The Molecular Epidemiology of Arsenic in Bladder Cancer

Karl Kelsey
Harvard School of Public Health
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Arsenic exposure and bladder cancer

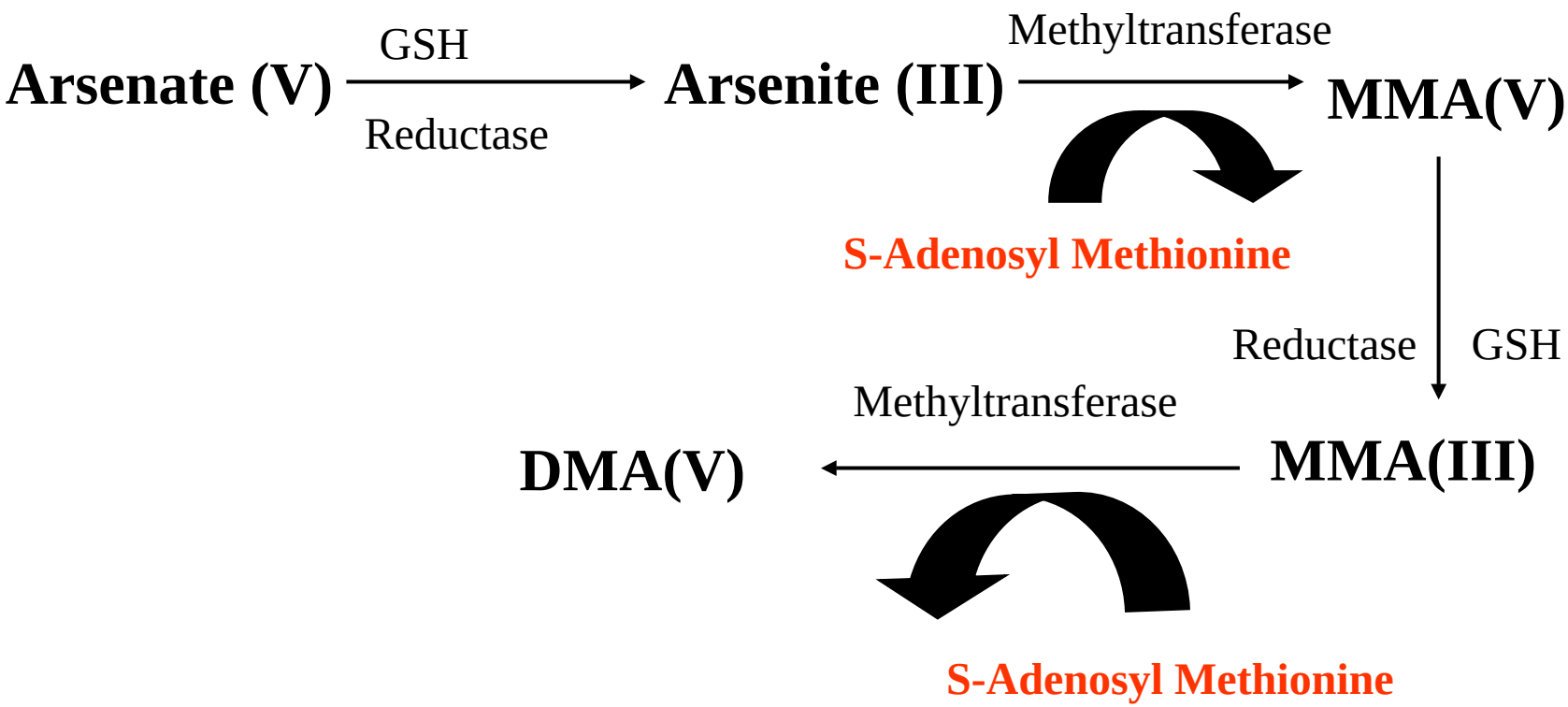
- **Ecological studies consistently report elevated mortality rates:**
  - ***Taiwan***
  - ***Argentina***
  - ***northern Chile***
- **Elevated incidence and mortality rates in cohort studies:**
  - ***in arsenic endemic areas (Taiwan)***
  - ***industrially contamination water (Japan)***
  - ***Fowler's solution (potassium arsenite)***

Arsenic exposure and bladder cancer – what is the mechanism?

- **Alter DNA repair**
  - **Co-carcinogen**
- **Oxidative stress**
- **Induction of apoptosis**
- **Alteration in DNA methylation**
  - **Epigenetic Carcinogen**

Arsenic Detoxication

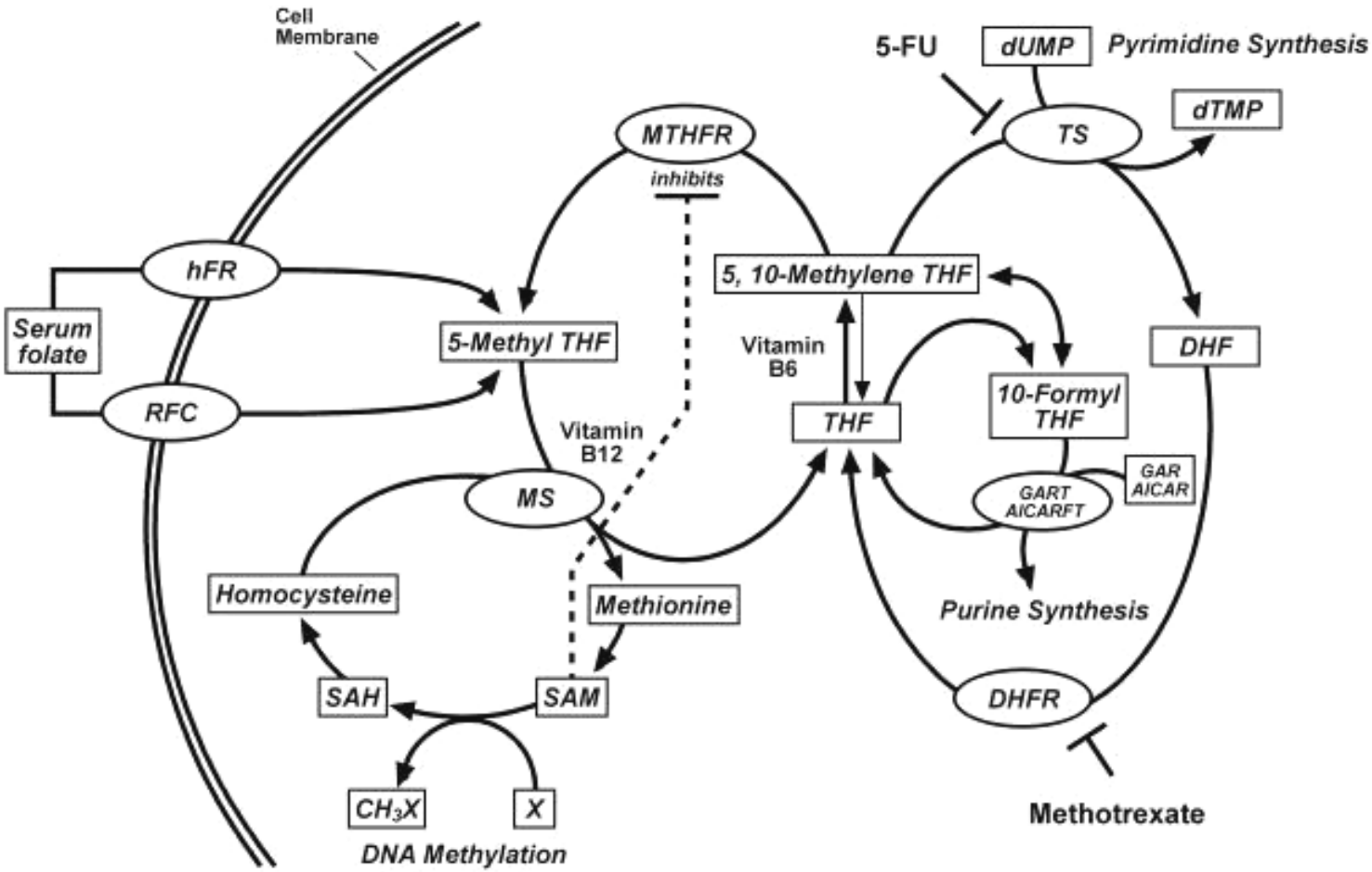
The importance of one carbon metabolism



Does Altered One Carbon Metabolism Contribute to Arsenic Carcinogenesis?

