Published in final edited form as:

Toxicol Lett. 2009 May 22; 187(1): 10–14. doi:10.1016/j.toxlet.2009.01.013.

DNA repair genotype interacts with arsenic exposure to increase bladder cancer risk

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Abstract

Drinking water arsenic exposure has been associated with increased bladder cancer susceptibility. Epidemiologic and experimental data suggest a co-carcinogenic effect of arsenic with exposure to DNA damaging agents, such as cigarette smoke. Recent evidence further supports the hypothesis that genetic variation in DNA repair genes can modify the arsenic – cancer relationship, possibly because arsenic impairs DNA repair capacity. We tested this hypothesis in a population-based study of bladder cancer with *XRCC3*, *ERCC2* genotype/haplotype and arsenic exposure data on 549 controls and 342 cases. Individual exposure to arsenic was determined in toenail samples by neutron activation. Gene-environment interaction with arsenic exposure was observed in relation to bladder cancer risk for a variant allele of the double-strand break repair gene *XRCC3* T241M (adjusted OR 2.8 (1.1–7.3) comparing to homozygous wild type among those in the top arsenic exposure decile (interaction p-value 0.01). Haplotype analysis confirmed the association of the *XRCC3* 241. Thus, double-strand break repair genotype may enhance arsenic associated bladder cancer susceptibility in the U.S. population.

Keywords

DNA repair; bladder cancer; arsenic; polymorphism; interaction

Introduction

Arsenic is an established carcinogen and is currently regulated by Federal and State standards for public water systems (Karagas *et al.* 1998b; Peters *et al.* 1999). Ingestion of high concentrations of inorganic arsenic is a cause of cancer of the skin, lung, and urinary bladder, and is a suspected cause of kidney cancer and other malignancies (Cantor *et al.* 2006). Drinking

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water arsenic exposure also has been associated with increased bladder cancer risk, possibly through a co-carcinogenic mechanism (Chen *et al.* 1986; Johansson and Cohen 1997; Karagas *et al.* 2004b). We hypothesized that individuals with impaired DNA repair capacity may represent a more sensitive subpopulation to arsenic carcinogenesis.

Arsenic's biologic effects and the extent of disease risk in the human population remains unknown at the exposure levels commonly encountered in U.S. drinking water (Abernathy *et al.* 1999). Studies, both clinical and experimental, have demonstrated that the biological effects of arsenic exist at levels below the new 10 µg/L standard (Kapaj *et al.* 2006). These effects include endocrine disruption, suppression of hormone regulation and hormone mediated gene transcription, alteration of cell cycle kinetics, and alterations in cellular proliferative response associated with carcinogenesis (Kapaj *et al.* 2006; Rossman 2003).

Approximately 2% of the drinking water serving US households contains 2 µg/L arsenic or more (IARC 2004). Chronic exposure to arsenic through drinking water is a major problem in several regions of the U.S., particularly the Northeast and Southwest (Ayotte *et al.* 2003). While this problem is primarily the result of geologic contamination, drinking water arsenic levels in these areas can be well above the current recommended maximum contaminant level of 10 µg/L (Ayotte *et al.* 2005). Adding to this, approximately 40% of households in New Hampshire are served by un-regulated privately supplied drinking water wells, 10% of which contain arsenic at levels greater than 10 µg/L (Karagas *et al.* 1998b). Moreover, until recent studies revealed the extent of geologic arsenic contamination of drinking water, arsenic was not part of the standard laboratory water-testing panel (Andrew *et al.* 2006a).

Studies of bladder and skin cancer conducted in the New Hampshire population have detected evidence of elevated cancer risks (Karagas *et al.* 2004a; Karagas *et al.* 2001). For bladder cancer, an excess risk was observed primarily among smokers exposed to arsenic in the drinking water, supporting the hypothesis that these levels of arsenic are co-carcinogenic (Karagas *et al.* 2004a). The population of New Hampshire is quite stable. Subjects in this study reported using the same water system for a mean of 15 years prior to diagnosis (Karagas *et al.* 2004a).

