Published in final edited form as:
Pancreas. 2012 January ; 41(1): 50–53. doi:10.1097/MPA.0b013e3182247625.

Genes related to diabetes may be associated with pancreatic cancer in a population-based case-control study in Minnesota

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Abstract

Objectives—Type 2 diabetes is associated with increased pancreatic cancer risk; however, the nature of this relation is not clear. We examined the link between ten diabetes-related single nucleotide polymorphisms (SNPs) and pancreatic cancer in a case-control study conducted in 1994–98.

Methods—Cases (n=162) were ascertained from hospitals in the Twin Cities and Mayo Clinic, Minnesota. Controls (n=540) from the general population were frequency matched by age, sex and race. Unconditional logistic regression provided odds ratios (OR) of pancreatic cancer and 95% confidence intervals (95% CI).

Results—In a multivariate-adjusted model, a significant association was observed only for rs780094 in the glucokinase regulator (GCKR) gene: ORs for pancreatic cancer were 1.00 for TT; 1.35 (95% CI, 0.71;2.58) for CT and 2.14 (95% CI, 1.12;4.08) for CC genotypes (p-trend=0.01), and did not change after adjustment for diabetes.

Conclusions—This study provides the first evidence that GCKR rs780094, a SNP related to diabetes, may be associated with pancreatic cancer risk. While the results from this analysis are preliminary, there is a biological plausibility for such an association.

Introduction

Pancreatic cancer is ranked as the 4th highest cause of cancer death in the United States with fatality rates almost equal to incidence rates. The vast majority (95%) of all pancreatic cancers arise in exocrine pancreas [1]. The etiology of this cancer is poorly understood, but risk factors include smoking, pancreatitis, meat intake, obesity, diabetes mellitus, and

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Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the Journal's Web site (www.pancreasjournal.com).

genetic factors [1, 2]. A meta-analysis reported that risk of pancreatic cancer is 82% higher for those with versus without diabetes [3]. It is unclear, however, whether diabetes is a cause or a consequence of pancreatic cancer or whether some factors increase the risk for both diabetes and pancreatic cancer. An issue that complicates studying this relation is that up to 30% cases of diabetes are undiagnosed in the United States [4]. To further investigate the link between type 2 diabetes (T2D) and pancreatic cancer, we examined 10 single-nucleotide polymorphisms (SNPs) in or near CDKALI, CDKN2A/B, FTO, GCKR, HHEX, IGF2BP2, KCNJ11, PPARG, SLC30A8, TCF7L2, which are associated with T2D in white Europeans in genome-wide association studies (GWAS) (Table 1) [5–7].

We hypothesized that the T2D risk-raising allele for each SNP is associated with an increased risk of pancreatic cancer in a case-control study in Minnesota (US). These associations may be through diabetes or due to an increase in susceptibility to pancreatic cancer because of abnormalities in beta-cell function.

Materials and Methods

Study design

A detailed description of this case-control study has been published [8, 9]. Briefly, cases (20 years or older) with newly diagnosed pancreatic cancer were recruited from hospitals in the Twin Cities metropolitan area and at the Mayo Clinic in Minnesota, 1994–98. Diagnoses of exocrine pancreatic cancer (*International Classification of Disease for Oncology, third edition, code C25*) were confirmed by pathologist. Because many patients with pancreatic cancer die soon after diagnosis, a rapid case-ascertainment system was used to enroll cases, so that the median number of days between diagnosis and the first contact for the study was 13 days [8]. Controls (20–64 y) were selected by stratified random sampling from the Twin Cities metropolitan area and were frequency matched to cases by age (within 5 years), sex, and race.

Data collection

All subjects were interviewed in-person regarding history of medical conditions, the age of the diabetes onset, insulin use, demographic factors and lifestyle behaviors. Subjects who self-reported a diabetes onset before 30 y of age were not considered as type 2 diabetic. In addition, to exclude diabetics whose disease reflected undiagnosed pancreatic cancer, subjects who had been diagnosed with diabetes within two years before their pancreatic cancer diagnosis (or index dates for controls) were also treated as non-diabetics. Anthropometric characteristics were not measured in participants as cases may have experienced rapid weight loss prior to diagnosis. Questions on previous weight and height in the questionnaire were not asked. The study was reviewed and approved by the University of Minnesota and the Mayo Clinic institutional review boards and written informed consent wasobtained from each participant prior to interview.

