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Polynucleotide kinase 3' phosphatase variant, dietary variables and risk of adenoma recurrence in the Polyp Prevention Trial

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Abstract

Polymorphisms in a number of genes encoding for DNA repair enzymes have been associated with altering the function of these enzymes and increasing risk of a number of cancers, including colon cancer. We have investigated the association between a common variant in polynucleotide kinase 3' phosphatase (PNKP), a putative DNA repair enzyme, and risk of adenoma recurrence in the Polyp Prevention Trial participants. We also investigated possible interaction or effect modification between carriage of the variant allele, dietary components and risk of adenoma recurrence. Unconditional logistic regression models were used to calculate the odds ratios and 95% confidence intervals for an association between the G/T polymorphism, PNKP T5644G and risk of adenoma recurrence. We observed no association between carriage of the variant allele and risk of adenoma recurrence. Furthermore, we found no effect modification between genotype, dietary components and risk of adenoma recurrence. The PNKP T5644G variant does not seem to be involved in adenoma recurrence in the Polyp Prevention Trial.

Keywords

adenoma; diet; DNA repair; Polyp Prevention Trial

Introduction

Development of colonic adenomas is widely acknowledged to be a precursor of colorectal carcinogenesis. Although known to increase the risk of colorectal cancer, not all of those individuals who develop adenomas progress to develop colorectal cancer. Recent guidelines

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from the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society suggest that the number, size, type and grade of dysplasia, all modify the risk (Winawer *et al.*, 2006). Genetics, environment and the interplay between the two, further modify risk of cancer development and progression (Rafter and Glinghammar, 1998). Single nucleotide polymorphisms (SNPs) in a number of genes have now been associated with increased risk of (sporadic) colorectal carcinogenesis (Roberts-Thomson *et al.*, 1999; Gaasenbeek *et al.*, 2006; Huang *et al.*, 2006; Kemp *et al.*, 2006; Sansbury *et al.*, 2006; Siezen *et al.*, 2006; Webb *et al.*, 2006).

Polynucleotide kinase 3' phosphatase (PNKP) is understood to act as a DNA repair enzyme, primarily restoring termini suitable for DNA polymerase in the wake of ionizing radiation or oxidative damage (Jilani *et al.*, 1999). A number of studies have reported mutations in DNA repair genes that seem to influence risk of colorectal cancer or adenoma (Hansen *et al.*, 2005; Hong *et al.*, 2005; Skjelbred *et al.*, 2006). Furthermore, antioxidant intake has previously been shown to interact with DNA repair genotypes to modulate cancer risk (Van Gils *et al.*, 2002; Shen *et al.*, 2005; Stern *et al.*, 2005; Goodman *et al.*, 2006; Reszka *et al.*, 2006).

To our knowledge this is the first time PNKP has been investigated in an observational-type study. We believe that PNKP may have a role to play similar to that of other DNA repair genes such as *XRCC1* and *XRCC3*. We therefore examined the association between the G/T polymorphism, PNKP T5644G (rs2305922), and a number of dietary components and risk of adenoma recurrence in a large randomized dietary intervention trial: the Polyp Prevention Trial (PPT).

Participants and methods

Study population

Participants in this study were from the PPT, a large multicenter study – randomized control trial to evaluate the effects of a high-fiber, high-fruit and vegetable, lowfat diet on the recurrence of colorectal adenomas. The trial has been outlined in detail elsewhere (Lanza *et al.*, 1996; Schatzkin *et al.*, 1996). Briefly, men and women 35 years of age or older were recruited at eight clinical centers in the United States. Participants had to have one or more histologically confirmed colorectal adenoma identified by complete colonoscopy in the 6 months before randomization. To be eligible, potential participants must not have had prior surgically resected adenomatous polyps, or diagnoses with colorectal cancer, inflammatory bowel disease, or a polyposis syndrome. A total of 1905 (91.6%) participants completed the study and received colonoscopy at 4 years (T4). The study was approved by the Institutional Review Boards of the National Cancer Institute and each of the participating centers. All participants provided written informed consent at entry into the study.

Participants completed an interviewer-administered questionnaire, including demographic, clinical, medication use, and dietary and supplementation information at baseline and at each of the four annual follow-up visits. Modified Food Frequency Questionnaires (FFQ) and 4-day food records (Block *et al.*, 1986; Mares-Perlman *et al.*, 1993) were also completed

at these visits. Those reporting regular nonsteroidal anti-inflammatory drug (NSAID) use at all five visits were coded as 'regular users'.

'Recurrence' in the context of this paper refers to any adenoma recurrence (after year 1) by the end of the 4-year study period. A particular strength of the PPT was that colonoscopy was used to detect adenoma recurrence.

