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Alcohol consumption and rectal tumor mutations and epigenetic changes

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Abstract

Purpose—An association between alcohol and rectal cancer has been reported in the epidemiological literature. In this study we further explore the association by examining specific tumor markers with alcohol consumption as well as types of alcoholic beverages consumed.

Methods—We assessed alcohol consumption with CpG Island Methylator Phenotype, *TP53* and *KRAS2* mutations in incident rectal cancer cases and compared them to population-based controls. We evaluated type, long-term, and recent alcohol consumption.

Results—We observed a trend toward increasing risk of CpG Island Methylator Phenotype positive tumors and long-term alcohol consumption. In contrast, after adjusting for recent total alcohol intake, recent high beer consumption significantly increased the odds of having a *TP53* mutation compared to those who did not drink beer (Odds Ratios 2.19 95% Confidence Interval 1.34, 3.57). We observed a non-statistically significant reduced risk of a *TP53* mutation among those who drank wine (particularly red wine) versus non-consumers of wine. The association between *TP53* mutations and recent beer consumption was strongest for transversion mutations.

Conclusions—These data suggest that both alcohol and specific constituents of alcoholic beverages contribute to rectal cancer risk among unique disease pathways.

Keywords

TP53; *KRAS2*; CpG Island Methylator Phenotype; rectal cancer; alcohol; beer; wine

Alcohol has been associated with increasing risk of colorectal cancer; however unresolved issues including the type of alcohol that influences risk, the amount and timing of alcohol consumed, as well as the impact of alcohol on specific CRC sites.¹ Some studies suggest that associations may be stronger for more distal or rectal cancer, while others show similar

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associations for both colon and rectal cancer.^{2, 3} Some studies show stronger associations with beer or liquor and rectal cancer, while others report stronger associations for alcohol overall with rectal tumors rather than colon tumors.^{1, 4, 5} Studies of colon cancer suggest that alcohol consumption could contribute to specific types of tumor mutations, with high levels of alcohol increasing the likelihood of a microsatellite unstable tumor.^{6, 7}

Unique associations between alcohol and types of alcoholic beverages consumed could contribute to specific tumor mutations. Long-term use of alcohol could contribute to DNA damage or epigenetic changes that cause the initiation of tumors while more recent use could influence tumor promotion from events such as oxidative stress or from specific antioxidants. Thus, evaluation of both long-term alcohol use and more recent use could help define the association between alcohol intake and rectal cancer. Associations with specific types of alcoholic beverages also could indicate that non-alcohol components of the alcoholic beverages alter risk rather than alcohol itself.

In this study, we evaluate consumption of total alcohol as well as alcoholic beverages, including wine, beer, and liquor over the 20 years prior to diagnosis and during the referent year (2 years prior to diagnosis) with specific epigenetic and genetic changes in rectal tumors. Data come from a population-based study of rectal cancer conducted in Northern California and Utah. We test the hypothesis that specific types of alcohol contribute to common epigenetic and genetic changes in rectal tumors.

Methods

Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California (KPMCP) and the state of Utah; study protocol and consent forms were approved by institutional review boards at the Kaiser Permanente Medical Care Program in Oakland, California and the University of Utah in Salt Lake City, Utah. Cases with a first primary tumor in the recto-sigmoid junction or rectum as determined by the Surveillance Epidemiology and End Results (SEER) Cancer Registries in Northern California and in Utah between May 1997 and May 2001 were identified. To be eligible for the study, participants had to be between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, could not have had previous colorectal cancer⁸, and could not have known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. All eligible cases within the study time period and geographic area were recruited for the study.

Tumor block retrieval involved obtaining biopsy prior to treatment as well as paraffin embedded tissue from the resection. In Utah, blocks were requested for all cases except those who refused release of blocks. At the KPMCP, samples were retrieved from persons who signed a consent form at the time of interview or who had died after the interview. A total of 1495 rectal cancer cases were identified; of those identified, 239 people had not given consent to have the tissue released (15.9%), and an additional 234 cases did not have adequate tumor tissue to obtain DNA. Tumor DNA was extracted from 81.4% of all rectal cancer cases identified, of which 750 cases had interview data. Controls were randomly selected from membership lists at KPMCP, and in Utah from social security lists and driver's license lists (for participants under 65 years); 1205 controls (68.8% of those selected) participated.

