Null Results in Brief

Cancer Epidemiology, Biomarkers & Prevention

Lack of Association for Reported Endocrine Pancreatic Cancer Risk Loci in the PANDoRA Consortium

Daniele Campa[1], Ofure Obazee[2], Manuela Pastore[1,2], Francesco Panzuto[3], Valbona Liço[4], William Greenhalf[5], Verena Katzke[6], Francesca Tavano[7], Eithne Costello[5], Vincenzo Corbo[8], Renata Talar-Wojnarowska[9], Oliver Strobel[10], Carlo Federico Zambon[11], John P. Neoptolemos[5], Giulia Zerboni[3], Rudolf Kaaks[6], Timothy J. Key[12], Carlo Lombardo[13,14], Krzysztof Jamroziak[15], Domenica Gioffreda[7], Thilo Hackert[10], Kay-Tee Khaw[16], Stefano Landi[1], Anna Caterina Milanetto[4], Luca Landoni[17], Rita T. Lawlor[8], Franco Bambi[18], Felice Pirozzi[7], Daniela Basso[19], Claudio Pasquali[4], Gabriele Capurso[3], and Federico Canzian[2]

Abstract

Background: Pancreatic neuroendocrine tumors (PNETs) are rare neoplasms for which very little is known about either environmental or genetic risk factors. Only a handful of association studies have been performed so far, suggesting a small number of risk loci.

Methods: To replicate the best findings, we have selected 16 SNPs suggested in previous studies to be relevant in PNET etiogenesis. We genotyped the selected SNPs (rs16944, rs1052536, rs1059293, rs1136410, rs1143634, rs2069762, rs2236302, rs2387632, rs3212961, rs3734299, rs3803258, rs4962081, rs7234941, rs7243091, rs12957119, and rs1800629) in 344 PNET sporadic cases and 2,721 controls in the context of the PANcreatic Disease ReseArch (PANDoRA) consortium.

Results: After correction for multiple testing, we did not observe any statistically significant association between the SNPs and PNET risk. We also used three online bioinformatic tools (HaploReg, RegulomeDB, and GTEx) to predict a possible functional role of the SNPs, but we did not observe any clear indication.

Conclusions: None of the selected SNPs were convincingly associated with PNET risk in the PANDoRA consortium.

Impact: We can exclude a major role of the selected polymorphisms in PNET etiology, and this highlights the need for replication of epidemiologic findings in independent populations, especially in rare diseases such as PNETs. *Cancer Epidemiol Biomarkers Prev; 26(8); 1349–51. ©2017 AACR.*

Introduction

Pancreatic neuroendocrine tumors (PNETs) are rare neoplasms, but their incidence has greatly increased in the past decades (1). In comparison with other solid tumors, little is known about PNET risk factors, and only a handful of association studies have been performed to uncover the genetic determinants of the disease (2–4). Berkovic and colleagues have performed studies on inflammation-related genes, such as *ILB1* and *TNFA* (2, 3), while Ter-Minassian and colleagues have selected a more comprehensive approach using a custom array containing almost 1,500 SNPs (4). Both teams suggested several interesting associations; however, due to the capricious nature of association studies and the relatively small sample size of these PNET studies, it is of uttermost importance to validate their findings in an independent population, such as the PANcreatic Disease ReseArch (PANDoRA) study (5).