There is also evidence that the process of arsenic carcinogenesis may be modulated by genetic differences in DNA repair. Specifically polymorphisms in the *XRCC3* and *ERCC2/XPD* repair genes have been associated with arsenic-related skin cancer risk (Applebaum *et al.* 2007; Thirumaran *et al.* 2006). In Bangladesh, individuals with the nucleotide excision repair (NER) pathway variant *ERCC2* genotype at codon 751 had an increased risk of arsenic-induced premalignant hyperkeratotic skin lesions (Ahsan *et al.* 2003).

The current study takes advantage of a population-based study of bladder cancer conducted in New Hampshire, U.S. to investigate the hypothesis that the *XRCC3* and *ERCC2* DNA repair gene polymorphisms interact with arsenic exposure to increase bladder cancer susceptibility.

Methods

Study group

We identified all cases of bladder cancer diagnosed among New Hampshire residents, ages 25 to 74 years, from July 1, 1994 to June 30, 1998 from the State Cancer Registry. Within 15 days of diagnosis, the state mandated rapid reporting system requires submission of an initial report of cancer, and a definitive report within 120 days. To be eligible for the study, subjects were required to have a listed telephone number and speak English. We sought physician consent before contacting eligible bladder cancer patients. We interviewed a total n = 459 bladder cancer cases, which was 85% of the cases confirmed to be eligible for the study. Non-

participants included (n = 10) whose physician denied patient contact, (n = 63) were reported as deceased by a household member or physician, (n = 3) no answer after 40 attempts distributed over day, evenings and weekends, (n = 75) declined participation and (n = 8) were too ill to take part. A standardized histopathology review was conducted by the study pathologist, and from this review we excluded eleven subjects who were initially reported to the cancer registry as bladder cancer (Karagas *et al.* 2004a).

For efficiency, we shared a control group with a study of non-melanoma skin cancer conducted covering a diagnostic period of July 1, 1993 to June 30, 1995 (Karagas *et al.* 1998a). We selected additional controls for bladder cancer cases for the intervening diagnostic period, frequency matched to these cases on age (25–34, 35–44, 45–54, 55–64, 65–69, 70–74 years) and gender. All controls less than 65 years of age were selected 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 (CMS) of New Hampshire. The method of control selection used in our study has been successfully employed in other case-control studies conducted in the region (e.g. Karagas *et al.* (Karagas *et al.* 1998a)). We interviewed a total n = 665 controls, which was 70% of the controls confirmed to be eligible for the study. Of the potential participants, (n = 18) were reported as deceased by a member of the household, (n = 17) no answer after 40 attempts distributed over day, evenings and weekends, (n = 261) declined, (n = 29) were mentally incompetent or too ill to take part.

Personal interview

Informed consent was obtained from each participant and all procedures and study materials were approved by the Committee for the Protection of Human Subjects at Dartmouth College. Consenting participants underwent a detailed in-person interview, usually at their home. Questions covered sociodemographic information (including level of education), lifestyle factors such as use of tobacco (including frequency, duration and intensity of smoking), family history of cancer, and medical history prior to the diagnosis date of the bladder cancer (cases) or reference date assigned to controls. Recruitment procedures for both the shared controls from the non-melanoma skin cancer study and additional controls were identical and ongoing concomitantly with the case interviews. Case-control status and the main objectives of the study were not disclosed to the interviewers. To ensure consistent quality of the study interviewer, interviews were tape recorded with the consent of the participants and routinely monitored by the interviewer supervisor. To assess comparability of cases and controls, we asked subjects if they currently held a driver's license or a Medicare enrollment card. Subjects were asked to provide a blood sample (buccal sample was requested in the case of a refusal) and a toenail sample. Samples were maintained at 4°C and sent via courier to the study laboratory at Dartmouth within 24 hours for processing and analysis. More than 90% of the toenail samples were collected at the time of interview (>95% within 12 months of interview) and the majority of cases were interviewed within 14 months of diagnosis.

