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SLC39A2 and *FSIP1* polymorphisms as potential modifiers of arsenic-related bladder cancer

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Abstract

Arsenic is a carcinogen that contaminates drinking water worldwide. Accumulating evidence suggests that both exposure and genetic factors may influence susceptibility to arsenic-induced malignancies. We sought to identify novel susceptibility loci for arsenic-related bladder cancer in a US population with low to moderate drinking water levels of arsenic. We first screened a subset of bladder cancer cases using a panel of approximately 10,000 non-synonymous single nucleotide polymorphisms (SNPs). Top ranking hits on the SNP array then were considered for further analysis in our population-based case–control study (*n* = 832 cases and 1,191 controls). SNPs in the fibrous sheath interacting protein 1 (*FSIP1*) gene (rs10152640) and the solute carrier family 39, member 2 (*SLC39A2*) in the ZIP gene family of metal transporters (rs2234636) were detected as potential hits in the initial scan and validated in the full case–control study. The adjusted odds ratio (OR) for the *FSIP1* polymorphism was 2.57 [95% confidence interval (CI) 1.13, 5.85] for heterozygote variants (AG) and 12.20 (95% CI 2.51, 59.30) for homozygote variants (GG) compared to homozygote wild types (AA) in the high arsenic group (greater than the 90th percentile), and unrelated in the low arsenic group (equal to or below the 90th percentile) (*P* for interaction = 0.002). For the *SLC39A2* polymorphism, the adjusted ORs were 2.96 (95% CI 1.23, 7.15) and 2.91 (95% CI 1.00, 8.52) for heterozygote (TC) and homozygote (CC) variants compared to homozygote wild types (TT), respectively, and close to one in the low arsenic group (*P* for interaction = 0.03). Our findings suggest novel variants that may influence risk of arsenic-associated bladder cancer and those who may be at greatest risk from this widespread exposure.

Introduction

Bladder cancer is responsible for significant morbidity worldwide. In the USA, an estimated 70,530 new cases of bladder cancer will occur in 2010, along with approximately 14,680 deaths from this malignancy (Jemal et al. 2009). Northern New England has among the highest bladder cancer mortality rates in the USA (Brown et al. 1995). This distinct cluster, that includes New Hampshire, is seen in both men and women, raising the possibility of an etiologic agent in the environment, such as arsenic exposure via drinking water or other sources.

Arsenic ingestion is associated with bladder cancer mortality in several regions of the world with highly arsenic contaminated drinking water supplies (IARC 2004). In the USA, Bates and colleagues found cumulative exposure to arsenic in drinking water related to an increased bladder cancer incidence, largely among smokers (Bates et al. 1995). In Finland (Kurttio et al. 1999), very low levels of well water arsenic (e.g., >0.5 microgram per liter (μg/L) vs. <0.1 μg/L) were associated with an increased risk of bladder cancer, likewise among smokers. In contrast, there was no evidence of elevated bladder cancer mortality

rates in a population of largely non-smokers from Utah with median drinking water concentrations ranging from 14 to 166 μg/L (based on five observed bladder cancer deaths) (Lewis et al. 1999). A cohort study in the northern region of Taiwan found a modest risk among those who drank water with 10.1–50.0 μg/L arsenic (RR = 1.9, CI = 0.1, 32.2) (Chiou et al. 2001a, b); however, no interaction was observed with smoking, raising the possibility of inter-population differences in risk.

In the rural state of New Hampshire, private, unregulated water supplies (i.e., bedrock wells) serve about 40% of the population, and arsenic concentrations above the current maximum contaminant level (MCL) of 10 μg/L have been detected in over 10% of private wells (Karagas et al. 2002). Previously, we reported an association between arsenic exposure, measured in toenail clipping samples, and incidence of bladder cancer among smokers (Karagas et al. 2004). We further found evidence of gene–environment interaction with polymorphisms in DNA repair gene X-ray repair complementing defective repair in Chinese hamster cells 3 (*XRCC3*) *241* and to a lesser extent in excision repair cross-complementing rodent repair deficiency, complementation group 2 (*ERCC2*) *312* (Andrew et al. 2009). Hsu et al. (2008) noted an interaction between a polymorphism in the base excision repair gene *XRCC1* and bladder cancer in a case–control study from Taiwan. These findings suggest the role of both environmental and genetic co-factors in arsenic-induced bladder cancer. Using an expanded study group from New Hampshire, we explored the possibility of variation in yet unidentified genes on susceptibility to arsenic-related bladder cancer.

