The association between common genetic variation in *STK11* (*LKB1*) and colorectal adenomas in the Health Professional's Follow-up Study and the Nurses' Health Study

Kazumi S. Tsukazawa

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Committee:

Polly A. Newcomb, Chair

Andrea N. Burnett-Hartman

Karen L. Edwards

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Abstract

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Kazumi S. Tsukazawa

Chair of the Supervisory Committee:
Professor Polly A. Newcomb
Department of Epidemiology

Germline mutations in serine-threonine kinase 11 (*STK11*) are hallmarks in hamartomatous polyps of the rare, hereditary disease, Peutz-Jeghers syndrome (PJS). PJS is associated with colorectal hamartomatous polyps and results in an increased risk of colorectal cancer and other malignancies. However, whether hamartomatous polyps contribute to high rates of neoplasm in carriers, or whether these patients progress through the conventional adenoma-carcinoma pathway is unknown. Single nucleotide polymorphisms (SNPs) in *STK11* are also observed to be polymorphic, so we hypothesized that common genetic variation in the *STK11* gene is positively associated with colorectal adenomas. We evaluated the association between common genetic variation in *STK11* and adenomas using adenoma cases and matched controls from Health Professional's Follow-up Study (HPFS) and Nurses' Health Study (NHS). Lymphocyte DNA was extracted and three tagSNPs in *STK11 (*rs2075604, rs3764640, rs8111699) were genotyped using the Illumina HumanOmniExpress platform. Logistic regression models adjusted for age, sex, and race using principal components, were used to compare adenoma cases to controls and estimate odds ratios and 95% confidence intervals for the association between adenomas and *STK11* SNPs. There was no association between colorectal adenomas and any of the SNPs evaluated; rs8111699 (OR=1.05, 95% CI (0.80, 1.37)), rs2075604 (OR=0.72, 95% CI (0.30, 1.75)), rs3764640 (OR=1.05, 95% CI (0.66, 1.66)). Exploratory analyses were conducted to assess effect modification of the association between *STK11* SNPs and adenomas by common risk factors for colorectal neoplasia. Two types of interaction were detected. Evidence was suggested for an interaction between SNP rs2075604 and family history (P=0.03). Also, evidence for another interaction was found with the same SNP and smoking (P=0.05).

To those who are family to me

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1. Introduction & Background

Colorectal cancer is the third most commonly diagnosed cancer and third leading cause of cancer death in both men and women in the United States [1]. Nearly 85% of sporadic colorectal cancers develop from adenomas [2]. The lifetime risk of developing a colorectal adenoma is approximately 19% in the U.S. population [3]. Thus, a history of adenoma(s) in an individual imposes an increased risk of developing colorectal cancer in comparison to individuals with no previous history [4]. The development of malignancy from adenomas is considered to have a long latency period estimated on average at 5 to 10 years [4].

Findings from hereditary cancer syndromes have contributed greatly to the uncovering of molecular events in tumorigenesis [5]. Germline mutations in the gene serine/threonine kinase 11 (*STK11*), also known as liver kinase B1 (*LKB1*), are associated with hamartomatous polyps of Peutz-Jeghers syndrome (PJS) [5 - 10]. PJS is a rare hereditary and autosomal dominant disorder. Multiple gastrointestinal hamartomatous polyps, which pose a heightened risk of various neoplasms including gastrointestinal cancers characterize PJS [11]. Importantly, it is the first cancer- susceptibility syndrome identified that is due to inactivating mutations in a protein kinase [5].

Some researchers hypothesize that hamartomatous polyps have malignant potential, enabling the transition from these lesions to adenomas, and eventually to carcinoma [12, 13]. Thus, in those with PJS, hamartomas are hypothesized to be precursors to adenomas, but there is controversy regarding the hamartoma-adenoma-carcinoma pathway paradigm. Adenomatous foci have been found within hamartomas [12]. However, the malignant transformation of hamartomas is rarely observed. Further, the polyps have been characterized as polyclonal rather than monoclonal, which suggests that they are less likely to have a high malignant potential [13].

Alternatively, the hamartomas may be a representation of abnormal mucosal prolapse caused by changes in cellular polarity induced by mutation in the *STK11* gene [11, 13]. Should this viewpoint be

valid, then the argument that colorectal cancer in those with PJS may arise on a background of mucosal instability, perhaps through an accelerated conventional adenoma-carcinoma pathways warrants consideration.

