Joint effects of genomic markers and urinary methylation capacity associated with inorganic arsenic metabolism on the occurrence of cancers among residents in arseniasis-endemic areas: A cohort subset with average fifteen-year follow-up

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ABSTRACT

Background: Chronic exposure to inorganic arsenic results in many cancers in susceptible persons. The metabolism of inorganic arsenic and genomic susceptibility are thought to be associated with cancer occurrence.

Methods: This study aims to examine the interaction of genomic susceptibility markers and urinary methylation capacity indicators involved in inorganic arsenic metabolism with all-cancer occurrence. This study conducted a follow-up on 96 residents to determine their urinary inorganic arsenic metabolites and genomic assay from an arseniasis area. Among them, 24 cancer developed. Multivariable Cox proportional hazards model was used to determine and estimate the candidate independent variables for cancer development.

Results: The residents with high inorganic arsenic exposure, high primary methylation index (PMI; MMA/InAs) (but lower secondary methylation index (SMI)), and non-...
heterogeneity type of genomic markers, including GSTO1, AS3MT, and MPO, tend to develop cancers. Subjects with higher PMI are at higher risk of developing cancers (HR = 1.66; 95% CI = 1.30–2.12). Cancer occurrence was greater among the CC type of GSTO1 (HR = 3.33; 95% CI = 1.11–10.00), CC type of AS3MT (HR = 19.21; 95% CI = 1.16–318.80), and AA type of MPO (HR = 13.40; 95% CI = 1.26–142.40). After adjusting confounders, a mutually moderating effect was revealed between genomic markers and methylation capacity on cancer occurrence.

Conclusions: This study found the hypermethylation responses to inorganic arsenic exposure and an array of genomic markers may increase the susceptibility of a wide range of organ cancers. The findings indicated a high-risk arsenic-exposed population to develop cancers. The phenotype of arsenic metabolism and genomic polymorphism suggested a potential preventive strategy for arsenic carcinogenesis.

At a glance of commentary

Scientific background on the subject

Inorganic arsenic exposure is associated with the risk of cancer.

What this study adds to the field

The combination of homozygosity type of selected genes including GSTO1, AS3MT, and MPO can predict the risk of cancer among arseniasis residents in which the inorganic arsenic methylation capacity will moderate the genomic effect on cancer occurrence.

Exposure to inorganic arsenic has been documented as an etiological factor of all-cause mortality in humans [1]. Cancers of the skin and other internal organ cancers, including lung, liver, bladder, and kidney, were associated with inorganic arsenic exposure [2,3]. Recently, studies from Bangladesh have also demonstrated the association between inorganic arsenic exposure and the development of various cancers [4,5]. Therefore, the WHO and the US EPA has adopted a new standard for arsenic in drinking water of 0.01 mg/l or 10 parts per billion (ppb), replacing the old standard of 50 ppb [6,7].

The central issue of inorganic arsenic toxicities is the role of inorganic arsenic metabolism in humans [8]. Studies have shown that humans exposed to environmental inorganic arsenic, by oxidation and methylation, will metabolize it to arsenite (As⁴⁺), arsenate (As⁵⁺), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in their urine [9,10]. Previous works have shown that residents with lower levels of methylation were likely to develop cancers of the skin, lung, and bladder [11–14]. Studies in the area of the northeastern coast of Taiwan have also demonstrated that adverse health effects were related to chronic arsenic exposure and methylation capacity. The first intermediate of the methylation capacity, monomethylarsonic acid levels in urine, was further defined as a biologically effective dose for inorganic arsenic exposure in humans [14,15].

