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E-cadherin polymorphisms and susceptibility to arsenic-related skin lesions in West Bengal, India

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ABSTRACT

Objectives: Although suppression of E-cadherin gene (CDH1) expression and exposure to arsenic have separately been associated with skin lesions, the combined effects of this "gene–environment" interaction have not been explored previously.
Study design: A population-based cross-sectional survey.
Method: This study involved 100 cases with skin lesions and 100 controls who were family members with no lesions. The subjects were recruited from villages and hamlets in northern Nadia Province, West Bengal. Each participant was required to undergo a detailed face-to-face interview; provide spot urine sample; provide saliva sample; and sign a consent form. The type and severity of skin lesions were assessed during a general medical examination of each participant in the field. The following 16 single nucleotide polymorphisms (SNPs) of the CDH1 were measured using DNA extracted from saliva samples: rs16260, rs5030625, rs155364, rs155808, rs155807, rs2303646, rs2059254, rs9925923, rs12919719, rs7188750, rs9989407, rs7196495, rs7196661, rs13689, rs12599393, and rs1862748.
Results: The main effects of SNPs on the risk for skin lesions were borderline for rs7196661 (p-value = 0.092), rs7196495 (p-value = 0.090), and rs12919719 (p-value = 0.065); the strongest association was found for rs9989407 (p-value = 0.058). Several SNPs, however, showed that the T>T genotype carriers are at higher relative risk for skin lesions compared to carriers of the C>C or C>T genotypes; these results need to be confirmed in a larger study. The main effects of some of the SNPs and genotype frequencies on the severity of skin lesions were found to be relatively weak.
Conclusions: This is the first study that indicates that CDH1 polymorphisms can contribute to the etiology of premalignant skin lesions in people chronically exposed to arsenic in drinking water, and that this gene may be a factor in individual susceptibility to cutaneous diseases.

1. Introduction

It is estimated that 26 million people in West Bengal have been chronically exposed to arsenic levels in drinking water that exceed the current guideline of 10 μg/L set by the World Health Organization (WHO, 2004; Chakraborti et al., 2009). Among the reported and suspected adverse health effects of arsenic, premalignant skin lesions (including melanosis, leucomelanosis, and keratosis) are generally recognized as the early manifestation of chronic exposure and hallmark of toxicity that may indicate increased future risk of arsenic-related cancer (Smith and Steinmaus, 2009; Ahsan et al., 2006; Ghosh et al., 2008). Unlike arsenic-related internal cancers that can have long latencies, premalignant skin lesions may appear with shorter periods of arsenic exposure (Saha, 2001). Only a small percentage of individuals exposed to comparable levels of arsenic in a given population ever develop premalignant skin lesions and the degree of an individual's susceptibility to arsenic-induced effects also varies among populations in different parts of the world (Steinmaus et al., 2005; Ghosh et al., 2008). The inter-individual variability that attends exposure–response relationships can be attributed partially to environmental factors, but genomic differences can also contribute to the differential susceptibility to arsenic exposure (Engstrom et al., 2009). It is now widely accepted that in areas where exposure to arsenic is high, the pathogenesis of skin lesions is a multi-factorial interaction of environmental triggers and genetic susceptibility. Although much work has been done on arsenic exposure and associated health effects, our understanding of the underlying genetic factors and the ways they interact with arsenic to influence susceptibility to skin lesions thus far remains quite limited.

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Cadherins are a family of transmembrane glycoproteins whose primary function is to mediate cell–cell and cell–solid tissue adhesion (they are the principal biological glue). They also play an important role in signal transduction, endothelial cell integrity and growth and vascular morphogenesis (Wang et al., 2008). The role of E-cadherin (CDH1) in pathogenesis of skin lesions is of interest because a multitude of clinical and experimental studies have shown that its adhesive function frequently is lost during the development of most, if not all, human epithelial cancers, including skin, bladder, breast, prostate, renal, ovarian, liver, kidney, colorectal, pancreatic, stomach, and lung carcinomas (Christofori and Semb, 1999; Hsu et al., 2000; Jiang and Mansel, 2000; Cavallaro et al., 2006; Baranwal and Alahari, 2009; Ranscht, 2010). The reduction or absence of CDH1 has been associated with loss of differentiation, invasion, and metastatic behavior of tumor cells (Birchmeier and Behrens, 1994; Yoshiura et al., 1995; Perl et al., 1998). Several different mechanisms appear to cause the loss of CDH1 function, including hypermethylation of its genome and by other epigenetic events that are part of normal homeostatic regulation that is modifiable by arsenic during development and differentiation of the human cutaneous system (Christofori and Semb, 1999; Furukawa et al., 1994; Tang et al., 1994; Engstrom et al., 2009). Although suppression of CDH1 expression and exposure to arsenic has separately been associated with skin lesions, the dermatological effects of this "gene–environment" interaction have not been explored previously. In this study, we used DNA from participants in a case–control study design to investigate the influence of polymorphisms in CDH1 on incidence of skin lesions among a population in Nadia Province, West Bengal that is exposed to high levels of arsenic in their water and food.

