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TERT gene harbors multiple variants associated with pancreatic cancer susceptibility

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A small number of common susceptibility loci have been identified for pancreatic cancer, one of which is marked by rs401681 in the *TERT–CLPTM1L* gene region on chromosome 5p15.33. Because this region is characterized by low linkage disequilibrium, we sought to identify whether additional single nucleotide polymorphisms (SNPs) could be related to pancreatic cancer risk, independently of rs401681. We performed an in-depth analysis of genetic variability of the telomerase reverse transcriptase (*TERT*) and the telomerase RNA component (*TERC*) genes, in 5,550 subjects with pancreatic cancer and 7,585 controls from the PANcreatic Disease ReseArch (PANDoRA) and the PanScan consortia. We identified a significant association between a variant in *TERT* and pancreatic cancer risk (rs2853677, odds ratio = 0.85; 95% confidence interval = 0.80–0.90, $p = 8.3 \times 10^{-8}$). Additional analysis adjusting rs2853677 for rs401681 indicated that the two SNPs are independently associated with pancreatic cancer risk, as suggested by the low linkage disequilibrium between them ($r^2 = 0.07$, $D' = 0.28$). Three additional SNPs in *TERT* reached statistical significance after correction for multiple testing: rs2736100 ($p = 3.0 \times 10^{-5}$), rs4583925 ($p = 4.0 \times 10^{-5}$) and rs2735948 ($p = 5.0 \times 10^{-5}$). In conclusion, we confirmed that the *TERT* locus is associated with pancreatic cancer risk, possibly through several independent variants.

Epidemiology

What's new?

Most pancreatic cancer patients do not survive long after diagnosis, and, so far, there are not many genetic markers to help screen for the disease. In search of genetic predictors of pancreatic cancer, the authors zoomed in on a region linked to susceptibility to the disease. They measured the frequency of different variants of two genes, telomerase reverse transcriptase and telomerase RNA component, among thousands of pancreatic cancer patients and controls. They identified several variants of the TERT gene that indicate a boosted pancreatic cancer risk, and which may develop into useful prognostic tools.

The majority of pancreatic cancer patients die within a year of diagnosis.[1] The poor prognosis is caused by various factors, including the lack of appropriate markers for early detection, the aggressiveness of the disease and the dearth of effective treatment possibilities available to the patients diagnosed at a late stage.[2] Therefore, the best hope to reduce mortality among patients is early diagnosis, and a possible strategy to increase chances of early diagnosis is to identify people at high risk in the population and subject them to enhanced surveillance.

Only a few epidemiologic risk factors have been established for pancreatic cancer, including cigarette smoking, heavy alcohol intake, diabetes mellitus (although diabetes or glucose intolerance diagnosed up to 3 years before diagnosis of cancer may be a result of the malignancy rather than a risk factor),[3] obesity, chronic pancreatitis and family history of pancreatic cancer.[4,5] Aside from ABO blood group,[6,7] even less is known about the genetic contribution to the disease, because only a rather small number of susceptibility loci have been identified through genome-wide association studies[8–11] and confirmed by follow-up studies.[12]

The *TERT-CLPTM1L* gene region on chromosome 5p15.33 is one of these few identified loci for pancreatic cancer risk.[10,13] The *TERT* gene encodes the telomerase reverse transcriptase, which, together with the telomerase RNA component (encoded by the *TERC* gene), constitute the telomerase complex.[14] A correctly functioning telomerase is required for accurate *de novo* synthesis of telomeric ends. Even moderate changes in TERT and TERC activity can profoundly affect telomere homeostasis.[15] Telomeres are highly specialized structures that have key roles in various cellular processes, such as chromosomal stability and cell growth,[16,17] and in proper segregation of chromosomes to daughter cells.[18] Overwhelming evidence suggests that telomere dysfunction, mediated by telomerase activation, is a driving force in cancer development.[15]

Both *TERT* and *TERC* contain pleiotropic risk loci because SNPs in both genes are associated with risks of developing a number of types of human tumors. For example, *TERT* rs2736100 is associated with glioma, testicular cancer and lung cancer,[19–22] whereas *TERT* rs401681 is associated with lung, bladder and pancreatic cancer,[10,23,24] *TERT* rs10069690 with estrogen receptor-negative breast cancer,[25] *TERT* rs2242652 with breast, prostate and ovarian cancer[26–28] and *TERC* rs10936599 with multiple myeloma and colorectal cancer.[29,30] The *TERT* locus is characterized by low linkage disequilibrium (LD), raising the possibility that additional SNPs could be, independently from rs401681 and rs2736098, related to pancreatic cancer risk, given the multiple polymorphic variants that are associated with other cancer types. To elucidate further the role of genetic variability in these two regions in pancreatic cancer risk, we examined 22 SNPs in *TERT* and seven in *TERC* in 5,550 pancreatic ductal adenocarcinoma (PDAC) case subjects and 7,585 controls.