During the past decades, various genomic markers were examined and were reported to be associated with both arsenic metabolism and the occurrence of specific cancers. The arsenic +3 oxidation state methyltransferase (AS3MT) was proven to involve the methylation of inorganic to MMA and MMA to DMA in rodents [16]. Researchers reported that AS3MT was associated with inorganic arsenic carcinogenesis through various methylation capabilities [17,18]. Glutathione S-transferase omega 1 (GSTO1) and GSTO2 polymorphisms were documented to be associated with various cancers, and both of them actively participated in arsenic metabolism [19]. One carbon metabolism-related enzymes were reported to be associated with DNA synthesis, repair, and methylation as well as carcinogenesis. Among them, 5-methylenetetrahydrofolate reductase (MTHFR), 10-MTHFR, and 5-methyltetrahydrofolic acid-homocysteine methyltransferase reductase (MTRR) were frequently reported as key players in the methylation of arsenic, which affects one’s susceptibility to arsenic toxicity [20]. In addition to these methylation-associated genes, oxidative stress has been proposed as one of the important mechanisms affecting inorganic arsenic metabolism and carcinogenesis. The literature has shown that manganese containing superoxide dismutase (MnSOD), endothelial nitric oxide synthase (eNOS), and myeloperoxidase (MPO) were associated with arsenic carcinogenesis in both mammal and epidemiological studies [21,22]. The tumor suppressor protein p53 mutation results in loss of control of cell growth and genomic instability. The p53 mutation is frequently documented as a factor related to arsenic carcinogenesis [23].

However, the evidence of association between inorganic arsenic metabolism patterns and cancers was limited to some specific cancer sites, such as the skin and bladder. In addition, mixed results were found regarding the pattern of inorganic arsenic metabolism (as PMI and SMI calculated by taking ratios of MMA to inorganic arsenic and DMA to MMA, respectively) and different manifestations of arsenic toxicities [10,15,24,25]. Hitherto, few studies were found to explore the association of genomic markers and methylation capacity with a wide range of cancers [26], which are important for depicting the progression of arsenic carcinogenesis. Therefore, this long-term follow-up study aims to examine the role of inorganic arsenic metabolic patterns and the associated genomic markers on.
the occurrence of various cancers among residents in an arseniasis area.

Materials and methods

Study subjects

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB #102-0710C). A total of 96 residents were eligible for both genomic markers assay and urinary inorganic arsenic speciation analysis. These subjects were, at that time, exposed to arsenic-contaminated water (early years of 1990s) and therefore recruited from an arseniasis area located in northeastern Taiwan for the study [9,14]. The length of this subset of cohort spanned September 1992 to December 2013 (a total of 21.3 years), while the average follow-up period of the cohort study was 15.55 years, after questionnaire administration and specimen collection. After the data collection, the local government implemented a tap-water supply program, which reached 100% tap-water accessibility in the study area after August 1995. According to the initial questionnaire and laboratory data, the residents had exposure to arsenic-contaminated drinking water for an average of 37 years with a mean arsenic concentration as high as 65.8 ppb, ranging from 0 ppb to 1547.9 ppb.

Data collection

Basic demographic variables and exposure data, including age, gender, marital status, education, occupation, cigarette smoking, alcohol drinking, and duration of well water exposure, were assessed using a questionnaire collected during 1990s when the participants were recruited. The diagnoses of cancers were based on the national cancer registration database and further confirmed with insurance claim data by linking the previously collected information with the national health insurance database thereafter. The ICD-9-CM codes 140–208 were used to define the cancers, while other cancers outside this range of codes were not included in this study. A total of 24 incident cases of cancers were found and counted: 2 (8.3%) cases of cancer of the oral and esophagus (ICD-9: 140–150), 2 (8.3%) of the stomach (ICD-9: 151), 1 (4.2%) of the colon/rectum (ICD-9: 153–154), 4 (16.7%) of the liver (ICD-9: 155), 5 (20.8%) of the lung (ICD-9: 162), 2 (8.3%) of skin cancer (ICD-9: 173), 3 (12.5%) of the cervix (ICD-9: 180), 3 (12.5%) of the prostate (ICD-9: 185), and 2 (8.3%) of the bladder (ICD-9: 188).

