

Polymorphisms in *XPD* (*Asp312Asn* and *Lys751Gln*) genes, sunburn and arsenic-related skin lesions

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Background: Single-nucleotide polymorphisms in genes related to DNA repair capacity and ultraviolet exposure have not been well investigated in relation to skin lesions associated with arsenic exposure. This population based case–control study, of 600 cases and 600 controls, frequency matched on age and gender in Pabna, Bangladesh, in 2001–2002, investigated the association and potential effect modification between polymorphisms in Xeroderma Pigmentosum complementation group D (*XPD*) (*Lys751Gln* and *Asp312Asn*) genes, tendency to sunburn and arsenic-related skin lesions. **Methods:** Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). **Result:** No significant association was observed between skin lesions and the *XPD* 312 *Asp/Asn* (adjusted OR = 0.87, 95% CI = 0.65–1.15) *Asn/Asn* (adjusted OR = 0.76, 95% CI = 0.50–1.15) (referent *Asp/Asp*); *XPD* 751 *Lys/Gln* (adjusted OR = 0.92, 95% CI = 0.69–1.23) *Gln/Gln* (adjusted OR = 0.98, 95% CI = 0.66–1.45) (referent *Lys/Lys*). While we did not observe any evidence of effect modification of these polymorphisms on the association between well arsenic concentration and skin lesions, we did observe effect modification between these polymorphisms and sunburn tendency and arsenic-related skin lesions. Individuals with the heterozygote or homozygote variant forms (*Asp/Asn* or *Asn/Asn*) had half the risk of skin lesions (OR = 0.45, 95% CI = 0.29–0.68) compared with those with the wild-type *XPDAsp312Asn* genotype (*Asp/Asp*) and individuals with heterozygote or homozygote variant forms (*Lys/Gln* or *Gln/Gln*) had half the risk of skin lesions (OR = 0.47, 95% CI = 0.31–0.72) compared with those with the wild-type *XPDLys751Gln* genotype (*Lys/Lys*), within the least sensitive strata of sunburn severity. We observed effect modification on the multiplicative scale for *XPD* 751 and *XPD* 312. **Conclusion:** *XPD* polymorphisms modified the relationship between tendency to sunburn and skin lesions in an arsenic exposed population. Further study is necessary to explore the effect of *XPD* polymorphisms and sun exposure on risk of arsenic-related skin lesions.

Abbreviations: BMI, body mass index; CI, confidence interval; LRT, likelihood ratio test; NER, nucleotide excision repair; OR, odds ratio; UV, ultraviolet; *XPD*, Xeroderma Pigmentosum complementation group D.

Introduction

Chronic arsenic exposure through drinking water impacts millions of people across the world, including Argentina, Bangladesh, Cambodia, Chile, China, Ghana, Hungary, Inner Mongolia, Mexico, Nepal, New Zealand, Philippines, Taiwan and Vietnam. Basal cell carcinoma and squamous cell carcinoma are the most commonly observed cancers associated with chronic elevated arsenic exposure through drinking water (1). The mechanism for arsenic carcinogenesis remains unclear; however, several proposed mechanisms do exist (2). Additionally the mechanism between arsenic-related skin cancer and pre-malignant arsenic-related skin lesions has not yet been elucidated. While the dose and magnitude of arsenic exposure necessary to result in skin lesions has not been established, the latency period is much shorter than for other cancer endpoints and limited evidence suggests that arsenic-related skin lesions may serve as markers of susceptibility for skin and other arsenic-related cancers (3). It has been documented that subjects with chronic arsenic exposure who develop basal or squamous cell non-melanoma skin cancers often develop these cancers at the site of keratoses (4). Skin lesions, hyperkeratosis and melanosis, are not required for the development of basal or squamous cell carcinomas.

