Coffee consumption, genetic susceptibility and bladder cancer risk

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Abstract

Objective—We evaluated the bladder cancer risk associated with coffee consumption in a case-control study in Spain and examined the gene-environment interactions for genetic variants of caffeine metabolizing enzymes.

Methods—The analyses included 1136 incident cases with urothelial carcinoma of the urinary bladder and 1138 controls. Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for area, age, gender, amount of cigarette smoking and years since quitting among former smokers.

Results—The OR (95%CI) for ever consumed coffee was 1.25 (0.95–1.64). For consumers of 1, 2, 3 and 4 or more cups/day relative to never drinkers, OR were, respectively: 1.24 (0.92–1.66), 1.11 (95%CI 0.82–1.51), 1.57 (1.13–2.19) and 1.27 (0.88–1.81). Coffee consumption was higher in smokers compared to never smokers. The OR for drinking at least 4 cups/day was: 1.13 (0.61–2.09) in current smokers, 1.57 (0.86–2.90) in former smokers, and 1.23 (0.55–2.76) in never

smokers. Gene-coffee interactions evaluated in NAT2, CYP1A2, and CYP2E1-02 and CYP1A1 were not identified after adjusting for multiple testing.

Conclusion—The modest increased bladder cancer risk among coffee drinkers supports the hypothesis that coffee is a weak carcinogen, although results may, in part, be explained by residual confounding by smoking. The findings from the gene-coffee interactions need replication in further studies.

Keywords

bladder cancer; coffee; genetic susceptibility; epidemiology

INTRODUCTION

Coffee is a complex mixture of chemicals. The carcinogenic potential of coffee has been examined in animal studies with inconsistent results (1). In humans, epidemiological studies suggest that coffee is possibly carcinogenic to the urinary bladder (1), although evidence remains controversial (2). A number of studies have reported a positive association (3–7) that was not replicated in other studies (8–11). The body of evidence excludes a strong effect and causality is questioned given the moderate association, a lack of a clear dose-response and the potential residual confounding by smoking (12–14).

Apart from a recent evaluation (15), the interplay between coffee consumption and genetic susceptibility on bladder cancer risk has not been examined. In humans, caffeine is mainly metabolised by cytochrome P450 (CYP) 1A2 enzyme in the liver. Additional CYPs isoforms (e.g. *CYP1A1*, *2E1*, *3A4*), N-acetyltransferase 2 (*NAT2*) and xanthine oxidase (*XO*) also appear to be involved in the formation of certain secondary metabolites (16–18). However, genetic variants of most of those genes have not been examined in relation to the bladder cancer risk by coffee consumption.

We evaluated the association between coffee consumption and bladder cancer risk in a case-control study conducted in Spain and examined the gene-environment interactions for variants of genes coding for caffeine metabolizing enzymes.

MATERIAL AND METHODS

Study design and population

We conducted a multicentre hospital-based case-control study of bladder cancer between June 1998 and June 2001 in Spain. Study subjects were recruited in 18 participating hospitals from 5 areas: Barcelona, Vallès/Bages (including two cities: Sabadell and Manresa), Alicante, Tenerife and Asturias. Cases were identified through the hospital urological services at diagnosis, and were patients with newly diagnosed histologically confirmed primary bladder cancer, aged between 20 and 80 years and living in the catchment area of the participating hospitals. In addition to registries from urological services, complete case ascertainment was secured by regular evaluations of hospital discharge records, pathology records and local cancer registries. Controls were patients with diagnoses unrelated to the bladder cancer risk factors under study, particularly tobacco use. They were individually matched to cases by gender, age group (5-year strata) and residence area. Controls were admitted to hospitals for hernias (37%), other abdominal surgery (11%), fractures (23%), other orthopaedic problems (7%), hydrocoele (12%), circulatory disorders (4%), dermatological disorders (2%), ophthalmologic disorders (1%), and other diseases (3%). The study was approved by the ethics committees of the participating centres, and subjects were enrolled after written informed consent.