Arsenic speciation and metabolism pattern classification

The underground water and urine were collected and stored at –20 °C. The determination of water arsenic concentration was done immediately upon collection. Urinary arsenic speciation was performed with high-performance liquid chromatography (HPLC) for separation of arsenic species and coupled with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for determination of the individual concentration of arsenic species. The detection limits of urinary inorganic arsenic metabolites included arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid were 0.4 ppb, 1.4 ppb, 1.4 ppb, and 1.0 ppb, respectively. Spiking analysis measured an average recovery rate ranging from

| Table 1 The distribution of sociodemographic variables and lifestyle variables and their effects on cancer occurrence. |
|---------------------------------------------------------------|
| **Person-years** | **Cancer cases** | **Incidence density (year⁻¹)** | **Univariate analysis** |
| **Sociodemographic variables** |  |  |  |
| Age |  |  |  |
| ≥59.47 years | 688.01 | 14 | 0.020 | 1.83 (0.81,4.15) |
| <59.47 years | 804.98 | 10 | 0.012 | 1.00 |
| Sex |  |  |  |
| Male | 895.42 | 19 | 0.021 | 2.64 (0.99,7.09) |
| Female | 597.58 | 5 | 0.008 | 1.00 |
| Education |  |  |  |
| Low | 475.64 | 4 | 0.008 | 0.46 (0.10,2.05) |
| Medium | 851.79 | 17 | 0.020 | 1.13 (0.33,3.86) |
| High | 165.57 | 3 | 0.018 | 1.00 |
| Occupation |  |  |  |
| Soldiers/government employees | 24.01 | 2 | 0.083 | 11.37 (2.08,62.24) |
| Farmers | 834.49 | 15 | 0.018 | 2.36 (0.78,7.12) |
| Laborers and trading | 126.46 | 3 | 0.024 | 3.19 (0.71,14.25) |
| Housekeeping and others | 508.03 | 4 | 0.008 | 1.00 |
| Marital status |  |  |  |
| Married | 1434.70 | 22 | 0.015 | 1.00 |
| Not married | 58.29 | 2 | 0.034 | 2.56 (0.60,10.95) |
| **Lifestyle Variables** |  |  |  |
| Cigarette smoking |  |  |  |
| Yes | 740.93 | 18 | 0.024 | 3.18 (1.26,8.02) |
| No | 752.06 | 6 | 0.008 | 1.00 |
| Alcohol drinking |  |  |  |
| Yes | 338.91 | 9 | 0.027 | 2.22 (0.97,5.11) |
| No | 1154.09 | 15 | 0.013 | 1.00 |
**Table 2 The metrics of arsenic exposure and urinary inorganic arsenic metabolites between cancer and noncancer groups.**