Single nucleotide polymorphisms (SNPs) in *STK11* are observed to be polymorphic. Consistent with the theory that mucosal instability induced by *STK11* mutation results in an accelerated adenoma-carcinoma pathway, we hypothesized that common genetic variation in the *STK11* gene is associated with adenomas. To test this hypothesis, we conducted a case-control study of adenomas using the Nurses' Health Study [14] and the Health Professionals Follow-up Study (HPFS) [15]. These prospective studies are a unique collection of colorectal adenoma cases and colonoscopy confirmed controls within the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO).

2. Material & Methods

2.1 Study Population

We obtained adenoma cases and controls from two prospective cohorts, the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The NHS cohort was initiated in 1976 with mailed-in response of questionnaires from 121,700 married female registered nurses aged 30 to 55 years [16, 17]. Details of the design and follow-up of this cohort have been described previously [14]. The HPFS is a parallel prospective study that began in 1986. It includes 51,529 U.S. male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians, ages 40 to 75 years, who responded to a mailed questionnaire [16]. Details of the design and follow-up of this cohort have been described previously [15].

Epidemiologic data and biospecimens from adenoma cases (HPFS N=313; NHS N=510) and colonoscopy-confirmed polyp-free controls (HPFS N=344; NHS N=577) were provided to the GECCO. DNA was extracted from blood samples. Illumina HumanOmniExpress was used for genotyping. Details and supplementary tables may be found for exclusion criteria, ascertainment, case-control set construction, and genotyping methods including quality control criteria for samples and SNPs, and SNP imputation methods [18, 19]. The Institutional Review Board at Harvard School of Public Health and at Fred Hutchinson Cancer Research Center approved this study.

2.2 SNP selection and imputation

We used information from the online genome variation server (GVS; www. http://gvs-p.gs.washington.edu/GVS) to generate a list of tagSNPs in *STK11*. We chose tagSNPs within GVS by setting the minor allele frequency (MAF) to 10%, linkage disequilibrium (LD) r^2 to 0.8, and the reference population to the CEPH (Centre d'Etude du Polymorphisme Humain) from Utah (CEU) HapMap population. Then we selected one tagSNP from each bin. Using this method, the following SNPs were

included in our analyses: rs2075604, rs3764640, and rs8111699. Of these, rs2065604 and rs3764640 were directly genotyped and rs8111699 was imputed to the CEU population in HapMap II release 24 using MACH [20]. Imputation accuracy was measured by calculation of R^2. The cutoff imposed was R^2 >0.3 since all SNPs in this sample were selected to have MAF ≥ 10% and thus satisfied MAF ≥ 1% used for analysis of typical imputed SNP data.

2.3 Statistical Analysis

The data for women and men were examined pooled. Individuals with a history of colorectal polyps or adenomas (N=5) were removed from the analysis. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between genotype and colorectal adenomas for each of the three SNPs. Each SNP was examined according to the codominant model, with genotype analyzed as a three-category variable with homozygous wild-type (common allele) as the reference. Commonly accepted colorectal cancer risk factors, age, race, and sex, was adjusted in the analyses for the main effect between genotype and colorectal adenoma risk. For controls, age at selection was used. For cases, age at diagnosis was used. The first three principal components of ancestry were used to adjust for race. Statistical tests for all analyses were two-sided.

In addition to these primary analyses, effect modification of the association between *STK11* SNPs and adenomas by commonly accepted colorectal cancer risk factors was assessed through exploratory analyses. These included family history of colorectal cancer, smoking, non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) use, aspirin use, obesity measured by body mass index (BMI) and sex. Family history was defined as having a first-degree relative with colorectal cancer. Smoking status was modeled as a three-level categorical variable (never, former, and current smoker). NSAIDs use, aspirin use, and sex was coded binary. NSAIDs and aspirin use was based on responses in the questionnaire (forms) of usage during referent time frame. BMI was modeled as a three-level categorical variable for normal (<25), overweight (25≤BMI<30), and obese (30≤) in reference to the World Health Organization (WHO) standards. For each of these risk factors, we reported the Wald p-value for the interaction term, which included the cross product between genotype and risk factor of interest.

3. Results

Each of the 3 SNPs (rs8111699, rs2075604, and rs3764640) was genotyped in 657 men and 1087 women with colorectal adenomas (N=823) and controls (N=921). The mean age of the cases and their frequency-matched controls was 67 years. A family history of colorectal cancer was more common among cases than controls (16.16% vs 13.46%), whereas regular aspirin use, NSAIDs use, and having never smoked or being a former smoker was more frequent among controls than cases (Table 1).

