Research Article

Cancer Prevention Research

Glucose Metabolism Gene Variants Modulate the Risk of Pancreatic Cancer

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Abstract

Long-term type 2 diabetes is a known risk factor for pancreatic cancer (PC). We hypothesized that genetic variants in glucose metabolism modify individual susceptibility to PC, especially those associated with diabetes. We retrospectively genotyped 26 single-nucleotide polymorphisms of 5 glucose metabolism genes: glucokinase (*GCK*), glutamine-fructose-6-phosphate transaminase 1 (*GFPT1*), glucose phosphate isomerase (*GPI*), hexokinase 2 (*HK2*), and O-linked *N*-acetylglucosamine transferase (*OGT*) in a case–control study of PC conducted at MD Anderson during 2004 to 2010. Initial genotyping was conducted in 706 patients with PC and 706 cancer-free controls by using the Sequenom method. A *HK2* genotype (R844K) with low frequency of homozygous variant was further examined in additional 948 patients and 476 controls. In the combined set of 1,654 cases and 1,182 controls, we showed a significant association of the *HK2* R844K GA/AA genotype with reduced PC risk (OR = 0.78; 95% CI, 0.64–0.94; $P = 0.009$) and a significant interaction with diabetes ($P_{interaction} < 0.001$). The *HK2* R844K GA/AA genotype was associated with a reduced risk of PC among nondiabetic individuals (OR = 0.68; 95% CI, 0.56–0.83) but with increased risk among diabetic patients (OR = 3.69; 95% CI, 2.34–5.82). These risk associations remained statistically significant when the analysis was restricted to whites or after exclusion of recent onset diabetes. No significant main effect of other genes or significant interaction of genotype with other risk factors was observed. The findings show a potential role of *HK2* gene, alone or in interaction with diabetes, in modifying the risk of PC. *Cancer Prev Res; 4(5); 758–66. ©2011 AACR.*

Introduction

Pancreatic cancer (PC) is the fourth most common cause of cancer mortality in the United States, with an estimated 43,140 new cases and 36,800 deaths in 2010 (1). Known or potential risk factors for PC include cigarette smoking, obesity, type 2 diabetes mellitus, family history of PC, heavy alcohol consumption, and chronic pancreatitis (2, 3). Diabetes and obesity are contributing factors in more than 30% of the PC cases (4). However, genetic factors that predispose individuals with obesity or diabetes to PC are not defined. A recent genome-wide association study (GWAS) has identified several gene variants, such as *ABO*, *NR5A2*, and *TERT* as PC susceptibility factors (5). Our previous studies using the candidate gene approach have also reported possible associations between genetic variation in antioxidant defense (6), and insulin-like growth factor axis signaling (7) and PC risk. However, there are probably more genetic factors, not yet identified, that contribute to the development of sporadic PC.

Whether mutations in metabolic pathways contribute to the pathogenesis of cancer has been controversial (8). Recent findings linking mutations of the isocitrate dehydrogenase, the succinate dehydrogenase, and the fumarate hydratase genes to several types of human cancer provided evidence that alterations in cellular metabolism contribute to the pathogenesis of human cancer (9). There is great advance in the understanding of how metabolism is tied to growth control and how its disruption contributes to tumorigenesis (10). Altered glucose metabolism is a hallmark of malignancies (11). Tumor cells utilize glycolysis instead of mitochondrial oxidative phosphorylation for glucose metabolism even with a normal oxygen supply (the Warburg effect; ref. 11). Some of the glucose metabolic genes, for example, hexokinase 2 (*HK2*), act as both facilitator and gatekeeper of malignancy (12). Glucose intolerance and diabetes are common manifestations of PC (13). The glucose metabolism pathway plays a crucial role in determining cell fate (14). Whether genetic variation in glucose metabolism affects PC risk is unknown. To fill this

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gap in knowledge, we studied patients with PC and healthy controls to evaluate possible associations with PC risk of 26 single-nucleotide polymorphisms (SNP) of 5 genes that encode for the rate-limiting enzymes in glucose metabolism: glucokinase (*GCK*), also known as hexokinase 4 (*HK4*); glutamine-fructose-6-phosphate transaminase 1 (*GFPT1*); glucose phosphate isomerase (*GPI*); hexokinase 2 (*HK2*); and O-linked *N*-acetylglucosamine transferase (*OGT*). Genetic susceptibility markers could be used to identify high-risk individuals for the primary prevention of PC.