Personal information and response rates

Trained interviewers administered a comprehensive computer assisted personal interview (CAPI) to the participants during their hospital stay. Collected information included socio-demographic characteristics, smoking habits, coffee consumption, occupational, residential and medical histories, and familial history of cancer. A food frequency questionnaire was self-administered. We identified 1,457 eligible cases and 1,465 eligible controls. Among these, 84% of cases (n=1219) and 88% of controls (n=1271) responded to the questionnaire. Biological samples for DNA analyses were obtained from 97% of cases and 91% of controls. Of these, 88.5% were based on blood samples (94% cases, 83% controls), and the rest were from buccal cell samples.

Coffee data

We ascertained whether participants had ever consumed coffee, age when started and quit coffee consumption and the average amount consumed per day during adult life. The actual questions were "*Did you ever drink at least one cup of coffee per week for a year or longer*?"; "*How old were you when you first had at least one cup of coffee per week*?"; "*How old were you when you last drank coffee*?"; and "*Thinking about all the years that you drank coffee over most of your adult life, which could be different from what you do now, how many cups of coffee did you usually drink per day*?". These questions were repeated separately for decaffeinated and regular coffee.

Genotyping

Single nucleotide polymorphisms (SNPs) were analysed in DNA extracted from leucocytes (1107 cases, 1032 controls) or mouthwash samples (43 cases, 117 controls). Genotyping was performed at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, US National Cancer Institute using TaqMan® (Applied Biosystems, Foster City, CA, USA) and GoldenGate® (Illumina®, San Diego, CA, USA) assays. Procedure of genotyping is detailed elsewhere (19;20). The description and methods for each assay can be found at: http://snp500cancer.nci.nih.gov. The 4 genes included in this analysis were selected from the literature based on their effect on caffeine metabolism (*CYP1A2, CYP1A1, CYP2E1* and *NAT2*). SNP selection favoured non-synonymous SNPs, those previously evaluated in relation to cancer risk or those with evidence of functional significance. All studied SNPs were under Hardy-Weinberg equilibrium in the control population and with minor allele frequency (MAF) &>0.05. Pairwise linkage disequilibrium (LD) between SNPs was estimated based on D' and r^2 values using *R*.

Statistical analysis

Ever coffee consumption was defined as having ever drunk at least one cup of coffee per week for one year or longer. Coffee consumers were grouped in categories according to cups of coffee consumed per day: 1, 2, 3 and 4 or more. We calculated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression adjusting for area, age, gender, amount of smoking (3 categories: never, 0–19 and 20+ cigarettes/day), and years since quitting among former smokers (continuous). Former and current smokers smoked 100 cigarettes or more in their lifetime and at least 1 cigarette/day during 6 months or longer. Former smokers who quit less than one year before interview were considered current smokers. Odds ratios were calculated separately for never, former and current smokers. The potential interactions of each individual SNP and high vs. low coffee intake (≥4 vs. <4 cups/day) were evaluated by introducing interaction terms in logistic regression models. Haplotype frequencies for genes with more than one SNP and their interaction with coffee were estimated using SNPstats (http://bioinfo.iconcologia.net/index.php?module=Snpstats). To adjust for multiple testing, the false discovery rate (FDR) test was applied (21). Analyses

of coffee main effects were limited to 1136 cases with urothelial carcinoma of the urinary bladder and 1138 controls with available information on amount of coffee consumption and smoking status. Genetic analyses were based in a subset of 1126 cases and 1117 controls with available DNA data.

RESULTS

Characteristics of the study population are shown in Table 1. Eighty seven percent of study subjects had ever consumed coffee (89% of cases, 85% of controls). By gender, 89% of men vs. of 79% women had ever consumed coffee. Coffee consumers drank on average 2.4 cups per day. Study subjects had difficulties at disentangling regular from decaffeinated coffee consumption when asked separately. Data on amount of regular and decaffeinated coffee consumption separately were missing in 15% and 28% of study subjects, respectively. Available data showed that ever consumption of decaffeinated coffee (72% of study population) was lower than regular coffee consumption (78%). Average consumption of regular and decaffeinated coffee was 2.5 cups/day (standard deviation, SD 3.2) and 1.6 (SD 1.1) cups/day among ever consumers, respectively.

Table 2 shows the relationship between coffee consumption and smoking status in our study subjects. Coffee consumption was correlated with smoking habits. Number of daily cups of coffee increased in former and current smokers, smokers of 20 cigarettes/day or more, former smokers quitting recently (<5 years) and blond tobacco smokers. However, coffee consumption was independent of duration of smoking.

