SESSION 4: DETOXIFICATION GENETICS AND HEALTH

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REVIEW FROM LECTURES 1-3

- Folate and MTHFR
- Iron overload
- Genome, Gene, Allele
- Vitamin A deficiency diet planning
- Lactose intolerance
- Gluten Intolerance
- Caffeine Metabolism
- Strength training genetics
- Sedentary lifestyle hypertension
- **Enhanced endurance benefit**
- **ROS and exercise**
- **Type 1 diabetes genetics**
- **Type 2 diabetes genetics**
- Dieting and HDL

SESSIONS 1 - 8

Session	Topic	Evaluations
1	INTRODUCTION TO MOLECULAR GENETICS, MOLECULAR BIOLOGY AND HUMAN GENETICS	Discussion – Participation
2	NUTRITIONAL AND DIETARY GENETICS: HOW DO OUR GENES REGULATE OUR NUTRITION AND NUTRITIONAL HEALTH?	Discussion - Participation
3.	FITNESS GENETICS AND GENETICS OF CHRONIC DISEASES: HOW DO OUR GENES REGULATE OUR RESPONSE TO EXERCISE AND HOW DO GENES REGULATE THE RISK OF CHRONIC METABOLIC DISORDERS?	Discussion - Participation
4.	DETOXIFICATION GENETICS: HOW DO OUR GENES REGULATE DETOXIFICATION WHICH INDIRECTLY IMPACTS OVERALL HEALTH AND DISEASE RISK?	Discussion - Participation
5.	NEUROGENETICS: HOW DO OUR GENES REGULATE THE SYNTHESIS AND BREAKDOWN OF NEUROTRANSMITTERS AND ITS IMPACT ON OUR HEALTH ?	Take home exam on sections 1-5, due during session 6
6.	GENETICS OF ENDOCANNABINOID PATHWAYS: HOW DO OUR GENES REGULATE THE RESPONSE TO CANNABIS?	Discussion - Participation
7.	SKIN GENETICS: HOW DO OUR GENES REGULATE OUR SKIN HEALTH ?	Take home assignment – due during session 8
8.	DISCUSSION AND PRACTICAL APPLICATIONS OF GENETIC TESTS DISCUSSED IN SESSIONS 2-7	Discussion - Participation

SESSION OBJECTIVES:

- What is detoxification ? Phase I and Phase 2
- Genetics of Phase I genetics and health implications
- Genetics of Phase 2 genetics and health implications
- Genetics of estrogen metabolism and modulation

WHAT IS DETOXIFICATION ?

Detoxification is not a liver cleanse / colon cleanse – no science behind it – an industry fuelled by populism and the human need for immediate solutions

Detoxification: Every organism has this

- A process that takes place in the liver
- Everyone has this for homeostasis
- Liver metabolizes every chemical, hormone, pollutant
- Enzymes are encoded by genes
- Variation in genes impacts detoxification
- Amenable to dietary intervention

Detoxification Enzymes

(or "Drug Metabolizing Enzymes," "Effector-Metabolizing Enzymes")

- Involved in detoxification of plant metabolites, dietary products, drugs, toxins, pesticides, carcinogens
- All DMEs have endogenous compounds as natural substrates (used in natural process of breaking down compounds)
- Located in every eukaryotic cell, most prokaryotes
- Many different types, many families, many alleles; each individual has a unique set of enzymes
- Selection result from variation in diet, climate, geography, toxins (pesticides)

Detoxification Enzymes

- Exogenous compounds (toxins, pesticides) compete with endogenous ligands (estrogen, other hormones)
  - for binding to receptors (estrogen, glucocorticoid)
  - channels (ion or other ligand)

  acting as agonists or antagonists.

- Such binding to receptors could result in toxicitiy, abnormal development, or cancer

- Detoxification enzymes act to break down these chemicals before they bind to receptors or channels

Partial list of detoxification enzymes

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Phase I (functionalization) reactions: oxidations and reductions

Cytochrome P450s, flavin-containing monooxygenases (FMOs), hydroxylases, lipooxygenases, cyclooxygenases, peroxidases, mononamine oxidases (MAOs)and various other oxidases, dioxygenases, quinone reductases, dihydrodiol reductases, and various other reductases, aldoketoreductases, NAD-and NADP-dependent alcohol dehydrogenases, aldehyde dehydrogenases, steroid dehydrogenases, dehalogenases.

Phase II (conjugation) reactions: transfer chemical moieties to water-soluble derivatives

UDP glucuronosyltransferases,GSH S transferases, sulfotransferases, acyltransferases,glycosyltransferases, glucosyltransferases, transaminases, acetyltransferases, methyltransferases

Hydrolytic enzymes

Glycosylases, glycosidases, amidases,glucuronidases, paraoxonases, carboxylesterases, epoxide hydrolase and various other hydrolases, acetylcholinesterases and various other esterases

CYPs (cytochrome P450s)

- At least 74 gene families
- 14 ubiquitous in all mammals

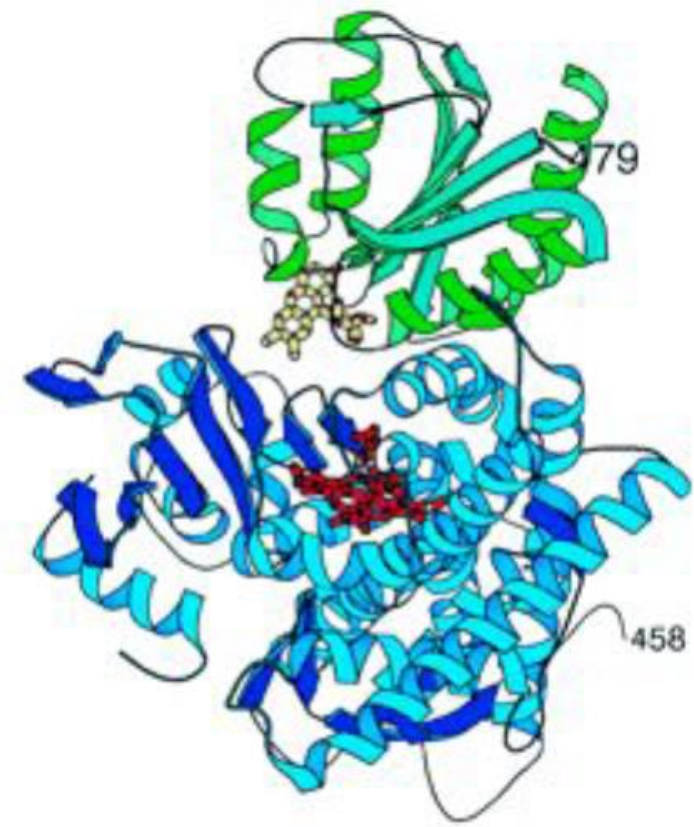
- CYP1, 2, 3, involved in detoxification of lipophilic, or nonpolar substances

- Other CYP families involved in metabolism of endogenous substances, such as fatty acids, prostaglandins, steroids, and thyroid hormones

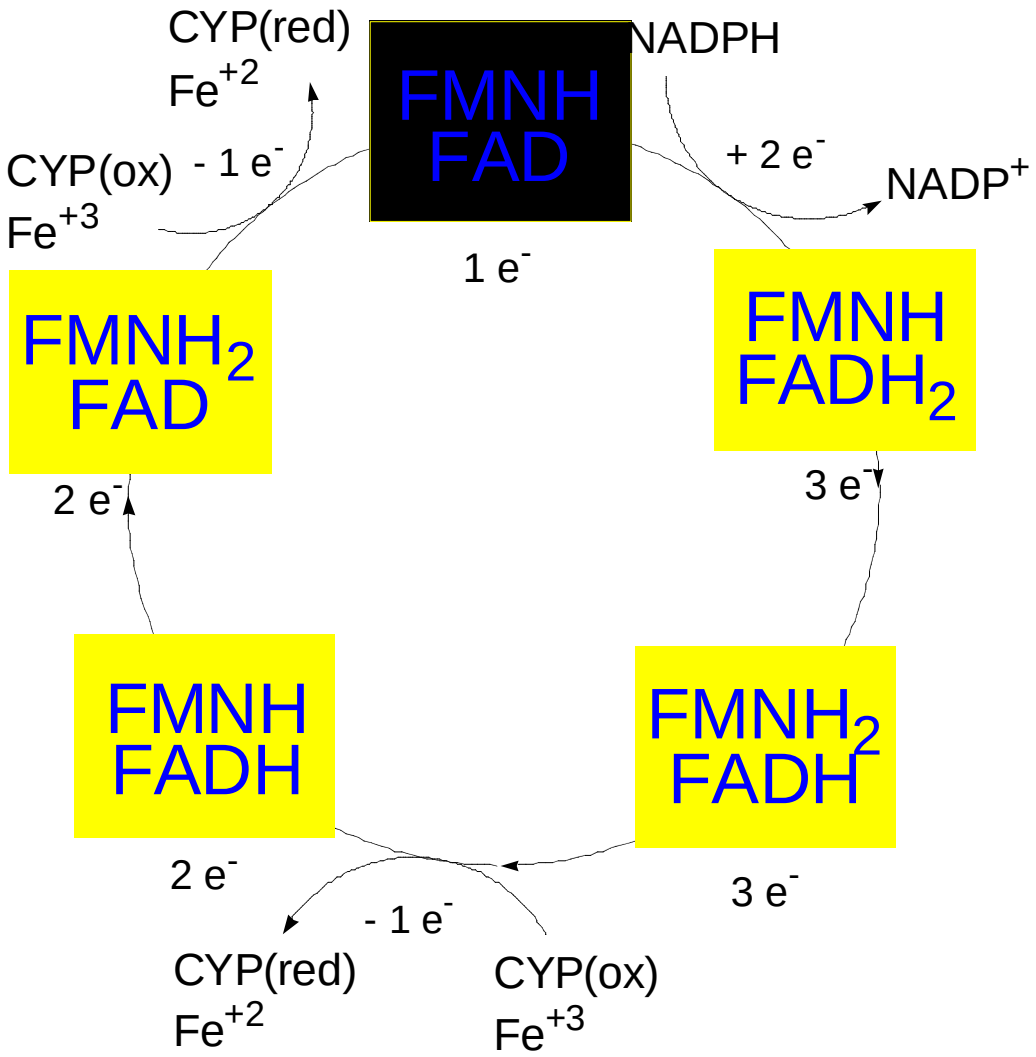
CYP450

- CYP catalyses a variety of reactions including epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation.

- A typical cytochrome P450 catalysed reaction is:
- NADPH + H+ + O2 + RH ==> NADP+ + H2O + R-OH



Electron Transfer in CYP Reductase

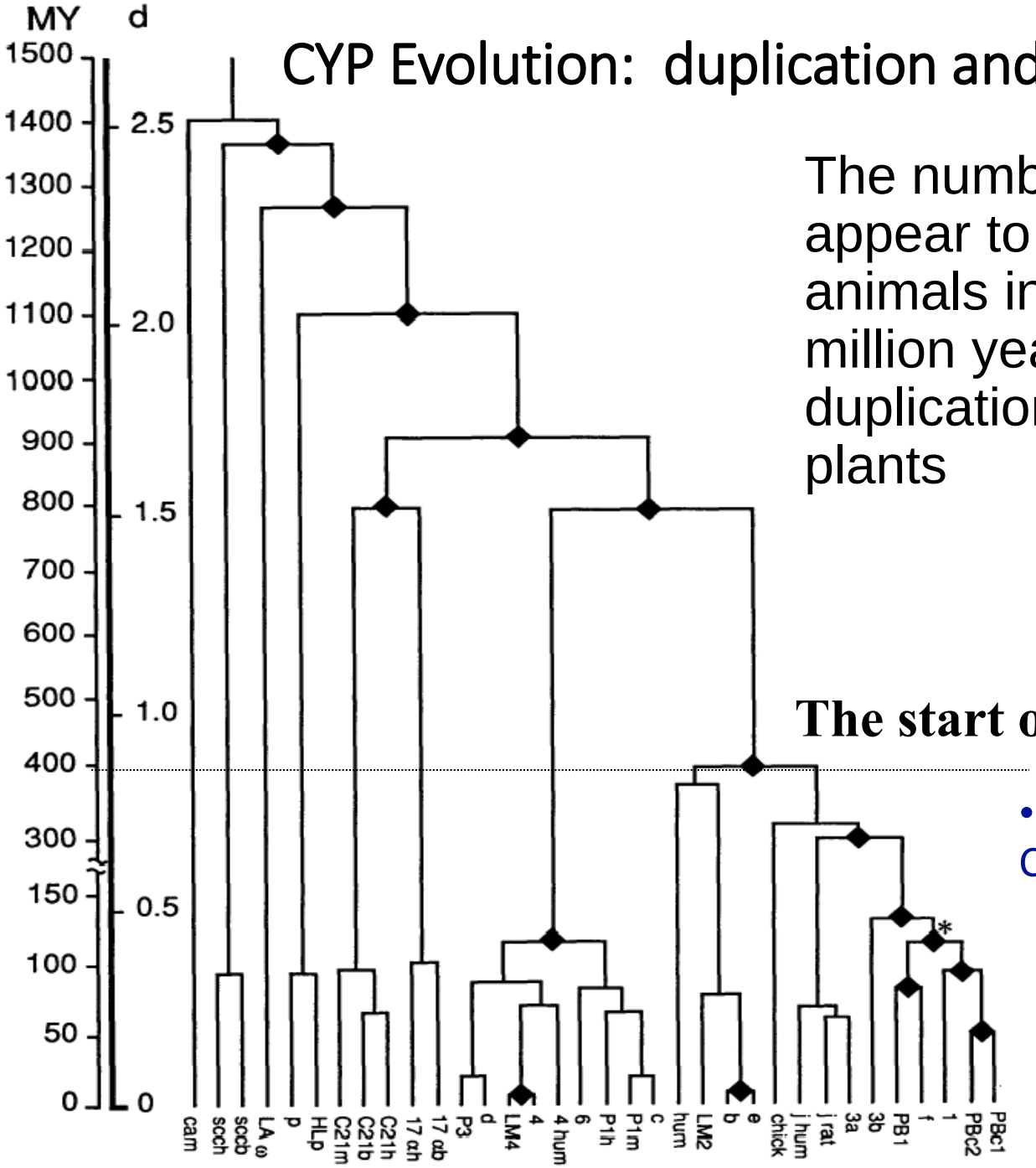


FAD is the electron acceptor from NADPH and the fully reduced **$FMNH_2$ is the electron donor to CYP.**

Evolutionary History of CYP450s

- Different types arose through gene duplication and differentiation
- The first CYP450s likely evolved in response to an increase in oxygen in the atmosphere (along with CAT and SOD)
- The massive diversity of these CYP is thought to reflect the coevolutionary history between plants and animals.
- Plants develop new alkaloids to limit their consumption by animals - the animals develop new enzymes to metabolize the plant toxins, and so on.

CYP Evolution: duplication and differentiation



The number of CYP2 genes appear to have exploded after animals invaded land, ~400 million years ago (50 gene duplications) and began eating plants

The start of the invasion of land

- Phylogenetic tree of 34 CYP450 proteins.
  - Black diamonds = gene-duplication events.
  - Unmarked branch points = speciation events.

Human population variation in detoxification allele frequencies

- Many different alleles (amino acid differences) at many DME genes
- Differences among populations might arise due to natural selection arising from Dietary differences, or differences in Climate and Geography
- There might also be differences arising from genetic drift (random loss of alleles in small populations)
- Maintenance of genetic variation might be explained by balancing selection (such as heterozygote advantage)

Human population variation in detoxification allele frequencies

Implications of genetic variation:

- Differences in dietary capacities
- Many drugs are plant derivatives, such that differences in response to plant compounds would affect drug responses
- Differences in drug metabolism, drug excretion rates and final serum drug concentrations

Humans have 18 gene families of cytochrome P450 genes and 43 subfamilies

- **CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)**
- **CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)**
- **CYP3 drug metabolism (1 subfamily, 4 genes, 2 pseudogenes)**
- **CYP4 arachidonic acid or fatty acid metabolism (5 subfamilies, 11 genes, 10**
- **pseudogenes)**
- **CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)**
- **CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1**
- **subfamily member)**
- **CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)**
- **CYP8A prostacyclin synthase (1 subfamily member)**
- **CYP8B bile acid biosynthesis (1 subfamily member)**
- **CYP11 steroid biosynthesis (2 subfamilies, 3 genes)**
- **CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase**
- **CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen**
- **CYP20 Unknown function (1 subfamily, 1 gene)**
- **CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)**
- **CYP24 vitamin D degradation (1 subfamily, 1 gene)**
- **CYP26A retinoic acid hydroxylase important in development (1 subfamily member)**
- **CYP26B probable retinoic acid hydroxylase (1 subfamily member)**
- **CYP26C probabvle retinoic acid hydroxylase (1 subfamily member)**
- **CYP27A bile acid biosynthesis (1 subfamily member)**
- **CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)**
- **CYP27C Unknown function (1 subfamily member)**
- **CYP39 7 alpha hydroxylation of 24 hydroxy cholesterol (1 subfamily member)**
- **CYP46 cholesterol 24-hydroxylase (1 subfamily member)**
- **CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol 14-alpha demethylase**

CYP450s important in Drug Metabolism

CYP3A4

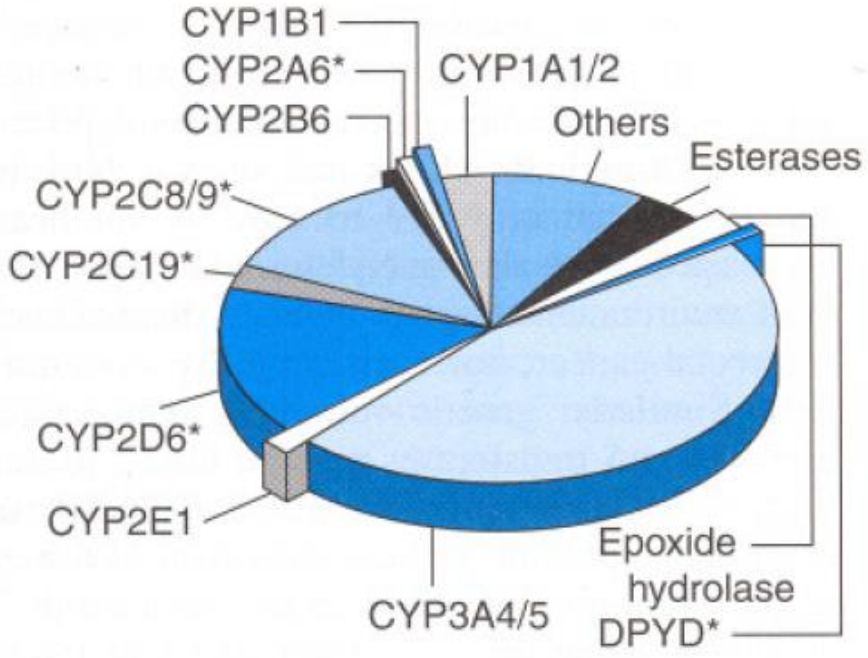
- most important (50%)
- inducible

CYP2D6

- next in line (20%)
- not inducible

CYP2C9 and 2C19

- next (15%)
- inducible



* Functional allelic variants. There are 70 identified functional variants of CYP2D6 alone

Some CYP enzymes involved in Drug Metabolism

TABLE 2. *Cytochrome P450 (CYP) enzymes and substrates*

Enzyme	Substrates
CYP1A2	Caffeine, clozapine, fluvoxamine, haloperidol, paracetamol, theophylline
CYP2C19	Amitriptyline, barbiturates (hexobarbital), clomipramine, diazepam, imipramine, mephenytoin, omeprazole, proguanil
CYP2D6	Antiarrhythmics, antihypertensives, β-blockers, tricyclic antidepressants, antipsychotics, selective serotonin reuptake inhibitors, morphine derivatives
CYP2C8–9	Ibuprofen, phenytoin, S-warfarin
CYP3A4	Carbamazepine, quinidine
CYP2E1	Acetone, ethanol, paracetamol

Ann Clin Biochem 1999: **36**

Pharmacological consequences of genetic variation at CYP

- Individual differences in the ability to breakdown different chemicals
- Inefficient drug metabolism: higher serum drug concentration, increase risk of concentration-dependent side-effects
- Over-efficient metabolism: failure to attain therapeutic doses

Human Liver Drug CYPs

CYP enzyme	Level (%total)	Extent of variability
1A2	~ 13	~40-fold
1B1	<1	
2A6	~4	~30 - 100-fold
2B6	<1	~50-fold
2C	~18	25-100-fold
2D6	Up to 2.5	>1000-fold
2E1	Up to 7	~20-fold
2F1		
2J2		
3A4	Up to 28 30-60*	~20-fold 90-fold*
4A, 4B		

S. Rendic & F.J. DiCarlo, Drug Metab Rev 29:413-80, 1997

**L. Wojnowski, Ther Drug Monit 26: 192-199, 2004*

Factors Influencing Activity and Level of CYP Enzymes

Nutrition	**1A1;1A2; 1B1, 2A6, 2B6, 2C8,9,19; 2D6, 3A4,5**
Smoking	**1A1;1A2, 2E1**
Alcohol	**2E1**
Drugs	**1A1,1A2; 2A6; 2B6; 2C; 2D6; 3A3, 3A4,5**
Environment	**1A1,1A2; 2A6; 1B; 2E1; 3A3, 3A4,5**
Genetic Polymorphism	**1A; 2A6; 2C9,19; 2D6; 2E1**

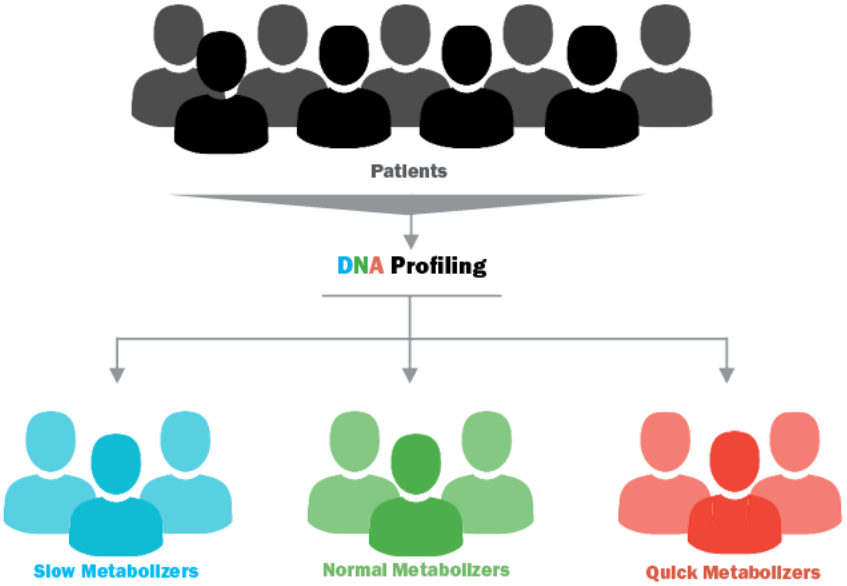
Red indicates enzymes important in drug metabolism

Adapted from: *S. Rendic Drug Metab Rev 34: 83-448, 2002*

Non-nitrogenous Substances that Affect Drug Metabolism

- Grapefruit juice - CYP 3A4 inhibitor; highly variable effects; fucocoumarins
  - Bailey, D.G. et al.; Br J Clin Pharmacol 1998, 46:101-110
  - Bailey, D.G et al.; Am J Cardiovasc Drugs 2004, 4:281-97.
- St John's wort, other herbal products
  - Tirona, R.G and Bailey, D.G. ; Br J Clin Pharmacol. 2006,61: 677-81
- Isosafrole, safrole
  - CYP1A1, CYP1A2 inhibitor; found in root beer, perfume

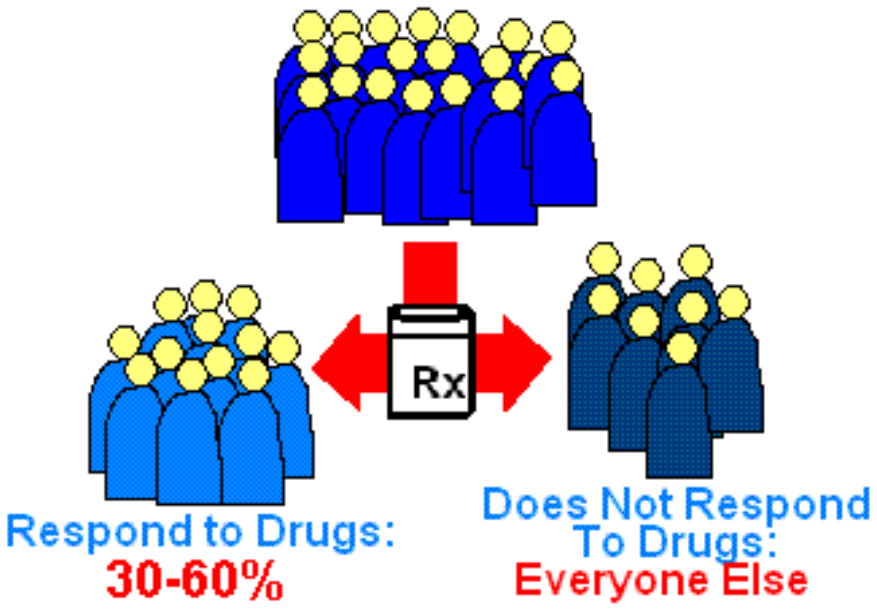
What is Pharmacogenetics?



People can metabolize drugs quickly, slowly or normally. Pharmacogenetics — the combination of pharmacology and genetics — uses information about a person's genetic make-up to help guide drugs and their doses for a particular person.

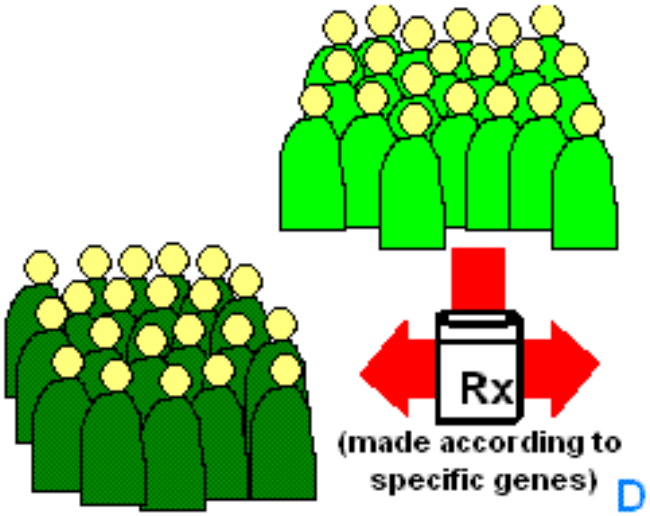
GOAL OF PHARMACOGENETICS

TODAY versus TOMORROW



Respond to Drugs:
30-60%

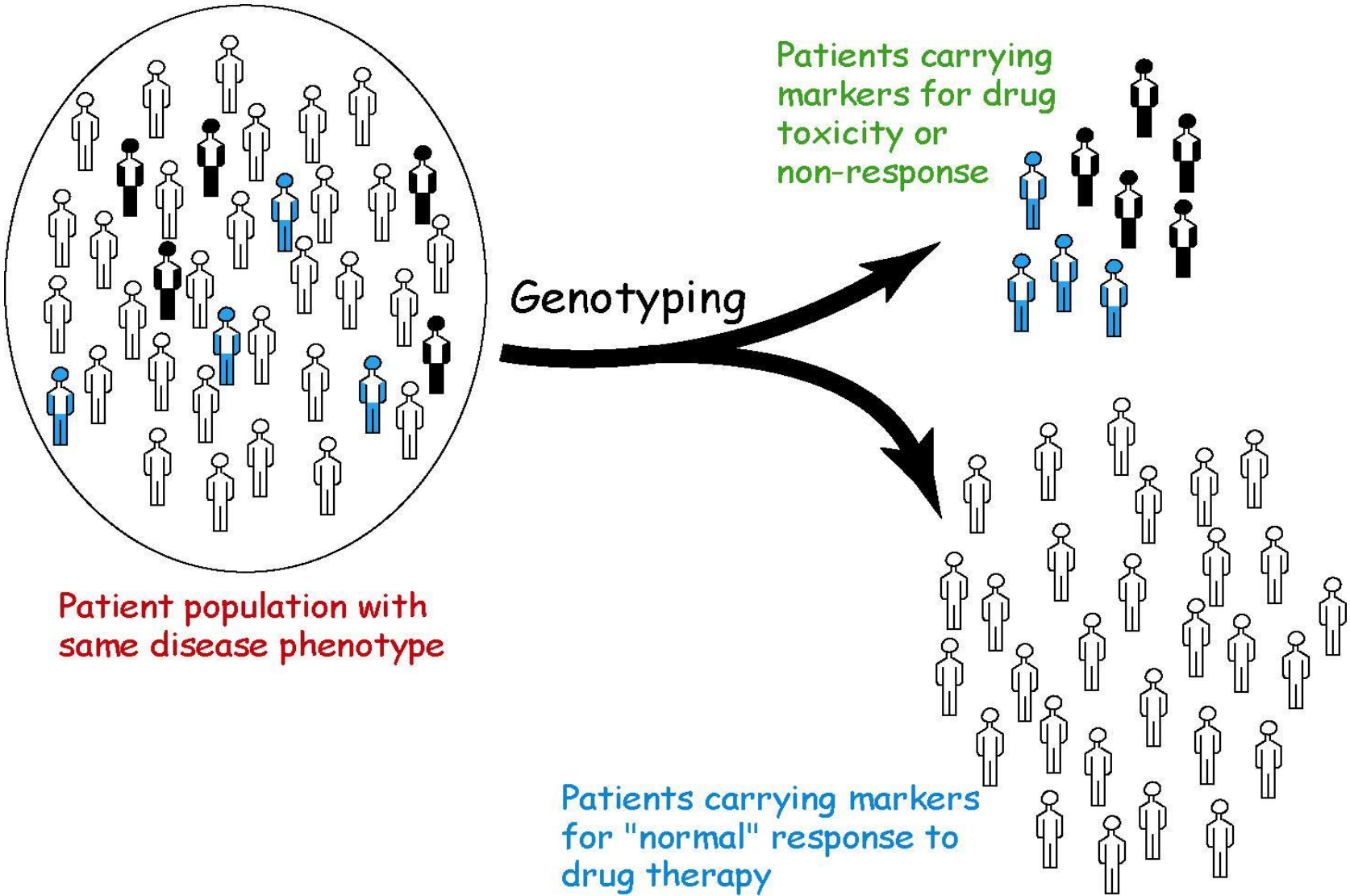
Does Not Respond To Drugs:
Everyone Else