Genetic Analysis

Tumor DNA obtained from paraffin-embedded tissue was characterized by their genetic profile that included sequence data for exons 5 through 8, the mutation hotspot exons of the *TP53* gene; sequence data for *KRAS2* codons 12 and 13; and five CIMP markers, methylated

in tumor (MINT)1, MINT2, MINT31, *CDKN2A* (*p16*), and *MLH1*.^{6, 9, 10} These tumor markers were selected because of their frequent alterations in colorectal tumors. CIMP positive tumors were defined as having two or more of 5 markers methylated. At this time there is no “consensus” as to the appropriate CIMP panel or method of detection. However, we have used our panel to demonstrate significant relationships between CIMP and numerous variables, including cigarette smoking and the *BRAF* V600E mutation, which were independent of microsatellite instability.^{11, 12} This work has helped to support the legitimacy of the CIMP concept¹³. For a small subset of cases, approximately 35, to evaluated both biopsy prior to treatment and resection after treatment and did not identify differences in CIMP status as a result of treatment modality.

Diet and Lifestyle Data

Trained and certified interviewers collected diet and lifestyle data as previously outlined.^{14, 15} Briefly, interviews were conducted in the participant's home or workplace using a laptop computer. All interviewers were thoroughly trained and all interviews were audio-taped for quality control purposes¹⁴. The referent year for the study was the calendar year approximately two years prior to date of diagnosis (cases) or selection (controls). Information was collected on demographic factors such as age, sex, and study center; physical activity; body size; cigarette smoking history; use of aspirin and non-steroidal anti-inflammatory drugs, family history of colorectal cancer in first degree relatives; medical and reproductive history including use of hormone replacement therapy (HRT).

Dietary intake was ascertained using an adaptation of the CARDIA diet history.^{15–17} Participants were asked to recall foods eaten, the frequency at which they were eaten, serving size, and if fats were added in the preparation. Recent alcoholic beverage intake was obtained for the referent year as part of the diet history questionnaire. We asked about specific types of alcohol consumed and usual amount consumed for the weekend days (Friday, Saturday, and Sunday) and week days (Monday through Thursday). Additionally, as part of the health and lifestyle questionnaire we obtained alcoholic beverage consumption patterns by asking participants to report their usual consumption patterns of liquor, wine, and beer for 10 and 20 years ago to get a better indication of long-term alcoholic beverage consumption patterns. Long-term consumption of alcohol was based on the average intake reported for those two time periods.

Statistical analysis

We assessed total alcohol reported, alcohol from liquor, wine, and beer during the referent year taken from the diet history questionnaire and long-term alcohol use as determined in the health and lifestyle history questionnaire. Alcohol consumption levels were based on consumption patterns in the population and literature reports of level of consumption and were classified as none, moderate (>0 to <20 grams per day for men, >0 to <10 grams per day for women), and high (≥20 grams per day for men, ≥10 grams per day for women). We assessed specific types of alcoholic beverage to help determine if other components in alcoholic beverages contributed to risk. Cut-points were based on the distribution of alcohol consumption; cut-points used for beer for men were 0 and 7 grams and for women were 0 and 2 grams of alcohol from beer; for wine were 0 and 3 grams for men and 0 and 5 grams for women, and for hard liquor were 0 and 10 grams for men and 0 and 5 grams for women. For recent alcohol consumption during the referent year we had more information on types of alcoholic beverage consumed and also were able to evaluate red and white wine separately. In addition to looking at associations based on grams of alcohol, we also assessed associations based on drinks of alcoholic beverage per day. Associations were evaluated with a referent group of non-drinkers as well as non-drinkers of specific type of

alcohol; associations were generally the same for both referent groups. We assessed associations for long-term use of 10 and 20 years ago as well as during the more recent referent year; additionally we assessed alcohol based on the combination of the long-term and recent use variables. Long-term alcohol consumption in individuals less than 40 years of age had their long-term alcohol consumption weighed by their reported intake 10 years ago.