The odds ratio of ever coffee consumption adjusted for age, sex, area and amount of smoking was 1.25 (95%CI 0.95–1.64). Additional adjustment for years of education, intake of fruits and vegetables, urbanicity of longest residence until age 18 and having ever worked in high-risk occupations led, respectively, to the following ORs: 1.24 (0.94–1.63), 1.21 (0.92–1.60), 1.25 (0.95–1.64, 1.23 (0.93–1.64). Adjusting additionally for total fluid consumption and average lifetime THM levels in the household (available for a subset) did not affect the risk estimates. The OR adjusted for fluids was 1.18 (0.83–1.68) vs. the unadjusted 1.18 (0.83–1.68) (N=1465). The OR adjusted by THM was 1.56 (1.09–2.21) vs. the unadjusted 1.57 (1.11–2.24). By smoking status, ever coffee consumption was associated with an increased risk of bladder cancer among smokers (Table 3). The interaction between coffee consumption and smoking (never/ever) was 0.043. By gender, OR for ever coffee consumption was 1.32 (95%CI: 0.97–1.79) among men and 1.06 (95%CI: 0.58–1.93) among women (p value for interaction 0.655). Among smokers, the OR for coffee consumption differed slightly in smokers by smoking patterns, but no evidence of effect modification was found. P-values for interaction between ever coffee consumption and (i) duration of smoking (<30 vs. 30+ years); (ii) among of smoking (1–19 vs. 20+ cigarettes/day); time since quitting among former smokers (<5 vs. 5+ years); and (iv) tobacco type (blond, black, both) were 0.382, 0.773, 0.615 and 0.282, respectively. We stratified subjects according to the lifetime residential THM level above or below the population median (26 μg/l). OR of bladder cancer for ever coffee consumption was 1.85 (95% CI 1.09–3.14) among subjects below the median and 1.38 (0.86–2.21) among subjects above the median THM level. There was no evidence of interaction between coffee consumption and THM level (p value 0.563).

We did not identify gene-coffee interactions for the evaluated SNPs in NAT2, CYP1A2 and CYP1A1. Although the interaction p-value for CYP2E1 (rs2070676) was statistically significant (0.03), it did not retain significance after adjusting for multiple comparisons (p-value 0.179). (Table 4). We found no evidence of interactions between CYP1A1 haplotypes, coffee intake (<4 vs. ≥4 cups/day) and bladder cancer risk (p-value 0.094). For CYP2E1

haplotypes, p-value was 0.033 but it did not retain significance as well after applying the FDR procedure.

DISCUSSION

Overall, bladder cancer risk among coffee drinkers was slightly increased, with no significant dose-response relationship (p trend = 0.082). Drinkers of four cups of coffee per day or more had an OR of 1.27 (95%CI 0.88–1.81). By smoking status, the OR among those drinking at least 4 cups of coffee per day compared to never drinkers was 1.23 (95%CI 0.55–2.76) in never smokers, 1.57 (95%CI 0.86–2.90) in former smokers and 1.13 (95%CI 0.61–2.09) in current smokers.

Coffee consumption was highly correlated with smoking habits, and residual confounding can not be ruled out in smokers. Among never smokers, although coffee consumption was moderate and the statistical power in heavy coffee drinkers was limited, we did observe a non significant increased risk of bladder cancer in heavy coffee consumers (4 daily cups of coffee or more). In addition, a marginally significant interaction between coffee and smoking was found (0.045), suggesting that the effect of coffee could be modified by smoking status.

Coffee intake in the study population is very similar to the consumption described in a study conducted in Italy during 1997–2000, where average coffee consumtpion was 2.6 cups/day among cases and 2.1 cups/day among controls (15). However, our consumption patters were modest compared to other studies and similar to consumption among non-smokers in previous studies. A pooled analysis of 10 case-control studies of bladder cancer conducted in 6 European countries including non-smokers reported an average coffee consumption of 2.1 cups/day in hospital controls and 2.9 cups/day in population controls (13). Higher intakes were found in a case-control study of bladder cancer conducted in the Netherlands (5), where average coffee consumption was 3.0 and 2.1 cups/day, respectively, in men and women classified as low coffee consumers and 6.5 and 5.1 cups/day, respectively, in men and women classified as heavy coffee consumers. In our study, seventy percent of study subjects consumed 2 cups of coffee per day or less and average consumption among controls was 2.2 cups/day.