Table 1. Description of the PANDoRA consortium population

	Cases	Controls	Total
Geographic origin			
Italy	789	1,630	2,419
Germany[1]	536	956	1,492
Czech Republic	249	745	994
Greece	70	88	158
Lithuania	57	192	249
Poland	99	320	419
United Kingdom[2]	101	175	276
Total	*1,901*	*4,106*	*6,007*
Gender[3]			
Male	1,093 (58%)	2,228 (53%)	3,321 (56%)
Female	787 (42%)	1,808 (47%)	2,595 (44%)
Median age (25%–75% percentiles)[4]			
PANDoRA	64 (19–98)	58 (17–98)	

[1]Cases from PANDoRA and controls from the ESTHER cohort.
[2]Cases from PANDoRA and controls from the European Prospective Investigation on Cancer cohort.
[3]Numbers do not add up to the total of subjects because of missing information.
[4]Age at diagnosis for cases and age at recruitment for controls.

Material and Methods

Study populations

We used a two-step strategy with a discovery phase consisting of biological samples from 1,885 PDAC case subjects and 4,048 controls collected in the context of the PANcreatic Disease ReseArch (PANDoRA) consortium, and a validation phase consisting of samples from 3,537 case subjects and 3,665 control subjects collected from studies participating in the PanScan consortium.

The PANDoRA consortium has been described in detail elsewhere.[31] Briefly, individuals with newly diagnosed PDAC were retrospectively identified in seven European countries (Italy, Poland, Germany, Czech Republic, England, Greece and Lithuania) between 1996 and 2012. Controls of Italian, Czech and Polish origin were recruited in the same hospitals, or at least the same geographical regions from where the case subjects were enrolled. British controls were selected from healthy volunteers recruited from the general population in the European Prospective Investigation on Cancer, an ongoing prospective cohort study in ten European countries (http://epic.iarc.fr/). The German controls were enrolled in ESTHER, a prospective cohort with 9,953 participants recruited during a general health check-up between July 2000 and December 2002 in Saarland (a state in Southwestern Germany). All subjects signed a written consent from. Relevant characteristics of the populations are shown in Table 1.

For the validation phase, we used data from the PanScan consortium. The PanScan study has been fully described elsewhere.[8,10] Briefly, case and control data and DNA samples were collected from 12 cohort studies and eight case–control

studies. Cases were defined as those individuals having primary adenocarcinoma of the exocrine pancreas. Controls were frequency matched with cases and were free of pancreatic cancer at the time of enrolment. Matching criteria varied according to the studies within PanScan. Additional information on the matching criteria are given in the original publications.[8,10] All subjects signed a written consent form.

SNP selection

Common genetic variability in the *TERT* gene region was investigated following a hybrid functional and tagging approach to identify candidate SNPs. Within the region of *TERT/CLPTM1L* (chr5: 1277490–1377121, NCBI36/hg18), all SNPs with a minor allele frequency > 5% in Caucasians (International HapMap Project, version 28; http://www.hapmap.org) were considered. Tagging SNPs were selected with the use of the Haploview Tagger Program (http://www.broad.mit.edu/mpg/haploview/ and http://www.broad.mit.edu/mpg/tagger/),[32] using pairwise tagging with a minimum r^2 of 0.8. We selected additional SNPs significantly associated at a genome-wide level with cancer risk or with telomere length.[26,28] For the *TERC* gene, we selected SNPs that have been previously associated with telomere length or cancer risk that also reside in chr3: 170974797–170984874 (NCBI36/hg18).[30,33,34] The final selection consisted of 29 SNPs; 22 in the *TERT* region and seven in *TERC*.