2. Materials and methods

This study included a total of 200 participants consisting of 100 cases with skin lesions and 100 controls who were family members with no lesions. To be eligible for the study, the case–control pair was required to have shared the same domicile for a minimum of 10 years prior to the time they were interviewed. The study design was intended to ensure that the each case–control pair was exposed to comparable levels of arsenic over an extended period of time.

The subjects were recruited from the following villages and hamlets in northern Nadia Province, West Bengal: Chhotaitna, Jompukur, Notipota, Purbapara, Sardarpara and Debagram. The cases were identified from a cohort of a cross-sectional survey that was conducted between August 2006 and December 2007 to investigate arsenic-associated skin lesions (mainly keratosis and hyperpigmentation), in the district of Nadia, West Bengal, India (Guha Mazumder et al., 2010). For that study, 10,469 participants were examined and interviewed, and the arsenic levels in their drinking water measured. We used a trusted community leader to identify the previous cohort and invite members of eligible household to participate in current study. The community leader also set up the time for the study staff to visit for interview and sample collection. Only one case–control pair was recruited from a household.

Each participant was required to (i) undergo a detailed face-to-face interview; (ii) provide spot urine sample; (iii) provide saliva sample; (iv) allow the project staff to visit their home to take water sample; and (v) sign a consent form. The survey questionnaire was used to collect demographic information (age, level of education, type of employment and marital status), the source of the household water supply (well or public) for each residence, dietary habits, bathing habits and the amount of water they consumed each day. Data were also collected on medical symptoms (pertaining to the eye, nose, ear, mouth, throat, muscle and joint, stomach, respiratory and nervous systems) and medication use; smoking habits; level of education, and type of employment. We also gathered information about birth outcomes (premature deliveries and children born underweight) from the female participants.

A general medical examination was given in the field, including a careful inspection for skin lesions, by a physician with long experience in diagnosing arsenic-related skin lesions in West Bengal. The criteria used in assessing the skin lesions (mainly keratosis and pigmentation) have been described previously (Guha Mazumder, 2000; Guha Mazumder et al., 1998). Keratosis was manifested primarily as diffuse bilateral thickening of palms and/or soles with or without nodules of various shapes and sizes while pigmentation was characterized by areas of mottled dark brown pigmentation (often in the form of finely freckled, "raindrop" patterns) that was bilaterally distributed on the trunk and extremities (Guha Mazumder et al., 2010). Depigmented spots were occasionally encountered but were not considered in the clinical assessment. A scoring system previously developed for classifying the severity of skin manifestations into three categories (Table 1) was adopted for this study. The height and weight of each participant was obtained after the medical examination.

About 2 mL of saliva for DNA analysis was obtained from each participant using an Oragene (saliva) collection and preservation kit. The saliva samples were kept in the fridge until analyzed. Spot urine sample was also collected from each participant in a wide-mouth plastic bottle, stored in an ice box and then taken to the lab for refrigeration. Water samples were obtained from (a) the well that was then used by the family as primary source of water for cooking and drinking; (b) any well that supplied the water for bathing, washing and cleaning purposes; and (c) well(s) where the family previously drew their drinking water. Household dusts were collected from a selected number of household.

Urine samples were analyzed by atomic absorption spectrophotometer (GFAAS) at the University of Kalyani. We used the Standard Reference Material (SRM) 2669 for urine from the US National Institute of Standards and Technology (NIST) in quality control. Since the initial survey between 2006 and 2007 when the cohort used in this study was created, most of the villagers have subsequently been provided with drinking water with considerably lower arsenic concentrations. In analyzing the results of this study, we decided to use the water data collected during the initial survey when the arsenic levels were very high rather than the arsenic levels in current water supply sources.

The extraction of DNA from saliva samples, genotyping and PCR measurements were made by BioServe (Beltsville, Maryland, USA). Briefly, the DNA was isolated from the Oragene saliva collection tubes using BioServe DNAQuik Kit (non-organic, non-column based, protein precipitation method). The SNPs were initially genotyped by Taqman system (Applied Biosystems, Foster City, CA, USA) using the MassARRAY Assay Design 3.1 that was custom developed by this BioServe. The results were checked using Sequenom's OligoCHECK software further processing. If any of the primers failed the QC, they were re-ordered and synthesized again. After all the primers were deemed good, they were used for setting up the validation of the assays. All PCR and extension primers were synthesized to appropriate concentrations and then spotted over a Sequenom's SpectroChip using a Robodesign Spectrospotter and analyzed by means of MALDI-TOF mass spectrometer. The resultant peaks were analyzed by automated software SpectroTYPER™ by Sequenom (San Diego, CA, USA).

The Institutional Review Boards at the University of Michigan and the DNGM Research Foundation, Kolkata approved the protocol for this study. Informed consent was obtained prior to participation.

2.1. Statistical analysis

Measured allele frequencies of CDH1 SNPs were examined for the Hardy–Weinberg equilibrium for both cases and controls by means of

Table 1
Criteria used in grading the severity of pre-cancerous skin lesion (from Mazumber, 2000).