Arsenic is well established as a human skin carcinogen, but its precise mechanism of action remains unknown and potential co-exposure to factors such as ultraviolet (UV) radiation contributes further to the complexity (2,5–8). One mechanism by which arsenic is thought to damage DNA is through an indirect pathway where it inhibits the DNA repair mechanisms necessary to repair DNA damage from other agents, resulting in increased mutations (9). It is also possible that arsenic acts as a co-carcinogen with another co-exposure by increasing positive growth signaling (8). Several experiments in mouse skin models, keratinocytes, *Escherichia coli*, Chinese hamster V79 cells and human lymphoblastoid TK6 cells have shown that arsenic and UV exposure are co-carcinogens (5,6,10–14). The dimethylarsinic acid metabolite of arsenic and UVB co-exposure resulted in carcinogenesis of the skin in mice (15). UV exposure, DNA repair genotype and arsenic exposure have been shown to be risk factors for non-melanoma skin cancer; however, these factors have not been investigated collectively in a population-based study (16–18).

DNA repair enzymes work in concert to protect the genome of the cell from carcinogenic exposure. The DNA repair Xeroderma Pigmentosum complementation group D (*XPD*, also known as *ERCC2*) is a nucleotide excision repair (NER) helicase. The enzyme has a dual role in the NER pathway: (i) uncoiling the double helix at the site of DNA lesions and (ii) transcription (19). The NER pathway is the major pathway for the removal of bulky DNA lesions, in particular UV-induced photoproducts, bulky monoadducts, cross-links and products of oxidative damage (20). The *XPD* protein is essential for NER, and this has been demonstrated through point mutations in the *XPD* protein that results in diseases of DNA repair deficiency such as xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome (21). It has been hypothesized that arsenic inhibits repair of UV-induced DNA damage, through inhibition of the NER pathway, and this may be a mechanism for the increase in mutations that result when cells are co-exposed to UV light and environmental arsenic exposure (9,22).

Several important single-nucleotide polymorphisms have been identified in the *XPD* locus. Among them, a *G* → *A* polymorphism

in codon 312 of exon 10 results in an *Asp* → *Asn* substitution in evolutionarily conserved region and another polymorphism, *C* → *A* in codon 751 of exon 23, produce a *Lys* → *Gln* substitution (23). These variant phenotypes have been associated with lower DNA repair capacity or higher risk for carcinogenesis in population-based studies (23–26). Other studies have reported no association. The functional roles of the polymorphisms of *XPB* 751 and 312 are not clear (1,21). The impact of these polymorphisms on repair phenotype and cancer susceptibility is uncertain (27). Recent meta-analysis has reported that the *XPB* 312 *Asp/Asn* and *Asn/Asn* alleles were associated with an increased risk of breast and lung cancer compared with *Asp/Asp*, whereas the *XPB* 751 *Lys/Gln* and *Gln/Gln* alleles were associated with increased risk of breast cancer compared with *Lys/Lys* (27).

To date, there are two studies related to *XPB* codon 751 that suggested a potential role for this gene in arsenic-related hyperkeratosis with limited sample size. One study, conducted in Bangladesh, reported that the risk of hyperkeratosis was elevated in individuals with the AA (*Gln/Gln*) genotype compared with the AC/CC (*Lys/Gln*, *Lys/Lys*) genotypes; however, the association was not significant after the model was adjusted for arsenic exposure (1). A second study in West Bengal also reported that the *Lys/Lys* genotype was associated with an elevated risk of hyperkeratosis; however, this analysis was also not adjusted for arsenic exposure (28). No other studies have investigated the main or modifying effects of *XPB* (*Asp312Asn*) on arsenic-related skin lesions. It has been suggested that larger studies investigate this relationship with combinations of other DNA repair genes and other environmental exposures. To our knowledge, there have been no published studies on polymorphisms in *XPB*, severity of sunburn and premalignant skin lesions in an arsenic-exposed population.

In this study, we examined the role of *XPB* as a candidate susceptibility gene for arsenic-related skin lesions, in a population-based case-control study in Pabna, Bangladesh. We estimated the main effects for *XPB* codon 312 and codon 751 genotypes, as well as modification of odds ratios (ORs) for tendency to sunburn and arsenic-related skin lesions.