The genotype distributions for these SNPs did not differ by sex. The minor allele frequencies for the SNPs (common allele/rare allele) rs8111699 (G/C), rs2075604 (G/T) and rs3764640 (G/T) were 35.8%, 25.8% and 35.8 % respectively in the HapMap CEU population. No evidence for a deviation from Hardy-Weinberg equilibrium was found in controls for each SNP. The gene frequencies of the three SNPs were 0.483 (0.283+0.5/2), 0.8755 (0.767+0.217/2), and 0.796 (0.628+0.336/2) respectively.

There was not a statistically significant trend in the association between genotype and adenoma risk for the any of the SNPs evaluated; rs8111699 (G/C) (OR=1.05, 95% CI (0.80, 1.37), P-trend = 0.716), rs2075604 (G/T) (OR=0.72, 95% CI (0.30, 1.75), P-trend = 0.278), and rs3764640 (G/T) (OR=1.05, 95% CI (0.66, 1.66), P-trend = 0.228) in reference to each common allele respectively (Table 2).

Among the commonly accepted risk factors for colorectal cancer (Table 3), colorectal adenomas were positively associated with smoking status (former OR=0.99, 95% CI (0.81, 1.21); current OR=1.67, 95% CI (1.18, 2.37); P-trend=0.043), and BMI (25≤BMI<30 OR=1.63, 95% CI (1.32, 2.02); 30≤ OR=1.38, 95% CI (1.03, 1.84); P-trend = 0.001). Colorectal adenomas were negatively associated with NSAIDs use (OR=0.71, 95% CI (0.54, 0.91)). Colorectal adenomas were not statistically significantly associated with family history of having a first-degree relative with colorectal cancer (OR=1.21, 95% CI (0.93, 1.59)), nor with aspirin use (OR=0.81, 95% CI (0.65, 1.01)).

There was no evidence for an interaction between SNP rs8111699 (G/C) and any of the risk factors (Table 4). Similarly, no evidence was found for an interaction between SNP rs3764640 (G/T) and any of the risk factors (Table 6). However, there was a statistically significant interaction between genotype and family history (P=0.03), and between genotype and smoking status (P=0.05), for rs2075604 (G/T) (Table 5).

4. Discussion

Although germline mutations in *STK11* are clearly associated with an increased risk of colorectal cancer, our results suggest that genotypic variation in the *STK11* gene at rs8111699, rs2075604, and rs3764640 were not associated with the most common precursor lesions for colorectal cancer, colorectal adenomas. These null results may be an indication that colorectal cancer development in PJS may not follow the conventional adenoma-carcinoma sequence. If hamartomas are the most salient precursor lesion in PJS patients, then genotypic variation in *STK11* may be associated with hamartoma-like lesions in the colon. In contrast with this hypothesis, it may also be that the genotypic variation in the *STK11* SNPs that we evaluated does not correlate with phenotypic variation in *STK11* gene expression. Further research is needed to determine the functional consequences of SNPs in *STK11* and to determine if SNPs in *STK11* are associated with non-adenomatous lesions in the colon, such as hamartomas.

Even though we did not identify a significant association between the each SNP and colorectal adenomas, there was evidence for a significant interaction between SNP rs2075604 and family history, and between this same SNP and smoking. Because our interaction analyses were exploratory, future studies are needed to replicate and validate these results. However, family history of colorectal cancer has been established as a risk factor for colorectal cancer, and our study results support previous findings for family history of colorectal cancer modifying risk for either colorectal adenoma or cancer through gene-environment interaction [21, 22]. Prior research has also demonstrated smoking as a risk factor for colorectal cancer [23, 24].

Although our study is the first study to evaluate the association between common genetic variation in *STK11* and colorectal adenoma risk, there is a prior study of *STK11* genotypic variation and colorectal cancer risk. This case-control study of colorectal cases (N=1665) and controls (N=2939) reported that

an intronic SNP in the *STK11* gene, rs741765, was associated with a 48% (OR=1.04, 95% CI (1.01, 2.08)) increased odds of rectal cancer for those with the AA genotype [25]. The rs741765 SNP was not evaluated in our study of adenomas, but a future study with this SNP as a candidate to be evaluated in our nested case-control study cohort may be informative.

