Associations of glutathione and arsenic methylation in Bangladesh

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Arsenic is methylated in the body
Arsenic is methylated via one-carbon metabolism

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

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

Methionine

SAM \xrightarrow{AS3MT} MMA^{V} \xrightarrow{DNMT} DNMT \xrightarrow{SAM} Methionine

Homocysteine

CBS \xrightarrow{CBS} \xrightarrow{MTHFR} 5 Methyl THF

Cystathionine

Cysteine + Glutamate \xrightarrow{\gamma-glutamylcysteine + Glycine} GSH \xrightarrow{GSH} GSSG

As(V) \xrightarrow{As(V)} As(III)
MMA(V) \xrightarrow{MMA(V)} MMA(III)
Oxidative stress may decrease arsenic methylation in two ways:

1. Upregulation of GSH production leads to decreased SAM production

2. Changes in redox inhibit methyltransferase enzymes
Glutathione and oxidative stress

$O_2^* \rightarrow *OH$

SOD

$H_2O_2$

GPx

2$H_2O$

GSH

GSSG

GR

↓GSH

↑GSSG

↓GSH/GSSG

Lipid peroxidation

Protein oxidation

Antioxidant depletion

DNA damage
One-carbon metabolism under pro-oxidant conditions

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

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

\textbf{Folate}
- \text{DHF} → \text{THF}
- \text{Glycine} → \text{5 Methyl THF} → 10 formyl THF
- Thymidylate synthesis
- Purine synthesis

\textbf{Methionine}
- \text{SAM} → \text{SAH}
- CBS
- Cystathionine
- Cysteine + Glutamate
- \text{y-glutamylcysteine + Glycine}

\textbf{Homocysteine}
- \text{SAM} → \text{SAH}
- CBS
- Cystathionine
- Cysteine + Glutamate
- \text{y-glutamylcysteine + Glycine}

\textbf{Transsulfuration pathway}
- GSH → GSSG
- As(V) → As(III)
- MMA(V) → MMA(III)

Figure courtesy of Dr. Mary Gamble
Redox state = energetic force for electron transfer; measures ability of compound to donate or receive electrons (reduction potential)

Nernst equation:

$$E_{(mV)} = E_0(-RT/nF) \times \ln([\text{reductant}] / [\text{oxidant}])$$

$$E_{(mV)} = -264 - 30 \times \log([\text{GSH}^2] / [\text{GSSG}])$$

- $E_0$ = standard reduction state at pH 7 (-264 mV for GSH)
- $R$ = gas constant
- $T$ = temperature in Kelvin
- $n$ = number of transferred electrons
- $F$ = Faraday’s constant

(Brewer, 2010)
Intracellular redox state influences enzyme activity

\[ \text{Cysteine (CYS)} \quad \text{Cystine (CYSS)} \]

\[ \text{GSH} \quad \text{GSSG} \]

SAM-dependent methyltransferases

\[ \text{AS3MT} \]

Fomenko et al., 2008
Hypothesis: Increased oxidative stress is associated with decreased arsenic methylation.

• H1. Decreased GSH is associated with decreased As methylation, which may be mediated by a decrease in SAM.

• H2. Increased GSSG and Eh(bGSH) (indicators of a more oxidized intracellular redox environment) are associated with decreased As methylation.
Folate and Oxidative Stress (FOX) Study

- Cross-sectional study of 379 arsenic-exposed adults in Araihaazar, Bangladesh

- Primary focus: examine dose-response relationships between arsenic exposure and oxidative stress
H1: Increased GSH production under conditions of oxidative stress leads to decreased SAM, which leads to decreased As methylation.

Is decreased GSH associated with decreased SAM?

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Folate-sufficient (n=266)</th>
<th></th>
<th>Folate-deficient (n=112)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B ± SE</td>
<td>p</td>
<td>B ± SE</td>
<td>p</td>
</tr>
<tr>
<td>bSAM</td>
<td>bGSH, unadjusted</td>
<td>0.042 ± 0.012</td>
<td>0.0007</td>
<td>0.010 ± 0.025</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>bGSH, full*</td>
<td>0.037 ± 0.014</td>
<td>0.0078</td>
<td>0.0086 ± 0.026</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Betas for bGSH represent 100-unit change in bGSH

*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betel nut use (log), BMI (log), vitamin B-12 (log), and television ownership
H1: Increased GSH production under conditions of oxidative stress leads to decreased SAM, which leads to decreased As methylation.