Grade	Severity	Scoring criteria
I	Mild	a) Diffuse melanosis. b) Suspicious spotty depigmentation/pigmentation over trunk/limbs. c) Mild diffuse thickening of soles and palms.
II	Moderate	a) Definite spotty pigmentation/depigmentation on the trunk and limbs, bilaterally distributed. b) Severe diffuse thickening (with/without wart like nodules of the palms and soles).
III	Severe	a) Definite spotty pigmentation/depigmentation as above with few blotchy pigmented/depigmented macular patches over trunks or limbs. b) Pigmentation involving the undersurface of tongue and/or buccal mucosa. c) Larger nodules over thickened palms and soles occasionally over dorsal aspect of hands and feet. Diffuse verrucous lesions of the soles with cracks and fissures and keratotic horns over palms/soles.

the Chi-square test. Genotype and allele frequencies between cases and controls were likewise examined by the same test. The type and severity of skin lesion on each participant were measured on a scale of 1 (few spotted lesions) to 3 (extensive and more penetrance). Association of the genotype with the scaled measure of the level of skin lesion was also evaluated by χ^2 test. The odds ratios (OR) and 95% confidence intervals (CI) were also calculated after controlling for important confounding variables. p-values less than 0.05 were considered significant. The statistical analysis was done using SPSS for Windows Version 18 by IBM (Armonk, NY, USA).

3. Results

Study participants ranged in age from 20 to 65 years and averaged 49 years. The average age of cases (46 years) was slightly higher than that for controls (40 years). Male participants made up 36% of the

Table 2
Association of demographic variables with skin lesions*.

A: Gender differences in study results

	Male		Female		Overall		χ^2	p
	n	%	n	%	n	%		
Tobacco smoker	2	3.17	0	0.00	2	1	7.2	0.027
Biri smoker	36	57.14	1	3.70	43	22	80.183	0.000
	Mean	S.D.	Mean	S.D.	Mean	S.D.	t	p
Age	46.5	6.5	44.7	6.5	46	8.4	−0.91	0.365
BMI	19.7	3.1	18.8	3.1	19.5	3	−1.195	0.235
Well water As	41.6	138	64.9	189	53.5	167	0.401	0.689

B: Demographic confounders

	Control		Case		Overall		χ^2	p
	n	%	n	%	n	%		
Gender							86.595	0.000
Male	4	4.2	63	70	67	36.2		
Female	91	95.8	27	30	118	73.8		
Tobacco smoker	0	0	2	2	2	1.1	4.082	0.130
Biri smoker	4	4.2	40	40	44	23.8	38.736	0.000
	Mean	S.D.	Mean	S.D.	Mean	S.D.	t	
Age	39.9	9	45.9	8.4	42.9	9.3	−4.828	0.000
BMI	20.3	3.3	19.5	3	19.9	3.2	1.933	0.055
Well water As	38.3	133	53.1	165	45.7	150	−0.609	0.544

C: Association of skin lesion severity with demographic variables

	No skin lesion	Mild skin lesion	Moderate and severe skin lesions	Total	χ^2	p
Gender	n	n	n	n	88.199	0.000
Male	4	21	42	67		
Female	91	13	14	118		
Total	95	34	56	185		
Current biri smoker				40.5	0.000	
No	95	19	39	153		
Yes	4	18	22	44		
Total	99	37	22	197		
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.		F	p
Age	39.9 ± 9.0	44.1 ± 9.5	46.9 ± 7.7		13.061	0.000
BMI	20.3 ± 3.3	19.1 ± 3.1	19.7 ± 2.9		2.27	0.106
Log urinary As	1.41 ± 0.76	1.93 ± 0.47	1.81 ± 0.57		9.629	0.000
Log As in well water	1.11 ± 0.46	1.21 ± 0.59	1.12 ± 0.50		0.545	0.581

*. Data do not always sum to 200 because of missing values in some of the variables

Table 3
Association of CDH1 polymorphism and arsenic-related skin lesions.

