Consequences of Pre- and Post-Natal Arsenic Exposure in Bangladesh

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<table>
<thead>
<tr>
<th>Carcinogenic Effects</th>
<th>Non-Carcinogenic effects</th>
</tr>
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<tbody>
<tr>
<td>Skin</td>
<td>Skin lesions</td>
</tr>
<tr>
<td>Lung</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>Bladder</td>
<td>Peripheral Vascular Disease</td>
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<tr>
<td>Liver</td>
<td>Lung Disease</td>
</tr>
<tr>
<td>Kidney</td>
<td>Neurologic deficits</td>
</tr>
<tr>
<td></td>
<td>Diabetes (?)</td>
</tr>
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</table>
Proposed Mechanisms of As Toxicity

- Enzyme inhibition
- Altered DNA repair
- Chromosomal instability
- Oxidative stress
- Altered DNA methylation
- Altered gene expression
Fifty-Year Study of Lung and Bladder Cancer Mortality in Chile Related to Arsenic in Drinking Water

Guillermo Marshall, Catterina Ferreccio, Yan Yuan, Michael N. Bates, Craig Steinmaus, Steve Selvin, Jane Liaw, Allan H. Smith
Fig. 1. Map of Chile, showing regions II and V. The country is administratively divided into regions that are numbered from north to south.
Fig. 2. Lung and bladder cancer mortality rate ratios comparing region II with region V for men and women aged 30 and above, separately, as estimated by Poisson regression with smoothing. The shading represents the 95% confidence bands. The circles represent the mortality rate ratios plotted at the midpoint of each successive 3-year period. Histograms (gray lines) of the population-weighted average arsenic water concentrations for region II, from 1950 to 1994 in 5-year increments, are also presented (vertical axes at right).
Unusual Cancer Excess After Neonatal Arsenic Exposure From Contaminated Milk Powder

Takashi Yorifuji, Toshihide Tsuda and Philippe Grandjean

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Mortality data: Excess skin and liver cancer, as well as pancreatic cancer and leukemia.
Activation of Inflammation/NF-κB Signaling in Infants Born to Arsenic-Exposed Mothers

Rebecca C. Fry\textsuperscript{1,2*}, Panida Navasumrit\textsuperscript{3*}, Chandni Valiathan\textsuperscript{1,2*}, J. Peter Svensson\textsuperscript{1,2}, Bradley J. Hogan\textsuperscript{1,2}, Manlin Luo\textsuperscript{1,2}, Sanchita Bhattacharya\textsuperscript{1,2a}, Krittinee Kandjanapa\textsuperscript{3}, Sumitra Soontararuks\textsuperscript{3}, Sumontha Nookabkaew\textsuperscript{3}, Chulabhorn Mahidol\textsuperscript{3}, Mathuros Ruchirawat\textsuperscript{3*}, Leona D. Samson\textsuperscript{1,2*}

1 Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, 2 Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, 3 Chulabhorn Research Institute, Bangkok, Thailand

The long-term health outcome of prenatal exposure to arsenic has been associated with increased mortality in human populations. In this study, the extent to which maternal arsenic exposure impacts gene expression in the newborn was addressed. We monitored gene expression profiles in a population of newborns whose mothers experienced varying levels of arsenic exposure during pregnancy. Through the application of machine learning–based two-class prediction algorithms, we identified expression signatures from babies born to arsenic-unexposed and exposed mothers that were highly predictive of prenatal arsenic exposure in a subsequent test population. Furthermore, 11 transcripts were identified that captured the maximal predictive capacity to classify prenatal arsenic exposure. Network analysis of the arsenic-modulated transcripts identified the activation of extensive molecular networks that are indicative of stress, inflammation, metal exposure, and apoptosis in the newborn. Exposure to arsenic is an important health hazard both in the United States and around the world, and is associated with increased risk for several types of cancer and other chronic diseases. These studies clearly demonstrate the robust impact of a mother’s arsenic consumption on fetal gene expression as evidenced by transcript levels in newborn cord blood.
A Subset of 11 Gene Transcripts Can Predict whether or not babies were exposed in utero with >80% accuracy.