Materials and methods

Study population

This case-control study was conducted in the Pabna district of Bangladesh and has been described previously (29). The source population included the general population of the 23 villages of Pabna. Community meetings held in 2001–2002 were used to recruit subjects for this study in conjunction with Pabna Community Clinic, an affiliate of Dhaka Community Hospital. Pabna Community Clinic provides healthcare service in Pabna. Briefly, eligible cases were Pabna residents, at least 16 years of age, with one or more type of skin lesion: diffuse/spotted melanosis, diffuse/spotted keratosis, hyperkeratosis or leuko-melanosis. Controls were healthy individuals diagnosed as free of arsenic-related disease randomly selected in a 1:1 ratio from Pabna, age of at least 16 years, living in the same village as cases but not sharing a tube well. Two trained physicians clinically diagnosed subjects by visual inspection, and treatment was provided at Pabna Community Clinic or Dhaka Community Hospital when necessary. Physicians were unaware of subjects' exposure status. Individuals found to have arsenic exposure greater than 50 µg/l were advised to seek alternative drinking water sources. Remediation efforts in the Pabna district have been discussed elsewhere (30).

Cases and controls were frequency matched on gender and age (± 3 years). The participation rate was 98.0%; a total of 24 subjects from 1224 declined to participate. The population is ethnically homogenous, and similar to the population of Bangla (West Bengal), India. Informed consent was obtained from all study participants. The study protocol was approved by the Institutional Review Boards at Dhaka Community Hospital, Bangladesh and Harvard School of Public Health Boston, MA, USA.

Interviews and sample collection

In 2001–2002, 1200 subjects were recruited and interview and sample collection have been described previously (29,31,32). Physicians, blinded to exposure status, examined potential cases and controls. Trained interviewers administered the questionnaire regarding arsenic exposure, occupation/lifestyle factors and collected toenail and individual well water samples. Data were collected on liters of water/liquid ingested per day, disease history and residential history including identification of current primary water source

(tube well), years of use and use of a previous tube well. Tendency to sunburn was assessed by self-report.

Two drops (0.2 ml) of pure nitric acid was added to each 100 ml water sample upon collection. The samples were stored in a cooler before storage in a 4°C refrigerated room. Environmental Protection Agency method 200.8 with inductively coupled plasma mass spectroscopy (Environmental Laboratory Services in North Syracuse, NY) (33). The method limit of detection was 1 µg arsenic/l.

Two 10 ml ethylenediaminetetraacetic acid tubes were used to collect blood and were stored in a cooler on ice until processed with cell lysis solution. Samples were sent to the Molecular Epidemiology Laboratory at Harvard School of Public Health for DNA extraction and genotyping.

Genotyping

DNA samples were stored at –80°C. Whole blood was collected from patients at the time of enrollment and DNA was extracted from these samples using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN). The genotyping methods for the *ERCC2 Asp312Asp* (exon 10) and *Lys751Gln* (exon 23) polymorphisms have been described in detail (34,35). Briefly, two separate polymerase chain reaction assays were used to detect the polymorphisms in exon 10 and exon 23 of *ERCC2* using published primer sequences. DpnII and MspI (for exon 10) and Mbo II enzyme (for exon 23) digestion (New England BioLabs, Ipswich, MA) were used for restriction fragment analyses.

Genotyping for *XPBAsp312Asn* and *XPDLys751Gln* was completed for 1115 subjects. Laboratory personnel were blinded to case status, and a random 5% of the samples were repeated to validate genotyping procedures. Two authors independently reviewed all results with 100% concordance.