- Folate deficiency is a cancer risk factor (many carcinogens deplete folate, incl. tobacco)
- SAM depletion can occur in-vivo
- Tumors are known to be hypomethylated
- Altered nucleotide pools associated with genomic instability

Folate metabolism



http://www.cdc.gov/genomics/hugenet/reviews/images/MTHFRL1.jpg

De novo methylation of the *p16*^{INK4A} gene in early preneoplastic liver and tumors induced by folate/methyl deficiency in rats

Igor P. Pogribny and S. Jill James

Division of Biochemical Toxicology, Federal Drug Administration, National Center for Toxicological Research, National Center for Toxicological Research, 3900 NCTR Road, Jefferson, AR 72079, USA

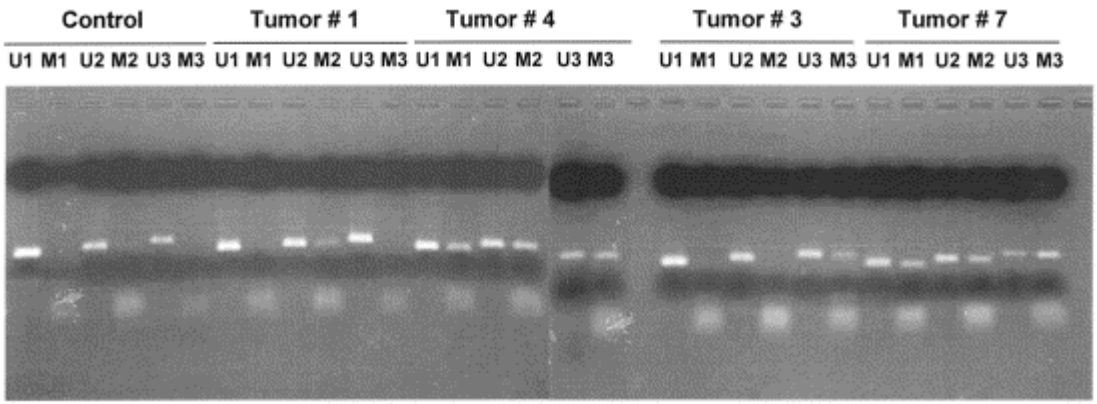
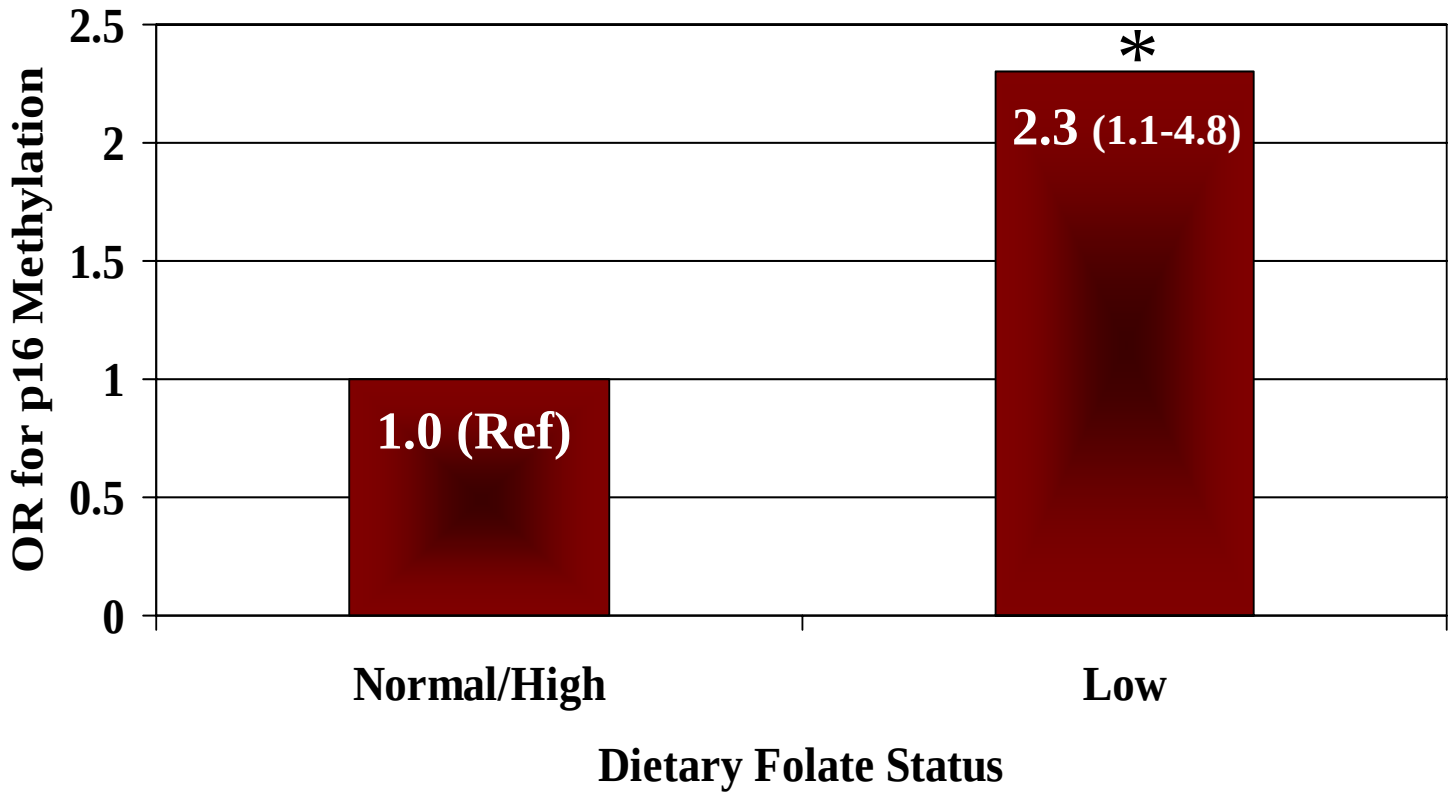
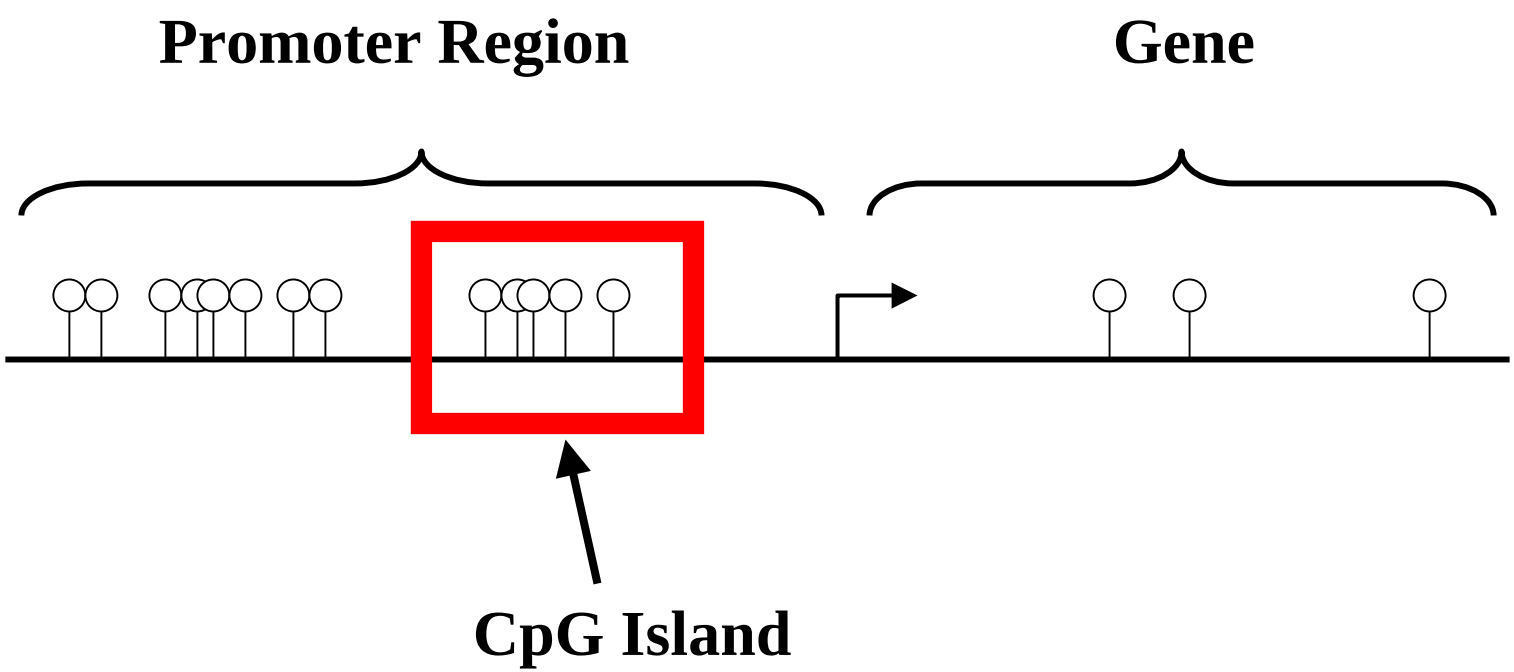


