Family Correlations of Arsenic Methylation Patterns in Children and Parents Exposed to High Concentrations of Arsenic in Drinking Water

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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 dimethylarsinate (DMA). Methylation activity is reflected in the ratios: InAs/methylated arsenic (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), 0.34–0.94; r = 0.82 for MMA/DMA, 95%CI, 0.43–0.95] 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 B_6, selenium, and vitamin B_{12}) 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. Larger studies with more extensive pedigrees will need to be conducted to confirm these findings.
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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. The application of family correlation studies such as this was used to determine whether there were genetic polymorphisms in drug methylation systems (*8,9*).

Ingested inorganic arsenic (InAs) is methylated in two steps, first to monomethylarsenate (MMA) and then to dimethylarsinate (DMA). The total of urinary InAs, MMA, and DMA is considered a biomarker of recent inorganic arsenic exposure (*10*). Methylation patterns can be assessed by the relative distributions in urine of inorganic arsenic and its metabolites (InAs, MMA, and DMA) (*7*). The activity of the first methylation step is indicated by the index InAs/methylated arsenic (metAs), the ratio of inorganic arsenic to the sum of methylated arsenic species (MMA + DMA). A high ratio of InAs/metAs indicates poor methylation at the first step. The activity of the second methylation step is represented by the ratio of MMA to DMA (MMA/DMA) (*11,12*). If the MMA/DMA ratio is high, there is poor methylation at the second step. Population studies have demonstrated that generally the relative distribution is in the range of 10–30% InAs, 10–20% MMA, and 60–80% DMA in populations exposed to arsenic (*2,13*). There is an emerging hypothesis that methylated forms of arsenic may play a larger role in arsenic toxicity (*14*).

The aim of this analysis was to determine whether arsenic methylation has a familial component. The conditions of Chiu Chiu, a small village in northern Chile, were ideal for evaluating familial correlations of arsenic methylation. Because of Chiu Chiu's extremely dry desert environment, all of its residents shared the sole drinking water supply to the village, which contained high levels of arsenic.

Materials and Methods

Study population. Selection of the Chiu Chiu study site in Chile is explained in detail elsewhere (*15*). For many years, Chiu Chiu's drinking water was piped in and until only recently had high arsenic content (750–800 μg/L). Chiu Chiu has approximately 250 permanent residents, who share this drinking water supply.

The 11 participating families in this cross-sectional study were selected if *a*) members of the family included one adult male (father), one adult female (mother), and two biologically related children older than 5 years of age, *b*) the two adults had lived in Chiu Chiu for at least 10 years, *c*) the children had been born in Chiu Chiu and resided there since birth, and *d*) the family's water source was the village supply. When a family had more than two children, the two children closest to 10 years of age were invited to participate. Initially we sought families in which both adults had lived and worked in Chiu Chiu for at least 20 years. When all the families meeting this criteria in the village were exhausted, we began admitting families in which one adult had 20 years of residence and the other had at least 10 years. The families selected were

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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 spectroscopy (HGAA) according to a method based on that described by Crecelius (*16*). In summary, inorganic arsenic (As^{3+} and As^{5+}), 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 spectroscopy 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 refers to the total of InAs, MMA, and DMA measured. The methylation indices, percentages of InAs, MMA and DMA (%InAs, %MMA, %DMA), and the ratios (InAs/metAs, MMA/DMA) were calculated.

Three water samples were obtained from different locations in Chiu Chiu and shipped to the University of Washington for analysis. They were analyzed for arsenic content by HGAA according to a procedure similar to that used for urine specimens (*17*).

Blood samples were obtained from all participants. Blood was aliquoted immediately and kept frozen until analysis. Pacific Biometrics, Inc. (Seattle, WA, USA) supervised and performed most serum and plasma nutrient analyses. Methods used for each nutrient are briefly presented. We measured plasma homocysteine by HPLC using an internal standard and monobromobimane derivatization (*18*). We determined vitamin B_6 (pyridoxal phosphate) by HPLC and fluorescence detection involving precolumn derivatization of plasma vitamers with sodium bisulfite (*19*). We analyzed serum levels of vitamin B_{12} and folate by CEDIA assays (*20*). Serum selenium was measured by graphite furnace atomic absorption spectrophotometry at the Nutrition Research Laboratory in the Department of Laboratory Medicine at the University of Washington, or by inductively-coupled plasma mass spectometry by the Associated Regional and University Pathologists' trace mineral laboratory at the University of Utah Health Science Center (Salt Lake City, UT). Plasma methionine was measured by amino acid analyzer using a cation-ion exchange column at the Scientific Research Consortium, Inc. (St. Paul, MN).

Statistical analysis. Statistical analyses were performed using SAS software (SAS Institute, Cary, NC, USA) for analysis of variance (ANOVA) and correlational analyses, and STATA for intraclass ANOVA (*21*). We compared the arsenic methylation indices across family members by ANOVA. We computed intraclass correlations to evaluate the correlation within families. This correlation is the proportion of variation of the methylation index that is explained by being in a specific family. The intraclass correlation is the Pearson product–moment correlation computed over all possible pairs of family members within the family.