Genotyping

De novo genotyping for the discovery phase was carried out on 1,885 PDAC case subjects and 4,048 controls within PANDoRA at the German Cancer Research Center in Heidelberg, Germany, on genomic DNA extracted from peripheral blood, using TaqMan (ABI, Applied Biosystems, Foster City, CA) and KASPar (KBioscence, Hoddesdon, UK) technologies. The order of DNA samples from case and control subjects was randomized on plates to ensure that similar numbers of cases and controls were analyzed in each batch. For quality control, duplicates of 10% of the samples were interspersed throughout the plates. Polymerase chain reaction plates were read on a ViiA7 real time instrument (Applied Biosystems). The ViiA7 RUO Software, version 1.2.2 (Applied Biosystems), was used to determine genotypes. The genotyping concordance between duplicate samples exceeded 99%, and the average SNP call fraction was 97.5% (93.6–99.8%), after all samples with a call fraction lower than 75% were discarded from the analysis. Genotype data used in the second phase were generated as part of PanScan at the National Cancer Institute Cancer Genomics Research Laboratory, using Illumina HumanHap550 and HumanHap550-Duo SNP arrays (PanScan-I) and Illumina Human 610-Quad arrays (PanScan-II). Only SNPs with call rates >94% and samples with call rates >94% were included in the analysis. Participants with <80% European ancestry were excluded from the analysis. The final numbers of cases and controls included in Stage 2 were 3,537 case subjects and 3,665 control subjects. An average discordance rate of 0.031% was observed for the 244 duplicate pairs used as quality control. Additional information on the genotyping performed in the PanScan studies is given in the original publications.[8,10]

Statistical analysis

Hardy Weinberg equilibrium was assessed in control subjects for each polymorphism. In the first phase, we included genotype data from 1,885 pancreatic cancer case subjects and 4,048 controls. Unconditional logistic regression methods were used to assess the main effects for the 29 selected genetic polymorphisms on PDAC risk, using allelic, co-dominant and dominant inheritance models. For each SNP, the more common allele in controls was assigned as the reference category. All analyses were adjusted for age (continuous), gender and geographic region of origin. In the validation phase, we examined SNPs that showed nominally statistically significant associations ($p < 0.05$) with PDAC risk. For the validation phase, we used the summary results that were calculated in the PanScan-I and II projects, in meta-analysis with our phase 1 data. Of the 29 initial SNPs, 10 had been genotyped in PanScan, whereas 19 were imputed. Imputation was performed using the 1,000 genomes reference dataset (1000G, Version 3, December 2012) (http://www.1000 genomes.org/) and IMPUTE2.[35] All 19 SNPs had quality scores (IMPUTE2 information score) >0.5. The significance threshold of the final analysis was adjusted, taking into account an estimate of the effective number of tests carried out as follows: because residual LD was possible, for each locus, we calculated the effective number of independent SNPs, M_{eff}, using the SNP Spectral Decomposition approach (simpleM method).[36] The study-wise M_{eff} obtained was 18. Additionally, we corrected for the different inheritance models tested (allelic, co-dominant and dominant). Thus, the threshold for statistical significance was 9.26×10^{-4} ($0.05/(18 \times 3)$).

Bioinformatic analysis

We used several bioinformatic tools to assess possible functional relevance for the three SNPs showing the most significant associations with risk of pancreatic cancer. RegulomeDB (http://regulome.stanford.edu/)[37] and HaploReg v2B[38] were used to identify the regulatory potential of the region nearby each SNP. Genevar (http://www.sanger.ac.uk/resources/software/genevar/)[39] was used to identify potential associations between the SNP and expression levels of nearby genes (eQTL).

Results

All analyzed SNPs were in Hardy Weinberg equilibrium in controls ($p > 0.05$), with the exception of rs16847897 that was then excluded from the following analysis.