| Arsenic Exposure                        | Cancer Median (Q1, Q3) | Noncancer Median (Q1, Q3) | p     | Univariate analysis HR (95% CI of HR) |
|----------------------------------------|-----------------------|---------------------------|-------|--------------------------------------|
| Concentration in well water (ppb)      | 27.10 (8.33,49.88)    | 19.45 (2.58,51.89)        | 0.42  | 1.00 (1.00,1.00)                      |
| Years of exposure                      | 34.50 (29.50,43.50)   | 36.00 (28.50,48.50)       | 0.57  | 1.00 (0.97,1.02)                      |
| Cumulative arsenic exposure (ppm-years) | 0.73 (0.30,1.86)      | 0.79 (0.21,2.62)          | 0.66  | 1.03 (1.00,1.07)                      |
| Urinary Inorganic Arsenic Metabolites   |                       |                           |       |                                      |
| Total (ppb)                            | 57.28 (47.33,107.67)  | 68.60 (38.44,105.14)      | 0.82  | 1.00 (1.00,1.01)                      |
| Inorganic arsenic (ppb)                | 6.30 (4.17,12.15)     | 8.05 (3.67,13.16)         | 0.77  | 0.99 (0.95,1.04)                      |
| MMA (ppb)                              | 9.16 (5.09,21.84)     | 7.55 (3.88,13.50)         | 0.32  | 1.01 (0.98,1.03)                      |
| DMA (ppb)                              | 46.47 (26.65,82.57)   | 49.39 (27.42,82.86)       | 0.95  | 1.00 (0.99,1.01)                      |
| AsI%                                   | 9.47 (4.82,20.98)     | 11.81 (6.41,18.73)        | 0.61  | 0.99 (0.94,1.05)                      |
| MMA%                                   | 14.89 (9.09,22.44)    | 11.77 (8.49,16.05)        | 0.11  | 1.06 (1.00,1.11)                      |
| DMA%                                   | 70.29 (60.42,83.35)   | 76.26 (63.81,84.58)       | 0.38  | 0.98 (0.95,1.02)                      |
| PMI                                    | 1.55 (0.79,2.95)      | 1.02 (0.61,1.47)          | 0.03  | 1.24 (1.11,1.39)                      |
| SMI                                    | 4.89 (2.72,8.75)      | 5.56 (3.93,10.39)         | 0.17  | 0.96 (0.89,1.04)                      |

95.18% to 100.03%. The Standard Reference Material, SRM 2670a, was used to perform the validity check and the values were calculated within the suggested range. According to the literature, the pattern of inorganic arsenic metabolism was further indicated as the primary arsenic methylation index (PMI: MMA/[As\textsuperscript{3+}\textsubscript{5} As\textsuperscript{5+}]) and secondary arsenic methylation index (SMI: DMA/MMA) [10,12,13,24].

**Genomic markers assay**

The extraction of DNA from urine followed the protocols of the QiAamp Blood Midi Kit (QIAGEN, Cat. No. C01-12143). A total of 4 ml urine was centrifuged 5 min at 20,000 g. The suspension fluid was discarded, and the remaining cells were mixed with 140 µl buffer AVL and carrier RNA. Nucleotides were extracted, and 50 µl were added to double distilled water for the subsequent analysis of PCR-RFLP. This study utilized The National Center for Biotechnology Information SNP database for the genomic target selection and primer design. Further confirmation of the appropriateness of primers used the SNP blast function. As a result, the following genomic markers were used in this study: MTRR (rs1532268), AS3MT (rs1046778), GSTO1 (rs4925), GSTO2 (rs156697), NOS3 (rs1799983), SOD2 (rs1799725), MPO (rs7208693), and TP53 (rs17882155). For DNA amplicons up to 500 bp, 25 µl reaction volumes (adding 2x distilled water) were used as follows: 0.125 µl Taq polymerase (1.25 U, Gibco); 2.5 µl 10 x PCR buffer minus Mg (1x); 0.75 µl 50 mM MgCl\textsubscript{2} (1.5 mM); 1 µl template DNA: 0.5 µl 10 mM dNTP mix (0.2 mM); and 1.25 µl 10 uM primer mix (0.5 µM). If DNA amplicons were >500 bp, the reactions were scaled up to a total volume of 100 µl. A program thermocycler was used to perform PCRs.

**Statistical analyses**

Numerical variables were displayed as the means ± standard deviations and categorical variables as frequencies and percentages. Two independent sample t-tests and chi-square tests were used to compare the differences between groups with respect to continuous variables and categorical variables, respectively. A Cox proportional hazards model was used to determine the strength of the association between the study variables and the occurrence of cancers while examining (and adjusting) the effects from other selected variables. The results were expressed as multivariate-adjusted hazard ratios and the corresponding 95% confidence intervals. The pattern of inorganic arsenic metabolism was further categorized into four groups, including low-PMI/low SMI, low-PMI/high-SMI, high-PMI/low-SMI, and high-PMI/high-SMI, according to medians of the PMI and SMI.