All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC). Tumors were defined by specific mutations detected as any *TP53* versus no *TP53* mutation, any *KRAS2* mutations versus no *KRAS2* mutation, or CIMP positive versus CIMP negative. For *TP53* and *KRAS2* mutations, we also examined transversion and transition mutations since specific types of mutations were assessed because other studies have shown specific mutations to have etiologic associations.^{10, 18} To compare specific types of mutations to controls while adjusting for the other tumor mutations simultaneously, a generalized estimating equation (GEE) with a multinomial outcome was used as case subjects could contribute from one to three outcome observations depending upon how many tumor mutations (CIMP, *KRAS2*, *TP53*) were present in a tumor.¹⁹ The GEE accounts for correlation introduced by including subjects multiple times and was implemented using the GENMOD procedure as described by Kuss et al.²⁰ All models were adjusted for age at diagnosis or selection and sex along with other factors that have been shown to be related to colon cancer including body mass index (BMI) in kg/m², long-term vigorous physical activity, pack-years of cigarettes smoked, dietary calcium, and total energy intake. We assessed associations with consumption of specific alcoholic beverages in models with and without adjustment for total grams of long-term and referent year alcohol intake used as a continuous variable. P for trend was assessed using ordered categories of variables and comparing the likelihood ratio of a model with the variable to the likelihood ratio of a model without the variable using the chi-squared test with one degree of freedom.

Results

Table 1 describes the study population. Of the 750 rectal cancer cases with tumor mutation information, 11.0 percent were CIMP positive, 28.9 percent had a *KRAS2* mutation, and 48.3 percent had a *TP53* mutation. The majority of both *KRAS2* and *TP53* mutations were transitions. The most common point mutations in *TP53* occurred at codon 175 and 248. The mean age of the study population was slightly over 61 years for both cases and controls. Participants reported consuming slightly less alcohol during the referent year than what they reported consuming 10 and 20 years ago for long-term alcohol consumption. For recent alcohol consumption beer contributed 48.7% and 37.8% of alcohol consumed for male cases and controls, respectively; beer contributed 16.9% and 15.9% of alcohol consumed by female cases and controls, respectively; wine contributed 28.4% and 37.4% for male cases and controls, respectively; wine contributed 57.1% and 57.9% for female cases and controls, respectively; liquor contributed 22.9% and 24.7% to male cases and controls, respectively; and liquor contributed 26.0% and 26.1% for female cases and controls, respectively.

To examine the association between components of the alcoholic beverages, we evaluated the associations with the beverages with and without adjusting for grams of alcohol intake for recent alcohol consumption during the referent year (Table 2). Associations for beer and wine became slightly stronger after adjusting for alcohol intake. Beer consumed during the referent period significantly increased risk of having a *TP53* tumor mutation; red wine was non-significantly inversely associated with *TP53* mutation, while white wine was not associated with *TP53* risk. We did not observe any effect modification by packyears of cigarettes smoked.

Long-term consumption of liquor increased the odds of having a CIMP positive rectal tumor (Table 3) after adjusting for alcohol. There were no significant associations with consumption of alcohol in the past from either beer or wine for any types of mutations

Evaluation of specific types of *TP53* mutations and alcohol showed that both long-term beer consumption and recent beer consumption were associated most strongly with transversion mutations (Table 4). Evaluation of common *TP53* point mutation (data not shown in table) showed that long-term liquor consumption increased the odds of having a mutation at codon 248 (OR 4.97 95% CI 1.28,19.38 for high versus no consumption) while high levels of recent beer consumption increased the odds of having a point mutation at codon 175 (OR 1.99 95% CI 0.87,4.55). The likelihood of a mutation at codon 245 was increased by recent total alcohol use (OR 3.00 95% CI 0.99,9.09 p linear trend 0.08) during the referent year.

