Importance of drug metabolizing enzymes in Toxicology:

some examples with chemical carcinogens

David L. Eaton, Ph.D.
Director, Center for Ecogenetics & Environmental Health
University of Washington
Fundamental concepts

• Drugs are nothing more than toxic chemicals that happen to have beneficial effects at the proper dose
• Everything is toxic -- ‘The dose makes the poison’
• The body doesn’t recognize the difference between chemicals that are drugs and chemicals that are not
• Therefore, the same drug metabolizing enzymes are responsible for the elimination of both drug and non-drug chemicals
DMEs and Evolution

• Animal models are of great value in both pharmacology and toxicology studies
• However, DMEs are subject to strong evolutionary pressures
  – Have evolved in part to ‘protect’ an organism from toxic substances in their environment
  – Probably heavily influenced by diet
  – Diets/environment of rats and mice are quite different than humans over evolutionary time scale
• Thus, the same genes (e.g., CYPs, GSTs) may have similar sequence, but potentially large functional differences
Basic aspects of chemical carcinogenesis

- Over 90% of known chemical carcinogens are ‘procarcinogens’
  - The ratio of ‘activation’ to ‘detoxification’ is more important than the absolute rate of either

- Mutagenesis is essential to ‘initiation’, but is likely involved at all stages
  - It is likely that ‘misrepair’, rather than persistent adducts, contributes most to initiation by adducting chemicals

- ‘promotion’ is strongly tied to enhanced cell proliferation – but not synonymous
Basic aspects of chemical carcinogenesis (2)

- Promotion / progression can occur by
  - Inhibition of apoptosis
  - Mitogenesis (enhanced cell proliferation)
  - Angiogenesis
  - Genomic instability / ‘mutator phenotype’
    » Loss of function of cell cycle checkpoints
    » Loss of fidelity of DNA repair

- ‘Classical’ chemical carcinogenesis
  - Initiation is irreversible (‘fixed’ mutation in oncogene)
  - Promotion is reversible, and requires continued presence of promoting agent
Basic aspects of chemical carcinogenesis

• **Genotoxic vs. ‘epigenetic’**
  – initiation vs. promotion vs. progression

• **Direct-acting**
  – only about 5% of carcinogens
  – some alkylating agents (drugs)

• **Indirect-acting** - require biotransformation
  – Most carcinogens are ‘pro-carcinogens’
  – biotransformation enzymes are involved in both activation and detoxification
  – subtle differences can make large differences in species and interindividual susceptibility
Types of carcinogens

• Reactive Oxygen / Nitrogen species
  - $\text{O}_2^-$, $\text{H}_2\text{O}_2$, hydroxyl radical, $\text{NO}^*$, $\text{ONOO}^- \times 10^5/\text{cell/day}$
  - Endogenous sources
    » Mitochondria:
    » Cytochrome P450
    » Inflammatory cells / macrophages
  - Exogenous sources
    » Metals – Fenton chemistry (produces $\cdot$ OH)
    » Redox cycling compounds
  - Antioxidants afford protection
    » GSH, TRX, GRX NADP CoE-Q; Vit E, C, $\beta$-carotine

• DNA Adduct formation
  - Alkylation reactions
  - Bulky adducts
Common gene families involved in carcinogen biotransformation

- PHASE I (Oxidation)
  - Cytochromes P450 (CYPs)
  - Flavin-dependent monooxygenases

- PHASE II (Conjugation & Hydrolysis)
  - Glutathione S-Transferases
  - N-acetyltransferases
  - Quinone Reductase
  - Epoxide hydrolases
Four examples of how DMEs determine species and interindividual differences in toxicity:

chemical carcinogenesis

- Aflatoxin B1
- Benzene
- Polyaromatic Hydrocarbons (PAHs)
Background - Aflatoxin B1

- A ‘natural’, potent hepatocarcinogen
- Produced by mold *Aspergillus flavus*
  - Exposure is almost totally via diet
  - Found in contaminated foodstuffs: corn, peanut etc.
- Contributes to liver cancer incidence
  - in China, SE Asia, Africa
  - In combination with hepatitis B virus
Mycotoxins: Structure of Aflatoxins