- Is decreased GSH associated with decreased As methylation? **NO**
- Is decreased SAM associated with decreased As methylation? **NO**

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<td>p</td>
</tr>
<tr>
<td>%uInAs</td>
<td>bSAM*</td>
<td>-1.39 ± 0.95</td>
<td>0.15</td>
<td>-1.79 ± 1.54</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>bGSH*</td>
<td>-0.093 ± 0.20</td>
<td>0.65</td>
<td>-0.34 ± 0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>%uMMA</td>
<td>bSAM*</td>
<td>0.19 ± 0.74</td>
<td>0.80</td>
<td>1.19 ± 1.33</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>bGSH*</td>
<td>0.17 ± 0.16</td>
<td>0.27</td>
<td>0.21 ± 0.34</td>
<td>0.54</td>
</tr>
<tr>
<td>%uDMA</td>
<td>bSAM*</td>
<td>1.20 ± 1.28</td>
<td>0.35</td>
<td>0.59 ± 2.21</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>bGSH*</td>
<td>-0.08 ± 0.28</td>
<td>0.77</td>
<td>0.14 ± 0.56</td>
<td>0.81</td>
</tr>
</tbody>
</table>

betas for bGSH represent 100-unit change in bGSH
*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betelnut use (log), BMI (log), vitamin B-12 (log), and television ownership
H2. Oxidative stress is associated with decreased As methylation due to a more oxidized intracellular redox environment.

1. Is increased bGSSG associated with decreased As methylation?

<table>
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<tbody>
<tr>
<td></td>
<td>B ± SE</td>
<td>p</td>
</tr>
<tr>
<td>%uInAs*</td>
<td>0.073±0.76</td>
<td>0.92</td>
</tr>
<tr>
<td>%uMMA*</td>
<td>-0.20±0.59</td>
<td>0.74</td>
</tr>
<tr>
<td>%uDMA*</td>
<td>0.12±1.02</td>
<td>0.90</td>
</tr>
</tbody>
</table>

2. Is a more positive Eh(bGSH) associated with decreased As methylation?

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<tr>
<td></td>
<td>B ± SE</td>
<td>p</td>
</tr>
<tr>
<td>%uInAs*</td>
<td>0.010±0.027</td>
<td>0.71</td>
</tr>
<tr>
<td>%uMMA*</td>
<td>-0.018±0.021</td>
<td>0.39</td>
</tr>
<tr>
<td>%uDMA*</td>
<td>0.008±0.037</td>
<td>0.83</td>
</tr>
</tbody>
</table>

a. log bGSSG

*Adjusted for total urinary As (log), urinary creatinine (log), sex, ever smoking, age (log), betelnut use, BMI (log), vitamin B-12 (log), and television ownership
Percent InAs, MMA, and DMA by quintile of bGSSG, in folate sufficient

**%InAs**

- bGSSG quintile 1: 16 ± 3%
- bGSSG quintile 2: 15 ± 3%
- bGSSG quintile 3: 15 ± 3%
- bGSSG quintile 4: 19 ± 3%
- bGSSG quintile 5: 14 ± 3%

**%MMA**

- bGSSG quintile 1: 13 ± 3%
- bGSSG quintile 2: 13 ± 3%
- bGSSG quintile 3: 15 ± 3%
- bGSSG quintile 4: 15 ± 3%
- bGSSG quintile 5: 14 ± 3%

**%DMA**

- bGSSG quintile 1: 70 ± 3%
- bGSSG quintile 2: 70 ± 3%
- bGSSG quintile 3: 70 ± 3%
- bGSSG quintile 4: 68 ± 3%
- bGSSG quintile 5: 72 ± 3%
Percent InAs, MMA, and DMA by quintile of bGSSG, in folate deficient

%InAs

%MMA

%DMA

*p<0.05, compared to quintiles 1, 2, and 3

*p<0.05, compared to quintiles 1, 2, and 3

*p<0.05, compared to quintiles 1, 2, 3, and 4
Global DNA methylation by quintile of blood GSSG, by folate nutritional status

Folate sufficient

Folate deficient

*p<0.05, compared to quintiles 1 and 2
Conclusion and future directions

- Increased bGSSG associated with decreased As methylation capacity in folate deficient
  - Mechanism: Inhibition of methyltransferases or other metabolic changes?