Materials and Methods

Study population and data collection

The study population was selected from a previous case–control study which is conducted at The University of Texas MD Anderson Cancer Center from February 1999 to December 2010 (3). The study design and data collection have been previously described in details (3). Each patient had a pathologically confirmed diagnosis of pancreatic adenocarcinoma which comprises more than 90% of PC. Recruitment of patients was not restricted with respect to age, race/ethnicity, or sex. Control subjects were recruited from the spouses, friends, and genetically unrelated family members of patients with various types of cancer other than lung cancer, head and neck cancer, and gastrointestinal cancer. Cases and controls were frequency-matched by age at the time of enrollment (±5 years), race/ethnicity, and sex. All study participants were residents of the United States and could communicate in English. The recruitment rate of eligible patients and controls was 80.6% and 76.9%, respectively (15). This study was conducted in an initial set of 706 case–control pairs randomly selected from the entire study population. Additional 948 cases and 476 controls were further tested for the *HK2* R844K SNP because of the low frequency of the homozygous variant. The inclusion criteria for this study were available DNA sample and risk factor information. Written informed consent for an interview and a blood sample donation was obtained from each study participant. The study was approved by the Institutional Review Board of MD Anderson Cancer Center and was conducted in accordance with all current ethical guidelines.

The following information was obtained by personal interview: cigarette smoking, alcohol consumption, medical history, family history of PC in first-degree relatives, height and body weight at different age periods. Diabetes was defined by self-reported diagnosis or use of antidiabetic medication and was verified from the medical records of cases. Recent onset of diabetes was defined as diabetes diagnosed 2 years or less before cancer diagnosis for cases or recruitment for controls. Cumulative smoking was calculated in "pack-years" (pack-years = packs smoked per day × years of smoking). Light and heavy smoking were defined as ≤20 pack-years and >20 pack-years, respectively. Alcohol consumption was calculated in grams of ethanol consumed daily; 12.0 oz of beer, 4.0 oz of wine, and 1.5 oz of hard liquor were each considered to be equivalent to approximately 12.0 g of ethanol. Light and heavy alcohol consumption were defined as ≤420 g/wk and >420 g/wk, respectively, averaged over the subject's lifetime of alcohol drinking. Body mass index (BMI, in kg/m^2) was calculated from the participant's self-reported weight and height at age 34 to 39. Our previous study has shown that obesity (BMI ≥ 30) at this age period was associated with the highest risk of PC compared with obesity at all other age periods (4). Because information on BMI was not collected before January 2004, 598 patients and 364 controls had missing values.

DNA extraction, SNP selection, and genotyping

DNA was extracted from peripheral lymphocytes by using the Qiagen DNA isolation kit and stored at 4°C for immediate use. We selected 17 tagging SNPs by using the SNPbrowser (Applied Biosystems, www.allsnps.com/snpbrowser) with a cutoff of $r^2 = 0.8$ and a minor allele frequency (MAF) ≥ 10% in non-Hispanic whites from the HapMap Project database (www.hapmap.org). The higher MAF (≥10%) was selected to ensure adequate statistical power. We also included 9 potentially functional SNPs located in the coding region (synonymous or nonsynonymous) or the untranslational region (UTR) with MAF ≥ 5% in non-Hispanic whites. The genes, chromosome regions, nucleotide substitutions, functions, reference SNP identification numbers, and MAFs of the 26 SNPs are given in Supplementary Table S1. The protein sequences, structures, homology models, mRNA transcripts, and predicted functions for the SNPs were evaluated by F-SNP (Queen's University, Kingston, Canada; ref. 16). For genotyping, we used the mass spectroscopy-based MassArray (Sequenom) method. We randomly selected 20% of the total samples and genotyped them in duplicate, and 99.5% concordance was observed. The inconsistent data were excluded from the final analysis.

Statistical analysis

The distribution of genotypes was tested for Hardy–Weinberg equilibrium with the goodness-of-fit χ^2 test. Genotype frequency and MAF of the SNPs were determined by direct gene counting. We used Pearson's χ^2 test to compare the distributions of categorical variables and genotype frequencies between cases and controls. Haplotype diversity and the linkage disequilibrium index (Lewontin's D' and r^2; ref. 17) were calculated by SNPAlyze (Dynacom) and Haploview 4.2 (Broad Institute, Cambridge, MA) software. We reconstructed the haplotypes by implementing the expectation–maximization algorithm by unphased genotype data (18).