Statistical analysis

Analysis was restricted to subjects who reported using the same well greater than 6 months to minimize potential for temporal variability in arsenic exposure. As described by Rothman *et al.* (36,37), variables used in frequency matching of subjects (age and sex) were included in all regression models. Other covariates were considered as potential confounders and were included in the models if they changed the ORs by >10% (38). Arsenic concentration and volume of liquid consumed per day were not combined as a dose variable, since liquid volume included juice, milk, soup, tea and water. Data exploration using generalized additive models, implemented in R (version 1.8.1), suggested that the log odds of case identification varied linearly with the arsenic concentration of well water; consequently, untransformed arsenic concentration was used as a continuous predictor of case status. Generalized additive models suggested that the log odds of case status had a non-monotonic relationship with body mass index (BMI); therefore, a quadratic term for BMI was included. To facilitate numerical stability, both linear and quadratic terms for BMI were centered at the median BMI value, 19.1. Consolidated categories for educational status, tendency to sunburn and age were established. A cumulative sun exposure variable was created for number of hours spent in the sun for each job reported and number of years at the job and for recreational time. Subjects were asked about the numbers of hours spent in the sun during work and recreational time. A variable was created for the number of hours per year in the sun, per year of reported work/recreational time spent in the sun for each subject.

Univariate analyses were performed to describe population characteristics and to identify possible data errors and/or outliers. Continuous variables were summarized using means, medians, standard deviations and ranges, whereas categorical variables were described using percentages. Bivariate analyses (χ^2 test or *t*-test, as appropriate) were conducted to explore differences between cases and controls prior to multivariate modeling.

Multiple unconditional logistic regression was used to evaluate the associations between DNA repair polymorphisms and arsenic-related skin lesions. Data exploration using generalized additive models, implemented in R (version 1.8.1), suggested that the log odds of case identification varied linearly with the arsenic concentration of well water; consequently, untransformed arsenic concentration was used as a continuous predictor of case status. ORs and other model-based association parameters were obtained from the parameter estimates of regression models, as were their 95% confidence intervals (CIs). Regression models were fit using Statistical Analysis Systems (SAS Institute, Cary, NC) version 8.2.

To estimate interaction on the multiplicative scale, adjusted ORs and 95% CIs were estimated in logistic regression models and with interaction terms for each genotype and arsenic exposure as a continuous variable. Interaction on a multiplicative scale was assessed in the same way for tendency to sunburn as a categorical variable with each genotype. Interaction of *XPB* genotypes with each other, with arsenic exposure or with self-reported sunburn tendency was evaluated by adding cross product terms to the logistic regression models and were evaluated using the likelihood ratio test (LRT). Multiplicative interaction was assessed using the LRT by comparing models including the cross-product

Table I. Characteristics of skin lesion cases and population-based controls in Pabna, Bangladesh

	Controls		Cases		P
		n		n	
Diffuse melanosis				377	
Leukomelanosis				342	
Spotted melanosis				145	
Diffuse keratosis				117	
Spotted keratosis				73	
Hyperkeratosis				40	
Mean age in years (SD)	33.7(12.6)	597	33.9 (12.7)	592	0.58
Mean BMI (kg/m ²) (SD)	20.4(3.1)	597	20.1 (3.1)	592	0.70
% Male	60.30%	360	60.30%	357	0.91
Mean duration of present well use (years) (SD)	10.1(9.0)	592	8.0 (7.2)	592	0.02
% Reported a previous well	2.84%	17	7.77%	45	<0.0001
Mean As level of current well (µg/l) (SD)	66.2 (149.6)	595	232.8 (315.7)	592	<0.0001
Mean As level in nail sample (µg/g) (SD)	2.8 (4.1)	583	5.9 (7.4)	589	0.0001
Mean daily total water/liquid consumption (l) (SD)	3.8(1.2)	595	3.7 (1.1)	592	0.84
% Ever used betel nuts	24.30%	145	27.70%	164	0.007
Mean years of betel nut use (SD)	10.8(8.9)	143	11.0 (9.5)	160	0.078
Mean number of betel nuts chewed per day (SD)	5.6(3.6)	158	5.7 (3.8)	149	0.53
% Chew tobacco leaves	16.40%	587	17.10%	590	<0.0001
Mean years of Tobacco leaves chewed (SD)	9.9(9.1)	95	10.9 (9.4)	95	1.0
% Smokes cigarettes currently	30.50%	597	26.70%	592	0.03
% Ever smoked	31.00%	597	28.70%	592	0.0007
Education level		597		592	0.8
% Illiterate	17.40%	104	22.90%	136	
% Literate (incomplete primary education)	23.80%	142	29.40%	174	
% Completed primary education	11.70%	79	11.80%	70	
% Completed middle school education	31.90%	191	23.50%	139	
% Completed secondary education or more	13.60%	81	26.00%	154	
Mean hours of sun exposure (SD)	25937.35(22 821)	597	26782.59 (22 343)	592	0.51