Materials and methods

Bladder cancer cases and controls were derived from two completed series of a population-based epidemiologic study as previously described (Andrew et al. 2009; Karagas et al. 1998; Dietrich et al. 2009) with current drinking water concentrations ranging from undetectable to 158 μg/L. The case group included New Hampshire residents, aged 25–74 years, first diagnosed with bladder cancer in July 1, 1994 through June 30, 1998 (Phase 1), and July 1, 1998 through December 31, 2001 (Phase 2) identified by the New Hampshire State Department of Health and Human Services' rapid reporting Cancer Registry. For efficiency, we shared a control group with a study of non-melanoma skin cancer covering a reference period of July 1, 1993 to June 30, 1995 and July 1, 1997, through March 31, 2000. We selected additional controls for the intervening period, frequency matched to cases on age (25–34, 35–44, 45–54, 55–64, 65–69, 70–74 years) and gender. We chose controls less than 65 years of age using population lists obtained from the New Hampshire Department of Transportation. The file contains the names and addresses of those holding a valid driver's license for the state of New Hampshire. Controls 65 years of age and older were chosen from data files provided by the Centers for Medicare & Medicaid Services. Controls were randomly assigned a comparable reference date to the cases' diagnosis dates. We successfully interviewed a total of 857 bladder cancer cases, of which 832 were considered cancerous (on histopathology re-review or, in about 10% with unavailable pathology materials, the original diagnosis) and 1,191 controls (Table 1). Participating subjects were approximately 85% of the cases and 70% of the controls confirmed to be eligible for the study.

Consenting subjects underwent a detailed in-person interview, usually at their home. The interview covered sociodemographic information (including level of education), occupational history, detailed information about use of tobacco products (e.g., history of cigarette smoking, cigars and smokeless tobacco) and medical history (including previous radiotherapy) prior to the reference date. Information relating to household water supply included type of water source used in their current residence (e.g., private well vs. public water), years of use of their current water system and use of water filters. For private,

domestic systems, we asked whether the water source was a dug/surficial well, spring, or deep/artesian well.

Venous blood samples of approximately 20–30 ml were collected in heparinized tubes. Blood was separated by centrifugation at 3,000 rpm for 20 min at 4°C and plasma, white blood cells and red blood cells (washed twice in saline) were stored separately at −80°C until analysis. Each specimen was labeled with a type code (plasma, red blood cells, buffy coat) and a unique identifier that did not reveal the subject's case–control status.

We did not disclose the case–control status and main objectives of the study to the interviewers. To ensure consistent quality, interviews were tape recorded with the consent of the participants and routinely monitored by the interviewer supervisor (<5% of participants refused to be taped). To assess comparability of cases and controls, we asked subjects if they currently held a driver's license or a Medicare enrollment card. All procedures and study materials were approved by the Committee for the Protection of Human Subjects at Dartmouth College.

Arsenic determinations

Subjects were mailed the instructions and materials to save a toenail-clipping specimen prior to the interview. Using an established protocol, samples were analyzed for arsenic using instrumental neutron activation analysis at the University of Missouri Research Reactor (Columbia, MO, USA) following careful washing to remove external contamination (Karagas et al. 2000). Each batch of analyses included quality control samples comprised of matrix-matched samples with known content and analytic blanks along with study samples and standards. The between assay coefficient of variability for matrix matched samples is about 8%. All samples were labeled with an identification number that did not disclose the case–control status of the study participants. Using this approach, arsenic values ranged from 0.009 microgram per gram (μg/g) to 7.63 μg/g (mean = 0.12 μg/g, standard deviation = 0.21 μg/g).

