported to be associated with PDAC risk, either as established loci (ie, significant at genome-wide level) or as suggestive ones. This implies that (a) our approach is valid because it successfully identifies known risk loci, and (b) it can shed light to associations previously reported but lacking possible functional explanations.

For example, rs9543325 ($P = 5.06 \times 10^{-19}$ in the discovery set), one of the lncSNPs discarded through LD filtering, maps to the locus 13q22.1, and is the same reported by Petersen et al in 2010 in the PanScan II GWAS.[35] The authors stated that the SNP maps to a nongenic region between *KLF2* and *KLF5* without explaining the function. Conversely herein we have identified that rs9543325 maps to the gene of lncRNA, *NONHSAG067118.1*, which gives a possible functional explanation to this finding.

Additional established PDAC risk loci where SNPs identified with our approach map are *NR5A2* (chromosome 1q32.1), *ETAA1* (2p14), *TERT-CLPTM1L* (5p15.33), *ABO* (9q34.2) and *BCAR*

(16q23.1). Additionally, one of the SNPs we selected for genotyping in PANDoRA (rs7663891) is in the region of *EDNRA* (chromosome 4q31.22), which was reported to be suggestively associated with PDAC risk,[11] although the association is not confirmed in our replication phase. One quarter of all known PDAC risk loci are linked to lncRNA function. In addition, gene-based analysis performed with MAGMA also supported the involvement of lncRNAs in PDAC etiology, considering that 3108 out of 11 857 lncRNA genes showed a statistically significant association with the disease. This clearly highlights the importance of these molecules in the pathology and emphasizes the need to further our knowledge on their interaction with miRNAs and other functional mechanisms.

Obvious strengths of our study are its large size and the rigorous two-phase approach that contribute to decreasing the possibilities of spurious findings. In addition, we performed a comprehensive analysis of the common genetic variability in human lncRNAs, an attempt that has never been tried before.

A possible limitation is that only individuals of Caucasian descent were included and therefore we used published data of a GWAS conducted in PDAC, in the Japanese population, to check the association of our top findings. Only rs7663891 showed a statistically significant association at the nominal *P* value level ($P = .0499$).[14] Different findings in genetic association studies conducted in population of different ethnicities are frequent and have already been documented in PDAC susceptibility.[16] In addition, the functional explanations that support our findings are derived from databases and not by direct evidence from experimental results.

In conclusion, we present here a novel PDAC risk locus supported by a genome-wide statistical significance and a plausible biological mechanism, pointing to the interplay of lncRNAs and miRNAs in the maintenance of cellular homeostasis and suggesting that subtle deregulation of these mechanisms by lncSNPs can lead to cancer. This finding improves our knowledge of genetic factors associated with PDAC risk and reinforces the role of lncSNPs in the susceptibility to PDAC.

ACKNOWLEDGMENTS

This work was supported by intramural funding of DKFZ, by Fondazione Tizzi (www.fondazionetizzi.it) and by Fondazione Arpa (www.fondazionearpa.it). M. L. was supported by Ministry of Health of Czech Republic, NV 19-03-00097, and NV 19-09-00088. F. T. was supported by Italian Ministry of Health grants (RC1803GA32) to the Division of Gastroenterology, Fondazione "Casa Sollievo della Sofferenza" IRCCS Hospital, San Giovanni Rotondo (FG), Italy and by the "5x1000" voluntary contribution. P. S. was supported by the Ministry of Health of the Czech Republic, Grant no. NV19-08-00113. V. H. was supported by the Charles University project no. UNCE/MED/006. P. V. was supported by grant no. NV18/03/00199, Ministry of Health, Czech Republic. The biosamples were obtained from the PancoBank (EPZ/Heidelberg, Germany; Ethical committee of the University of Heidelberg case numbers 301/2001 and 159/2002; Prof M. W. Büchler, Dr N. A. Giese, E. Soyka, M. Stauch, M. Meinhardt) supported by BMBF grants (01GS08114,01ZX1305C, 01KT1506), Heidelberger Stiftung Chirurgie and Biomaterial Bank Heidelberg (Prof P. Schirmacher; BMBF grant 01EY1101).

CONFLICT OF INTEREST

Dr Neoptolemos reports grants from NUCANA, grants from Heidelberger Stiftung Chirurgie, grants from Stiftung Deutsche Krebshilfe, outside the submitted work. The other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

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). 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 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.