OR and 95% CI were calculated by unconditional logistic regression adjusted for smoking status (never, ≤20 pack-years, and >20 pack-years), alcohol consumption (never, ≤420 g ethanol/wk, and >420 g ethanol/wk), diabetes (yes or no), and family history of cancer in first-degree relatives (yes or no). Because diabetes could be a manifestation of subclinical PC, a total of 173 cases and 30 controls with recent onset diabetes were excluded from

the genotype–diabetes interaction analyses to reduce reversal causality. Because information on BMI was not collected before 2004, 598 cases and 364 controls had missing BMI. BMI was not included in the multivariate model.

We explored potential gene interactions with smoking, alcohol, diabetes, or BMI. For example, for the risk factor of diabetes, nondiabetic individuals without the at-risk genotype were used as the reference group, and adjusted ORs were estimated by using unconditional logistic regression for the following groups: nondiabetic individuals with the at-risk genotype (OR_{10}), diabetic patients without the at-risk genotype (OR_{01}), and diabetic patients with the at-risk genotype (OR_{11}). $OR_{11} > OR_{10} + OR_{01}$ indicates a more-than-additive effect, and $OR_{11} > OR_{10} \times OR_{01}$ indicates a more-than-multiplicative effect.

The cross-product term of genotype with risk factors was generated by using logistic regression models. The significance of the interaction term ($P_{interaction}$) was obtained by the likelihood ratio test; the full model contained the interaction term, the main effect of genotype, and the exposure variable; and the reduced model lacked the interaction term. All statistical analyses were carried out by SPSS and Stata 10.0 (Stata) software. The Bonferroni correction method was used to address multiple comparison (19). A P value of 0.0019 (=0.05/26) was considered statistically significant after adjusting for multiple comparisons.

Results

Characteristics of the study population

The study population's demographics and potential risk factors for PC are given in Table 1. As a result of frequency matching, there were no significant differences between cases and controls in the distribution of sex and race/ethnicity in the original set of 706 case–control pairs. Controls were younger than cases and were underrepresented with minorities in the additional study set. As previously reported, diabetes, heavy smoking, family history of cancer, and obesity (BMI $\geq$ 30 kg/m^2) were independent risk factors for PC in this study population (15). The risk estimates for PC in relation to smoking, alcohol, diabetes, family history of cancer, and BMI were comparable between the original set and the additional set of the study population.

Genotype distribution and allele frequency

The observed MAFs of the 26 SNPs in this study population were comparable to those reported in the general population (Supplementary Table S1). All genotype distributions were in Hardy–Weinberg equilibrium except for *OGT* IVS18-424A>G in cases, and *GCK* IVS6+87A>C, *OGT* IVS8-72G>A, and IVS18-424A>G in controls ($P < 0.05$). Linkage disequilibrium data (D' values) are presented in Supplementary Table S2. As expected, genotype distributions are significantly different between racial/ethnic groups. For example, the frequency of *HK2* Ex17-79G>A (R844K) AA genotype was 40.2% for blacks and 2.4% for whites (data for other SNPs are not shown). Thus, further analysis on the association of genotype/haplotype and risk of PC was conducted in the entire study population with adjustment for race/ethnicity as well as in whites only.

Association of genotype with PC risk

In the 706 case–control pairs, after adjusting for confounders, *HK2* Ex17-79G>A (R844K) GA/AA was associated with decreased risk of PC in all study subjects (OR = 0.76; 95% CI, 0.59–0.97; $P = 0.03$) or in white only (OR = 0.74; 95% CI, 0.57–0.94; $P = 0.02$; Table 2). In the additional 948 cases and 476 controls, the protective trend of the variant allele was observed but the difference was not statistical significant (Table 2). When the data were combined in a total of 1,654 cases and 1,182 controls, the association between *HK2* R844K GA/AA genotype and reduced risk of PC was statistically significant (OR = 0.78; 95% CI, 0.64–0.94; $P = 0.009$). The following genotypes showed nonsignificant associations in the initial set: *HK2* Ex7+62T>C (D251D; $P = 0.05$) and Ex18+407T>G; *GFPT1**4058A>G; and *OGT* IVS18-424A>G and IVS8-72G>A ($P \leq 0.15$; Supplementary Table S3).