Discussion

A major consideration of studies of alcoholic beverages is determining if associations are due to the alcoholic content of the beverage or other non-alcoholic components of these drinks. Our results suggest that alcohol may contribute to increased likelihood of a CIMP positive tumor, while non-alcoholic components of wine and beer may influence *TP53* mutations in rectal tumors. Further assessment of specific types of *TP53* mutations showed that beer was more likely to influence transversion mutations in the *TP53* gene. Associations with beer were stronger after adjusting for grams of alcohol, further suggesting that non-alcohol components of beverages are contributing to rectal cancer risk. The literature on the association between alcohol and rectal cancer is extensive, although inconclusive.^{1, 4, 21–25} Consistent with others, we observed few significant findings with *KRAS2* mutations.⁵

In these data we saw significant associations between total grams of alcohol consumption and CIMP positive rectal tumors. However, we were unable to evaluate associations with microsatellite instability (MSI) given few rectal tumors with MSI.²⁶ We have reported that CIMP positive colon tumors were not associated with alcohol.^{6, 7} Liquor, the alcoholic beverage with the most concentrated alcohol content, was associated with CIMP positive rectal tumors. Others have shown that alcohol is associated with methylated head and neck tumors.²⁷ Given the association with total alcohol and liquor, the most concentrated source of alcohol, we interpret this as an indication that alcohol itself, rather than some non-alcohol component in alcoholic beverages, is associated with CIMP positive rectal tumors.

The pattern of risk associated with beer and wine suggests that a non-alcoholic component of these beverages may be influencing risk, especially when the associations between beer and wine and *TP53*-mutated tumors appears to be stronger after adjusting for the effects of grams of alcohol consumed. Furthermore, the trend towards an association of decreased risk with wine appears to be restricted to red wine, which contains polyphenols that have antioxidant properties.^{28, 29} Studies have shown that *TP53* mutations are associated with oxidative stress³⁰, thus hypothesizing a protective effect from flavonoid-containing beverages is reasonable. Findings for liquor were similar to those observed for red wine, suggesting either a similar mechanism or a chance finding for both wine and liquor consumption and *TP53* mutations

Epidemiological studies have reported direct association between beer consumption and rectal cancer with associations varying by amount and time of consumption.^{4, 23, 25} While the association between beer consumption and rectal tumors overall was null in our study, we observed a significant association between beer and *TP53* mutations. *TP53* mutations are the most common mutation in a number of cancers and are one of the most commonly detected mutations in rectal tumors. Interestingly, we also observed that transversion

mutations were associated with the greatest risk from recent consumption of beer and alcohol; there was also an indication that specific point mutations may also be influenced by beer intake. Point mutation hotspots within *TP53* are in the evolutionarily conserved areas of the gene.³¹ An arginine residue at codon 248 in the L3 loop of the core domain of the gene is thought to play a critical role in DNA binding; an arginine at codon 175 is located in the L2 loop in the vicinity of the zinc binding site; a glycine at codon 245 also in the L3 loop allows the L3 loop to assume unique conformations.³² Furthermore, mutations in specific sites have been linked with specific exposures that alter cancer risk. For instance, polycyclic aromatic hydrocarbons and benzo[a] pyrene has been associated with G>T transversions and *TP53* mutations at codons 157, 248, and 273^{31, 33}; aflatoxin has been associated mutations at codon 249.³¹

Studies have shown that the guanine base (G) is highly susceptible to oxidative stress because it has the lowest oxidation potential of the four DNA nucleotide bases, thus transversion mutations involving a G:C<>T:A change occur more frequently under oxidative stress conditions.^{34, 35} Nitric oxide can cause DNA damage; it has been shown to induce *TP53* accumulation and post-translational modification^{30, 36} which can lead to the selective expansion of mutated *TP53* cells.³⁰ Studies have shown that nitric oxide, in an inflammatory condition such as ulcerative colitis, can increase frequency of *TP53* mutations.³⁷

Our data suggest that some non-alcohol component of beer may contribute to *TP53* risk, with the greatest risk for more recent beer consumption during the referent year. It is possible that some component of beer contributes to oxidative stress resulting in the observed increased risk of *TP53* mutations, especially transversions. Beer contains isohumulones, the bitter acids from hops³⁸, which has many chemo preventive properties. Isohumulones have been shown to reduce insulin resistance, activate the peroxisome proliferator receptor alpha, and inhibit angiogenesis by suppressing cyclooxygenase-2.³⁸ Hops also have been shown to exhibit estrogen-like activities.³⁹ Studies conducted in rats further suggest the chemo-preventive constituents of beer protect against heterocyclic amine-induced mutagenesis.^{40, 41} While our results do not support a chemo preventive role for beer, as much of the literature might suggest, our observed associations of increased risk are consistent with other epidemiological research findings.^{1, 4, 5, 25, 42}

