

No association between phosphatase and tensin homolog genetic polymorphisms and colon cancer

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Abstract

AIM: To investigate the association between single nucleotide polymorphisms (SNPs) in the phosphatase and tensin homolog (PTEN) tumor suppressor gene and risk of colon cancer.

METHODS: We utilized a population-based case-control study of incident colon cancer individuals (n

= 421) and controls ($n = 483$) aged ≥ 30 years to conduct a comprehensive tagSNP association analysis of the PTEN gene.

RESULTS: None of the PTEN SNPs were statistically significantly associated with colon cancer when controlled for age, gender, and race, or when additionally adjusted for other known risk factors ($P > 0.05$). Haplotype analyses similarly showed no association between the PTEN gene and colon cancer.

CONCLUSION: Our study does not support PTEN as a colon cancer susceptibility gene.

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Key words: Colon cancer; Phosphatase and tensin homolog; Candidate gene; Genetic polymorphisms; Single nucleotide polymorphism association

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INTRODUCTION

The phosphatase and tensin homolog (PTEN) tumor suppressor gene is second only to p53 in mutation frequency in human cancers^[1]. PTEN utilizes multiple mechanisms to control cellular growth, the most important of which is by inhibiting phosphoinositide 3-kinase (PI3K) activation^[2]. The PI3K signaling cascade has been shown to play an important role in the development of colon tumors and other neoplasia^[3-5], suggesting that genetic variations in this pathway might confer susceptibility to colon cancer. PTEN is a likely candidate, because mutations^[6-9], deletions^[10], and loss of heterozygosity^[11] in PTEN have been found in a variety of tumors. Although PTEN alterations were

initially discovered in glioma, prostate, kidney, and breast carcinomas, more recent discoveries in gastrointestinal cancers suggest a likely association between PTEN and colorectal cancers^[12-14]. PTEN expression is decreased in over 50% of colon tumors, and PTEN loss is associated with increased risk of local colon cancer recurrence^[3,15]. In addition, PTEN missense and nonsense mutations occur in Cowden Disease, a hereditary cancer susceptibility syndrome involving multiple hamartomas of the gastrointestinal tract and other tissues^[16,17].

To date, three published studies have examined PTEN as a potential candidate susceptibility gene for four cancer types: breast and prostate cancer^[18], meningiomas^[19], and colorectal cancer^[20]. These studies found no association with PTEN genetic polymorphisms. However, the colorectal cancer study included a relatively small number of predominantly Jewish patients and examined only two PTEN single nucleotide polymorphisms (SNPs) that did not cover the full PTEN gene region. Therefore, a more thorough investigation of PTEN and colon cancer is warranted. Here we carried out a comprehensive tagSNP association analysis to further clarify the relationship between PTEN genetic polymorphisms and the risk of colon cancer in a population-based case-control study.

MATERIALS AND METHODS

Study design and data collection

The study design, data collection and study population have been described in detail elsewhere^[21]. Briefly, between 2003 and 2006, incident colon cancer cases identified from the Surveillance, Epidemiology, and End Results (SEER) Kentucky Cancer Registry (KCR) and population controls were recruited for an incident case-control of colon cancer. Histopathologically confirmed colon cancer cases were identified through regular queries of the KCR database. Random digit dialing using the same area codes and exchanges as cases was used to recruit population controls who were ≥ 30 years and had no personal history of cancer, with the exception of non-melanoma skin cancer. Those with known inflammatory bowel disease, a family history of familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer, were excluded. Participants provided a blood sample collected at approved medical facilities after an overnight fast. They also completed a self-administered questionnaire (http://epi.grants.cancer.gov/CFR/about_questionnaires.html), which collected detailed information on personal and family history of colon (and other) cancers, lifestyle, and behavioral risk factors. Participation rates were 72.2% for cases and 62.5% for eligible controls. The study was approved by the Institutional Review Boards of Case Western Reserve University/University Hospitals of Cleveland, the University of Kentucky, Lexington, and the University of Southern California, Los Angeles.