Arsenic analysis

Toenail arsenic levels are an internal biomarker of exposure over a 6–12 month period that correlates with drinking water arsenic concentration (Karagas *et al.* 2000; Slotnick *et al.* 2007). Toenail clipping samples collected at the time of interview were analyzed for arsenic and other trace elements by Instrumental Neutron Activation Analysis (INAA) at the University of Missouri Research Reactor, using a standard comparison approach as described previously (Karagas *et al.* 1998b; Wang *et al.* 2007). The detection limit for arsenic measured by INAA is approximately 0.001 µg/g. A total of 1068 participants provided toenail samples for analysis. Data from our previous analyses of cancer risk in this U.S. population suggested that the risk

did not increase linearly across the observed arsenic concentrations. Rather the graphs suggested that risk elevation began in the more highly exposed individuals (Karagas *et al.* 2004a; Karagas *et al.* 2001). Thus, groups were created for high versus low arsenic exposure analyses using the 90th percentile as a cutoff, which is a toenail arsenic level of 0.191 µg/g. The mean toenail arsenic level in the lower 90th percentile was 0.085 (range 0.009–0.191), while the upper 10th percentile mean was 0.363 (range 0.192–2.484).

Genotyping

DNA was isolated from peripheral circulating blood lymphocyte specimens harvested at the time of interview using Qiagen genomic DNA extraction kits (QIAGEN Inc., Valencia, CA). Genotyping for non-synonymous SNPs *XRCC3* C/T at position 241, *ERCC2* A/C at position 751 and G/A at position 312 was performed by Qiagen Genomics using their SNP mass-tagging system (Kelsey *et al.* 2004). Genotyping for other SNPs was performed using the GoldenGate Assay system through Illumina's Custom Genetic Analysis service (Illumina, Inc., San Diego, CA). Of the 1113 participating cases with confirmed bladder cancer and controls, we genotyped DNA on 915 (82%) for the current study.

Statistical analysis

Analysis was performed on subjects with both genotype and toenail arsenic data (549 controls and 342 cases). Characteristics of this population are similar to that of the overall study population. To assess the relation between DNA repair gene SNPs and bladder cancer risk, we conducted logistic regression analyses for individuals with one or two variant alleles in comparison to those homozygous wild type for each individual SNP. We assessed linkage disequilibrium in homozygotes using a chi-square test and inferred haplotypes using PHASE 2.1 (Stephens *et al.* 2001). Analyses were stratified by age, gender, and smoking status (never, former, current). Interactions were evaluated by including interaction terms in the regression model. Statistical significances of the interactions were assessed using likelihood ratio tests comparing the models with and without interaction terms.

Results

Demographic characteristics of the study population are shown in Table 1. A large percentage of the subjects were male. In addition, participants tended to be mainly Caucasian with a mean age of 62. Our population-based study enrolled cases diagnosed at a variety of facilities in the state of New Hampshire with 71% of the participants being diagnosed with non-invasive tumors. Current smoking was more common among cases than controls.

Table 2 shows bladder cancer risk stratified by toenail arsenic exposure level for *XRCC3* and *ERCC2*. Elevated bladder cancer risk was associated with toenail arsenic levels >90th percentile in *XRCC3* heterozygotes/variants compared with wildtype (adjusted OR 2.8 (1.1–7.3), but not among those with low arsenic exposure (OR 0.8 (0.6–1.1)) (gene-environment interaction p-value 0.01). The risk associated with variant *XRCC3* genotype was even more elevated in the top 5th percentile, but with limited statistical precision (toenail arsenic level above 0.278 µg/g adjusted OR 3.5 95%CI (0.9–14)). Haplotype analysis (Table 3) further supported the hypothesis that *XRCC3* genotype modifies arsenic-related bladder cancer risk. The risk for the 100 haplotype varies by arsenic exposure level above and below the 90th percentile (interaction p-value = 0.04). Results were not altered when our analysis was restricted to Caucasians which comprised 97% of our study population.

We further explored arsenic-genotype relationships stratified by smoking status. Risk elevation for *XRCC3_241* was appeared largely restricted to current and former smokers ($\geq 90^{\text{th}}$ percentile toenail arsenic level, adjusted OR 2.9 (1.0– 8.2)) as compared to never smokers

(($\geq 90^{\text{th}}$ percentile toenail arsenic level, adjusted OR 1.3 (0.1– 12))(Table 4). With limited statistically precision, we additionally observed a 2.3 fold higher risk associated with the *ERCC2* D312N polymorphism in arsenic exposed individuals (OR 2.3 (0.8–7.0) (Table 2), that also appeared higher among smokers (OR 2.7 (0.8–9.0)) than non-smokers (OR 1.4 (0.1–19)). Odds ratios for the other *XRCC3* or *ERCC2* SNPs did not appear to differ by arsenic exposure status above or below the top decile.