Genotyping

DNA was isolated from peripheral circulating blood lymphocyte specimens (buffy coat) using Qiagen genomic DNA extraction kits (QIAGEN Inc., Valencia, CA, USA). In our initial analysis, we detected an increased risk of bladder cancer at higher levels of toenail arsenic concentrations, and the association was confined to smokers (Karagas et al. 2004). Therefore, as an initial screen, we sampled from among those with a history of cigarette smoking from the extremes of high and low arsenic i.e., above the 90th percentile in the original analyses of >0.194 μg/g) (n = 25) and a random sample of those with the lowest concentrations (<0.06 μg/g) in toenails (n = 25). Genotyping on isolated DNA from lymphocytes on these subjects was performed using an approximately 10,000 nonsynonymous coding polymorphisms cSNP MegAllele™ genotyping system developed by ParAllele (acquired by Affymetrix) (Hardenbol et al. 2003). 10% of samples were genotyped in duplicate, and 1 positive kit control (an unknown DNA from a blood bank) was genotyped 16 times. The average call rate was 97.7% and the median was 98.7%. The repeatability of the genotypes was 99.7%. With this sample size and estimated genotype prevalence of 30%, we had roughly 80% power (alpha = 0.10) to detect a fourfold odds ratio. Genes were ranked for their association with arsenic exposure according to their Fisher's exact test P value. The top five SNPs were selected for genotyping in the full case–control study using a 96-plex GoldenGate Assay system by Illumina's Custom Genetic Analysis service (Illumina, Inc., San Diego, CA, USA) custom designed for this study and other validation efforts. Of those selected, two were successfully genotyped: *FSIP1* C402R (cystine to arginine amino acid change; rs10152640) and *SLC39A2* F115L (phenylalanine to

leucine amino acid change; rs2234636). Two SNPs in the otoconin 90 gene (rs7386783 and rs7386782) and another SNP in an unknown gene (rs7709531) failed in the design phase of the custom Illumina panel. DNA samples on 670 bladder cancer cases and 974 controls were processed and run on the custom Illumina 96-plex Golden Gate assay. For quality control purposes, DNA samples from three individuals were repeated across multiple genotyping plates and genotype calls were compared within a sample. For each of the SNP assays on the panel that successfully produced genotypes for>50% of subjects, the genotype calls were 100% identical within each of the three quality control DNA samples. Individuals with genotype data had similar characteristics to the overall study group with respect to age (61 vs. 62 years), gender (67 vs. 69% men) and smoking status (24 vs. 23% current smokers).

We computed odds ratios and 95% confidence intervals using unconditional logistic regression to assess the association between each SNP (wild type, heterozygous, and homozygous variant) among those with high (above the 90th percentile) versus low (below the 90th percentile) toenail arsenic—the cutpoint for high arsenic used in the initial screen as well as previous analyses of arsenic–gene interactions and DNA methylation in our study population (e.g., Applebaum et al. 2007; Andrew et al. 2009; Wilhelm et al. 2010). Models were adjusted for age, gender and smoking status (never, current, former). We further conducted analyses restricted to ever smokers to follow the design of the initial scan. We tested the statistical significance of the interaction terms using a log likelihood test (Breslow and Day 1980).

Results

Using a SNP panel of approximately 10,000 cSNPs, we screened a subset of ever smoker bladder cancer cases with high (>90th percentile, 0.194 μg/g) versus low (<0.006 μg/g) toenail arsenic, and generated an association table ranked by p-value (top 50 SNPs in supplemental Table 1). SNPs in *FSIP1* C402R (rs10152640, allelic frequency = 0.41) and *SLC39A2* F115L (rs2234636), allelic frequency = 0.37) fell below a Fisher's exact *P* value of 1 in 10,000 (<0.0001) and were selected for further investigation in the case–control study.

In the case–control study analysis, elevated odds ratios were observed for heterozygous and homozygous variants for *FSIP1* C402R among those with high arsenic levels (OR for heterozygotes = 2.57; 95% CI 1.13, 5.85 and OR for homozygotes = 12.20; 95% CI 2.51, 59.30) (*P* for trend = 0.001). In contrast, the odds ratios were close to one among those in the low arsenic group (*P* for interaction = 0.002) (Table 2). We likewise found elevated odds ratios both for heterozygous and homozygous variants in *SLC39A2* F115L (OR for heterozygotes = 2.96; 95% CI 1.23, 7.15 and OR for homozyotes = 2.91; 95% CI 0.99, 8.52) (*P* for trend = 0.028), whereas no such associations were observed among those in the low arsenic group (*P* for interaction = 0.03) (Table 2). For both SNPs, associations were present among smokers (Table 2).