Respond To Drugs:
100%

Does Not Respond To Drugs:
0%

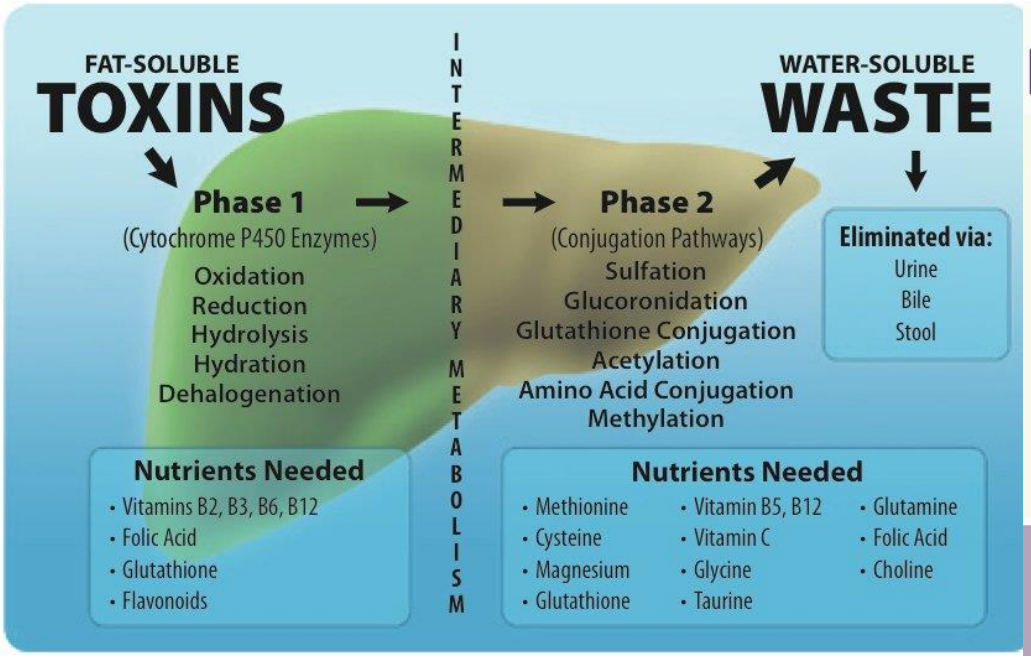
Manipulating Therapeutic Outcomes

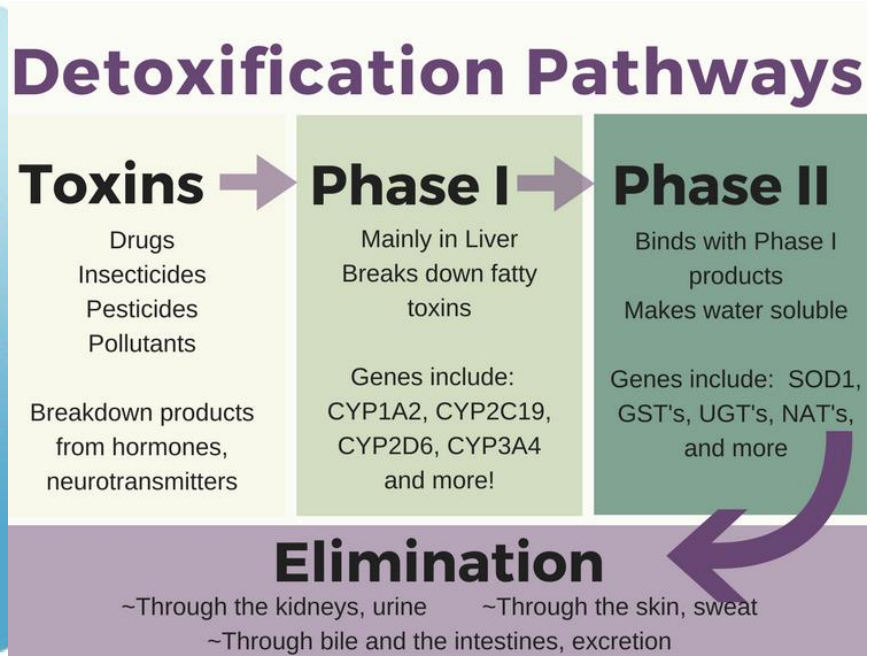


DETOXIFICATION AND GENETICS

Liver is the site of detoxification of all chemicals, alcohol, drugs, pollutants, insecticides, medicines, hormones including estrogen

Cytochrome P450 Complex enzymes play a role in Phase I

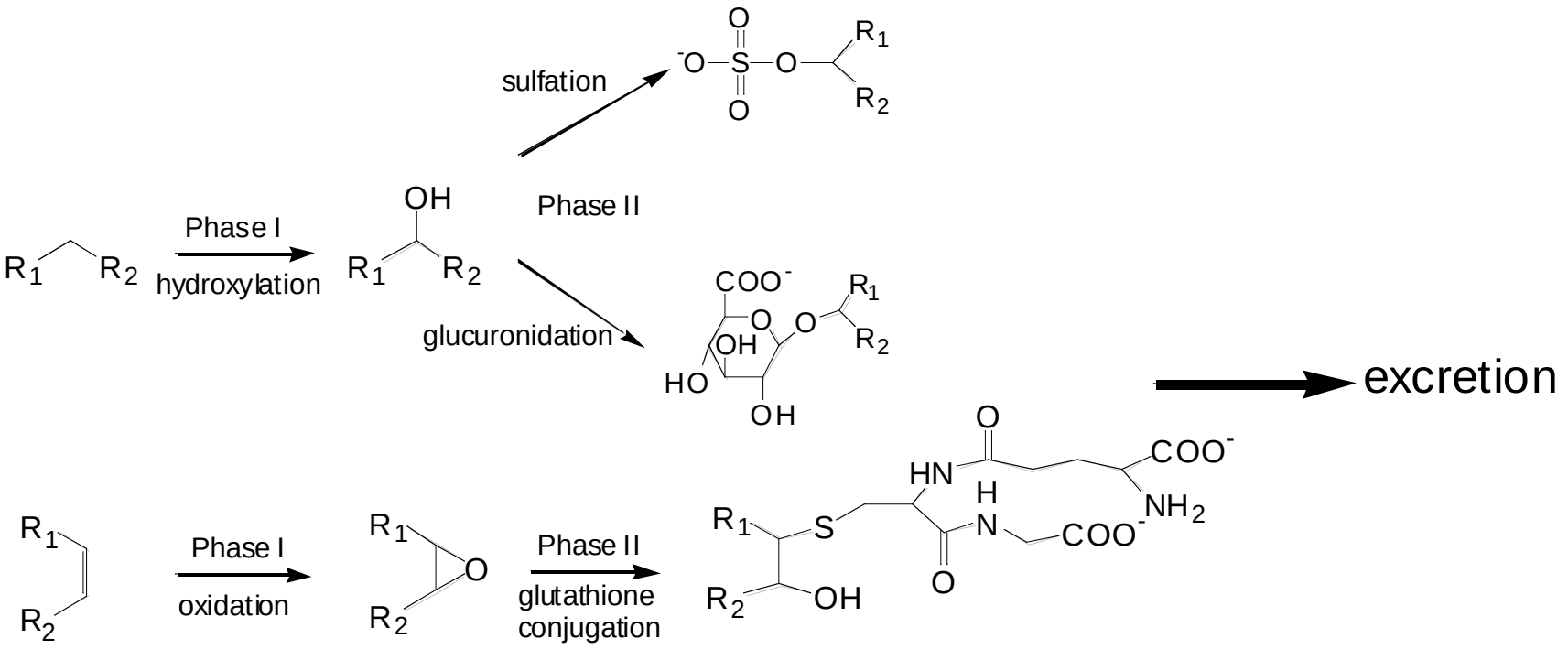




Phase I metabolism

Reactions catalyzed by xenobiotic biotransforming enzymes are generally divided into two groups: Phase I and phase II.

1. Phase I reactions involve hydrolysis, reduction and oxidation, exposing or introducing a functional group (-OH, -NH_2, -SH or –COOH) to increase reactivity and slightly increase hydrophilicity.

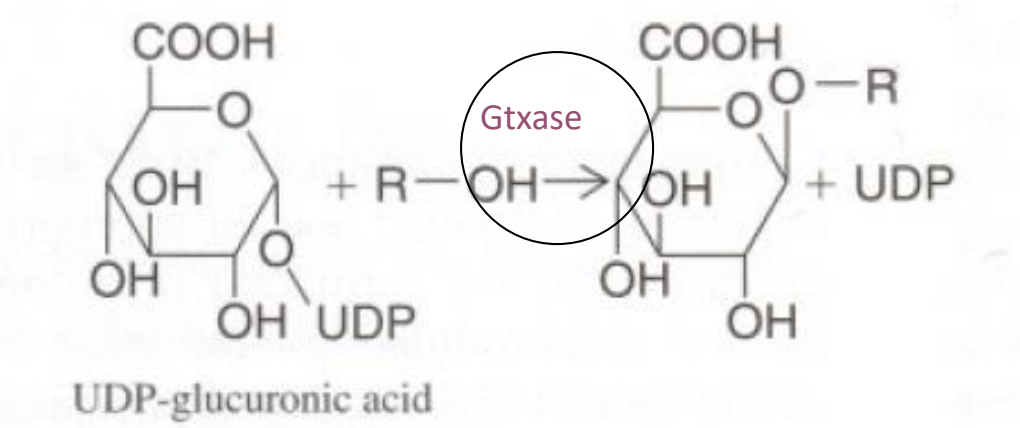


Phase II metabolism (conjugation)

Substrate is phase I reaction product, or other endogenous compound (eg. steroid hormones)

Conjugaton of highly polar glucuronide enhances water solubility/decreases lipid solubility and thereby promotes excretion

Fewer genes and functional variants than P450s

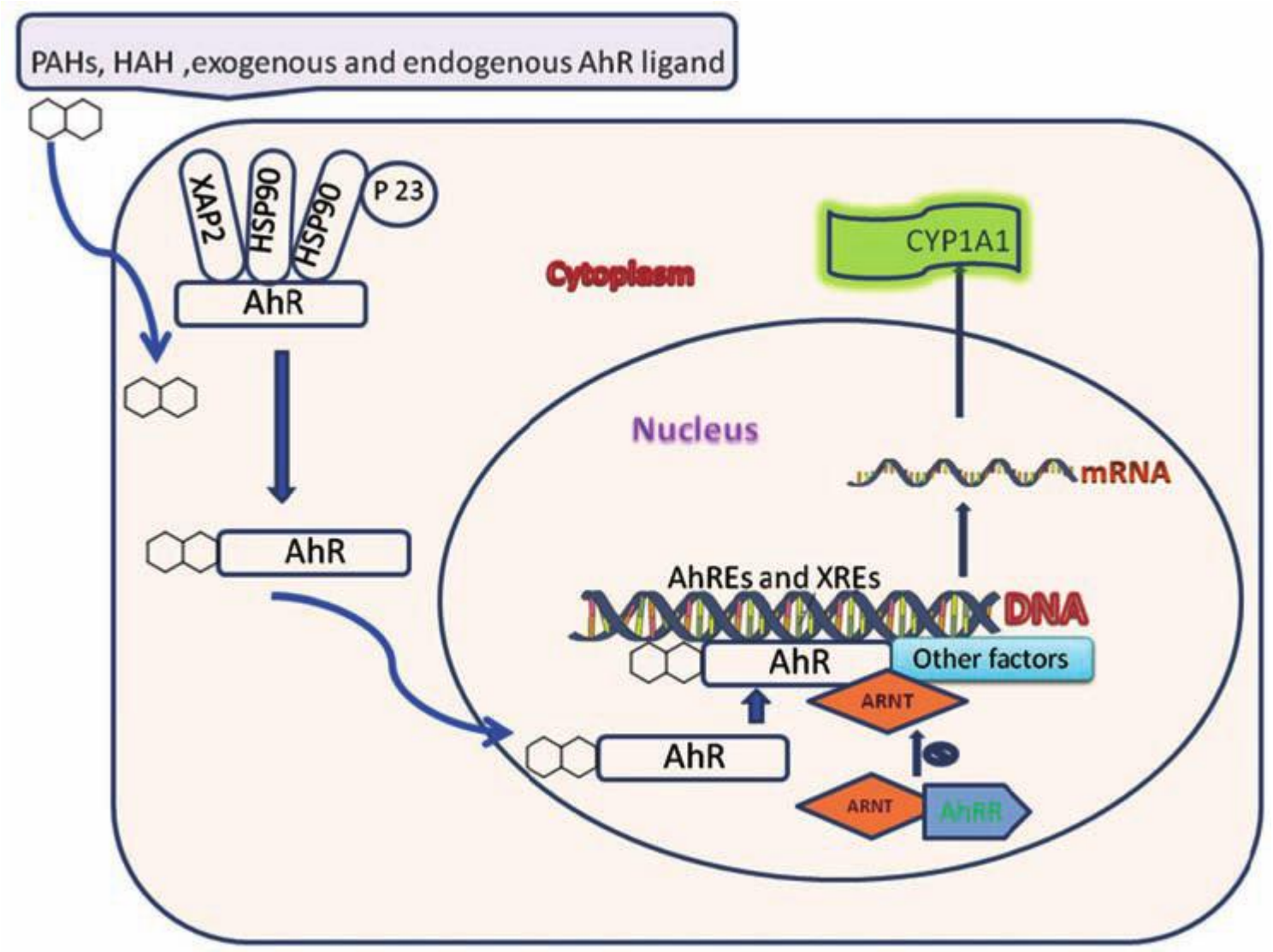


UDP-glucuronic acid

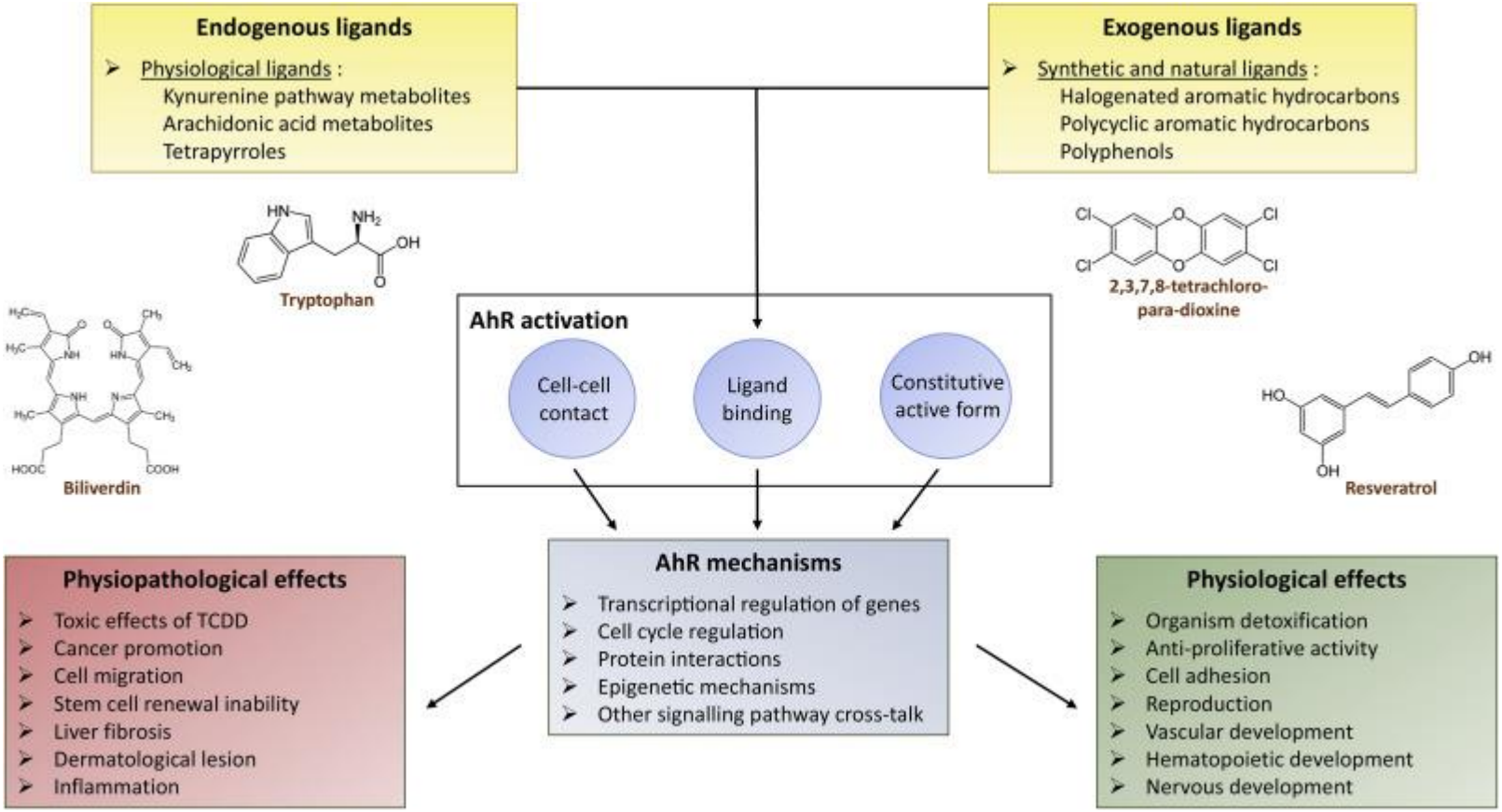
CYP1A1 FUNCTION

- The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids.
- Cytochrome P450, family 1, subfamily A, polypeptide 1
- This protein localizes to the endoplasmic reticulum and its expression is induced by some polycyclic aromatic hydrocarbons (PAHs), some of which are found in cigarette smoke.
- The enzyme's endogenous substrate is unknown; however, it is able to metabolize some PAHs to carcinogenic intermediates.

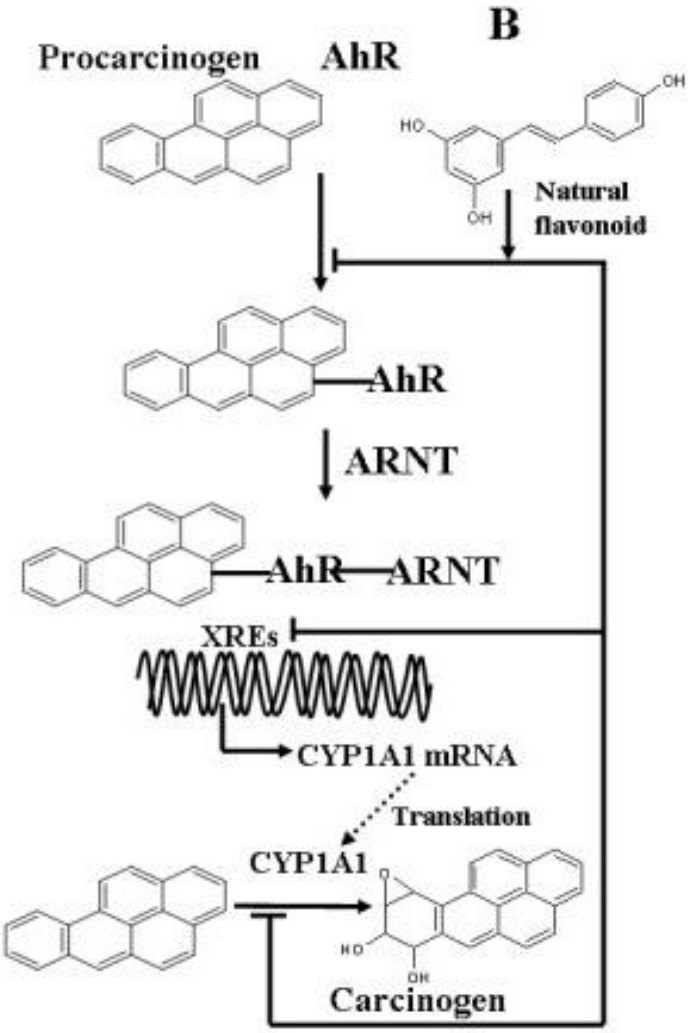
CYP1A1 FUNCTION



ARYL HYDROCARBON RECEPTOR – CYP1A1



CYP1A1 PROCARCINOGEN - CARCINOGEN



CYP1A1 POLYMORPHISMS – CANCER RISK

TABLE 2. Association Between Metabolic Polymorphisms and Risk of Lung Cancer

Polymorphism	Cases n (%)	Controls n (%)	Adjusted* Odds Ratio (95% Confidence Interval)	p
CYP1A1 rs4646903 TT (ancestral†)	148 (32.0)	169 (44.6)	1.0 (reference)	
TC	218 (47.2)	167 (44.1)	1.50 (1.06–2.11)	0.021
CC	96 (20.8)	43 (11.4)	2.63 (1.61–4.28)	< 0.001
	‡<0.0001	§p = 0.857	p_{trend} < 0.0001	
TC + CC vs. TT			1.72 (1.25–2.38)	0.001

CYP1A1 POLYMORPHISMS – LARYNGEAL CANCER RISK

Table 3 Summary ORs for various contrasts of CYP1A1 rs1048943 and rs4646903 polymorphisms in this meta-analysis

	Number of studies	(AG + GG) *versus* AA OR (95% CI) *P* (*Q*-test)	GG *versus* AA OR (95% CI) *P* (*Q*-test)
rs1048943			
Total	6	1.86 (1.45–2.40) 0.000	1.77 (1.28–2.81) 0.007
rs4646903			
Total	7	1.33 (1.04–1.71) 0.029	1.53 (1.31–2.21) 0.012

- CYP1A1*2A allele
- Associated with C variant at RS1048943
- Increased risk of lung cancer, hepatocellular cancer, laryngeal cancer, leukemia
- Naringenin shown to inhibit CYP1A1

CYP1B1

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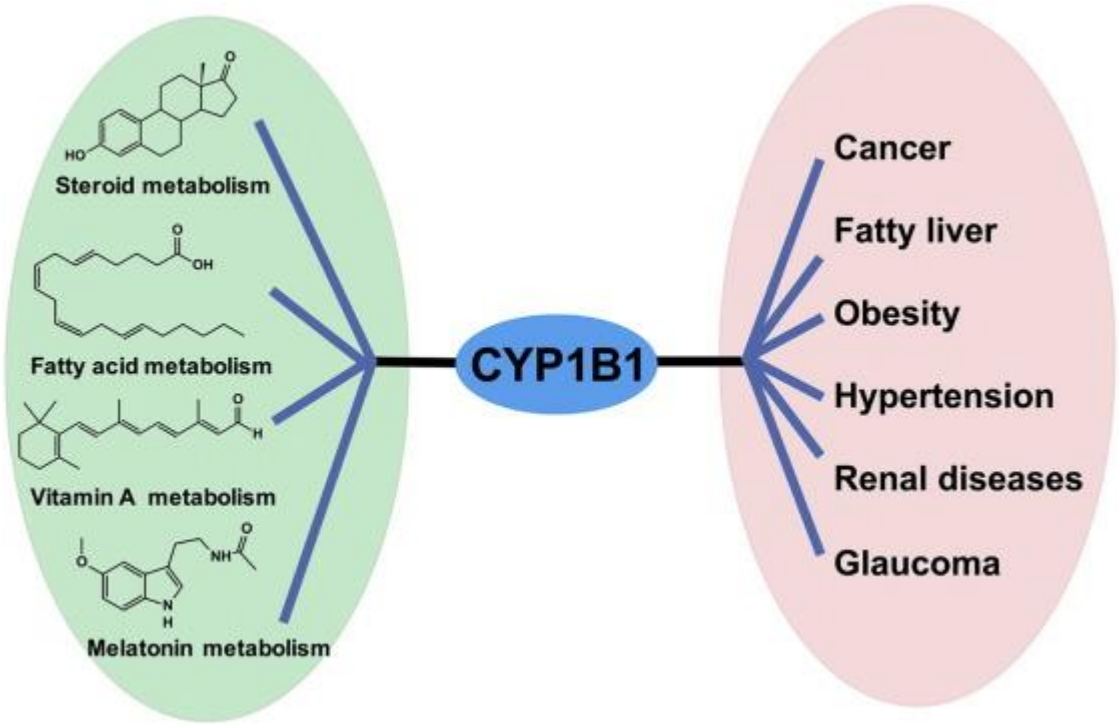
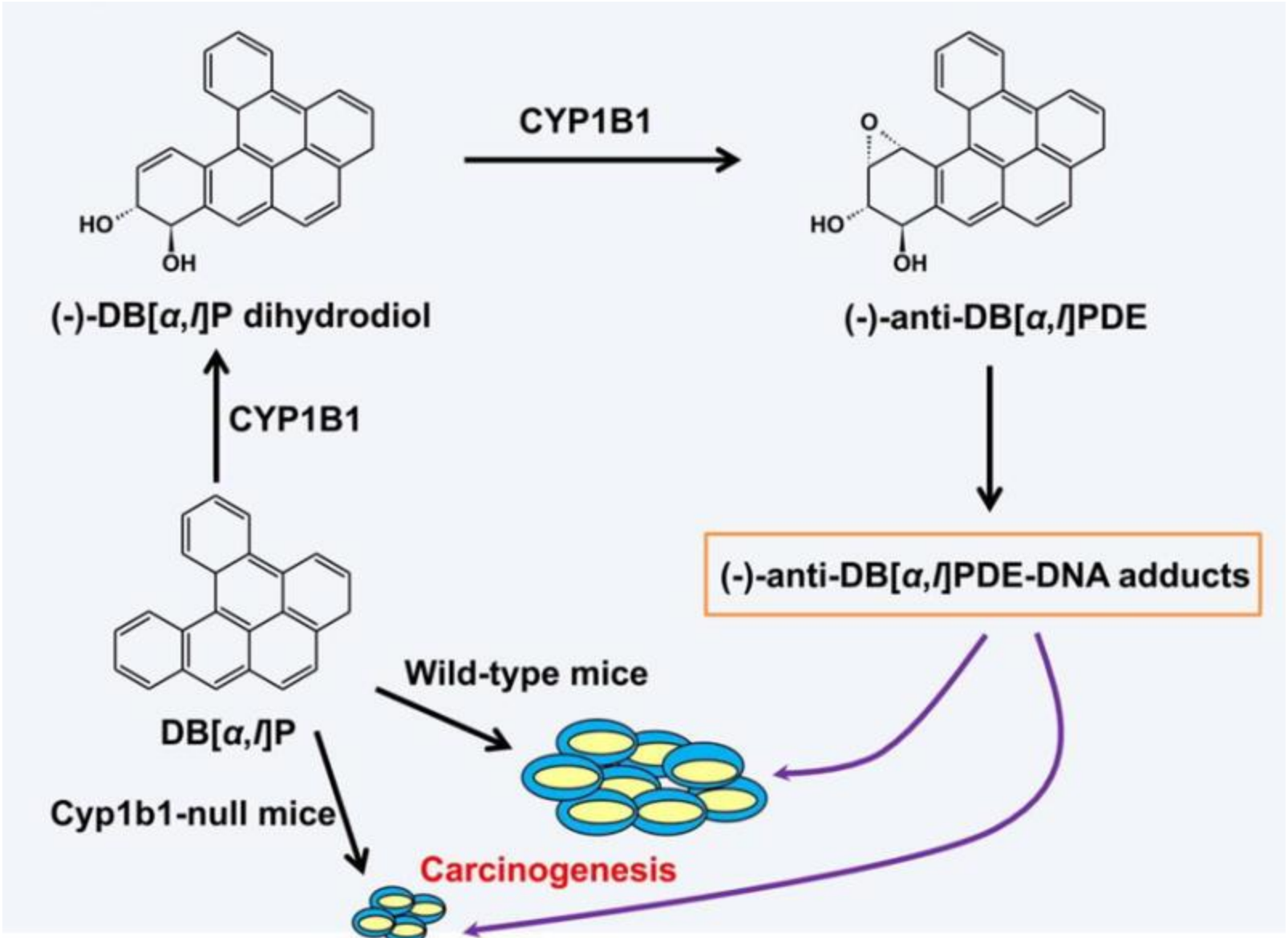
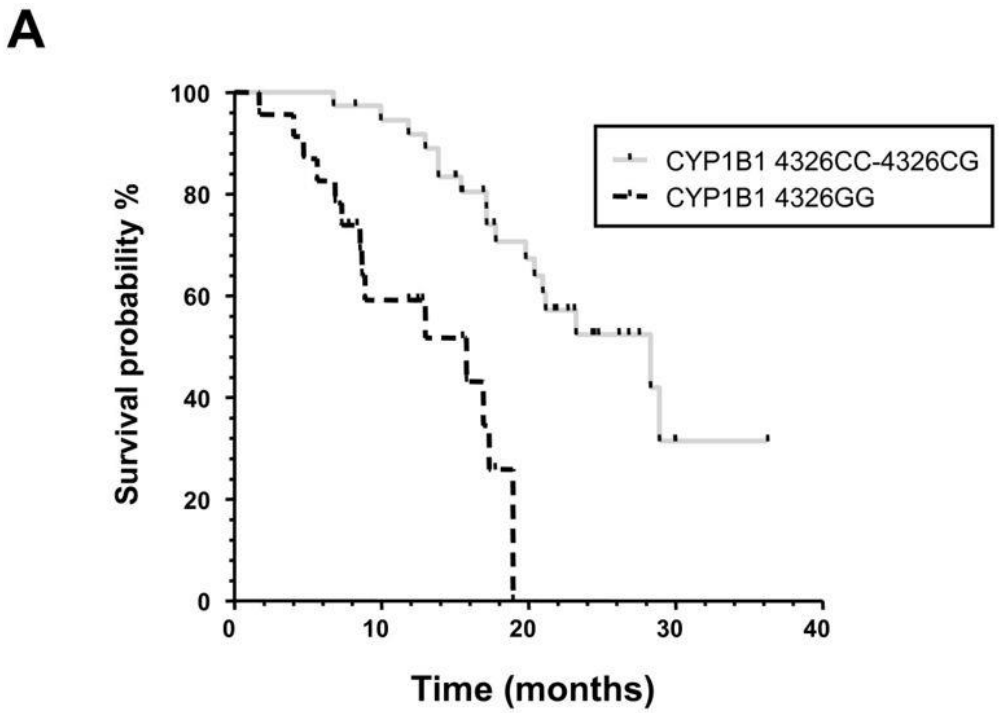
- Cytochrome P450, family 1, subfamily B, polypeptide 1
- CYP1B1 loss of function mutations are the most common cause of autosomal recessive congenital glaucoma worldwide



CYP1B1 AND CANCER

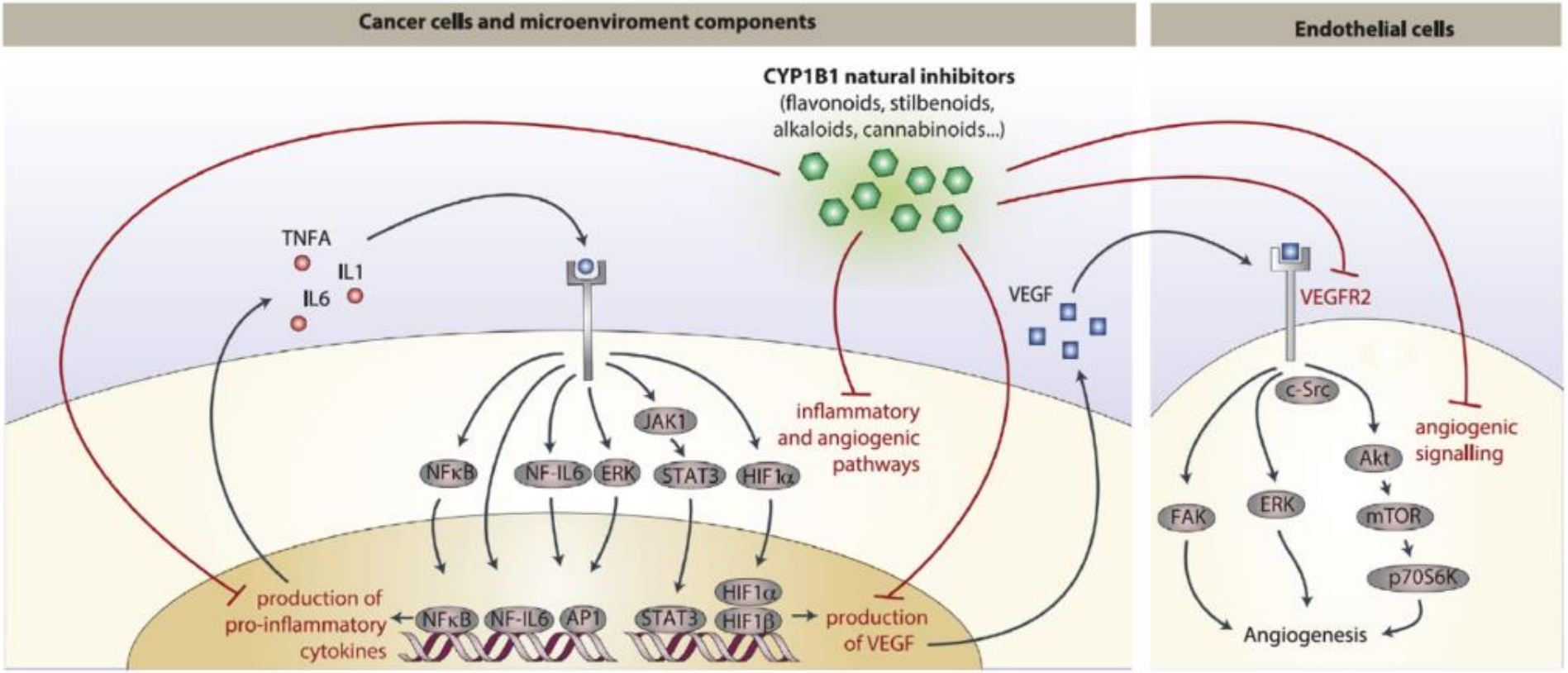


CYP1B1 POLYMORPHISMS – AGGRESSIVE PROSTATE CANCER



- CYP1B1*3-allele
- 4326C>G SNP (rs1056836), leading to the 432LeuVal (432LV) amino-acid transition, is associated with increased catalytic activity of CYP1B1

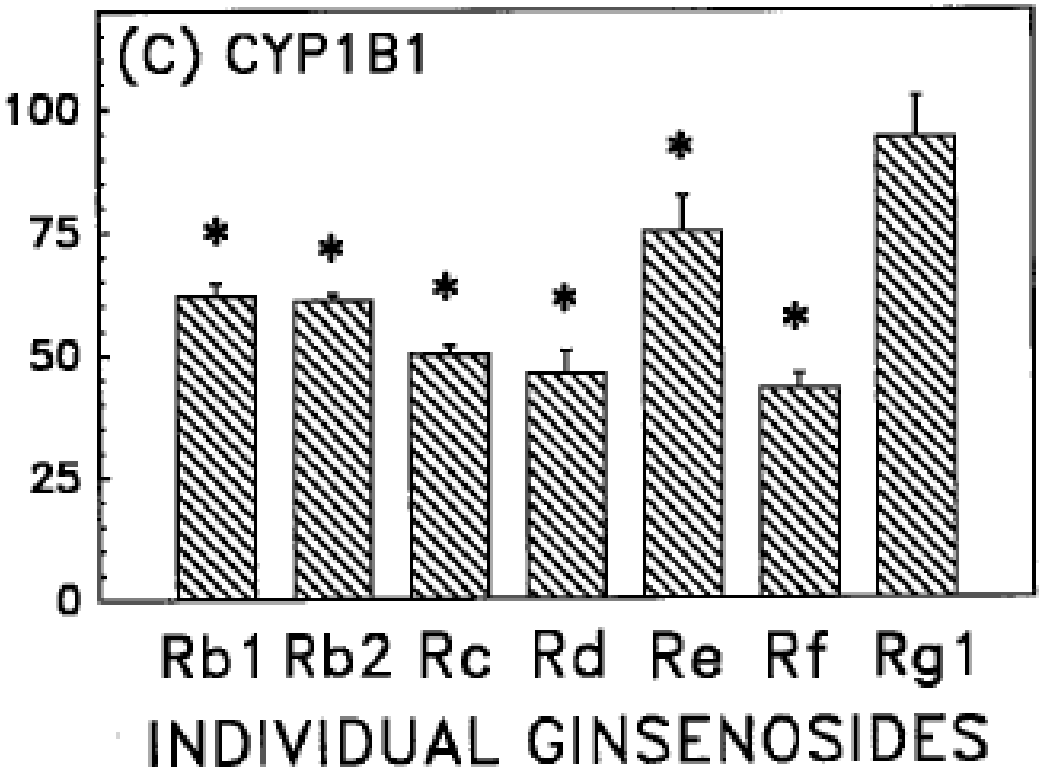
MODULATION OF CYP1B1 ACTIVITY AND CANCER



- Gingko biloba extract shown to inhibit CYP1B1 activity

GINSENG INHIBITS CYP1B1

- In vitro studies indicate ginseng inhibits CYP1B1
- In vivo studies – Ginseng modulates CYP450 pathway
- If increased activity -> increased cancer risk -> Need intervention for activity reduction



CYP2A6 AND CIGARETTES

- ✓ *CYP2A6 – metabolizer of nicotine – key ingredient in cigarettes and tobacco*
- ✓ *1.2* billion people worldwide are known to smoke tobacco daily.
- ✓ *5.6* trillion cigarettes are smoked per year.
- ✓ *4.2* million people die annually from tobacco-related disease.
- ✓ Tobacco-related diseases will be about 10 million in 2020.

