Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water.

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Long-term ingestion of inorganic arsenic causes various health effects, including cancers of the bladder, skin, and lung and development of skin lesions (1). The biotransformation of arsenic in humans occurs through the methylation process. Few data exist that link methylation patterns to arsenic-induced disease (2,3). Although it has been suggested that genetic polymorphisms cause variation in arsenic methylation (4–7), little evidence has been found to substantiate this hypothesis.

Family correlation studies assist in determining whether variations in methylation patterns may be caused by genetic polymorphisms. If genetic factors contribute to arsenic methylation capacity, family studies should demonstrate that siblings have a higher correlation of methylation activity than their parents (2,3). Family correlation studies assist in determining whether variations in methylation patterns are due to a genetic basis for the variation in arsenic methylation. Larger studies with more extensive pedigrees will need to be conducted to confirm these findings.

Key words: arsenic, family correlations, metabolism, methylation, susceptibility, urine.

The evidence of familial correlation was investigated in two studies: one in the United States and the other in Chile. In the United States, the 11 participating families in the study were selected for high prevalence of arsenic-induced effects. Participants from this family had low InAs/metAs values, which is consistent with increased toxicity of trivalent methylated arsenic species. Despite our small sample size, we observed that methylation patterns aggregate in families and are correlated in siblings, providing evidence of a genetic basis for the variation in arsenic methylation.

We investigated the evidence of a familial contribution to urinary methylation patterns in families ingesting arsenic in drinking water. Arsenic methylation can be assessed by measuring urinary levels of inorganic arsenic (InAs) and its methylated metabolites, monomethylarsonate (MMA), and dimethylarsonate (DMA). Methylation activity is reflected in the ratios: InAs/metAs and MMA/DMA. Eleven families from Chile were selected because of their long-term exposure to very high levels of arsenic in drinking water (735–762 µg/L). Each family consisted of a father, a mother, and two children. We measured urinary arsenic and its methylated metabolites for each participant (n = 44). The intraclass correlation coefficients showed that 13–52% of the variations in the methylation patterns were from being a member of a specific family. Family correlations were calculated for father–mother, parent–child, and sibling–sibling pairs. Methylation patterns correlated strongly between siblings (r = 0.78 for InAs/metAs, 95% confidence interval (CI), 0.54–0.94; r = 0.82 for MMA/DMA, 95% CI, 0.78–0.89) compared to lower correlations in father–mother pairs (r = 0.18, r = 0.01, respectively), after adjustment for total urinary arsenic, age, and sex. Family correlations were not notably altered when adjustments were made for specific blood micronutrients (methionine, homocysteine, folate, vitamin B12, selenium, and vitamin B6) potentially related to methylation. We also report on a family pedigree with high prevalence of arsenic-induced effects. Participants from this family had low InAs/metAs values, which is consistent with increased toxicity of trivalent methylated arsenic species. Despite our small sample size, we observed that methylation patterns aggregate in families and are correlated in siblings, providing evidence of a genetic basis for the variation in arsenic methylation.

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the only families we could find in the entire village that met these criteria. Each participant was interviewed for residential and smoking histories and examined by four physicians for arsenic-induced skin lesions (keratoses, hypopigmentation, and hyperpigmentation). Informed consent was obtained from all participants.

**Sampling and chemical analysis.** Spot urine samples were collected for each participant and kept frozen at −20°C until they were transported to the University of Washington in Seattle for arsenic content analysis. Urinary concentrations of InAs, MMA, and DMA were determined using hydride generation atomic absorption spectrometry (HGAA) according to a method based on that described by Crecelius (16). In summary, inorganic arsenic (As\(^{\text{III}}\) and As\(^{\text{V}}\)), MMA, and DMA are reduced to the corresponding arsine in a batch reactor using sodium borohydride using 5-mL samples. The volatile reduction products (arsine, methyl arsine, and dimethyl arsine, respectively) are removed by sparging with helium. Entrained arsines are concentrated in a chromosorb-filled cryogenic trap at liquid nitrogen temperatures until all arsine-forming arsenic in the sample has reacted. The cryotrap is then allowed to warm, and the collected arsines are separated on the basis of differential volatilization. The detection of the separated volatile arsenic species is accomplished by atomic absorption spectrophotometry using a hydrogen microburner combustion cell to convert arsines to elemental arsenic. Detection limits for InAs, MMA, and DMA were 0.5, 1.0, and 2.0 µg/L, respectively. In this paper, total urinary arsenic concentrations (µg/L) were 0.5, 1.0, and 2.0 µg/L, respectively. In this paper, total urinary arsenic concentrations (µg/L) were 0.5, 1.0, and 2.0 µg/L, respectively. In this paper, total urinary arsenic concentrations (µg/L) were 0.5, 1.0, and 2.0 µg/L, respectively. In this paper, total urinary arsenic concentrations (µg/L) were 0.5, 1.0, and 2.0 µg/L.