Interactions of genotypes with known risk factors

Next, we examined the potential interaction between genotypes and known risk factors: diabetes (no vs. yes), smoking status (nonsmoker vs. smoker), alcohol consumption (nondrinker vs. drinker), and BMI (<25 kg/m^2 vs. $\geq$25 kg/m^2). The heterozygous and homozygous genotypes were combined if the homozygous variant had a very low frequency (number of homozygote < 5) or if the homozygous and heterozygous genotypes exerted a similar effect (increased or reduced the risk) on susceptibility to PC. In the 706 case–control pairs, we observed possible interactions of diabetes with *GCK* IVS1+9652C>T, *GFPT1* Ex19-115G>T, and *HK2* Ex17-79G>A (R844K) in modifying PC risk ($P_{interaction} \leq 0.05$, Table 3). After adjusting for multiple comparisons, only *HK2* R844K had a statistically significant interaction ($P_{interaction} < 0.001$). However, the interaction of *HK2* R844K and diabetes was not confirmed in the additional samples (Table 3). To exclude any experimental error in genotyping, we used Taqman method to confirm the *HK2* R844K genotype in diabetic controls and conducted direct DNA sequencing on 9 samples. Both efforts confirmed the accuracy of the *HK2* R844K genotype (data not shown). When the data of the original and additional samples were combined, the interaction of *HK2* R844K with diabetes remained highly significant before and after exclusion of recent-onset diabetes or minorities (Table 3). We observed a possible interaction of BMI with *HK2* Ex16-78A>G (L766L) and *GPI* IVS9+2363C>G in the original set ($P_{interaction} = 0.04$ and 0.05, respectively; Table 4). No potential interaction of genotype with alcohol consumption or smoking was observed ($P_{interaction}$ >0.05, data not shown).

Association of haplotype with PC risk

Four haplotypes of *GFPT1* and *HK2* were significantly associated with PC risk ($P < 0.05$, Table 5). The associations