Fig. 2. MSP analysis of *p16* methylation in control livers and liver tumors induced by folate/methyl deficient diet. Bisulfite modified DNA was amplified with two sets of primers specific to unmethylated (U) and methylated (M) cytosine residues. Each set contains one common sense primer, which was paired with one of three different antisense primers, 1, 2, or 3 to examine methylation status of CpG sites within exon 1 of *p16* gene

Low Dietary Folate is Associated with p16*INK4A* Hypermethylation



Where epigenetic silencing happens!

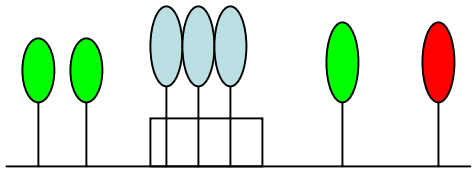


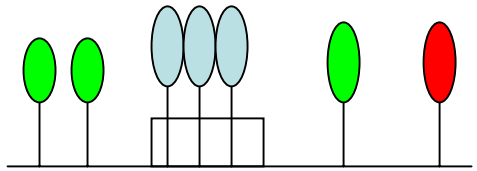
= CpG

CpG dinucleotides are under-represented in the genome, but over-represented in promoter regions

DNA Methylation in Cancer

- Aberrant
  - Occurs in promoters of tumor suppressors
- Tumor specific & Clonal
- Silences transcription of a gene – equivalent to mutation or deletion
- Alternative “hit” to inactivation of tumor suppressor
- Targeting and specificity unclear
  - Not a “global” phenomenon
  - Carcinogens driving this alteration?



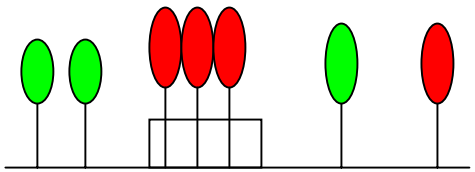


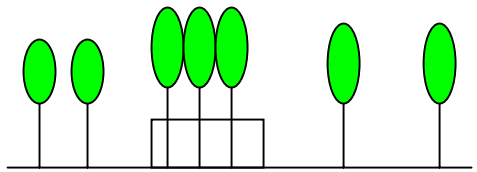
HYPERMETHYLATION

(SILENCE TSGs)

HYPOMETHYLATION

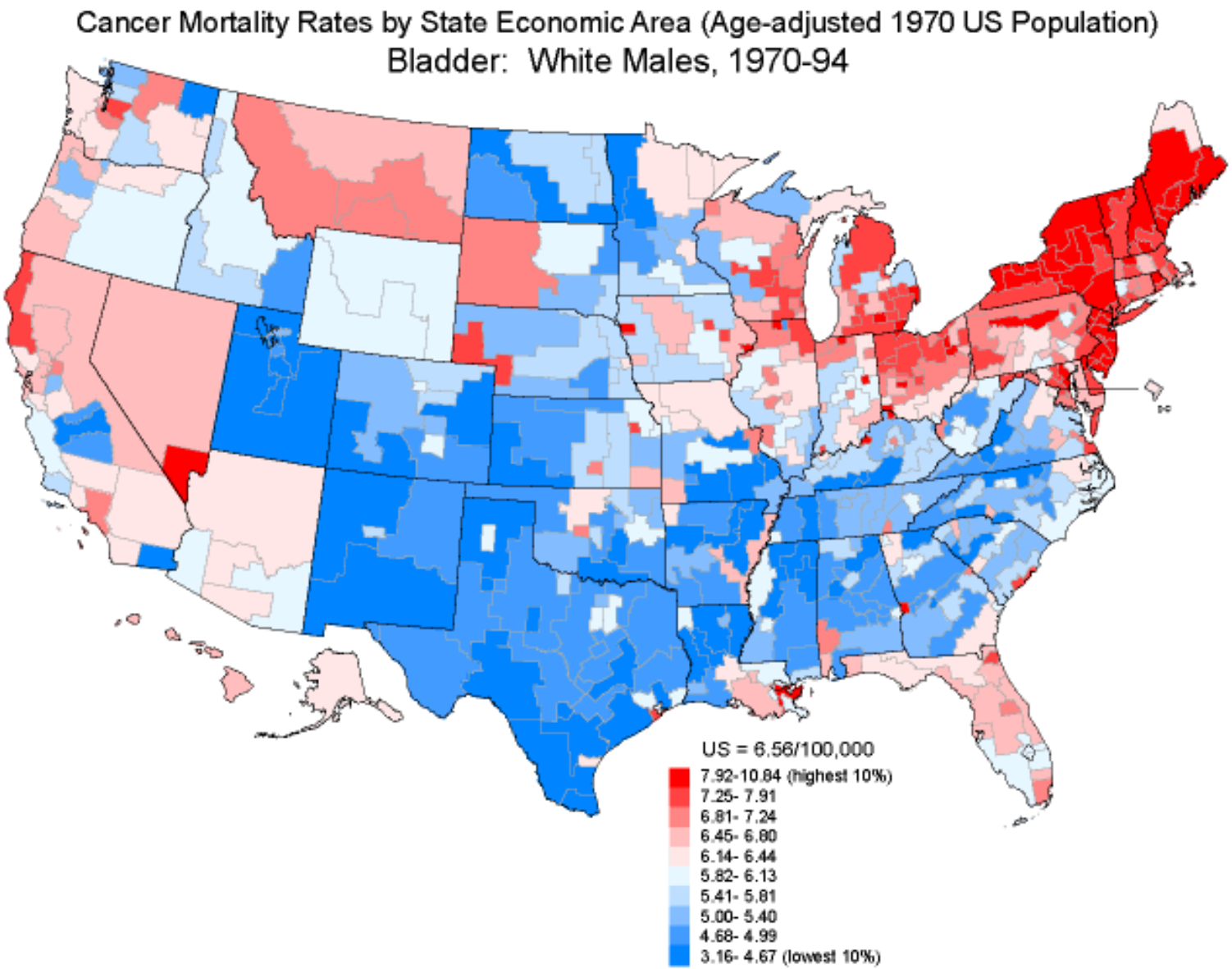
(GENOMIC INSTABILITY)