Thirty milliliters of venous blood were drawn from each consenting participant. DNA was isolated by phenol-chloroform method and storedat −70°C until further analysis [10]. The SNPs were genotyped using the TaqMan system (Applied Biosystems, Foster City, CA). The percent of missing data for SNPs was <5.3%. For each SNP, there were 10 quality control (QC) samples. Each of these QC samples was genotyped 4 times and 100% consistency was achieved in replicates. Genotyping was conducted for 822 people. Due to small number of non-Caucasians (< 6%), only Caucasians who completed the survey were included in this analysis, resulting in 162 cases and 540 controls.

Statistical analysis

Using the t-test for continuous variables and the chi-square test for categorical variables, we compared demographic, lifestyle and other characteristics between pancreatic cancer cases and controls and between diabetics and non-diabetics. Of note, diabetes cases were identified from both the pancreatic cancer cases and controls.

For each SNP, allele frequencies and departures from Hardy-Weinberg equilibrium (HWE) were determined (chi-square test, df =1). All measured SNPs were in HWE. Because the biological function of most of the polymorphisms is unknown, SNP genotypes were modeled as three-level variables -- homozygous AA, heterozygous AB, and homozygous BB genotype, where the low-risk allele for diabetes was the referent category. Unconditional logistic regression was used to calculate the odds ratios (ORs) of pancreatic cancer and diabetes and 95% confidence intervals (CI) for each SNP. Because heterozygous genotypes often have intermediate effects, a test for linear trend was also conducted. ORs for pancreatic cancer were adjusted for age and sex in model 1 and additionally adjusted for smoking status and pack-years, alcohol, and physical activity in model 2. In addition, we adjusted for history of diabetes and for insulin use to test if the potential effect of genotypes on pancreatic cancer was independent of these traits.

Given the recognized highly significant association of all 10 SNPs with prevalent diabetes in the general population, we chose to use a p-value of $p < 0.05$ as an indicator of a significant SNP association with pancreatic cancer. Statistical analyses were conducted using SAS software (version 9.2; SAS Institute, Cary, NC).

Results

The mean age of pancreatic cancer cases (n=162) was 65.0 y and controls (n=540), 65.7 y; 62% of the cases and 57% of the controls were males. Pancreatic cancer cases were more likely to smoke and to have a lower education level than controls. More cases (n=31; 14%) than controls (n=21; 6%) reported a T2D history (n=52) (see Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/MPA/A95). The age-adjusted OR of pancreatic cancer for diabetes versus non-diabetes was 2.62 (95% CI, 1.45–4.74).

The observed allele frequencies were similar to those reported in GWAS for European populations [5-7, 11, 12], which offers further support for genotyping validity. Table 1 presents ORs of diabetes and pancreatic cancer for each SNP adjusted for age and sex. Two SNPs, CDKALI rs7754840 and TCF7L2 rs12255372, were statistically significantly associated with diabetes. For the remaining SNPs, ORs were similar to those observed in GWAS studies of diabetes [5–7, 11, 12], but did not reach statistical significance, which may be explained by limited power and the nesting of the analysis within a pancreatic cancer case-control study. For pancreatic cancer, a statistically significant association was observed only for GCKR rs780094. Compared with TT genotype, multivariate odds ratios were 1.35 (95% CI, 0.71; 2.58) for CT genotype and 2.14 (95% CI, 1.12; 4.08) for CC genotype (p-trend=0.01) (Table 2). Since the mechanism of the rs780094 polymorphism is unknown, we also examined dominant and recessive models: ORs were 1.46 (95% CI, 0.87; 2.47) and 1.43 (95% CI, 1.00; 2.05), respectively. The associations did not markedly change after adjustment for diabetes (Table 2) or insulin use (not presented). Similar associations were observed in a subset of subjects without diabetes.

Discussion

In this population-based case-control study, there is suggestive evidence that the CC genotype of GCKR rs780094 is associated with increased pancreatic cancer risk compared

to the TT genotype. The test for trend was statistically significant (p-trend = 0.01), adding further evidence of an association. Interestingly, the association was also found among individuals without diabetes.