We examined family resemblance patterns in arsenic methylation by estimating Pearson correlation coefficients between pairs of family members. We examined interclass correlations between father and mother, parent and child, and between siblings. Parent–child pairs were evaluated with each parent to each child. To evaluate whether there were independence concerns with including both children, we also used averages of the siblings in analyses. These results are not reported because they did not differ notably from the results including all pairs.

The family correlations were also conducted with residual values. We adjusted the urinary arsenic methylation indices by multiple regression for age and sex, and then including total urinary arsenic for some analyses:

$\hat{y}$(methylation index predicted) = b_1(age) + b_2(sex) + [b_3(total arsenic)].

We obtained the residual values from the difference of the actual value and the predicted value estimated from the regressions of the methylation indices:

residual = y_i (observed) – $\hat{y}$ (predicted) (i = 1,2,…,44 for each participant).

These residual values reflect the variation after the effects of the regression variables have been removed. Smoking and body mass index (kilograms per square meter) were added to the models, but did not change the results.

To evaluate whether nutritional factors may modify the correlations between family members, we adjusted for specific blood micronutrients and biochemical indicators known to be involved in the arsenic methylation pathway. We adjusted for methionine, homocysteine, folate, vitamin B_6, selenium, and vitamin B_{12} (cobalamin) separately. Each nutrient or indicator was added to the regression, and interclass correlations were computed with the residual values.

Results

Information on the 11 families is presented in Table 1. Four adult men had arsenic-induced skin lesions (*15*). In one family, both the daughter and her father had skin lesions. Most smokers were men. All ex-smokers had stopped smoking more than 5 years before the study. We measured arsenic concentrations at 735, 762, and 763 µg/L

Table 1. Information on 11 families participating in study.

Characteristics	Fathers (n = 11)	Mothers (n = 11)	Sons (n = 14)	Daughters (n = 8)
Mean age, years (range)	38 (30–50)	36 (26–47)	10 (6–14)	11 (9–14)
Mean BMI, kg/m^2 (range)	25.8 (22.1–27.0)	26.6 (20.7–37.3)	19.2 (15.3–30.3)	18.3 (15.7–22.3)
No. with skin lesions	4	0	0	1
No. of current/ex-smokers	4/3	1/1	0	0

BMI, body mass index.

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)	466 (161–773)	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)	0.22
%MMA	14 (3–25)	18 (11–25)	11 (4–17)	13 (3–19)	14 (10–20)	< 0.01
%DMA	69 (49–86)	62 (49–75)	74 (58–83)	72 (59–86)	68 (55–81)	0.02
InAs/metAs	0.21 (0.07–0.47)	0.25 (0.10–0.47)	0.18 (0.07–0.33)	0.18 (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)	0.19 (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 %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.

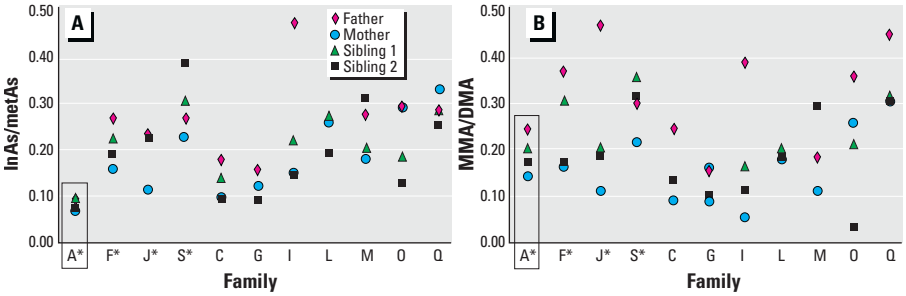

Figure 1. Urine-based arsenic methylation indices plotted for each participant by family. (*A*) ratio of inorganic arsenic to methylated arsenic (InAs/metAs) and (*B*) ratio of MMA/DMA.
*Families that had at least one family member diagnosed with arsenic-induced skin lesions. Family A is boxed (see Figure 3).

Table 3. Intraclass correlations (r) and SE for families of urine-based methylation indices.

Methylation index	Intraclass correlation ($r \pm$ SE)	p-Value[a]
%InAs	0.52 ± 0.16	< 0.01
%MMA	0.13 ± 0.15	0.16
%DMA	0.32 ± 0.17	0.01
InAs/metAs	0.45 ± 0.16	< 0.01
MMA/DMA	0.38 ± 0.17	< 0.01

[a]For ANOVA.

Table 4. Interclass correlation coefficients (r) and 95% CI between pairs of family members for urine-based methylation indices.