Discussion

Consistent with previous studies in other cancers, we observed evidence of arsenic modification of bladder cancer risk by DNA repair gene polymorphisms. In particular we found evidence of gene-environment interaction between the *XRCC3* variant genotype and high toenail arsenic levels on bladder cancer risk, especially among smokers. *ERCC2* variants with toenail arsenic levels in the top decile also had a slightly higher risk of bladder cancer, but our estimates were imprecise. These data are consistent with our previous report of an elevation in overall bladder cancer risk among smokers with high levels of arsenic exposure (Karagas *et al.* 2004b) and extend our earlier finding of an increased risk of bladder cancer among those with a variant *XRCC3* genotype (Andrew *et al.* 2007). Together these data support the hypothesis that other sources of DNA damage may interact with arsenic to induce tumorigenesis. This damage can be caused by high levels of smoking-induced lesions or an accumulation of lesions due to polymorphisms that decrease DNA repair function.

The importance of DNA damage and repair pathways for arsenic carcinogenicity is highlighted by its co-carcinogenic potential in the presence of UV irradiation and other mutagenic-carcinogens (Karagas *et al.* 2004b; Pott *et al.* 2001; Rossman *et al.* 2001). Arsenic has also been shown to inhibit DNA repair enzymes, possibly through a mechanism that involves displacement of zinc ions from zinc fingers (Andrew *et al.* 2003; Hartwig 1998; Hartwig *et al.* 1997; Li and Rossman 1989; Yager and Wiencke 1997) (Andrew *et al.* 2006b; Andrew *et al.* 2003). DNA repair pathways may have some redundancy and be able to compensate for one another to ensure that lesions are repaired despite impaired activity of a single enzyme. An interaction could occur between a polymorphism that hampered DNA repair gene function combined with arsenic inhibition of other DNA repair capabilities. In a study of human lymphocytes, *XRCC3* 241 variants had higher levels of damage measured as the frequency of micronuclei (Mateuca *et al.* 2008). Additional mechanistic data and replication in other populations are needed to clarify the nature of the interaction between arsenic exposure and genetic variations in DNA repair genes, particularly with respect to the ability to repair tobacco associated DNA damage. Benzo(a)pyrene and arsenic synergize to induce DNA adducts in the lungs and skin of exposed mice providing evidence of arsenic – polycyclic aromatic hydrocarbon (PAH) interactions (Evans *et al.* 2004).

Limitations of this study include the fact that there were a small number of highly exposed individuals. While our arsenic measurements does not indicate the specific source of the exposure, we believe that toenail arsenic serves as a good indicator for arsenic exposure because it integrates all sources of exposure and metabolic differences between individuals over a long period of time. Future studies are needed to replicate these findings in other arsenic exposed populations and elucidate the mechanism by which arsenic interacts with the DNA double-strand break repair gene *XRCC3*.

Acknowledgements

Grant support: This publication was funded in part by grant numbers CA102327, CA121382, CA099500, CA82354, CA57494, 5 P42 ES05947, RR018787, CA078609, and ES007373 from the National Cancer Institute, NIH and from the National Institute of Environmental Health Sciences, NIH.

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Table 1
Population characteristics

	Controls N (%)	Cases N (%)
Sex		
Males	355 (65)	266 (78)
Females	194 (35)	76 (22)
Reference age		
<40	23 (4)	7 (2)
40–55	101 (18)	52 (15)
5–70	288 (52)	184 (54)
>70	137 (25)	99 (29)
Race		
White	533 (97)	332 (97)
Non-white	16 (3)	10 (3)
Smoking status^a		
Never	166 (30)	69 (20)
Former	286 (52)	173 (51)
Current	97 (18)	100 (29)
Stage		
Non-invasive, low grade	-	207 (64)
Non-invasive, high grade	-	23 (7)
Invasive	-	84 (26)
Carcinoma In Situ	-	9 (3)

^aFormer smoker defined as smoking stopped >1 year prior to the diagnosis date.