Previous studies showed that the major C-allele of GCKR rs780094 was associated with higher levels of several metabolic traits: fasting glucose, insulin level, insulin resistance, incident diabetes, and lower C-reactive protein. It was also associated with impaired beta-cell function, lower levels of HDL cholesterol and triglyceride [5, 13–15].

In liver cells and, to a lesser extent, in pancreatic islet cells, GCKR regulates activity of glucokinase (GCK), a susceptibility gene for maturity-onset diabetes of the young [16]. When glucose concentration is low, GCKR produces GCK protein that inhibits GCK activity, and consequently glycolysis [13, 17]. A high degree of inhibition of glucokinase in CC carriers may result in high fasting glucose and higher insulin levels [13], which may have a mitogenic effect on the cells of exocrine pancreas. Thus, GCKR may affect pancreatic carcinogenesis through higher insulin levels and/or diabetes. In our study the association of rs780094 with pancreatic cancer did not markedly change after adjustment for type 2 diabetes, suggesting that the association is not mediated through diabetes or that there are too few diabetics to detect modification of the association by this trait. Mutation in rs780094 may also be associated with reduced beta-cell function originating as early as during pancreas development [18]. The origin of exocrine pancreatic cancer is not unknown. Since several studies demonstrated that exocrine pancreatic cancer may derive from both exocrine ducts and from stem cells within endocrine Langerhans islets and, specifically from beta cells, impairments in beta-cell function may contribute to carcinogenesis of exocrine pancreas [19]. Also, since rs780094 is located in a large haplotype block of 500 kb that includes several other genes, intronic rs780094 may be in linkage disequilibrium with nearby functional variants which could cause pancreatic cancer [15]. Finally, the observed association may be due to chance.

To our knowledge, no other study has examined the GCKR rs780094 in relation to pancreatic cancer. Recently, Murad et al [2011] reported an association of GCK rs1799884 with diabetes and prostate cancer [20]. It was demonstrated that SNPs rs1799884 and rs780094 interact to increase fasting plasma glucose; they are similarly associated with diabetes and beta-cell function [21], and may be involved in a similar way in the development of pancreatic cancer. We did not observe any associations of pancreatic cancer with SNPs other than with GCKR rs780094, which may reflect a true absence of associations or low power to detect modest associations. This is in contrast to the findings of a nested case-control study in smokers that reported an association of PPARG Pro12Ala with pancreatic cancer (OR=1.79 (95%CI, 0.96–3.33)); however, that association may have been solely the result of a subgroup analysis, i.e. the association of PPARG with pancreatic cancer was observed only among high-dose vitamin A users [22].

This study has strengths and limitations. The advantage of measuring SNPs as an exposure is that genes are assorted at random at conception, so there is no question of temporality between exposure and outcome and there is little expectation of confounding. Further, the cases were identified through a rapid case-ascertainment system to avoid mortality bias. All subjects were interviewed in person by trained interviewers. However, as in any case-control study, recall bias could arise because diabetes and other covariates were self-reported. In addition, our study had limited power to detect associations of SNPs with pancreatic cancer risk and type 2 diabetes. Finally, although these SNPs have been specifically identified for testing association with pancreatic cancer, our significance threshold for SNP associations may have been too high given the number of SNPs tested.

In spite of these limitations, this study provides preliminary evidence that variation in GCKR is associated with pancreatic cancer risk. This association should be examined in larger populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Lori Strayer (Division of Epidemiology and Community Health, University of Minnesota, Minneapolis) for her technical assistance during preparation of the manuscript.

Financial support: This study was supported by National Cancer Institute grant RO1-CA-58697. A.E. Prizment was supported as a post-doctoral fellow by training grant T32CA132670 from the National Cancer Institute.

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Pancreas. Author manuscript; available in PMC 2013 January 1.