SNP main effects

In the discovery phase, in which we genotyped DNA samples in the PANDoRA consortium, we noted 12 *TERT* and five

Table 2. Associations between pancreatic cancer risk and SNPs in the *TERT* gene regions (Phase 1)

SNP	Alleles (M/m)[1]	Cases/controls[2]			M *vs.* m[3]	p_{allele}	Mm *vs.* MM	p_{het}	mm *vs.* MM	p_{hom}	Mm + mm *vs.* MM	p_{dom}	p_{trend}
		MM	Mm	mm									
rs10069690	C/T	1,025/2,117	643/1,429	127/257	0.95 (0.87–1.05)	0.299	0.91 (0.80–1.03)	0.123	0.99 (0.78–1.25)	0.908	0.92 (0.82–1.03)	0.16	0.8009
rs10078761	A/T	719/1,478	804/1,744	251/569	0.93 (0.85–1.01)	0.081	0.92 (0.81–1.05)	0.199	0.86 (0.72–1.03)	0.111	0.91 (0.80–1.02)	0.107	0.2013
rs13190087	T/G	1,764/3,445	118/154	3/5	1.52 (1.18–1.94)	0.001	1.57 (1.21–2.04)	0.001	1.30 (0.28–6.01)	0.74	1.56 (1.2–2.02)	0.001	0.028
rs2075786	C/T	839/1,767	799/1,689	239/409	1.10 (1.01–1.19)	0.036	1.03 (0.91–1.16)	0.676	1.28 (1.06–1.55)	0.011	1.07 (0.96–1.21)	0.223	0.0769
rs2242652	C/T	1,254/2,551	496/1,170	80/150	0.89 (0.80–0.99)	0.034	0.82 (0.72–0.94)	0.003	1.01 (0.75–1.36)	0.967	0.84 (0.74–0.95)	0.007	0.2155
rs2735948	C/T	564/1,199	861/1,735	403/635	1.13 (1.04–1.23)	0.005	1.03 (0.90–1.18)	0.696	1.30 (1.10–1.54)	0.002	1.10 (0.97–1.25)	0.137	0.0205
rs2736098	G/A	980/1,839	584/1,307	126/251	0.96 (0.87–1.06)	0.381	0.89 (0.78–1.01)	0.08	1.04 (0.81–1.32)	0.778	0.91 (0.81–1.03)	0.147	0.1929
rs2736100	G/T	418/932	861/1,763	445/817	1.11 (1.02–1.21)	0.013	1.09 (0.94–1.26)	0.259	1.24 (1.05–1.47)	0.013	1.14 (0.99–1.30)	0.071	0.008
rs2736109	C/T	664/1,277	740/1,700	242/600	0.91 (0.83–0.99)	0.03	0.87 (0.76–1.00)	0.047	0.84 (0.70–1.01)	0.067	0.86 (0.76–0.98)	0.024	0.0332
rs2736122	C/T	1,099/2,311	671/1,483	114/259	0.95 (0.86–1.04)	0.245	0.96 (0.85–1.08)	0.472	0.88 (0.69–1.11)	0.276	0.94 (0.84–1.06)	0.327	0.3092
rs2853676	G/A	1,012/2,147	712/1,606	136/296	0.95 (0.87–1.05)	0.32	0.92 (0.82–1.04)	0.202	0.96 (0.77–1.21)	0.737	0.93 (0.83–1.04)	0.217	0.2447
rs2853677	A/G	581/1,021	804/1,729	273/659	0.83 (0.76–0.91)	4.3×10^{-5}	0.81 (0.70–0.93)	0.002	0.70 (0.58–0.84)	0.0001	0.78 (0.68–0.88)	0.0001	8.1×10^{-5}
rs2853690	C/T	1,272/2,411	435/908	53/95	0.92 (0.82–1.04)	0.182	0.90 (0.78–1.03)	0.121	0.96 (0.67–1.37)	0.806	0.90 (0.79–1.03)	0.13	0.1892
rs2853691	T/C	866/1,772	695/1,646	141/342	0.89 (0.81–0.98)	0.014	0.87 (0.77–0.98)	0.026	0.82 (0.66–1.02)	0.078	0.86 (0.76–0.97)	0.013	0.0169
rs401681	C/T	497/1,260	923/1,977	437/811	1.15 (1.06–1.25)	0.001	1.17 (1.02–1.34)	0.023	1.32 (1.12–1.55)	0.001	1.21 (1.07–1.38)	0.003	0.0015
rs4246742	A/T	1,265/2,788	491/924	47/90	1.18 (1.06–1.32)	0.004	1.24 (1.09–1.42)	0.001	1.13 (0.77–1.65)	0.524	1.23 (1.08–1.4)	0.002	0.0091
rs4583925	G/A	1,537/3,101	243/386	6/15	1.31 (1.10–1.55)	0.002	1.38 (1.15–1.66)	0.001	0.81 (0.30–2.17)	0.679	1.36 (1.13–1.63)	0.001	0.0051
rs4635969	C/T	1,075/2,482	571/1,302	85/168	1.01 (0.92–1.12)	0.811	0.99 (0.87–1.12)	0.823	1.10 (0.83–1.45)	0.501	1.00 (0.89–1.13)	0.991	0.7027
rs4975605	C/A	505/1,080	783/1,750	326/810	0.93 (0.85–1.01)	0.075	0.99 (0.86–1.14)	0.878	0.85 (0.71–1.01)	0.06	0.94 (0.83–1.08)	0.385	0.2505
rs655475	G/A	1,367/2,653	452/887	38/71	0.99 (0.87–1.11)	0.809	0.97 (0.84–1.11)	0.641	1.07 (0.70–1.63)	0.767	0.97 (0.85–1.11)	0.708	0.6902
rs7705526	C/A	820/1,492	819/1,630	195/406	0.91 (0.83–1.00)	0.045	0.91 (0.80–1.03)	0.147	0.83 (0.68–1.02)	0.076	0.90 (0.79–1.01)	0.07	0.0589
rs7726159	C/A	817/1,472	784/1,641	234/465	0.91 (0.83–0.99)	0.028	0.85 (0.75–0.96)	0.011	0.87 (0.72–1.05)	0.148	0.85 (0.76–0.96)	0.009	0.071