Smoking prevalence and reason for concern

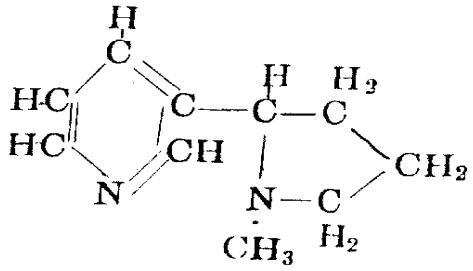
- *Health consequences of smoking*
- ✓ Respiratory disease
- ✓ Cardiovascular disease
- ✓ Cerebrovascular disease

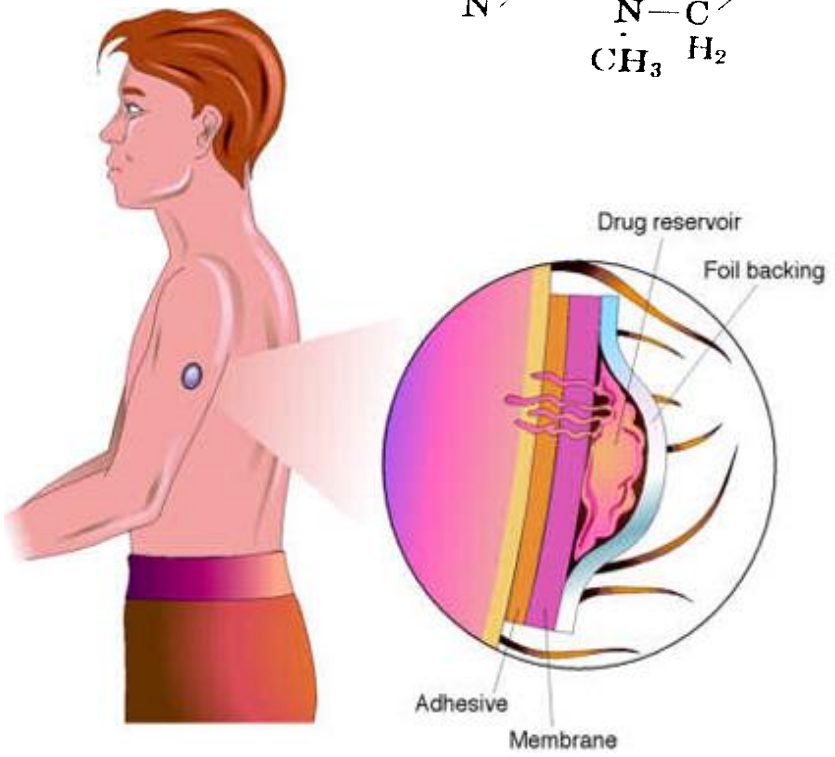
cancer

- *Cancers*
- ✓ Lung
- ✓ Oral cavity
- ✓ Pharynx
- ✓ Pancreas
- ✓ Kidney
- ✓ Urinary tract

- **15% of all cancers**
- **80-90%of lung cancers**

Pharmacokinetics of Nicotine





- Readily absorbed from all over the body, including
  - Lungs (smoked)
  - Mucosa (cigar, chewing tobacco, gum, nasal spray)
  - Skin (patch)
  - Gastrointestinal tract (uncommon)

Pharmacokinetics

- Absorption
  - The most common way to get nicotine into your bloodstream is through inhalation
  - Your lungs are lined by millions of alveoli, which are the tiny air sacs where gas exchange occurs
    - These alveoli provide an enormous surface area, 90 times greater than that of your skin, and thus provide ample access for nicotine and other compounds
  - Nicotine taken in by cigarette or cigar smoking takes only 10-15 seconds to reach the brain but has a direct effect on the body for only ~30 minutes

Pharmacokinetics

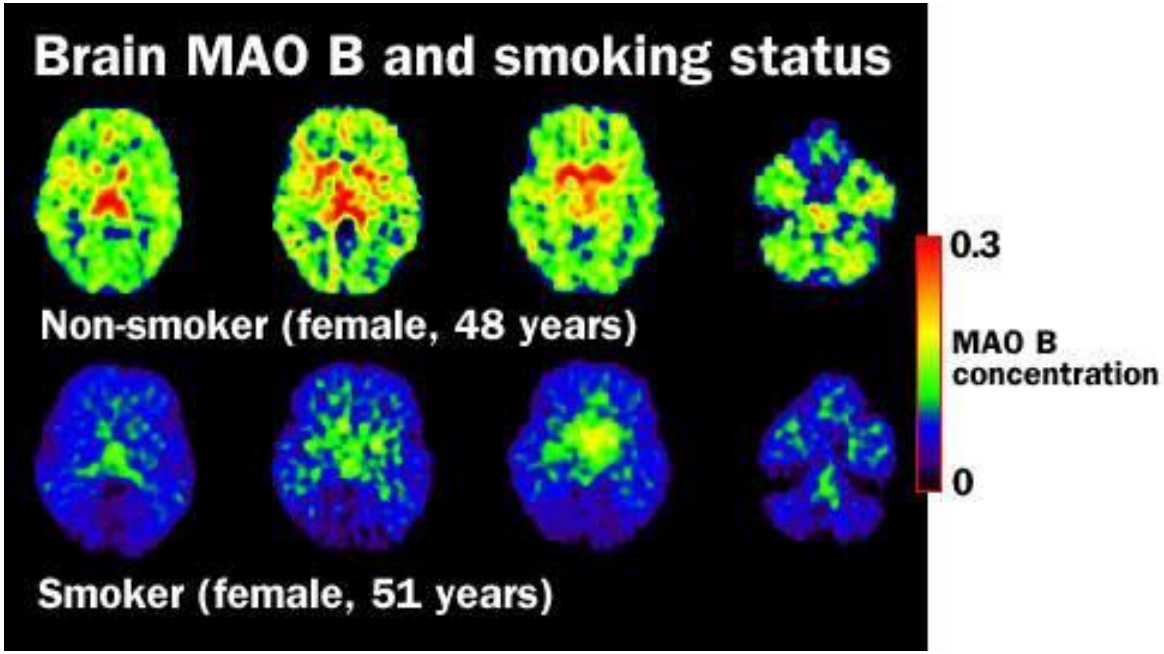
- Nicotine in smoke peaks in brain very rapidly, despite relatively slow increase in blood concentration
- A typical cigarette contains 20 mg of nicotine
- ~2.5 mg of nicotine is absorbed
- Half-life: ~ 2 hours
- 80-90% metabolized in liver

Pharmacokinetics

- Metabolism & Elimination
  - About 80 percent of nicotine is broken down to cotinine by enzymes in your liver (e.g., CYP2A6)
  - Nicotine is also metabolized in your lungs to cotinine and nicotine-N-oxide
  - Cotinine and the remaining nicotine is filtered from the blood by your kidneys and excreted in the urine

Smoking and MAO levels

Something in cigarette smoke seems to slow the breakdown of dopamine by affecting MAO levels

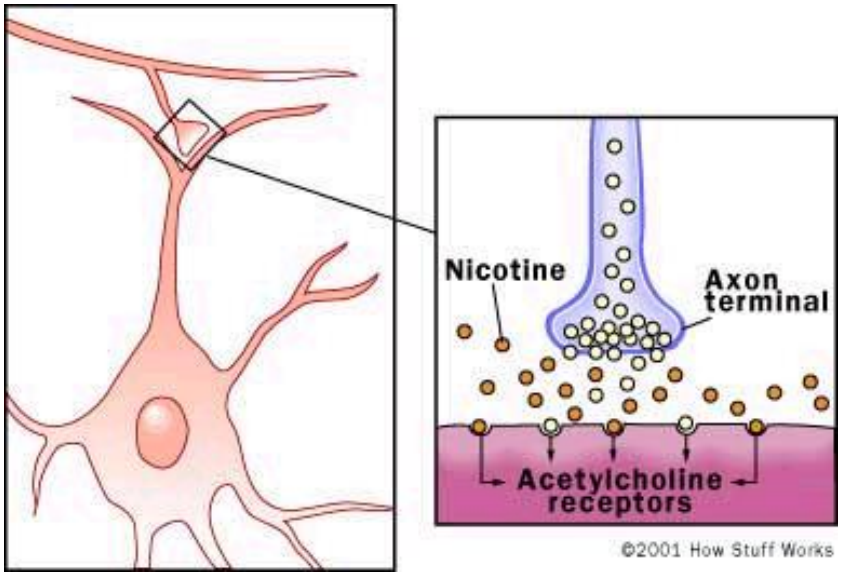


Novel treatment strategies based on genetic manipulation

- CYP2A6
  - ✓ Hepatic enzyme
  - ✓ 90% of inactivation of nicotine to cotinine
  - ✓ 17 allelic variants
  - ✓ The allelic variants vary in the ability to metabolize CYP2A6 substances
  - ✓ Population variability
  - ✓ The metabolic ratio of cotinine to nicotine varies among populations due to differences in CYP2A6 variants

Pharmacodynamics

- Nicotine is a direct agonist for nicotinic ACh receptors
- Nicotine initially causes a rapid release of adrenaline, the "fight-or-flight" hormone



Public health implications of CYP2A6 polymorphism

- ✓ Decreases risk of smoking initiation and dependence
- ✓ Decreases amount smoked
- ✓ Decreases risk of tobacco-related cancers and mutations

Risk of smoking initiation and tobacco dependence

- Null or defective CYP2A6 allele(s) or slow metabolizers [SMs].
  - ✓ CYP2A6*2 & CYP2A6*4
  - ✓ Use of greater levels of tobacco when they learn smoking.
  - ✓ Feeling of nicotine overdose.
  - ✓ Slower acquisition of withdrawal and tolerance.
  - ✓ Greater addiction
- Extensive metabolizers [EMs].
  - ✓ CYP*1/*1
  - ✓ Use as much as SMs use but it is normal for them.
  - ✓ don't feel nicotine overdose.
  - ✓ faster tolerance and withdrawal.

The number of cigarettes smoked and other smoking indices

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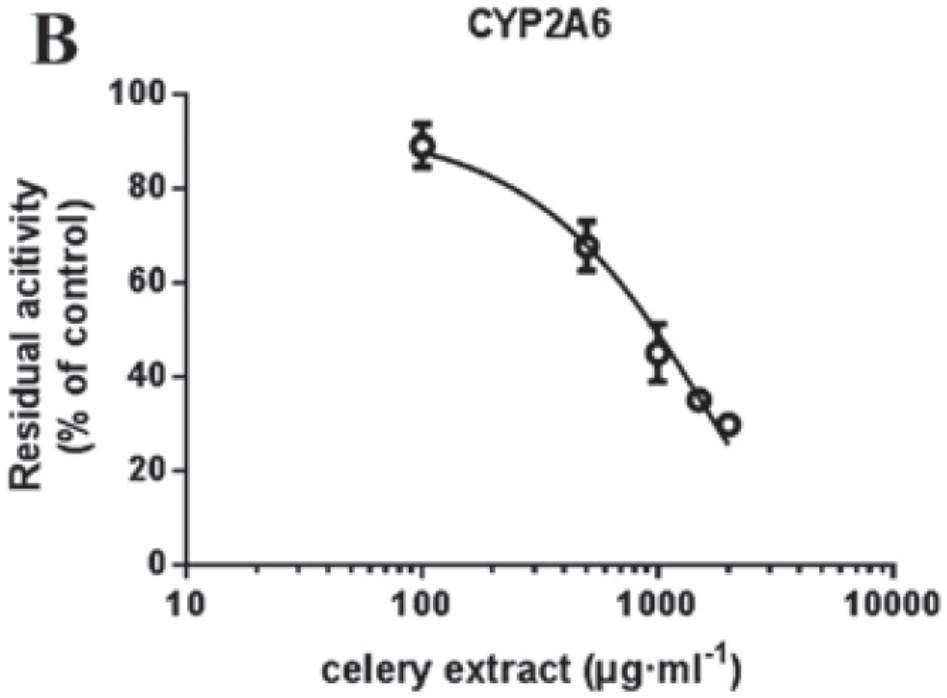
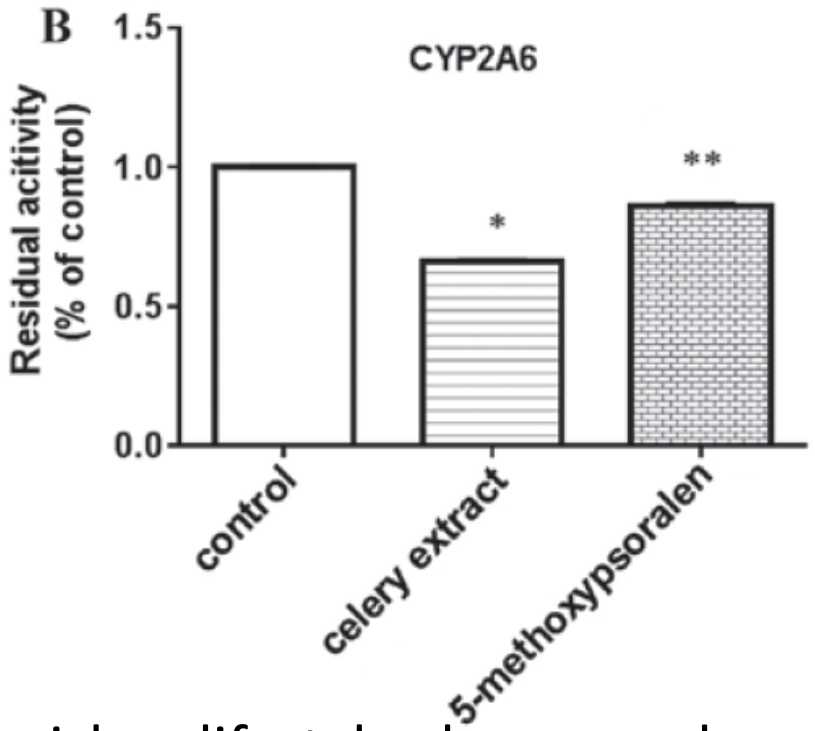
- SMs
  - ✓ lower CO breath conc.
  - ✓ lower plasma cotinine
    - (low metabolism of nicotine to cotinine)
- EMs or FMs
  - ✓ more CO breath conc.
  - ✓ more plasma cotinine
    - (high metabolism of nicotine to cotinine)
  - ✓ smoke each cigarette more intensely

Risk of tobacco-related cancers and mutations

- Carriers of one or more defective CYP2A6 alleles are in lower risk of lung cancer development
  - ✓ Decreased ability to bioactivate certain tobacco–specific pro-carcinogens.
- Carriers of active CYP2A6 alleles are in higher risk of lung cancer development
  - ✓ Increased ability to bioactivate certain tobacco–specific pro-carcinogens.
  - ✓ These pro carcinogens impact DNA

CYP2A6 INHIBITION BY CELERY





- Increased CYP2A6 -> increased cancer risk -> lifestyle change and incorporation of components that inhibit CYP2A6

CYP2C9 AND GENETIC POLYMORPHISM

Allele	SNP	CYP2C19 Function
*1	N/A	Normal function
*2	681G>A	Loss of function
*3	636G>A	Loss of function
*17	-808C>T	Gain of function

- Makes up almost 18% of cyp 450 associated proteins in liver
- Responsible for clearance of 15-20% of clinically used drugs

Warfarin Dosing - Background

- Commonly prescribed oral anti-coagulant and acts as an inhibitor of the vitamin K cycle

- Prescribed following MI, atrial fibrillation, stroke, venous thrombosis, prosthetic heart valve replacement, and following major surgery

- Warfarin (Coumadin) >20 million US prescriptions (2007)

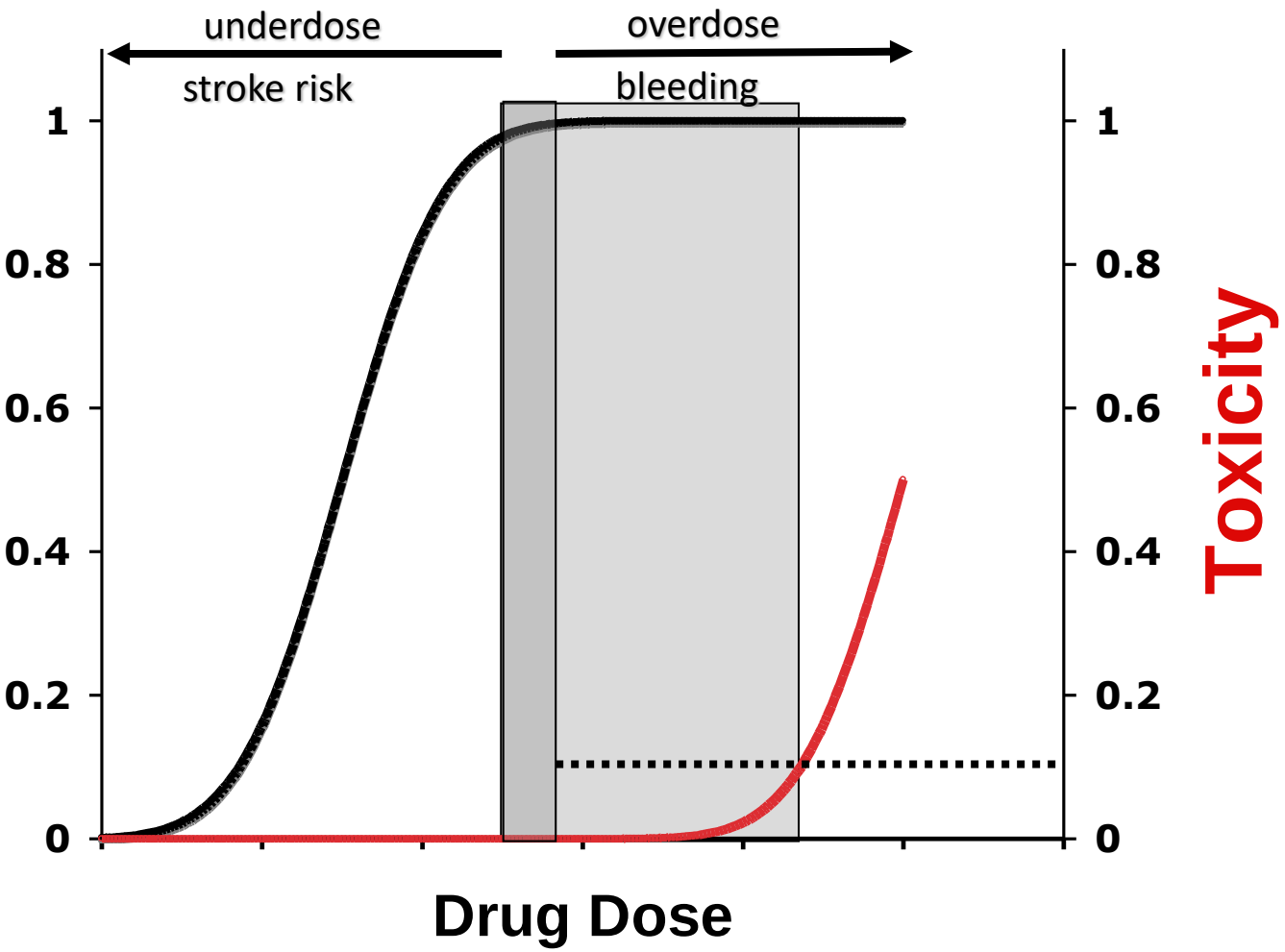
(-) Difficult to determine effective dosage
- Narrow therapeutic range
- Large inter-individual variation

(-) Major bleeding episodes in 1-2% of all patients

11% of all adverse events (Gurwitz et al. JAMA 2003)

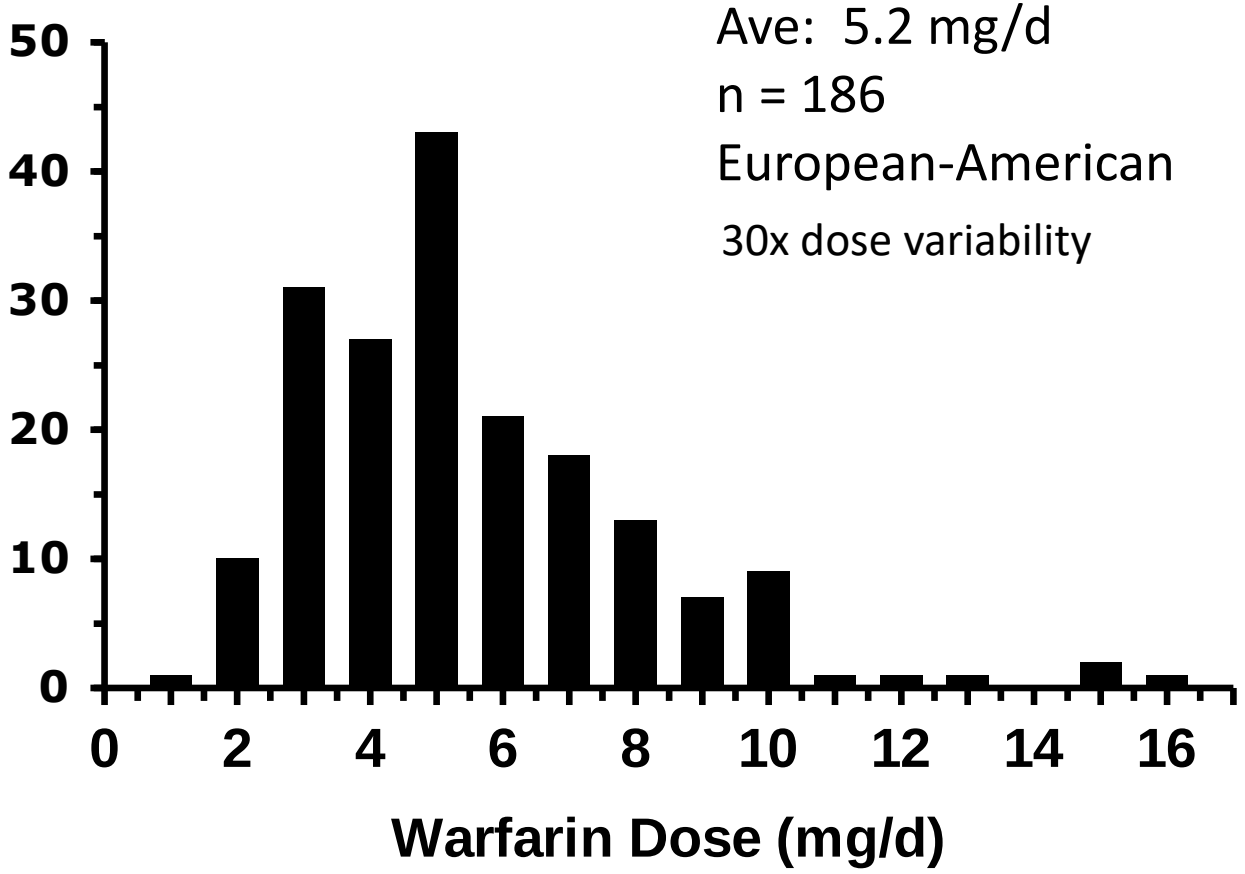
(++) Prevents 20 strokes for each bleeding event

Warfarin Dosing – Narrow therapeutic range



- Narrow therapeutic range
- Monitoring of INR (2.0 - 3.0) = Maintenance Dose

Warfarin Dosing – Narrow therapeutic range



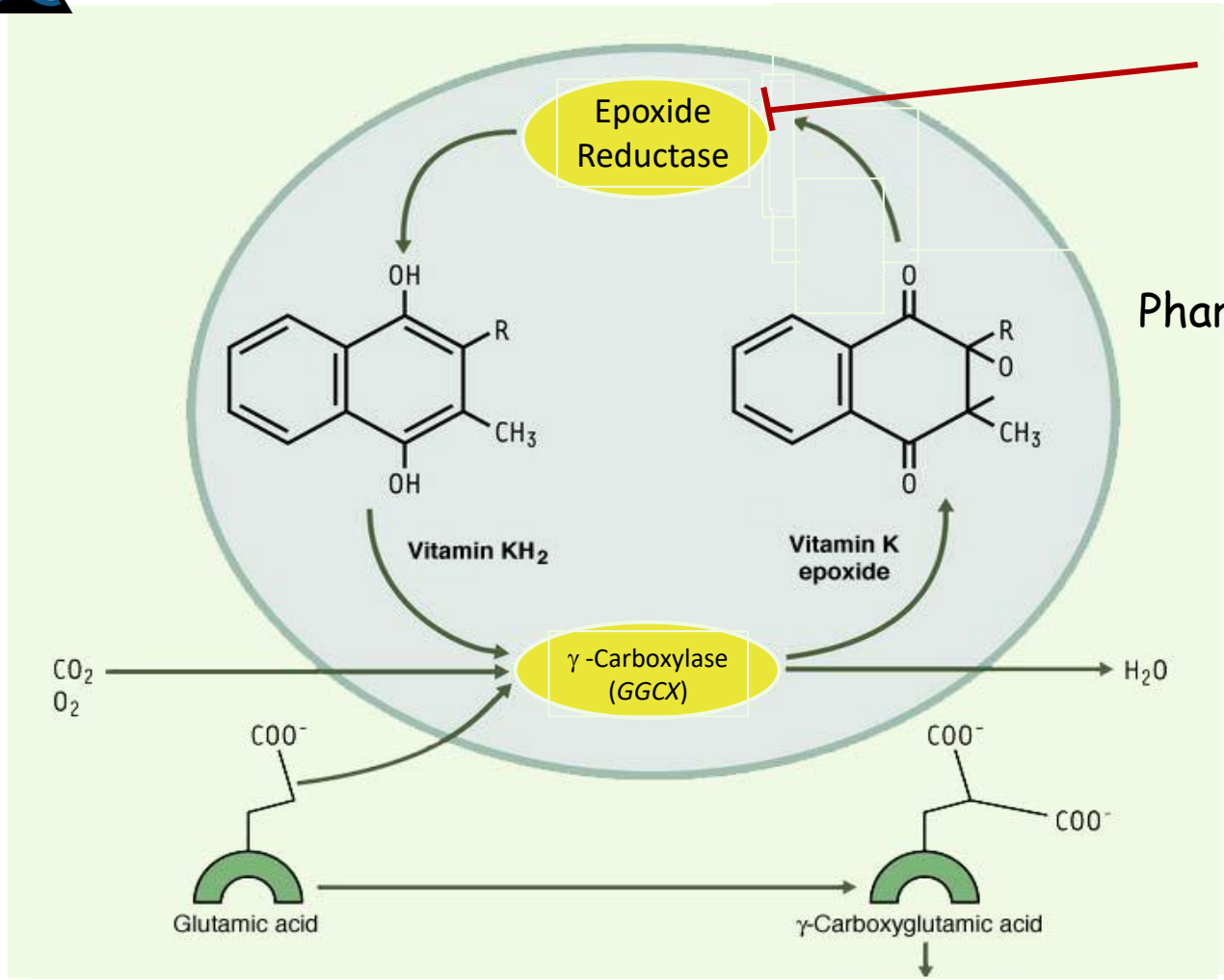
✓Patient/Clinical/Environmental Factors

✓Pharmacokinetic/Pharmacodynamic - Genetic

Vitamin K Cycle

- Vitamin K synthesized by plants and bacteria
  e.g., leafy green vegetables and intestinal flora
- Vitamin K - discovered from defects in blood "koagulation"
- Vitamin K - required coenzyme for the conversion of
  glutamic acid (Glu) to carboxyglutamic acid (Gla)
- Glu --> Gla modification needed for Ca^{2+} binding, clot formation
- Vitamin K administration is the antidote for warfarin toxicity

Warfarin inhibits the vitamin K cycle



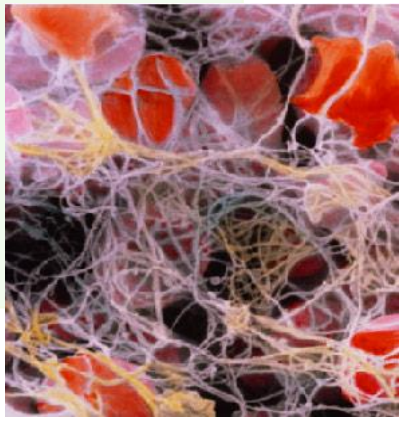
Warfarin

↓ CYP2C9

Inactivation

Pharmacokinetic

Vitamin K-dependent clotting factors
(FII, FVII, FIX, FX, Protein C/S/Z)



Warfarin drug metabolism

- Major pathway for termination of pharmacologic effect is through metabolism of S-warfarin in the liver by CYP2C9
- CYP2C9 SNPs alter warfarin metabolism:
  CYP2C9*1 (WT) - normal
  CYP2C9*2 (Arg144Cys) - intermediate metabolizer
  CYP2C9*3 (Ile359Leu) - poor metabolizer
- CYP2C9 alleles occur at:
  European: *2 - 10.7% *3 - 8.5 %
  Asian: *2 - 0% *3 - 1-2%
  African-American: *2 - 2.9% *3 - 0.8%

Effect of CYP2C9 Genotype on Anticoagulation-Related Outcomes

(Higashi et al., *JAMA* 2002)

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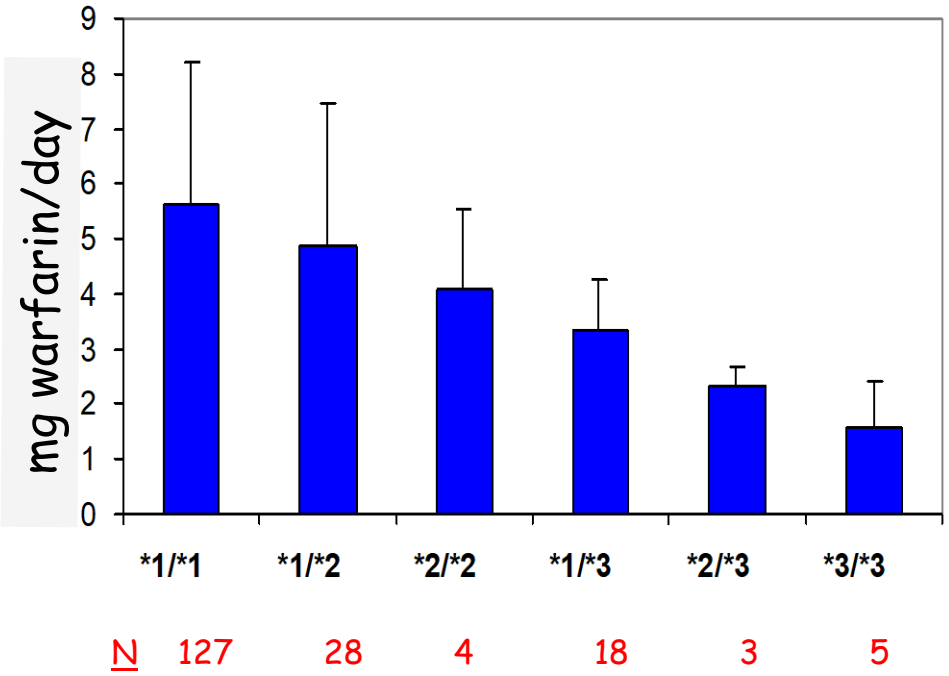
WARFARIN MAINTENANCE DOSE



TIME TO STABLE ANTICOAGULATION

CYP2C9-WT ~90 days

CYP2C9- Poor metabolizer Variant ~180 days
*2 or *3 carriers take longer to reach stable anticoagulation

- Variant alleles have significant clinical impact
- Need to understand CYP2C9 to offer therapeutic efficacy
- Low CYP2C9 metabolism -> Reduce Warfarin dose -> increased toxicity risk

CYP2C19

- Key metabolizer of drugs – 10% of drugs
- Plavix
- Anti-ulcer drugs

CYP2C19 Phenotypes

Genotype	Phenotype
*1/*1	Normal Metabolizer (NM)
*1/*2, *1/*3	Intermediate Metabolizer (IM)
*2/*2, *2/*3	Poor Metabolizer (PM)
*1/*17	Rapid Metabolizer (RM)
*17/*17	Ultra-rapid Metabolizer (UM)

Race	PMs	IMs	RM/UM
Whites	2%	25%	40%
Blacks	4%	30%	45%
Asian	14%	50%	<5%

CYP2C19 – PROTON PUMP INHIBITORS – ULCER DRUGS

Phenotype	Phenotype details	Examples of diplotypes	Therapeutic recommendations for omeprazole
Ultrarapid metabolizer	Normal or increased CYP2C19 activity	**17/*17*	Be extra alert to insufficient response. For the eradication of *H. pylori* , increase dose by 100–200%. For other conditions, consider dose increase by 100–200%.
Extensive metabolizer	Normal CYP2C19 activity	**1/*1*	Dose recommended by drug label
Intermediate metabolizer	Decreased CYP2C19 activity	**1/*2* **1/*3* **2/*17* **3/*17*	Dose recommended by drug label
Poor metabolizer	Markedly reduced or absent CYP2C19 activity	**2/*2* **2/*3* **3/*3*	Dose recommended by drug label

Clopidogrel (Plavix)

1. An antiplatelet drug used in patients with cardiovascular disease to reduce risk for heart attack, stroke, unstable angina, and cardiovascular death. The liver's cytochrome P450 (CYP) system converts it to its active metabolite. Several genotypes of the liver enzyme exist in humans: CYP2C19* 2,*3, *4, *5, *6, *7, and *8.

