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Association of Breast Cancer Susceptibility Variants with Risk of Pancreatic Cancer

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Abstract

Background—A number of susceptibility genes are common to breast and pancreatic cancer. Recently, several breast cancer susceptibility loci have been identified through genome-wide association studies. Here we evaluated possible associations between these single nucleotide polymorphisms (SNPs) and pancreatic cancer risk.

Methods—Ten SNPs from FGFR2, TOX3, MAP3K1, H19, LSP1, chromosome 8q24, CASP8 and LUM were investigated for associations with pancreatic cancer risk following genotyping in 1143 caucasian individuals with pancreatic adenocarcinoma and 1097 unaffected controls from a clinic-based pancreatic cancer case-control study.

Results—CASP8 rs1045485 (Odds ratio (OR)= 0.78; 95% confidence interval (95%CI), 0.65-0.9; p=0.005) and MAP3K1 rs889312 (OR= 0.85; 95%CI, 0.74-0.97; p=0.017)) showed evidence of association with risk of pancreatic cancer. The CASP8 rs1045485 association was evident in ever-smokers (p=0.002), but not in non-smokers (p=0.55), and the effect was strongest in heavy smokers (OR, 0.52; 95% CI, 0.29- 0.93; p=0.03). In contrast the MAP3K1 rs889312 association was only evident in non-smokers (OR, 0.78; 95%CI, 0.64-0.95; p=0.01). In addition, evaluation of the influence of the ten SNPs on survival detected significant associations between outcome for locally advanced pancreatic cancer cases and both 8q rs6983561 (p=0.045) and LUM rs2268578 (p=0.02).

Conclusion—Association studies in a large pancreatic case-control study indicates that SNPs associated with breast cancer may also be associated with pancreatic cancer susceptibility and survival.

Keywords

SNP; Pancreatic cancer; breast cancer; cancer risk

Introduction

Pancreatic cancer is a common cancer affecting both men and women that ranks fourth as a cause of death from cancer in the USA. Familial clustering or a family history of pancreatic cancer is a significant risk factor for the disease (1, 2), suggesting that susceptibility to

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pancreatic cancer can be inherited. It has been estimated that 10–20% of pancreatic cancers arise due to a significant inherited component (3). Several high penetrance susceptibility genes including *p16* (Familial atypical melanoma mole syndrome) (4), *STK11* (Peutz-Jeghers syndrome) (5), *hMLH1* (hereditary nonpolyposis colon cancer) (6), *FANCC* (7, 8), *PRSS1* (hereditary pancreatitis) (9), BRCA2 (10) and PalB2 (11) have been identified. However, mutations in these genes account for <5% of all pancreatic cancers. This suggests that low and moderately penetrant inherited variants may account in part for the remaining familial risk of pancreatic cancer.

Genome wide association studies (GWAS) and candidate gene studies of large sets of cases and controls have successfully identified a number of low penetrance commonly inherited variants associated with a number of different diseases. Few commonly inherited variants associated with risk of pancreatic cancer have been identified to date, although this will likely change when GWAS for pancreatic cancer are completed. Another method for identifying SNPs associated with pancreatic cancer susceptibility is to evaluate SNPs that predispose to other forms of cancer. This approach is based on the observation that certain SNPs have been associated with risk of more than one cancer. For instance, rs6983561 in the 8q24 region (12) and rs7931342 in the 11q13 region (13) have been associated with breast and prostate cancer, whereas rs2660753 in the 3p12 region has been associated with both prostate and ovarian cancer (13). Furthermore, the involvement of several pancreatic cancer predisposition loci, including BRCA2, p53 and MLH1, in breast and other cancers, suggests that these different types of cancer have common predisposition mechanisms that may include commonly inherited SNPs.

Here we evaluated seven SNPs previously associated with breast cancer risk (14-18) for influence on pancreatic cancer risk using 1,143 caucasian pancreatic cancer cases recruited through a rapid ascertainment protocol and 1,097 Caucasian unaffected controls. In addition, three SNPs from the 8q24 region, including one associated with breast cancer risk alone and two with prostate cancer risk were studied (12,14,19).

Materials and Methods

Pancreatic cancer case-control study

From October 2000 through March 2007, patients with pancreatic adenocarcinoma were consecutively recruited under an ultra-rapid recruitment protocol (recruitment at the time of clinic visit for the initial work up for pancreatic cancer) to a registry during their visit to the Mayo Clinic. Of those approached, 71% consented to participate in the study. A total of 1,203 individuals with pancreatic adenocarcinoma with completed risk factor and family history questionnaires and available blood samples for DNA analysis, representing 62% of all pancreatic adenocarcinoma patients identified at Mayo Clinic during this time period, were selected for genotyping. The median time from initial diagnosis to enrollment on the study was 14 days (25%-75% 2-40 days). The median/mean age at diagnosis for all pancreas adenocarcinoma cases at Mayo Clinic over the same time period was 66/65.6 years, versus 67/65.5 for cases included in this study.