Although coffee is a complex mixture, the selection of genes in our analyses was focused on caffeine. This is a vast oversimplification and SNPs that could be relevant for coffee metabolism may well have not been analyzed. In addition, we had available a limited number of SNPs for CYP1A2, the most consistently associated with caffeine metabolism. The previous study evaluating gene-coffee interactions was not specifically focused on coffee-metabolizing enzimes and results are only comparable and consistent for NAT2. This is the first study evaluating gene-coffee interactions for bladder cancer for most of the genes we evaluate and results remain preliminary.

In conclusion, coffee consumption was highly correlated with smoking habits and the increased risk of bladder cancer among coffee drinkers could partly be explained by residual confounding among smokers. Among never smokers, an increased risk was only observed in the highest category of coffee drinking (4+ cups/day). These results support the hypothesis that coffee is a weak carcinogen.

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Table 1

Characteristics of the study population and coffee consumption

	Cases N=1,136		Controls N=1,138	
	N	**%**	**N**	**%**
Gender				
Men	1001	88.1	992	87.2
Women	135	11.9	146	12.8
Age (years)				
<55	171	15.0	195	17.1
55–64	246	21.7	280	24.6
65–69	255	22.5	262	23.0
70–74	249	21.9	219	19.3
≥75	215	18.9	182	16.0
Region				
Asturias	462	40.7	463	40.7
Barcelona	212	18.6	227	19.9
Tenerife	203	17.9	201	17.7
Vallès-Bages	174	15.3	168	14.8
Alicante	85	7.5	79	6.9
Smoking				
Never	165	14.5	357	31.4
Ex	457	40.2	467	41.0
Current	514	45.3	314	27.6
Coffee consumption				
Never	120	10.6	166	14.6
Ever	1016	89.4	972	85.4
1 cups/day	336	29.6	352	30.9
2 cups/day	303	26.7	321	28.2
3 cups/day	223	19.6	165	14.5
4+ cups/day	154	13.5	134	11.8
Mean (SD)*, cups/day	2.4 (2.2)		2.2 (1.7)	
Percentiles 25, 50, 75, 90	1, 2, 3, 4		1, 2, 3, 4	
N	1016		972	

*among ever coffee consumers

Table 2

Coffee consumption by smoking status.

	Coffee consumption											
	Never		1 Cup/day		2 Cups/day		3 cups/day		≥ 4 cups/day		Overall	
	N	%	N	%	N	%	N	%	N	%	N	%
Smoking status												
Never	118	41.3	176	25.6	126	20.2	64	16.5	38	13.2	522	23.0
Former	93	32.5	298	43.3	266	42.6	164	42.3	103	35.8	925	40.6
Current	75	26.2	214	31.1	232	37.2	160	41.2	147	51.0	828	36.4
Duration of smoking, years												
<30	35	21.5	103	20.7	102	20.9	74	23.3	54	21.9	368	21.5
30+	128	78.5	395	79.3	387	79.1	243	76.7	193	78.1	1346	78.5
Amount of smoking (cigarettes/day)												
1–19	68	39.8	197	38.7	149	30.3	79	24.4	39	15.8	532	30.5
20+	103	60.2	312	61.3	343	69.7	245	75.6	208	84.2	1211	69.5
Time since quitting in former smokers, years												
<5	11	11.8	38	12.8	41	15.4	30	18.3	20	19.4	140	15.2
5+	82	88.2	260	87.2	225	84.6	134	81.7	83	80.6	784	84.8
Type of tobacco												
Blond	17	14.5	48	12.2	56	13.7	41	15.4	50	23.5	212	15.1
Black	76	65.0	228	57.7	207	50.5	114	42.7	67	31.4	692	49.4
Both	24	20.5	119	30.1	147	35.8	112	41.9	96	45.1	498	35.5

Table 3

Odds ratios (OR) and 95% confidence intervals (CI) of bladder cancer for coffee consumption, by smoking status and for the whole sample.