2. There are subgroups of patients (2-14% of the population) who are *poor* metabolizers of clopidogrel because of genetic differences (genetic polymorphisms) in this enzyme. Racial background is also a factor. As a result, these patients do not get the drug's full benefit and have a higher risk for cardiac, cerebrovascular, and peripheral arterial events.

CYP2C19and Clopidogrel

Clopidogrel is a prodrug that requires bioactivation by CYP2C19

CYP2C19 loss-of-function (LOF) alleles

- Lead to reduced or absent enzyme activity
- Impair ability to activate clopidogrel
- Reduce effectiveness of clopidogrel after PCI

CYP2C19 - Clopidogrel

Outcomes Based on RCT and Registry Post-Hoc Analyses

Meta-analysis of 9 trials and 9685 clopidogrel-treated high risk patients

Outcome	LOF vs non-LOF
MACE*	HR 1.57 (1.13-2.16)
Stent Thrombosis	HR 2.81 (1.81-4.37)

*Major adverse cardiovascular events (CV death, MI, or stroke)

LOF=Loss of function

Mega JL, et al. *JAMA* 2010;304:1821-30.

CYP2C19

- Ultrarapid metabolizer – Need to increase proton pump inhibitor dose for efficacy
- Poor metabolizer – Higher risk of Clopidogrel adverse effects and at the same time drug does not have efficacy since CYP2C19 converts the drug into the bioactive form
- Induction or inhibition need depends on the needs and the health condition of the individual
- Ginger inhibits CYP2C19

CYP2D6

- CYP2D6 is responsible for the metabolism and elimination of approximately 25% of clinically used drugs, via the addition or removal of certain functional groups
- Certain prodrugs are activated by CYP2D6
- Largest variability among drug metabolizing enzymes due to genetic variation
- Almost 7-10% Caucasians lack complete activity of CYP2D6

Human Polymorphism at CYP2D6

- Oxidative metabolism of over 40 common drugs
- More than 50 different alleles have been identified
- 5-10% Caucasians have null alleles, and no function
- 7% Caucasians have duplication causing excessive function due to excessive expression of the enzyme
- Many intermediate levels of functioning

Various CYP alleles in Caucasians

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TABLE 3. *Cytochrome P450 (CYP) allele subgroups, characteristic mutation(s), enzyme activity among Caucasians*

Designation	Characteristic mutation(s)	Enzyme activity	Allelic frequency (%)	Reference
*CYP2D6*1*	Wild type	Normal		
*CYP2D6*2*	$G_{1749}C$, $C_{2938}T$, $G_{4268}C$ substitutions	Normal	30	11
*CYP2D6*3*	A_{2637} deletion	Deficient	2	12
*CYP2D6*4*	$G_{1934}A$ substitution	Deficient	22	13
*CYP2D6*5*	Gene deletion	Deficient	2	14
*CYP2D6*6*	T_{1795} deletion	Deficient	2	15
*CYP2D6*7*	$A_{3023}C$ substitution	Deficient	0·1	16
*CYP2D6*8*	$G_{1846}T$ substitution	Deficient	0·1	9
*CYP2D6*9*	(A_{2701}–A_{2703}) or (G_{2702}–A_{2704}) deletion	Decreased	1·5	17
*CYP2D6*10*	$C_{188}T$, $G_{1749}C$, $G_{4268}C$ substitutions	Decreased	1·5	18
*CYP2D6*11*	$G_{971}C$ substitution	Deficient	0·1	19
*CYP2D6*12*	$G_{212}A$ substitution	Deficient	0·1	20
*CYP2D6*13*	Hybrid: *2D7* exon 1, *2D6* exons 2–9	Deficient	0·1	21
*CYP2D6*14*	$G_{1846}A$ substitution	Deficient	0·1	9
*CYP2D6*15*	T_{226} insertion	Deficient	0·1	22
*CYP2D6*16*	Hybrid: *2D7* exons 1–7, *2D6* exons 8–9	Deficient	0·1	21
*CYP2D6*1* × 2	Gene duplication	Increased	1	23
*CYP2D6*2* × 2	Gene duplication	Increased	1·5	11
*CYP2D6*4* × 2	Gene duplication	Deficient	0·5	24
*CYP2C19*1*	Wild type	Normal		
*CYP2C19*2*	$G_{681}A$ substitution exon 5	Deficient	15	25
*CYP2C19*3*	$G_{636}A$ substitution	Deficient	0·3	26
*CYP2C19*4*	A_1G substitution	Deficient	0·6	27

Extra copy of CYP 2D6 (gene duplication)

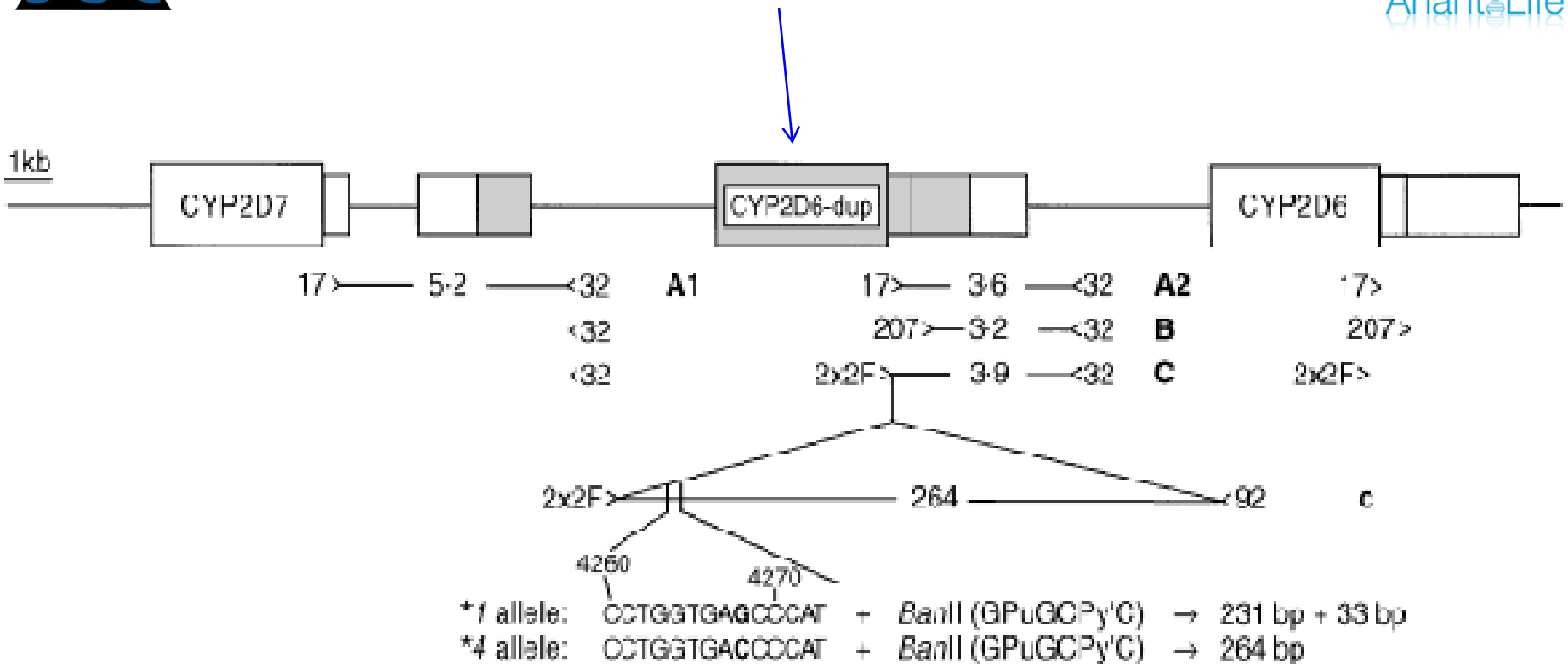
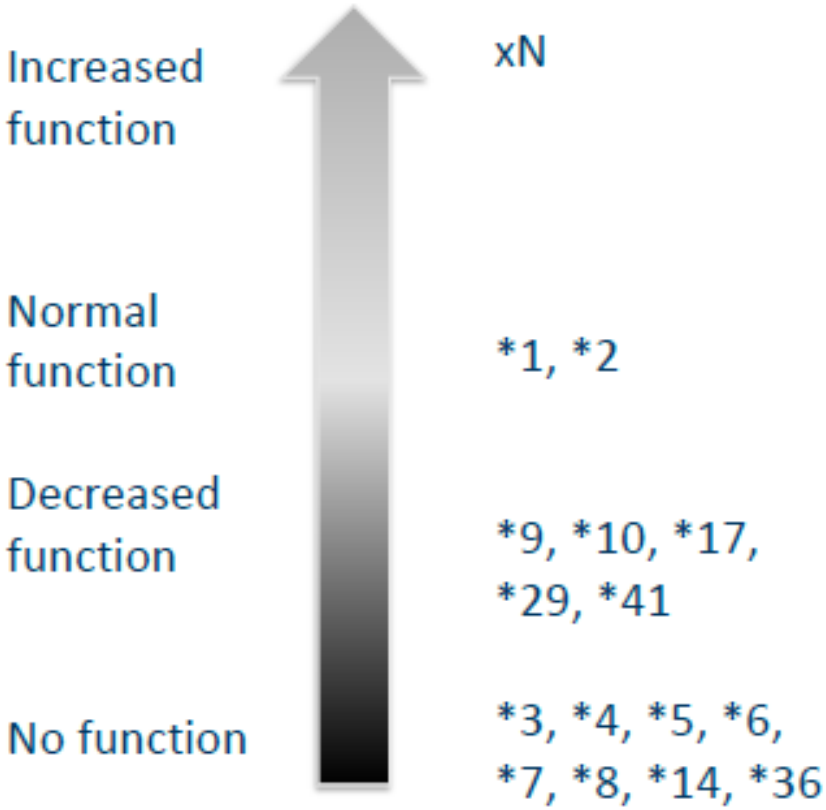


FIGURE 1. *Genomic organization of an allele with duplicate* CYP2D6 *genes. The shaded area indicates the 'extra' sequence compared with a 'normal' allele. The polymerase chain reaction-based method for identification of* CYP2D6 *gene duplication is depicted schematically: A1 = internal control product; A2 = product indicative for gene duplication; B = positive control product; C and c = identification of *4 allele duplication (only in the case of the *1/*4 genotype). Reprinted in part from Lovlie* et al.,[50] *with permission from Elsevier Science.*

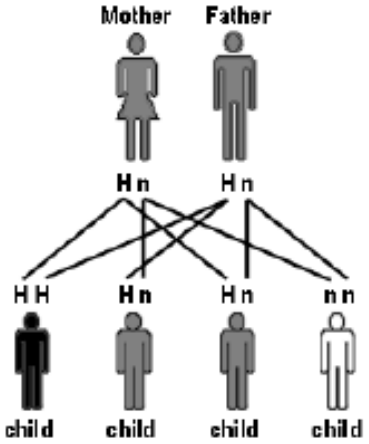
Ann Clin Biochem 1999: **36**

CYP2D6 ALLELES



CYP2D6 ALLELES

Allele 1	Allele 2	Gene duplication	Phenotype
Normal function	Normal function	Yes	Ultra-rapid metabolizer
Normal function	Increased function	No	Rapid metabolizer
Normal function	Normal function	No	Normal metabolizer
No function	Decreased function	No	Intermediate metabolizer
No function	No function	No/Yes	Poor metabolizer



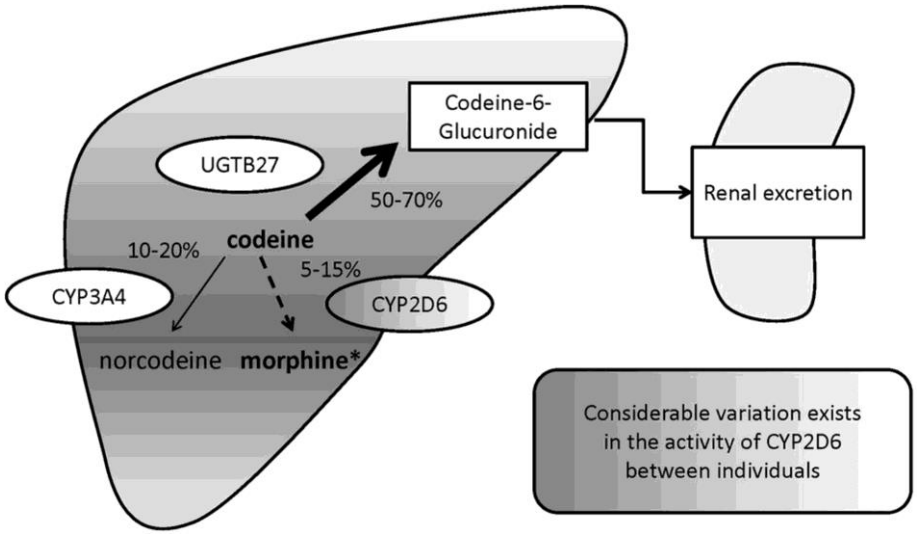
Caudle et al, *Genetics in Medicine*, 2016

CYP2D6 – CODEINE METABOLISM

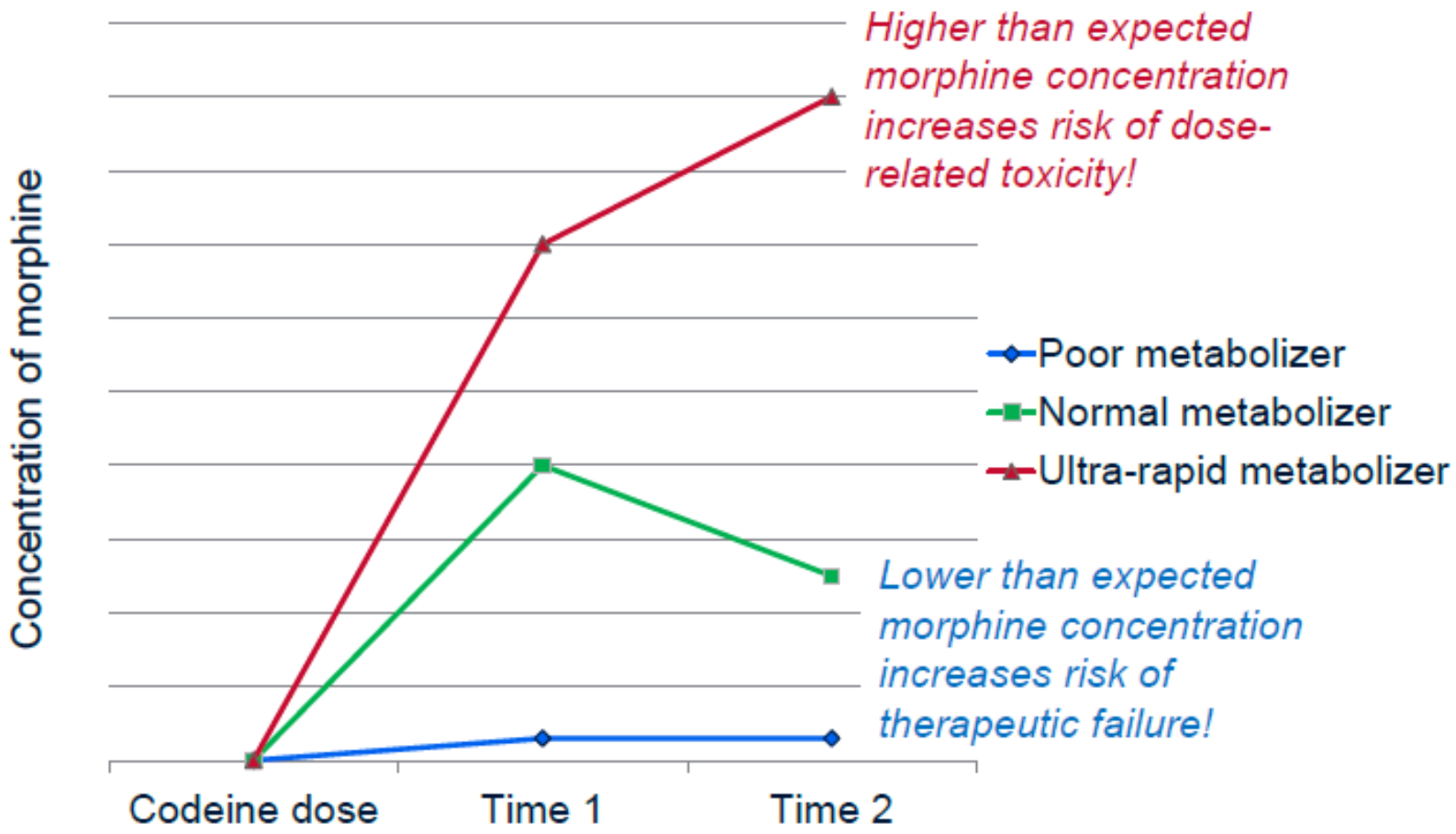
CYP2D6 enzyme converts codeine into morphine (active drug)

Individual can have increased/decreased CYP2D6 activity based on their genetics

Increased CYP2D6 activity -> Greater conversion of Codeine to Morphine -> Risk of morphine overdose with codeine use



CYP2D6 – CODEINE METABOLISM



CYP2D6 AND ANTI-DEPRESSANTS

- Ultra rapid metabolizers of CYP2D6 associated with greater rates of suicides
- Rapid metabolism of anti-depressants
- CYP2D6 also expressed in brain and impacts serotonin and dopamine pathways
- Poor metabolism of CYP2D6 -> increased risk of adverse effects with anti-depressants use
- Lower medicine dose for poor metabolizers

CYP3A4

- The CYP3A4 protein localizes to the endoplasmic reticulum, and its expression is induced by glucocorticoids and some pharmacological agents.
- Most drugs undergo deactivation by CYP3A4, either directly or by facilitated excretion from the body. Also, many substances are bioactivated by CYP3A4 to form their active compounds, and many protoxins being converted into their toxic forms
- Intestinal expression of CYP3A4 plays a major role in drug metabolism for certain drugs
- Important for drug metabolism
- Genetic polymorphisms impact activity

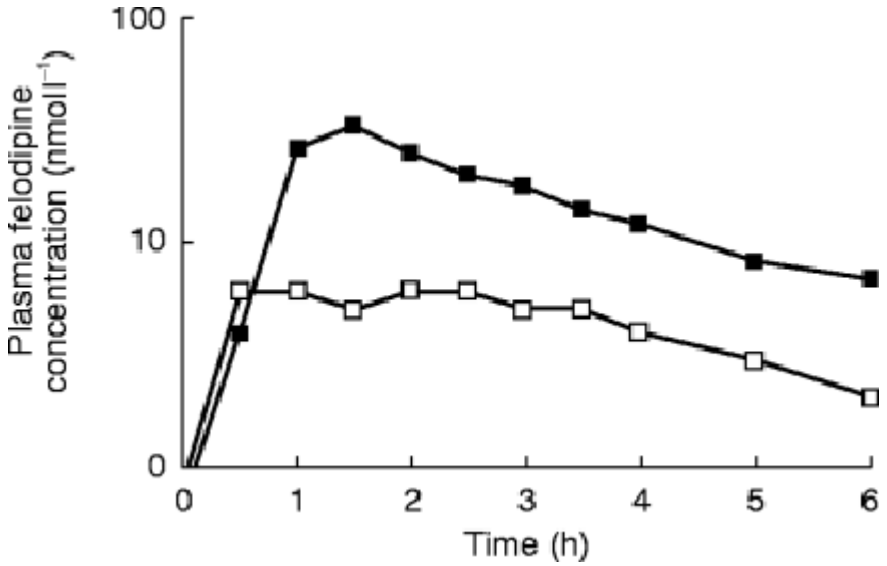
CYP3A4 POLYMORPHISM

- Polymorphisms associated with reduced activity
- Several cancer drugs have toxic effects if not broken down rapidly
- Reduced CYP3A4 activity -> Increases bioavailability of cancer drugs -> Increased toxicity
- CYP3A4 - metabolizes anaesthetics such as Midazolam
- Reduced CYP3A4 activity -> Increases bioavailability of anaesthetic -> Increases sedation

GRAPEFRUIT JUICE CYP3A4

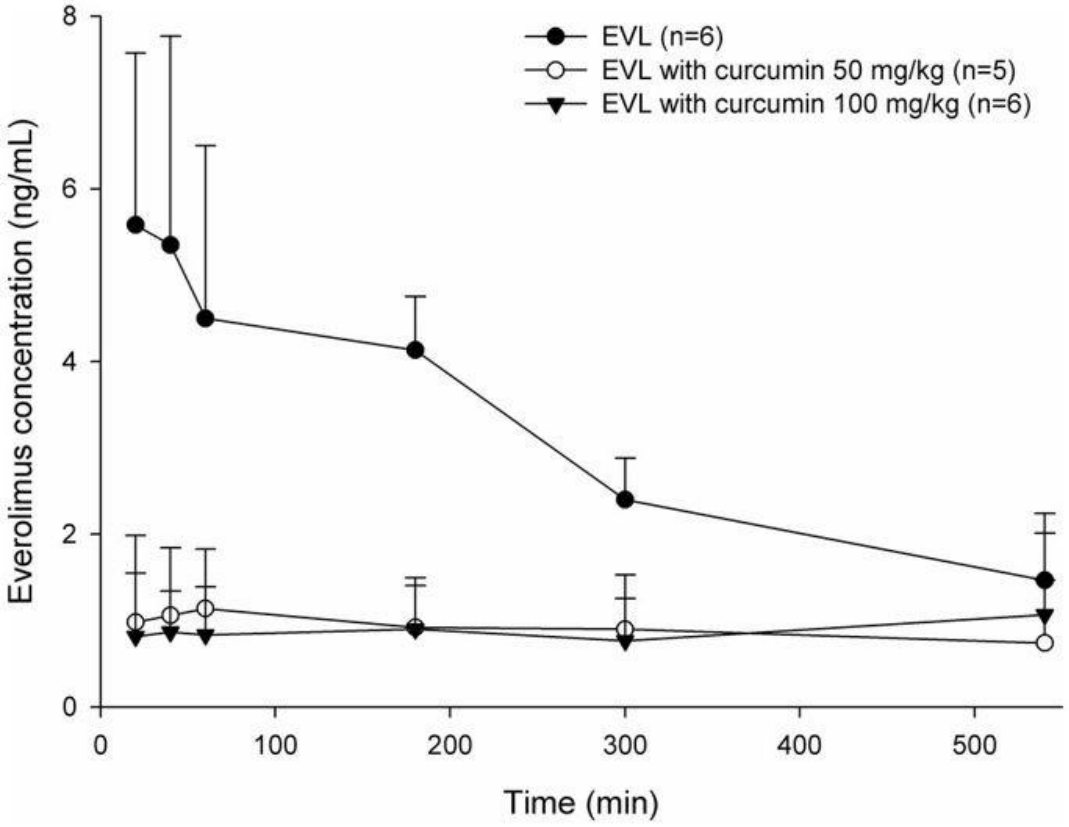
- Potent inhibitor of CYP3A4
- Increases bioavailability of drugs which are metabolized by CYP3A4
- Felodipine – drug for high blood pressure – bioavailability increases by upto 280 % if combined with Grapefruit Juice
- Impacts intestinal enterocyte CYP3A4 activity

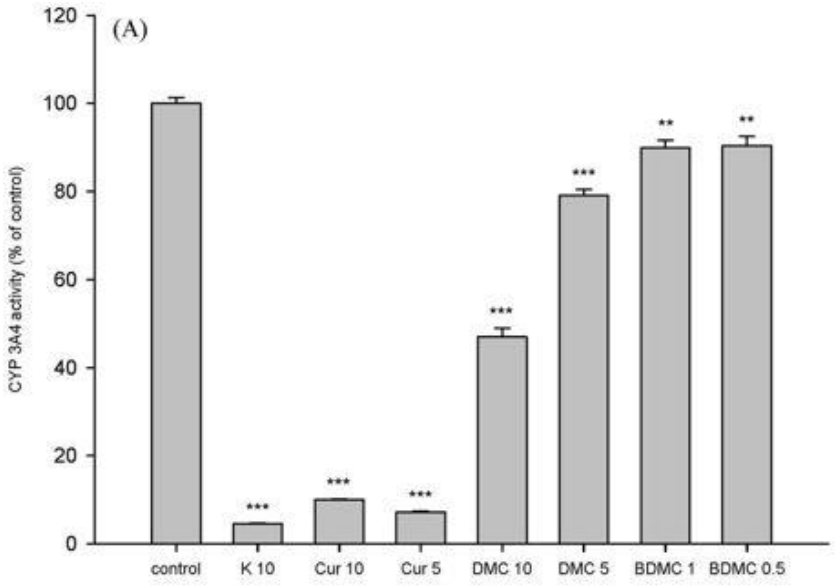
Individuals with reduced CYP3A4 activity – further prone to effects of grapefruit juice



CYP3A4 AND CURCUMIN

- Oral curcumin intake induces CYP3A4
- Demethoxy curcumin can suppress CYP3A4 inhibition
- Low CYP3A4 activity associated with cancer in a few studies





CYP3A5

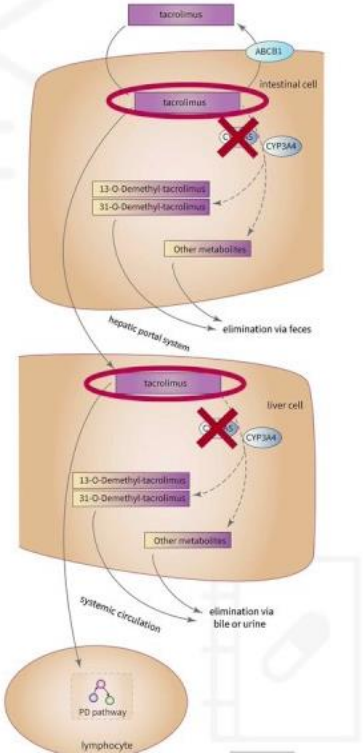
- One of the most prominently expressed enzyme in the liver
- This enzyme is involved in an NADPH-dependent electron transport pathway.
- It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics.
- Important for metabolism of tacrolimus and immune modulatory drugs
- Major allele is the one which results in reduced enzymatic activity.

CYP3A5 AND TACROLIMUS

- Tacrolimus - immunosuppressive drug used mainly after allogeneic organ transplant to lower the risk of organ rejection.