Slide courtesy of Leona Samson, MIT
PRENATAL Arsenic Exposure Modulates Genes Involved in Inflammatory Response and Cellular Stress Responses

Robust genome-wide response to prenatal arsenic exposure

We have identified arsenic-associated gene sets that classify prenatal arsenic exposure

These genes map onto ontologies that include numerous processes including inflammation, cell signaling, stress response and apoptosis

Slide courtesy of Leona Samson, MIT
Arsenic Contamination in Bangladesh

Percentage of Tubewell Contamination:
- Below 20%
- 20 - 40
- >40 - 60
- >60 - 80
- Above 80%
Arsenic in 5,966 wells
Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study

Cohort Recruitment and Follow-up

HEALS Original Cohort
- ~12,000 adults
- 9/2002-11/2004 Follow-up 1
- 113 deaths

HEALS Expanded Cohort
- ~8,000 adults
- 4/2010-
- 113 deaths
- 120 deaths
- 174 deaths

A validated verbal autopsy was used to classify deaths using WHO’s ICD-10

The predominant cause of arsenic-related deaths was cardiovascular disease!
Association Between Respiratory Symptoms and Baseline Water and Urinary Arsenic

**RRs were adjusted for age, gender, body mass index, education, and smoking**

Parvez et al., Thorax 65:528-533, 2010
Relationship Between Water Arsenic Concentrations and Intellectual Function

Arsenic Metabolism

\[ \text{As}^{\text{V}} \quad \text{As}^{\text{III}} \]

- OH \quad \text{GSN} \quad \text{GSSG} \quad \text{OH}
- O = \text{As}^{\text{V}} - \text{OH}
- O

\[ \text{As}^{\text{III}} \quad \text{HO} - \text{As}^{\text{III}} - \text{OH} \]

\[ \text{MMAs}^{\text{V}} \quad \text{MMAs}^{\text{III}} \]

- \text{Trx-(SH)}_2 \quad \text{Trx-(S)}_2
- \text{OH}
- O = \text{AsIII} - \text{CH}_3
- \text{OH}

\[ \text{DMAs}^{\text{V}} \]

- \text{CH}_3
- O = \text{As}^{\text{V}} - \text{CH}_3
- \text{OH}
One-Carbon Metabolism

Substrate Examples:
- InAs\textsuperscript{III}
- MMA\textsuperscript{III}
- cytosine (CpG)

Respective Products:
- MMA\textsuperscript{V}
- DMA\textsuperscript{V}
- methyl-cytosine (DNA methylation)

GSH $\rightarrow$ GSSG
As(V) $\rightarrow$ As(III)
MMA(V) $\rightarrow$ MMA(III)
Prevalence of Folate Deficiency and Hyperhomocysteinemia in Araihazar, Bangladesh (N = 1650)

Total Blood Arsenic: Maternal Blood vs. Cord Blood

EHP (2007) 115(10):1503-09
% InAs, %DMA and % MMA in Mother's Urine

Number of Samples

% InAs = 9.3
% DMA = 82.5
% MMA = 8.2
Arsenic Metabolites: Maternal Blood vs. Cord Blood

Maternal Blood:
- %InAs = 20.2
- %MMA = 31.6
- %DMA = 45.1

Cord Blood:
- %InAs = 18.6
- %MMA = 34.5
- %DMA = 43.5

EHP (2007) 115(10):1503-09
Normal DNA methylation and changes associated with cancer, environmental exposures, and/or folate deficiency

CpG island

Normal

Cancer/Environmental Exposures

region-specific hypermethylation

Inactivation of Tumor suppressor genes

CpG

m^5CpG

genomic hypomethylation

Genomic instability
Environmental Influences on DNA Methylation

- Diet: Folate, methionine, genistein, etc.
- Cigarette Smoking
- Depression
- Grooming behavior
- Heavy Metals, e.g. Cadmium, Lead
- Arsenic

TRL 1215 transformed rat liver epithelial cells

(A) DNA methylation status in cells treated with arsenic for 19 weeks and cultured in arsenic-free medium for 6 more weeks.

(B) DNA methylation status in cells exposed to 0.5 μM arsenic at times indicated. All data are normalized to the control.