AFB₁

AFB₂

AFG₁

AFG₂
AFQ, AFM, AFP (detoxification)
Genetic Sensitivity to Aflatoxin B1: animal models

- Very potent liver carcinogen in rats
  - 15 ppb in diet produced high level of liver cancer
- Not a liver carcinogen in mice
  - 100,000 ppb caused no liver cancer
- Difference in susceptibility due to differences in biotransformation
  - Mice activate AFB to AFBO equally well as rats
  - Mice constitutively express a form of glutathione S-transferase, mGSTA3, that efficiently detoxifies AFBO
- Relevance to humans?
## GST activity toward AFB-8,9-oxide

<table>
<thead>
<tr>
<th>GST Enzyme</th>
<th>CDNB Activity (µmol/min/mg)</th>
<th>AFBO Activity (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse GSTA3-3</td>
<td>12</td>
<td>192</td>
</tr>
<tr>
<td>Rat GSTA3-3</td>
<td>16</td>
<td>0.3</td>
</tr>
<tr>
<td>Rat GSTA5-5</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>Human GSTA1-1</td>
<td>54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Human GSTM1a-1a</td>
<td>110</td>
<td>.09</td>
</tr>
</tbody>
</table>

From: Buetler et al., Cancer Res. 1997.
Human Susceptibility to AFB

• Human CYPs (1A2, 3A4) roughly comparable to rodent CYPs in rate of formation of AFBO
  – Are genetic polymorphisms in CYP1A2 and/or 3A4 important determinants of AFB sensitivity?

• Are polymorphic Human GSTs important?

• Is Human microsomal epoxide hydrolase important?
Molecular Epidemiology of AFB and liver cancer: genetic sensitivity?

- AFB alone increases liver cancer risk (~3x) (1)
- When combined with Hep. B virus, 60x (1)
- Does GSTM1 homozygous null genotype increase risk?
- Does mEH variant increase risk?

1. Qian et al., Cancer Epi. Biomark. Prev. 3: 3-10, 1994
## GST and AFB Sensitivity

McGlynn et al., PNAS 92: 2384-2387, 1995

<table>
<thead>
<tr>
<th></th>
<th>GSTM1 null</th>
<th>GSTM1 +</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>29 (56%)</td>
<td>23 (44%)</td>
<td>52 (100%)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>47 (41%)</td>
<td>69 (59%)</td>
<td>116 (100%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>76</td>
<td>92 (54%)</td>
<td>168</td>
</tr>
</tbody>
</table>

OR (95% CI) = 1.9 (0.94-3.63)
### GST and AFB Sensitivity


<table>
<thead>
<tr>
<th>Serum AFB-alb</th>
<th>GSTM1 Null Case</th>
<th>Control</th>
<th>Adj OR</th>
<th>GSTM1 Non-Null Case</th>
<th>Control</th>
<th>Adj OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetect.</td>
<td>5</td>
<td>27</td>
<td>1 (ref)</td>
<td>9</td>
<td>17</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>14</td>
<td>4.1 (1-17)</td>
<td>4</td>
<td>10</td>
<td>.7 (.2-3.2)</td>
</tr>
<tr>
<td>High</td>
<td>4</td>
<td>2</td>
<td>12.4 (1.7-93)</td>
<td>2</td>
<td>3</td>
<td>1.4 (.2-11)</td>
</tr>
</tbody>
</table>

All cases and controls were HBsAg+
mEH and AFB Sensitivity

McGlynn et al., PNAS 92: 2384-2387, 1995

<table>
<thead>
<tr>
<th></th>
<th>mEH 1/1</th>
<th>mEH1/2m</th>
<th>EH 2/2</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>5 (10%)</td>
<td>18 (35%)</td>
<td>29 (56%)</td>
<td>52 (100%)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>30 (26%)</td>
<td>46 (40%)</td>
<td>40 (34%)</td>
<td>116 (100%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>35</td>
<td>64</td>
<td>69</td>
<td>168</td>
</tr>
</tbody>
</table>