Classic genetic models assume biallelic inactivation of tumor suppressors as necessary for tumorigenesis [26]. However, our study focused on *STK11*, a haploinsufficient tumor suppressor, which deviates from these models in that abnormal phenotype results with the loss of function of one allele. The precise molecular mechanisms of haploinsufficieny in tumor suppressor genes remain unknown. Inflammatory mediators (derived from cyclooxygenase and lipoxygenase pathways) have been shown to directly inactivate *STK11* [25]. Strong interaction between haploinsufficient tumor suppressor genes has been suggested using network analyses *in silico* and that slight perturbation in a network could contribute to tumorigenesis [27]. A central pathway converged of hormones, inflammation and energy-related factors (CHIEF) has been proposed as a hypothesized pathway of colorectal cancer risk based upon epidemiological data [25]. This pathway is comprised of two major arms that regulate insulin sensitivity, one of which involves STK11 [28].

Lowered insulin sensitivity that characterizes diabetes has been implicated as a risk factor in colorectal adenoma and cancer. Primarily liver-produced insulin growth factor-1 (IGF-1) and insulin growth factor binding partner-3 (IGFBP-3) are up-regulated by growth hormone(s), and have been shown to be associated independently with an elevated risk of large or tubulovillous/villous colorectal adenomas and cancer in the NHS cohort [29]. Thus, studies of STK11 and diabetes or insulin sensitivity may inform possible mechanisms for how *STK11* may mediate colorectal cancer risk. The mutated G allele in *STK11* gene SNP rs8111699 has been found to be associated with higher insulin and IGF-1 levels in a study of 85 Caucasian, Northern Spanish hyperinsulinemic girls due to androgen excess, despite the rather heterogeneous condition [30]. It was also associated with differences in metformin response [30]. The beneficial effect of metformin in diabetic patients (type II diabetes) who are at high risk of in colorectal adenoma development was found in an American population receiving colonoscopy (N=3456)

[31]. In contrast, our study population comprised of healthy individuals who did not have diabetes reporting no association between rs8111699 genotype and colorectal adenoma. We thus cannot rule out a possibility that colorectal adenoma development in healthy individuals, who are not subject to androgen excess or diabetic symptoms, may still be influenced by low insulin sensitivity exerted by rs8111699 genotype but also require other factors that perturb the normal endocrine-metabolic features of energy homeostasis.

Our study population was large, including over 1,744 men and women from two well-characterized cohorts. Given the large sample-size and excellent epidemiologic data, we were able to explore important gene-environment interactions in our analyses. However, power may still have been limited to detect weak associations. At the same time, the large number of comparisons in our exploratory interaction analyses increased the probability of a type I error. Also, we used an agnostic approach to tag the entire *STK11* gene, because the functional significance of *STK11* SNPs is unclear. However, this approach may miss other SNPs that result in phenotypic changes in the expression of *STK11*. Genotyping results of available tag SNPs were all located in the intronic region of the gene. Thus, we could not have evaluated the association between non-synonymous mutations and colorectal adenomas. Also, epidemiologic factors evaluated in our analyses were based on self-report, and therefore subject to recall bias. However, because we used a nested case-control approach, epidemiologic factors were evaluated prior to adenoma development, and any recall bias is likely to be non-differential between adenoma cases and controls.

In summary, in this population of health professionals, we did not identify a correlation between *STK11* common genetic variation and colorectal adenomas. The functional role of *STK11* as a tumor suppressor is extensive given both its ubiquitous expression in embryonic and adults tissues [32], as well as the consequences of its best characterized substrate, 5' adenosine monophosphate -activated protein kinase (AMPK), a sensor of cellular energy status [33]. It is still unknown what functional alterations in *STK11* contribute to hamartomas or adenocarcinomas. Further, *STK11* interacts with many molecules to exert functional effects. The study design and analysis would not have been able to

capture all these possibilities. Further research is necessary to understand the mechanisms and potential genetic effect modifiers of the STK11 pathway in the relationships between colorectal hamartomas, adenomas, and cancers. The incorporation of additional detailed information in future research will likely improve mechanistic insight.