- Opportunities for intervention
  - Antioxidant supplementation
  - Folate supplementation
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  – Julie Oka

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[3’]-methyl incorporation (DPM) by quintile of bGSSG, by folate nutritional status

*Increased DPM = Decreased global DNA methylation

*\(p<0.05\), compared to quintiles 1 and 2
Demographic and clinical data of subjects in the current study

<table>
<thead>
<tr>
<th>Baseline variables</th>
<th>Folate-deficient (&lt;9 nmol/L) (n=100)</th>
<th>Folate-sufficient (≥9 nmol/L) (n=222)</th>
<th>Group difference</th>
<th>Overall (n=322)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood GSH (uM)</td>
<td>505.2 ± 149.9</td>
<td>484.5 ± 185.7</td>
<td>0.35(^2)</td>
<td>491 ± 173</td>
</tr>
<tr>
<td>Blood GSSG (uM)</td>
<td>34.4 ± 19.0</td>
<td>38.6 ± 18.4</td>
<td>0.019(^2)</td>
<td>37.2 ± 18.6</td>
</tr>
<tr>
<td>Eh bGSH:GSSG (mV)</td>
<td>-200.6 ± 11.7</td>
<td>-196.6 ± 13.9</td>
<td>0.034(^2)</td>
<td>-197.9 ± 13.4</td>
</tr>
<tr>
<td>Plasma GSH (uM)</td>
<td>2.5 ± 0.71</td>
<td>2.6 ± 0.72</td>
<td>0.40(^2)</td>
<td>2.6 ± 0.72</td>
</tr>
<tr>
<td>Plasma GSSG (uM)</td>
<td>2.06 ± 0.66</td>
<td>2.16 ± 0.57</td>
<td>0.052(^2)</td>
<td>2.13 ± 0.60</td>
</tr>
<tr>
<td>Eh pGSH:GSSG (mV)</td>
<td>-98.4 ± 6.7</td>
<td>-98.3 ± 7.4</td>
<td>0.97(^2)</td>
<td>-98.3 ± 7.2</td>
</tr>
<tr>
<td>Plasma Cys (uM)</td>
<td>3.55 ± 2.27</td>
<td>3.89 ± 2.57</td>
<td>0.26(^2)</td>
<td>3.78 ± 2.49</td>
</tr>
<tr>
<td>Plasma CySS (uM)</td>
<td>53.1 ± 14.7</td>
<td>57.5 ± 13.1</td>
<td>0.0037(^2)</td>
<td>56.2 ± 13.4</td>
</tr>
<tr>
<td>Eh pCys:CySS (mV)</td>
<td>-46.8 ± 15.8</td>
<td>-47.6 ± 17.3</td>
<td>0.61(^2)</td>
<td>-47.2 ± 16.8</td>
</tr>
<tr>
<td>Blood SAM (uM)</td>
<td>1.35 ± 0.54</td>
<td>1.23 ± 0.47</td>
<td>0.038(^2)</td>
<td>1.27 ± 0.50</td>
</tr>
<tr>
<td>Blood SAH (uM)</td>
<td>0.33 ± 0.15</td>
<td>0.30 ± 0.17</td>
<td>0.012(^2)</td>
<td>0.31 ± 0.17</td>
</tr>
</tbody>
</table>

1. Mean ± SD (all such values); 2. P-values determined by Wilcoxon’s rank sum test; 3. P-values determined by chi-square test
## E for various cell processes

<table>
<thead>
<tr>
<th>E(mV)</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>-165</td>
<td>Necrosis</td>
</tr>
<tr>
<td>-185</td>
<td>G0/differentiated G1</td>
</tr>
<tr>
<td>-195</td>
<td>Dephosphorylation threshold of phosphoproteins on serine residues</td>
</tr>
<tr>
<td>-205 to &lt;-260</td>
<td>Proliferation</td>
</tr>
</tbody>
</table>

Hoffman, 2009
Glutathione

- L-cysteine, L-glutamic acid, and glycine
- Thiol (SH) group acts as proton donor
- Intracellular - high (1-10 mM, liver 5-10 mM)
  - GSH
  - GSSG: < 1% of GSH
  - 3 major reservoirs: ~90% in the cytosol, ~10% in the mitochondria, small percentage in the ER
- Extracellular - lower (1-10 uM)
- GSH/GSSG usually > 10
Methods

• Glutathione processing:
  – Blood collected and immediately transferred to Eppendorf tubes containing either
    • 5% perchloric acid (PCA), 0.1 M boric acid and γ-glutamyl glutamate as internal standard (for whole blood GSH), or
    • 100 mM serine borate, 60 units heparin, 0.75 mg bathophenanthroline, 4.5 mg iodoacetic acid, and γ-glutamyl glutamate
  – Samples centrifuged for 1 min.; 200 µl of supernatant transferred into Eppendorf tubes containing an equal volume of 10% PCA, 0.2M boric acid
  – Samples sent to Columbia for derivatization and HPLC analysis
  – Measure Cys/CySS, GSH/GSSG