Table II. Adjusted ORs (95% CIs) of XPD genotypes on risk of skin lesions

Genotype	Controls (N) (%)	Cases (N) (%)	Total	Crude OR	95% CI	Adjusted OR	95% CI
XPD							
<i>Asp312Asn</i>				1115			
<i>Asp/Asp</i> (GG)	239 (42.7%)	253 (45.7%)		1.0		1.0	
<i>Asp/Asn</i> (AG)	244 (43.6%)	227 (40.8%)		0.91	(0.69–1.19)	0.87	(0.65–1.15)
<i>Asn/Asn</i> (AA)	77 (13.8%)	75 (13.5%)		0.86	(0.58–1.28)	0.76	(0.50–1.15)
XPD							
<i>Lys751Gln</i>				1115			
<i>Lys/Lys</i> (AA)	86 (15.4%)	88 (15.9%)		1.0		1.0	
<i>Lys/Gln</i> (AC)	259 (46.3%)	251 (45.2%)		0.99	(0.75–1.31)	0.92	(0.69–1.23)
<i>Gln/Gln</i> (CC)	215 (38.4%)	216 (38.9%)		1.10	(0.75–1.60)	0.98	(0.66–1.45)

Crude OR adjusted for As (arsenic) concentration of well liters of liquid/day.

Adjusted for sunburn, As concentration of well liters of liquid/day, educational status, smoking status, chewing tobacco use, betel nut use, sex, age and previous well use.

term with a model containing only the main effects. Stratified analysis by tendency to sunburn was also conducted for each genotype. Co-dominant models were used for *XPD*Lys751Gln and *XPD*Asp312Asn.

Results

Characteristics of cases and controls were presented in Table I and have been presented previously (29). No significant departures were observed from Hardy–Weinberg equilibrium among cases and controls, *XPD* codon 751 SNP (A → C) (χ^2 ; $P = 0.45$) and *XPD* codon 312 SNP (G → A) (χ^2 ; $P = 0.11$) (Table II).

The results from the logistic regression models show that the *XPD* genotypes were not significantly associated with risk of skin lesions (Table II), nor was there evidence of effect modification between arsenic exposure and the genotypes on skin lesions [*XPD*Lys751Gln (LRT, $P = 0.10$); *XPD*Asp312Asn (LRT, $P = 0.73$)]. There was no

evidence of effect modification between tendency to sunburn and arsenic exposure (LRT, $P = 0.18$).

The adjusted main effects of the tendency to sunburn as associated with arsenic-related skin lesions are shown in Table III. A significant test for trend was observed with increasing sensitivity to sunburn; however, a significant association was only noted in individuals that reported painful burn or blisters after sun exposure (P -trend = 0.003). Those who reported the highest category of sun sensitivity had twice (OR = 2.17, 95% CI = 1.20–3.92) the odds of skin lesions than those who reported no tendency to sunburn after exposure.

We investigated whether the association for arsenic-related skin lesions and DNA repair genotypes differed by tendency to sunburn (Table IV). In stratified analysis, we found that in individuals who reported almost no tendency to sunburn, individuals with heterozygote or homozygote variant forms (*Asp/Asn* or *Asn/Asn*) had half the risk of skin lesions (OR = 0.45, 95% CI = 0.29–0.68) compared with

Table III. Adjusted OR for tendency to sunburn and arsenic-related skin lesions

	Cases (n) (%)	Controls (n) (%)	OR	95% CI
Practically no tendency	207 (35%)	256 (43%)	1.0 (referent)	
Some redness only	222 (37.5%)	204 (34.2%)	1.23	(0.91–1.66)
Burn	124 (21%)	113 (19%)	1.33	(0.94–1.89)
Painful burn or blisters	39 (6.5%)	23 (3.8%)	2.17	(1.20–3.92)
P-trend	0.003			

Model adjusted for well arsenic concentration, total liquids per day, age, gender, previous well use, smoking, chewing tobacco and betel nut use.