Cross Sectional Study of 294 Adults in Bangladesh, with a wide range of folate status

*a priori* Hypotheses:

- Arsenic exposure is associated with genomic hypomethylation of leukocyte DNA
- Hypomethylation of leukocyte DNA is exacerbated by folate deficiency
[3H]-Methyl-Incorporation Assay

- CpG Islands
- Transposable Elements
- Aging/Cancer
  - region-specific hypermethylation
  - global hypomethylation

Methylated Cytosine

Unmethylated Cytosine
Cross Sectional Study; N=294 Adults
Folate Deficiency $\rightarrow$ ↓ DNA Methylation

Methyl Incorporation (DPM/µg DNA)

<table>
<thead>
<tr>
<th>Plasma Folate</th>
<th>&lt; 9 nM</th>
<th>≥ 9 nM</th>
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<tbody>
<tr>
<td>p = 0.03</td>
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Am J Clin Nutr 2007;86:1179-86
[3H]-methyl incorporation of leukocyte DNA by Age Quartiles in adults and 6 yrs

Mean DPM ± SE adjusted for urinary arsenic, urinary creatinine, gender, and smoking
Test for trend (adults only) p = 0.001
# T-test p < 0.0001 between six year old children and first quartile in adults
N = 165 children and 294 adults

Am J Clin Nutr 2007;86:1179-86
[3H]-methyl incorporation of leukocyte DNA by Urinary As Quartiles (N=294)

Mean DPM ± SE adjusted for age, urinary creatinine, gender, and smoking.

Test for trend; $p = 0.98$

Test for trend; $p = 0.009$

Am J Clin Nutr 2007;86:1179-86
Hypotheses:

Folate deficiency, hyperhomocysteinemia and/or alterations in DNA methylation are associated with increased risk for arsenic-induced premalignant skin lesions.
Nested Case Control Study of Arsenic-Induced Premalignant Skin Lesions

Cohort 2-Yearly Follow-Up Visits:

- 317 Incident Skin Lesion Cases Identified; 273 with serum
- 273 Controls individually matched to cases for gender, age (within 5 years) and water As (within 100 µg/L)

Biological Samples Collected
Folate Deficiency, Hyperhomocysteinemia and Hypomethylation of DNA Independently associated with increased risk for Arsenic-Induced Skin Lesions

<table>
<thead>
<tr>
<th>Predictors of Risk:</th>
<th>Odd Ratio (95% C.I.)</th>
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<tbody>
<tr>
<td>Low Hcys/ High folate</td>
<td>1</td>
</tr>
<tr>
<td>Low Hcys/ Low folate</td>
<td>2.44 (1.32 – 4.52) **</td>
</tr>
<tr>
<td>High Hcys/ High folate</td>
<td>2.69 (1.14 – 6.32) *</td>
</tr>
<tr>
<td>High Hcys/ Low folate</td>
<td>2.84 (1.54 – 5.24) ***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA methylation</th>
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</thead>
<tbody>
<tr>
<td>1st tertile</td>
<td>1</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>2.24 (1.30 – 3.848) **</td>
</tr>
<tr>
<td>3rd tertile</td>
<td>1.81 (1.02 – 3.22) *</td>
</tr>
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<tr>
<td>Urinary Creatinine (fold)</td>
<td>0.57 (0.44 – 0.74) ***</td>
</tr>
<tr>
<td>Urinary As (fold)</td>
<td>1.35 (1.03 – 1.56) **</td>
</tr>
<tr>
<td>Age</td>
<td>1.29 (1.11 – 1.51) **</td>
</tr>
<tr>
<td>Betel nut Use</td>
<td>1.32 (0.84 – 2.07)</td>
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</table>

* p < 0.05, ** p < 0.01, *** p < 0.001; Adjusted ORs derived from a logistic model for all predictors of skin lesions (n = 231 case-control pairs)

EHP (2009), 117(2):254-60
Conclusions

• Pre- and post-natal As exposure is associated with a myriad of effects, but pre-natal exposure appears to be particularly problematic.
• The fetus is exposed to a suite of toxic As metabolites.
• In adults, As exposure is associated with a dose-dependent increase in global DNA methylation, but only when folate is adequate. (Four separate studies)
• In adults, hypomethylation of DNA is associated with increased risk for As-induced skin lesions.
• Pre-natal As exposure is associated with a dose-dependent increase in methylation of cord blood DNA.
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