DNA for genotyping was available from a total of 1723 of the 1905 PPT participants. This analysis is limited to those participants identifying themselves as African American or Caucasian. Those participants who selfidentified race as 'other' were excluded from the current analysis owing to small numbers ($n = 43$) to give a final genotyped cohort of $n = 1680$.

Genotyping assay

Genotyping was performed by BioServe Biotechnologies, Ltd (Laurel, Maryland, USA). A detailed description of the protocol for this analysis was previously reported (Sansbury *et al.*, 2006). Briefly, BioServe Biotechnologies, Ltd, used a two-step PCR process and mass spectrometry (Masscode, Qiagen Genomics, Bothel, Washington, USA) as described by Kokoris *et al.* (2000), using both touchdown PCR and an identical locus-specific PCR. Allele-specific PCR products were pooled, subjected to photolysis and analyzed by mass spectrometry. Datagen (Qiagen Genomics, Bothel, Washington, USA) software was used to call the SNP alleles (manually or automatically). Concordance rate for the (10%) duplicate samples was 99%.

Data analysis

Univariate and multivariate odds ratios (ORs) and 95% confidence intervals were determined using unconditional logistic regression models for the association between genotype (or allele carriage) and risk of any recurrence, multiple adenoma recurrence or advanced adenoma recurrence using the PROC LOGISTIC function of SAS (version 8.1, SAS Institute, Cary, North Carolina, USA). Multivariate ORs were adjusted for age (as a continuous variable), sex and regular use of NSAID.

Homozygosity for the more frequent TT allele was set as the referent category for analysis of polymorphism association. A two-sided significance level of 5% was used for all analyses. Dietary variables are known to affect DNA repair and as such were investigated here for possible interaction with the PNKP SNP. We investigated the possible interaction using the 4-year average energy-adjusted (per 1000 kcal/day) median intake of dietary/vitamin intake to generate the dichotomous variable '< median intake' and 'median intake'. The referent group for the interaction model was defined as those homozygous for the T allele consuming below the PPT median intake of the dietary factor in question.

Results

Comparison of demographic and nongenetic factors between those found to have multiple polyps at T4 and those who had no or one polyp at T4 are presented in Table 1. The proportion of male participants to female participants differed significantly according to

number of adenomas at T4, with a higher proportion of male participants in the multiple adenoma group ($P < 0.0001$). The multiple adenoma group was also significantly older than the 'none or one' adenoma group ($P < 0.0001$).

The frequency of the PNKPT5644G SNP alleles was consistent with that previously reported for European populations and the population was found to be in Hardy-Weinberg equilibrium ($\chi^2 = 0.926$). No difference in genotype frequency was noted across the T4 outcome groups (any, multiple or advanced recurrence, data not shown). The unadjusted OR for any adenoma recurrence with carriage of the G allele as either homozygote or heterozygote was 0.89 (95% confidence interval: 0.71,1.12).

We then investigated the joint effects of PNKPT5644G genotype and intake of fruit, vegetables and a number of vitamins. As outlined already, 4-year average energy-adjusted (per 1000 kcal/day) median intake of dietary/vitamin intake was used to generate the dichotomous variable '< median intake' and 'median intake'. The models were also adjusted for the effects of age, sex and NSAID use.

The results of this analysis and the overall odds of multiple adenoma recurrence according to genotype are outlined in Table 2. Those with the TT genotype and less than the median intake of dietary variables formed the referent group. Carriers of the G allele, either as heterozygotes or homozygotes were the comparative group. No significant associations were observed between genotype carriage, vitamin C or E intake, folate intake, total carotenoids intake or total fruit and vegetable intake and risk of multiple adenoma recurrence. Furthermore, we did not observe any significant interactions between genotype, dietary intake and risk of multiple or advanced adenoma recurrence.

Discussion

The purpose of this study was to investigate the overall association and possible interactions between a polymorphism in the gene encoding PNKP, a putative DNA repair enzyme, and a number of dietary variables in determining risk of adenoma recurrence. We observed no association between the PNKP T5644G polymorphism and adenoma recurrence in the PPT, and there were no significant interactions between carriage of the polymorphism, intake of a number of dietary factors and adenoma recurrence. Significant interactions have been reported earlier between SNPs in DNA repair genes, dietary intake of antioxidants and cancer (Van Gils *et al.*, 2002; Shen *et al.*, 2005; Stern *et al.*, 2005; Goodman *et al.*, 2006; Reszka *et al.*, 2006). Stern *et al.* (2005) reported that the same type of interaction might modify risk of colorectal adenoma.