Genotyping

Fifty validated SNPs have been identified within the PTEN gene in HapMap (NCBI Build 36). Four tagging SNPs (rs2299939, rs12357281, rs2248293, and rs926091)

Table 1 Descriptive characteristics of the CWRU/kentucky colon cancer genetic epidemiology study population

	Cases (n = 421)	Controls (n = 483)	P ¹
Age ² (yr)	62.7 \pm 10.6	57.6 \pm 11.2	< 0.0001
Gender (%)			
Female	214 (50.8)	307 (63.6)	0.0001
Male	207 (49.2)	176 (36.4)	
Race (%)			
Caucasian	394 (93.6)	450 (93.2)	0.3
African-American	22 (5.2)	21 (4.4)	
Other	5 (1.2)	12 (2.5)	
BMI ^{3,4} (kg/m ²)	29.2 \pm 6.2	28.2 \pm 6.0	< 0.0001
Family History ^{3,5}			
Yes	94 (26.8)	72 (17.1)	0.0015
No	257 (73.2)	349 (82.9)	
NSAID use ^{3,6} (%)			
Yes	235 (64.2)	306 (68.9)	0.18
No	131 (35.8)	138 (31.1)	
Physical activity ³ (%)			
Vigorous	165 (42.7)	247 (53.8)	0.006
Moderate	106 (27.5)	98 (21.4)	
Light	115 (29.8)	114 (24.8)	

¹P-value of significance difference between cases and controls in a χ^2 test (discrete variables and genotypes) or *t*-test (continuous); ²Age (mean \pm SD) at diagnosis for cases and age at recruitment for controls; ³Calculations based on cases and controls with available information; ⁴BMI (mean \pm SD); ⁵Family history of first-degree relatives with colorectal cancer; ⁶NSAID: Yes = either ibuprofen or aspirin use in the last 6 mo.

were selected for genotyping in our study. These tag SNPs were selected based on the following criteria: (1) minor allele frequency $\geq 5\%$; (2) pair-wise $r^2 \geq 0.8$; (3) spanning 5 kb upstream of the 5' end and 2 kb downstream of the 3' end of the PTEN gene. Genotyping was done using the TaqMan allelic discrimination assay with pre-designed primer/probe sets (Applied Biosystems). The failure rate for genotyping was less than 0.1%. For quality assurance, assays were repeated on 2% of random samples, with a concordance call rate of 100%.

In our analysis, in addition to age, gender, and race, we also included risk factors known to be associated with colon cancer. Body mass index (BMI) was computed by dividing self-reported weight in kilograms (kg) by height in meters squared (m²). A positive family history of colon cancer was defined as having at least one first-degree relative with colon cancer. Non-steroidal anti-inflammatory drug (NSAID) use was defined as using any NSAID at least twice a week for > 6 mo. Physical activity was quantified using metabolic equivalents of energy expenditure units (METS), with light activity < 3.0 METS, moderate 3-6 METS, and vigorous activity > 6 METS.

Statistical analysis

Unconditional logistic regression analyses were performed to test the association of each individual SNP with the risk of colon cancer. The higher frequency allele was considered the referent for each SNP. Association was assessed for dominant, additive, and recessive modes of inheritance by number of copies of the risk allele. For the dominant model, participants with at least one copy of the risk allele were coded as 1 and those with no risk allele

Table 2 Associations of PTEN SNPs with colon cancer *n* (%)

SNP	Cases	Controls	Base model ¹			Full model ²		
			OR	95% CI	P	OR	95% CI	P
rs926091								
CC	289 (69.1)	351 (72.8)	1.0			1.0		
CT	122 (29.2)	122 (25.3)	1.18	0.87-1.60	0.61	1.12	0.79-1.60	0.73
TT	7 (1.7)	9 (1.9)	1.12	0.40-3.13	0.71	0.99	0.29-3.41	0.92
rs2299939								
CC	270 (64.6)	325 (67.4)	1.0			1.0		
CA	134 (32.1)	147 (30.5)	1.15	0.85-1.55	0.56	1.01	0.72-1.42	0.18
AA	14 (3.3)	10 (2.1)	1.96	0.82-4.66	0.25	2.13	0.84-5.38	0.11
rs2248293								
TT	181 (43.3)	212 (44.0)	1.0			1.0		
TC	196 (46.9)	210 (43.6)	1.04	0.78-1.39	0.21	1.04	0.47-1.45	0.52
CC	41 (9.8)	60 (12.4)	0.77	0.48-1.22	0.23	0.86	0.51-1.46	0.51
rs12357281								
GG	353 (84.4)	408 (84.7)	1.0			1.0		
GC/CC ³	65 (15.6)	74 (15.3)	0.93	0.64-1.37	0.98	0.99	0.65-1.53	0.98