[1]M = major allele (*i.e.*, more common in controls); m = minor allele (less common in controls).
[2]Numbers may not add up to 100% due to genotyping failure, DNA depletion or covariate missing values.
[3]M *vs.* m = quantitative additive (allelic) model; Mm *vs.* MM = heterozygous carriers *vs.* common homozygous; mm *vs.* MM = rare homozygous *vs.* common homozygous; Mm + mm *vs.* MM = heterozygous carriers + rare homozygous *vs.* common homozygous (dominant model). Odds ratio (95% confidence interval). All analyses were adjusted for age at diagnosis/age at recruitment, gender and country of origin.

Table 3. Associations between pancreatic cancer risk and SNPs in the *TERC* gene regions (Phase 1)

SNP	Alleles (M/m)[1]	Cases/controls[2]										
		MM	Mm	mm	M *vs.* m[3]	p_{allele}	Mm *vs.* MM	p_{het}	mm *vs.* MM	p_{hom}	Mm + mm *vs.* MM	p_{dom}	p_{trend}
rs10936599	C/T	1,126/2,152	578/1,395	101/235	0.84 (0.76–0.93)	0.001	0.78 (0.69–0.89)	0.0001	0.82 (0.64–1.06)	0.139	0.79 (0.70–0.89)	0.0001	0.0089
rs10936603	G/T	1,045/2,036	573/1,364	119/280	0.87 (0.79–0.96)	0.005	0.81 (0.71–0.92)	0.001	0.86 (0.68–1.09)	0.205	0.82 (0.73–0.92)	0.001	0.1148
rs11709840	A/C	1,009/1,918	656/1,498	150/346	0.87 (0.80–0.96)	0.004	0.83 (0.73–0.93)	0.003	0.83 (0.67–1.03)	0.085	0.83 (0.73–0.93)	0.001	0.1714
rs12696304	G/C	1,001/1,714	629/1,284	124/262	0.89 (0.81–0.99)	0.025	0.85 (0.75–0.97)	0.015	0.87 (0.68–1.1)	0.244	0.85 (0.76–0.97)	0.012	0.0867
rs16854453	G/A	971/2,092	553/1,387	105/284	0.87 (0.79–0.96)	0.007	0.84 (0.74–0.96)	0.009	0.82 (0.64–1.05)	0.112	0.84 (0.74–0.95)	0.005	0.1399
rs1920116	G/A	1,014/1,935	702/1,486	152/344	0.91 (0.83–1.00)	0.049	0.89 (0.79–1.01)	0.064	0.87 (0.70–1.08)	0.194	0.89 (0.79–1.0)	0.042	0.5162

[1]M = major allele (*i.e.*, more common in controls); m = minor allele (less common in controls).
[2]Numbers may not add up to 100% due to genotyping failure, DNA depletion or covariate missing values.
[3]M *vs.* m = quantitative additive (allelic) model; Mm *vs.* MM = heterozygous carriers *vs.* common homozygous; mm *vs.* MM = rare homozygous *vs.* common homozygous; Mm + mm *vs.* MM = heterozygous carriers + rare homozygous *vs.* common homozygous (dominant model). Odds ratio (95% confidence interval). All analyses were adjusted for age at diagnosis/age at recruitment, gender and country of origin.