This investigation is one of the largest population-based studies of rectal tumor mutations reported to date; however, it is not without limitations. Other than the reported cited above, other reports on alcohol and risk factors for rectal tumor mutations have not been reported. Although the numbers of cases and controls were large, we were still limited in our ability to test associations for mutations or epigenetic changes that are uncommon in rectal tumors, such as CIMP, MSI, or specific point mutations. While we were able to detect significant associations with total long-term total alcohol consumption and CIMP positive tumors, other types of alcoholic beverages that may have had a smaller effect could have been missed because of lack of power. There is potential error in subject recall of alcohol consumption, especially when asked details of their consumption patterns. We have attempted to evaluate the impact of recalling alcohol in the setting which the study took place, including the potential for differential recall in the presence of a third party.⁴³ Additionally, we sequenced only the mutation hot spots of the *TP53* and *KRAS2* genes and therefore some samples may have been misclassified as not having a *TP53* or *KRAS2* mutation. In order to thoroughly test our study hypothesis, many comparisons were made. It is possible that associations detected were the result of chance.

In summary, our data suggests that not all alcoholic beverages uniformly contribute to epigenetic and genetic changes in rectal tumors. Furthermore it appears that non-alcoholic

components of beer and wine may alter risk of developing a *TP53* mutation. Replication of these findings is needed. Our evaluation of alcoholic beverages as they relate to specific types of rectal mutations provides additional insight into potential biological mechanisms, as well as an explanation for some of the differences reported between alcohol and rectal cancer in the literature. These findings re-enforce the previous work that shows alcohol consumption could influence risk of rectal cancer.

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Table 1

Summary of study population

	Cases		Controls	
	n	(%)	n	(%)
Total	951	44.1	1205	55.9
Tumor Mutation Data Available	750	78.9		
CIMP Ki- <i>ras</i>	74	11.0		
Overall	215	28.9		
Transitions	140	21.0		
Transversions	75	12.4		
<i>p53</i>	340	48.3		
Overall	248	40.5		
Transitions	69	15.9		
Transversions	37	9.2		
Codon 175	19	5.0		
Codon 245	36	9.0		
Codon 248	29	7.4		
Codon 273	11	2.9		
Codon 282				

	Mean	SD	Mean	SD	P value
Age	61.2	10.9	61.6	11.1	
Longterm alcohol (g/day)	14.4	31.3	11.3	36.9	0.04
Longterm beer (g/day)	7.1	21.1	4.8	14.5	<.01
Longterm wine (g/day)	2.0	5.4	2.4	7.3	0.16
Longterm liquor (g/day)	5.4	17.1	4.3	31.8	0.29
Reference year alcohol (g/day)	12.2	30.5	9.9	23.2	0.04
Reference year beer (g/day)	5.0	18.2	2.7	10.4	<.01
Reference year wine (g/day)	3.3	8.4	3.9	13.0	0.26
Reference year red wine (g/day)	1.2	0.6	1.3	0.6	0.68
Reference year white wine (g/day)	1.3	0.7	1.3	0.7	0.87
Reference year liquor (g/day)	3.8	18.5	3.3	13.3	0.51

Table 2

Associations between types of alcohol consumed during the referent year and rectal tumor mutations¹.