¹Base model adjusted for age, gender and race; ²Full model further adjusted for BMI, family history of colorectal cancer, NSAID use, and physical activity based on 328 cases and 390 controls; ³Only three cases and two controls were of genotype CC, so they were combined with the heterozygotes to ensure validity of model fit.

Table 3 PTEN haplotype associations with colon cancer *n* (%)

Haplotype ³	Frequency	Cases	Controls	Base model ¹			Full model ²		
				OR	95% CI	P	OR	95% CI	P
A-G-T-C	0.180	146 (48.2)	157 (51.8)	1.16	0.865-1.550	0.32	1.05	0.751-1.456	0.79
C-C-T-C	0.079	53 (48.2)	57 (51.8)	0.98	0.643-1.485	0.91	1.04	0.642-1.669	0.89
C-G-C-C	0.184	88 (42.1)	121 (57.9)	0.76	0.548-1.060	0.11	0.94	0.645-1.356	0.72
C-G-C-T	0.151	71 (52.6)	64 (47.4)	1.44	0.979-2.106	0.06	1.31	0.849-2.029	0.22
C-G-T-C	0.398	58 (41.1)	83 (58.9)	0.75	0.510-1.089	0.13	0.70	0.449-1.082	0.11

¹Base model adjusted for age, gender and race; ²Full model further adjusted for BMI, family history of colorectal cancer, NSAID use, and physical activity based on 329 cases and 390 controls; ³Five other haplotypes representing a total of six participants were removed due to rarity.

copies were coded as 0. Number of risk alleles present (0, 1, or 2) determined coding for the additive model. For the recessive model, participants with two risk alleles were coded as 1, and all others were 0.

Haplotypes and their frequencies were estimated using PROC HAPLOTYPE in SAS/Genetics version 9.1. Due to the high certainty of haplotype pairs for each individual (>98%), each haplotype was coded as 1 if estimated to be present in that individual (1 or 2 copies) and 0 otherwise. Haplotypes with a frequency < 5% in our study population were excluded from analyses due to small sample sizes. All univariate and multivariate analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC) with an $\alpha = 0.05$ cutoff for statistical significance.

RESULTS

Data from 904 participants (421 cases and 483 controls), of which 93% were Caucasian, were included in the analyses. Table 1 summarizes the descriptive characteristics of the study population. Cases were evenly split by gender, whereas controls were more likely to be female. A higher percentage of cases than controls reported a positive family history of colon cancer.

All SNPs were in Hardy-Weinberg equilibrium in both the case ($P > 0.10$) and the control ($P > 0.15$)

groups. None of the four PTEN SNPs were statistically significantly associated with colon cancer when adjusted for age, gender, and race (Table 2). Further adjustment for family history, BMI, non-steroidal anti-inflammatory drug use, and physical activity did not alter the results. The additive model results are shown for the three SNPs that had sufficient numbers. The dominant model results are displayed for rs12357281, as only five participants had the rare genotype for this SNP.

Five haplotypes represented 99.2% of the variants for PTEN in this population (Table 3). Similar to the SNP results, none of the haplotypes were associated with colon cancer in the base model (adjusted for age, gender, and race) or the full model (further adjusted for BMI, family history, NSAID use, and physical activity). Odds ratios for the base model ranged from 0.75 (95% CI = 0.510-1.09) for the most frequent haplotype (C-G-T-C) to 1.44 (95% CI = 0.979-2.11) for the haplotype with the fourth highest frequency in our population (C-G-C-T).