ORCID

Manuel Gentiluomo https://orcid.org/0000-0002-0366-9653
Francesca Tavano https://orcid.org/0000-0002-8831-7349
Pavel Soucek https://orcid.org/0000-0002-4294-6799
Maria Gazouli https://orcid.org/0000-0002-3295-6811
Federico Canzian https://orcid.org/0000-0002-4261-4583
Daniele Campa https://orcid.org/0000-0003-3220-9944

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. 2019;144:1941-1953.
2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res*. 2014;74:2913-2921.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA A Cancer J Clin*. 2020;70:7-30.
4. Kleeff J, Korc M, Apte M, et al. Pancreatic cancer. *Nat Rev Dis Primers*. 2016;2:16022.
5. Zhu H, Li T, Du Y, Li M. Pancreatic cancer: challenges and opportunities. *BMC Med*. 2018;16:214.
6. Midha S, Chawla S, Garg PK. Modifiable and non-modifiable risk factors for pancreatic cancer: a review. *Cancer Lett*. 2016;381:269-277.

7. Maisonneuve P, Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. *Int J Epidemiol*. 2015;44:186-198.
8. Carreras-Torres R, Johansson M, Gaborieau V, et al. The role of obesity, type 2 diabetes, and metabolic factors in pancreatic cancer: a Mendelian randomization study. *J Natl Cancer Inst*. 2017;109(9):djx012.
9. Langdon RJ, Richmond RC, Hemani G, et al. A phenome-wide Mendelian randomization study of pancreatic cancer using summary genetic data. *Cancer Epidemiol Biomarkers Prev*. 2019;28:2070-2078.
10. Lu Y, Gentiluomo M, Lorenzo-Bermejo J, et al. Mendelian randomisation study of the effects of known and putative risk factors on pancreatic cancer. *J Med Genet*. 2020;57(12):820-828.
11. Klein AP, Wolpin BM, Risch HA, et al. Genome-wide meta-analysis identifies five new susceptibility loci for pancreatic cancer. *Nat Commun*. 2018;9:556.
12. Campa D, Pastore M, Gentiluomo M, et al. Functional single nucleotide polymorphisms within the cyclin-dependent kinase inhibitor 2A/2B region affect pancreatic cancer risk. *Oncotarget*. 2016;7:57011-57020.
13. Gentiluomo M, Lu Y, Canzian F, Campa D. Genetic variants in taste-related genes and risk of pancreatic cancer. *Mutagenesis*. 2019;34(5-6):391–394. http://doi.org/10.1093/mutage/gez032.
14. Lin Y, Nakatochi M, Hosono Y, et al. Genome-wide association meta-analysis identifies GP2 gene risk variants for pancreatic cancer. *Nat Commun*. 2020;11:3175.
15. Campa D, Rizzato C, Stolzenberg-Solomon R, et al. TERT gene harbors multiple variants associated with pancreatic cancer susceptibility. *Int J Cancer*. 2015;137:2175-2183.
16. Campa D, Rizzato C, Bauer AS, et al. Lack of replication of seven pancreatic cancer susceptibility loci identified in two Asian populations. *Cancer Epidemiol Biomarkers Prev*. 2013;22:320-323.
17. Gentiluomo M, Peduzzi G, Lu Y, Campa D, Canzian F. Genetic polymorphisms in inflammatory genes and pancreatic cancer risk: a two-phase study on more than 14 000 individuals. *Mutagenesis*. 2019;34(5-6):395-401.
18. Xu X, Qian D, Liu H, et al. Genetic variants in the liver kinase B1-AMP-activated protein kinase pathway genes and pancreatic cancer risk. *Mol Carcinog*. 2019;58:1338-1348.
19. Feng Y, Liu H, Duan B, et al. Potential functional variants in SMC2 and TP53 in the AURORA pathway genes and risk of pancreatic. *Cancer*. 2019;40:521-528.
20. Yang W, Liu H, Duan B, et al. Three novel genetic variants in NRF2 signaling pathway genes are associated with pancreatic cancer risk. *Cancer Sci*. 2019;110:2022-2032.
21. Gentiluomo M, Canzian F, Nicolini A, Gemignani F, Landi S, Campa, D. Germline genetic variability in pancreatic cancer risk and prognosis. *Seminars in Cancer Biology*. 2020. http://doi.org/10.1016/j.semcancer.2020.08.003.
22. Galeotti AA, Gentiluomo M, Rizzato C, et al. Polygenic and multifactorial scores for pancreatic ductal adenocarcinoma risk prediction. *Journal of Medical Genetics*. 2020;jmedgenet–2020. http://doi.org/10.1136/jmedgenet-2020-106961. Online ahead of print.
23. Kumar V, Westra H-J, Karjalainen J, et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. *PLoS Genet*. 2013;9:e1003201.
24. Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet*. 2015;47:199-208.
25. Dhanoa JK, Sethi RS, Verma R, Arora JS, Mukhopadhyay CS. Long non-coding RNA: its evolutionary relics and biological implications in mammals: a review. *J Anim Sci Technol*. 2018;60:25.