SNP	Control		Case		χ^2	p	$\chi^2_{\text{Hardy–Weinberg}}$
	n	%	n	%			
rs2059254					2.283	0.319	1.421
CC	70	72	64	67			
TT	2	2.1	6	6.2			
CT	25	26	26	27			
rs1862748					2.725	0.256	0.501
CC	74	78	65	709			
TT	1	1.1	4	4.3			
CT	20	21	24	26			
rs12599393					4.526	0.104	0.333
CC	78	79	64	67			
TT	1	1.0	4	4.2			
TC	19	19	27	28			
rs13689					2.484	0.289	0.118
CC	2	2.0	0	0.00			
TT	75	76	79	81			
TC	22	22	18	18			
rs7196495					4.817	0.09	1.758
CC	4	4.1	5	5.4			
TT	46	47	57	61			
TC	48	49	31	33			
rs7196661					4.767	0.092	1.008
CC	4	4.1	5	5.3			
TT	48	49	60	63			
TC	46	47	30	32			
rs9925923					2.069	0.355	0.250
CC	69	72	61	64			
TT	2	2.1	5	5.3			
TC	25	26	29	31			
rs16260					0.055	0.973	0.056
AA	3	3.2	3	3.3			
CC	63	68	63	69			
CA	27	29	25	27			
rs9989407					5.693	0.058	1.252
CC	4	4.1	5	5.3			
TT	46	47	60	63			
CT	47	48	30	32			
rs155364					2.851	0.24	0.190
AA	31	33	39	43			
TT	14	15	15	17			
AT	49	52	36	40			
rs155808					1.096	0.578	0.006
AA	34	38	39	45			
GG	12	13.3	11	13			
AG	44	49	36	42			
rs12919719					2.851	0.065	0.956
CC	74	79	58	65			
GG	1	1.1	4	4.5			
GC	18	19	27	30			
rs9001					0.683	0.711	1.117071133
AA	76	81	71	83			
CC	1	1.1	2	2.3			
CA	17	18	13	15			
rs9836592					1.367	0.505	0.06212221
CC	5	5.1	2	2.2			
TT	64	65	59	63			
TC	30	30	32	34			

Table 4
Logistic regression results (adjusted by gender, age, BMI and Biri smoking) showing association of CDH1 genotypes with skin lesions in the study population.

	Control		Case		Estimate	Odd ratio	95% C.I.	p-value
	n	%	n	%				
SNP and genotype								
rs2059254								
CC	70	72.2	64	66.7		1		
TT	2	2.1	6	6.3	1.816	6.144	0.743–50.786	0.092
CT	25	25.8	26	27.1	0.328	1.389	0.523–3.684	0.509
TT + CT	27	27.8	32	33.3	0.53	1.698	0.674–4.279	0.261
rs1862748								
CC	74	77.9	65	69.9		1		
TT	1	1.1	4	4.3	2.831	16.955	1.260–228.172	0.033
CT	20	21.1	24	25.8	0.351	1.42	0.516–3.912	0.497
TT + CT	21	22.1	28	30.1	0.629	1.876	0.720–4.888	0.198
rs12599393								
CC	78	79.6	64	67.4		1		
TT	1	1.0	4	4.2	2.715	15.106	1.153–197.942	0.039
TC	19	19.4	27	28.4	0.282	1.325	0.474–3.708	0.592
TT + TC	20	20.4	31	32.6	0.577	1.78	0.679–4.667	0.241
rs13689								
CC	2	2.0	0	0.0				
TT	75	75.8	79	81.4		1		
TC	22	22.2	18	18.6	−1.202	0.301	0.091–0.993	0.049
CC + TC	24	24.2	18	18.6	−1.599	0.202	0.049–0.838	0.208
rs7196495								
CC	4	4.1	5	5.4		1		
TT	46	46.9	57	61.3	1.358	3.89	0.358–42.182	0.264
TC	48	49.0	31	33.3	1.211	3.36	0.300–37.525	0.326
TT + CT	94	95.9	88	94.6	1.34	3.82	0.367–39.785	0.262
rs7196661								
CC	4	4.1	5	5.3		1		
TT	48	49.0	60	63.2	0.711	2.037	0.256–16.222	0.502
TC	46	46.9	30	31.6	0.25	1.284	10.753	0.818
TT + TC	94	95.9	90	94.7	0.456	1.577	0.206–12.088	0.661
rs9925923								
CC	69	71.9	61	64.2		1		
TT	2	2.1	5	5.3	1.849	6.352	0.756–53.395	0.089
TC	25	26.0	29	30.5	0.529	1.697	0.641–4.493	0.287
TT + TC	27	28.1	34	35.8	0.701	2.016	0.796–5.102	0.139
rs16260								
AA	3	3.2	3	3.3		1		
CC	63	67.7	63	69.2	0.909	2.482	0.153–49.282	0.523
CA	27	29.0	25	27.5	0.651	1.917	0.111–33.191	0.655
CC + CA	90	96.8	88	96.7	0.829	2.29	0.145–36.12	0.556
rs9989407								
CC	4	4.1	5	5.3	−0.715	0.489	0.062–3.881	0.499
TT	46	47.4	60	63.2		1		
CT	47	48.5	30	31.6	−0.509	0.601	0.245–1.475	0.266
CC + CT	51	52.6	35	36.8	−0.533	0.587	0.247–1.394	0.227
rs155364								
AA	31	33.0	39	43.3		1		
TT	14	14.9	15	16.7	−0.342	0.711	0.189–2.670	0.613
AT	49	52.1	36	40.0	−0.701	0.496	0.192–1.286	0.149
TT + AT	63	67.0	51	56.7	−0.616	0.54	0.221–1.323	0.178
rs155808								
AA	34	37.8	39	45.3		1		
GG	12	13.3	11	12.8	−0.042	0.959	0.244–3.764	0.952
AG	44	48.9	36	41.9	−0.382	0.683	0.268–1.738	0.423
GG + AG	56	62.2	47	54.7	−0.414	0.661	0.246–1.779	0.412
rs12919719								
CC	74	78.7	58	62.4		1		
GG	1	1.1	4	4.3	2.741	15.507	1.168–205.934	0.038
GC	18	19.6	27	31.8	0.443	1.558	0.560–4.330	0.395
GG + GC	19	20.6	31	36.1	0.725	2.065	0.783–5.441	0.143