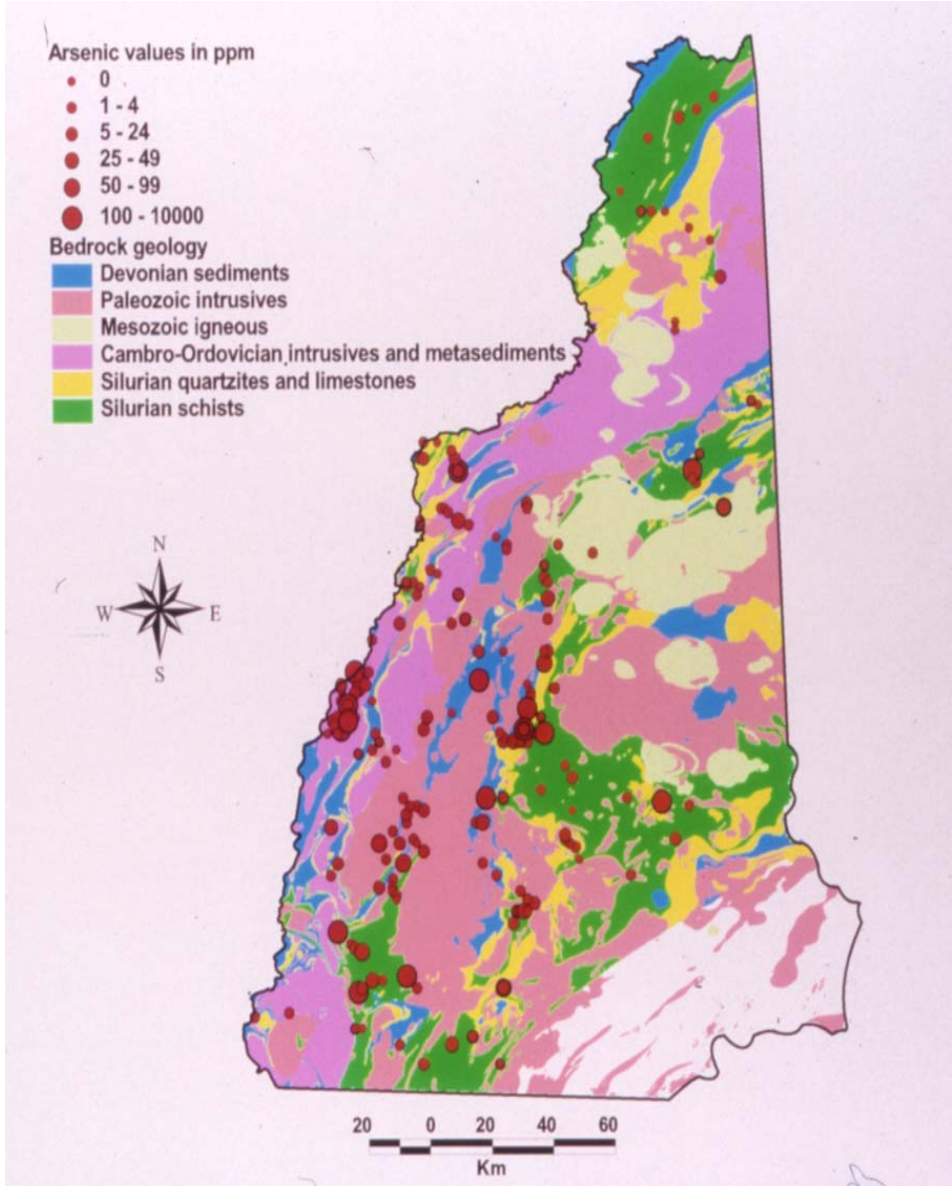
STOP

START



from: Devesa S et al. *Atlas of Cancer Mortality for U.S. Counties: 1950-94*. Vol DHEW Publ. No. (NIH) 99-4564; 1999

New Hampshire



Bladder Cancer Case-Control Study

P.I. – Margaret Karagas

Dartmouth SBRP

NH residents 25-74 years

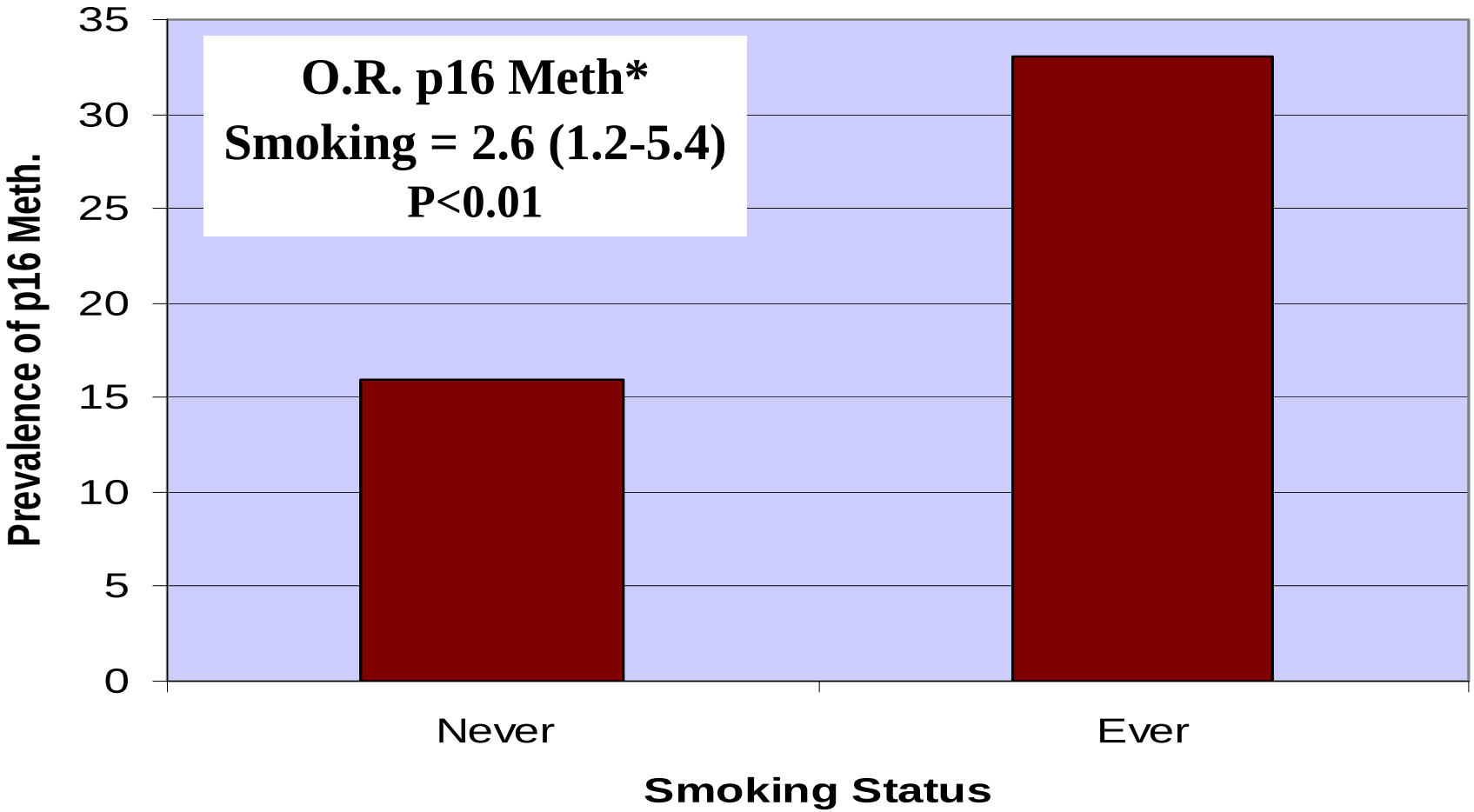
– Cancer Cases:

- *bladder cancer* *n~450/850+*

Smoking and Arsenic Exposure are Major Risk Factors

p16 Methylation is More Prevalent in Smokers

case-only analysis



*controlled for age, sex

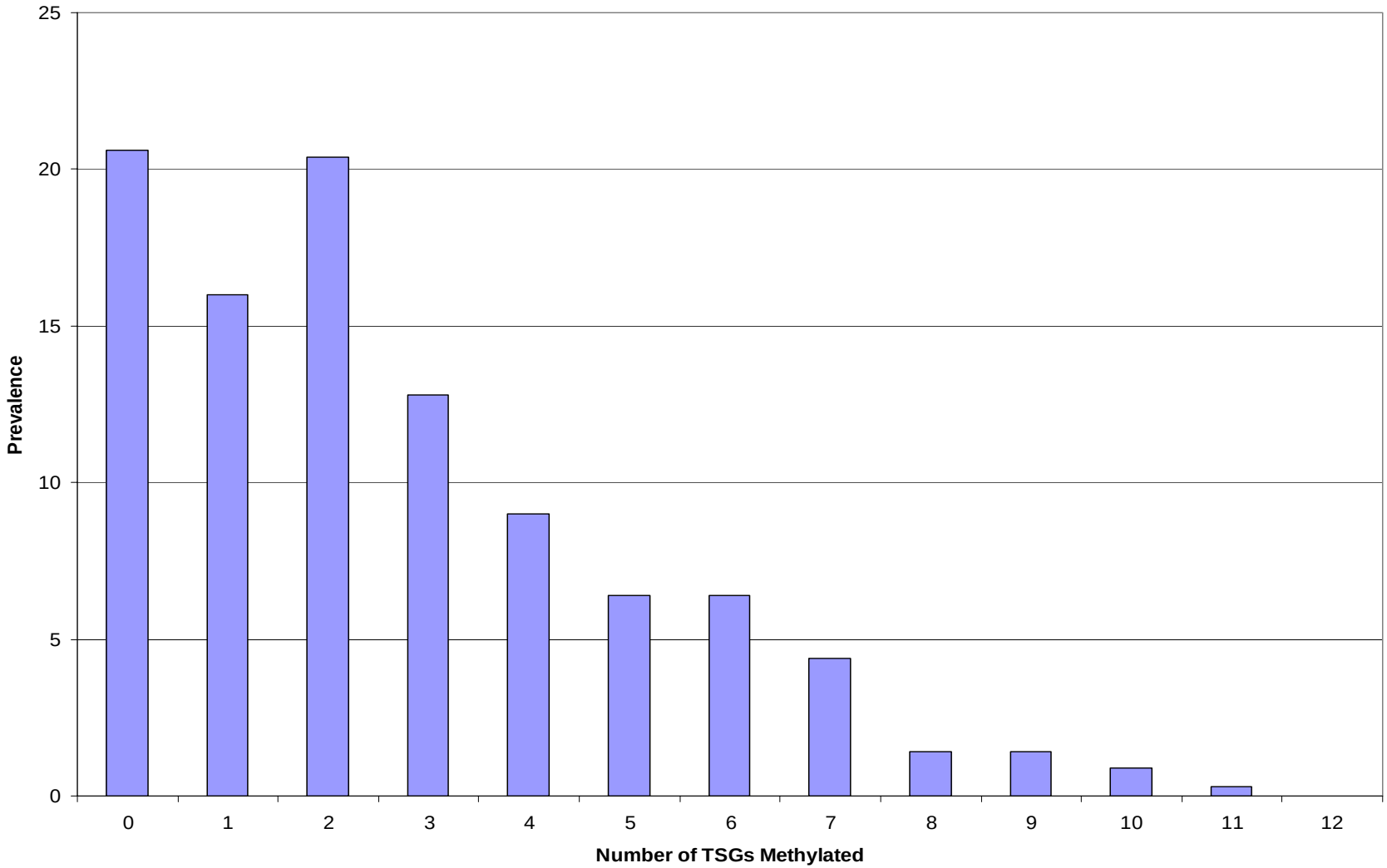
PRSS3 and *RASSF1A* Methylation is Associated with Arsenic Exposure