OR (to 1/1) 2.3 4.3
OR (1/2+2/2) 3.3 (1.2-9.2)

mEH effect was more pronounced in HBsAg+ subjects:
OR of 15 for mEHwt/ HBsAg+ vs. mEH wt, HBsAg-
OR of 77 for mEHvar/HBsAg+ vs. mEH wt, HBsAg-

*But very small sample size*
CYP1A2 mediated AFB mutagenicity, ± mEH

From: Kelly et al., Toxicol. Sci., 65:35-42, 2002
Attenuation of AFB-DNA Adduct Formation by mEH

From: Kelly et al., Toxicol. Sci., 65:35-42, 2002
Relevance of enzyme ‘induction’: Diet and other environmental factors can shift the balance of activation to detoxification

Many, but not all, xenobiotic metabolizing enzymes are inducible, and PMs in regulatory regions might alter differential induction
Can diet modify AFB biotransformation in human liver?

• Dietary/chemo-intervention in rats
• Intervention trial with Oltipraz in China
  – Induction of GSTA vs GSTM1
  – Inhibition (or induction?) of CYP1A2?
• Cruciferous vegetables - glucosinolates
  – Indole 3-carbinol --> diindolylmethane (dioxin like)
    » Induces CYP1A1 and 1A2
  – Isothiocyanates: sulforaphane (SFN), PEITC
    » Activates the Keap1/Nrf2/ARE pathway
• Rat vs. human?
  – No ARE in human GSTA1
• Use human hepatocytes, phytochemicals
Keap1/NRF2/ARE Pathway


- Homeostatic conditions:
  - Keap1 binds Nrf2
  - Cullin 3 (Cul3) with E2 and Ub

- Rapid degradation of Nrf2 by the Ubiquitin-Proteasome System:
  - Oxidants or Electrophiles activate Keap1
  - Nrf2 ubiquitinated and degraded by proteasome

- Accumulation of Stabilized Nrf2 by Oxidants & Nuclear Translocation of Nrf2:
  - Oxidant-Cys, Oxidant-Oxidant-Cys
  - Nrf2 in the nucleus
Effects of phytochemicals on AFB-DNA adduct formation in human hepatocytes
General Effects of SFN, PEITC, DIM on Global Gene Expression

- Differential expression (>2x, and p<.05):
  - SFN: 1,538 genes
  - PEITC: 363 genes
  - DIM: 281 genes

- SFN increased: AHR, GCLC/M, NQ01, thioredoxin reductase 1; decreased: CYPs 2D6, 3A7, 3A4; GSTs T1, Z1; FMO 1, 5.

- DIM increased: CYPs 1A1, 1A2, 1B1, 2B6, GSTA3, glutathione peroxidase 2, FMO 4.

- PEITC increased: CYPs 2B6, 2E1, 2C8, 3A7, GSTA3 and GCLC.
Effects of SFN mRNA levels for AFB detoxification pathways

- **GSTM1**: ctri 1.0, 100 0.8, 500 0.6, 1000 0.5
- **GSTT1**: ctri 1.0, 100 1.2, 500 0.7, 1000 0.5
- **mEH**: ctri 1.0, 100 1.4, 500 2.0, 1000 2.2
- **AFAR**: ctri 1.0, 100 0.5, 500 0.5, 1000 0.9
Effects of GSTM1 Genotype on AFB-DNA Adducts

![Bar chart showing effects of GSTM1 genotype on AFB-DNA adducts.]

- **GSTM1 Null**
- **GSTM1 +**

**AFB-DNA Adducts (per 10^7 NT)**

- DMSO
- SFN
Effects of DIM and SFN on CYP Gene Expression

Bar charts showing the effects of DIM and SFN on the expression of CYP3A4, CYP3A5, CYP1A1, and CYP1A2. The x-axis represents the concentration of DIM and SFN in [µM], ranging from 0 to 50, with control (ctrl) at 1.0. The y-axis for CYP3A4 and CYP3A5 is linear, while the y-axis for CYP1A1 and CYP1A2 is logarithmic.