**Results**

The median age of the 96 study subjects upon data collection was 57.3 years, with a range from 41.5 to 76.2 years [Table 1]. The likelihood of developing cancers was greater in higher age groups (HR = 1.83; 95% CI = 0.81–4.15) compared with lower age groups but was not statistically significant. The occurrence of cancers was higher among male subjects (HR = 2.64; 95% CI = 0.99–7.09) compared with female subjects. The association between marital status or education level and the occurrence of cancers was not statistically significant. A higher likelihood of developing cancers was found in soldiers/government employees (HR = 11.37; 95% CI = 2.08–62.24).

Cigarette smoking was positively associated with the occurrence of cancers (HR = 3.18; 95% CI = 1.26–8.02) [Table 1].

Among parameters of water arsenic exposure and urinary arsenic metabolites, cumulative arsenic exposure (HR = 1.03; 95% CI = 1.00–1.07), MMA% (HR = 1.06; 95% CI = 1.00–1.11), and PMI (HR = 1.24; 95% CI = 1.11–1.39) were significantly positively associated with the occurrence of cancers [Table 2]. In univariate analysis, the TT type of GSTO2 was found to be significantly associated with the occurrence of cancers (HR = 2.80; 95% CI = 1.04–7.54) compared with the CT type of GSTO2. Other genomic markers did not exhibit statistically significant associations with the occurrence of cancers. However, a higher risk of cancer occurrence was observed among the CC type of GSTO1 (HR = 1.47; 95% CI = 0.65–3.32), CT type of GSTO2 (HR = 1.25; 95% CI = 0.55–2.84), TT type of SOD2 (HR = 2.58; 95% CI = 0.88–7.54), GG type of NOS3 (HR = 1.48; 95% CI = 0.55–3.98), AA (HR = 4.06; 95% CI = 0.57–28.85) and CC (HR = 2.64; 95% CI = 0.62–11.32) types of MPO, AG (HR = 2.54; 95% CI = 0.56–11.60) and GG (HR = 1.82;
In Cox regression analyses, cumulative arsenic exposure was positively associated with the occurrence of cancers with hazard ratios ranging from 1.02 to 1.03 in different models. A significantly positive association was found between PMI (HR = 1.66; 95% CI = 1.30–2.12) and the occurrence of cancers while adjusting for age, gender, education level, occupation category, cigarette smoking, alcohol drinking, and cumulative arsenic exposure. A multivariate-adjusted hazard ratio of 3.37 (95% CI = 3.64) in developing cancers was observed among subjects with above median value of PMI but below median value of SMI. In addition, a significantly monotonic trend of cancer development by multivariable Cox regression was found among subjects with inorganic arsenic metabolism pattern appearing in the following order: low-PMI/high SMI, low-PMI/low-SMI, high-PMI/high-SMI, and high-PMI/low-SMI. However, only three genomic markers (GSTO1, AS3MT, and MPO) were statistically associated with the occurrence of cancers in the multivariable regression analyses. The CC type of GSTO1 (HR = 3.33; 95% CI = 1.11–10.00), CC (HR = 19.21; 95% CI = 1.16–318.80) and TT (HR = 5.96; 95% CI = 1.47–24.12) types of AS3MT, and AA type of MPO (HR = 13.40; 95% CI = 1.26–142.40) were at higher risk of developing cancers as opposed to their respective homozygosity type in the multivariable regression analyses [Table 4].