26. Huang X, Zhi X, Gao Y, Ta N, Jiang H, Zheng J. LncRNAs in pancreatic. *Cancer*. 2016;7:57379-57390.
27. Slack FJ, Chinnaiyan AM. The role of non-coding RNAs in oncology. *Cell*. 2019;179:1033-1055.
28. Gao P, Wei G-H. Genomic insight into the role of lncRNA in cancer susceptibility. *Int J Mol Sci*. 2017;18(6):1239.
29. Kim JO, Jun HH, Kim EJ, et al. Genetic variants of *HOTAIR* associated with colorectal cancer susceptibility and mortality. *Front Oncol*. 2020;10:72.
30. Deng Y, Zhou L, Li N, et al. Impact of four lncRNA polymorphisms (rs2151280, rs7763881, rs1136410, and rs3787016) on glioma risk and prognosis: a case-control study. *Mol Carcinog*. 2019;58:2218-2229.
31. Yuan H, Liu H, Liu Z, et al. A novel genetic variant in long non-coding RNA gene NEXN-AS1 is associated with risk of lung cancer. *Sci Rep*. 2016;6:34234.
32. Ji X, Zhang J, Liu L, et al. Association of tagSNPs at lncRNA MALAT-1 with HCC susceptibility in a southern Chinese population. *Sci Rep*. 2019;9:10895.
33. Lu Y, Beeghly-Fadiel A, Wu L, et al. A transcriptome-wide association study among 97,898 women to identify candidate susceptibility genes for epithelial ovarian cancer risk. *Cancer Res*. 2018;78:5419-5430.
34. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic. *Cancer*. 2009;41:986-990.
35. Petersen GM, Amundadottir L, Fuchs CS, et al. A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. *Nat Genet*. 2010;42:224-228.
36. Wolpin BM, Rizzato C, Kraft P, et al. Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. *Nat Genet*. 2014;46:994-1000.
37. Childs EJ, Mocci E, Campa D, et al. Common variation at 2p13.3, 3q29, 7p13 and 17q25.1 associated with susceptibility to pancreatic cancer. *Nat Genet*. 2015;47:911-916.
38. Campa D, Rizzato C, Capurso G, et al. Genetic susceptibility to pancreatic cancer and its functional characterisation: the PANcreatic Disease ReseArch (PANDoRA) consortium. *Dig Liver Dis*. 2013;45:95-99.
39. Zhao Y, Li H, Fang S, et al. NONCODE 2016: an informative and valuable data source of long non-coding RNAs. *Nucleic Acids Res*. 2016;44:D203-D208.
40. Xie C, Yuan J, Li H, et al. NONCODEv4: exploring the world of long non-coding RNA genes. *Nucleic Acids Res*. 2014;42:D98-D103.
41. Miao Y-R, Liu W, Zhang Q, Guo A-Y. lncRNASNP2: an updated database of functional SNPs and mutations in human and mouse lncRNAs. *Nucleic Acids Res*. 2018;46:D276-D280.
42. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol*. 2015;11:e1004219.
43. Ghazavi F, De Moerloose B, Van Loocke W, et al. Unique long non-coding RNA expression signature in ETV6/RUNX1-driven B-cell precursor acute lymphoblastic. *Leukemia*. 2016;7:73769-73780.
44. DiStefano JK. The emerging role of long noncoding RNAs in human disease. *Disease Gene Identification*. Methods in Molecular Biology, Vol. 1706. New York: Humana Press; 2018:91-110. https://doi.org/10.1007/978-1-4939-7471-9_6.
45. Yang T, Zhang Z, Zhang J, et al. The rs2147578 C > G polymorphism in the lnc-LAMC2-1:1 gene is associated with increased neuroblastoma risk in the Henan children. *BMC Cancer*. 2018;18:948.
46. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*. 2012;491:399-405.
47. Campa D, Capurso G, Pastore M, et al. Common germline variants within the CDKN2A/2B region affect risk of pancreatic neuroendocrine tumors. *Sci Rep*. 2016;6:39565.
48. Ter-Minassian M, Wang Z, Asomaning K, et al. Genetic associations with sporadic neuroendocrine tumor risk. *Carcinogenesis*. 2011;32:1216-1222.
49. Ang S-F, Zhao Z-S, Lim L, Manser E. DAAM1 is a formin required for centrosome re-orientation during cell migration. *PLoS ONE*. 2010;5(9):e13064. http://doi.org/10.1371/journal.pone.0013064.

50. Xiong H, Yan T, Zhang W, et al. miR-613 inhibits cell migration and invasion by downregulating Daam1 in triple-negative breast cancer. *Cell Signal*. 2018;44:33-42.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Corradi C, Gentiluomo M, Gajdán L, et al. Genome-wide scan of long noncoding RNA single nucleotide polymorphisms and pancreatic cancer susceptibility. *Int. J. Cancer*. 2021;148:2779–2788. https://doi.org/10.1002/ijc.33475