When the above analyses were restricted to Caucasian only, the results did not substantially change (data not shown).

DISCUSSION

Recent studies have shown changes in the PTEN

gene in colon cancer tumors, including mutations^[6], loss of heterozygosity^[12], and low or absent gene expression^[15,22,23], making it a strong candidate susceptibility gene for colon cancer. In the present study, we selected four tag SNPs covering the entire PTEN region to examine association between PTEN genetic variation and risk of colon cancer. We found no evidence for association for the individual SNPs or the haplotypes. Our results indicate that common inherited variations in PTEN are unlikely to predispose to colon cancer, despite the reported high frequency of somatic mutations of the PTEN gene in colon tumors^[6,12].

The importance of PTEN as a tumor suppressor and protector of chromosomal stability is well documented^[24-28]. However, its particular function and mechanism in specific cancers is unknown. While PTEN might be necessary to prevent Akt from being phosphorylated in the PI3K pathway, its loss might not be sufficient for tumorigenesis. Recent studies suggest that mutations in other parts of the PI3K signaling pathway, such as PIK3CA and PIK3CB, might be more important in leading to tumor growth^[21,23,29,30]. In addition, PTEN might be more influential in affecting local recurrence^[15] or metastases^[31] than primary tumors. These avenues warrant further investigation with regard to PTEN and colon cancers.

Although we only genotyped four tagging SNPs out of the total 61 (50 validated) possible SNPs on PTEN, they were spaced to cover the entire length of the gene, including both upstream and downstream regions, and the entire PTEN gene is in a single LD block for Caucasians. Analyses, excluding African Americans or other minorities, yielded similar results, indicating that population stratification is unlikely to have confounded our results. Our study has over 90% power to detect an odds ratio of 1.7 and > 80% power to detect an odds ratio of 1.5 with a type I error rate of 0.05, assuming a dominant model and a minor allele frequency of 16%.

To our knowledge, this is the first population-based study to examine PTEN genetic polymorphisms with risk of "sporadic" colon cancer. Taken together with other studies^[18-20], our results do not support PTEN as a colon cancer susceptibility gene.

COMMENTS

Background

Colon cancer is the third leading cause of cancer death in the United States and worldwide for men and women. Up to 30% of all colon cancer cases may be due to heritable factors, but only five percent are associated with known genes. The phosphatase and tensin homolog (PTEN) tumor suppressor gene is a likely candidate for association with colon cancer.

Research frontiers

While the phosphoinositide 3-kinase (PI3K) signaling cascade has been shown to play an important role in the development of colon tumors, it is unclear which elements are controlling this association. PTEN controls cellular growth by inhibiting the PI3K pathway. In addition, PTEN expression is decreased in over 50% of colon tumors, and PTEN loss is associated with increased risk of local colon cancer recurrence. Therefore, PTEN is a promising candidate gene for colon cancer.

Innovations and breakthroughs

This study has provided further insight into the role of the PTEN gene in colon cancer risk.

Applications

In the process of identifying genetic causes of cancer, it is important to determine precisely which elements of a biologic pathway are responsible for affecting tumor suppression or development. Then, treatments and preventive measures can be tailored to those who would benefit most. The PTEN gene directly impacts a known signaling mechanism associated with colon cancer but has not been well-evaluated in a genetic association study. This study found that PTEN is not a likely colon cancer candidate gene, and future research should focus on other parts of the PI3K signaling pathway to understand its role in colon cancer risk.

Terminology

PTEN is a gene located on chromosome 10 known to affect tumor cell growth.

Peer review

Various genetic changes of PTEN gene in colon cancer have been described so far in the literature. In the present study, the authors examined the possibility of PTEN as susceptibility gene for sporadic colon cancer. However there was no association of individual single nucleotide polymorphisms or the haplotypes examined and therefore they concluded that common inherited variations in PTEN are unlikely to predispose to colon cancer. Although results of this study show no association of PTEN expression or susceptibility to sporadic colon cancer, and no-association results are not as attractive as positive associations, I feel they should be presented to the scientific audience in order not to create a literature bias towards publishing only studies with positive associations.

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