[1]Department of Biology, University of Pisa, Pisa, Italy. [2]Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Digestive and Liver Disease Unit, S. Andrea Hospital, "Sapienza" University of Rome, Rome, Italy. [4]Pancreatic and Digestive Endocrine Surgery, Department of Surgery, Oncology and Gastroenterology -DiSCOG, University of Padova, Padua, Italy. [5]Institute of Translational Medicine, Cancer Research UK Liverpool Cancer Trials Unit, Liverpool, United Kingdom. [6]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [7]Division of Gastroenterology and Research Laboratory, Department of Surgery, IRCCS Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy. [8]ARC-NET: Centre for Applied Research on Cancer, University and Hospital Trust of Verona, Verona, Italy. [9]Department of Digestive Tract Diseases, Medical University of Lodz, Lodz, Poland. [10]Department of General, Visceral and Transplantation Surgery, University Hospital Heidelberg, Heidelberg, Germany. [11]Department of Medicine - DIMED, University of Padova, Padua, Italy. [12]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, United Kingdom. [13]Division of General and Transplant Surgery, University of Pisa, Pisa, Italy. [14]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, University of Pisa, Pisa, Italy. [15]Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland. [16]University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom. [17]The Pancreas Institute, Department of Surgery, University and Hospital Trust of Verona, Verona, Italy. [18]Blood Transfusion Service, Azienda Ospedaliero Universitaria Meyer, Florence, Italy. [19]Department of Laboratory Medicine, University-Hospital of Padova, Padua, Italy.

Corresponding Author: Federico Canzian, German Cancer Research Center, INF 580, Heidelberg 69210, Germany. Phone: 4962-2142-1791; Fax: 4962-2142-1810; E-mail: f.canzian@dkfz.de

doi: 10.1158/1055-9965.EPI-17-0075

Table 1. Study population

	Cases	Controls
Region		
Germany	32	768
Northern Italy	159	520
Central Italy	68	559
Southern Italy	13	509
Poland	19	189
United Kingdom	53	176
Total	344	2,721
Sex		
Male	174	1,455
Female	167	1,239
Median age (interquartile range)	59 (48–68)	59 (47–68)

Materials and Methods

The PANDoRA consortium has been described elsewhere (5). In this study, 344 PNET cases and 2,721 controls have been genotyped (Table 1). Cases were sporadic, that is, not observed in the context of genetic syndromes associated with PNET, such as multiple endocrine neoplasia (MEN)-1, MEN-2, Von Hippel–Lindau or tuberous sclerosis. Controls were selected in the same geographic areas as the cases. We selected 16 SNPs that represent all the polymorphic variants so far identified as risk loci for PNETs but not replicated yet. In addition to the SNPs reported in this article, we have recently performed a study on the *CDKN2A* gene variability in relation to PNET susceptibility, and therefore, all the variants of this gene are not reported here (6). Genotyping was performed using the KASPar SNP genotyping system (KBiosciences). The order of DNA samples from cases and controls was randomized on plates to ensure that similar numbers of cases and controls were analyzed simultaneously. For quality control purpose, around 8% of the samples were duplicated and genotype concordance was checked. Statistical analysis was performed using an unconditional logistic regression setting the more common allele for each polymorphism as reference and adjusting for age, gender, and country of origin. Given that in the original article on *IL1B* (3), an analysis was performed combining the alleles of the two SNPs, we also performed it (using the same criteria as Berkovic). We also used online bioinformatic tools such as HaploReg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php), RegulomeDB (http://www.regulomedb.org/), and GTEx (http://www.gtexportal.org/home/) to explore potential functions of the SNPs.

Results

The average call rate of the 16 genotyped SNPs was 96.63% (92.06–99.04), and the concordance rate of duplicated genotypes was higher than 99%. The genotype distributions at all loci were in Hardy–Weinberg equilibrium in controls, with nonsignificant χ^2 values. Table 2 shows the frequencies and distribution of the genotypes, the ORs and 95% confidence intervals for the association with PNET risk. None of the SNPs showed any statistically significant association considering a P value threshold of 0.05. The only potentially interesting effect was a trend for the carriers of the minor allele (A) of the *IL1B*-rs1143634 SNP and a decreased risk of developing PNET ($P_{\text{homozygous}} = 0.082$). The combined diplotype analysis for the *IL1B* gene, as done in the original article by Berkovic and colleagues (3), did not reveal any statistically significant associations (data not shown). For *IL1B*-rs1143634, HaploReg showed two possible eQTLs (with expression of gene *CHCHD5*, $P = 4.1 \times 10^{-5}$, and of gene *SLC20A1*, $P = 0.0014$), RegulomeDB showed a score of 5 (minimal binding evidence), and GTEx showed no statistically significant results.