study population. The smaller number of male participants was due to the fact that that the prevalence of skin lesions was higher in men and by design the controls were the women in the same households. The cases were more likely than controls to smoke tobacco (biri, bidi or beedi in Hindu); this observation might have been confounded by gender because more that 80% of biri smokers were male. Only two participants were tobacco smokers while 38 smoked biri; no female participant reported being a cigarette smoker and only one was a biri smoker. There were no gender differences with respect to age, BMI, or the source of drinking water among the cases and controls (Table 1).

We found significant age (p-value<0.001), gender (p-value<0.001) and BMI (p-value<0.055) differences related to prevalence of skin lesions among the cases and controls (Table 2). As to be expected based on the study design, there was no significant difference in arsenic concentrations in wells previously used for drinking water by cases and controls (p-value = 0.544; Table 2). The difference in severity of skin lesions was statistically different for age (p-value<0.001), but not for BMI (p-value = 0.106) or arsenic levels in previous wells used for drinking water (Table 2).

The following 16 SNPs of CDH1 were investigated in the study: rs16260, rs5030625, rs155364, rs155808, rs155807, rs2303646,

Table 5
CDH1 polymorphism and severity of arsenic-related skin lesions.

	Control		Case (mild)		Case (moderate)		Case (severe)		χ^2	p
	n	%	n	%	n	%	n	%		
SNP and genotype										
rs2059254									3.522	0.73
CC	70	72.2	22	64.7	36	66.67	6	75.0		
TT	2	2.06	2	5.88	4	7.41	0	0.00		
CT	25	25.8	10	29.4	14	25.93	2	25.0		
rs1862748									4.578	0.573
CC	74	77.9	23	67.6	36	70.59	6	50.0		
TT	1	1.05	1	2.94	3	5.88	0	0.00		
CT	20	21.1	10	29.4	12	23.53	6	50.0		
rs12599393									6.707	0.292
CC	78	79.6	24	64.8	33	67.35	7	77.8		
TT	1	1.02	1	2.70	3	6.12	0	0.00		
TC	19	19.4	12	32.4	13	26.53	2	22.2		
rs13689									4.315	0.666
CC	2	2.02	0	0.00	0	0.00	0	0.00		
TT	75	75.7	31	68.9	41	77.36	7	77.8		
TC	22	22.2	14	31.1	12	22.64	2	22.2		
rs7196495									7.353	0.251
CC	4	4.08	1	2.94	4	7.84	0	0.00		
TT	46	46.9	19	55.9	33	64.71	5	62.5		
TC	48	48.9	14	41.2	14	27.45	3	37.5		
rs7196661									9.005	0.14
CC	4	4.08	0	0.00	5	9.43	0	0.00		
TT	48	48.9	24	70.6	30	56.60	6	75.0		
TC	46	46.9	10	29.4	18	33.96	2	25.0		
rs9925923									3.331	0.761
CC	69	71.9	21	61.7	34	65.38	6	66.7		
TT	2	2.08	2	5.88	3	5.77	0	0.00		
TC	25	26.0	11	32.3	15	28.85	3	33.3		
rs16260									1.153	0.988
AA	3	3.23	1	2.94	2	4.08	0	0.00		
CC	63	67.7	24	70.6	34	69.39	5	62.5		
CA	27	29.0	9	26.5	13	26.53	3	37.5		
rs9989407									9.91	0.099
CC	4	4.12	0	0.00	5	9.43	0	0.00		
TT	46	47.4	24	70.6	30	56.60	6	75.0		
CT	47	48.4	10	29.4	18	33.96	2	25.0		
rs155364									5.548	0.46
AA	31	32.9	11	33.3	24	48.00	4	57.1		
TT	14	14.9	7	21.2	7	14.00	1	14.3		
AT	49	52.1	15	45.4	19	38.00	2	28.5		
rs155808									6.602	0.335
AA	34	37.8	11	37.9	25	50.00	3	42.9		
GG	12	13.3	7	24.1	3	6.00	1	14.3		
AG	44	48.9	11	37.9	22	44.00	3	42.8		
rs12919719									7.706	0.207
CC	74	79.6	21	61.8	32	66.67	5	83.3		
GG	1	1.08	1	2.94	3	6.25	0	0.00		
GC	18	19.3	12	35.3	13	27.08	1	16.7		

rs2059254, rs9925923, rs12919719, rs7188750, rs9989407, rs7196495, rs7196661, rs13689, rs12599393, and rs1862748. These SNPs have been explored in various studies with respect to cancers of various organs. Three of these (rs5030625, rs155807, and rs7188750) were found to have very low minor allele frequencies and could not be analyzed reproducibly; they were not included in subsequent analysis.