Table 1:
Characteristics of study participants by adenoma status (N=1744) in the Health Professional Follow-up Study [a] and Nurse's Health Study [b]

	Controls (N=921)	(%)	Adenomas (N=823)	(%)
Age				
<50	3	0.3	2	0.2
50 - 59	181	19.6	152	18.5
60 – 69	359	38.9	341	41.5
70 - 79	345	37.4	294	35.8
80<	34	3.7	33	4
Sex				
Men	344	37.4	313	38
White, non-Hispanic				
Yes	908	99.7	802	99.5
Family History †				
Yes	124	13.46	133	16.16
BMI				
<25	487	52.9	357	43.4
25≤BMI<30	305	33.1	346	42.04
30≤	105	11.4	99	12.03
Smoker Status				
Never Smoker	442	48.7	376	46.7
Former Smoker	398	43.8	338	41.9
Current Smoker	68	7.5	92	11.4
Aspirin use ‡				
Yes	295	32	230	27.9
NSAIDs use ‡				
Yes	178	19.3	124	15.1

[a] Men only. [b] Women only. † Family history of colorectal cancer. ‡ During referent time period.

Table 2:
Adjusted odds ratios (ORs) [c] for colorectal adenoma in relation to *STK11* polymorphisms

	Controls	Adenomas		
	N	**N**	**OR[c]**	**95% CI**
rs8111699 genotype				
G/G (ref)	291	253	1	(reference)
G/C	448	405	1.04	(0.83, 1.29)
C/C	182	165	1.05	(0.80, 1.37)
P-trend				0.716
rs2075604 genotype				
G/G (ref)	752	651	1	(reference)
G/T	148	158	1.23	(0.96, 1.58)
T/T	13	8	0.72	(0.30, 1.75)
P-trend				0.278
rs3764640 genotype				
G/G (ref)	590	500	1	(reference)
G/T	288	286	1.17	(0.96, 1.42)
T/T	42	37	1.05	(0.66, 1.66)
P-trend				0.228

[c] Adjusted for age at selection for controls, age at diagnosis for cases, race by principal components analysis pc1, pc2, pc3 and sex.

Table 3:
Adjusted odds ratios (ORs)[d] for colorectal adenomas and family history, cigarette smoking, NSAIDs use, aspirin use, and BMI respectively

	OR[d]	95% CI	P-value
Family History			
No	1	(reference)	
Yes	1.2	(0.92, 1.58)	0.176
Smoker Status			
Never	1	(reference)	
Former	0.99	(0.81, 1.21)	
Current	1.67	(1.18, 2.37)	
			0.043
NSAIDs use			
No	1	(reference)	
Yes	0.71	(0.54, 0.92)	0.012
Aspirin use			
No	1	(reference)	
Yes	0.81	(0.65, 1.01)	0.052
BMI			
<25	1	(reference)	
25≤BMI<30	1.63	(1.32, 2.02)	
30≤	1.38	(1.03, 1.84)	
			0.001
Sex			
Female	1	(reference)	
Male	1.02	(0.83, 1.26)	0.82

[d] Adjusted for age at selection for controls, age at diagnosis for cases, race by principal components analysis pc1, pc2, pc3, sex, family history of colorectal polyps, smoker status, NSAIDs use, aspirin use, and BMI.

Table 4:
Logistic regression analyses results of the association between *STK11* polymorphism rs8111699 (G/C) and colorectal adenomas stratified by family history, cigarette smoking, NSAIDs use, aspirin use, BMI and sex

rs8111699	GG		G/C		C/C		
	OR[c]	95% CI	OR[c]	95% CI	OR[c]	95% CI	P interaction
Family History							
No	1	(reference)	1.05	(0.83, 1.33)	1.16	(0.87, 1.55)	0.093
Yes	1	(reference)	0.92	(0.52, 1.61)	0.55	(0.25, 1.19)	
Smoker							
Former	1	(reference)	1.05	(0.77, 1.45)	1.03	(0.69, 1.54)	0.555
Current	1	(reference)	0.96	(0.69, 1.34)	0.94	(0.62, 1.42)	
NSAIDs use							
No	1	(reference)	0.97	(0.77, 1.23)	0.98	(0.73, 1.32)	0.24
Yes	1	(reference)	1.40	(0.81, 2.40)	1.43	(0.74, 2.76)	
Aspirin use							
No	1	(reference)	1.00	(0.77,1.29)	0.92	(0.67,1.27)	0.154
Yes	1	(reference)	1.17	(0.78, 1.75)	1.46	(0.89, 2.42)	
BMI							
<25	1	(reference)	1.12	(0.82, 1.53)	1.10	(0.75, 1.62)	0.944
25≤BMI<30	1	(reference)	0.92	(0.64, 1.32)	0.87	(0.55, 1.37)	
30≤	1	(reference)	1.03	(0.57,1.84)	1.18	(0.58, 2.39)	
Sex							
Male	1	(reference)	0.87	(0.61, 1.24)	1.11	(0.70, 1.74)	0.949
Female	1	(reference)	1.19	(0.90, 1.56)	1.03	(0.73, 1.45)	

[c] Adjusted for age at selection for controls, age at diagnosis for cases, race by principal components analysis pc1, pc2, pc3 and sex.