	Control			All Cases ²			CIMP High			TP53 Mutation			KRAS2 Mutation		
	n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)		
Total	None	650	499	1.00		40	1.00		175	1.00		112	1.00		
	Moderate	294	240	1.03 (0.83, 1.28)		13	0.66 (0.34, 1.27)		95	1.12 (0.86, 1.46)		71	1.34 (0.98, 1.84)		
	High	248	201	0.92 (0.72, 1.16)		20	1.38 (0.78, 2.43)		67	0.94 (0.68, 1.30)		29	0.58 (0.38, 0.91)		
	P trend			0.56		0.49			0.89			0.10			
Beer ³	None	865	657	1.00		52	1.00		226	1.00		152	1.00		
	Moderate	259	196	1.03 (0.80, 1.31)		17	1.20 (0.66, 2.19)		73	1.15 (0.83, 1.59)		41	0.91 (0.61, 1.35)		
	High	67	85	1.45 (0.98, 2.15)		4	0.85 (0.27, 2.69)		37	1.97 (1.24, 3.12)		19	1.16 (0.67, 2.02)		
	P trend			0.14		0.94			0.02			0.86			
Red Wine ³	None	993	785	1.00		61	1.00		283	1.00		181	1.00		
	Moderate	145	116	1.03 (0.78, 1.37)		7	0.80 (0.36, 1.79)		42	1.05 (0.72, 1.52)		19	0.72 (0.44, 1.17)		
	High	53	37	0.83 (0.52, 1.31)		5	1.72 (0.68, 4.39)		11	0.64 (0.35, 1.18)		12	1.21 (0.66, 2.24)		
	P trend			0.63		0.59			0.39			0.82			
White Wine ³	None	931	742	1.00		55	1.00		264	1.00		167	1.00		
	Moderate	193	136	0.91 (0.70, 1.18)		10	0.90 (0.44, 1.85)		51	0.97 (0.69, 1.36)		32	0.92 (0.61, 1.39)		
	High	67	60	1.10 (0.74, 1.63)		8	1.98 (0.83, 4.77)		21	0.98 (0.60, 1.61)		13	0.89 (0.48, 1.66)		
	P trend			1.00		0.28			0.89			0.66			
Liquor ³	None	937	738	1.00		53	1.00		257	1.00		160	1.00		
	Moderate	187	151	0.97 (0.75, 1.26)		17	1.48 (0.81, 2.73)		64	1.12 (0.82, 1.53)		39	1.11 (0.76, 1.64)		
	High	67	49	0.66 (0.43, 1.01)		3	0.68 (0.20, 2.25)		15	0.61 (0.33, 1.12)		13	0.97 (0.51, 1.83)		
	P trend			0.13		0.84			0.41			0.86			

¹ Adjusted for age, sex, BMI, long-term vigorous physical activity, pack-years of cigarette smoked, dietary calcium, and total energy intake.

² Includes cases without tumor marker data; numbers vary slightly from missing data.

³ Also adjusted for total long-term alcohol use

Table 3

Associations between long-term alcohol use and rectal tumor mutations

	Control	All Cases*			CIMP High			TP53 Mutation			KRAS2 Mutation		
		n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	
Long-term alcohol	None	545	401	1.00	30	1.00		143	1.00		93	1.00	
	Moderate	409	296	0.91 (0.73, 1.12)	22	1.03 (0.58, 1.86)		108	0.97 (0.73, 1.27)		69	0.97 (0.69, 1.35)	
	High	237	241	1.16 (0.90, 1.49)	21	1.60 (0.87, 2.96)		85	1.17 (0.86, 1.59)		50	1.03 (0.69, 1.52)	
	<i>P</i> trend			0.37		0.19			0.43			0.94	
Long-term beer ^I	None	729	564	1.00	44	1.00		196	1.00		138	1.00	
	Moderate	244	159	0.77 (0.58, 1.02)	13	1.09 (0.53, 2.23)		58	0.92 (0.63, 1.35)		35	0.70 (0.45, 1.08)	
	High	216	214	1.05 (0.77, 1.41)	16	1.37 (0.65, 2.85)		81	1.34 (0.92, 1.95)		39	0.67 (0.43, 1.06)	
	<i>P</i> trend			0.88		0.46			0.18			0.10	
Long-term wine ^I	None	759	607	1.00	44	1.00		222	1.00		140	1.00	
	Moderate	217	165	0.97 (0.74, 1.28)	13	1.26 (0.59, 2.65)		58	0.94 (0.66, 1.33)		31	0.78 (0.50, 1.21)	
	High	214	166	0.94 (0.71, 1.25)	16	1.41 (0.65, 3.04)		56	0.81 (0.57, 1.16)		41	0.99 (0.65, 1.50)	
	<i>P</i> trend			0.67		0.37			0.30			0.84	
Long-term liquor ^I	None	783	576	1.00	44	1.00		205	1.00		130	1.00	
	Moderate	288	227	1.08 (0.84, 1.38)	12	0.84 (0.39, 1.78)		85	1.16 (0.84, 1.62)		49	1.08 (0.72, 1.62)	
	High	120	133	1.37 (0.96, 1.95)	17	2.74 (1.23, 6.11)		45	1.11 (0.71, 1.73)		32	1.36 (0.79, 2.34)	
	<i>P</i> trend			0.11		0.05			0.54			0.32	

* Includes cases without tumor marker data.