Metabolism of tacrolimus by CYP3A5 → inactivation of drug

CYP3A5 poor metabolizers → decreased metabolism of tacrolimus compared to normal and intermediate metabolizers → improved chance of reaching target tacrolimus concentrations



CYP3A5 POLYMORPHISM

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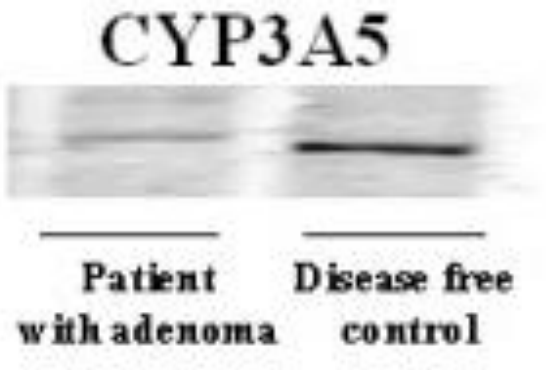
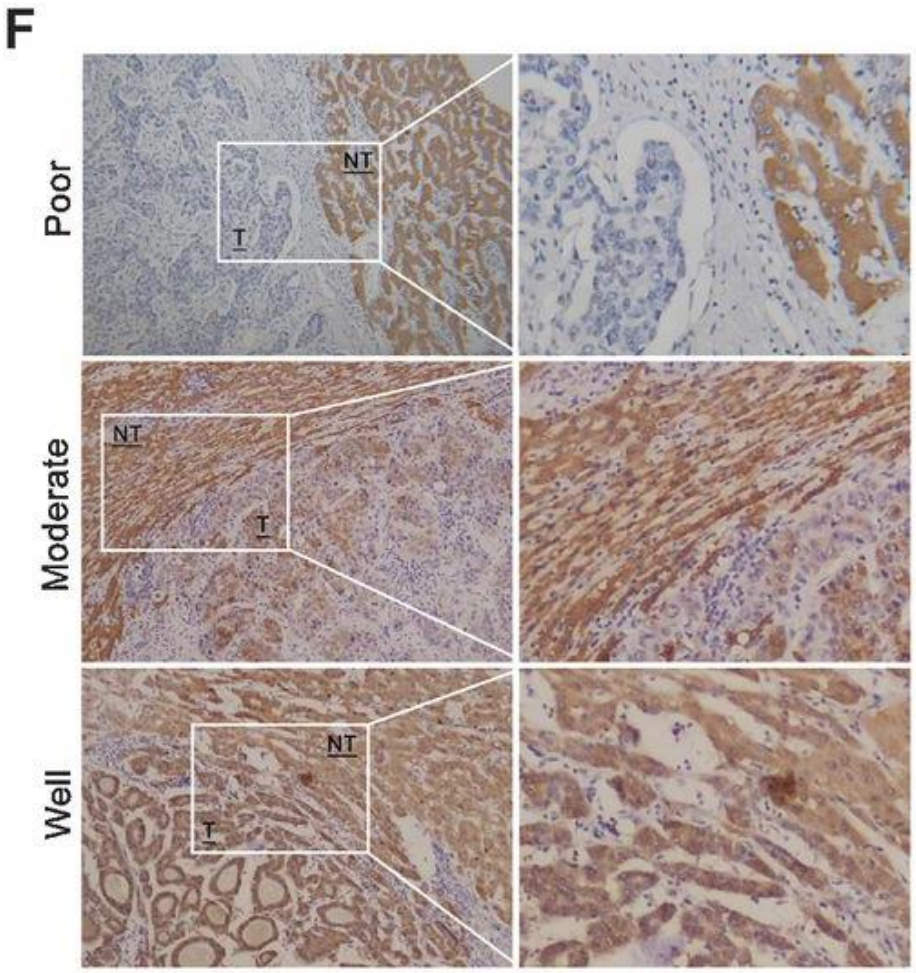
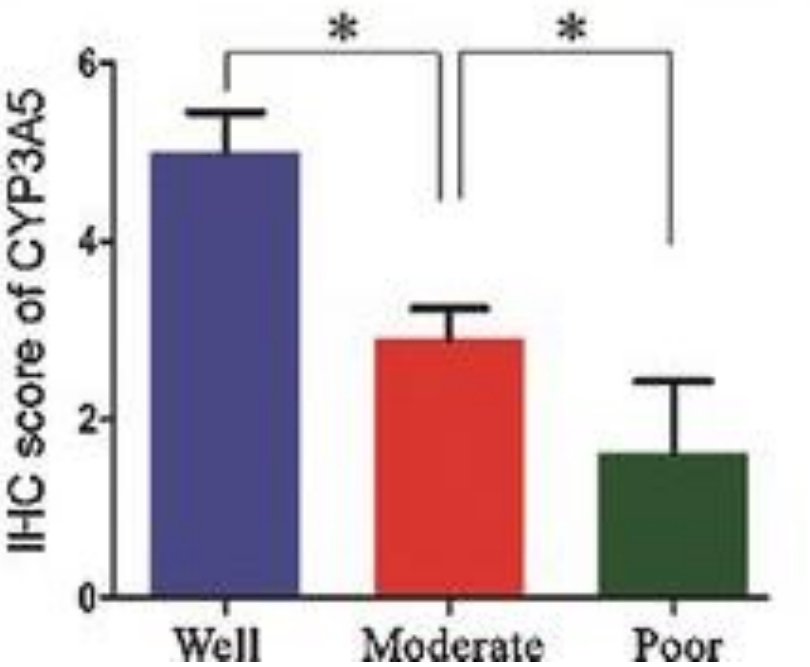
- CYP3A5 associated polymorphisms with reduced activity – Slower breakdown of tacrolimus
- Lower therapeutic dose
- At high dose, tacrolimus stays in blood stream – toxic effects
- Similar consequences with other immune modulatory drugs
- Drugs for suppressing inflammation
- Low activity alleles also associated with increased risk of colon cancer and leukemia
- Risk reduction

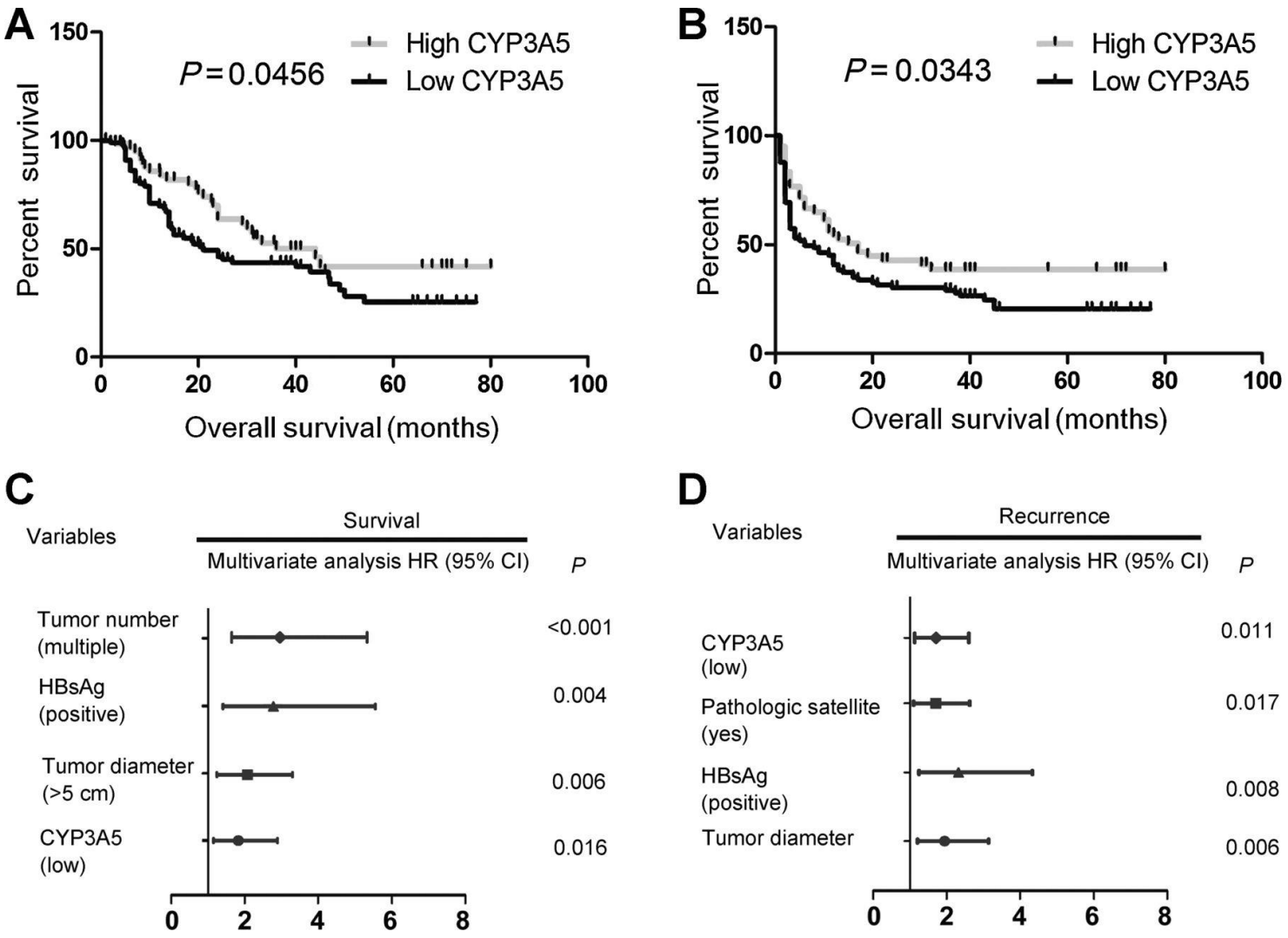


CYP3A5 AS TUMOR SUPPRESSOR

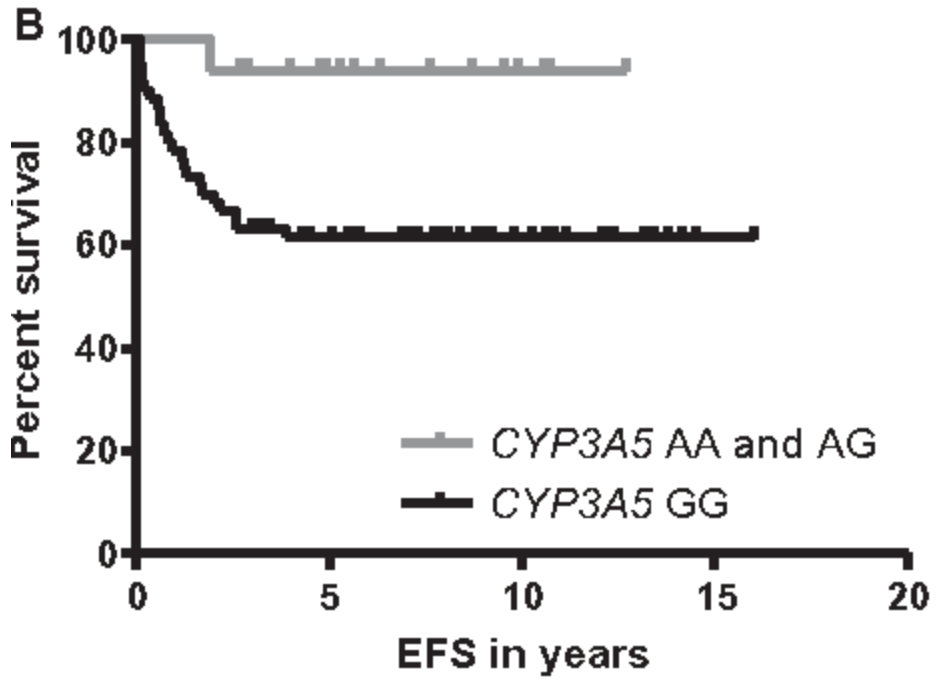




CYP3A5 AS TUMOR SUPPRESSOR



CYP3A5 AND LEUKEMIA



- If poor metabolizer -> increased cancer risk -> Avoidance of toxins and lifestyle change

CYP3A5 genotype	Patients (frequency)	Controls (frequency)
AA	6 (0.010)	0 (0)
AG	89 (0.144)	21 (0.103)
GG	521 (0.846)	182 (0.897)

Phase II: Conjugation

- In phase Ⅰ reactions, xenobiotics are generally converted to more polar, hydroxylated derivatives.
- In phase Ⅱ reactions, these derivatives are conjugated with molecules such as glucuronic acid, sulfate, or glutathione.
- This renders them even more water-soluble, and they are eventually excreted in the urine or bile.
- Acetylation, Glutathione conjugation, Methylation

Glutathione (GSH) Conjugation

- DETOXIFICATION of electrophiles!
- Electrophilic chemicals cause:
  - Tissue necrosis
  - Carcinogenicity
  - Mutagenicity
  - Teratogenicity
- The thiol (SH group) ties up potent electrophiles

Glutathione S-transferase

(+)benzo[a]pyrene-
7,8-dihydrodiol-
9-10-epoxide

DNA reactive; lung and skin tumors

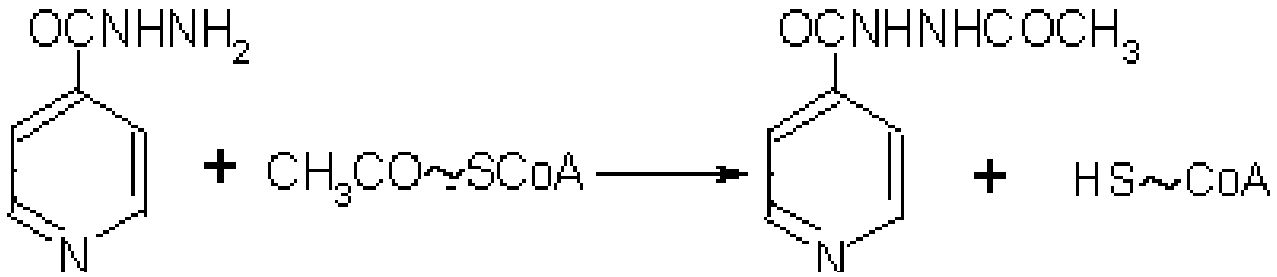
Inactive

DETOXIFICATION

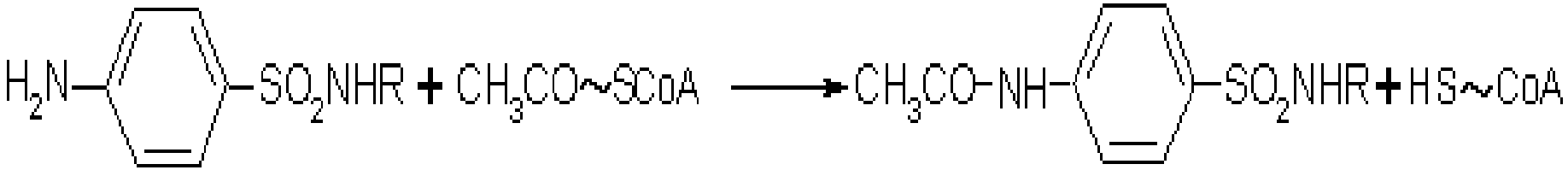
Acetylation

X + Acetyl-CoA - - - - >Acetyl-X + CoS
where X represents a xenobiotics.
(for: aromatic amines)

- **Enzyme: acetyltransferases**
  - **present in the cytosol of various tissues, particularly in liver.**



isoniazid



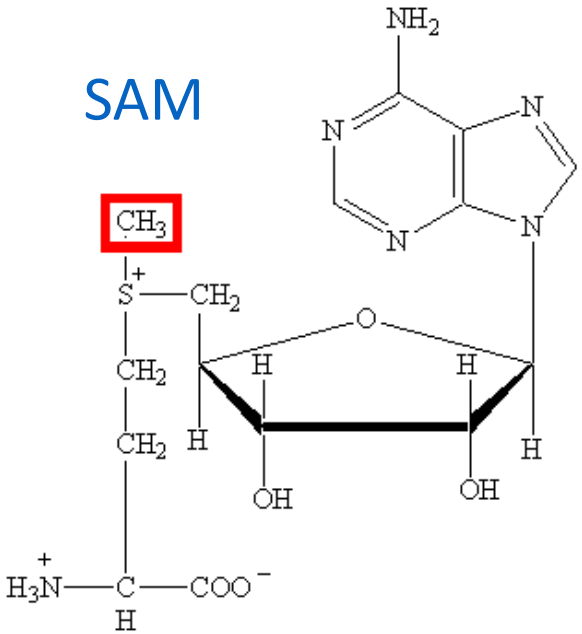
sulfanilamide

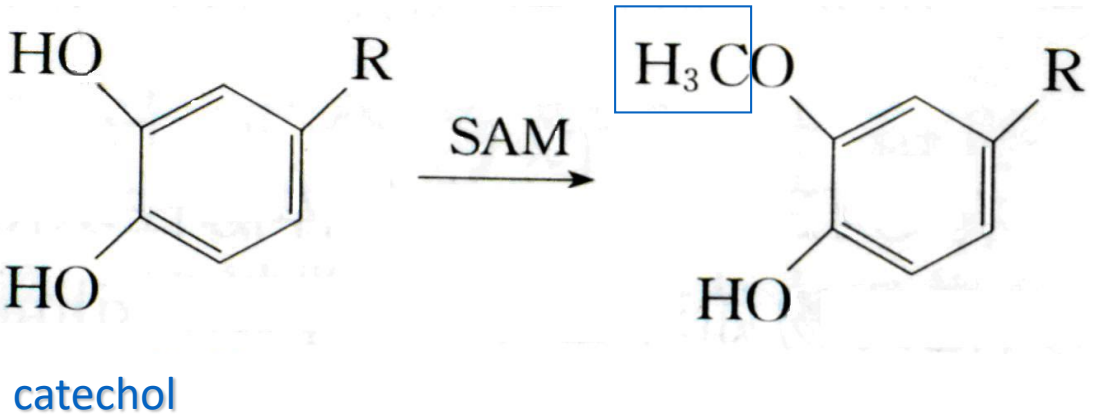
- Important for drugs with primary amino groups
- Generally, metabolites are nontoxic and inactive
- Acetylation does NOT increase water solubility
- Detoxification or termination of drug activity

Methylation

- A few xenobiotics are subject to methylation by methyltransferase, emplyoing S-adenosylmethione(SAM) as the methyl donor.

SAM





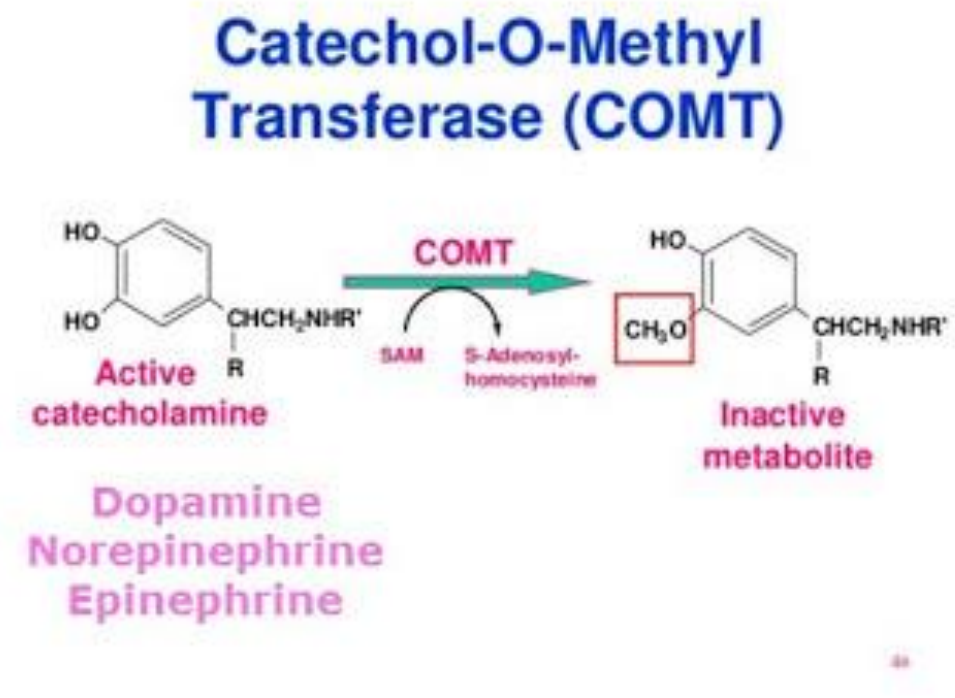
catechol

Metabolism via Methylation

- Key for biosynthesis of many compounds
- Important in the inactivation of physiologically active biogenic amines → neurotransmitters
  - norepinephrine, dopamine, serotonin, histamine
- *Minor* pathway in the metabolism of drugs
- Methylation does NOT increase water solubility
- Most methylated products are inactive

COMT

Codes for Catechol-O-Methyl Transferase, a enzyme that degrade catecholamines (such as dopamine, epinephrine, and norepinephrine), catecholestrogens, and various drugs and substances having a catechol structure



COMT: How do we get there from here ?

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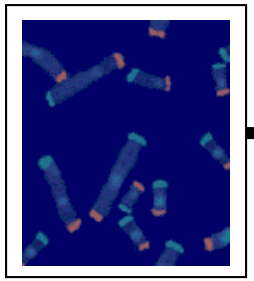
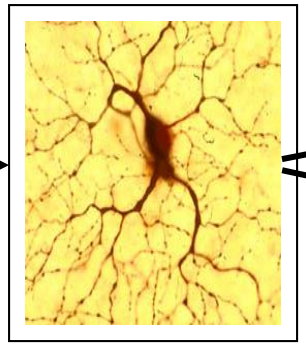
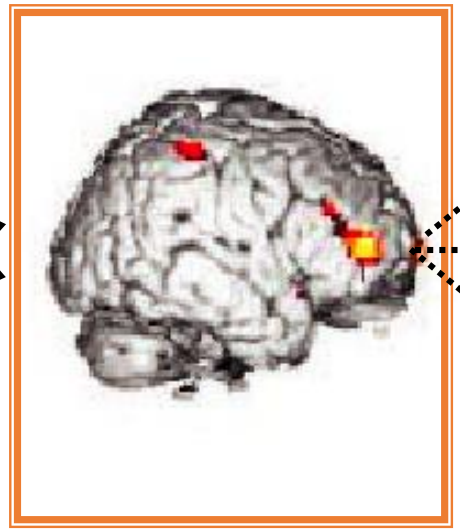






psychosis,
OCD, anxiety,
drug abuse,
bipolar disorder,
aggression,
impulse control,
pain sensitivity…

COMT:

val[158]met polymor-phism

Valine - WT

Met - Mut – Low activity

Cells:

Systems:

Behavior:

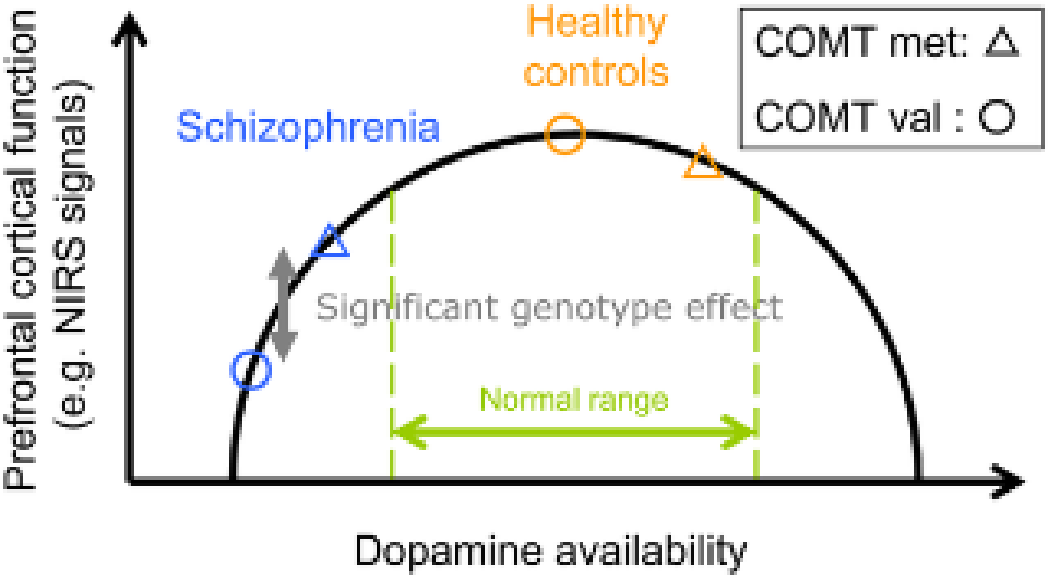
complex functional interactions and emergent phenomena

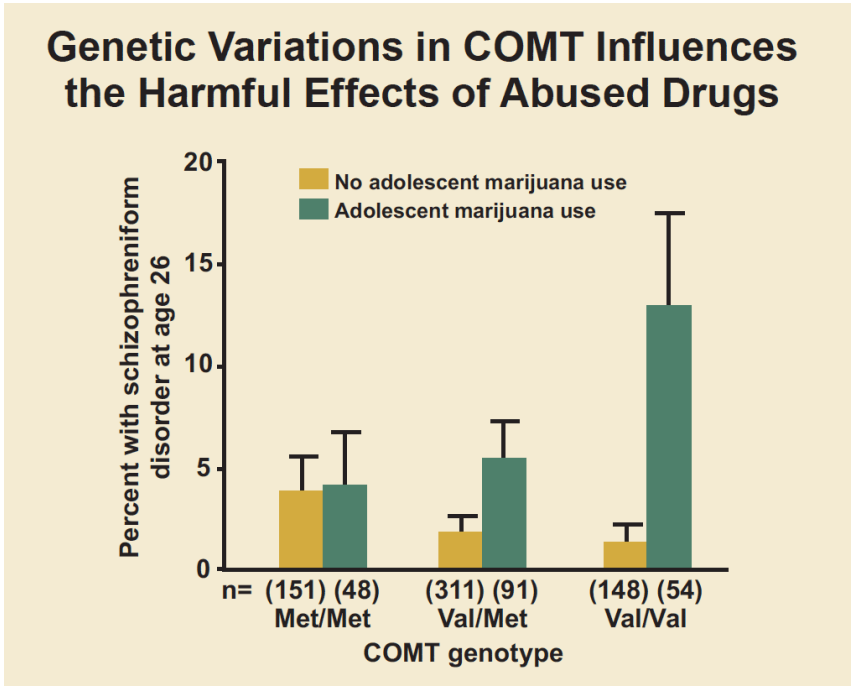
COMT Polymorphisms

Low activity – Increased dopamine levels
Increased stress sensitivity but improved memory and attention to detail

High activity – Reduced dopamine levels capacity to deal with stress , at the expense of a reduction in cognitive performance in non-stressful environments

COMT Polymorphisms





Val/Val - Hyperefficient COMT

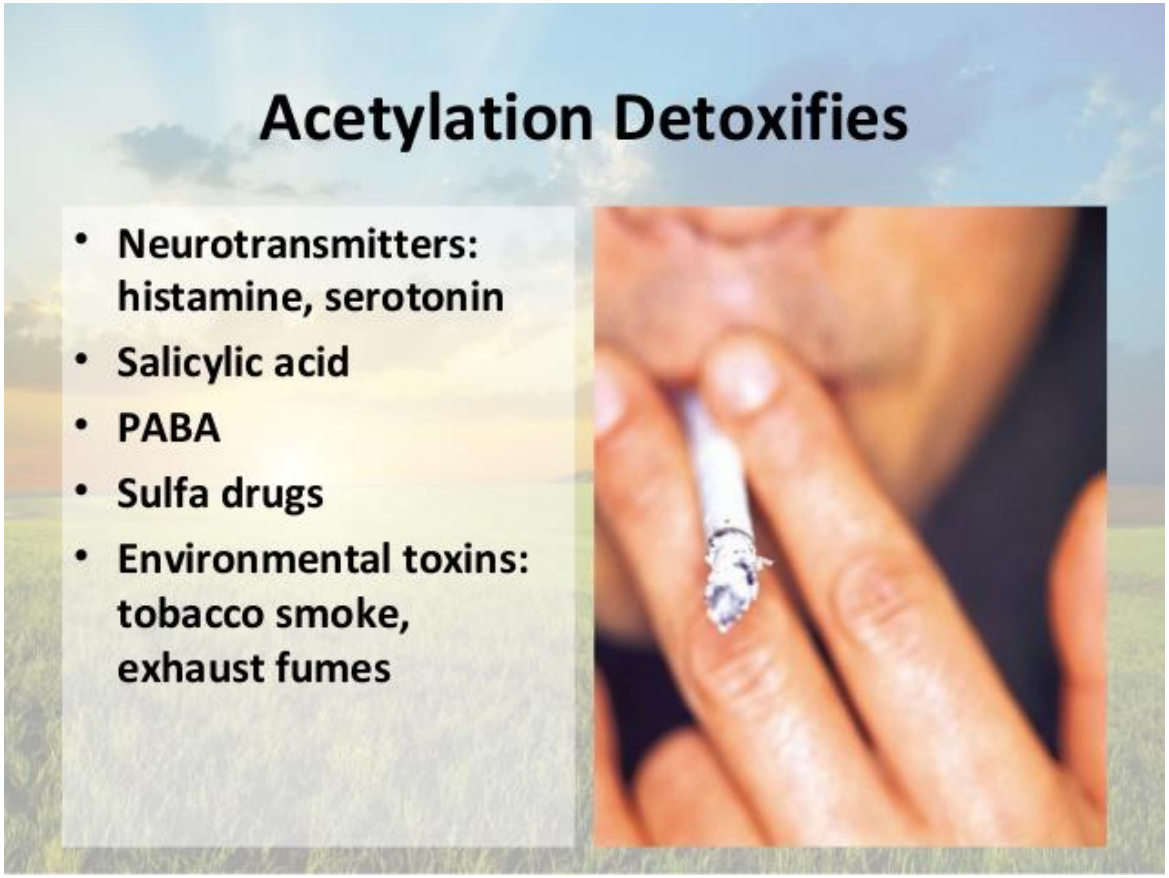
Met/Met Reduced COMT

MODULATION OF COMT ACTIVITY

- Magnesium is a co-factor for COMT activity
- Low COMT activity – increasing magnesium intake may help increase COMT activity
- Green tea is an inhibitor of COMT activity
- COMT Met/Met at increased risk of Cannabis induced effects – Reduce cannabis consumption and further try to inhibit COMT

ACETYLATION - DETOXIFICATION

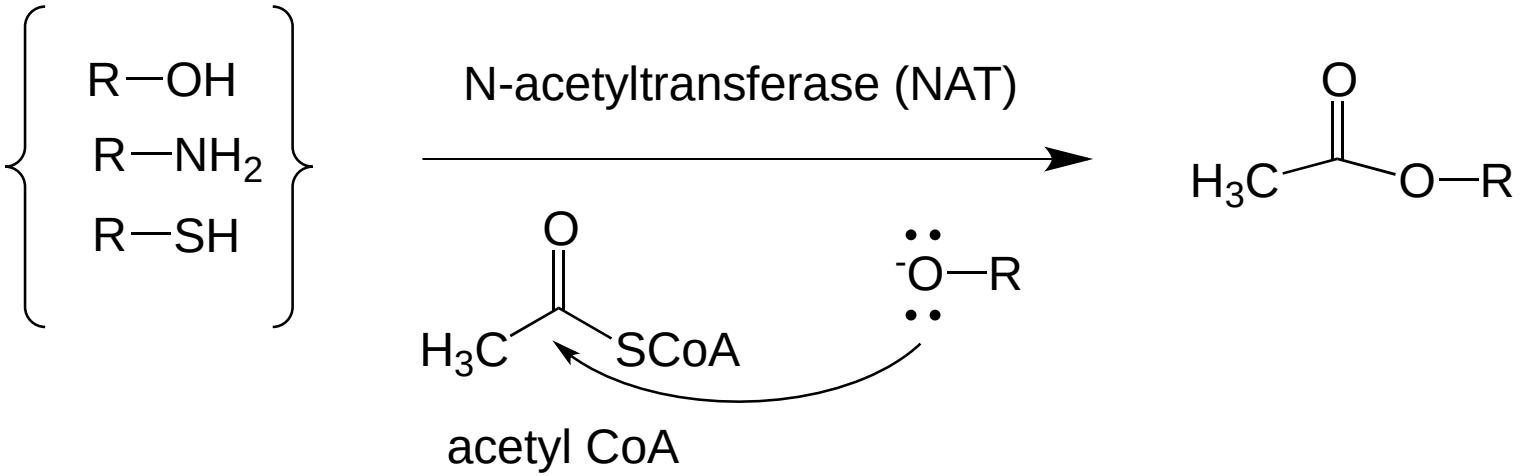
- Attachment of acetyl-CoA to toxic metabolites
- Acetyl CoA – a byproduct of glycolysis



N-Acetyltransferases (NAT)

- N-acetylation of xenobiotics is performed by N-acetyltransferases (NAT)
- N-acetylation is a major route of biotransformation for xenobiotics containing an aromatic amine (R-NH2).
- Unlike other Phase II reactions, acetylation masks an amine with a nonionizable group and are less water soluble than the parent compound.
- NAT uses the co-factor acetyl-Coenzyme A (acetyl CoA)

products of Phase I



- There are two N-acetyltransferases NAT1 and NAT2

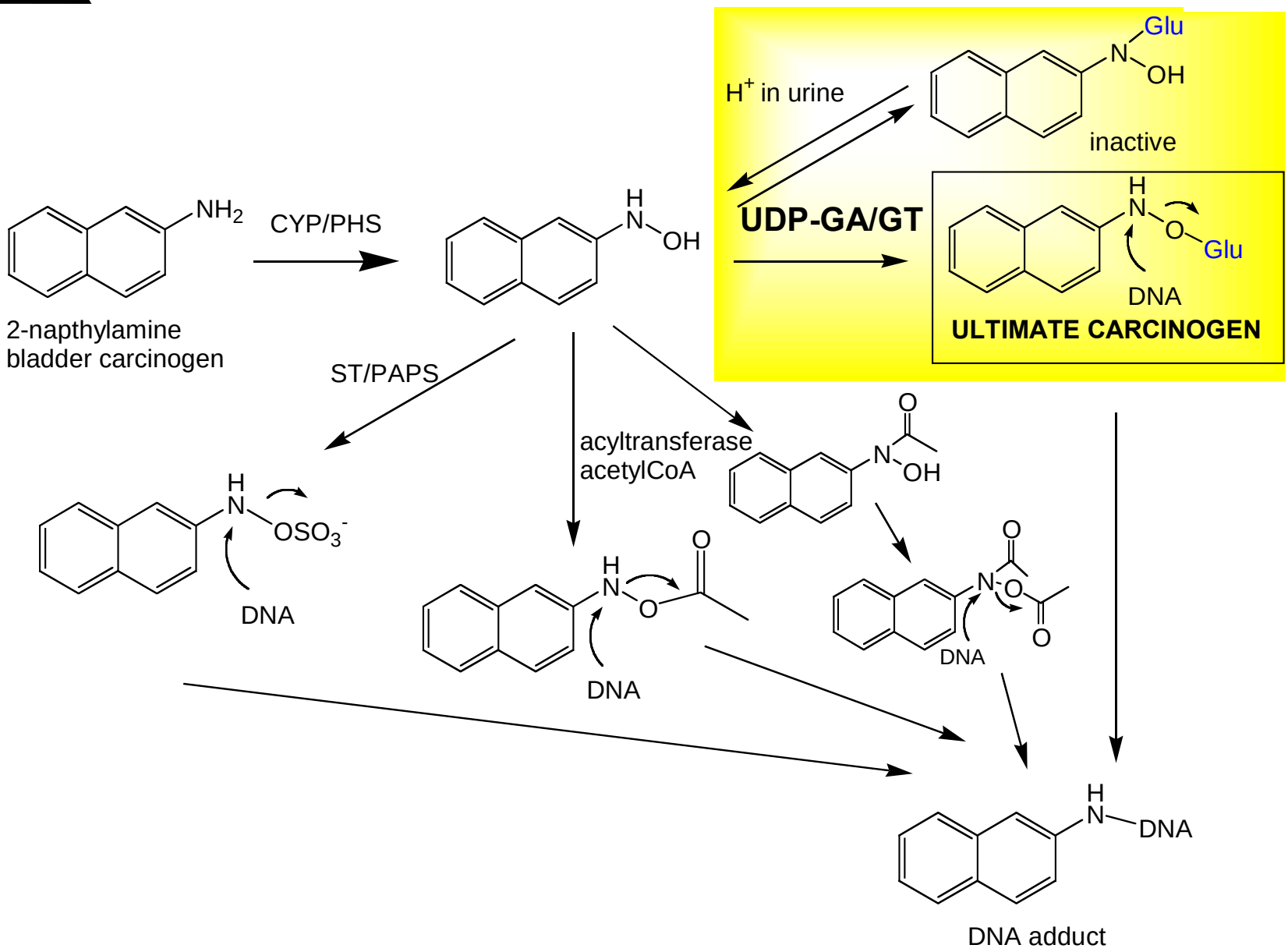
Polycyclic aromatic amines:

β-napthylamine

2-Naphthylamine (BNA) is an aromatic amine used to make azo dyes. It is a known human bladder carcinogen and has largely been replaced by less toxic compounds.