	Never smokers N=522		*Former smokers* N=924		*Current smokers* N=828		*All* N=2274
Coffee consumption	**cases/controls**	**OR (95% CI)** †	**cases/controls**	**OR (95% CI)** ‡*	**cases/controls**	**OR (95% CI)** ‡	**OR (95% CI)** ‡
Never	40 / 78	1.00	34 / 59	1.00	46 / 29	1.00	1.00
Ever	125 / 279	0.85 (0.53–1.35)	423 / 408	1.85 (1.16–2.95)	468 / 285	1.20 (0.72–2.01)	1.25 (0.95–1.64)
1 cup/day	54 / 122	0.91 (0.53–1.56)	152 / 146	1.92 (1.16–3.17)	130 / 84	1.14 (0.65–2.00)	1.24 (0.92–1.66)
2 cups/day	32 / 94	0.61 (0.34–1.10)	128 / 138	1.62 (0.97–2.70)	143 / 89	1.20 (0.68–2.09)	1.11 (0.82–1.51)
3 cups/day	24 / 40	1.06 (0.53–2.13)	94 / 70	2.36 (1.36–4.11)	105 / 55	1.39 (0.77–2.53)	1.57 (1.13–2.19)
4+ cups/day	15 / 23	1.23 (0.55–2.76)	49 / 54	1.57 (0.86–2.90)	90 / 57	1.13 (0.61–2.09)	1.27 (0.88–1.81)
P-trend		0.961		0.176		0.559	0.082

† Adjusted for age (5 categories), gender and area.

‡ Adjusted additionally for intensity of smoking (cigarettes/day).

* Adjusted additionally for years since quitting smoking

Table 4

Odds ratio (OR) of bladder cancer for coffee consumption by selected genetic polymorphism and gene-cofffee interaction p-values.

	Coffee consumption				OR (95%CI)	Coffee-SNP interaction *p*-value[†]
	<4 cups/day		≥4 cups/day		<4 vs. ≥4 cups coffee/day	
SNP	**Controls**	**Cases**	**Controls**	**Cases**		
NAT2						
Rapid/intermediate	388	327	55	55	1.08 (0.70 – 1.66)	
Slow	504	587	66	93	1.03 (0.72 – 1.47)	0.7123
CYP1A2-03 (rs762551)						
AA	332	397	48	61	0.95 (0.62 – 1.47)	
AC	361	395	51	68	1.12 (0.74 – 1.69)	
CC	111	98	8	15	1.30 (0.49 – 3.47)	0.2766
CYP1A1-15 (rs4646421)						
CC	621	664	91	109	0.98 (0.71 – 1.35)	
CT	153	182	16	26	1.19 (0.58 – 2.47)	
TT	11	17	0	4	-	0.2208
CYP1A1-78 (rs2198843)						
GG	561	552	75	93	1.12 (0.79 – 1.59)	
GC	218	288	32	41	0.77 (0.45 – 1.30)	
CC	30	36	3	8	8.25 (0.66 – 103.74)	0.9661
CYP1A1-81 (rs2472299)						
CC	330	386	48	63	1.02 (0.66 – 1.57)	
CT	340	381	50	62	0.93 (0.61 – 1.43)	
TT	113	94	9	14	1.33 (0.50 – 3.55)	0.5763
CYP2E1-02 (rs2070676)						
CC	592	657	85	97	0.87 (0.63 – 1.22)	
CG	203	207	25	41	1.28 (0.72 – 2.28)	
GG	13	12	0	4	-	0.0255
CYP2E1-31 (rs8192766)						
TT	661	718	90	111	1.01 (0.73 – 1.38)	
TG	140	152	18	30	1.33 (0.65 – 2.69)	

	Coffee consumption				OR (95%CI)	Coffee-SNP interaction *p*-value†
	<4 cups/day		≥4 cups/day		<4 vs. ≥4 cups coffee/day	
SNP	Controls	Cases	Controls	Cases		
GG	8	5	2	1	-	0.6305

†Interaction p-values after multiple comparison adjustment were: 0.8310 for NAT2, 0.6454 for CYP1A2-03, 0.6454 for CYP1A1-15, 0.9661 for CYP1A1-78, 0.8310 for CYP1A1-81, 0.1785 for CYP2E1-02 and 0.831 for CYP2E1-31