Stage of disease at surgery was abstracted from the medical record and categorized as resectable, locally advanced, metastatic and not specified. When grouping study cases by stage, 29% were resected, 33% locally advanced, and 38% metastatic. In contrast, when grouping all pancreatic cancer cases at Mayo Clinic during the same time period, 24% were resected, 33.5% were locally advanced, and 42.5 % metastatic. Thus a slightly higher proportion of patients undergoing surgery participated in the study. Overall, the participating cases were representative of the overall Mayo Clinic pancreatic adenocarcinoma patient population.

From May 2004 to February 2007, healthy controls were recruited from the General Internal Medicine clinic at Mayo Clinic (Rochester) (20). Peripheral blood was collected for DNA analysis and risk factor questionnaires were administered. For this study, 1,203 controls frequency matched to cases on gender, residence (three-state (MN, WI, IA); five state area (MN, WI, IA, SD, ND); or outside of area), age at recruitment (in 5-year increments), and race/ethnicity were selected.

Study participants provided information about age at initiation and cessation of smoking and the number of packs smoked per day or smoking information was extracted from the participant's medical record. Smoking data were available for 99.7% of study participants. Subjects were categorized as “never smokers” and “ever smokers” (≥100 cigarettes in their lifetime). Ever smokers were further stratified by current and former smoking status and by number of pack-years of smoking (≤20 pack-years, >20-40 pack-years, and >40 pack-years).

Overall survival data was obtained from the medical record death certificates, online resources (Accurint), and direct contact with next of kin. The median survival time was 271 days. This study was approved by the Mayo Clinic Institutional Review Board.

Genotyping

All DNA samples were genotyped in the Mayo Clinic Genotyping Shared Resource on an Illumina Golden Gate® Custom 768-plex OPA panel as part of a combined effort to genotype SNPs in pancreatic cases and controls. Ten SNPs were selected for this study and 758 SNPs were selected by other investigators to address other hypotheses. BeadStudio II software was used to analyze the data and prepare reports. Cases and controls were intermixed on plates. Genotyping was successful for 1,189 cases (1,143 Caucasian) and 1,126 controls (1,097 Caucasian) with average SNP call rate and sample success rates >99%. Forty-seven duplicate pairs displayed 99.9% concordance.

Statistical Analysis

Cases and controls were similar in age but differed in BMI, gender (despite attempted frequency matching), percent of ever-smokers and percent reporting a first degree relative with pancreatic cancer as shown in Supplementary Table 1. All SNPs were in Hardy-Weinberg equilibrium ($p>0.05$). The association between each SNP and disease was assessed using unconditional logistic regression under a log-additive model using SAS (SAS software, version 9.1.2, Cary, North Carolina). Multivariate logistic analyses adjusted for age, gender, smoking status (ever/never), family history of pancreas cancer in a first degree relative (yes/no) and body mass index (BMI) were also performed. These analyses were adjusted for multiple testing using a Bonferroni correction. In addition, analyses were conducted following stratification of cases and controls by gender and extent of smoking (ever/never and pack-years). Associations with overall survival (based on date of diagnosis to date of death or last contact) were assessed using a Cox proportional hazards model adjusted for age at diagnosis, gender and pancreatic cancer stage (resectable, locally advanced and metastatic).

Results and Discussion

Ten SNPs were evaluated for an influence on pancreatic cancer risk in a large pancreatic cancer case-control study in which the cases were recruited through a rapid-ascertainment protocol. Five of the ten SNPs were selected because of associations with breast cancer risk in breast cancer GWAS. These included SNPs in the MAP3K1, LSP1 and H19 loci (14), a SNP in FGFR2 (14,18), and a SNP in the TOX3/TNRC9 locus in the 16q12 region (14,15). In addition, a SNP in CASP8, validated as a protective factor against breast cancer by the

Breast Cancer Association Consortium (BCAC) (16), three SNPs in the 8q24 region associated with breast cancer risk alone (rs13281615) (12,14), or prostate cancer risk (rs6983561, rs13254738) (12,19), and a SNP in LUM identified as a candidate risk factor for breast cancer (17), were studied. Genotypes from a total of 1,143 pancreatic cancer cases and 1,097 unaffected controls were obtained for each of the SNPs.