Table 1. Characteristics of the study population

Variables	Original set				Additional set				Combined set			
	No. of cases (%)	No. of controls (%)	OR[a] (95% CI)	*P*	No. of cases (%)	No. of controls (%)	OR[a] (95% CI)	*P*	No. of cases (%)	No. of controls (%)	OR[a] (95% CI)	*P*
Total	706 (100)	706 (100)			948 (100)	476 (100)			1,654 (100)	1,182 (100)		
Sex			Matching factor				Matching factor				Matching factor	
Female	282 (39.8)	269 (38.1)			392 (41.4)	217 (45.6)			674 (40.7)	486 (41.1)		
Male	424 (60.2)	437 (61.9)			556 (58.6)	259 (54.4)			980 (59.3)	696 (58.9)		
Race/ethnicity							Matching factor				Matching factor	
White	624 (88.4)	630 (89.2)			806 (85.0)	448 (94.1)			1,430 (86.5)	1,078 (91.2)		
Hispanic	43 (6.1)	46 (6.5)			53 (5.6)	13 (2.7)			96 (5.8)	59 (5.0)		
Black	27 (3.8)	25 (3.5)			69 (7.3)	12 (2.5)			96 (5.8)	37 (3.1)		
Asian/others	12 (1.7)	5 (0.7)			20 (2.1)	3 (0.6)			32 (1.9)	8 (0.7)		
Age at recruitment, y			Matching factor				Matching factor				Matching factor	
Mean ± SD	62.5 ± 10.0	61.1 ± 10.0			61.6 ± 10.1	61.1 ± 10.0			62.0 ± 10.1	61.1 ± 10.0		
<50	77 (10.9)	99 (14.0)			119 (12.6)	101 (21.2)			196 (11.9)	200 (16.9)		
50–60	172 (24.4)	197 (27.9)			265 (28.0)	156 (32.8)			437 (26.4)	353 (29.9)		
60–70	265 (37.5)	251 (35.6)			351 (33.2)	149 (31.3)			616 (37.2)	400 (33.8)		
≥70	192 (27.2)	159 (22.6)			213 (22.5)	70 (14.7)			405 (24.5)	229 (19.4)		
Diabetes												
No	518 (73.4)	627 (88.8)	1.0		706 (74.4)	418 (88.9)	1.0		1,224 (74.0)	1,045 (88.9)	1.0	
Yes	188 (26.6)	79 (11.2)	2.77 (2.04–3.76)	<0.001	242 (25.6)	52 (11.1)	2.77 (2.01–3.82)	<0.001	430 (26.0)	131 (11.1)	2.79 (2.26–3.45)	<0.001
Smoking status												
Nonsmoker	285 (40.4)	360 (51.0)	1.0		401 (42.3)	248 (54.4)	1.0		686 (41.5)	608 (52.3)	1.0	
≤20 pack-years	175 (24.8)	175 (24.8)	1.32 (0.99–1.76)	0.057	230 (24.3)	97 (21.3)	1.44 (1.08–1.91)	0.01	405 (24.5)	272 (23.4)	1.31 (1.09–1.58)	0.005
>20 pack-years	246 (34.8)	171 (24.2)	1.68 (1.27–2.22)	<0.001	316 (33.4)	111 (24.3)	1.79 (1.37–2.34)	<0.001	562 (34.0)	282 (24.3)	1.77 (1.48–2.12)	<0.001
Alcohol consumption[b]												
Nondrinker	319 (46.9)	325 (46.2)	1.0		402 (41.6)	185 (40.5)	1.0		721 (43.8)	510 (44.0)	1.0	
≤420 g/wk	284 (41.8)	324 (46.1)	1.05 (0.81–1.35)	0.72	459 (47.5)	235 (51.4)	0.86 (0.68–1.09)	0.21	743 (45.1)	559 (48.2)	0.94 (0.80–1.09)	0.41
>420 g/wk	77 (11.3)	54 (7.7)	1.44 (0.94–2.20)	0.09	106 (10.9)	37 (8.1)	1.32 (0.88–2.00)	0.18	183 (11.1)	91 (7.8)	1.41 (1.07–1.86)	0.01
0–420 g/wk vs. >420 g/wk			1.48 (1.03–2.13)	0.03			1.43 (0.97–2.12)	0.07			1.46 (1.12–1.90)	0.005
Family history of cancer[c]												
No	262 (37.3)	318 (45.4)	1.0		360 (38.2)	226 (49.6)	1.0		622 (37.8)	544 (47.1)	1.0	
Yes	441 (62.7)	382 (54.6)	1.56 (1.24–1.96)	<0.001	583 (61.8)	230 (50.4)	1.58 (1.26–1.98)	<0.001	1,024 (62.2)	612 (52.9)	1.57 (1.26–1.91)	<0.001
BMI[d], kg/m^2												
<25	188 (51.8)	254 (59.8)	1.0		357 (51.4)	242 (61.6)	1.0		545 (51.6)	496 (60.6)	1.0	
25–30	130 (35.8)	144 (33.9)	1.22 (0.90–1.65)	0.20	254 (36.6)	122 (31.0)	1.41 (1.08–1.85)	0.01	384 (36.3)	266 (32.5)	1.31 (1.08–1.60)	0.007
≥30	45 (12.4)	27 (6.4)	2.25 (1.35–3.76)	0.002	83 (12.0)	29 (7.4)	1.94 (1.23–3.05)	0.004	128 (12.1)	56 (6.8)	2.08 (1.49–2.91)	<0.001

[a]OR was adjusted for sex, race, age, diabetes, smoking, alcohol consumption, BMI, and family history of cancer.

[b]Information was missing for 26 cases and 3 controls.

[c]Information was missing for 12 cases and 7 controls.

[d]Information was available for only 906 cases and 798 controls.

Table 2. Association of HK2 R844K genotype with PC risk

Genotype	Original set			Additional set			Combined set		
	Cases/controls (%)	OR[a] (95% CI)	*P*	Cases/controls (%)	OR[a] (95% CI)	*P*	Cases/controls (%)	OR[a] (95% CI)	*P*
All study subjects									
GG	61.7/55.2	1.0		58.9/55.7	1.0		60.2/55.7	1.0	
GA	34.5/39.6	0.77 (0.60–0.99)	0.04	37.2/40.8	0.87 (0.63–1.21)	0.42	35.9/39.7	0.79 (0.65–0.97)	0.02
AA	3.8/5.2	0.69 (0.40–1.19)	0.18	3.9/3.6	0.60 (0.28–1.28)	0.19	3.9/4.5	0.64 (0.42–0.98)	0.04
GG vs. GA/AA		0.76 (0.59–0.97)	0.03		0.85 (0.62–1.18)	0.34		0.78 (0.64–0.94)	0.009
White only									
GG	64.1/57.0	1.0		63.5/57.1	1.0		63.8/57.0	1.0	
GA	33.0/39.2	0.74 (0.56–0.97)	0.03	35.7/40.4	0.91 (0.65–1.29)	0.59	34.5/39.7	0.76 (0.62–0.94)	0.01
AA	2.9/3.8	0.74 (0.39–1.40)	0.35	0.7/2.5	0.39 (0.14–1.13)	0.08	1.7/3.3	0.52 (0.30–0.90)	0.019
GG vs. GA/AA		0.74 (0.57–0.96)	0.02		0.88 (0.63–1.24)	0.46		0.74 (0.61–0.91)	0.004

