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Susceptibility locus for lung cancer at 15q25.1 is not associated with risk of pancreatic cancer

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Abstract

Objectives—Four genome-wide association (GWA) studies have found that variation in a region of strong linkage disequilibrium on the long arm of chromosome 15 (15q24-25.1), containing nicotinic acetylcholine receptor genes, contributes to lung cancer risk. Since cigarette smoking is a major risk factor for developing both lung cancer and pancreatic cancer, we hypothesized that variation in this region may also modify individual susceptibility to pancreatic cancer.

Methods—We conducted a case-control study of 532 patients with pathologically confirmed pancreatic adenocarcinoma and 1046 age-, sex-, ethnicity-, and smoking behavior-matched cancer-free controls.

Results—We found that the two risk single nucleotide polymorphisms (SNPs) reported in the lung cancer GWA studies, rs8034191: A>G and rs1051730: G>A, located in this 15q24-25.1 region, were not associated with risk of pancreatic cancer.

Conclusions—The results of our study suggest that the two SNPs at 15q25.1 do not modify pancreatic cancer risk.

Keywords

nicotinic acetylcholine receptor; single nucleotide polymorphisms; pancreatic cancer; case-control study and chromosome 15

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Conflict of interest statement None declared

1. Introduction

Genetic predisposition to pancreatic cancer has become a subject of intense research. High-penetrance germline mutations in *BRCA1*,¹ *BRCA2*,² *p53*,³ *p16*,^{4,5} *PRSS1*,⁶ *STK11*,^{6,7} and mismatch repair genes¹ explain only a small proportion of cases. Much of the remaining variation in genetic risk is probably explained by combinations of more common, lower-penetrance variants. Recently, four genome-wide association studies for lung cancer identified two single nucleotide polymorphisms (SNPs) rs1051730 and rs8034191 associated with increased lung cancer risk in the same region of the long arm of chromosome 15 (15q24-25.1) containing nicotinic acetylcholine receptor genes.^{8–11}

The two SNPs rs1051730 and rs8034191 mapped to a region of strong linkage disequilibrium within 15q24-25.1. The SNP rs1051730 is a synonymous SNP with a G-to-A substitution at codon 215 of exon 5 of *CHRNA3* (nicotinic acetylcholine receptor alpha subunits 3), while rs8034191 is an A-to-G substitution in intron 2 of *LOC123688* (a hypothetical gene). Both rs1051730 and rs8034191 map to a 100-kb region of strong linkage disequilibrium (LD) on chromosome 15 extending from 76,593,078 base pairs to 76,681,394 base pairs. Four genes map to this region: *CHRNA3*, *CHRNA5*, part of *CHRNA4* (nicotinic acetylcholine receptor alpha subunits 3 and 5a and beta subunit 4) and *PSMA4* (proteasome alpha 4 subunit isoform 1), as well as the hypothetical gene *LOC123688* isoform 1.

Although the SNPs identified above are known to contribute independently or directly to lung cancer risk, it is not known whether they also contribute to risk in other smoking-related cancers. Since genetic variants in *CHRNA3*, *CHRNA5*, and *CHRNA4* may play a role in nicotine dependence^{12–14} and cigarette smoking is one of the major risk factors for developing pancreatic cancer, we hypothesized that these two SNPs may modify individual susceptibility to pancreatic cancer. To test this hypothesis, we conducted a case-control study of 532 patients with pathologically confirmed pancreatic adenocarcinoma and twice as many age-, sex-, and ethnicity-matched cancer-free controls. To minimize confounding effects from cigarette smoking and increase the power to detect genetic effects, we also frequency matched controls to cases according to smoking behavior (never, former, or current).

2. Materials and Methods

2.1 Study subjects

This study included 532 patients with newly diagnosed and histopathologically confirmed primary pancreatic adenocarcinoma. They were consecutively recruited from the Gastrointestinal Center at The University of Texas M. D. Anderson Cancer Center in Houston, Texas from February 1999 to May 2007. Control subjects were 1046 healthy individuals seen for routine care at Kelsey-Seybold Clinics; the largest physician group-practice plan in the Houston Metropolitan area, consisting of 23 clinics and more than 300 physicians. Controls had no cancer history (with the exception of nonmelanoma skin cancer) and were frequency matched to cases by age (± 5 years), sex, and ethnicity. We also frequency matched controls to cases according to smoking behavior (never, former, or current). Informed consent was obtained from all study participants by trained MD Anderson staff interviewers before epidemiological data and blood samples were collected. The study was approved by the MD Anderson Institutional Review Board.