**Table 2.** Distribution of urine-based arsenic methylation indices by family membership, mean (range).

| Methylation index | All (n = 44) | Fathers (n = 11) | Mothers (n = 11) | Sons (n = 14) | Daughters (n = 8) | p-Value\(^{a}\) |
|-------------------|-------------|----------------|----------------|-------------|----------------|----------------|
| Mean total arsenic (µg/L) | 490 (55–1,320) | 464 (184–1,026) | 486 (161–733) | 566 (55–1,320) | 427 (260–736) | 0.60 |
| %InAs | 17 (6–32) | 20 (9–32) | 15 (6–25) | 15 (8–22) | 18 (7–28) | 0.68 |
| %MMA | 14 (3–25) | 18 (11–25) | 11 (4–17) | 13 (3–19) | 14 (10–20) | < 0.01 |
| %DMA | 89 (49–86) | 62 (49–75) | 74 (58–83) | 72 (59–86) | 69 (55–81) | 0.02 |
| InAs/metAs | 0.21 (0.07–0.47) | 0.25 (0.10–0.47) | 0.18 (0.07–0.33) | 0.16 (0.09–0.29) | 0.22 (0.07–0.39) | 0.20 |
| MMA/DMA | 0.22 (0.03–0.46) | 0.30 (0.15–0.46) | 0.16 (0.05–0.29) | 0.15 (0.03–0.31) | 0.22 (0.03–0.35) | < 0.01 |

\(^{a}\)For ANOVA.
from three samples of Chiu Chiu drinking water, confirming high arsenic content.

Chiu Chiu participants had elevated total urinary arsenic levels (average 490 µg/L), which confirms high exposure to arsenic (Table 2). The average distributions of urinary arsenic metabolites were 17% InAs, 14% MMA, and 69% DMA. The average for InAs/metAs was 0.21, and for MMA/DMA 0.22. Overall, the distributions did not differ between fathers, mothers, sons, and daughters for total arsenic levels, %InAs, and the ratio of InAs/metAs. However, fathers have higher %MMA and MMA/DMA ratio and lower %DMA. InAs/metAs and MMA/DMA ratios were plotted for each individual by family in Figure 1.

The intraclass correlations for Chiu Chiu indicate familial aggregations of methylation patterns (Table 3). The intraclass correlations for families were \( r = 0.52 \) for %InAs, 0.13 for %MMA, 0.32 for %DMA, 0.45 for InAs/metAs, and 0.38 for MMA/DMA. Between 13 and 52% of the variations of all the methylation indices can be explained by being a member of a specific family.

Results of the family resemblance correlation analyses are presented in Table 4. The unadjusted and adjusted interclass correlation coefficients (\( r \)), 95% confidence intervals (CI), and \( p \)-values are shown. High correlations were found between siblings for all variables (\( r = 0.82 \) for %InAs; 0.62 for %MMA; 0.82 for %DMA; 0.80 for InAs/metAs; 0.76 for MMA/DMA). The parent–child correlations were much lower (0.16–0.50). The correlations between the fathers and mothers were also lower (0.21–0.46).

Correlations of the residual values from specific regression models adjusting for total urinary arsenic, age, and sex are further defined (Table 4). The correlations between siblings remained high (\( r = 0.74 \) for %InAs, 95% CI, 0.26–0.93; \( r = 0.69 \) for %MMA, 95% CI, 0.15–0.91; \( r = 0.83 \) for MMA/DMA, 95% CI, 0.47–0.96; \( r = 0.72 \) for InAs/metAs, 95% CI, 0.22–0.92; \( r = 0.78 \) for MMA/DMA, 95% CI, 0.43–0.94). However, the parent–child (\( r = -0.01 \)–0.31) and father–mother (\( r = -0.02 \)–0.26) correlations were lower. The adjusted correlations are presented graphically in Figure 2.

Even after adjusting for individual blood levels of micronutrients and biochemical indicators (methionine, homocysteine, folate, vitamin B\(_6\), selenium, vitamin B\(_12\)), the sibling correlations remained high. After adjusting for folate and homocysteine, the father–mother correlations for InAs/metAs increased. The correlations and regression coefficients are shown for InAs/metAs and MMA/DMA in Table 5.

We also report on characteristics of family A. Both the father and daughter had skin lesions. We then discovered that the son, who had not participated in the study, also had arsenic-induced skin cancer. Upon further investigation of their family pedigree (Figure 3), we found among three generations of this family that six out of 10 members who lived in Chiu Chiu have developed arsenic-related health effects (squamous cell carcinoma, Bowen’s disease, and skin lesions). Additionally, the InAs/metAs ratios for all four members of Family A are among the lowest six values of the entire study (see Figure 1A). The probability that four participants who rank this low are in one particular family, by chance alone, is < 0.001.