Table IV. Adjusted ORs for skin lesions stratified by tendency to burn

Tendency to Sunburn	Cases (n) (%)		Controls (n) (%)		OR	95% CI
<i>XPDAsp312Asn</i>						
	<i>Asp/Asp</i>	<i>(Asp/Asn + Asn/Asn)</i>	<i>Asp/Asp</i>	<i>(Asp/Asn + Asn/Asn)</i>		
Model 1						
Practically no tendency	94 (37.2%)	82 (27.2%)	100 (41.8%)	128 (39.9%)	0.48	(0.30–0.79)
Some redness only	88 (34.8%)	133 (44.0%)	83 (34.7%)	120 (37.4%)	1.10	(0.70–1.74)
Burn	58 (22.9%)	62 (20.5%)	49 (20.5%)	58 (18.1%)	1.08	(0.60–1.94)
Painful burn or blisters	13 (5.1%)	25 (8.3%)	7 (2.9%)	15 (4.7%)	3.63	(0.40–33.25)
<i>XPDLys751Gln</i>						
	<i>Lys/Lys</i>	<i>(Lys/Gln + Gln/Gln)</i>	<i>Lys/Lys</i>	<i>(Lys/Gln + Gln/Gln)</i>		
Model 2						
Practically no tendency	82 (38%)	96 (28.4%)	85 (39.5%)	143 (41.5%)	0.53	(0.33–0.86)
Some redness only	76 (35.1%)	145 (42.9%)	80 (37.2%)	123 (35.7%)	1.27	(0.79–2.01)
Burn	47 (21.8%)	70 (20.7%)	44 (20.5%)	63 (18.3%)	1.15	(0.63–2.12)
Painful burn or Blisters	11 (5.1%)	27 (8.0%)	6 (2.8%)	16 (4.6%)	2.84	(0.32–25.37)

Model 1 adjusted for well arsenic concentration, total liquids per day, XPD 312, age, gender, previous well use, smoking, chewing tobacco and betel nut use.

Model 2 adjusted for well arsenic concentration, total liquids per day, XPD 751, age, gender, previous well use, smoking, chewing tobacco and betel nut use.

those with the wild-type *XPDAsp312Asn* genotype (*Asp/Asp*). On the multiplicative scale, we found significance between the *XPDAsp312Asn* genotype and tendency to sunburn (LRT, $P = 0.03$).

In the stratified analysis, we also observed that individuals with heterozygote or homozygote variant forms (*Lys/Gln* or *Gln/Gln*) had half the risk of skin lesions (OR = 0.47, 95% CI = 0.31–0.72) compared with those with the wild-type *XPDLys751Gln* genotype (*Lys/Lys*), within the least sensitive strata of sunburn severity (Table IV). When evaluated on the multiplicative scale, we found a significant association between the *XPDLys751Gln* genotype and tendency to sunburn (LRT, $P = 0.05$). When stratified, we observed that individuals with the combined *XPD 751 Lys/Lys* genotype and *XPD 312 (Asp/Asn* or *Asn/Asn)* genotype had a lower risk of skin lesions compared with individuals with wild type for both of these alleles (OR = 0.34, 95% CI = 0.16–0.73) (Table V). We did observe evidence of interaction between the *XPDAsp312Asn* and *XPDLys751Gln* genes (LRT, $P = 0.03$). We did not have adequate sample size to study interaction between both polymorphisms and arsenic or both polymorphisms and sunburn sensitivity on the risk of skin lesions.