### Discussion

This subset analysis of residents in an arseniasis-endemic area shows that inorganic arsenic metabolic pattern, as demonstrated by the primary methylation index (PMI) and secondary methylation index (SMI), is associated with an increased risk of various cancers in adults. Our findings extend the results of previous reports on the association between the pattern of inorganic arsenic metabolism and skin, lung, or bladder cancer [11–14]. They provide evidence that the high inorganic arsenic methylation response at the primary stage (but low response to the secondary arsenic methylation stage) may promote the onset of various cancers in a dose-response manner. In addition, the study found that residents in arseniasis-endemic regions with homozygosity of three genomic markers (GSTO1, AS3MT, and MPO) have a higher risk of developing various cancers. Researchers have proposed that high activity in the primary arsenic methylation process may increase the accumulation of methyl methacrylate (MMA) and trigger carcinogenesis [8]. Although this is consistent with our major findings, a high methylation capacity is also believed to be a protective mechanism from carcinogenesis in other studies [8,14]. The contradiction can also be explained by the diverse data sources and variations in biological systems as reflected

| Genomic Markers | Cancer | Noncancer | p | Univariate analysis |
|-----------------|--------|-----------|---|---------------------|
| GSTO1 (glutathione S-transferase omega 1; rs4925) | | | | |
| AA | 1 (4.17%) | 5 (6.94%) | 0.61 | 0.72 (0.09,5.58) |
| AC | 11 (45.83%) | 39 (54.17%) | | 1.00 |
| CC | 12 (50%) | 28 (38.89%) | | 1.47 (0.65,3.32) |
| GSTO2 (glutathione S-transferase omega 2; rs156697) | | | | |
| CC | 1 (4.17%) | 4 (5.56%) | 0.75 | 0.87 (0.11,6.64) |
| CT | 10 (41.67%) | 24 (33.33%) | | 1.25 (0.55,2.84) |
| TT | 13 (54.17%) | 44 (61.11%) | | 1.00 |
| SOD2 (superoxide dismutase 2; rs1799725) | | | | |
| CC | 0 (0%) | 4 (5.56%) | 0.09 | – |
| CT | 4 (16.67%) | 25 (34.72%) | | 1.00 |
| TT | 20 (83.33%) | 43 (59.72%) | | 2.58 (0.88,7.54) |
| NOS3 (nitric oxide synthase 3; rs1799983) | | | | |
| GG | 18 (75%) | 49 (68.06%) | 0.14 | 1.48 (0.55,3.98) |
| GT | 5 (20.83%) | 23 (31.94%) | | 1.00 |
| TT | 1 (4.17%) | 0 (0%) | | – |
| MPO (myeloperoxidase; rs7208693) | | | | |
| AA | 2 (8.33%) | 4 (5.56%) | 0.36 | 4.06 (0.57,28.85) |
| AC | 2 (8.33%) | 15 (20.83%) | | 1.00 |
| CC | 20 (83.33%) | 53 (73.61%) | | 2.64 (0.62,11.32) |
| MTRR (5-methyltetrahydrofolate-homocysteine methyltransferase reductase; rs1532268) | | | | |
| AA | 2 (8.33%) | 13 (18.06%) | 0.32 | 1.00 |
| AG | 10 (41.67%) | 20 (27.78%) | | 2.54 (0.56,11.60) |
| GG | 12 (50%) | 39 (54.17%) | | 1.82 (0.41,8.12) |
| AS3MT (arsenic +3 oxidation state methyltransferase; rs1046778) | | | | |
| CC | 1 (4.17%) | 2 (2.78%) | 0.07 | 2.33 (0.27,19.99) |
| CT | 5 (20.83%) | 34 (47.22%) | | 1.00 |
| TT | 18 (75%) | 36 (50%) | | 2.80 (1.04,7.54) |
| TP53 (tumor protein p53; rs17882159) | | | | |
| CC | 0 (0%) | 5 (6.94%) | 0.41 | – |
| GG | 14 (58.33%) | 38 (52.78%) | | 1.04 (0.46,2.34) |
| TG | 10 (41.67%) | 29 (40.28%) | | 1.00 |
in human data, in vitro experiments, and in vivo studies [10,27,28]. Our data also show that subjects with a higher PMI and a lower SMI are likely to develop cancers, which is partly consistent with the notion that MMAIII accumulation is a biomarker of health indices [8]. Additionally, a few studies have suggested the negative association between SMI and cancers of the skin and bladder [12,13].