Results from tests for association are shown in Table 1. Two SNPs showed evidence of significant ($p<0.05$) associations with pancreatic cancer risk under a log-additive model (Table 1). The CASP8 rs1045485 was associated with a decreased risk of pancreatic cancer (OR, 0.79; 95%CI, 0.67-0.94; p=0.008), in keeping with the effect of the SNP on breast cancer risk. The MAP3K1 SNP rs889312 was also associated with a reduced risk of pancreatic cancer (OR, 0.85; 95%CI, 0.75- 0.97; p=0.017). This is the opposite effect to that seen for breast cancer and may reflect a non-specific association or opposite effects on complex signaling pathways in different tissue types. None of the other eight SNPs displayed significant associations with pancreatic cancer. Analyses were repeated using a multivariate model in which ORs were adjusted for age at diagnosis or consent, gender, ever/never smoking, BMI and family history of pancreatic cancer. The effects of these SNPs on risk were not substantially altered when accounting for these covariates, although CASP8 rs1045485 displayed a more highly significant association with pancreatic cancer risk (p=0.0048) (Table 1). Importantly, this SNP maintained significance even after Bonferroni correction for multiple testing. In further exploratory studies, evaluation of gender specific associations detected the strongest association for the CASP8 SNP (OR, 0.72; 95%CI, 0.55-0.93; p=0.011) among females and for the MAP3K1 SNP among males (OR, 0.81; 95% CI, 0.68-0.97; p=0.02) (Table 2). None of the other SNPs displayed gender specific significant associations.

Subsequently, the influence of smoking on these associations was assessed. The CASP8 SNP displayed strongest effects among ever-smokers (OR,0.69; 95%CI 0.55-0.87; p=0.0018), and heavy smokers (Pack-years >=40) (OR, 0.52; 95%CI, 0.29-0.93; p=0.028) (Table 2). In contrast the MAP3K1 SNP displayed the strongest effect among non-smokers (OR, 0.78; 95%CI, 0.64-0.95; p=0.013) (Table 2). Stratification by smoking status also identified significant associations between H19 rs2107425 (OR, 0.82; 95%CI, 0.69-0.98; p=0.033) and risk in ever-smokers and between LUM rs2268578 (OR, 1.31; 95%CI, 1.03-1.68; p=0.03) and risk in non-smokers, despite an absence of significance in the overall case-control study.

Next the influence of the ten SNPs on overall survival among the pancreatic cancer cases was evaluated. A total of 1,030 cases were assessed as a group and also when categorized as resectable (n=304), locally advanced (n=347) or metastatic (n=379) cancers. Analyses showed that MAP3K1 rs889312 was marginally significant (p=0.05) overall, but not in any of the individual categories (Table 3). Similarly, 8q rs6983561 displayed a marginally significant association with overall survival in the locally advanced cases (p=0.045). However, LUM rs2268578 was more significantly associated with outcome in the locally advanced cases (HR, 0.72; 95%CI, 0.55-0.95; p=0.02) (Table 3).

In summary, eight SNPs associated with breast cancer risk and two additional SNPs (from the 8q24 region) associated with prostate cancer risk were evaluated for effects on pancreatic cancer risk in a large case-control study. Two SNPs in the CASP8 and MAP3K1 loci displayed significant associations with pancreatic cancer risk. Importantly the CASP8 rs1045485 retained significance after adjusting for multiple testing. This is consistent with the recently reported association between a -652 6N del polymorphism in CASP8 and a reduced risk of pancreatic cancer in the Han Chinese population (21). It is also interesting to note that both the direction and the strength of the effects were consistent with those

observed in the initial breast cancer studies that identified these SNPs as risk factors for breast cancer. Together these findings suggest that SNPs in high linkage disequilibrium with these SNPs in the CASP8 locus may influence the risk of pancreatic cancer in the general population. Additional studies of pancreatic cancer cases and controls are needed to further establish the relevance of these findings.

Smoking, a major source of carcinogen exposure, is an established risk factor for pancreatic cancer (22). The results from this study indicated that rs1045485 in CASP8 showed the strongest protective effect against pancreatic cancer in ever-smokers. Furthermore, the effect size was highly correlated with the amount of smoking. We speculate that the modification of caspase 8 activity or expression by SNPs in the CASP8 locus may alter the cellular response to smoking. Similarly, the finding that MAP3K1 rs889312 was only significantly associated with risk in non-smokers and that H19 rs2107425 and LUM rs2268578 only exhibited significant effects on risk in the presence and absence of smoking, respectively, suggests that additional studies should be conducted to determine whether these SNPs are important modifiers of the smoking associated risk of pancreatic cancer.

Finally, the influence of the breast cancer associated SNPs on overall survival of pancreatic cancer cases was evaluated. Only MAP3K1 rs889312 showed evidence of even a marginally significant association with survival. This SNP was positively associated with overall survival (Table 3) consistent with a protective influence on risk (Table 1). Similarly, the effects of 8q24 rs6983561 and LUM rs2268578 on survival, albeit in locally advanced cancer patients only, were in keeping with the influence of these SNPs on risk. None of these findings maintained significance after adjustment for multiple testing. These limited findings were not unexpected since none of the SNPs displayed significant associations with breast cancer survival in recent studies by the breast cancer association consortium (23).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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