2.2 Genotyping

Genomic DNA was extracted with an AUTOPURE LS Automated DNA Purification Instrument (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. Alternately, DNA was extracted using Qiagen (Chatsworth, Calif) kits.

Genotyping was performed using the Taqman method with a 7900 HT sequence detector system (Applied Biosystems). The primer and probe sequences for each SNP are available upon request. Amplification mixes (5 μ l) contained sample DNA (5 ng), 1 $\times$ TaqMan buffer A, deoxynucleotide triphosphate (200 μ M), MgCl₂ (5 mM), AmpliTaq Gold (0.65 units), each primer (900 nM), and each probe (200 nM). The thermal cycling conditions consisted of 1 cycle for 10 min at 95°C, 40 cycles for 15 s at 95°C, and 40 cycles for 1 min at 60°C. SDS version 2.1 software (Applied Biosystems) was used to analyze end-point fluorescence, in accordance with the allelic discrimination technique. Water control, ample internal controls, and previously genotyped samples were included in each plate to ensure accuracy of the genotyping. In addition, 5% of the samples were randomly selected and run in duplicate on each genotyping assay with no observed discrepancy in the genotypes of the duplicate samples.

2.3 Statistical analysis

A χ^2 test was used to assess the differences between cases and controls with regard to categorical variables such as sex and smoking status. Student's *t* test was used to test for differences between the cases and controls for continuous variables. Hardy-Weinberg equilibrium (HWE) for each SNP was tested using a goodness-of-fit test. Unconditional logistic regression analysis was performed to estimate odds ratios (ORs) and the 95% confidence intervals (CIs). Statistical analyses were performed using Stata 10.1 (Stata Corporation, College Station, TX). Haplotypes were inferred using the expectation-maximization algorithm implemented in the HelixTree software (Golden Helix, Bozeman, MT). Haplotype and diplotype frequencies were analyzed using the HelixTree Software. All *P* values were two sided and *P* $\leq$ 0.05 was considered the threshold of statistical significance.

3. Results

3.1 Subject characteristics

There were a total of 1,569 study subjects recruited for this study with 532 pancreatic cancer patients and 1046 cancer-free controls (Table 1). The population consisted of 1461 Caucasians (93.1%), 51 Hispanics (3.3%), 36 African Americans (2.3%), and 21 others (1.3%). There were more males (63%) than females in this study. There was no statistically significant difference in the distributions of age, sex, ethnicity, and smoking status between cases and controls, suggesting that our frequency-matching on these variables was satisfactory.

3.2 Genotypes and cancer risk

Genotype distributions of the two SNPs in this study were in agreement with that expected under the Hardy-Weinberg equilibrium in the cases and controls, (all *P* > 0.55). The distribution of the genotype frequencies between cases and controls is shown in Table 2. The two SNPs rs8034191: A>G and rs1051730: G>A, were not associated with risk of pancreatic cancer, when the heterozygous or homozygous variant genotypes were compared with the homozygous wild-type genotypes in the additive or dominant models (Table 2). As shown in Table 3, when we stratified the groups by smoking status, there was no evidence for an association between cancer risk and the two SNPs within the strata.

3.3 Haplotypes, diplotypes and cancer risk

Although rs8034191 and rs1051730 are separated by 88 kb, significant linkage disequilibrium was found between the two SNPs ($R^2 = 0.83$, $D' = 0.923$, $P < 0.0001$). There were four possible haplotypes derived from the two known genotypes, but none was

significantly associated with risk of pancreatic cancer (data not shown). Likewise, no diplotype was associated with risk of pancreatic cancer (data not shown).

4. Discussion

In this case-control study of pancreatic cancer, we found that two SNPs rs1051730 and rs8034191 in the region of 15q25.1 containing nicotinic acetylcholine receptor genes (CHRNA3 and CHRNA5) were not associated with risk of pancreatic cancer. Furthermore, none of the haplotypes or diplotypes was significantly associated with risk of pancreatic cancer. On the basis of our findings, we conclude that the SNPs rs1051730 and rs8034191 do not modify individual susceptibility to pancreatic cancer