In the present study, urinary arsenic metabolites were found to be 9.47 ± 1.11%, 11.77 ± 1.49%, and 70.29 ± 7.62%, respectively, for InAs, MMA, and DMA, which is within the previously reported range [20]. Although our subjects had a history of long-term exposure to high concentrations of arsenic, this suggests that their methylation profile is consistent with values reported by other studies. Nevertheless, the question of why the range of inter-individual variability of inorganic arsenic methylation causes diverse carcinogenic effects needs to be elucidated. Some studies have shown that MMA is more cytotoxic and genotoxic than AsIII and AsV, suggesting that the oxidation state of methylated arsenicals is important for the manifestation of their toxic and/or genotoxic effects. A recent study showed that MMA% might be a potential marker of cancer-associated mortality [29]. In the present study, however, we found that a high PMI and a low SMI seemed to be associated with a high risk of developing cancers. It is possible that arsenic aids the carcinogenesis by mechanisms linked to methyl donor and glutathione (GSH) depletion [28] as well as their subsequent genotoxic effects such as induction of oxidative stress, interference with signal transduction, or gene expression [30].

Many studies have explored alternative models. For instance, whether the trivalent methylated arsenic species per se are more toxic compared to the premethylated inorganic compounds. Based on these models, MMA (III) and DMA (III) may cause cellular toxicity, genotoxicity, and clastogenic inhibition on cysteine-containing enzymes [31]. Studies have shown that the DNA damage may directly or indirectly be induced by methylated trivalent arsenicals [32]. In animal models, MMA (III) or DMA(V) exposure led to carcinogenesis in the bladder, lungs, and skin [28,33,34]. These findings shed some insights on our results about the high cancer risk among participants with a high PMI but a low SMI.

We also found an independent and slightly moderating effect between selected genomic markers and the pattern of inorganic arsenic metabolism on the risk of cancers. The analyses showed that the association between inorganic arsenic methylation patterns and the occurrence of cancers was stronger when three significant genomic markers (GSTO1, AS3MT, and MPO) are considered. This phenomenon implies that the three genomic markers are important in modulating the carcinogenic effects of inorganic arsenic through the methylation process. An early preventive strategy can be deduced from this observation, especially among high risk populations with certain methylation patterns to inorganic arsenic exposure.

The present study shows that subjects with the AA genotype of GSTO1 have a higher cancer risk (HR = 4.26) but this association was not statistically significant. The frequency of the AA genotype of GSTO1 was 6.1% in the present community.

### Table 4 Multivariable Cox regression models for arsenic exposure, arsenic methylation capacity, and genomic markers.

|                        | Model I       | Model II      | Model III     | Model IV      |
|------------------------|---------------|---------------|---------------|---------------|
| **Arsenic Exposure**   |               |               |               |               |
| Cumulative Arsenic Exposure (ppm-years) | 1.03 (1.00,1.06) | 1.03 (1.00,1.06) | 1.03 (1.00,1.06) | 1.02 (0.99,1.05) |
| **Arsenic Methylation Capacity** |               |               |               |               |
| PMI                    | 1.66 (1.30,2.12) | 0.97 (0.89,1.06) |               |               |
| SMI                    |               |               |               |               |
| **PMI SMI (median low/high)** | 1.00*        | 1.00*         |               |               |
| Low Low                | 1.15          |               | 2.74          | 3.37          |
|                         | (0.22,6.18)   |               | (0.64,11.78)  | (0.85,13.35)  |
| High High              | 1.03          |               | 2.85          | 3.64          |
|                         |               |               | (0.15,6.95)   | (0.57,14.30)  |
| High Low               |               |               |               |               |