BNA also is present in cigarette smoke.

NAT turns this into a potent carcinogen


AnantLife
Glu
N
OH
H+ in urine
inactive
UDP-GA/GT
H
N
O
Glu
DNA
ULTIMATE CARCINOGEN
NH2
CYP/PHS
H
N
OH
2-napthylamine
bladder carcinogen
ST/PAPS
acyltransferase
acetylCoA
O
N
OH
H
N
OSO3-
DNA
H
N
O
O
DNA
N
O
O
O
DNA
H
N
DNA
DNA adduct

NAT and Hearing Loss

- The NAT2*6A AA genotype encodes a slow acetylator, which slows down the detoxification mechanisms.
- This might lead to a higher concentration of xenobiotics in the inner ear, which in turn might increase the number of acquired mitochondrial mutations, eventually leading to cell damage and hearing loss.
- 10% prevalence among Europeans

	Z_{low}	Z_{high}
	General European population	
GSTM1	1.00	1.00
GSTT1	0.37	0.80
NAT2*5A	1.00	0.68
NAT2*6A	0.21	**0.013**
NAT2*7A	0.72	1.00

NAT AND CANCER CANCER

- Colon Cancer and Lung Cancer – Fast acetylator
- Breast Cancer – Slow Acetylation
- Bladder Cancer – Slow Acetylation

RED MEAT AND CANCER

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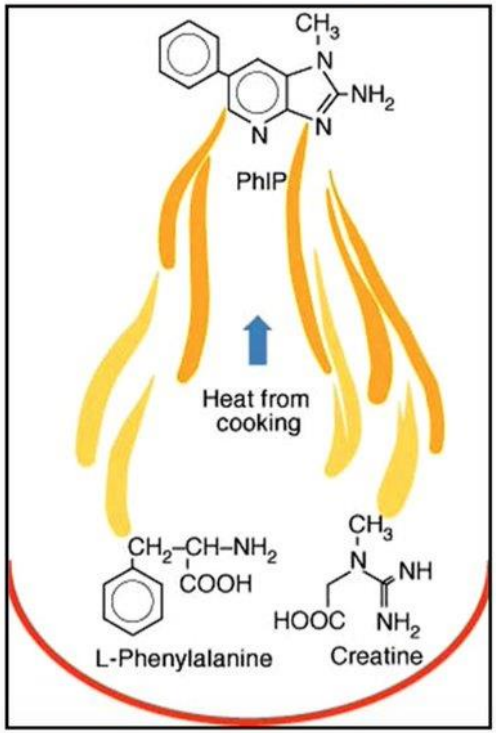
Carcinogens Associated With Cooked Meat: Heterocyclic Amines

Cooking muscle meats (beef, pork, fowl, fish) at high temperatures causes amino acids to react with creatine, forming carcinogenic compounds called **heterocyclic amines.**

PhIP=phenylimidizo[4,5]pyridine



Heterocyclic amines (HAs) can form DNA adducts and chromosomal breaks, introducing mutations into cells of the digestive system

Temperature is the main factor in the formation of HAs: Frying, grilling, and barbecuing produce the most HAs, oven roasting and baking produce the least.

Meats partially cooked in the microwave have lower HA levels than those that are not.

HAs enter the body as precarcinogens and are metabolized by the P450 system into carcinogens.

NAT2 RAPID METABOLIZER AND COLON CANCER

- 9-fold increase in CRC risk for ever-smokers who preferred their red meat well-done and had a rapid metabolic phenotype for NAT2
- exposure to carcinogens through consumption of well-done meat increases the risk of CRC only in genetically susceptible individuals
- N-hydroxylated HAA metabolites are substrates for O-acetylation primarily by NAT2 to form the reactive N-acetoxy species which bind to DNA.

NAT GENETICS AND HEALTH

- Acetylation and cancer risk (increased NAT)
- Acetylation and red meat consumption (increased NAT)
- Acetylation and age related hearing loss (reduced NAT)
- Genetics of NAT-1 and NAT-2 is predictive

MODULATION OF NAT ACTIVITY

- Diallyl disulfide – found in garlic is inhibitor of NAT activity
- Decreased protein expression through an impact on RNA levels

- Androgens activate NAT activity
- NAT gene promoter is induced by androgens

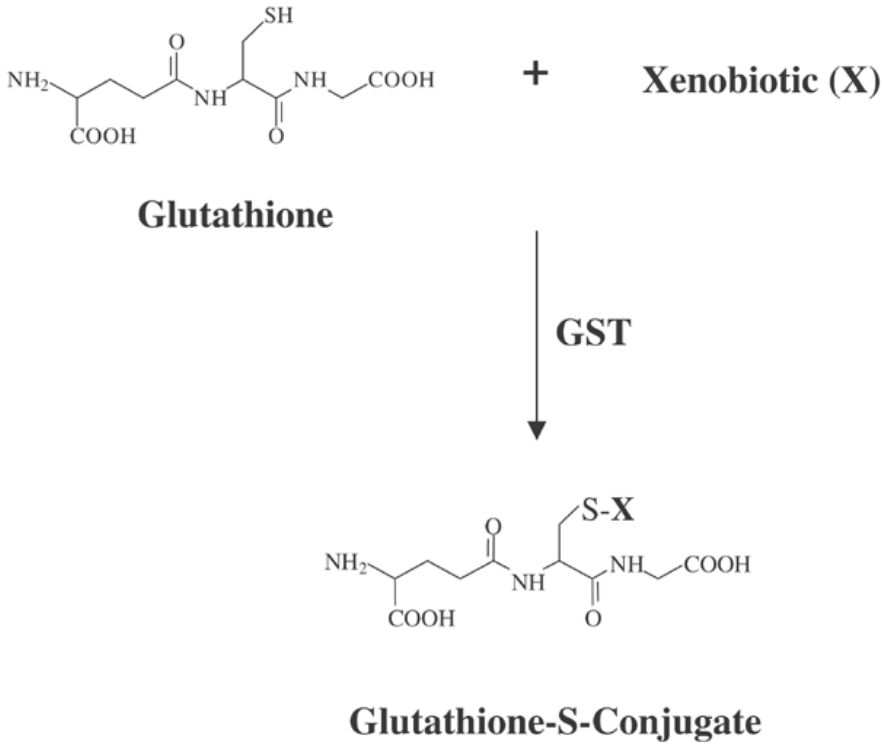
QUESTIONS

- NAT – reduced activity – increased hearing loss risk – What to do?

- NAT – increased activity – What to do ? What are there risks?

GLUTATHIONE CONJUGATION AND DETOXIFICATION

- Glutathione – antioxidant, tripeptide
- Electrophilic xenobiotics, carcinogens etc are conjugated with Glutathione
- Important for toxin elimination from liver, lungs, intestinal tract and kidney
- High levels of toxin exposure rapidly depletes glutathione levels



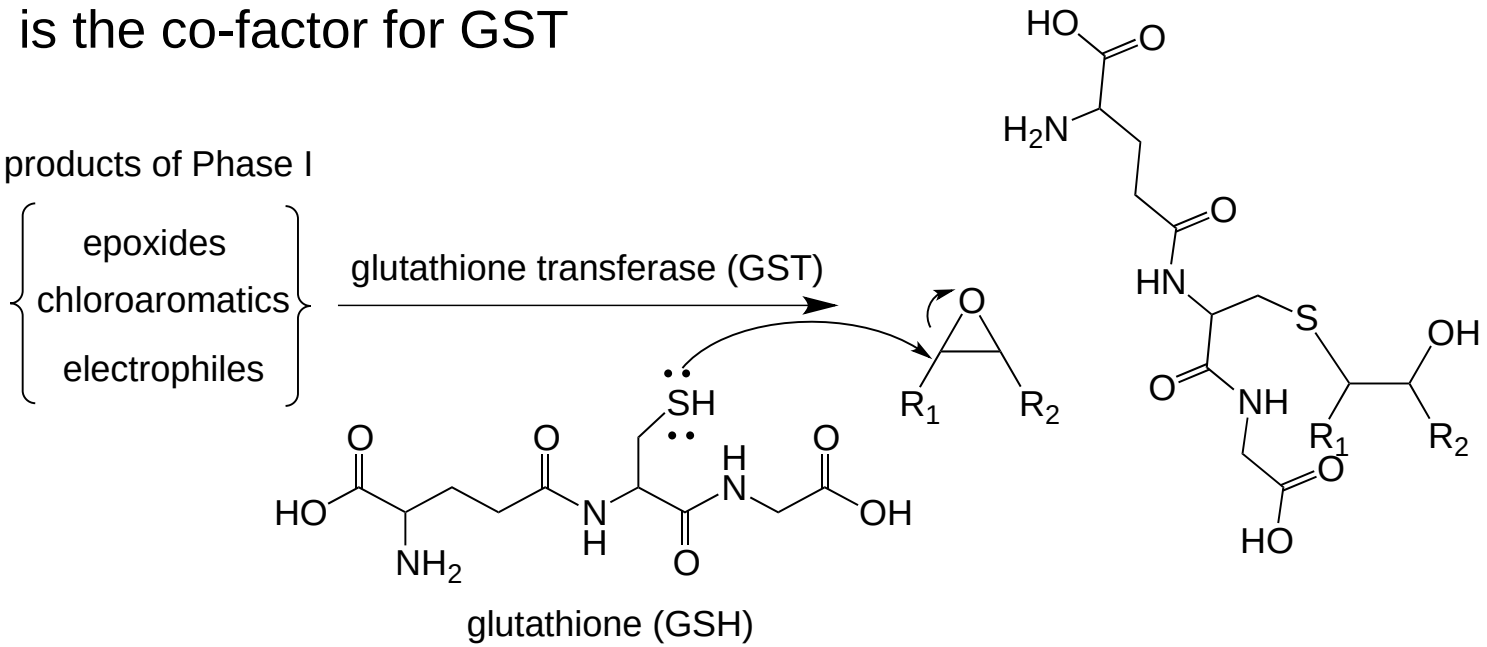
Glutathione Conjugation

Substrates for glutathione conjugation include an enormous array of electrophilic xenobiotics, or xenobiotics biotransformed to electrophiles.

Substrates for glutathione S-transferase (GST) share 3 common features: 1) hydrophobic; 2) electrophilic; 3) react nonenzymatically with glutathione (GSH) at a measurable rate.

The concentration of GSH is very high in liver (10 mM) and GST makes up 10 % of total cellular protein.

GSH is the co-factor for GST

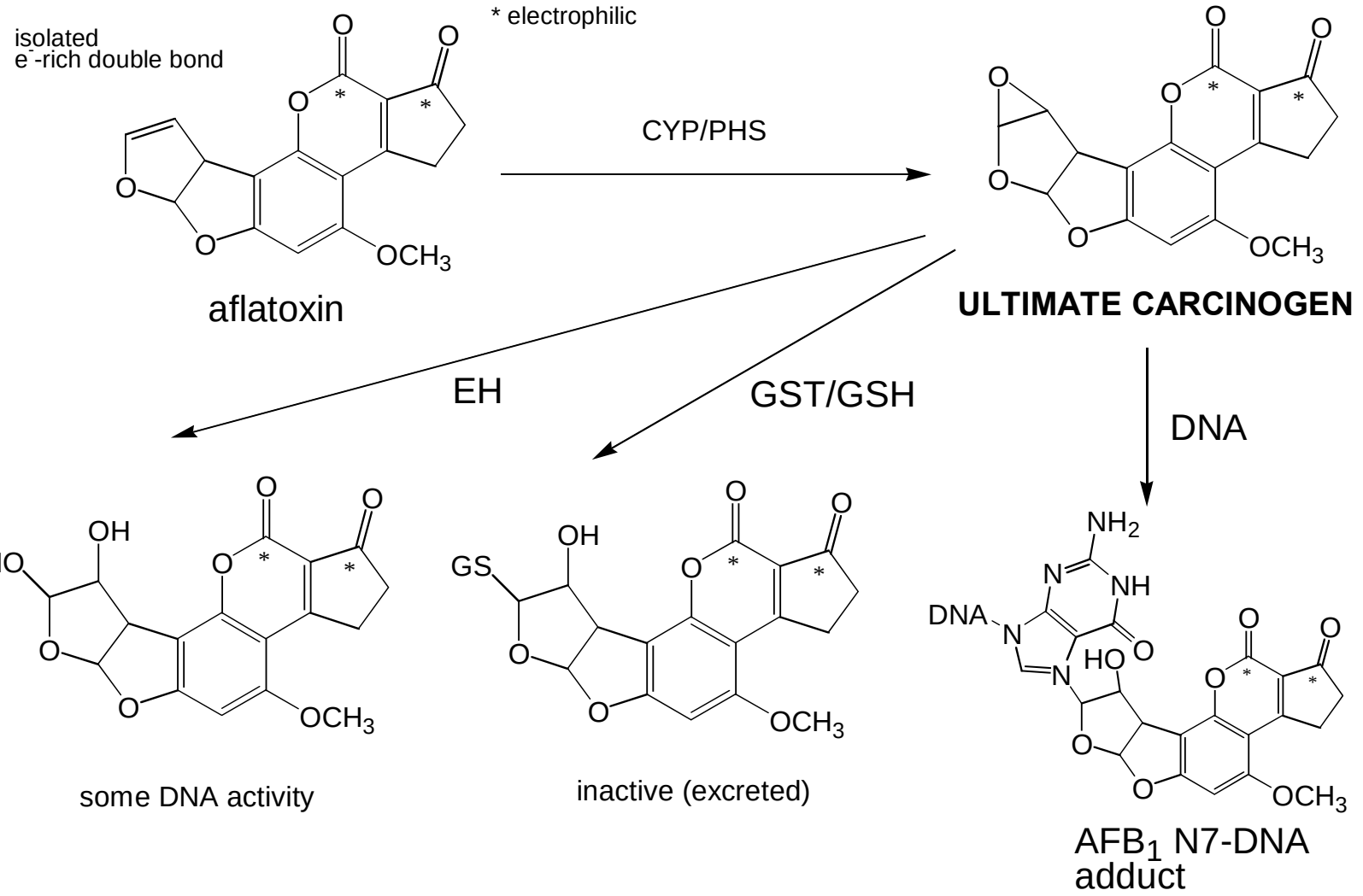


Aflatoxin

Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus.

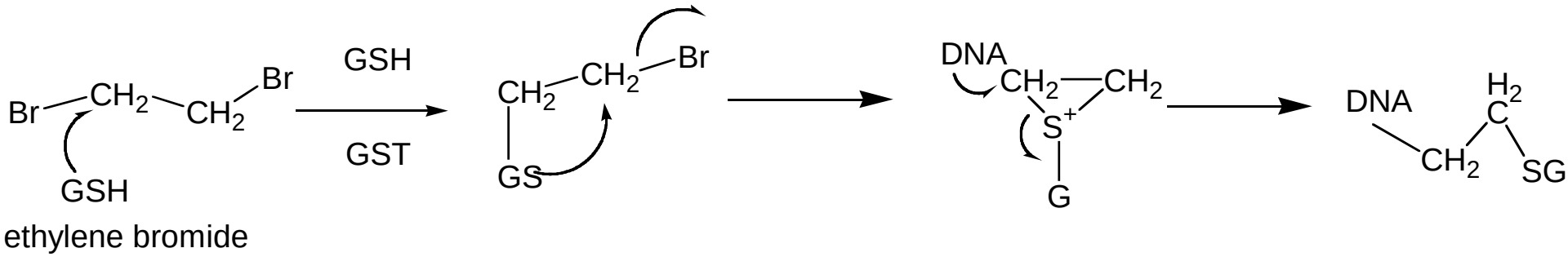
They can be found on moldy peanuts, corn and other crops.

Aflatoxin B1 is the most potent liver carcinogen.



Glutathione (GSH) plays an essential role in deactivation (protective mechanism of AFB_1); mice have higher GST levels than rats and rats are more susceptible to cancer from AFB_1.

Rare Example of GST/GSH-Mediated Bioactivation



ethylene bromide

1,2-Dibromoethane is a manufactured chemical and also occurs naturally in small amounts in the ocean where it is formed. – teratogen, carcinogen

1,2-Dibromoethane has been used as a pesticide in soil, and on citrus, vegetable, and cereal crops.

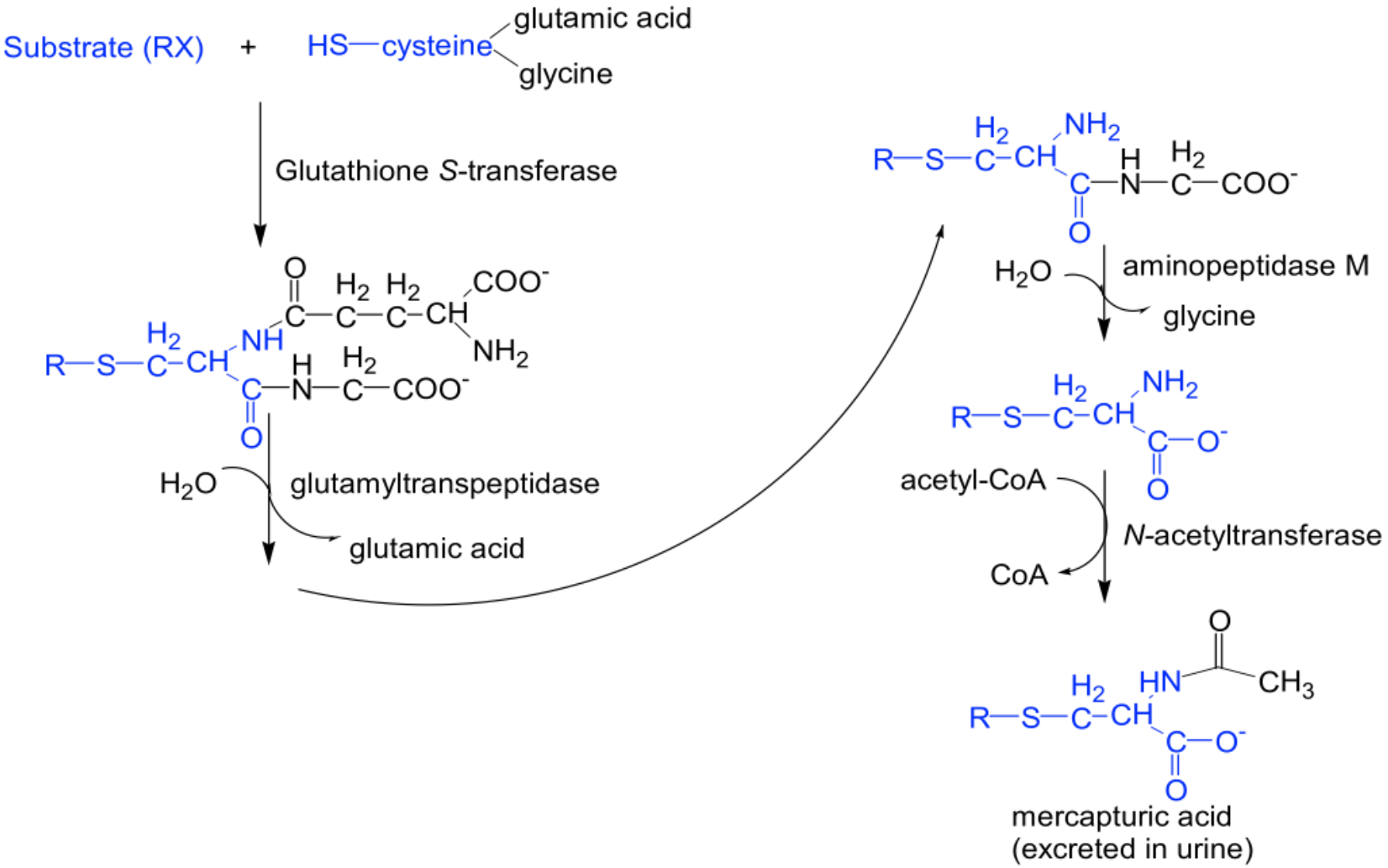
Most of these uses have been stopped by the US EPA since 1984.

Another major use was as an additive in leaded gasoline.

Uses today include as a fumigant for treatment of logs for termites and beetles, control of moths in beehives and for the preparation for dyes and waxes.

Excretion of Glutathione Conjugates

Glutathione conjugates can be formed in the liver and can be excreted intact in bile or can be converted to mercapturic acids in the kidney and excreted in the urine.

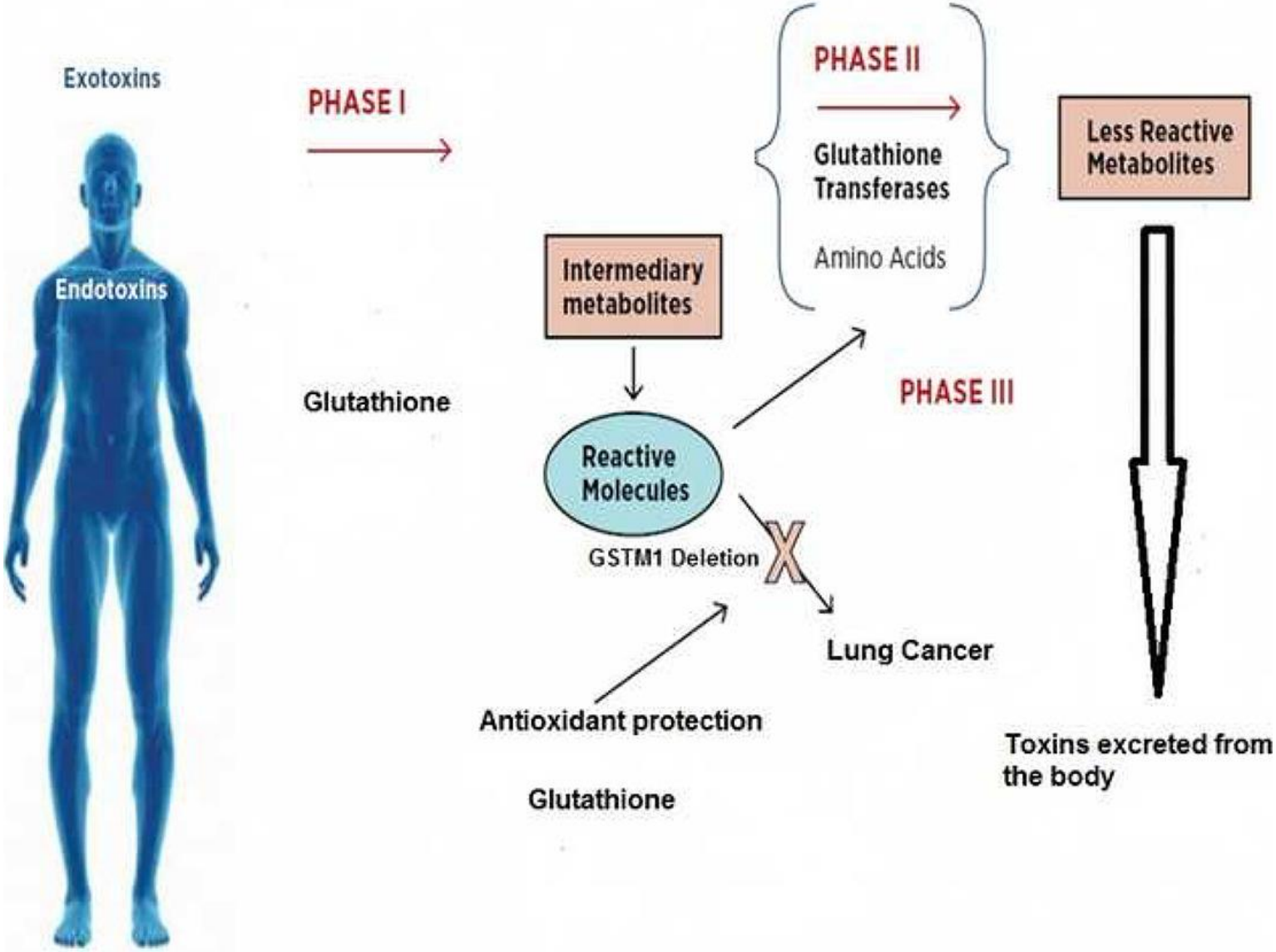


GST POLYMORPHISMS AND HEALTH

- Genetic variation dictates the levels of GST activity
- Low activity associated with increased risk of cancer and diabetes
- High prevalence of alleles with low or no activity

GST genotypes	Frequency (%)
GSTM1	
Null	49 (58.3%)
Present	35 (41.7%)
GSTT1	
Null	45 (53.6%)
Present	39 (46.4%)
GSTM1/GSTT1	
Both null	23 (27.4%)
Null/present	48 (57.1%)
Both present	13 (15.5%)

GSTM1 & LUNG CANCER DEVELOPMENT



GSTM1 MUTATIONS

Groups	GSTM1 genotypes (%)		OR	95% CI	*P*
	Wild-type	Null			
Control	128 (52.68)	115 (47.32)			
IBD	41 (38.68)	65 (61.32)	1.76	1.10-2.80	0.01
UC	38 (40.43)	56 (59.57)	1.64	1.01-2.65	0.045
CD	3 (25)	9 (75)	3.33	0.88-12.63	0.076

UC: Ulcerative colitis, CD: Crohn's disease, IBD: Inflammatory bowel diseases, OR: Odds ratio, CI: Confidence interval, GST: Glutathione S-transferases

Reduced GSTM1 activity -> Increased cancer risk
Gingko biloba induces GSTM1

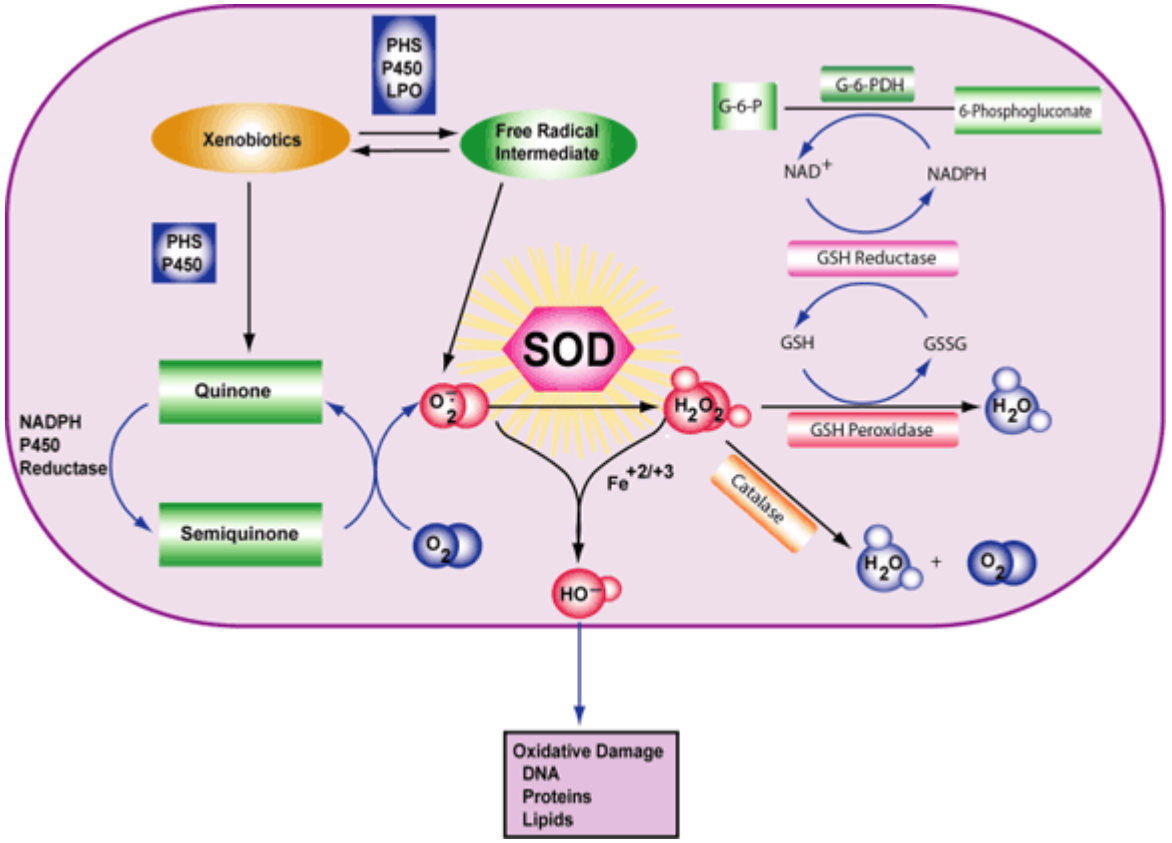
Only in rare cases, need lowered GSTM1 activity

DETOXIFICATION OF SUPEROXIDE

- Superoxides are a major causative agent for oxidative damage
- Reactive oxygen species
- Generated by immune cells as they kill infected cells
- Generated during mitochondrial respiration
- Generated by environmental toxins
- Associated with cancer, inflammation, aging and chronic diseases
- Superoxide dismutase

Superoxide Dismutase

- Superoxide dismutase (SOD) is one of the primary antioxidant enzymes.
- SOD catalyzes the conversion of superoxide ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2).