Discussion

Overall, the *XPD 312 (Asp/Asn + Asn/Asn)* and *XPD 751 (Lys/Gln + Gln/Gln)* were associated with a reduced risk of skin lesions in those individuals with no tendency to sunburn after 2 h of sun exposure. Since there are no previous results of modification by these genotypes of sunburn and pre-malignant hyperkeratosis and melanosis, we can compare our results with findings related to non-melanoma skin cancer. It is unclear whether individuals with the *XPD Lys/Lys* genotype have an increased or a decreased risk of non-melanoma skin cancer (39,40). Arsenic-related hyperkeratosis and melanosis are only associated with non-melanoma skin cancers; however, a study reported that the *751Gln* allele was associated with melanoma and squamous cell carcinoma and that this relationship was modified by the number of sunburns and a measure of cumulative sun exposure

(41). It was hypothesized by these authors that when challenged with high sun exposure melanocytes with the variant form of *XPD 751* and impaired DNA repair capacity may accumulate more mutations and have a higher rate of apoptosis and thus a lower risk of melanoma (41). It is possible that a similar mechanism is responsible for the observed effects in our study.

While the functional role of *XPD Lys/Lys* (wild type) is not clear, it is thought that a polymorphism at 751 has the largest effect on enzyme activity (1,21). Other investigators have found that the *Lys/Lys751* genotype was associated with a lower level of DNA repair activity (25). Individuals with the *XPD Gln751Gln* genotype also had 20% lower ($P = 0.004$) DNA repair capacity compared with the *XPD Lys751Lys* (wild type) phenotype measured by a Luciferase assay (42). Our findings are consistent with a previous study that found that modifications of the *XPD* gene also modify the effect of arsenic on hyperkeratosis, which is considered a pre-cursor to non-melanoma skin cancer. Prior to adjustment for arsenic exposure, a previous study observed that individuals with the AA genotype of *XPD 751* had an increased risk of arsenic-related hyperkeratosis (1). While we did not observe a main effect of the genotype, we observed potential modification that would suggest those with the *Lys/Lys* genotype had an increased rate of skin lesions after adjustment for arsenic exposure and tendency to sunburn. We found that the prevalence of the *Lys/Lys* (AA) genotype was slightly lower in our Bangladesh study population (15.8%, 15.9%) than other populations (21 to almost 50%); however, our study was population based and this distribution is representative (1,43–45).

The functional role of *XPD 312* has yet to be elucidated (21). While some studies have found no association with the *Asp312Asn* *XPD* polymorphism and DNA repair ability (25), other studies have reported that the *Asp/Asp* genotype of *XPD 312* was associated with a decrease in apoptosis in response to UV exposure and this mechanism could be related to increased survival and cloning of cells with DNA damage (46). This finding is consistent with our finding that those with the (*Asp/Asn + Asn/Asn*) alleles had a reduced risk of skin

Table V. Estimated adjusted ORs and 95% CIs of *XPD* 751 and *XPD* 312 polymorphisms and skin lesions

Genotype						
<i>XPD</i> 751	<i>XPD</i> 312	Controls (N)	Cases (N)	OR	95% CI	P-value
<i>Lys/Lys</i> (wild type)	<i>Asp/Asp</i> (wild type)	187	201	1.0 (referent)		
<i>Lys/Gln</i> + <i>Gln/Gln</i>	<i>Asp/Asp</i>	52	51	0.76	(0.47–1.23)	0.26
<i>Lys/Lys</i>	<i>Asp/Asn</i> + <i>Ans/Asn</i>	28	15	0.34	(0.16–0.73)	0.006
<i>Lys/Gln</i> + <i>Gln/Gln</i>	<i>Asp/Asn</i> + <i>Ans/Asn</i>	293	286	0.77	(0.59–1.02)	0.06

Model adjusted for well arsenic concentration, total liquids per day, age, gender, previous well use, smoking, chewing tobacco and betel nut use.

lesions compared with the *Asp/Asp* genotype. It is possible that failure to undergo apoptosis allows cells to replicate and further mutate, first resulting in skin lesions and potentially later resulting in arsenic-related cancer. Additionally, it is possible that the effect is only observed among those with the least sensitivity to sunburn, because they may have continually experienced greater unprotected UV exposure since they have less tendency to sunburn. This information is not possible to assess from our data.