Table 5:
Logistic regression analyses results of the association between *STK11* polymorphism rs2075604 (G/T) and colorectal adenomas stratified by family history, cigarette smoking, NSAIDs use, aspirin use, BMI and sex

rs2075604	G/G		G/T		T/T		
	OR[c]	95% CI	OR[c]	95% CI	OR[c]	95% CI	P interaction
Family History							
No	1	(reference)	1.36	(1.04, 1.78)	0.86	(0.32, 2.28)	0.033
Yes	1	(reference)	0.63	(0.33, 1.20)	0.4	(0.04, 4.12)	
Smoker							
Former	1	(reference)	0.98	(0.67, 1.44)	0.26	(0.06, 1.22)	0.048
Current	1	(reference)	1.5	(1.04, 2.16)	1.58	(0.42, 5.96)	
NSAIDs use							
No	1	(reference)	1.13	(0.86, 1.48)	0.61	(0.22, 1.66)	0.113
Yes	1	(reference)	0.91	(1.01, 3.61)	1.45	(0.20, 10.61)	
Aspirin use							
No	1	(reference)	1.21	(0.90, 1.62)	0.82	(0.26, 2.60)	0.889
Yes	1	(reference)	1.27	(0.79, 2.04)	0.68	(0.17, 2.79)	
BMI							
<25	1	(reference)	1.01	(0.70, 1.46)	0.81	(0.24, 2.82)	0.61
25≤BMI<30	1	(reference)	1.65	(1.08, 2.50)	0.81	(0.16, 4.20)	
30≤	1	(reference)	1.16	(0.61, 2.18)	0.37	(0.04,3.60)	
Sex							
Male	1	(reference)	1.11	(0.74, 1.66)	0.63	(0.15, 2.74)	0.467
Female	1	(reference)	1.31	(0.96, 1.81)	0.75	(0.24, 2.30)	

[c] Adjusted for age at selection for controls, age at diagnosis for cases, race by principal components analysis pc1, pc2, pc3 and sex.

Table 6:
Logistic regression analyses results of the association between *STK11* polymorphism rs3764640 (G/T) and colorectal adenomas stratified by family history, cigarette smoking, NSAIDs use, aspirin use, BMI and sex

rs3764640	G/G		G/T		T/T		
	OR[c]	95% CI	OR[c]	95% CI	OR[c]	95% CI	P interaction
Family History							
No	1	(reference)	1.23	(0.98, 1.53)	1.26	(0.76, 2.08)	0.057
Yes	1	(reference)	0.88	(0.51, 1.50)	0.42	(0.12,1.49)	
Smoker							
Former	1	(reference)	1.07	(0.79, 1.43)	0.93	(0.48, 1.79)	0.222
Current	1	(reference)	1.19	(0.87, 1.63)	0.86	(0.41, 1.79)	
NSAIDs use							
No	1	(reference)	1.09	(0.87,1.37)	0.93	(0.57, 1.53)	0.055
Yes	1	(reference)	1.66	(1.02, 2.70)	2.11	(0.61, 7.28)	
Aspirin use							
No	1	(reference)	1.23	(0.97, 1.57)	1.05	(0.61, 1.80)	0.684
Yes	1	(reference)	1.06	(0.73, 1.54)	1.11	(0.46, 2.66)	
BMI							
<25	1	(reference)	1.09	(0.81, 1.47)	0.9	(0.44, 1.84)	0.444
25≤BMI<30	1	(reference)	1.18	(0.85, 1.66)	1.16	(0.54, 2.47)	
30≤	1	(reference)	1.49	(0.87, 2.56)	1.05	(0.36, 3.08)	
Sex							
Male	1	(reference)	0.95	(0.69, 1.32)	1.15	(0.49, 2.68)	0.347
Female	1	(reference)	1.36	(1.05, 1.76)	1.02	(0.59, 1.77)	

[c] Adjusted for age at selection for controls, age at diagnosis for cases, race by principal components analysis pc1, pc2, pc3 and sex.

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