**Genomic Markers**

|                      | Model I       | Model II      | Model III     | Model IV      |
|----------------------|---------------|---------------|---------------|---------------|
| GSTO1                |               |               |               |               |
| AA                   | 4.26 (0.33,54.89) |               |               |               |
| AC                   | 1.00          |               |               |               |
| CC                   | 3.33 (1.11,10.00) |               |               |               |
| AS3MT                |               |               |               |               |
| CC                   | 19.21 (1.16,318.80) |               |               |               |
| CT                   | 1.00          |               |               |               |
| TT                   | 5.96 (1.47,24.12) |               |               |               |
| MPO                  |               |               |               |               |
| AA                   | 13.40 (1.26,142.40) |               |               |               |
| AC                   | 1.00          |               |               |               |
| CC                   | 4.77 (0.68,33.72) |               |               |               |

All models were adjusted for age, sex, education, occupation, smoking, and alcohol drinking.

*p < 0.05, test for monotonic trend by multivariable Cox regression model.
cohort subset, which is right in-between that of 2.7% in a Taiwanese study and 9.8% in a US hospital-based study [35,36]. A significantly increased risk for various cancers was observed among subjects with the CC genotype of GSTO1 (HR = 3.33), which is coherent with studies that reported that the CC genotype of GSTO1 may result in defective protection against cellular oxidative stress [37]. The polymorphisms of AS3MT are involved in the methylation of inorganic arsenic [38]. The TT genotype of AS3MT was shown to be significantly associated with the development of cancers (HR = 2.80). The frequency of the TT genotype of AS3MT was found to be 56.3%, which is similar to that of 50.9% in a Swedish study [39]. The literature has indicated that polymorphisms in AS3MT significantly predicted arsenic metabolism and its related health effects across different populations. The polymorphism variation of myeloperoxidase (MPO) is generally recognized as a factor associated with oxidative state transformation. MPO is involved in the formation of a variety of ROS and activating carcinogens [40]. Although researchers have proposed that the oxidative stress related to arsenic exposure is associated with arsenic adverse effects, limited evidence was found in epidemiological studies demonstrating the effect of the MPO genotype on arsenic carcinogenesis. This study found that the less frequent haplotype AA of MPO was significantly associated with cancers. All the three genomic markers suggest that arsenic carcinogenesis, with regard to its metabolism, may be comprehensively related to oxidation, phase I conjugation, and methylation. As reported in the extant literature, the three enzymatic components are generally regarded as key steps for arsenic metabolism and, in turn, the variations affect an individual’s risk of cancer [8]. To the best of our knowledge, this study is the first to comprehensively investigate genomic biomarker effects on arsenic carcinogenesis, focusing on various cancers in an epidemiological cohort follow-up. The findings are important for future arsenic carcinogenesis research.

Although these findings are innovative and significant in the field of cancer research and arsenic carcinogenesis, this study has some limitations. First, a prospective design that uses repeated measurements of the subjects’ urinary inorganic arsenic metabolites would be required to confirm the reliability of such a dosimeter. Second, the samples were primarily from Chinese/Taiwanese individuals. Although the results can be generalized, applying the results to people of other ethnicities should be done cautiously and with in-depth studies for confirmation. Third, the pattern of inorganic arsenic methylation seems to be an indicator for the early detection of a high-risk group, though further confirmation with biomarkers in the related biological pathways is suggested. Fourth, this subset of study samples was randomly selected from the community cohort. However, a larger sample size to confirm the findings is suggested in the future.

Conclusions

This study found the hypermethylation responses to inorganic arsenic exposure and an array of genomic markers may increase the susceptibility of a wide range of organ cancers. Residents in arseniasis-endemic regions with homozygosity of three genomic markers (GSTO1, AS3MT, and MPO) have a higher risk of developing various cancers. The phenotype of arsenic metabolism and genomic polymorphism suggested a potential preventive strategy for arsenic carcinogenesis.

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Conflicts of interest

All authors declare they have no conflicts of interest.

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