Genotype and allele frequencies for cases and controls are presented in Table 2. There was no evidence that the genotype frequencies deviated from those expected under Hardy–Weinberg Equilibrium (Table 3). The main effects of the polymorphisms on skin lesion risk are also shown in Table 3. The association of increased risks for skin lesions with genotype frequencies was borderline for rs7196661 (p-value = 0.092; $\chi^2 = 4.77$), rs7196495 (p-value = 0.090; $\chi^2 = 4.82$), and rs12919719 (p-value = 0.065; $\chi^2 = 2.85$). The strongest association of genotype and skin lesions was found for rs9989407 (p-value = 0.058; $\chi^2 = 5.69$).

Table 4 shows the genotype and allele-specific risks for skin lesions for CDH1 polymorphisms after controlling for age, gender, BMI and tobacco (biri) smoking. Several SNPs suggest that the T>T genotype carriers are at higher relative risk for skin lesions compared to carriers of the C>C or C>T genotypes; these mutants occur in rs1862748 (Odd ratio (OR) = 16.9; Confidence Interval (CI) = 1.26–228; p-value = 0.033), rs12599393 (OR = 15.1; CI, 1.15–198; p-value = 0.039). Marginally higher risks were found for T>T mutants in rs2059254 (OR = 6.14; CI, 0.74–51; p-value = 0.092), and rs9925923 (OR = 6.35, CI, 0.76–53; p-value = 0.089). The very low frequencies of the homozygous T>T variant genotype for these SNPs should be noted; the rs1862748T>T and rs12599393T>T mutants were detected in only 5.3% of the study population (Table 4). A larger samples size would be needed to confirm whether the T>T genotypes in these SNPs can modify the risk of skin lesions in carriers. The low frequency of the homozygous G>G genotype in rs12919719 compared to the G>C or C>C (Table 4) also places some doubt on the observation showing that it is a risk factor for skin lesions (OR = 15.5; CI, 1.17–206; p-value = 0.038). The heterozygous T>C genotype, a major allele in rs13689, was found to be protective factor (OR = 0.301; CI, 0.091–0.993; p-value 0.049) for skin lesions (Table 4).

The main effects of SNPs and genotype frequencies on the severity of skin lesions are shown in Table 5. A weak association was found in only

rs9989407 (p-value = 0.099; $\chi^2 = 9.91$). A number of genotypes, however, showed non-significant associations with increased severity of skin lesions; these include the C>T genotype in rs1862748 (21% of cases, 29% of mild cases and 50% of severe cases), the C>C genotype in rs9989407 (47% in controls and 75% in severe cases), and the A>A genotype in rs155364 (33% in controls, 48% in moderate cases and 57% in severe cases). These data cannot rule out the fact that the (tested) genetic variants in CDH1 may influence the progression of skin lesions into more severe phenotypes. The evidence from this study is weakened by the small sample size, however.

4. Discussion

Our results are generally consistent with those of previous studies on the modifiable determinants of skin lesions in areas where people are exposed to high levels of arsenic in their drinking water (reviewed recently Chen et al., 2009). We found that males and older participants were more likely to be afflicted with skin lesions compared to females and younger adults (see Table 2). The association of tobacco smoking with risk of skin lesions reported in men in Bangladesh (Chen et al., 2006) is also consistent with the results of this study. The observation that comparatively higher BMI is associated with reduced risk for skin lesion in Bangladesh (Ahsan et al., 2006) is also confirmed to a limited extent in our study population (p-value = 0.055). Studies in various Asian countries have reported dose-dependent associations between arsenic exposure and risk of skin lesions (Guha Mazumder et al., 1998; Fewtrell et al., 2005; Rahman et al., 2006; Ahsan et al., 2007; Chen et al., 2009). We also found a strong association (p-value<0.001) between urinary arsenic levels (a good surrogate for arsenic exposure) and the risk of skin cancer in our study population (Table 2). No correlation was found, however, between the prevalence of skin lesion and concentrations of arsenic in wells used previously for drinking water, presumably because the current burden of skin lesion is not related to exposures during the 2006/2007 period.

In the past few years, a number of studies have been done on the associations between CDH1 gene polymorphisms and susceptibility to different types of cutaneous diseases in humans (Cattaneo et al., 2006; Wang et al., 2008; Zhang et al., 2008; Chen et al., 2011). To the best of our knowledge, this is the first investigation of the associations between CDH1 gene polymorphisms and precancerous skin lesions in an area with known environmental tiger (exposure to high levels of arsenic in drinking water).