Though the interactions reported here are not significant, there is a general trend toward a protective effect of the polymorphism for those individuals consuming below the median intake of fruit and vegetables, vitamin C and folate. Those consuming above the median intake of vitamin E seem to have some protection against multiple polyp recurrence with carriage of the variant allele. Stern *et al.* (2005) investigated the association between two SNPs in *XRCC1* and one in *XRCC3*, the two genes involved in the repair of damage induced by unsaturated fatty acid. In that study, significant interactions (P for interaction =

0.022) were noted between carriage of a number of the XRCC polymorphisms and antioxidant intake. Odds of adenoma development were of similar magnitude to those reported here and were also nonsignificant. Though each of the interactions reported here are nonsignificant, we looked at a single SNP in what is undoubtedly a complex pathway. Looking at a number of SNPs within PNKP or indeed a panel of SNPs or haplotypes across a number of DNA repair genes may provide some insight as to possible dietary interactions that may confer a protective effect.

The strengths of our study lie in our relatively large numbers of study participants from a prospective dietary intervention trial, for which we have both genotype and detailed food frequency questionnaire data. Furthermore, there is a paucity of studies investigating PNKP or the putative effects of the PNKP T5644G polymorphism.

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Table 1

Baseline characteristics of participants individuals who with multiple adenomas at T₄ and those with none or one adenoma at T₄

Characteristics T ₀	One adenoma T ₄ n (%)	Multiple adenomas T ₄ n (%)	P value
SexGender			
Male	865 (61.8)	213 (75.8)	
Female	534 (38.2)	68 (24.2)	<0.0001
Age (mean) ^a	60.4 (SD: 9.9)	64.9 (SD: 9.2)	<0.0001
Race			
Caucasian	1280 (91.5)	265 (94.3)	
Other	119 (8.5)	16 (5.7)	0.11
BMI			
<25kg/m ²	368 (26.3)	59 (21.0)	
25–29 kg/m ²	674 (48.2)	132 (47.0)	
30 kg/m ²	357 (25.5)	90 (32.0)	0.04
Smoking			
Never	557 (39.8)	108 (38.4)	
Former	666 (47.6)	133 (47.3)	
Current	176 (12.6)	40 (14.3)	0.736
Alcohol			
Ever	808 (42.2)	160 (56.9)	
Never	591 (57.8)	121 (43.1)	0.801
Regular NSAIDs use (use at all five visits)			
No	1204 (86.1)	244 (86.8)	
Yes	195 (13.9)	37 (13.2)	0.732
Education			
>HS	1063 (76.0)	204 (72.6)	
HS	335 (24.0)	77 (27.4)	0.222

^a Age is shown with the mean values for each group and the standard deviation.

P values are the result of χ^2 (proportion) or *t*-test (mean), as appropriate.

BMI, body mass index; HS, high school; NSAIDs, nonsteroidal anti-inflammatory drugs.

Table 2

ORs and 95% CIs for multiple adenoma recurrence

	PNKP T5644G genotype	< Median intake		Median intake		<i>P</i> _{Interaction} (LR)
		No recurrence <i>n</i> (%)	Multiple recurrence <i>n</i> (%)	OR (95% CI) ^{a,b}	Multiple recurrence <i>n</i> (%)	
Total fruit, vegetable and juice intake	TT	122 (25.4)	43 (35.8)	1.0	41 (28.7)	0.152
	GT+GG	359 (74.6)	77 (64.2)	0.61 (0.39, 0.95)	102 (71.3)	
Vitamin C	TT	127 (26.0)	45 (36.9)	1.0	39 (27.7)	0.113
	GT+GG	361 (74.0)	77 (63.1)	0.6 (0.39, 0.93)	102 (72.3)	
Vitamin E	TT	137 (29.1)	49 (34.8)	1.0	35 (28.7)	0.805
	GT+GG	334 (70.9)	92 (65.2)	0.76 (0.5, 1.15)	87 (71.3)	
Folate	TT	123 (25.4)	45 (36.6)	1.0	39 (27.9)	0.145
	GT+GG	362 (74.6)	78 (63.4)	0.62 (0.4, 0.95)	101 (72.1)	
Carotenoids	TT	129 (27.1)	40 (33.1)	1.0	44 (31.0)	0.838
	GT+GG	347 (72.9)	81 (66.9)	0.75 (0.48, 1.17)	98 (69.0)	
Overall effect of genotype		Multiple recurrence <i>n</i> (%)		Multiple recurrence OR (95% CI) ^a		
		TT		1.0 (reference)		
		GT+GG		0.79 (0.59, 1.07)		

Also shown are ORs and 95% CIs for the combined effect of dietary fruit, vegetable or juice or single vitamin intake on risk of developing multiple adenoma at T4. Median dietary/vitamin intake is compiled from a 4-year average energy-adjusted (per 1000 kcal/day) median intake.

LR, likelihood ratio; PNKP, Polynucleotide kinase 3' phosphatase.

^a Odds ratio (OR) and 95% confidence interval (CI).

^b Adjusted for age, sex and NSAIDs use.