[a]OR was adjusted for sex, race, age, diabetes, smoking, alcohol consumption, and family history of cancer.

Table 3. Effect of interaction of genotype with diabetes on PC risk

Genotype	Diabetes	No. of cases/controls	OR (95% CI)		
			Model A[a]	Model B[b]	Model C[c]
GCK IVS1+9652C>T					
CC	No	363/410	1.0	1.0	1.0
CT/TT	No	152/203	0.92 (0.70–1.20)	0.91 (0.70–1.19)	0.90 (0.68–1.18)
CC	Yes	128/62	2.26 (1.59–3.22)	1.63 (1.07–2.50)	2.20 (1.52–3.20)
CT/TT	Yes	58/16	4.23 (2.34–7.67)	3.69 (1.74–7.86)	4.50 (2.27–8.92)
$P_{interaction}$			0.04	0.05	0.036
GFPT1 Ex19–115G>T					
TT	No	187/226	1.0	1.0	1.0
GT/GG	No	331/371	1.07 (0.83–1.39)	1.08 (0.83–1.40)	1.07 (0.67–1.13)
TT	Yes	119/59	2.33 (1.58–3.43)	1.78 (1.12–2.82)	2.19 (1.49–3.23)
GT/GG	Yes	69/19	4.19 (2.38–7.36)	3.13 (1.60–6.12)	3.44 (1.87–6.30)
$P_{interaction}$			0.05	0.09	0.03
HK2 Ex16–78A>G (L766L)					
GG	No	206/228	1.0	1.0	1.0
GA/AA	No	312/392	0.96 (0.74–1.24)	0.96 (0.74–1.24)	0.92 (0.71–1.18)
GG	Yes	61/35	2.01 (1.25–3.26)	1.26 (0.70–2.28)	1.75 (1.06–2.86)
GA/AA	Yes	127/43	3.27 (2.15–4.98)	2.83 (1.72–4.67)	3.43 (2.16–5.43)
$P_{interaction}$			0.09	0.01	0.09
HK2 Ex17–79G>A (R844K)[d]					
GG	No	720/550	1.0	1.0	1.0
GA/AA	No	502/490	0.68 (0.56–0.83)	0.66 (0.54–0.80)	0.65 (0.53–0.81)
GG	Yes	274/103	1.85 (1.43–2.39)	1.44 (1.07–1.95)	1.33 (0.99–1.87)
GA/AA	Yes	155/26	3.69 (2.34–5.82)	2.67 (1.59–4.47)	2.12 (1.25–3.61)
$P_{interaction}$			<0.001	<0.001	<0.001

[a]OR was adjusted for sex, race, age, smoking, alcohol consumption, and family history of cancer.
[b]Recent-onset diabetes (duration <2 years) was excluded from the model.
[c]Analysis was conducted among whites only.
[d]Analysis was conducted in combined (original + additional) set.

represented the effect of the *GFPT1* *4058A>G, IVS14-3094T>C, IVS12-1764C>T, and Ex19-115G>T; and the *HK2* Ex17-79G>A (R844K) genotypes on PC risk. After adjusting for multiple comparisons, 2 of the 4 haplotypes remained significant risk predictors ($P \leq 0.001$).

Discussion

In this study, we observed a weak protective effect and a significant interaction of a *HK2* nonsynonymous coding SNP (R844K) with diabetes in affecting PC risk. To the best of our knowledge, this is the first study to show a potential role of glucose metabolism gene variants in modulating susceptibility to PC.