TERC SNPs that were nominally associated with pancreatic cancer risk ($p < 0.05$) considering any genotype comparison. The most significant finding was the association of the minor (G) allele of *TERT* rs2853677 with decreased pancreatic cancer risk ($OR_{homozygous} = 0.70$; 95% confidence interval (CI) = 0.58–0.84; $p = 1.1 \times 10^{-4}$; $p_{trend} = 8.1 \times 10^{-5}$). We also confirmed the previously described association between rs401681 and pancreatic cancer risk ($OR_{homozygous} = 1.32$; 95% CI = 1.12–1.55; $p = 1.1 \times 10^{-3}$; $p_{trend} = 1.1 \times 10^{-3}$). The complete results for analysis of the *TERT* SNPs are shown in Table 2. For the *TERC* gene, the most significant association was for the minor allele (T) of rs10936599 and decreased PDAC risk ($OR_{heterozygous} = 0.78$; 95% CI = 0.69–0.89; $p = 10^{-4}$, $p_{trend} = 8.9 \times 10^{-3}$). The complete results for analysis of *TERC* SNPs are shown in Table 3. Supporting Information Table S4 shows stratified analysis divided by country of origin.

As a second step, we performed a meta-analysis between our discovery phase and previously generated PanScan data. We considered associations supported by $p < 9.26 \times 10^{-4}$ as statistically significant. We identified one SNP in the *TERT* gene, rs2853677, which was significantly associated with PDAC risk ($OR_{allele} = 0.85$; 95% CI = 0.80–0.90; $p = 8.3 \times 10^{-8}$). A second SNP in *TERT*, rs2736100, was associated with PDAC risk ($OR_{allele} = 0.90$; 95% CI = 0.85–0.94; $p = 3 \times 10^{-5}$). In addition, we observed another statistically significant association with pancreatic cancer risk in *TERT*: rs2735948 ($OR_{homozygous} = 1.27$; 95% CI = 1.13–1.43; $p = 5 \times 10^{-5}$). We also replicated the association between rs401681 and pancreatic cancer. A tendency for some SNPs to be associated with pancreatic cancer risk only in cohorts or only in case–control studies has already been observed in the context of PanScan.[8,10] Therefore, we performed an additional meta-analysis for rs4583925 excluding the cohorts. This analysis showed that the association with pancreatic cancer risk was stronger in the meta-analysis using only the case–control studies for rs4583925 ($OR_{meta\text{-}case\ controls} = 1.36$; 95% CI = 1.17–1.57; $p = 4.0 \times 10^{-5}$) and for rs13190087 ($OR_{meta\text{-}case\ controls} = 1.41$; 95% CI = 1.17–1.71; $p = 0.0003$). Table 4 shows results for all SNPs that reached study-wise significance ($p < 9.26 \times 10^{-4}$). The results for the meta-analyses of PANDoRA and PanScan for all SNPs that were significant in Phase 1 are shown in Supporting Information Table S1.

The rs2853677 and rs2736100 polymorphisms were moderately linked to each other ($r^2 = 0.53$) and in very low LD with the previously identified rs401681 PDAC risk locus ($r^2 = 0.07$ and $r^2 = 0.01$, respectively). rs4583925 and rs2735948 are not correlated with each other ($r^2 = 0.003$ and $D' = 0.277$), and rs2735948 showed moderate LD with rs401681 ($r^2 = 0.371$ and $D' = 0.663$). The last SNP rs13190087 has a moderate LD with all the other SNPs, and its association with pancreatic cancer risk is probably only a reflection of this (Supporting Information Table S2 shows the LD between the SNPs as calculated by the SNAP software[40]).