Discussion

Using a non-synonymous genome wide scan approach, we identified coding SNPs in *SLC39A2* and *FSIP1* that appeared to modify the association between bladder cancer and arsenic exposure. Among the top ranking SNPs was a polymorphism in *SLC39A2*, a member of the ZIP gene family of metal transporters first identified in plants (Guerinot 2000) that encodes the human protein, hZIP2. The name derives from Zrt-, Irt-like protein. Both ZRT1 and ZRT2 are zinc-regulated transporters with high/low affinity for zinc and IRT1 is the major iron transporter responsible for iron uptake from the soil. hZIP2 is responsible for

cellular uptake of zinc and is regulated by intracellular zinc concentrations (Gaither and Eide 2000).

While a metal transporter, hZIP2 is not known to bind arsenic. However, arsenic does interfere with zinc motifs in proteins, for example by binding to cysteines in zinc fingers such as those found in oncogenic proteins (Zhang et al. 2010). Thus, in theory, if the *SLC39A2* polymorphism disrupts hZIP2 function, and consequently zinc uptake, this, in turn, could facilitate arsenic replacement of zinc in key pathway moieties. In addition, normal prostate epithelium accumulates high concentrations of zinc, but this does not occur in malignant prostate cells where hZIP2 appears to be down regulated (Desouki et al. 2007). This suggests a potential tumor suppressive function of zinc in prostate tissue. Still, as yet there are no reports of hZIP2 being expressed in either normal bladder epithelium or tumorous tissue.

To our knowledge, the role of the *SLC39A F115L* polymorphism (rs2234636) in human disease has not been investigated previously nor has a specific function been identified. A different SNP in *SLC39A2* (rs2234632) was inversely associated with carotid artery disease in a hospital-based study from Italy (Giacconi et al. 2008). Both arsenic and zinc have putative effects on oxidative signaling, and potentially atherosclerosis. Zinc is required for normal immune function, whereas arsenic impairs immune function, even at low levels of exposure (Prasad et al. 2010). In a small human study, hZIP2 mRNA levels were overexpressed in leukocytes of children with asthma ($n = 9$) compared to children without asthma ($n = 9$) (Xu et al. 2009). Whether our findings are due to altered oxidative signaling, immune function or other pathways will require further mechanistic studies.

Linkage disequilibrium with another “causal” SNP is an alternative explanation for our results. We examined linkage disequilibrium in the region of rs2234633 in the HapMap project data (downloaded on March 20, 2010) from the CEPH population made up of Utah residents with ancestry from northern and Western Europe, which most closely resembles that of our study population (The International HapMap Project 2003). In the Haploview analysis (Barrett et al. 2005), there does not appear to be linkage disequilibrium in the region of rs2234633 (Fig. 1). This suggests that our observation of the *SLC39A2* SNP with arsenic-associated bladder cancer may indeed be due to functional polymorphisms in *SLC39A2* rather than neighboring genes. But again, this will need to be investigated in functional studies.

We observed a gene-dosing effect of the *FSIP1* polymorphism on arsenic-related bladder cancer. *FSIP1* encodes a protein that was first identified as binding to a protein component of the fibrous sheath of sperm flagellum (Brown et al. 1995). There is evidence that *FSIP1* is a direct target of steroid receptor coactivator-3 (SRC-3/A1B1) (Labhart et al. 2005), which is a known oncogene associated with breast cancer (Anzick et al. 1997; Shou et al. 2004; Torres-Arzayus et al. 2004) and a coactivator for nuclear receptors, including estrogen receptor-α (ER-α) (McKenna et al. 1999). A recent study has shown that arsenic modifies ER-α mediated gene regulation in vivo and in cell culture at low, environmentally relevant concentrations (Davey et al. 2007). Though speculative, it is possible that regulation of *FSIP1* by SRC-3/ER-α is associated with bladder cancer and that this regulatory activity is modified by arsenic.

Alternatively, unlike *SLC39A2*, the *FSIP1* SNP is in strong linkage disequilibrium with SNPs in another gene, Thrombospondin 1 (*THBS1*) (Fig. 2). Expression levels of *THBS1* have been previously linked to invasion and differentiation of bladder carcinomas (Donmez et al. 2009). Furthermore, interactions of polymorphisms in this gene with polymorphisms in the vascular endothelial growth factor (*VEGF*) gene have been associated with susceptibility

to other types of cancer such as prostate cancer (Sfar et al. 2009). Thus, polymorphisms in *THBS1* also may be potential candidates for further exploration.