Table 2
Bladder cancer risk associated with XRCC3 and ERCC2 polymorphisms by toenail arsenic level dichotomized at the 90th percentile.

	Controls N (%)	Cases N (%)	Toenail arsenic Low ($<90^{\text{th}}$)	Toenail arsenic High ($\geq 90^{\text{th}}$)	Interaction p-value
<i>XRCC3_241</i> rs861539 p. T241M					
Wildtype	208 (38)	137 (40)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	338 (62)	205 (60)	OR 0.8 (0.6–1.1)	OR 2.8 (1.1–7.3)	0.01
<i>XRCC3_03</i> rs1799796 g.IVS7–14A>G					
Wildtype	237 (46)	141 (47)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	278 (54)	161 (53)	OR 1.0 (0.8–1.4)	OR 1.3 (0.5–3.7)	0.8
<i>XRCC3_04</i> rs1799794 g.Ex2+2A>G					
Wildtype	333 (65)	190 (63)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	180 (35)	112 (37)	OR 1.1 (0.8–1.5)	OR 0.7 (0.3–2.0)	0.4
<i>ERCC2_03</i> rs28365048 p.K751Q					
Wildtype	204 (40)	114 (38)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	312 (60)	187 (62)	OR 1.1 (0.8–1.5)	OR 1.2 (0.4–3.3)	0.8
<i>ERCC2_09</i> rs1799787 g.IVS19–70C>T					
Wildtype	250 (49)	135 (45)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	264 (51)	167 (55)	OR 1.2 (0.9–1.6)	OR 1.1 (0.4–2.9)	0.9
<i>ERCC2_312</i> rs7199793 p.D312N					
Wildtype	200 (40)	110 (39)	1.0 (ref)	1.0 (ref)	
Het /Var - risk	298 (60)	174 (61)	OR 0.9 (0.7–1.2)	OR 2.3 (0.8–7.0)	0.2

Odds ratios (OR) adjusted for age, gender, smoking status (former, current). Genotype data were unavailable for *XRCC3_241* on 3 subjects, *XRCC3_03* on 74 subjects, *XRCC3_04* on 76 subjects, *ERCC2_03* on 74 subjects, *ERCC2_09* on 75 subjects, *ERCC2_312* on 109 subjects.

Table 3

Haplotypes and interaction p-values by gene.

Haplotypes	Frequency Controls (N%)	Frequency Cases (N%)	Toenail arsenic Low ($<90^{\text{th}}$)	Toenail arsenic High ($\geq 90^{\text{th}}$)	Interaction p-value
<i>XRCC3^d</i>					
2 000	102 (62)	62 (38)	1.0 (ref)	1.0 (ref)	Ref
1 010	378 (61)	237 (39)	1.0 (0.7–1.5)	3.2 (0.6–17.7)	0.2
3 001	218 (59)	153 (41)	1.1 (0.7–1.7)	2.6 (0.4–15.1)	0.4
5 100	419 (62)	258 (38)	0.9 (0.6–1.4)	5.6 (1.1–30.1)	0.04

^dOrder: XRCC3_ 241 XRCC3_03 XRCC3_04. Other rare haplotypes n=3.
Adjusted for age, gender, smoking.

Table 4
Bladder cancer risk associated with XRCC3 241 and toenail arsenic by smoking status.

Toenail arsenic	XRCC3_241 rs861539 p. T241M	Controls N (%)	Cases N (%)	Never smoker	Former, Current Smoker
Low (<90 th)	Wildtype	178 (36)	124 (41)	1.0 (ref)	1.0 (ref)
	Het /Var	314 (64)	182 (59)	0.7 (0.4–1.3)	0.8 (0.6–1.1)
High (≥90 th)	Wildtype	30 (56)	13 (36)	1.0 (ref)	1.0 (ref)
	Het /Var	24 (44)	23 (64)	1.3 (0.1–12)	2.9 (1.0–8.2)

Genotype data were unavailable for XRCC3_241 on 3 subjects. Adjusted for age, gender.