SOD POLYMORPHISMS

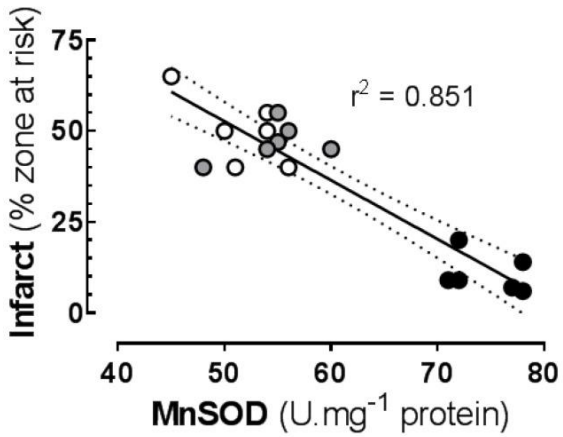
- Polymorphisms associated with reduced SOD activity
- Associated with reduced anti-oxidant activity
- Prone to oxidative damage
- Increased risk of cancer
- Type 1 diabetics – Kidney damage associated with reduced SOD activity

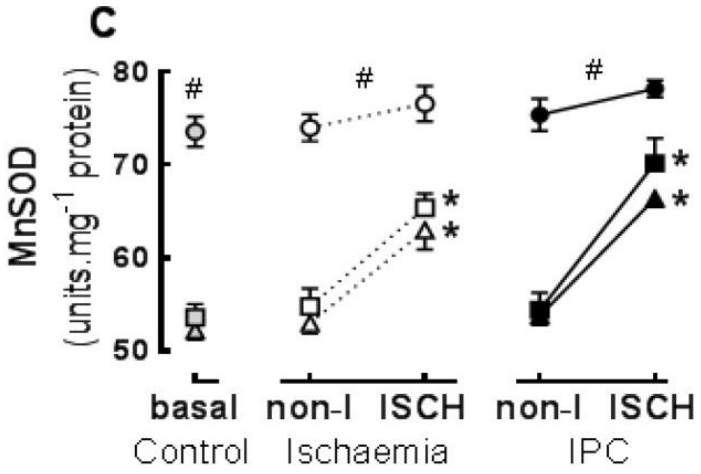
SOD POLYMORPHISMS AND BPA

- BPA binds to catalytically active site of SOD
- Irreversible binding
- Compromises SOD function
- Compromises anti-oxidant potential -> increased free radicals
- BPA associated toxicity
- If low SOD activity -> significantly greater inhibition of SOD by BPA
- Low SOD polymorphisms – avoid BPA

MODULATION OF SOD

- Fish oil induces SOD activity – 7% diet





- Reduced SOD activity -> Greater cancer risk -> Greater risk of BPA dependent toxicity -> Avoidance of xenobiotics and induction of SOD

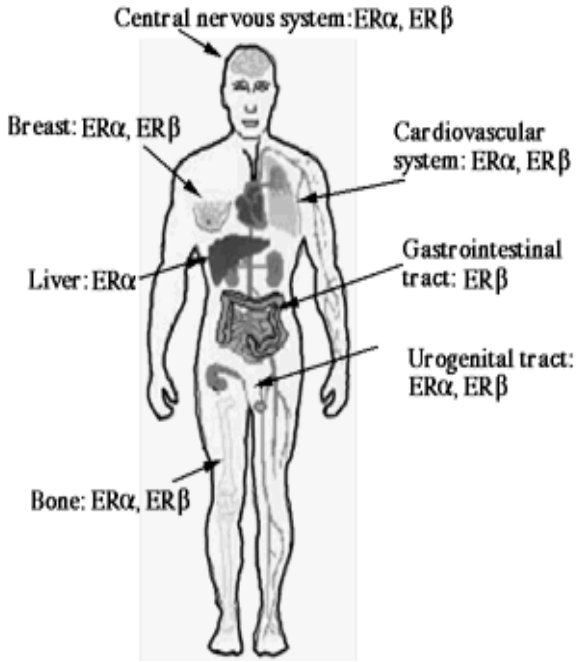
GENETICS OF ESTROGEN METABOLISM

- Estrogen – a risk factor for breast cancer
- Cytochrome p450 complex plays a central role
- Variants within Cytochrome p450 genes impact estrogen metabolism
- Impairment in estrogen metabolism -> breast cancer
- Genetics can help identify impairment
- Dietary/nutritional plan to induce enzymatic activity to help reduce the associated risks

ESTROGEN METABOLISM

- Estrogens are a class of sex steroid hormones that are synthesized from cholesterol and are secreted primarily by the ovaries, with contributions from placenta, adipose tissue, and adrenal glands.

- Estrogen (17β-estradiol, E_2) is more than just the female hormone. It is essential and in both sexes and it has functions in:
  - -the musculoskeletal system
  - -central nervous system
  - -hypothalamic pituitary axes
  - -reproductive system
  - -cardiovascular system
  - -immune system



- Despite its beneficial effects in women's health, E_2 is implicated in development and progression of breast cancer.
- Estrogen promotes proliferation of breast epithelial cells

Breast Cancer

- Breast cancer was first recognized to be an estrogen-dependent disease in 1896, when the British physician George Beatson demonstrated that oophorectomy induced regression of mammary tumors in a subset of premenopausal patients.

- Breast cancer is the second leading cause of cancer deaths in women today and the most common cancer (excluding nonmelanoma skin cancers) among women in North America.

- Above 75% of breast cancers are positive for *ER* and/or *PgR*, with E_2 being the main stimulant.

- Endocrine therapy is the preferred first-line therapy in patients with nonaggressive, receptor-positive, metastatic breast cancer.

ESTROGEN METABOLISM AND BREAST CANCER

- Exposure to genotoxic chemicals a risk for breast cancer
- Mammary carcinogenesis begins with proliferation of ductal calls
- Hormones provide the stimulus for the proliferating ductal cells to form tumors
- Environment -> Estrogen -> Genetics -> Cancer

ESTROGEN LIKE COMPOUNDS IN ENVIRONMENT

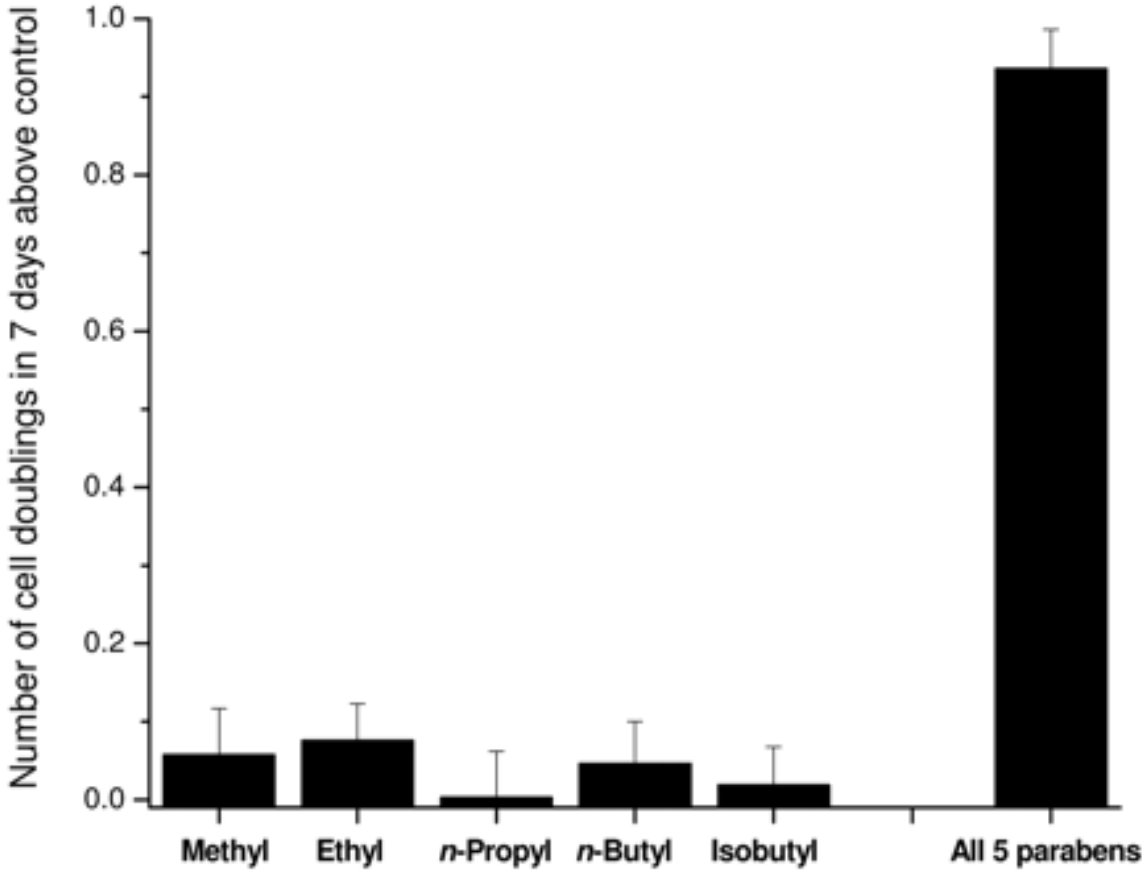
- Estrogen-mimicking endocrine disruptors (EEDs) such as polychlorinated biphenyls (PCBs), bisphenol A (BPA), and phthalates have been found ubiquitously throughout our environment.
- Several studies have shown exposure to PCBs as being a risk factor for breast cancer development
- Environmental oestrogens may enter the human breast from dietary consumption of residues of organochlorine pesticides or polychlorinated biphenyls (PCBs) and of phytoestrogens or from exposure in the domestic environment to bisphenol A and phthalates of plastics, alkyl phenols of detergents, or polybrominated diphenylethers (PBDEs) in soft furnishing

ENVIRONMENT AND ESTROGEN

Table I. *Evidence for the presence of cosmetic chemicals with oestrogenic activity in the human breast.*

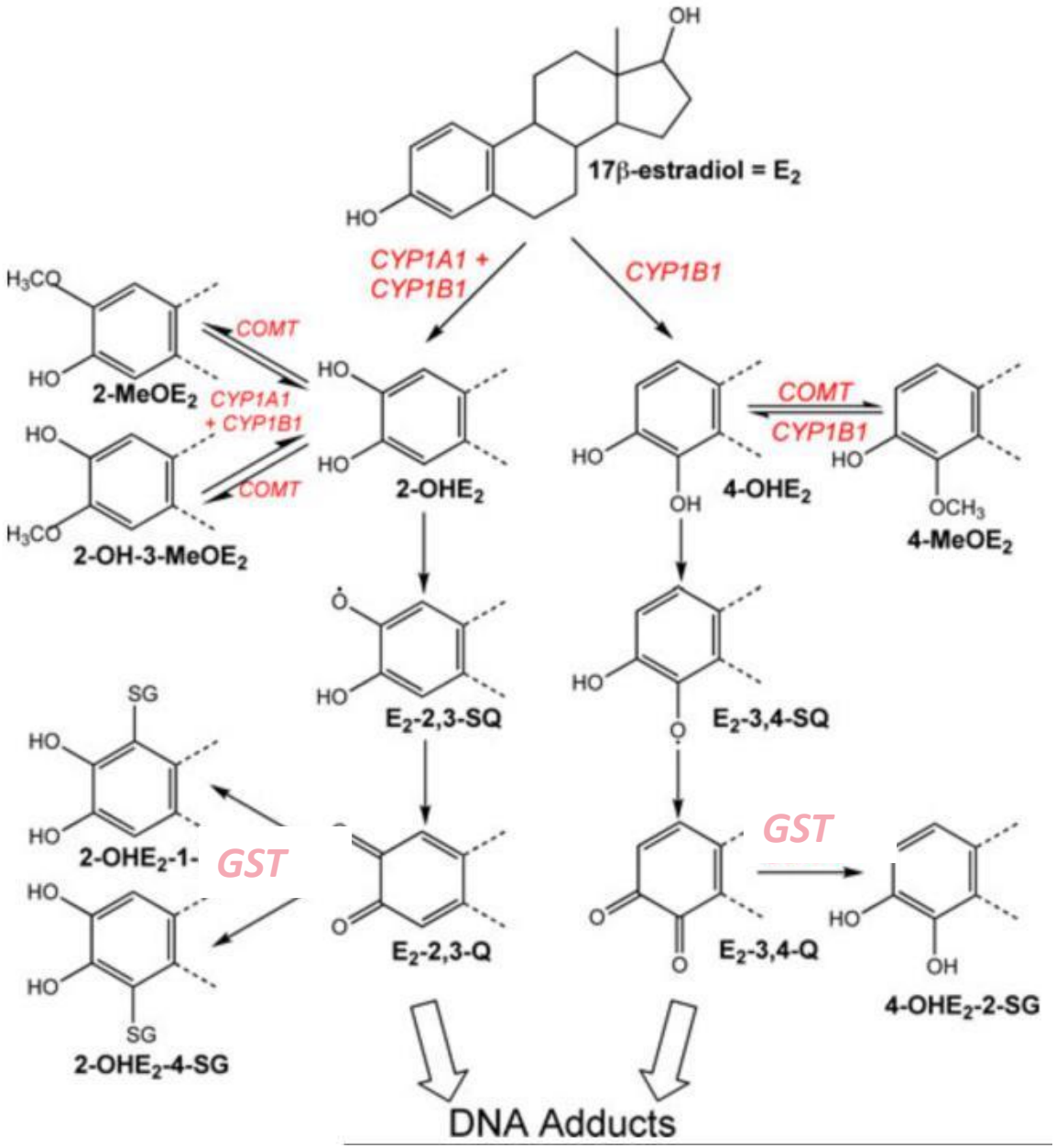
Component	Function in cosmetic	Evidence for oestrogenic activity (agonist and/or antagonist)	Measurement in human breast tissue or breast milk	Systemic absorption into humans from topical application
Aluminium chlorhydrate	Antiperspirant	Interferes with binding of oestrogen to ER and oestrogen regulated gene expression (37)	Tissue (38)	Underarm skin into blood (39, 40)
Parabens (methyl-, ethyl-, *n*-propyl-, isopropyl, n-butyl-, isobutyl, benzyl)	Preservative	*In vitro* and in rodent uterotrophic assay (reviewed in 41 and 42)	Tissue (43)	Dorsal skin into blood (44) and urine (45)
Triclosan	Deodorant/preservative	*In vitro* (46)	Milk (47-48)	Cosmetic use into plasma/milk (48)
Benzophenone-3, benzophenone-2, octyl-methoxycinnamate, 3-(4-methylbenzylidene)-camphor, homosalate, octyl-dimethyl-*p*-aminobenzoic acid	Absorb ultraviolet light	*In vitro* and in rodent uterotrophic (49-53)	Milk (54)	Dorsal skin into blood (55, 56) and urine (56)
Polycyclic musks (HHCB, AHTN)	Fragrance	*In vitro* (57-61)	Milk (62, 63)	
Nitromusks (musk xylene, musk ketone)	Fragrance	*In vitro* (61)	Milk (63)	
Benzyl salicylate, butylphenyl methylpropional	Fragrance	*In vitro* (64)		
Benzyl benzoate	Fragrance fixer, acaricide	*In vitro* (64)		
Octamethylcyclotetrasiloxane	Conditioning, spreading	*In vitro* and in animal models (65-67)		
Nonylphenol	Surfactant	*In vitro* and in rodent uterotrophic assay (68, 69)	Milk (30)	
Dibutylphthalate, diethylphthalate, di(2-ethylhexyl)phthalate, butylbenzylphthalate	Plasticizer	*In vitro* (70-72)	Milk (28, 29)	Cosmetic use into urine (73); dorsal skin into blood (44) and urine (45)
Ethynylestradiol	Anti-ageing	Synthetic estrogen	Physiological effect on human breast (74, 75)	Physiological effect from cosmetic cream (74, 75)

ENVIRONMENT AND ESTROGEN



Estrogen like compounds can together induce breast cell proliferation at concentrations at which individual compound will have no effect

ESTROGEN METABOLISM

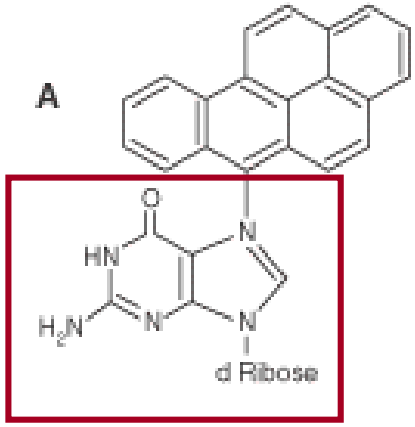


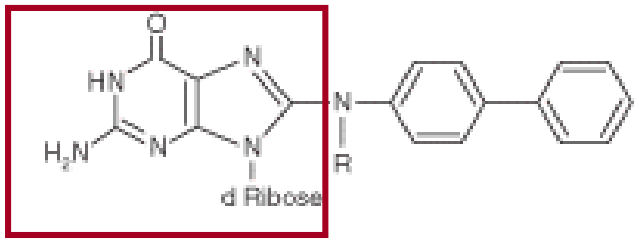
DNA adducts and cancer

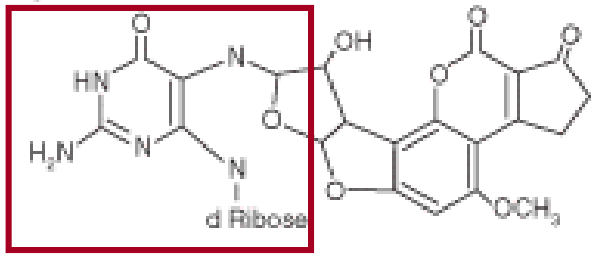
Since most chemical carcinogens react with DNA and are mutagenic, interactions with DNA have been viewed as the most important reactions of these agents.

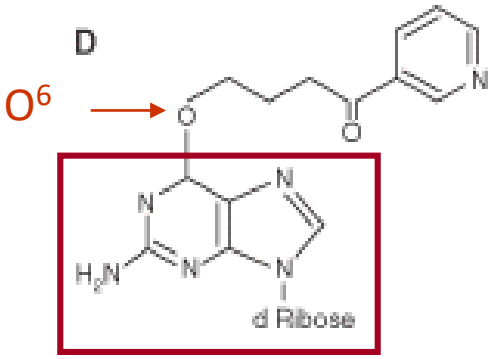
The principal reaction products of the nitrosamines and similar alkylating agents with DNA are N^7 and O^6 guanine derivatives. Reactions also occur with other DNA bases.

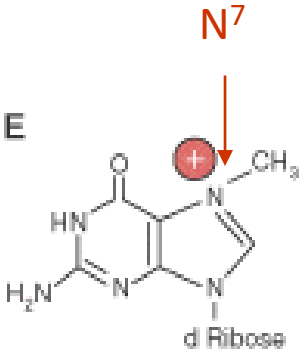
Examples of carcinogen- DNA adducts











deoxyguanosine

A. N-7 (benzo[a]pyren-6-yl)guanine **B.** *N*-(deoxyguanosin-8-yl)-{acetyl}aminobiphenyl
C. 8,9-dihydro-8-(N5-formyl- 2 , 5 , 6 -triamino-4 -oxo-N5-pyrimidyl)-9-hydroxy-aflatoxin B1
D. O^6-[4-Oxo-4(3-pyridyl)butyl]guanine, a mutagenic lesion formed by the metabolism of the tobacco-specific nitrosamine, NNK **E.** N-7-methyldeoxyguanosine

Potential biological consequences of DNA-adduct formation

a. An insertion of the flat planar rings of a polycyclic hydrocarbon between the stacked bases of double-helical DNA may distort the helix, leading to a frame-shift mutation during DNA replication past the point of the intercalation.

b. Alkylated bases in DNA can mispair with the wrong base during DNA replication – for example, O^6 methyguanine pairs with thymine instead of cytosine during DNA replication, leading to a base transition (i.e., GC→AT) type of mutation during the next round of DNA replication.

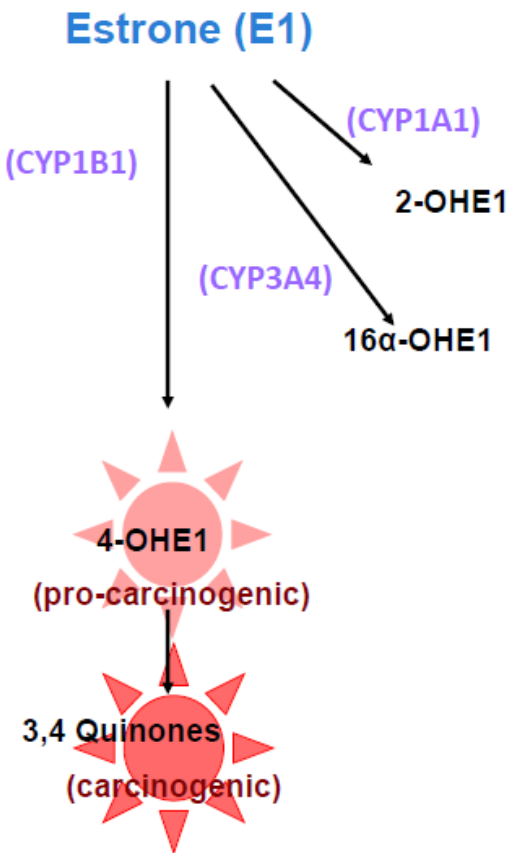
c. Many of the base adducts formed by carcinogens involve modifications of N-3 or N-7 positions on purines that induce an instability in the glycosidic bond between the purine base and deoxyribose. This destabilized structure can then undergo cleavage by DNA glycosylase, resulting in loss of the base.

ESTROGEN METABOLITES AND BREAST CANCER

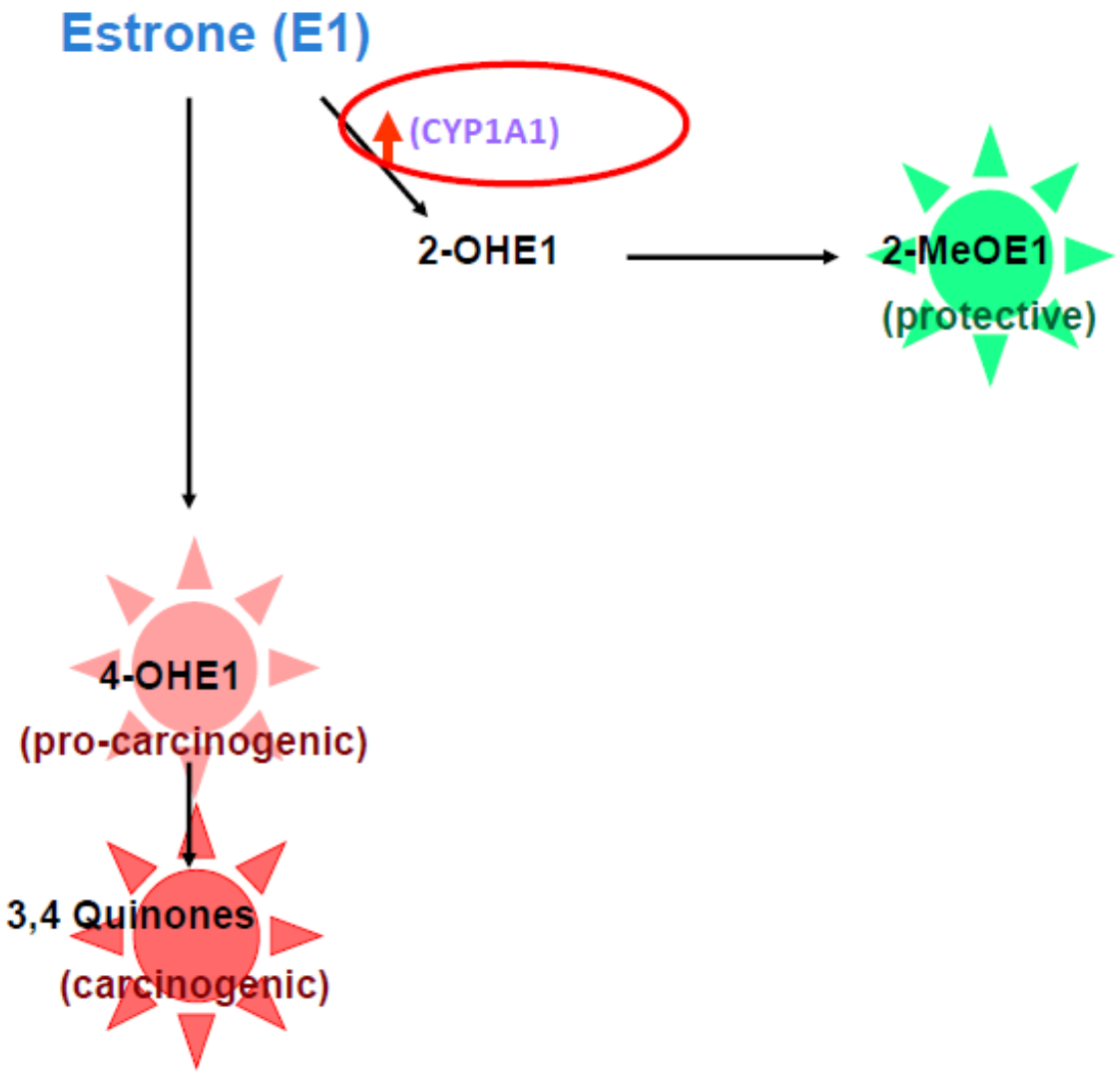
Genotoxic metabolites of estradiol contribute to breast cancer development.

Both estrogen receptor-mediated and estrogen receptor-independent effects of estradiol contribute to breast cancer development.

ESTROGEN METABOLISM



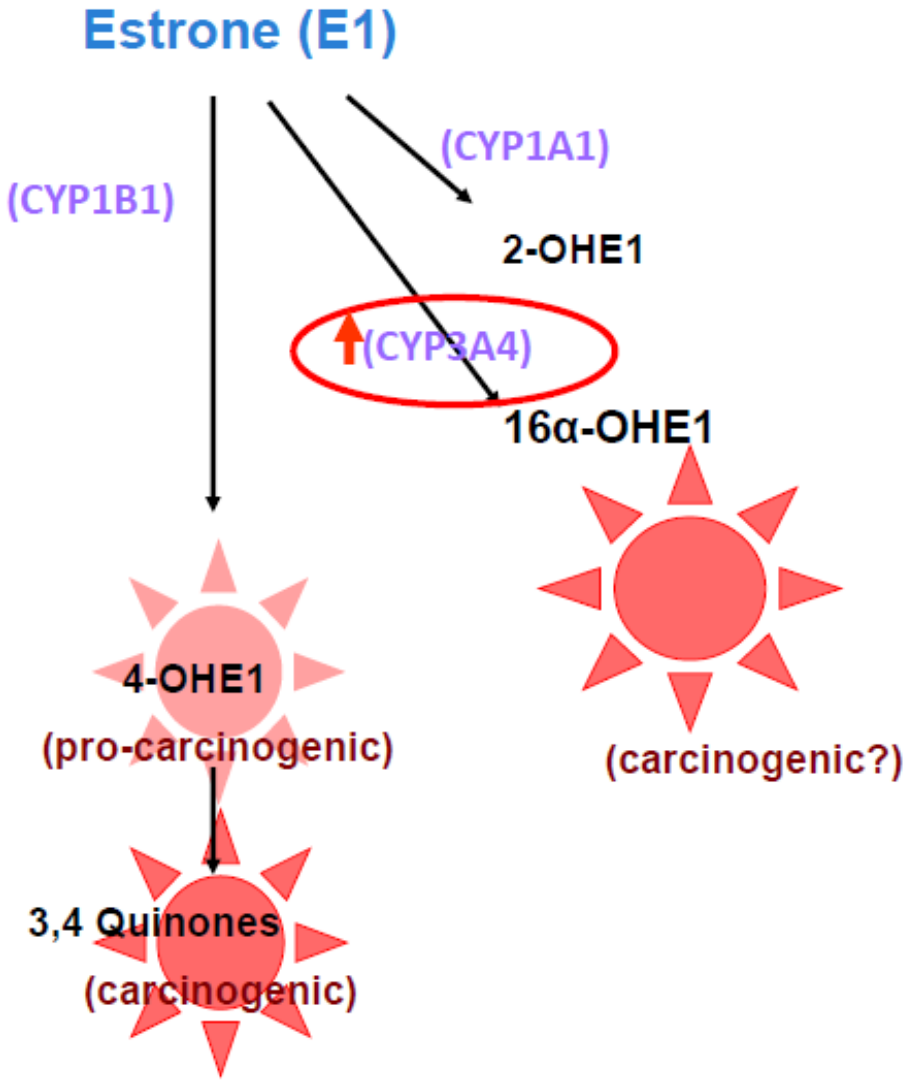
ESTROGEN METABOLISM



CYP1A1 AND 2-OHE-1

- 2-Hydroxyesterone – 2-OHE-1 has weak activity
- Weak binding to estrogen receptor
- Does not induce proliferation of cells and may in fact induce suppression of proliferation
- Increased CYP1A1 activity - preferentially drives conversion of estrogen to 2-OHE-1 which is non-cancerous
- 2-OHE-1 may have anti-proliferation effects

ESTROGEN METABOLISM



16-OHE-1

- 16-alphaHydroxyesterone – 16-OHE-1
- Preferential and potent binding to estrogen receptor
- Drives cell proliferation
- Can increase proliferation by upto 60% -> greater mutation risk
- Carcinogenic
- Increased CYP3A4 activity – drives 16-OHE-1

16-OHE-1 and Breast Cancer

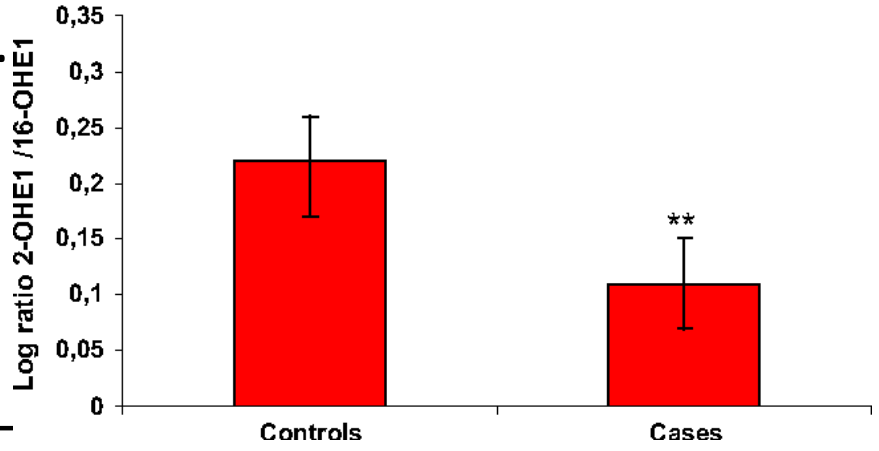
Table 3 Distribution of intratissue hydroxyestrogens according to the ER status of breast tumors[a]

ER status[b]	Hydroxyestrogens			
	$16\alpha OHE_1$	$2OHE_1$	$4OHE_1$	$2OHE_1$:$16\alpha OHE_1$
Positive ($n = 12$)	2.285 (1.961–2.609)	2.673 (2.346–3.001)	2.773 (2.596–2.949)	1.25 (0.983–1.511)
Negative ($n = 9$)	1.823 (1.423–2.224) $P = 0.058$	2.856 (2.653–3.058) $P = 0.366$	2.575 (2.256–2.895) $P = 0.210$	1.65 (0.324–2.916) $P = 0.048$
	$2OHE_2$	$4OHE_2$	$4OHE_2$:$2OHE_2$	
Positive ($n = 12$)	2.530 (2.088–2.972)	3.027 (2.587–3.467)	5.10 (1.005–9.199)	
Negative ($n = 9$)	2.234 (1.753–2.716) $P = 0.333$	2.314 (1.704–2.924) $P = 0.100$	3.42 (0.151–6.686) $P = 0.380$	

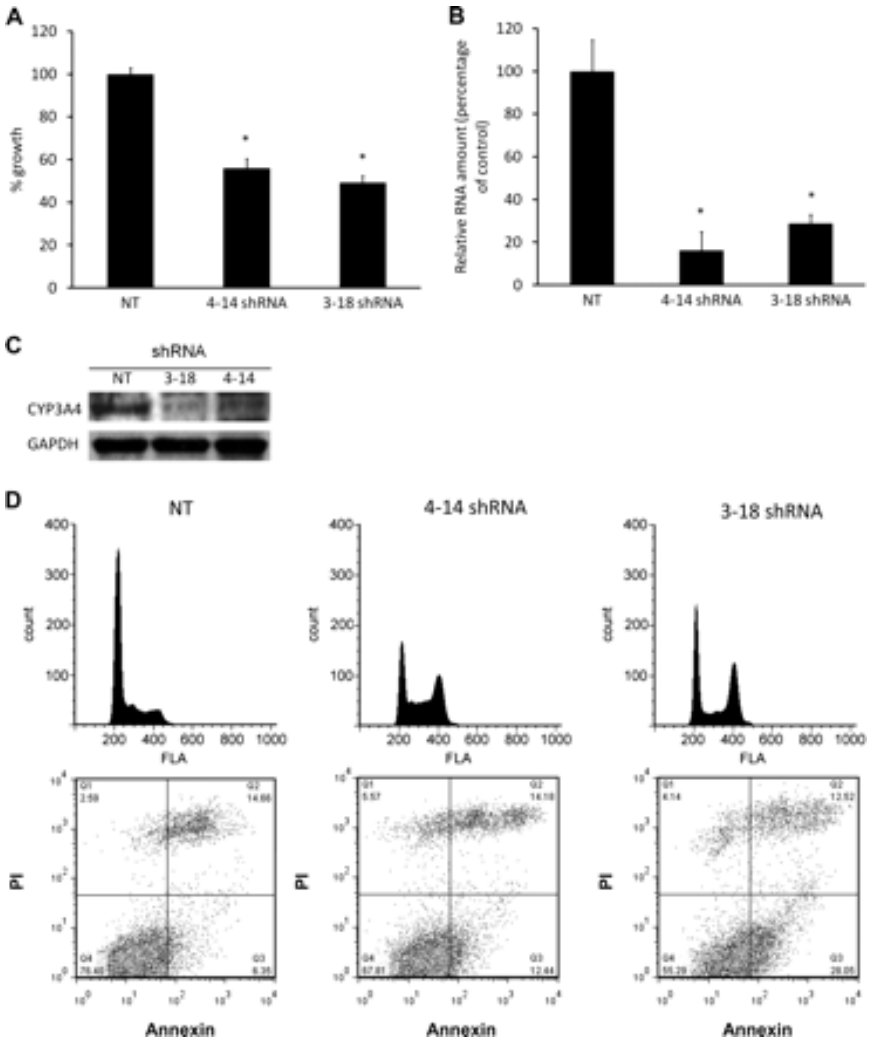
[a] Means and (95% confidence intervals) of logarithmically transformed data.
[b] ER-positive group aggregates ER +/+ and −/+ tumors; ER-negative group includes ER −/− tumors only.