Table 1

Diabetes-related single-nucleotide polymorphisms (SNP) and their associations with diabetes and pancreatic cancer in the case-control study in Minnesota

Genes	Function	SNP	MAF[a]	OR (95%CI)[a] for diabetes[b]	OR (95%CI)[a] for pancreatic cancer[b]
1. CDKALI	Cyclin dependent kinase regulatory subunit associated protein 1-like 1	rs7754840	C: 0.29	reference 2.10 (1.05;4.19) 3.33 (1.40;7.95) p-trend = 0.004	reference 1.32 (0.90;1.93) 0.93 (0.49;1.78) p-trend = 0.58
CDKN2A/B	Cyclin-dependent kinase inhibitor; tumor suppressor	rs10811661	C: 0.17	reference 1.11 (0.59;2.08) 2.12 (0.45;9.19) p-trend =0.47	reference 1.39 (0.95;2.02) 1.15 (0.37;3.60) p-trend = 0.13
FTO	Fat mass and obesity associated protein	rs8050136	A: 0.40	reference 0.67 (0.33;1.35) 1.61 (0.77;3.35) p-trend =0.32	reference 1.62 (1.08;2.45) 1.22 (0.71;2.08) p-trend = 0.25
GCKR	Glucokinase regulator	rs780094	T: 0.35	reference 0.66 (0.28;1.56) 1.29 (0.58;2.87) p-trend =0.24	reference 1.27 (0.73;2.21) 1.70 (0.97;2.97) p-trend = 0.04
HHEX	Hematopoietically expressed homeo box	rs1111875	C: 0.58	reference 1.19 (0.52;2.73) 1.13 (0.47;2.71) p-trend =0.85	reference 1.21 (0.73;1.99) 1.11 (0.66;1.88) p-trend = 0.81
IGF2BP2[c]	Insulin-like growth factor 2 mRNA binding protein	rs4402960	T: 0.31	reference 1.37 (0.74;2.55) 0.98 (0.35;2.72) p-trend =0.66	reference 0.70 (0.48;1.01) 0.93 (0.52;1.68) p-trend = 0.31
KCNJ11	Integral membrane protein in a K+ signaling channel	rs5219	T: 0.35	reference 1.66 (0.86;3.21) 1.92 (0.78;4.75) p-trend =0.09	reference 1.00 (0.68;1.47) 1.11 (0.63;1.96) p-trend = 0.79
PPARG	Nuclear ligand-dependent transcription factor	rs1801282	G: 0.15	reference 0.38 (0.04;3.51) 1.23 (0.15;9.91) p-trend =0.16	reference 0.62 (0.18;2.12) 0.64 (0.19;2.13) p-trend = 0.84
SLC30A8	Zinc transporter	rs13266634	T:0.28	reference 2.29 (0.52;10.10) 2.07 (0.47;9.12) p-trend =0.66	reference 0.93 (0.47;1.85) 0.74 (0.37;1.48) p-trend = 0.20
TCF7L2[c]	Transcription factor activated in Wnt pathway	rs12255372	T: 0.27	reference 1.53 (0.82;2.87) 2.43 (0.80;5.84) p-trend =0.04	reference 1.17 (0.80;1.79) 0.90 (0.47;1.74) p-trend = 0.83

[a]MAF -minor allele frequency, OR-odds ratio, CI-confidence interval.

[b] Adjusted for age and sex.

[c] Two pairs of SNPs in linkage disequilibrium had been genotyped within TCF7L2 and IGF2BP2: rs7903146 and rs12255372 in TCF7L2 (r^2=0.75) and rs1470579 and rs4402960 in IGF2BP2 (r^2=1.00) [23]. Associations with diabetes and pancreatic cancer for the pairs of SNPs in each gene were very similar, so only one of the two SNPs in each gene was included into the final table. These similar findings further confirm genotyping quality.

Table 2

Odds ratio (OR) and 95% confidence interval (CI) for pancreatic cancer in relation to GCKR rs780094[a] in the case-control study in Minnesota

Geno- type	Cases, n (%)(n=162)	Controls, n (%)(n=531)	OR[b](95CI%)	OR[c] (95CI%)	No diabetes OR[b] (95CI%)
TT	20 (12.4)	92 (17.3)	reference	reference	reference
CT	69 (42.6)	245 (46.1)	1.35 (0.71;2.58)	1.27 (0.65;2.47)	1.34 (0.67;2.66)
CC	73 (45.1)	194 (36.5)	2.14 (1.12;4.08)	2.10 (1.08;4.07)	2.01 (1.09;4.02))
			p-trend =0.01	p- trend =0.01	p- trend =0.03

[a] Nine people have missing data on rs780094

[b] Adjusted for age, sex, smoking status, pack-years, alcohol use, education, and physical activity

[c] Additionally adjusted for diabetes