Discussion and Conclusion

Because of the rarity of PNETs, there have been only a small number of studies investigating the genetic susceptibility to this disease. The most interesting findings have been polymorphic variants in genes involved in inflammatory response, cell-cycle control, and DNA repair mechanisms. This is one of the largest studies on PNETs to date, and our sample size was by far larger than those used in the original studies, giving us more than 95% statistical power to detect the previously reported associations. The *IL1B*-rs1143634 variant is a synonymous, possibly functional, SNP that has been widely studied in relation to a variety of human diseases and conditions, making it an attractive candidate for PNET risk. Our results however suggest, at best, only a minor

Table 2. Associations between endocrine pancreatic cancer risk and the selected SNPs

SNP	Gene	Alleles M/m	Genotype groups Cases/controls[b]			Mm vs. MM[a] OR (95% CI)[c]	P_{het}	mm *vs.* MM[a] OR (95% CI)[c]	P_{hom}	Original publication
rs2069762	*IL2*	A/C	138/1,128	148/1,099	45/285	1.01 (0.77–1.34)	0.913	1.31 (0.89–1.94)[d]	0.169	2
rs16944	*IL1B*	G/A	148/1,078	142/1,157	47/279	0.82(0.63–1.07)	0.155	1.22 (0.82–1.81)	0.321	3
rs1143634	*IL1B*	G/A	208/1,551	120/978	12/155	0.87(0.68–1.12)	0.289	0.58 (0.31–1.07)	0.082	3
rs1059293	*IFNGR2*	C/T	114/846	170/1,323	54/492	0.94 (0.72–1.22)	0.639	0.83 (0.58–1.19)	0.303	4
rs1136410	*ADPRT*	A/G	246/1,753	78/611	9/63	0.86 (0.65–1.15)	0.311	1.03 (0.50–2.15)	0.934	4
rs1052536	*LIG3*	C/T	114/799	151/1,230	69/459	0.87 (0.65–1.16)	0.330	1.12 (0.78–1.59)	0.546	4
rs2236302	*MMP14*	C/G	274/1,980	62/492	4/41	0.89 (0.65–1.23)	0.503	0.46 (0.11–1.93)	0.287	4
rs2387632	*VEGFR1*	C/T	146/1,133	155/1,130	34/335	1.00 (0.78–1.29)	0.974	0.75 (0.50–1.13)	0.171	4
rs3212961	*ERCC1*	G/T	244/1,368	79/410	7/38	0.99 (0.72–1.36)	0.938	0.98 (0.41–2.36)	0.967	4
rs3734299	*PERP*	T/C	158/1,035	144/1,117	39/348	0.80 (0.61 1.04)	0.104	0.75 (0.50–1.12)	0.169	4
rs3803258	*SLC10A2*	T/C	241/1,738	89/711	7/66	0.99 (0.76–1.30)	0.953	0.96 (0.42–2.18)	0.425	4
rs4962081	*TSC1*	C/A	293/2,335	49/249	2/22	1.11 (0.80–1.55)	0.515	0.68 (0.15–3.03)	0.921	4
rs7234941	*BCL2*	C/T	240/1,932	87/696	10/59	0.96 (0.73–1.25)	0.760	1.34 (0.67–2.72)	0.408	4
rs7243091	*BCL2*	G/A	212/1,664	110/857	15/130	0.96 (0.74–1.24)	0.763	0.87 (0.49–1.53)	0.625	4
rs12957119	*BCL2*	A/C	227/1,799	86/698	18/94	0.92 (0.70–1.21)	0.535	1.38 (0.80–2.39)	0.253	4
rs1800629	*TNFA*	G/A	261/2,280	66/558	6/42	0.96 (0.70–1.32)	0.819	0.61 (0.18–2.00)	0.413	4

Abbreviations: M, major allele; m, minor allele.

[a]Mm vs. MM = heterozygous vs. common homozygous; mm vs. MM = rare homozygous vs. common homozygous (in both cases using a codominant model).