case-only analysis

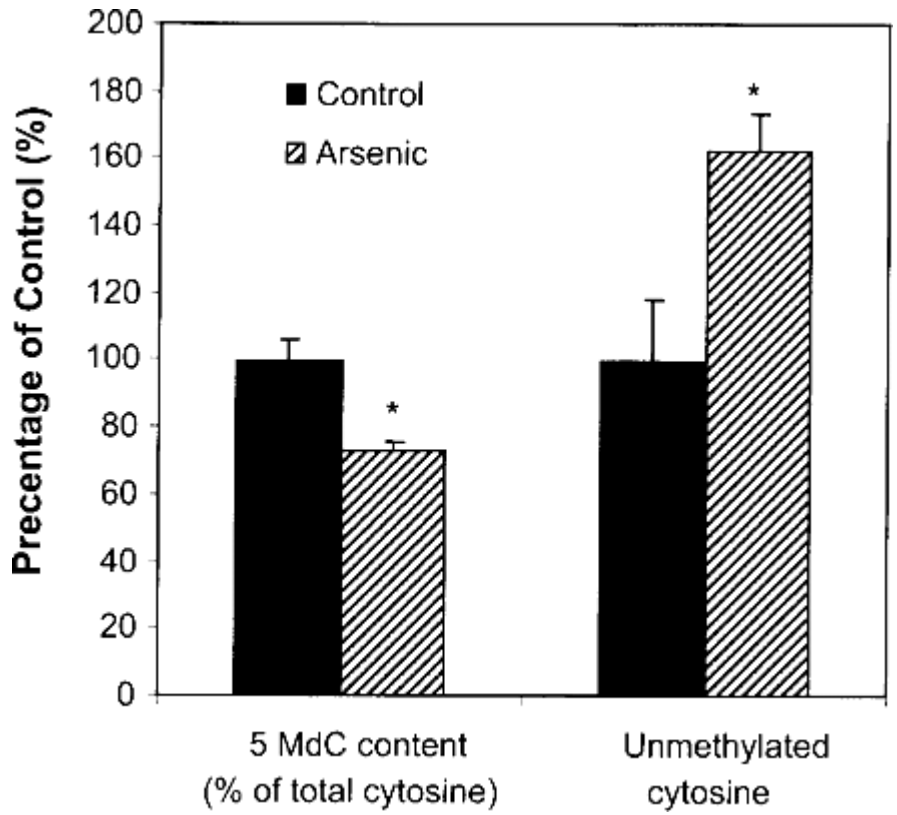
Co-variate	N (351)	***PRSS3* Methylation** OR (95% CI)	*P*	***RASSF1A* Methylation** OR (95% CI)	*P*
Smoking Status					
Never smoker	55	1.0 (ref)		1.0 (ref)	
Former, Quit <10 yrs	42	0.7 (0.3-1.8)	0.5	0.6 (0.3-1.5)	0.3
Former, Quit 10+yrs	131	1.0 (0.5-2.1)	1.0	0.5 (0.2-1.0)	0.06
Current Smoker	117	1.4 (0.7-2.9)	0.4	0.5 (0.3-1.1)	0.1
Toenail Arsenic					
<0.26 μg/g	318	1.0 (ref)		1.0 (ref)	
0.26+ μg/g	18	2.8 (1.0-7.6)	0.05	3.5 (1.2-10.0)	0.02

Note: Model is controlled for age, gender, tumor stage, TP53 IHC staining intensity, and both exposures in the table. Missing values were coded as missing and included in the model.

Distribution of TSG Methylation in Bladder Cancer



Arsenic Exposure is Associated with Global Hypomethylation in Animals



Chen et al., *Carcinogenesis*. 2004 Sep;25(9):1779-86

Are *Targeted Hypermethylation* and *Global Hypomethylation* Related?

- **Present in virtually all tumors**
- **SAM depletion (folate deficiency) cause:**
  - **Chromosomal fragility & Genomic instability**
  - **expression of normally repressed DNA repetitive elements and introns.**
  - **Are these sites of repressed small RNAs?**
- **In plants, epigenetic silencing is targeted by small RNAs.**

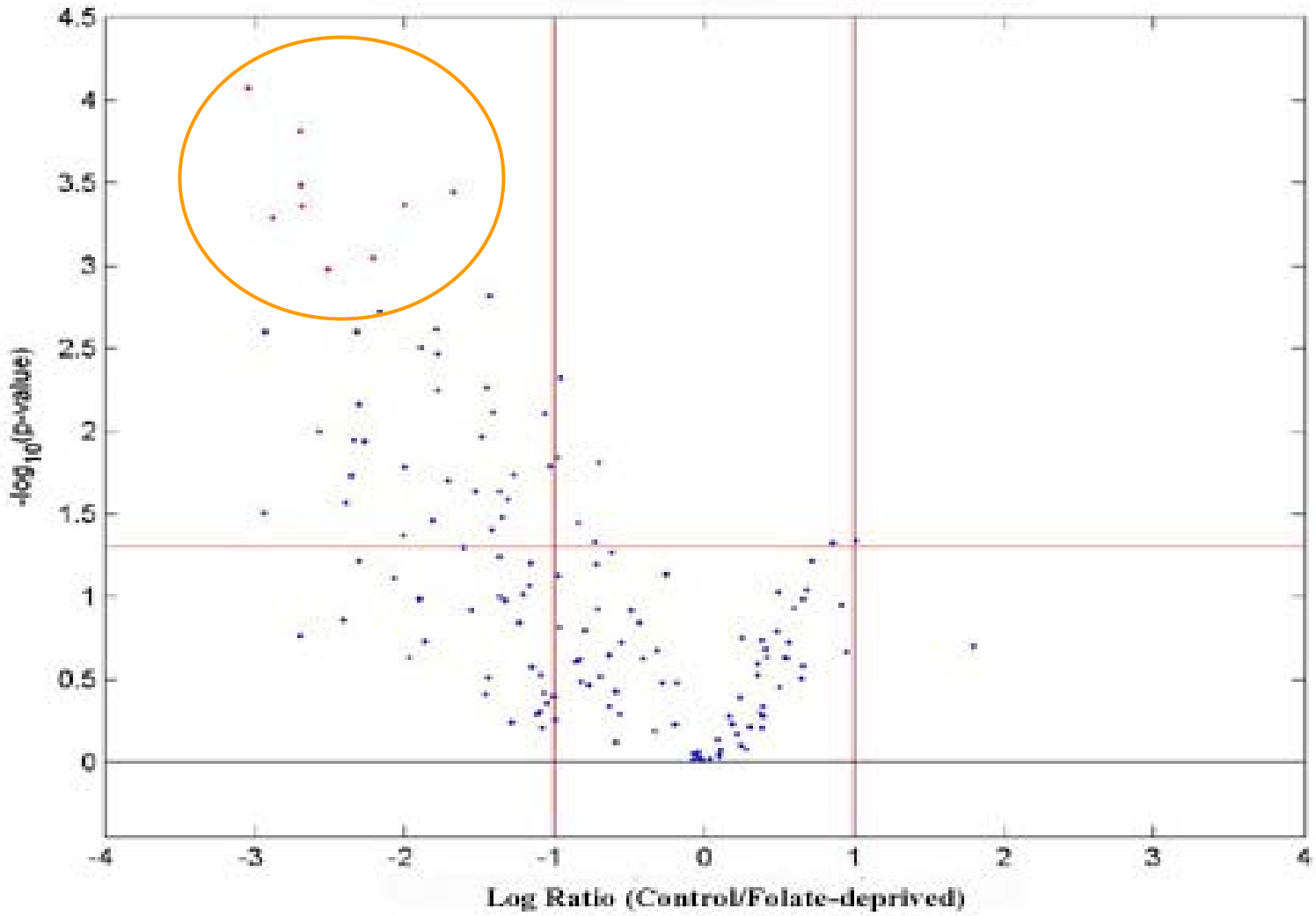
MicroRNAs and Epigenetic Silencing

- **Known to target methylation silencing in plants**
- **Altered expression in tumors**
- **Approximately 400 known to exist in humans**
- **Known to alter protein/gene expression in mammalian cells**
  - **Binds mRNA, likely influences DNA methylation perhaps in tandem with siRNA**

Does hypomethylation alter the human cell microRNA profile?