Table 4. Polymorphisms significantly associated with pancreatic cancer risk after adjustment for multiple testing

Gene	SNP	Study	OR	95% CI[1]	*p*-value[2]
TERT	rs401681[3]	PANDoRA	1.32	1.12–1.55	0.001
		PanScan I + II	1.40	1.23–1.60	8×10^{-7}
		Meta-analysis	1.37	1.24–1.42	1.9×10^{-9}
TERT	rs2853677[4]	PANDoRA	0.83	0.76–0.91	4.3×10^{-5}
		PanScan I + II	0.86	0.79–0.93	1.2×10^{-4}
		Meta-analysis	0.85	0.80–0.90	8.3×10^{-8}
TERT	rs2736100[4,5]	PANDoRA	0.90	0.83–0.98	0.013
		PanScan I + II	0.90	0.84–0.96	0.0014
		Meta-analysis	0.90	0.85–0.94	3×10^{-5}
TERT	rs4583925[3,6]	PANDoRA	1.38	1.15–1.66	0.001
		PanScan I + II	1.11	0.96–1.30	0.16
		PanScan I + II (case/control studies)	1.32	1.04–1.70	0.02
		Meta-analysis	1.21	1.08–1.36	0.001
		Meta-analysis (case/control studies)	1.36	1.17–1.57	4×10^{-5}
TERT	rs2735948[3]	PANDoRA	1.30	1.10–1.54	0.002
		PanScan II	1.25	1.06–1.47	0.01
		Meta-analysis	1.27	1.13–1.43	5×10^{-5}
TERT	rs13190087[3,5]	PANDoRA	1.57	1.21–2.04	0.001
		PanScan I + II	1.04	0.85–1.27	0.68
		PanScan I + II (case/control studies)	1.26	0.96–1.66	0.099
		Meta-analysis	1.27	0.85–1.90	0.251
		Meta-analysis (case/control studies)	1.41	1.17–1.71	0.0003

[1]CI, confidence interval.
[2]Because no heterogeneity was observed for the selected polymorphisms between the studies, we used a fixed-effects meta-analysis; for every SNP in the meta-analysis, we considered the most significant association observed in Phase 1 (*i.e.*, homozygotes (co-dominant model) for the rare allele for rs401681 and rs2735948, carriers of the rare allele (allelic model) for rs2853677 and rs2736100 and heterozygotes (co-dominant model) for rs4583925).
[3]SNP imputed in PanScan.
[4]SNP genotyped in PanScan.
[5]The reference allele in PANDoRA and in PanScan are inverted; therefore, we changed it in PANDoRA to perform a correct meta-analysis.
[6]Results reported in PanScan[8,10] prompted us to analyze separately the cohorts and case–control studies for all SNPs that, after phase 1, were associated with risk at $p < 0.05$. The complete results are reported in the Supporting Information Table S1.

Possible functional effects

We used several bioinformatic tools to predict possible functional relevance of the SNPs showing the most significant associations. Using Genevar, we observed that the A allele of rs2853677 was associated with increased gene expression of two genes *in cis*: the solute carrier family 6 member 18 (*SLC6A18*) and the zinc finger DHHC domain-containing protein 11 (*ZDHHC11*). These associations ($p = 0.014$), however, were not below the threshold suggested by Genevar for significance ($p < 10^{-3}$). RegulomeDB showed a score of 5, indicating the possible presence of a transcription factor binding motif or a DNase sensitivity peak. For rs4583925, HaploReg suggested the presence of DNase sensitivity peak in pancreatic islets and in pancreatic adenocarcinoma tissues. In addition, this SNPs showed an association, statistically significant, with *ZDHHC11* gene expression ($p = 10^{-4}$). Bioinformatics approaches did not reveal possible functional explanations for rs2735948 (Supporting Information Table S3 shows the results from HaploReg).

Discussion

Multiple independent polymorphic variants in the 5p15.33 region, which includes the *TERT* and the *CLPTM1L* genes, are associated with the development of cancer in various organs.[10,13,19,22,23,25,27,28,41,42] This region is characterized by a low degree of LD, which allows for the possibility that several independent variants might be simultaneously associated with individual cancer sites, as has been shown for lung, prostate and bladder cancer.[20] Thus, we sought to analyze this region in detail in a large-scale study, to determine whether multiple variants associate with risk of pancreatic cancer. Indeed, we report reduced risk associated with the G allele of rs2853677 ($p = 8.3 \times 10^{-8}$). This SNP has previously been associated with glioma in Chinese subjects[43] and with lung cancer in

Japanese subjects,[44] although the allele associated with the increase in risk of the disease is the other one (A), a phenomenon that has been observed for other SNPs of this region. We found this SNP to be independent of rs401681 and rs2736098, the previously identified pancreatic cancer susceptibility loci, as clearly shown by the low LD between them ($r^2 = 0.07$ between rs401681 and rs2853677; $r^2 = 0.23$ between rs2736098 and rs2853677).