2-OHE-1: 16-OHE-1 RATIO AND BREAST CANCER

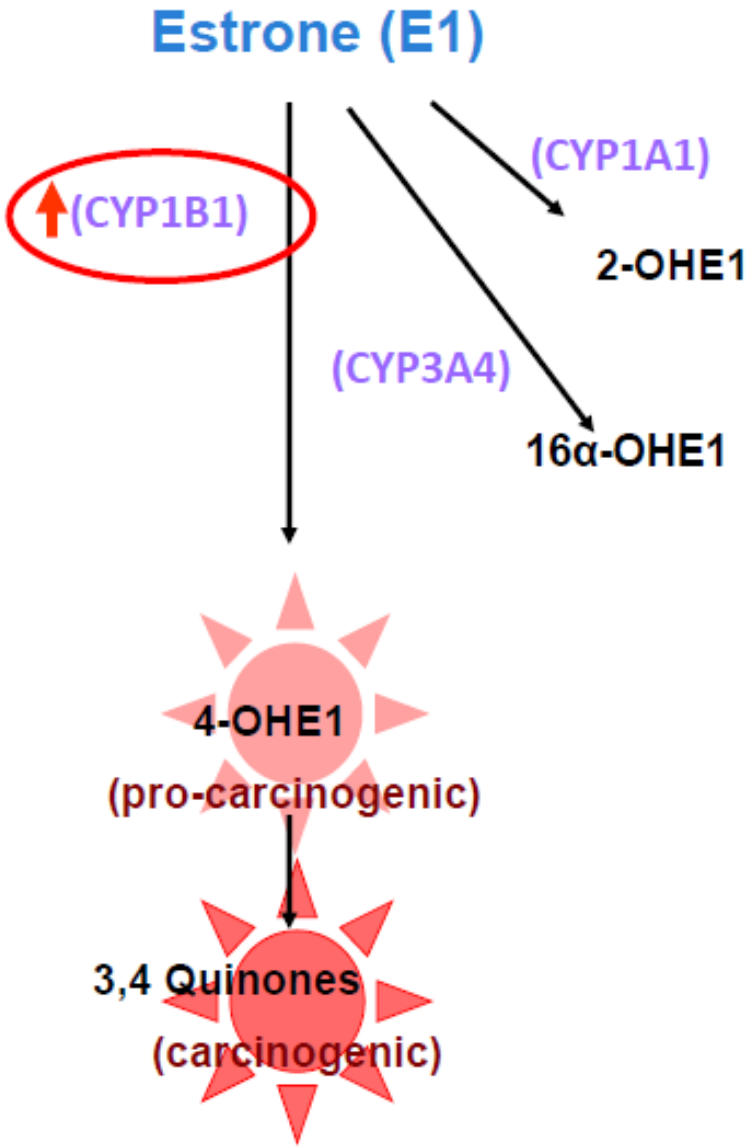
- the metabolite ratio of 2OHE1 to 16αOHE1 reflects the relative dominance of one pathway over the other.
- High ratio -> more 2-OHE1 and less 16-OHE-1
- Low ratio -> more 16-OHE1 – associated with breast cancer
- Low ratio -> Poor prognosis



CYP3A4 INHIBITION – BREAST CANCER



ESTROGEN METABOLISM



4-OHE-1

- 4-Hydroxyesterone – 4-OHE-1
- 4-OHE1 can be converted to 3,4-Quinones which are pro-carcinogenic meaning that they cause cancer
- DNA damage – oxidative damage – mutations
- Increased 4-OHE-1 production in uterine fibroids
- Conjugated equine estrogen – previously used for hormone therapy – undergoes hydroxylation to 4-OHE-1

Catechol Estrogen Quinones to DNA Adducts

- Endogenous Estrogens can become carcinogenic via formation of catechol estrogen quinones, which react with DNA to form specific depurinating estrogen-DNA adducts. (4-OHE-1 and 16-OHE-1)
- The mutations resulting from these adducts can lead to cell transformation and the initiation of breast cancer.
    - Irrespective of ER status
    - Mechanism: Sheer volume of DNA “apurinic” sites – DNA repair enzymes make mistakes, leading to single nucleotide polymorphisms (SNP)

Catechol Estrogen Quinones to DNA Adducts

- 4-OH-estrone induces DNA Adduct formation in normal breast epithelial cells
- 4-OH induced DNA adducts – marker for prostate cancer risk

Prostate Cancer

- Small study of urine estrogen metabolites in men with prostate cancer vs. benign urological d/o vs. healthy controls
- 4-OHE1-DNA Adducts detected at higher levels in samples from subjects with prostate cancer and benign urological conditions compared to healthy males
- This is the first demonstration that CEQ-derived DNA adducts are present in urine samples from subjects with prostate cancer.
- Markushkin Y et al. Potential biomarker for early risk assessment of prostate cancer. Prostate. 2006 Oct 1;66(14):1565-71

Extension to Other Cancers

- This mechanism is also involved in
  - Initiation of leukemia by benzene
  - Olfactory tumors by naphthalene
  - Neurodegenerative diseases such as Parkinson's disease by dopamine.
    - Estrogens and Human Diseases. Volume 1089 published November 2006 Ann. N.Y. Acad. Sci. **1089:** 286–301 (2006). doi: 10.1196/annals.1386.042

CYP1B1 AND 4-OHE-1

- CYP1B1 drives generation of 4-OHE-1
- Faster activity drives more generation of 4-OHE-1
- Increased CYP1B1 activity associated with lower 2-OHE-1:16-OHE-1 ratio
- Increased risk of breast cancer with faster CYP1B1 activity
- CYP1B1 expression observed in tissues which give rise to hormone responsive tumors eg breast, cancer, uterus
- Also association with prostate cancer

XENOBIOTICS AND ESTROGEN METABOLISM

- DTT, Polychlorinated biphenyls (PCBs), BPA, Polybrominated diphenyl ethers , perfluorinated chemicals
- DNA methylation – epigenetic modification to impact cyp450 enzymes
- Impact on the activity of Estrogen receptor
- Preferentially produce more of carcinogenic 4-OHE1 and 16-OHE1
- Mutations in CYP1B1, CYP1A1 AND CYP3A4 modulate risk further

Cyp1B1 inhibition (ie: reduction of DNA adducts)

- Reduce xenobiotic pollutant exposure
- N-acetyl Cysteine
- Sulforaphane (glucosinolate from broccoli) induces quinone reductase, which takes CEQs back to catechol estrogens, reducing the potential for the creating of DNA adducts.
    - Hwang. J Med Food. 2005 Summer;8(2):198-203
- Glutathione conjugates

CYP 1B1 Inhibition to decrease DNA Adduct Formation

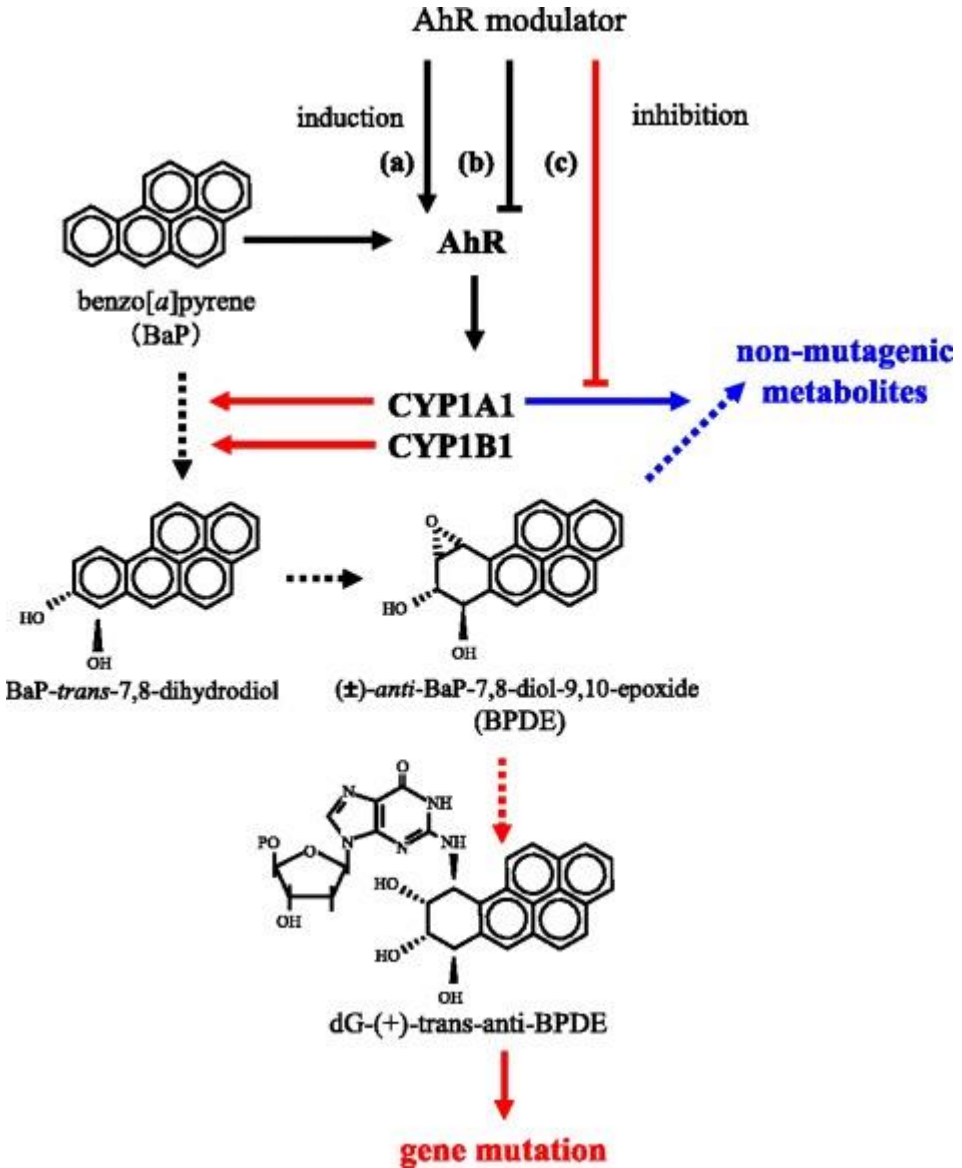
- Reduced Lipoic Acid
- N-acetyl Cysteine
- Resveratrol – particularly shown to inhibit CYP1B1 activity in response to estrogenic compounds
- Melatonin (minimal but positive effect)

Synergism b/t Paclitaxel and Broccoli Glucosinolate

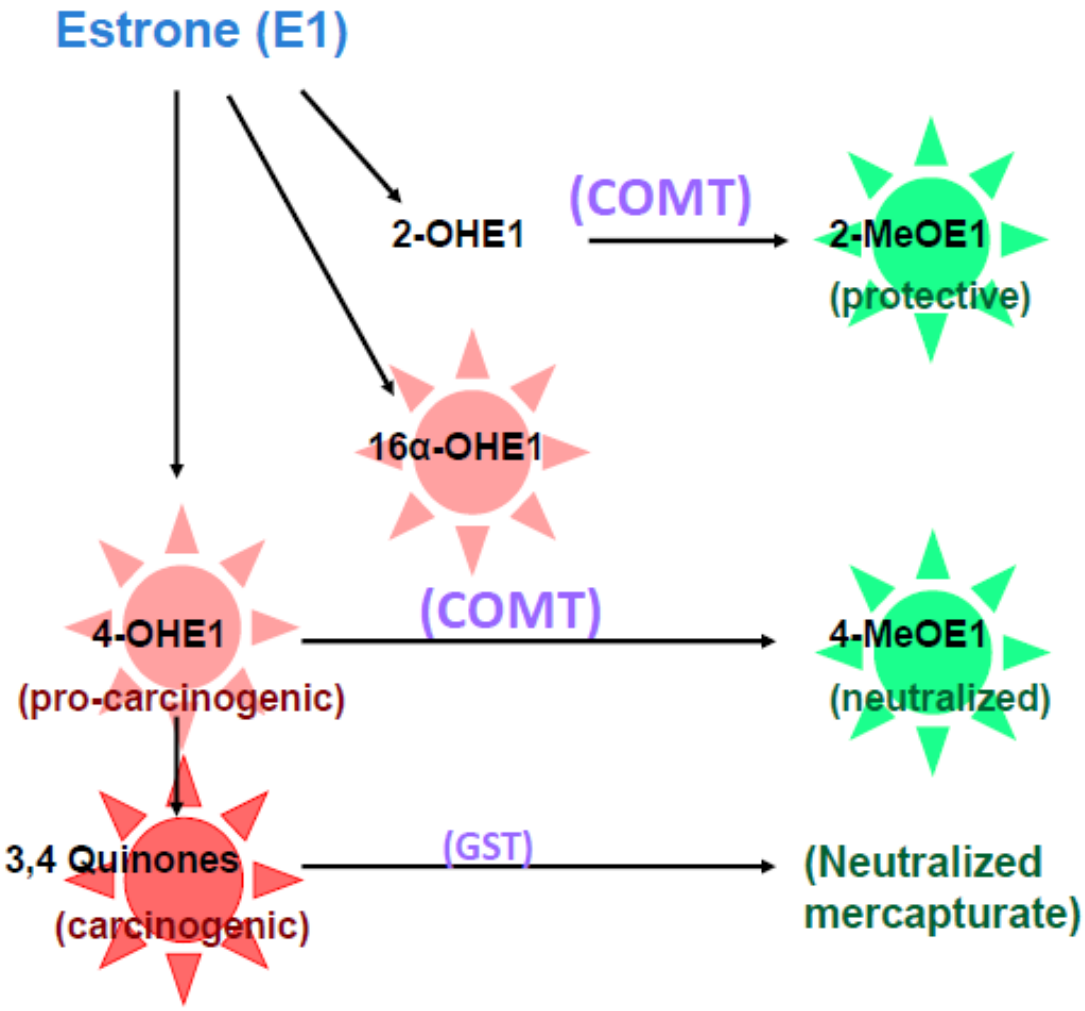
- Diindolylmethane in combination with paclitaxel synergistically inhibits growth of Her2 / neu human breast cancer cells through G2M phase cell-cycle arrest and induction of apoptosis / necrosis
- Diindolylmethane produced upon breakdown of indole-3-carbinol found in cruciferous vegetables – CYP1B1 inhibitor
- Paclitaxel – chemotherapy drug

Cyp 1B1 Inhibition to decrease DNA Adduct Formation

- Increased methylation of catechol estrogens leads to feedback inhibition of Cyp1B1



ESTROGEN METABOLISM



COMT and CE (Catechol Estrogen)

- Quantitatively, the most active CE conjugative pathway is methylation. CE methylation is catalyzed by COMT
- Catechol-O-methyltransferase (COMT) a classical phase II enzyme, catalyzes the transfer of methyl groups from *S*-adenosyl methionine, the enzyme cofactor, to hydroxyl groups of a number of catechol substrates, including the CEs.
- Under normal circumstances, CEs are, for the most part, promptly *O*-methylated by COMT to form 2- and 4-*O*-methylethers, which are then excreted.
- While virtually all catechols are substrates for COMT, the highest affinities for the enzyme are exhibited by the CEs
    - Journal of the National Cancer Institute Monographs No. 27, 2000

Low Functioning COMT and Breast Cancer Risk

- Genetic epidemiology studies have proposed a possible correlation between the low activity allele ($COMT^{LL}$) and increased breast cancer risk

- Lavigne JA, et al. An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. Cancer Res 1997;57:5493–5497.
- Huang CS, et al. Breast cancer risk associated with genotype polymorphism of the estrogen metabolizing genes CYP17, CYP1A1, and COMT: A multigenic study on cancer susceptibility. Cancer Res 1999;59:4870–4875.
- Yim D-S, et al. Relationship between the val158met polymorphism of catechol *O*-methyl transferase and breast cancer. Pharmacogenetics 2001;11:1–8.

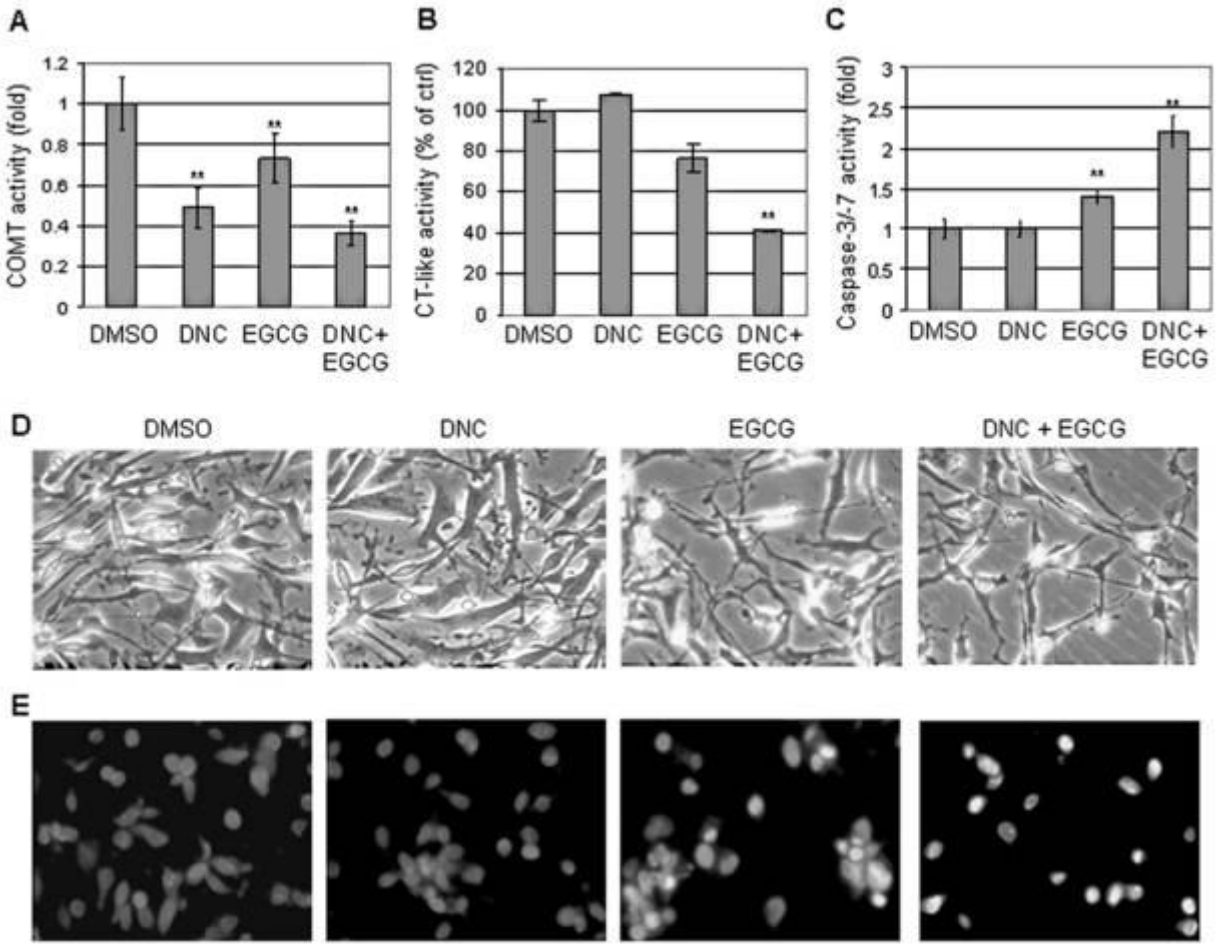
COMT and Breast Cancer

- COMT protects cells from the genotoxicity and cytotoxicity of catechol estrogens, by preventing their conversion to quinones
    - Adds methyl group ($-CH_3$) at the -OH site that would otherwise be oxidized by peroxidase enzymes
- Low activity of COMT leads to higher levels of depurinating estrogen-DNA adducts that can induce mutations and initiate cancer.
    - MCF-10F (human breast epithelial cells that are ER neg)
    - Estrogen-DNA adducts' carcinogenicity independent of ER status

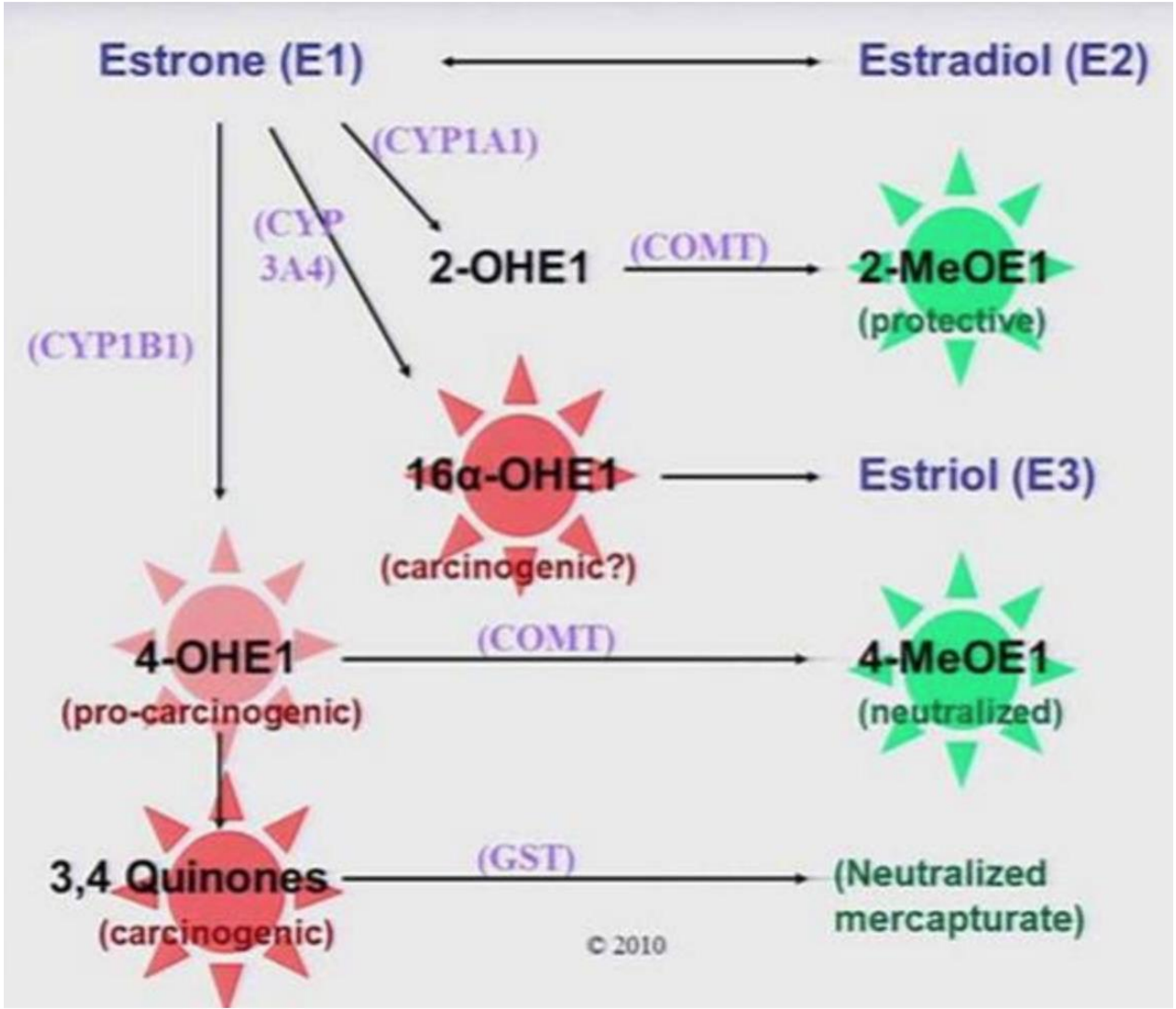
Low Functioning COMT Common

- 25% of US Caucasians are homozygous for the val108/158met polymorphism in the COMT gene
  - Lachman HM, et al. Pharmacogenetics 1996;6:243–250.
  - Scanlon PD, et al. Science 1979;203:63–65.
- 27% Chinese Americans and 34% Japanese Americans
  - Wu A et al. Cancer Res 2003;63: 7526–7529
- Val108/158Met SNP associated with 3-4x reduction in functional enzymatic rate of COMT.
  - Zhu BT. Curr Drug Metab 2002;3: 321–349

Green tea induces death of breast cancer cells with low COMT levels



GST ENZYMES AND ESTROGEN



GENETICS & DETOXIFICATION ENZYMES

GSTM1 enzymes functions in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione.

Impaired GSTM1 activity is associated with increased risk of cancers due to impaired elimination of environmental toxins and carcinogens.

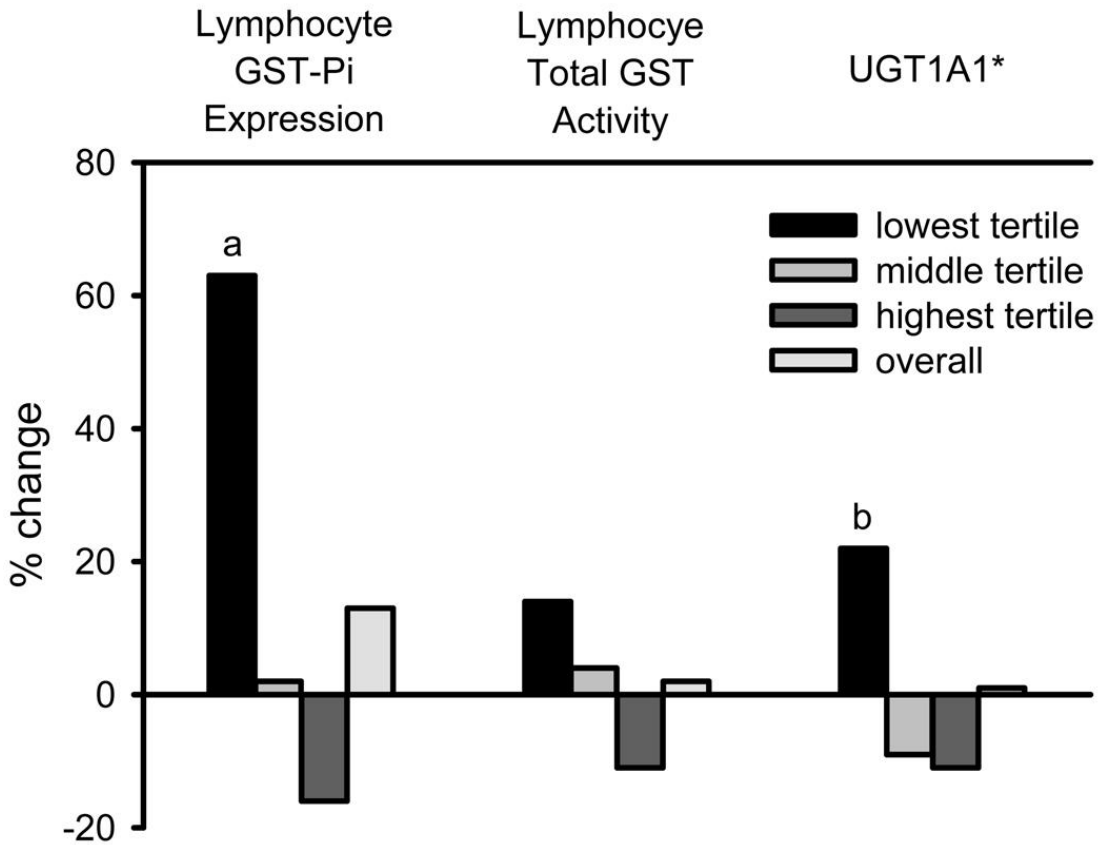
Impaired GSTM1 activity impairs clearance of cancerous estrogen metabolites – Health concern

gene	allele	change/ phenotype
GSTM1	*GSTM1*0*	gene deleted: no enzyme
	*GSTM1*A*	G519: active
	*GSTM1*B*	C519: active
	*GSTM1*1X2*	gene duplicated:high activity

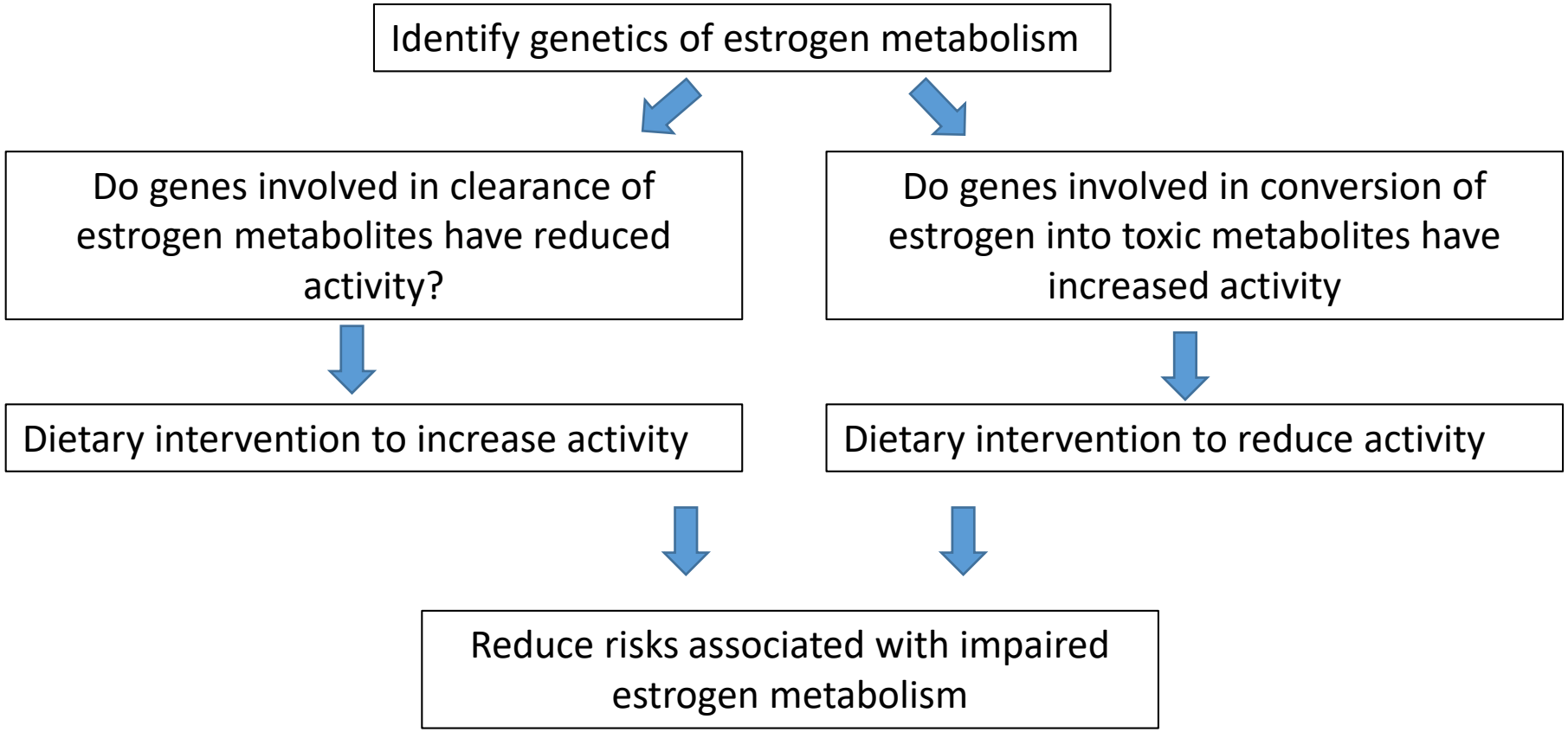
DIETARY INTERVENTION TO BOOST DETOX ENZYMES

GSTM1 plays a key role in inactivation of toxic estrogen metabolites

Dietary intervention with resveratrol boosts GSTM1 activity – Clinical evidence



WHAT DOES THIS MEAN?



CASE STUDY

PHASE I GENE	YOUR GENOTYPE	ENZYME ACTIVITY	IMPACT
CYP1A1	rs1048943 (TT) rs1799814 (GG)	Normal	Normal conversion of estrogen to 2-OHE-1
CYP1B1	rs4680 (CG) rs1056827 (AC) rs1056836 (CG)	Increased	Faster conversion of estrogen into cancerous 4-OHE-1
CYP3A4	rs35599367 (GG) rs12721627 (GG) rs28371759 (AA) rs4987161 (AA)	Decreased	Reduced conversion of estrogen into cancerous 16-OHE-1
COMT	rs4680 (AG)	Normal	Normal excretion of estrogen
GSTM1	rs366631 (AA)	Decreased	Reduced conversion of toxic estrogen metabolites into non-toxic forms

Which enzymatic pathways need to be induced or repressed?