We found that increasing severity of sensitivity to sun exposure was associated with an increased risk of arsenic-related skin lesions among an arsenic exposed population. Increased sun exposure has been associated with an increased risk of non-melanoma skin cancer (47). Risk of arsenic-related skin lesions has been reported to be greater in men with excessive sun exposure (48). We did not find a significant association between cumulative sun exposure and risk of skin lesions. Cases and controls reported similarly high number of hours with sun exposure either working or in recreational activities (Table I). It is also possible that there is some recall bias; however, this study population was from a rural area and the majority of subjects reported occupations that required significant outdoor work.

The study measured tendency/severity of sunburn after sun exposure for two hours and hours spent in the sun for occupation and recreational activities by self-report. This measure does not take into account the darkness of skin pigmentation, which may be associated with the risk of sunburn and the dose of UV radiation to the dermis. Cumulative sun exposure was self-reported as was the tendency to sunburn; however, similar measures such as self-reported number of lifetime sunburns have been employed in other large population based studies (39,49,50). There is potential for recall bias; however, there is no established association between severity of sunburn history and arsenic-related skin lesions so there is no reason to believe that cases would have differentially reported tendency to sunburn differently than controls. A recent study validated that self-reported measures of sun sensitivity and self-reported history of non-melanoma skin cancer were reasonably reliable measures to use in screening subjects for studies and found that all groups were equally reliable when reporting sun sensitivity (51). We did not observe a significant difference between length of cumulative estimate of hours spent in the sun between cases and control subjects ($P = 0.52$), nor did we observe an association between cumulative sun exposure and risk of skin lesions (OR = 1.00, 95% CI = 1.00–1.0) in a multivariate adjusted model. It was possible that there was exposure misclassification in the number of hours spent working/recreationally outdoors; however, the majority of occupations in this rural area involved outdoor work. Any misclassification in sun exposure is likely to be non-differential and could bias results in either direction. Arsenic exposure was measured by individual well measurements in this study. Exposure misclassification was possible if current arsenic exposure from individual wells does not represent past exposure. Evidence exists that well arsenic concentration in Bangladesh is relatively stable over time, though shallow wells are somewhat more variable (52). Well depth was not measured by the field team in this study. In our population, the mean duration of current well use is 10.1 years for controls and 8.0 years for cases, which indicates that subjects have had a relatively long exposure period that is adequate for the development of skin lesions (3). It is possible that our findings are due to linkage to another significant

polymorphism or by differential effects of the polymorphism by other modifying factors, such as skin pigmentation. Unmeasured confounders are possible. In order for the unmeasured confounder to explain the interaction results between genotype and sunburn tendency among an arsenic-exposed population, the confounder would have to be differentially distributed in the separate genotype–exposure strata. A strength of this study was that it was conducted on an ethnically homogenous population from Pabna, Bangladesh, and the study was of a large enough sample size to investigate gene–environment interactions. Reliability of genotyping and arsenic concentration of drinking water was high due to previously described quality control procedures. Hence, measurement error in genotyping and analysis of drinking water arsenic concentration was possible but unlikely. Arsenic concentration of wells was determined by a private lab in the USA without knowledge of case–control status. Genotyping was also conducted without indicators of study outcome status. Any measurement error would have been independent of case–control status, and any bias would have been non-differential. Limited numbers did not allow us to assess potential interaction between both *XPD* polymorphisms, co-exposure to arsenic and sunburn and skin lesions.

Polymorphisms in *XPD* 312 and 751 may play a role in the NER phenotype and therefore to genetic susceptibility to skin lesions that have been associated with increased risk for skin cancer. Further investigations are needed to examine the effect of multiple *XPD* polymorphisms and co-exposure to arsenic and sun associated with risk of skin lesions.

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