In *TERT*, rs2853677 is located in the first intron, a region that may play a role in the regulation of the gene expression, because it lies in a DNase I hypersensitive region. Bioinformatic analysis of rs2853677 using functional data from the Encyclopedia of DNA Elements Project[45] obtained through HaploR, *e.g.*, Regulome DB and Genevar, suggested that the A allele may be associated with increased expression of two genes: the solute carrier family 6 member 18 (*SLC6A18*), a neutral amino acid transporter, and the zinc finger DHHC domain-containing protein 11 (*ZDHHC11*), the function of which is not clear yet. This suggestive association should be validated in an independent sample set.

On the other hand, rs2853677 is associated with leukocyte telomere length (LTL) and in particular the A (risk allele) allele is associated with longer LTL.[46] It is interesting to note that in two recent prospective studies, longer LTL have been shown to be associated with increased risk of pancreatic cancer.[47,48] This is consistent with our finding that the G allele, which is associated with decreased pancreatic cancer risk in our study, is also associated with shorter telomeres in the study by Melin *et al.*[46] Thus, it is possible that the link between rs2853677 and pancreatic cancer occurs *via* the variation of telomere length and, in particular, that the A allele leads to constitutively longer telomeres, which may, in turn, be responsible for the increase in pancreatic cancer risk. On the other hand, in another study, based on a retrospective case–control study, shorter telomeres were associated with increased risk of pancreatic cancer.[49] The functional relevance of the association between rs2853677 and pancreatic cancer is, therefore, currently unclear, and additional research is required.

Another SNP, rs2736100, which has been associated with risk of multiple cancer types,[50] is in moderate LD ($r^2 = 0.538$, $D' = 0.798$) with rs2853677. In our study, rs2736100 shows an association with pancreatic cancer risk ($p = 3.0 \times 10^{-5}$). These two SNPs are very close to each other (678 bp) and the fact that both are strongly associated with the disease but that their clear functional effects cannot be demonstrated opens the possibility that there might be a yet unknown variant that is in LD with both SNPs and underlies the increased risk of the disease.

Another finding of potential significance is the association between the minor allele of rs4583925 and increased pancreatic cancer risk. This SNP is completely independent of both rs401681 and rs2853677, and bioinformatic analysis suggests that this SNP might also be involved in the regulation of *ZDHHC11*. The fact that two pancreatic susceptibility SNPs that are completely independent of each other (rs2853677 and rs4583925) could both influence the expression of the same gene suggests the possible involvement of *ZDHHC11* in pancreatic cancer, although functional studies are needed to validate and better characterize this suggestive association. Moreover, for rs4583925, HaploReg shows that the SNP may lie in a pancreas-specific DNAse sensitivity region. This finding, if confirmed by functional studies, could be of importance in identifying a novel regulatory region for the *TERT* gene.

The major strength of this study is its size, with a total of 5,550 subjects with PDAC and 7,585 control subjects; this is the largest genetic analysis of pancreatic cancer risk published to-date. Additionally, our selection of SNPs provides an extensive coverage of genetic diversity in the regions of interest, because we have represented, through tagging, >90% of common genetic variability in the *TERT* and *TERC* loci. Possible limitations of the study may be the fact that the vast majority of the subjects included were of Caucasian origin, and therefore, we cannot extend the findings to other populations and that patients and controls in PANDoRA were recruited in various centers across Europe, and therefore, there might be some population stratification. Additionally, we used only bioinformatic tools to assess the possible functional effect of the SNPs.

In conclusion, our results suggest that the TERT locus is significantly associated with pancreatic cancer risk, likely through more than one variant. We observed a possible new association between rs2853677 and risk of pancreatic cancer.

However, we were not able to find mechanistic link between the association and the disease apart from a possible role in determination of telomere length, and therefore, our results have to be taken with caution. The next logic step to confirm the findings would be to perform functional studies to characterize the described associations.

Epidemiology

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