- **CYP3A4**: No significant changes are observed with DIM or SFN treatment.
- **CYP3A5**: No significant changes are observed with DIM or SFN treatment.
- **CYP1A1**: Treatment with DIM at 50 [µM] and SFN at 50 [µM] results in a significant increase in expression, indicated by **,** with expressions of 113 and 625, respectively.
- **CYP1A2**: Treatment with DIM at 10 [µM] results in a significant increase in expression, indicated by **,** with an expression of 25.
Effects of SFN on CYP3A4 mRNA in human hepatocytes

(Quantitative real time RT-PCR)
How does SFN down-regulate CYP3A4 gene?
How is CYP3A4 expression regulated?

- **Constitutive Androstan Receptor (CAR)**
  - Important in constitutive expression, and some inducible
  - Phenobarbital activates

- **Glucocorticoid Receptor**
  - Corticosterone and other glucocorticoids

- **Vitamin D Receptor**
  - Dihydroxy Vitamin D

- **Steroid and Xenobiotic Receptor (SXR)**
  - Also called ‘PXR’ – rodent different than human
  - Many drugs act as activating ligands
    - Rifampicin
    - RU 486
    - Numerous antiretroviral drugs
    - Some cancer chemotherapeutic agents
  - Induction of CYP3A4 is a major problem – drug interactions
Effects of SFN on SXR-mediated CYP3A4 induction in LS180 cells

CYP3A4 mRNA expression

RIF
- - + + + - - - - -
RU486
- - - - - - + + + +
SFN (µM)
- - 1 10 25 - 1 10 25
CYP3A4 Reporter Promoter with SXR expression in HepG2

HepG2 cells were transfected with a CYP3A4 promoter linked to a luciferase reporter and CMX-β-galactosidase transfection control plasmid in the absence or presence of a human SXR expression construct. (A) After transfection, cells were treated with control or 10 μM RIF or RU486 for 24 hrs with or without SFN at indicated concentrations. (B) After transfection, cells were treated with 10 μM RIF and SFN at indicated concentrations.
Effects of SFN on ligand binding to SXR binding site

His6-SXR LBD was co-expressed with the SRC-1 receptor interaction domain and purified. The receptor complex was bound to nickel chelate Flash-Plates and incubated with 50 nM of 3H-SR12813 in the presence of indicated concentration of SFN or clotrimazole.
Ecogenetics of Benzene Poisoning: CYP2E1 and NQO1
Rothman et al., Cancer Res. 57: 2839-42, 1997

- Nested case-control study
  - 11,177 workers employed in benzene occupations, Shanghai, China (subset of retrospective cohort study)
  - Benzene-exposed workers periodically screened for BP
  - BP met strict diagnostic criteria (e.g., repeated leuko-penia; documented Benzene exposures > 6 mo, etc.)

- 50 cases, 50 matched controls
- Phenotyped and genotyped for CYP2E1
- Genotyped for NQO1
Functional Significance of PMs

- **NQO1*2** is a cSNP → C to T change at position 609 → a pro to ser change at aa position 187.
  - The mutant NQO1*2 protein is rapidly degraded → lack of NQO1 protein in NQO1*2/*2 genotype.

- **CYP2E1 genotype**: RFLP PM- *Rsal*
  - 5’ flanking region
  - Little effect on expression in vitro

  » CYP2E1 phenotype
  - Measures fractional excretion of chlorzoxazone
## Benzene Poisoning Risk
### Significance of CYP2E1 and NQO1 PMs Individually

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>adjOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype</td>
<td><strong>Cases</strong></td>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td>CYP2E1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c2c2</td>
<td>5 (10%)</td>
<td>8 (17%)</td>
<td>0.6 (0.2-1.9)</td>
</tr>
<tr>
<td>c1c1/c1c2</td>
<td>43 (90%)</td>
<td>40 (83%)</td>
<td>1.0</td>
</tr>
<tr>
<td>rapid</td>
<td>34 (71%)</td>
<td>23 (48%)</td>
<td>2.5 (1.1-6.0)</td>
</tr>
<tr>
<td>slow</td>
<td>14 (29%)</td>
<td>25 (52%)</td>
<td>1.0</td>
</tr>
<tr>
<td>NQO1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- -</td>
<td>20 (41%)</td>
<td>11 (25%)</td>
<td>2.6 (1.1-6)</td>
</tr>
<tr>
<td>++/+ -</td>
<td>29 (59%)</td>
<td>37 (77%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