Drinking water contamination with arsenic represents a major global health concern, affecting the human lifespan (Argos et al. 2010). Aside from the known carcinogenic effects of arsenic on bladder cancer incidence in highly exposed populations, data are beginning to emerge that observable elevations in risk among certain subgroups of the population may occur at lower levels of exposure. Although results are imprecise, studies from Utah (Bates et al. 1995), Nevada and California (Steinmaus et al. 2003) and New Hampshire (Karagas et al. 2004) in the USA and from Finland (Kurttio et al. 1999; Michaud et al. 2004) suggest a relation between bladder cancer incidence and arsenic exposure among smokers, and in some of these studies, for specific latency periods (Bates et al. 1995; Steinmaus et al. 2003; Bates et al. 2004). A trend of increasing bladder cancer risk was noted in a cohort study from the northeast of Taiwan, based on a small number of cases (Chiou et al. 2001b). In contrast, no association was found in a GIS analysis from Denmark where levels are largely below 2 μg/L (Baastrup et al. 2008), or in a mortality study of a predominantly nonsmoking population in Utah (Lewis et al. 1999).

Indeed, there is earlier evidence that genetic factors may be involved in susceptibility to arsenic-related bladder cancers. In southwest Taiwan (221 cases, 223 controls), Hsu et al. (2008) reported a gene–environment interaction between a polymorphism in a DNA repair gene, *XRCC1*, arsenic exposure and bladder cancer risk, whereas no interaction was found with polymorphisms in glutathione-S-transferase (*GST*) genes *GSTM1*, *GSTT1*, *GSTP1* or in the epoxide hydrolase gene. Although not statistically significant, another hospital based study from southwest Taiwan (170 cases, 402 controls) observed stronger trends in bladder cancer risk with urinary arsenic among those variant for *p53* codon 72, cyclin D1 (*CCND1) G870A* and *p21* codon 31 (Chung et al. 2008). An earlier, similarly designed case–control study (53 cases, 101 controls), found no association with N-acetyltransferase 2 (*NAT2)* slow acetylator genotypes and bladder cancer risk among the arsenic exposed (Su et al. 1998). Notably, these relatively small studies were limited to populations with unusually high exposure levels. To our knowledge, at lower levels of exposure, genetic modifiers of arsenic-related cancer have been explored only in our initial study from New Hampshire. Using a targeted SNP approach we found potential interactions with *XRCC3* 241 and possibly *ERCC2* 312 (342 cases, 549 controls) (Andrew et al. 2009).

By screening nearly 10,000 non-synonymous coding polymorphisms and validating them in an expanded group of cases and controls, we identified SNPs in previously unrecognized genes that may be involved or linked with susceptibility loci for arsenic-induced bladder cancer. While high exposure levels of arsenic are unquestionably carcinogenic to the bladder and other organs, effects at lower levels of exposure remain less clear despite their importance from a public health perspective. Accumulating data indicate that the effects of arsenic at lower exposure levels may be enhanced by environmental or host (i.e., genetic) factors. Our findings suggest novel variants that may influence susceptibility to arsenic-related bladder cancer in populations with relatively low levels of exposure, and may help identify those at greatest risk from this widespread exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FSIP1	Fibrous sheath interacting protein 1
SLC39A2	Solute carrier family 39, member 2
OR	Odds ratio
CI	Confidence interval
MCL	Maximum contaminant level
GST	Glutathione-S-transferase
μg/g	Microgram per gram
μg/L	Microgram per liter
SNP	Single nucleotide polymorphism
XRCC3	X-ray repair complementing defective repair in Chinese hamster cells 3
ERCC2	Excision repair cross-complementing rodent repair deficiency, complementation group 2
VEGF	Vascular endothelial growth factor
THBS1	Thrombospondin 1
CCND1	Cyclin D1
NAT2	*N*-acetyltransferase 2