These two SNPs were reported to be associated with increased lung cancer risk in genome-wide association studies.⁸⁻¹¹ Amos and colleagues analyzed 315,450 tagging SNPs in 1,154 current and former smoking cases of European ancestry and 1,137 frequency-matched, ever-smoking controls from Texas and replicated in an additional 711 cases and 632 controls from Texas and 2,013 cases and 3,062 controls from the UK. Their study indicated that the candidate SNPs and smoking have independent effects on lung cancer risk.⁸ Liu *et al* identified the association between these SNPs on the q arm of chromosome 15 and the increased risk of familial lung cancer in a small case-control study (case=194, control=219).¹⁰ No association between smoking quantity and the high-risk alleles was found. The small sample size may have limited the ability to detect this association. Hung and colleagues analyzed 317,139 SNPs in 1,989 lung cancer cases and 2,625 controls from six central European countries and replicated in an additional 2,513 lung cancer cases and 4,752 controls. They observed an association between the candidate SNPs and increased lung cancer risk in never-smokers and ever-smokers. There was no association between these SNPs and any of the measures of nicotine addiction.⁹ However, Thorgeirsson *et al* suggested that the association between rs1051730 and lung cancer risk is mediated through nicotine dependence.¹¹ Recently, Spitz *et al* found a statistically significant association of the variants with both nicotine dependence as well as lung cancer risk.¹⁵ They did not find evidence that the variants were associated with elevated risk in 547 never-smoking lung cancer case subjects. They suggested that the variants may be associated with nicotine dependence and also have a direct role in lung carcinogenesis.

We did not observe any significant change in risk of pancreatic cancer associated with rs1051730 or rs8034191. With the given sample size of our study, we had ample power (>80%) to detect odd ratios as low as 1.25 at a significance level of 0.05. As discussed above, all of these studies on lung cancer observed an association between these SNPs and increased lung cancer risk, although they differ on whether the association is direct or mediated through smoking behavior. Our results suggest that the SNP effect may be cancer specific. Hung and colleagues also analyzed rs8034191 and rs16969968 (a marker in strong linkage disequilibrium with rs1051730) in two separate studies of head and neck cancer and observed no effect in either of the two studies separately or combined or in any of the cancer subgroups;⁹ likewise Spitz *et al* did not find evidence that the variants were associated with elevated risk in other smoking-related cancers (bladder and renal),¹⁵ further suggesting that the effect is cancer specific. The nicotinic acetylcholine receptor genes have a defined role in nicotine dependence and have been reported to have a direct role in downstream signaling pathways that promote lung carcinogenesis.^{16,17} Pancreatic cancer may have adopted a different carcinogenic pathway.

Interestingly, in HapMap data, these two SNPs are extremely rare in Asians. Wu *et al* also confirm that the two SNPs were very rare in Chinese population by conducting a case control study consisting of 1,152 cases with lung cancer and 1,152 controls. Furthermore,

these SNPs were not significantly associated with risk of lung cancer in Chinese population.¹⁸ The study underscored the difference in genetic markers among different ethnic populations.

These observations highlight different effects of the causal gene in this region on disease risks for cancer and behavioral traits, although it is possible that the locus influencing susceptibility to lung cancer is not the same as that responsible for the effect on pancreatic cancer risk.

In conclusion, we have studied the two SNPs, rs1051730 and rs8034191, and found no evidence for association with risk of pancreatic cancer. Our results in combination with those of other studies indicate that these genetic variants may play no or only a minor role as modifiers of pancreatic, bladder, renal, and head and neck cancer risks. Furthermore, they may play different roles in different ethnic groups.

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Reference

1. Lal G, Liu G, Schmock B, et al. Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. *Cancer Res.* 2000; 60:409–416. [PubMed: 10667595]
2. Goggins M, Schutte M, Lu J, et al. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res.* 1996; 56:5360–5364. [PubMed: 8968085]
3. Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat.* 2003; 21:313–320. [PubMed: 12619118]
4. Bartsch DK, Sina-Frey M, Lang S, et al. CDKN2A germline mutations in familial pancreatic cancer. *Annals of surgery.* 2002; 236:730–737. [PubMed: 12454511]
5. Goldstein AM. Familial melanoma, pancreatic cancer and germline CDKN2A mutations. *Hum Mutat.* 2004; 23:630. [PubMed: 15146471]
6. Lochan R, Daly AK, Reeves HL, et al. Genetic susceptibility in pancreatic ductal adenocarcinoma. *The British journal of surgery.* 2008; 95:22–32. [PubMed: 18076020]
7. Su GH, Hruban RH, Bansal RK, et al. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. *Am J Pathol.* 1999; 154:1835–1840. [PubMed: 10362809]
8. Amos CI, Wu X, Broderick P, et al. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat Genet.* 2008; 40:616–622. [PubMed: 18385676]
9. Hung RJ, McKay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature.* 2008; 452:633–637. [PubMed: 18385738]
10. Liu P, Vikis HG, Wang D, et al. Familial aggregation of common sequence variants on 15q24-25.1 in lung cancer. *J Natl Cancer Inst.* 2008; 100:1326–1330. [PubMed: 18780872]
11. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature.* 2008; 452:638–642. [PubMed: 18385739]
12. Berrettini W, Yuan X, Tozzi F, et al. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Molecular psychiatry.* 2008; 13:368–373. [PubMed: 18227835]
13. Saccone SF, Hinrichs AL, Saccone NL, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet.* 2007; 16:36–49. [PubMed: 17135278]