From: Rothman et al., Cancer Res. 57: 2839-42, 1997
## Benzene Poisoning Risk

**Significance of CYP2E1 and NQO1 PMs Combined**

<table>
<thead>
<tr>
<th>CYP2E1 Phenotype</th>
<th>NQO1 Genotype</th>
<th>OR adj (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>++ / +-</td>
<td>1.0</td>
</tr>
<tr>
<td>Slow</td>
<td>- -</td>
<td>2.7 (0.6-11.8)</td>
</tr>
<tr>
<td>Rapid</td>
<td>++ / +-</td>
<td>2.7 (0.9-8.0)</td>
</tr>
<tr>
<td>Rapid</td>
<td>- -</td>
<td>7.8 (1.9-32.5)</td>
</tr>
</tbody>
</table>

From: Rothman et al., Cancer Res. 57: 2839-42, 1997
Benzene and Hematotoxicity
Lan et al., *Science*, Nov. 2004

- 250 workers – exposure assessment
  
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>&lt; 1 ppm</th>
<th>1 – 10 ppm</th>
<th>&gt; 10 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6480</td>
<td>5540</td>
<td>5660</td>
<td>4770</td>
</tr>
<tr>
<td>Urine</td>
<td>0.38 (1.24)</td>
<td>13.4 (18.3)</td>
<td>86 (130)</td>
<td>847 (1250)</td>
</tr>
</tbody>
</table>

- Multiple measures of hematotoxicity
  
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>6480</td>
<td>5540</td>
<td>5660</td>
<td>4770</td>
</tr>
<tr>
<td>Gran</td>
<td>4110</td>
<td>3360</td>
<td>3480</td>
<td>2790</td>
</tr>
<tr>
<td>B cells</td>
<td>218</td>
<td>186</td>
<td>170</td>
<td>140</td>
</tr>
</tbody>
</table>
• CYP2E1 genotype – no effect
• MPO genotype and NQ01 variants associated with ‘increased risk’, based on WBC count
  - At < 1ppm:
    » No risk genotype:  5980 (1420)
    » Either ‘at risk’ genotype:  5480 (1120)
    » Both ‘at risk’ genotypes:  4900 (1240)
    » No effect of genotype in non-exposed
miRNA regulation of CYP2E1

- CYP2E1 mRNA isoform is highly abundant in the liver
- In human liver protein expression of CYP2E1 often disassociated from mRNA levels.
- The lack of correlation attributed to the translational repression by miR-378 in the CYP2E1 30UTR
- miR-378-mediated regulation of CYP2E1 is specific in humans
  - CYP2E1 30UTR region harboring miR-378 MRE is poorly conserved in rat and mouse
Summary of PMs and Benzene

Dougherty et al. NQO1, MPO, CYP2E1, GSTT1 and GSTM1 polymorphisms and biological effects of benzene exposure—a literature review. Toxicol Lett. 2008 Nov 10;182(1-3):7-17

- Multiple polymorphic enzymes involved: NQO1, CYP2E1, GSTT1, GSTM1 and MPO.
- Twenty two reports were included in this review
- A modest effect of the studied gene polymorphisms on the analyzed biomarkers was observed.
  - GSTM1 and GSTT1 showed some consistent associations with both biomarkers of exposure and effect.