**Discussion**

This investigation is the first of its kind to evaluate the degree of familial resemblance for arsenic methylation capacities. Chiu Chiu families were an ideal population to study because the residents consumed the same highly arsenic-contaminated water for many years, the only water source in this extremely dry desert region. High intraclass correlations for the participating families indicate that methylation is more similar among relatives than among unrelated individuals. We found significant correlations of urinary methylation indices between siblings, which suggests that the capacity to metabolize arsenic has a familial component.
Genetic polymorphisms have explained differences in other methylation systems (22), so it is likely that arsenic methylation may also be genetically determined. If so, individual susceptibility caused by differences in arsenic methylation capacity may be inherited. Few studies have investigated familial patterns and genetic factors in development of arsenic health effects and metabolism. In Taiwan, a population-based study found that patients with blackfoot disease, an arsenic-induced peripheral vascular disease, were three times more likely to have a family history of blackfoot disease than community controls (23). However, exposure variation could account for this pattern. A case report from the Netherlands described an entire family exposed to a pesticide containing arsenite (24). Among all the family members exposed, only the young female in the family, with a 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency, had elevated homocysteine in the plasma and urine and showed neurotoxic symptoms after exposure (24). A specific genetic polymorphism for MTHFR which may be involved in arsenic methylation could have contributed to her susceptibility to arsenic.

Another study from Taiwan found that genetic polymorphisms of the detoxification enzymes glutathione S-transferases M1 and T1 were associated with varying arsenic methylation patterns (6). The researchers found that those with the null GSTM1 genotype had a higher percentage of %InAs, and those with the null GSTT1 genotype had increased %DMA in the urine. Studies conducted in native Andean populations from Argentina have found unusually low fractions of urinary MMA (~2%) (5,25) compared to levels in populations elsewhere. It has been postulated that a genetic polymorphism controlling arsenic-methylating enzymes accounts for the interindividual variation across populations (4,12).

In this investigation, we reported on family A, which presents an interesting family history of arsenic-related health effects. Family members who resided in Chiu Chiu had a high prevalence of arsenic-induced skin lesions. It had been thought that methylation reduced the toxicity of arsenic (12), but recent laboratory evidence suggests that methylated trivalent MMA may be more toxic than the inorganic forms (14,26). The low values of InAs/metAs found in family A could indicate that the bio-transformation of arsenic through methylation may be associated with increased health effects. The particular characteristics of family A and its extended pedigree may be an example of a genetic predisposition in methylation resulting in susceptibility to the effects of arsenic.

The children’s methylation indices were consistent with those measured in children in Mexico and Belgium (27,28). In contrast, they were not consistent with the low %MMA reported in one study from Argentina (25). The high sibling–sibling correlations of all methylation indices are intriguing. Variation in arsenic methylation is likely caused by both genetic factors and environmental effects. The children in this

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**Table 5.** Interclass correlations (r) between pairs of family members adjusted for blood levels of specified nutritional factors, total urinary arsenic, age, and sex.

| Methylation index/nutritional factor | Father–mother | Parent–child | Sibling–sibling |
|-------------------------------------|----------------|--------------|-----------------|
| lnAs/metAs                          |                |              |                 |
| No nutritional adjustment           | 0.18           | 0.26         | 0.78            |
| Folate (mg/mL)                      | 0.33           | 0.21         | 0.65            |
| Methionine (µmol/L)                 | 0.14           | 0.27         | 0.73            |
| Vitamin B6 (nmol/L)                 | 0.26           | 0.22         | 0.67            |
| Vitamin B12 (µg/mL)                 | 0.09           | 0.22         | 0.65            |
| Selenium (µmol/L)                   | 0.20           | 0.27         | 0.70            |
| Homocysteine (µmol/L)               | 0.55           | 0.27         | 0.67            |
| MMA/DMA                             |                |              |                 |
| No nutritional adjustment           | –0.01          | –0.01        | 0.82            |
| Folate (mg/mL)                      | 0.06           | –0.02        | 0.77            |
| Methionine (µmol/L)                 | –0.03          | 0.03         | 0.76            |
| Vitamin B6 (nmol/L)                 | –0.11          | –0.09        | 0.73            |
| Vitamin B12 (µg/mL)                 | –0.01          | –0.01        | 0.79            |
| Selenium (µmol/L)                   | 0.04           | 0.01         | 0.76            |
| Homocysteine (µmol/L)               | 0.26           | 0.00         | 0.80            |
study were born into environments with arsenic exposure. The sibling–sibling correlations may be influenced by environments shared only by the children, such as home, school, or play areas, or there may have been a cohort effect. The lower parent–child correlations may be a result of genetic effects associated with metabolism being more important and pronounced at younger ages.

An important limitation of this study involves its size. Although the population is unique because there was only one drinking water source for these families, the small sample size precludes definitive differentiation between genetic and environmental contributions to family correlations. Examination of the confidence intervals in Table 4 suggests that the sibling–sibling correlations are indeed real. However, the confidence interval limits for the father–mother and parent–child correlations are quite high, suggesting caution is needed in interpretation of these particular findings.

Despite the small sample size, the present study provides evidence of a familial component, supporting a genetic basis for arsenic methylation. This study is a first step in establishing familial patterns. In this analysis, adjustments were made for blood levels of micronutrients and biochemical indicators to control for nutritional factors that may be involved in methylation. We are not able to conclude whether there is an effect from nutritional factors on methylation or on any gene–environment interaction. The relative contributions of heredity and shared environment to the familial resemblance and possible modes of inheritance will require more research.

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