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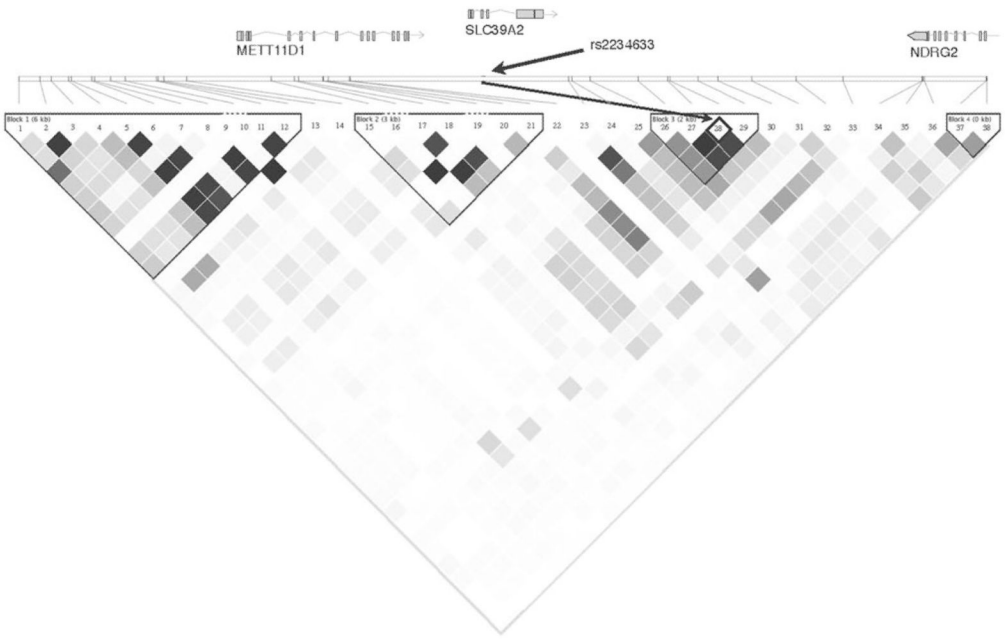

Fig. 1.

Linkage disequilibrium (LD) between pairs of SNPs around rs2234633 as determined by Haploview. Relative distances between SNPs in the region are marked as *vertical hash marks* in the *white bar* above the LD plot. The relative positions of the genes in the region are shown in the *top level. Gray boxes* in the genes represent exons, while *grey lines* represent introns. The name of each gene is written below its graphical representation. *Black triangular outlines* in the LD plot mark areas of high LD. The *borders* of these LD blocks were determined by Haploview. The position of rs2234633 is marked by *black arrows* below the gene in which it is located (SLC39A2) and by a *black diamond* in the LD plot (SNP 27). *Darker areas* in the LD plot indicated higher levels of LD

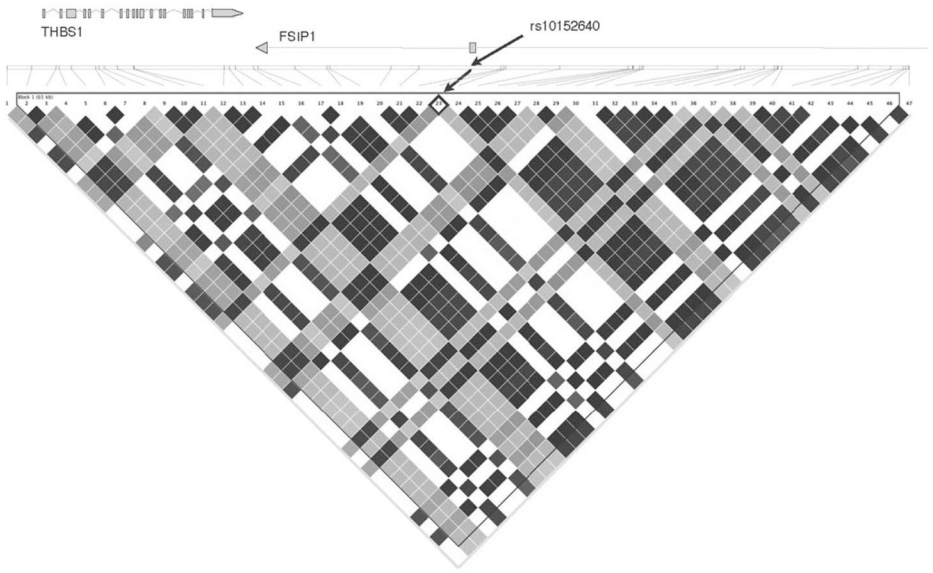

Fig. 2.