Conclusions
- Genetic polymorphisms on the benzene metabolism pathway should be taken into account
- Unique combinations of genetic polymorphisms may increase susceptibility of individuals and/or population subgroups.
- However, gene–gene interactions, and the biological effects of long-term and low-level exposure to benzene are not yet analyzed with well-designed studies that incorporate multiple biological end-points and multiple genes.
NQO1*2 PM and childhood leukemia

- meta-analysis of 7 case-control studies
  - a family-based study previously demonstrated overtransmission of this allele among childhood acute lymphoblastic leukemia cases
  - meta-analysis showed that the NQO1*2 variant allele had no significant effect on childhood leukemia
- Was an increased risk associated with having at least 1 copy of the NQO1*2 allele in a subset of cases with MLL translocations
  - summary OR = 1.39 (95% CI: 0.98, 1.97)
  - Heterogeneity between studies may be due to:
    » differences in population exposures to NQO1 substrates
    » small sample sizes
    » potential population stratification in non-family-based studies

ALL and AML were not associated with either maternal smoking during pregnancy or candidate polymorphisms in CYP1A1, CYP2E1, EPHX1, and NQO1.

Carrying two NAT2*5 alleles was significantly associated with ALL (OR = 1.8 [1.3-2.5]).

BUT the analyses suggested an interaction between three genes involved in benzene metabolism CYP2E1, NQO1, and EPHX1.

There was no interaction between maternal smoking and any of the polymorphisms under study.

CONCLUSIONS:
No evidence of the interaction between CYP1A1*2A/2B and maternal smoking suggested previously.

The association with NAT2*5 and the gene-gene interactions need to be replicated.
Polyaromatic Hydrocarbons

The K-region epoxide of BaP

Bay region epoxides of PAHs
Formation of Bay Region Diol-epoxide From Benzo(a)pyrene
Stereochemistry of Bay Region epoxide formation

CYP1A1

- [(+)-anti]BaP-7R,8R-diol-9S,10R-epoxide (R,S,S,R)
- [(+-)syn]BaP-7R,8S-diol-9R,10S-epoxide (R,S,R,S)
- [(+)-anti]BaP-7S,8R-diol-9R,10S-epoxide (S,R,S,S)
- [(+)-syn]BaP-7S,8S-diol-9S,10R-epoxide (S,R,R,S)

BaP

- (+)-anti-BPDE
- (-)-anti-BcPhDE
- (+)-syn-BcPhDE
- anti-DMBade
- syn-DMBade
- (-)-anti-DB[a,i]PDE
- (+)-syn-DB[a,i]PDE
Summary: Key DMEs involved in PAH metabolism

- **CYP1A1** - activation
  - Not normally constitutively expressed
  - Highly inducible via the AhReceptor

- **Other CYPS** – non-bay region hydroxylation

- **Microsomal Epoxide Hydrolase**
  - Two steps – 7,8-epoxide to 7,8-dihydrodiol
  - Diol epoxide sterically hindered – low mEH activity

- **Glutathione S-transferases**
  - GSTM1, GSTP1

- **UDPGs, SULTs** – elimination of hydroxylated metabolites, but role in protecting from DNA damage less clear
Specific activities of hGSTs A1-1, M1-1 and P1-1 toward (+)-BPDE

Table 7. GSH Conjugation and DNA Adduct Formation in Control and Human GST Overexpressing V79MZ Cells Incubated with (+)-anti-BPDE

<table>
<thead>
<tr>
<th>cell lines</th>
<th>(+)-anti-BPDE conjugates&lt;sup&gt;a&lt;/sup&gt; (pmol/10&lt;sup&gt;6&lt;/sup&gt; cells)</th>
<th>(+)-anti-BPDE adducts&lt;sup&gt;a&lt;/sup&gt; (pmol/10&lt;sup&gt;6&lt;/sup&gt; cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V79MZ</td>
<td>2.46 ± 1.19</td>
<td>0.82 ± 0.13</td>
</tr>
<tr>
<td>V79MZhA1-1</td>
<td>11.6 ± 2.99</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>V79MZhM1-1</td>
<td>18.7 ± 3.68</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>V79MZhP1-1</td>
<td>78.3 ± 19.0</td>
<td>0.20 ± 0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> The results are expressed as the mean ± SE (n = 3).