HKs catalyze the phosphorylation of glucose to form glucose-6-phosphate (G6P), which is the first, rate-limiting step in glucose metabolism. HK2, which localizes to the outer mitochondrial membrane, is the major HK isoform expressed in cancer cells (20). HK2 works with 4 key protein partners to ensure rapid and efficient production of G6P. G6P serves as the precursor not only for glycolysis but also for the biosynthesis of key metabolites via the pentose phosphate pathway and the mitochondrial tricarboxylic acid cycle, both essential for the growth and proliferation of cancer cells (20). In addition, binding of HK2 to the voltage-dependent anion channel 1 inhibits mitochondria-induced apoptosis and suppresses cell death (21). HK2 is not expressed in most normal mammalian tissues. However, in the early stages of liver and pancreas tumorigenesis, HK2 is expressed and HK4 (GCK) expression is silenced because the affinity of HK2 for glucose is approximately 250 times that of HK4 (21). Overexpression of *HK2* induced by AMP-activated protein kinase and hypoxia-inducible factor 1 alpha subunit has been suggested to play a pivotal role in pancreatic carcinogenesis (22).

In this study, we observed a protective effect of *HK2* variants alone or a differential effect in interaction with diabetes on PC risk. Nondiabetic individuals carrying the variant K allele of the *HK2* R844K had significantly decreased PC risk whereas diabetic patients carrying the

Table 4. Joint effect of genotype with BMI on PC risk

Genotype	BMI (kg/m^2)	No. of cases/controls	OR[a] (95% CI)	
			Model A[b]	Model B[c]
HK2 Ex16–78A>G (L766L)				
GG/GA	<25	174/216	1.0	1.0
AA	<25	14/32	0.69 (0.56–0.85)	0.67 (0.55–0.83)
GG/GA	≥25	146/148	0.85 (0.25–2.85)	0.83 (0.22–2.80)
AA	≥25	29/22	2.12 (1.64–2.74)	2.07 (1.61–2.71)
$P_{interaction}$		0.04	0.05	
GPI IVS9+2363C>G				
CC/GC	<25	183/237	1.0	1.0
GG	<25	5/7	1.65 (1.20–2.27)	1.60 (1.12–2.20)
CC/GC	≥25	161/158	3.28 (1.16–9.24)	3.01 (1.14–8.99)
GG	≥25	13/6	4.95 (2.63–9.30)	4.89 (2.56–9.12)
$P_{interaction}$			0.05	0.05

[a]OR was adjusted for sex, race, age, smoking, diabetes, alcohol consumption, and family history of cancer.
[b]Analysis was conducted in all subjects.
[c]Analysis was conducted among whites only.

K allele had increased risk of PC. *HK2* R844K is located at the C terminus of HK2 protein and is an evolutionarily conserved nonsynonymous coding SNP. The K variant is predicted to deleteriously change the solvent accessibility and hydrophobicity of the protein and to regulate RNA splicing (16). Thus, the K variant may confer a dysfunctional HK2 enzyme or lower its activity, decrease the glycolysis rate, and, because an energy supply is lacking, dampen tumor development. This notion is also supported by our previous finding that the K variant was associated with better overall survival in patients with PC (23). Notably, in this study we found that the K variant was associated with increased PC risk in diabetic patients. We speculate that lower activity of HK2 in diabetic patients may aggravate hyperglycemia and insulin resistance-mediated hyperinsulinemia, which together increase the risk of carcinogenesis. We also found that a haplotype containing the *HK2* R844K was significantly associated with reduced PC risk. Further study is warranted to show the functional significance of these SNPs and to confirm our findings in other study populations.

We also observed a possible association between *GFPT1* haplotypes containing the 3′-UTR Ex19-115G>T G allele and an increased PC risk. *GFPT1* encodes the enzyme that catalyzes the first, rate-limiting step in the hexosamine biosynthesis pathway (HBP) and controls the glucose flux into the HBP. This pathway shuttles glucose to cellular glycosylation (24). Glucose flux into the HBP initiates

Table 5. Association of haplotype with PC risk

Haplotype[a]	Frequency in cases/controls	OR[b] (95% CI)	*P*[b]	OR[c] (95% CI)	*P*[c]
GFPT1					
GTTT	0.4143/0.4328	1.0		1.0	
GTTG	0.0377/0.0121	3.33 (1.79–6.18)	<0.001	4.14 (2.38–8.17)	<0.001
ACTG	0.0167/0.0090	2.67 (1.00–7.09)	0.048	2.85 (1.14–6.65)	0.03
ACCG	0.0059/0.0034	12.5 (1.52–100)	0.018	11.8 (1.49–90.9)	0.02
HK2					
TG	0.6585/0.6269	1.0		1.0	
TA	0.1255/0.1683	0.67 (0.53–0.85)	<0.001	0.65 (0.52–0.83)	<0.001