Methylation index correlation for family pair	Unadjusted	Adjusted for age and sex	Adjusted for total urinary arsenic, age, and sex	95% CI	p-Value
%InAs					
Father–mother	0.46	0.47	0.26	–0.40–0.75	0.43
Parent–child	0.50	0.49	0.31	–0.01–0.55	0.04
Sibling–sibling	0.82	0.83	0.74	0.26–0.93	< 0.01
%MMA					
Father–mother	0.21	0.14	0.01	–0.59–0.61	0.97
Parent–child	0.16	0.14	0.02	–0.28–0.32	0.89
Sibling–sibling	0.62	0.63	0.69	0.15–0.91	< 0.01
%DMA					
Father–mother	0.25	0.26	–0.02	–0.61–0.59	0.96
Parent–child	0.33	0.32	0.12	–0.18–0.40	0.44
Sibling–sibling	0.82	0.82	0.83	0.47–0.96	< 0.01
InAs/metAs					
Father–mother	0.38	0.39	0.18	–0.47–0.70	0.60
Parent–child	0.43	0.42	0.26	–0.04–0.52	0.08
Sibling–sibling	0.80	0.81	0.72	0.22–0.92	< 0.01
MMA/DMA					
Father–mother	0.21	0.18	–0.01	–0.60–0.60	0.98
Parent–child	0.18	0.15	–0.01	–0.31–0.29	0.95
Sibling–sibling	0.76	0.75	0.78	0.43–0.94	< 0.01

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*-tranferases 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 biotransformation 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

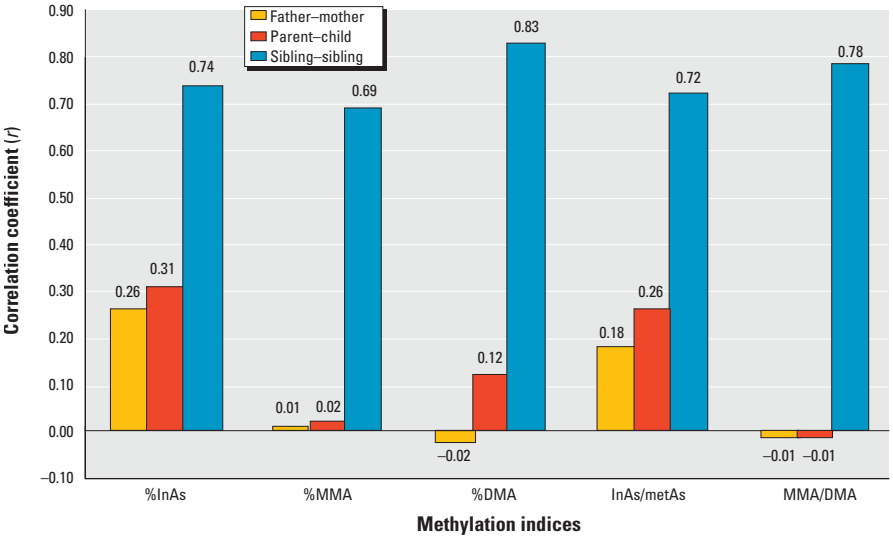

Figure 2. Family resemblance correlations. Bar graph of the interclass correlation coefficients (*r*) of each urine-based arsenic methylation index between family pairs (father–mother, parent–child, and sibling–sibling) adjusted for total urinary arsenic, age, and sex (*n* = 11 families).

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
InAs/metAs			
No nutritional adjustment	0.18	0.26	0.78
Folate (ng/mL)	0.33	0.21	0.65
Methionine (μmol/L)	0.14	0.27	0.73
Vitamin B_6 (nmol/L)	0.26	0.22	0.67
Vitamin B_{12} (pg/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 (ng/mL)	0.06	–0.02	0.77
Methionine (μmol/L)	–0.03	0.03	0.76
Vitamin B_6 (nmol/L)	–0.11	–0.09	0.73
Vitamin B_{12} (pg/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

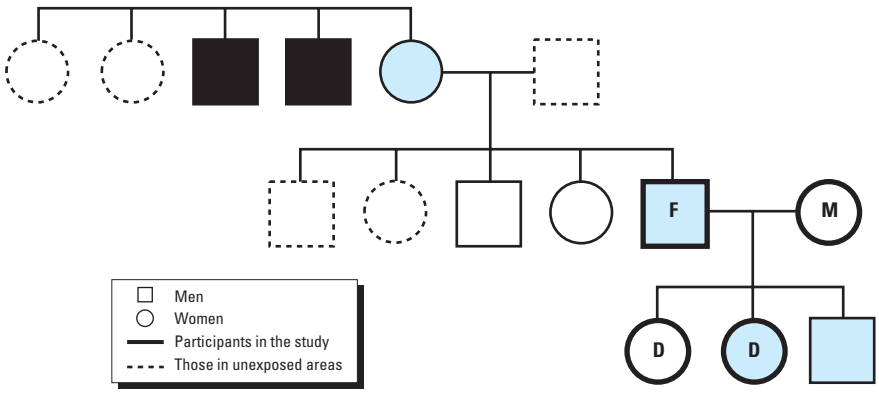

Figure 3. Pedigree of family A. D, daughter; F, father; M, mother. Some individuals lived in areas considered unexposed to arsenic; all other individuals lived in Chiu Chiu. Skin lesions are represented in blue. Skin cancers (squamous cell carcinoma and Bowen's disease) are represented in black.

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 interactions. 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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