Linkage disequilibrium (LD) between pairs of SNPs around rs10152640 as determined by Haploview. Relative distances between SNPs in the region are marked as *vertical hash marks* in the *white bar* above the LD plot. The relative positions of the genes in the region are shown in the *top level. Gray boxes* in the genes represent exons, while *grey lines* represent introns. The name of each gene is written below its graphical representation. *Black triangular* outlines in the LD plot mark areas of high LD. The *borders* of these LD blocks were determined by Haploview. The position of rs10152640 is marked by *black arrows* below the gene in which it is located (FSIP1) and by a *black diamond* in the LD plot (SNP 23). *Darker areas* in the LD plot indicated higher levels of LD

Table 1

Characteristics of bladder cancer case–control study participants

Characteristic	Controls (*n* = 1,191)	Bladder cancer cases (*n* = 832)
Subject age (years), median (range)	64 (28–74)	64 (25–74)
Gender, *n* (%)		
Men	728 (61.1%)	631 (75.8%)
Women	463 (38.9%)	201 (24.2%)
Smoking history[a]		
Never	399 (33.7%)	141(17.2%)
Former	578 (48.9%)	407 (49.8%)
Current	206 (17.4%)	270 (33.0%)

[a] Smoking history was missing on 8 controls and 14 cases

Table 2

Bladder cancer odds ratios (95% confidence intervals) for selected genotypes and toenail arsenic concentrations, overall and stratified by cigarette smoking history

	Toenail arsenic concentrations				
	Low (≤90th percentile)		High (> 90th percentile)		
	N cases/*N* controls[a]	OR (95% CI)	*N* cases/*N* controls[a]	OR (95% CI)	
FSIP1 C402R(rs10152640)					
All subjects					
AA	241/363	1.00 (ref)	18/47	1.00 (ref)	
AG	226/356	0.94 (0.73, 1.19)	26/30	2.57 (1.13, 5.85)	*P* for interaction = 0.002
GG	50/69	1.04 (0.68, 1.57)	10/3	12.20 (2.51, 59.30)	
		P for trend = 0.858		*P* for trend = 0.001	
Non-smokers					
AA	50/124	1.00 (ref)	2/14	1.00 (ref)	
AG	33/120	0.71 (0.42, 1.18)	1/15	0.22 (0.01, 5.1)	*P* for interaction = 0.004
GG	10/21	1.08 (0.47, 2.49)	3/0	NA	
		P for trend = 0.565		*P* for trend = 0.038	
Ever smokers					
AA	191/239	1.00 (ref)	16/33	1.00 (ref)	
AG	193/236	1.01 (0.77, 1.33)	25/15	3.40 (1.38, 8.38)	*P* for interaction = 0.004
GG	39/48	1.02 (0.64, 1.62)	7/3	4.61 (1.00, 21.17)	
		P for trend = 0.928		*P* for trend = 0.006	
SLC39A2 F115L(rs2234636)					
All subjects					
TT	222/317	1.00 (ref)	12/37	1.00 (ref)	
TC	227/357	0.92 (0.72, 1.18)	28/29	2.96 (1.23, 7.15)	*P* for interaction = 0.03
CC	66/107	0.94 (0.65, 1.35)	13/14	2.91 (1.00, 8.52)	
		P for trend = 0.589		*P* for trend = 0.028	
Non-smokers					
TT	34/110	1.00 (ref)	2/12	1.00 (ref)	
TC	43/117	1.14 (0.68, 1.94)	3/10	1.84 (0.24, 13.87)	*P* for interaction = 0.027

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	Toenail arsenic concentrations				
	Low (≤90th percentile)		High (> 90th percentile)		
	N cases/*N* controls[a]	OR (95% CI)	*N* cases/*N* controls[a]	OR (95% CI)	
CC	16/36	1.48 (0.73, 3.02)	1/7	0.86 (0.06, 11.69)	
		P for trend = 0.295		*P* for trend = 0.982	
Ever smokers					
TT	187/207	1.00 (ref)	10/25	1.00 (ref)	
TC	184/240	0.85 (0.64, 1.12)	25/19	3.13 (1.20, 8.19)	*P* for interaction = 0.027
CC	50/71	0.80 (0.53, 1.21)	12/7	3.96 (1.19, 13.25)	
		P for trend = 0.188		*P* for trend = 0.015	

[a]DNA samples from a total of 670 cases and 974 controls were processed and run on the Illumina 96-plex Golden Gate assay (see text). The results exclude those with missing *SLC39A2* genotype (62 cases, 90 controls), and *FSIP1* genotype (59 cases, 83 controls). Of those with available genotype data the results exclude missing values for toenail arsenic (40 cases, 23 controls), smoking status (1 cases, 0 controls). We adjusted for age, gender, and smoking status in the overall models, and age and gender in analyses stratified by smoking status