- **Is folate deficiency associated with an altered pattern of miRNA?**
  - List of miRNAs is incomplete and growing
- **Human TK6 lymphoblasts grown in folate-deficient media for 6 days**
  - **Cells were hypomethylated**
  - **Spotted arrays used to interrogate miRNA**

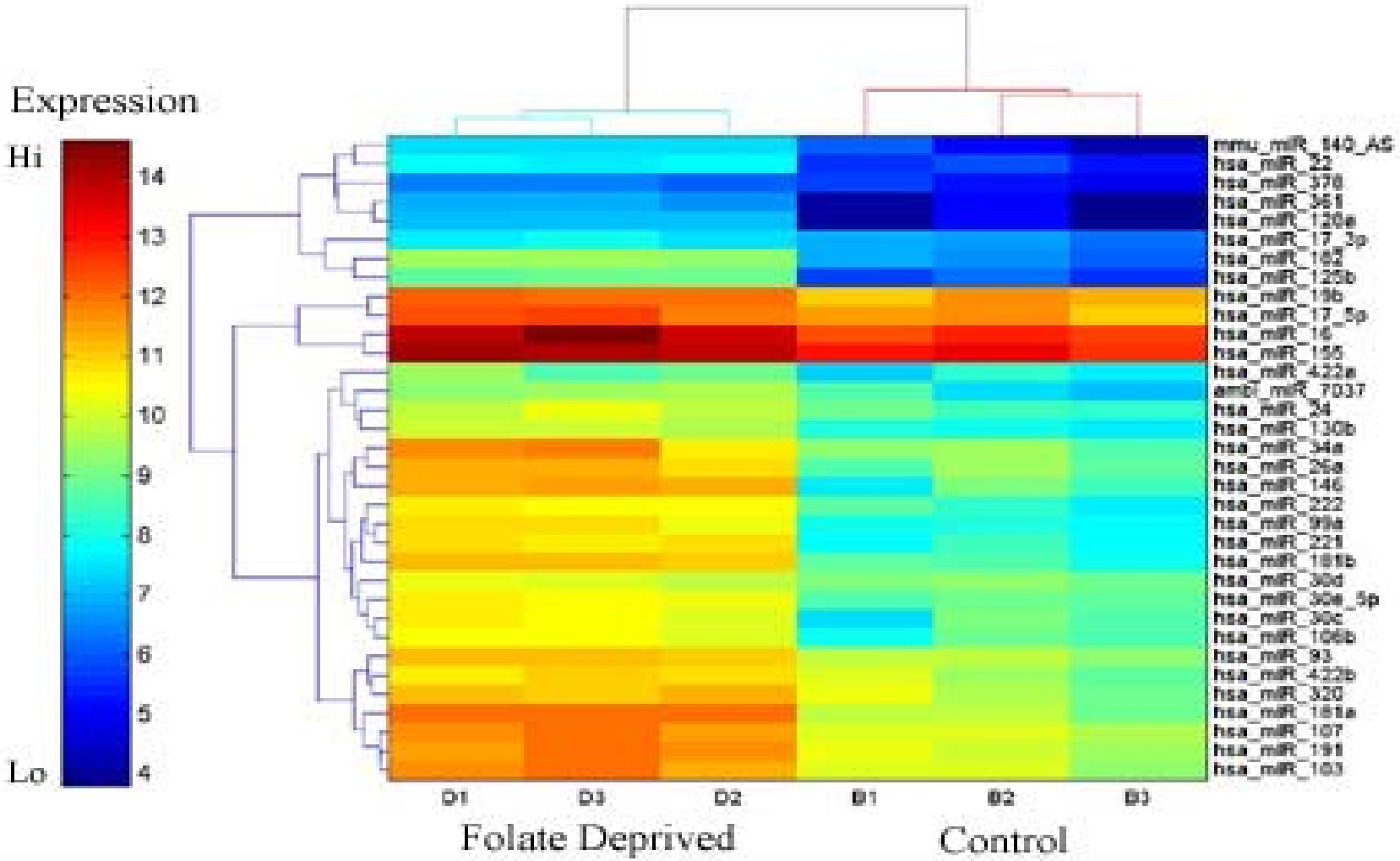
Volcano Plot



Expressed in Folate-deprived

Expressed in Control

Pairwise Cluster of miRNAs Significantly Altered by Folate Deficiency



Future Studies

- **Examine hypo/hypermethylation in tumors *and* in peripheral blood**
  - *Assoc with diet?*
  - *Greater in Arsenic exposed?*
  - *Assoc with gene-specific hypermethylation?*
- **In-vitro Studies to examine the mechanism**
  - *Targeting – specific microRNA arrays in arsenic exposed cells*

Acknowledgements

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  (Env. Biostats Core)