The present study has identified four SNPs in CDH1 that may affect the development of skin lesions in arsenic-exposed residents of Nadia District, India. The four SNPs suggesting increased risk for skin lesion are rs7196495 (p-value = 0.09), rs7196661 (p-value = 0.092), rs9989407 (p-value = 0.058) and rs12919719 (p-value = 0.065). These results represent the first evidence that CDH1 polymorphisms can influence human susceptibility to arsenic-related lesions and presumably the cutaneous metabolism of arsenic as well. We also found that many individuals who carry minor T>T allele variants had increased susceptibility for skin lesions. Genotypes in CDH1 SNPs that were associated with increased risk for skin lesion even after controlling for age, BMI, gender and tobacco smoking included rs1862748T>T (OR = 17.0; p-value = 0.033), rs12599393T>T (OR = 15.1; p-value = 0.039), rs2059254T>T (OR = 6.14; p-value = 0.092) and rs9925923T>T (OR = 6.35; p-value = 0.089). The G>G genotype, a minor variant in rs12919719 SNP was also found to be significantly associated with increased odds for skin lesions (OR = 15.5; p-value = 0.038). By contrast, carriers of the T>C genotype in the rs13689 SNP may have reduced risk for skin lesions (OR = 0.301; p-value = 0.049). In other words, this variant may be a factor in reducing the susceptibility of controls to arsenic-related skin lesions. The protective versus promotive roles of cadherin on skin lesions suggest that the effect of arsenic exposure on this gene is allele specific. These findings indicate that the contribution of E-cadherin gene to individual susceptibility to arsenic-related skin lesions and other health effects may be more pervasive than has generally been realized.

A number of studies have shown that the C>A mutant in the rs16260 is an important adjuvant in the suppression of CDH1 expression and hence is implicated in the increased susceptibility to different types of cancer (Wang et al., 2008). A meta-analysis of 26 case–control studies involving 7042 cases and 7011 controls was conducted to assess the susceptibility of the rs16260A allele carriers to seven types of cancers (Wang et al., 2008). This analysis showed that compared to non-carriers, the rs16260A allele carriers had about a 17–19% increased risk of several invasive cancers. Among the Europeans, the rs16260AA homozygote was associated with an increased risk of urothelial cancer while carriers of A allele were at increased risk of lung, prostate and gastric cancer (Wang et al., 2008). No evidence, however, was found linking differences in alleles or genotypes of rs16260 with increased susceptibility to cancers in the Asian population (Wang et al., 2008). A subsequent meta-analysis reviewed the results in 423 papers on gastric cancer (GC) available from a total of 17 case–control studies with 3511 GC patients and 4826 controls (Chen et al., 2011). The second study likewise found that the rs16260A allele carriers had a significantly increased risk of GC among Caucasians (AA vs.CA + CC: OR = 1.50, 95% CI = 1.03–2.19, p = 0.03), but not among Asians (AA vs. CA + CC: OR = 0.87, 95% CI = 0.56–1.37, p = 0.56). We found no association between any of the genotypes in rs16260 in the Indian population, which is consistent with the previous suggestion that this SNP is an ethnicity-dependent risk factor for some disease phenotypes.

A number of studies have reported that loss of or decreased expression of CDH1 is involved in the transition from well-differentiated adenoma to invasive carcinoma (Siitonen et al., 1996; Christofori and Semb, 1999). Others have associated the levels of CDH1 in cancer tissues with histological types and even with a higher probability of cancer progression and death (Umbas et al., 1994; Shariat et al., 2011). We did not find any strong association between CDH1 SNPs and the stages of skin lesions in our study population (Table 5). Although there were differences in allele and genotype frequencies between the cases and controls (Table 5), these did not reach statistical significance. Whether carriers of specific mutants in CDH1 gene are at increased risk for developing skin cancers from their skin lesions cannot be inferred from this study.

The role of cadherins in cutaneous biology has been explored in several studies (Furukawa et al., 1994; Danen et al., 1996; Papadavid et al., 2002; Haass et al., 2005). In human epidermis, homeostatic balance is maintained between keratinocytes and melanocytes. At the epidermal/dermal junction, melanocytes adhere to adjacent basal keratinocytes through expression of CDH1 (Tang et al., 1994; Hsu et al., 2000). The keratinocytes maintain control over cell growth and dendricity, as well as expression of melanoma-associated cell surface molecules of normal melanocytes (Hsu et al., 2000, 2002). By contrast, transformed melanoma cells are outside the keratinocyte-mediated regulation. The loss of regulatory dominance by keratinocytes has been associated with down-regulation of CDH1 expression in melanoma cells (Hsu et al., 2000). Melanoma cells can escape from this control by keratinocytes through three major mechanisms involving cadherins: (i) down-regulation of receptors important for communication with keratinocytes such as CDH1, P-cadherin, desmoglein and connexins; (ii) up-regulation of receptors and signaling molecules not found on melanocytes but important for melanoma-melanoma and melanoma-fibroblast interactions such as N-cadherin; and (iii) loss of anchorage to the basement membrane because of an altered expression of the extracellular-matrix binding by the cadherin family of biologic glue (Haass et al., 2005). Disruption of the CDH1-mediated, normal regulatory control from keratinocytes may represent one of the mechanisms that can account for melanocyte transformation into disease phenotypes (Hsu et al., 2002). We hypothesize that the down-regulation of cadherin expression by arsenic exposure may be mediating the escape of melanoma cells from the microenvironment created by epidermal keratinocytes and

consequently may be enabling them to develop new and altered spatial relations that favor uncontrolled proliferation, migration, and formation of skin lesion.