14. Weiss RB, Baker TB, Cannon DS, et al. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. *PLoS genetics*. 2008; 4:e1000125. [PubMed: 18618000]
15. Spitz MR, Amos CI, Dong Q, et al. The CHRNA5-A3 region on chromosome 15q24-25.1 is a risk factor both for nicotine dependence and for lung cancer. *J Natl Cancer Inst*. 2008; 100:1552–1556. [PubMed: 18957677]
16. Lam DC, Girard L, Ramirez R, et al. Expression of nicotinic acetylcholine receptor subunit genes in non-small-cell lung cancer reveals differences between smokers and nonsmokers. *Cancer Res*. 2007; 67:4638–4647. [PubMed: 17510389]
17. West KA, Brognard J, Clark AS, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *The Journal of clinical investigation*. 2003; 111:81–90. [PubMed: 12511591]
18. Wu C, Hu Z, Yu D, et al. Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. *Cancer Res*. 2009; 69:5065–5072. [PubMed: 19491260]

Table1

Selected characteristics of cases and controls

Variables	Cases N=523 (%)	Controls N=1046 (%)	P-value
Ethnicity			1.00
Caucasian	487 (93.1)	974 (93.1)	
Hispanic	17 (3.3)	34 (3.3)	
African American	12 (2.3)	24 (2.3)	
Other	7 (1.3)	14 (1.3)	
Sex			1.00
Men	324 (62.0)	648 (62.0)	
Women	199 (38.0)	398 (38.0)	
Age, Mean (SD)	60.8 (10.2)	61.0 (10.4)	0.77
Smoking status			1.00
Never	221 (42.2)	442 (42.2)	
Former	219 (41.9)	438 (41.9)	
Current	83 (15.9)	166 (15.9)	

Table2

Genotype frequencies in cases and controls and odds ratio estimates for pancreatic cancer

Genotypes	All subjects		OR (95% CI)	P value	Caucasian		OR (95% CI)	P value
	Cases N =523	Controls N =1046			Cases N =487	Controls N =974		
rs8034191								
AA	244	484	reference		222	433	reference	
AG	223	461	0.96 (0.77–1.20)	0.715	212	442	0.94 (0.74–1.18)	0.570
GG	56	101	1.10 (0.77–1.58))	0.605	53	99	1.04 (0.72–1.51)	0.819
AG/GG	279	562	0.98 (0.80–1.22)	0.886	265	541	0.96 (0.77–1.19)	0.682
rs1051730								
GG	240	479	reference		215	427	reference	
AG	225	462	0.97 (0.78–1.21)	0.802	216	445	0.96 (0.77–1.21)	0.756
AA	58	105	1.10 (0.77–1.57)	0.591	56	102	1.09 (0.76–1.57)	0.642
AG/AA	283	567	1.00 (0.81–1.23)	0.971	272	547	0.99 (0.79–1.23)	0.911

Table3

Genotype frequencies in cases and controls and odds ratio estimates for pancreatic cancer

Genotypes	Non-smokers		OR (95% CI)	P value	Ever-smokers		OR (95% CI)	P value
	Cases N =221	Controls N =442			Cases N =302	Controls N =604		
rs8034191								
AA	102	202	reference		142	282	reference	
AG	99	200	0.98 (0.70–1.38)	0.908	124	261	0.94 (0.70–1.27)	0.698
GG	20	40	0.99 (0.55–1.78)	0.974	36	61	1.17 (0.74–1.85)	0.498
AG/GG	119	240	0.98 (0.71–1.36)	0.912	160	322	0.99 (0.75–1.30)	0.925
rs1051730								
GG	104	193	reference		136	286	reference	
AG	92	205	0.83 (0.59–1.17)	0.295	133	257	1.09 (0.81–1.46)	0.571
AA	25	44	1.05 (0.61–1.82)	0.849	33	61	1.14 (0.71–1.82)	0.591
AG/AA	117	249	0.87 (0.63–1.21)	0.408	166	318	1.10 (0.83–1.45)	0.510