Dartmouth SBRP

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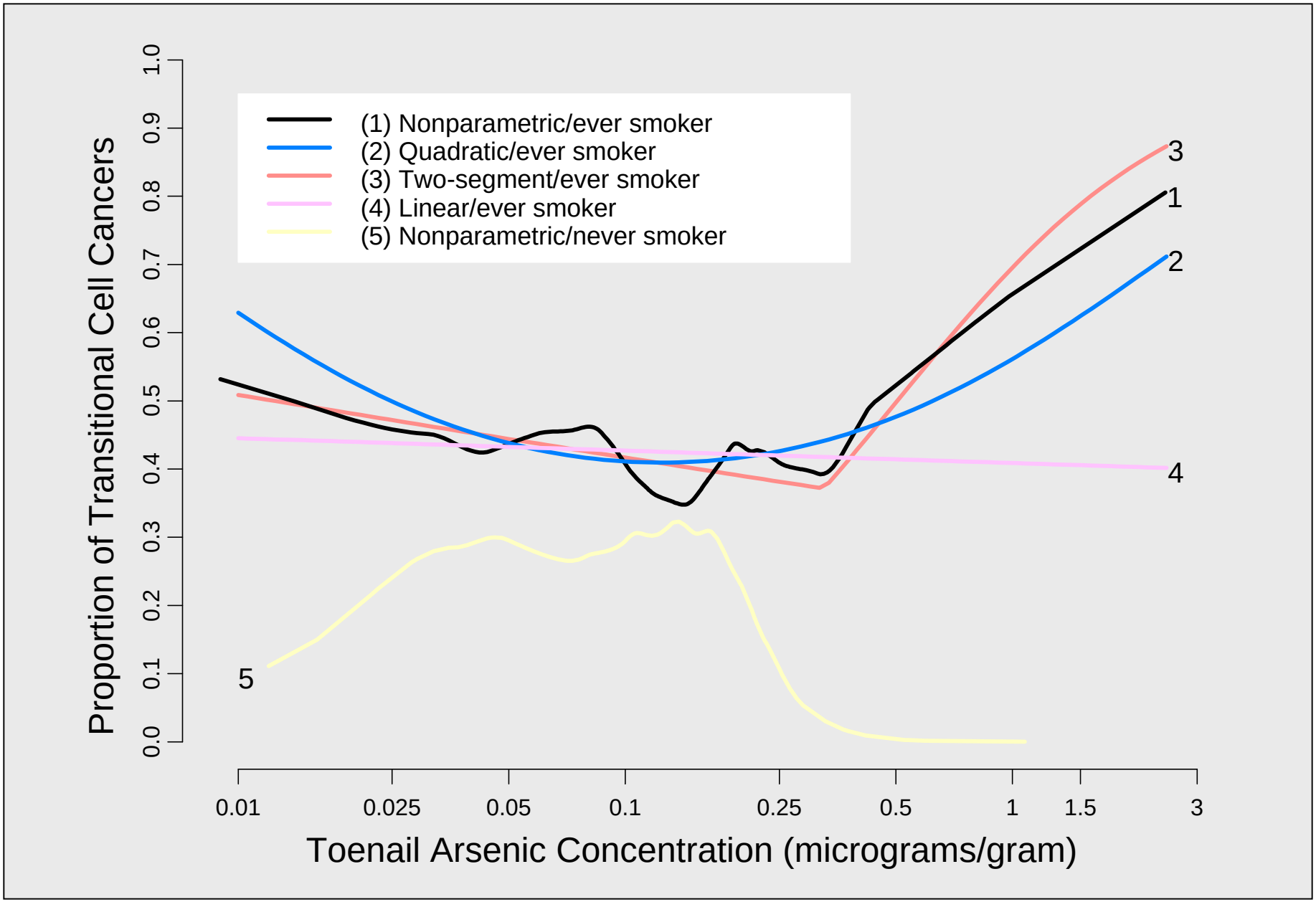
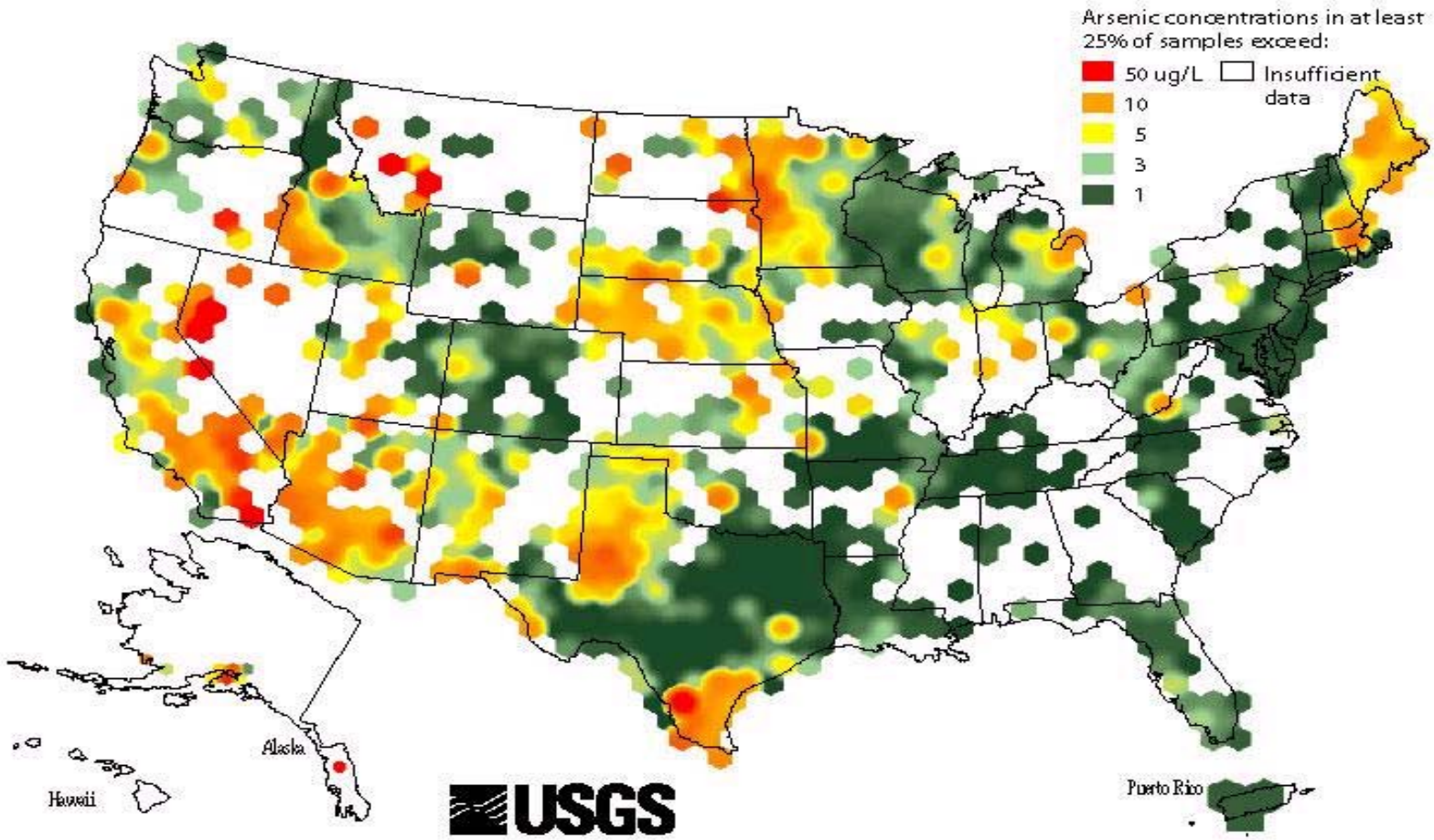
- Margaret Karagas


(1) Nonparametric/ever smoker
(2) Quadratic/ever smoker
(3) Two-segment/ever smoker
(4) Linear/ever smoker
(5) Nonparametric/never smoker
Proportion of Transitional Cell Cancers
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.01
0.025
0.05
0.1
0.25
0.5
1
1.5
3
Toenail Arsenic Concentration (micrograms/gram)
1
2
3
4
5

Arsenic in drinking water



Gene Promoters Studied

- RASSF1A
- p16
- SFRP1
- SFRP2
- SFRP4
- SFRP5
- PRSS3

Questions

- Does carcinogen exposure induce methylation?
- Are genes coordinately silenced?
  - What is the distribution of methylation silencing?
- Are all tumors the same?
- Does this cluster?

Methylation Prevalence

N=355

- SFRP2 52%
- SFRP5 37%
- PRSS3 33%
- RASSF1A 32%
- P16 31%
- SFRP1 18%
- SFRP4 9%

Methods

- DNA extraction
  - De-crosslinking and extraction from 450 formalin-fixed embedded tumor samples
- Bisulfite modification
  - Converts unmeth. C → U
  - Meth. C → C
- Methylation-specific PCR
  - Amplifies Methylated DNA specifically & sensitively
  - Primers specific to methylated sequence
  - Control for DNA presence using modified actin primers
- Analysis
  - Use unconditional logistic regression to
    - examine associations between methylation and predictors of methylation (odds of being methylated)
    - Examine associations between methylation and characteristics of tumors (odds of tumor have a characteristic)

Categorical Analysis: Odds Ratios for Transitional Cell Cancers

	Percentile of Toenail Arsenic Concentration*						
	≤25	**25.1-50**	**50.1-75**	**75.1-90**	**90.1-95**	**95.1-97**	**97.1-100**
All	1.0	1.4 (1.0, 2.0)	1.1 (0.7, 1.6)	1.0 (0.7, 1.6)	1.3 (0.7, 2.5)	0.4 0.1, 1.5)	1.4 (0.6, 2.9)
Never smokers	1.0	0.9 (0.4, 1.9)	1.2 (0.5, 2.7)	1.1 (0.4, 2.9)	0.5 (0.1, 2.1)	--	--
Ever smokers	1.0	1.5 (1.0, 2.3)	1.0 (0.7, 1.6)	1.0 (0.7, 1.6)	1.8 (0.6, 1.7)	0.5 (0.9, 3.7)	2.2 (0.9, 5.1)

* Based on control group distribution

Drinking Water in New Hampshire

	Type of	Water	
Arsenic concentration	**Private (40%)**	**Public (60%)**	**Total (100%)**
undetectable	<1%	<1%	<1%
≤1 μg/L	64%	91%	80%
1.1 – 10 μg/L	23%	7%	14%
10.1 – 50 μg/L	**10%**	**1%**	**5%**
>50 μg/L	**3%**	**0%**	**1%**