NOTE: OR was adjusted for sex, race, age, diabetes, smoking, alcohol consumption, and family history of cancer.
[a]Haplotype of *GFPT1*: *4058A>G, IVS14-3094T>C, IVS12-1764C>T, and Ex19-115G>T; *HK2*: Ex7+62T>C (D251D), and Ex17-79G>A (R844K). Haplotypes with $P > 0.05$ are not shown.
[b]Analysis was conducted in all subjects.
[c]Analysis was conducted among whites only.

posttranslational modification of nuclear and cytoplasmic proteins to regulate transcription, protein degradation, and signal transduction (25). Thus, altered GFPT1 enzyme activity could modify the risk of cancer by affecting these important cellular functions. The 3′-UTR variant may harbor sequence motifs critical for regulating transcription, mRNA stability, mRNA cellular localization, or microRNA targeting (26).

In this study, we observed a possible interaction of *GCK* IVS1+9652C>T with diabetes on PC risk. GCK (HK4), a member of the HK family, catalyzes the ATP-dependent phosphorylation of glucose. In contrast to other HKs, GCK is not inhibited by its product G6P, but remains active when glucose is abundant. GCK maintains glucose homeostasis by functioning as the glucose sensor and glycolysis pacemaker in regulating insulin secretion (27). *GCK* was associated with type 2 diabetes in a recent GWAS (28). The *GCK* −515G>A glucose-raising allele alone was reported to be associated with reduced β-cell function (29) and to interact with glucose-6-phosphatase catalytic subunit 2 to exert an additive effect that increased fasting glucose level and decreased insulin secretion (30). In our study, the *GCK* IVS1+9652C allele is in linkage with the −515A allele (Supplementary Table S2). We found that the IVS1+9652T allele was associated with decreased PC risk in nondiabetic individuals but increased PC risk in diabetic patients. We previously reported that the *GCK* IVS1+9652T allele was associated with reduced overall survival in patients with PC (23). The *GCK* −515G>A allele was reported to be associated with a higher fasting glucose level (29). However, we did not observe any significant association of −515G>A with PC risk. The interaction of IVS1+9652C>T with diabetes in increasing PC risk that we observed may have been due to chance.

Although the number of study participants with BMI information was limited, we did observe a weak interaction of *GPI* IVS9+2363C>G CG/GG with BMI in increasing PC risk ($P_{interaction} = 0.05$). GPI catalyzes the reversible isomerization of G6P and fructose-6-phosphate, and plays a role in glycolysis and gluconeogenesis. GPI guides the glucose flux into the pentose phosphate pathway, which produces pentose and NADPH (31). GPI also acts as an autocrine motility factor secreted by the tumor cells to promote tumor progression, migration, and metastasis (32, 33). GPI enables tumor cells to survive and proliferate under nutrient-deprived or hypoxic conditions (34). Obesity is known to promote tumor growth and progression in PC; pancreatic tumor cells grew larger and faster and metastasized more frequently in obese mice than in lean animals (35). GPI may act in synergy with obesity in promoting tumor development. However, we previously found that the *GPI* IVS9+2363C>G CG/GG genotype was predictive of better overall survival in patients with PC (23). Thus, we cannot exclude the possibility that the risk association observed in this study was caused by a survival bias.

The strength of our study includes a hypothesis-driven selection of genes and a relatively large sample size. The study's limitations include the limited number of genes and SNPs evaluated, the limited coverage of the tagging SNP, and the potential for false-positive findings associated with multiple comparisons. We applied the Bonferroni corrections with a stringent *P* value to address the multiple comparisons. Because the frequencies of some homozygotes are relatively low, we cannot exclude the possibility that certain homozygote exerted effect on modified PC risk is underevaluated because of type 2 error (false-negative). Additional studies with larger samples in different populations are needed to confirm our findings. Furthermore, showing the functional significance of these gene traits is pivotal in understanding their role in PC. A recent GWAS identified several gene variants associated with PC risk (5, 36), but no gene–environment interaction has yet been examined. Our study has provided strong evidence for an important role of the *HK2* gene variant alone or in conjunction with diabetes in influencing PC risk. These genetic markers may help to identify individuals with a high risk of PC among those with diabetes. If our observations are confirmed in other study populations, these findings may provide opportunities for the primary prevention of PC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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