Sundberg K et al., Chem. Res. Toxicol., 2002
GSTM1 and Lung Cancer

• Meta analysis yielded OR of 1.41
  - 1593 cases, 2135 controls
  - 95% CI 1.23 - 1.61, p<0.0001

• More recent meta analysis found lower (1.17) OR

• Estimated attributable risk, 17%

• No large differences with histological type
  - squamous 1.49 (1.22-1.80, n=591)
  - adenocarcinoma 1.53 (1.26 - 1.85, n=482)
  - small cell carcinoma 1.90 (1.27-1.85, n=122)

From: McWilliams et al., Cancer Epi Biomark Prev 4: 589-94, 1995
Smith et al, – Polymorphisms in Xenobiotic Conjugation, IN: Gene-Environment Interactions, Chp 8, Wiley Press, 2006
GSTM1 and Lung Cancer: Interactions with other PMs

• Two common PM in CYP1A1:
  
  – a) MspI downstream of polyA tail → increased CYP1A1 protein
  
  – b) Ile462Val increased CYP1A1 activity

• In Japanese, strong interaction between CYP1A1 Val/Val and MspI PMs and GSTM1 null genotypes for lung cancer risk

• Not seen in Scandinavian studies, but frequency of variant allele about 10x lower than in Japanese

• Issues of cigarette dose, ethnic differences in allele frequency, population size, and multiple comparisons complicate studies
GSTM1 and Lung Cancer Risk-Biological Plausibility?

- **Supportive:**
  - some activity toward PAH epoxides
  - Good in vitro DNA protection
  - high amounts in liver, site of much activation
  - Smokers’ urine more mutagenic in GSTM1 null
  - lymphocyte SCE increased in GSTM1 null smokers
  - interaction with CYP1A1

- **Not Supportive**
  - Very low expression in lung
  - Catalytic activity toward BPDE relatively low, compared with GSTP1
  - inconsistencies in results across many studies
  - association evident in adenocarcinoma
GSTP1 Polymorphism

- Two Single nucleotide polymorphisms
  - Isoleucine ---> Valine at codon 105 (GSTP1*B)
  - Alanine ---> Valine at codon 113 (GSTP1*C -- both)
  - P1*C linked with P1*B; GSTP1*B+1*C allele frequency ~ 30%; homozygotes (B+C) ~ 7%
  - P1*D (I105, V113) recently identified
  - B*/C* variant, but not D*, has altered activity: Val105 form has HIGHER activity toward (+)-anti- BPDE

- Ethnic differences in allele frequencies
GSTP1 Polymorphism - Epi studies

• For GSTP1*B (Val105), Possible association:
  – Testicular cancer (~3X increased risk)
  – Breast cancer (esp. with GSTM1 and T1 PMs)
  – Oral / Pharyngeal & Laryngeal cancer (~2x incr. risk)
  – Bladder cancer (~3X increased risk)

• But probably not:
  – Lung Cancer (Several studies, no significant association)
  – Colorectal Cancer

• Possibly protective for prostate cancer (?)

• GSTP1 exon 6 variant genotypes associated with improved survival among patients with Stage III and IV NSCLC

Lu et al., Cancer. 2006 Jan 15;106(2):441-7
GST / CYP interaction chemotherapy for breast cancer

• low-drug genotype group retained a significantly poorer DFS (hazard ratio [HR] = 4.9; 95% CI, 1.7 to 14.6;
• “Combined genotypes at CYP3A4, CYP3A5, GSTM1, and GSTT1 influence the probability of treatment failure after high-dose adjuvant chemotherapy for node-positive breast cancer”

– cohort of 90 node-positive breast cancer patients who received anthracycline-based adjuvant chemotherapy followed by high-dose multiagent chemotherapy with stem-cell rescue

GSTM1, isothiocyananates and lung cancer risk in smokers

• population-based case-control study of 933 African Americans and Caucasians (311 cases; 622 controls).

• Broccoli, cauliflower, greens, and cabbage food-frequency variables represented isothiocyanates.

• Isothiocyananes were protective for lung cancer risk. Adjusted odds ratio (OR) for the uppermost quartile > 80 micro mol isothiocyanates/wk, compared to lowest, was 0.65 (CI = 0.41-1.00, trend P = 0.02).

• Association was stronger among subjects with homozygous deletion of GSTM1 (OR = 0.52, CI 0.31-0.86) than subjects with at least one GSTM1 copy (OR = 0.77; CI 0.49-1.21).