A large number of experimental and clinical studies show that, in addition to skin carcinomas, down-regulation of CDH1 is associated with the development of most human epithelial cancers, including bladder, breast, prostate, renal, ovarian, liver, kidney, colorectal, pancreatic, stomach, and lung carcinomas (Christofori and Semb, 1999; Hsu et al., 2000; Jiang and Mansel, 2000; Cavallaro et al., 2006; Baranwal and Alahari, 2009; Ranscht, 2010). The reduction or absence of CDH1 is generally associated with loss of differentiation, invasion, and metastatic behavior of tumor cells. Conversely, activation of CDH1 has been shown to result in tumor growth retardation (Birchmeier and Behrens, 1994; Yoshiura et al., 1995; Perl et al., 1998) and inhibition of the invasive and metastatic phenotype in carcinoma cells (Frixen et al., 1991; Hermiston et al., 1996). Altered expression of CDH1 has also been well documented in precancerous lesions as well as in melanoma and non-melanoma skin cancer (Tada et al., 1996; Fuller et al., 1996; Koseki et al., 1999; Wu et al., 2000; Papadavid et al., 2002). A study of the role of CDH1 in cutaneous cell carcinoma and its precursors showed that normal skin and skin with mild or moderate solar elastosis strongly expressed membranous CDH1. The expression of CDH1 was found to be progressively reduced in the epidermis of skin with severe solar elastosis through solar keratosis to squamous cell carcinoma (Lyakhovitsky et al., 2004).

Among the many processes that can induce aberrant expression of CDH1, the best documented mechanisms by which CDH1 expression is lost or inappropriately reduced is through the hypermethylation of its DNA promoter region (Chiles et al., 2003; Lim et al., 2008; Ren et al., 2011). Lin et al. (2010) found that CDH1 of tumor DNA and DNA from urine samples are frequently methylated in bladder cancer and have even proposed the hypermethylation of CDH1 in urine sediment DNA as a potential biomarker for detecting superficial and low grade cancer. A likely mechanism by which arsenic can suppress the CDH1 promoter activity is therefore through the epigenetic methylation process (Pilsner et al., 2007; Majumdar et al., 2010). Arsenic-mediated hypermethylation of promoters of some genes has been reported in human skin cancer (Chanda et al., 2006) and bladder cancer (Chen et al., 2007; Marsit et al., 2006). Hypermethylation of promoter genes has also been observed in humans, animals and cell lines exposed to arsenic (Chanda et al., 2006; Chen et al., 2007; Marsit et al., 2006; Zhang et al., 2008; Ren et al., 2011). The linkage between epi-methylation of arsenic and CDH1 is an interesting topic that has yet to be explored.

The manifold roles of cadherin in development and disease require that its functions be regulated at multiple levels, including gene transcription, proteolysis, endocytosis, and interactions with intracellular proteins (Strathdee, 2002; Berx and van Roy, 2009; Ranscht, 2010). The highly conserved cytoplasmic domain of CDH1 provides selective target sites and opportunities for arsenic to moderate its function, such as at the adherens junction, the growth factor receptors and associated signaling pathways; during phosphorylation and dephosphorylation of cadherins and β-catenin; and during cleavage by extracellular and intracellular metalloproteases. At these potential homeostatic checkpoints, the cadherins are a sensitive gene that may be far more promiscuous in their binding specificities than previously assumed (Niessen and Gumbiner, 2002). This suspicion is based on the fact that arsenic itself is not a classic carcinogen since it is not efficient at inducing point mutations or initiating and promoting the development of tumors in experimental animals (Rossman et al., 2004; Klein et al., 2007). Mechanisms by which exposure to arsenics can induce skin lesions and cancers include alteration in the expression of genes involved in arsenic metabolism, stress response, damage response and apoptosis, cell cycling, cell signaling and growth factor signaling (Nriagu and Bernstam, 2003; Wang et al., 2005; Tapio and Grosche, 2006; Ren et al., 2011). The metabolic cycles of CDH1 and arsenic thus clearly involved many similar processes and pathways where interactions can occur. Little is currently known about such homeostatic regulation of CDH1 function by arsenic exposure, however. The results of this study beg for a detailed investigation of this issue.

In conclusion, this is the first study that indicates that CDH1 polymorphisms can contribute to the etiology of premalignant skin lesions in people chronically exposed to arsenic in drinking water. This gene–environment interaction may account for some of the observed inter-individual variability in the incidence of skin lesions among the study participants. The results call for further exploration of the molecular etiology by which development of skin lesion phenotypes is linked to functional alteration of the CDH1 expression. There is a need to explore whether this polymorphism is a genetic factor for susceptibility to arsenic-related health effects in different ethnic populations. Although this study is somewhat underpowered, the results can nevertheless help in guiding the selection of factors that should be further investigated in future larger studies. Such research can also facilitate the discovery of genomic biomarkers of arsenic exposure and early effects, and may help to identify subpopulations that are particularly susceptible to arsenic-associated diseases.

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