[b]Numbers may not add up to 100% due to genotyping failure, DNA depletion, or missing covariate data.

[c]OR (95% confidence interval). All analyses were adjusted for age at diagnosis/recruitment, gender, and country of origin.

influence of the variant in the etiology of the disease. In addition, we found the minor allele to be associated with decreased risk, whereas in the original publication, it was the opposite. The three selected bioinformatic tools did not reveal a clear-cut indication about the functional effect of the SNP. In conclusion, in this study, we can exclude a major role of the selected polymorphisms in PNET etiology and highlight, on one hand, the importance of finding genetic markers for the disease, ideally through a genome-wide association study approach, and on the other hand the need of replication of epidemiologic findings in independent populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Campa, E. Costello, O. Strobel, J.P. Neoptolemos, G. Capurso, F. Canzian

Development of methodology: M. Pastore, J.P. Neoptolemos

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): O. Obazee, F. Panzuto, V. Liço, W. Greenhalf, R. Talar-Wojnarowska, O. Strobel, C.F. Zambon, J.P. Neoptolemos, G. Zerboni, R. Kaaks, T.J. Key, C. Lombardo, K. Jamroziak, D. Gioffreda, T. Hackert, K.-T. Khaw, S. Landi, A.C. Milanetto, L. Landoni, R.T. Lawlor, F. Bambi, F. Pirozzi, D. Basso, C. Pasquali, G. Capurso

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Pastore, F. Canzian

Writing, review, and/or revision of the manuscript: D. Campa, O. Obazee, F. Panzuto, V. Katzke, E. Costello, R. Talar-Wojnarowska, O. Strobel, J.P. Neoptolemos, R. Kaaks, T.J. Key, K. Jamroziak, T. Hackert, K.-T. Khaw, G. Capurso, F. Canzian

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): O. Obazee, M. Pastore, V. Liço, W. Greenhalf, F. Tavano, V. Corbo, O. Strobel, C. Lombardo, T. Hackert, K.-T. Khaw

Study supervision: T. Hackert, F. Canzian

Grant Support

This work was partially supported by CRUKC8221/A19170 and MRCMR/M012190/1 grants.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 24, 2017; revised April 21, 2017; accepted April 21, 2017; published online August 1, 2017.

References

1. Viudez A, De Jesus-Acosta A, Carvalho FL, Vera R, Martin-Algarra S, Ramirez N. Pancreatic neuroendocrine tumors: challenges in an underestimated disease. Crit Rev Oncol Hematol 2016;101:193–206.
2. Berkovic MC, Jokic M, Marout J, Radosevic S, Zjacic-Rotkvic V, Kapitanovic S. IL-2 -330 T/G SNP and serum values-potential new tumor markers in neuroendocrine tumors of the gastrointestinal tract and pancreas (GEP-NETs). J Mol Med 2010;88:423–9.
3. Cigrovski Berkovic M, Catela Ivkovic T, Marout J, Zjacic-Rotkvic V, Kapitanovic S. Interleukin 1beta gene single-nucleotide polymorphisms and susceptibility to pancreatic neuroendocrine tumors. DNA Cell Biol 2012; 31:531–6.
4. Ter-Minassian M, Wang Z, Asomaning K, Wu MC, Liu CY, Paulus JK, et al. Genetic associations with sporadic neuroendocrine tumor risk. Carcinogenesis 2011;32:1216–22.
5. Campa D, Rizzato C, Capurso G, Giese N, Funel N, Greenhalf W, et al. Genetic susceptibility to pancreatic cancer and its functional characterisation: the PANcreatic Disease ReseArch (PANDoRA) consortium. Dig Liver Dis 2013;45:95–9.
6. Campa D, Capurso G, Pastore M, Talar-Wojnarowska R, Milanetto AC, Landoni L, et al. Common germline variants within the CDKN2A/2B region affect risk of pancreatic neuroendocrine tumors. Sci Rep 2016; 6:39565.