^I Adjusted for age, sex, BMI, long-term vigorous physical activity, pack-years of cigarette smoked, dietary calcium, and total energy intake, and long-term and recent alcohol intake

Table 4

Associations between alcohol and *TP53* transition and transversion mutations.

		Control		Transition*		Transversion*		Transition vs. Control		Transversion vs. Control	
		n	n	n	n	n	n	OR	(95% CI)	OR	(95% CI)
Long-term consumption	Total Alcohol	545	104	30	1.00	1.00	1.00	1.00		1.00	
	None	545	104	30	1.00	1.00	1.00	1.00		1.00	
	Moderate	409	82	20	1.01	1.01	0.84	0.84	(0.72, 1.41)	0.84	(0.45, 1.56)
	High	237	59	18	1.19	1.19	1.22	1.22	(0.80, 1.78)	1.22	(0.61, 2.44)
	<i>P</i> trend				0.42	0.42	0.67	0.67			
Beer [†]	None	729	146	35	1.00	1.00	1.00	1.00		1.00	
	Moderate	244	44	11	0.78	0.78	1.73	1.73	(0.50, 1.23)	1.73	(0.64, 4.67)
	High	216	54	22	1.04	1.04	3.50	3.50	(0.65, 1.68)	3.50	(1.35, 9.08)
	<i>P</i> trend				0.93	0.93	<.01	<.01			
Wine [†]	None	759	163	44	1.00	1.00	1.00	1.00		1.00	
	Moderate	217	39	13	0.78	0.78	1.21	1.21	(0.50, 1.21)	1.21	(0.55, 2.64)
	High	214	43	11	0.84	0.84	0.75	0.75	(0.54, 1.31)	0.75	(0.33, 1.73)
	<i>P</i> trend				0.38	0.38	0.56	0.56			
Liquor [†]	None	783	154	41	1.00	1.00	1.00	1.00		1.00	
	Moderate	288	57	20	0.99	0.99	1.47	1.47	(0.66, 1.48)	1.47	(0.71, 3.05)
	High	120	34	6	1.33	1.33	0.65	0.65	(0.77, 2.30)	0.65	(0.22, 1.90)
	<i>P</i> trend				0.41	0.41	0.71	0.71			
Recent consumption	Total Alcohol	650	124	37	1.00	1.00	1.00	1.00		1.00	
	None	650	124	37	1.00	1.00	1.00	1.00		1.00	
	Moderate	294	63	16	1.09	1.09	0.98	0.98	(0.78, 1.54)	0.98	(0.52, 1.83)
	High	248	59	15	1.16	1.16	1.00	1.00	(0.80, 1.68)	1.00	(0.52, 1.95)
	<i>P</i> trend				0.42	0.42	1.00	1.00			
Beer [†]	None	865	167	42	1.00	1.00	1.00	1.00		1.00	
	Moderate	183	34	9	0.98	0.98	1.55	1.55	(0.62, 1.54)	1.55	(0.64, 3.72)
	High	143	44	17	1.54	1.54	3.18	3.18	(0.97, 2.44)	3.18	(1.45, 6.99)
	<i>P</i> trend				0.11	0.11	<.01	<.01			
Red wine [†]	None	993	206	58	1.00	1.00	1.00	1.00		1.00	
	Moderate	87	20	8	1.07	1.07	1.62	1.62	(0.63, 1.83)	1.62	(0.70, 3.73)

	Control	Transition*	Transversion*	Transition vs. Control	Transversion vs. Control
High	111	19	2	0.79 (0.46, 1.35)	0.30 (0.07, 1.31)
<i>P</i> trend				0.47	0.26
None	937	185	56	1.00	1.00
Moderate	128	34	5	1.33 (0.85, 2.08)	0.68 (0.25, 1.82)
High	126	26	7	0.90 (0.53, 1.52)	0.55 (0.22, 1.38)
<i>P</i> trend				0.95	0.15

[/] Adjusted for age, sex, BMI, long-term activity, pack-years of cigarette smoking, dietary calcium and energy intake, and long-term and referent year alcohol use.