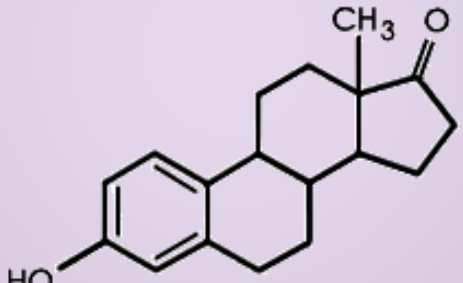
Estrogen Metabolism, Epigenetics & Breast Cancer



Estrogen

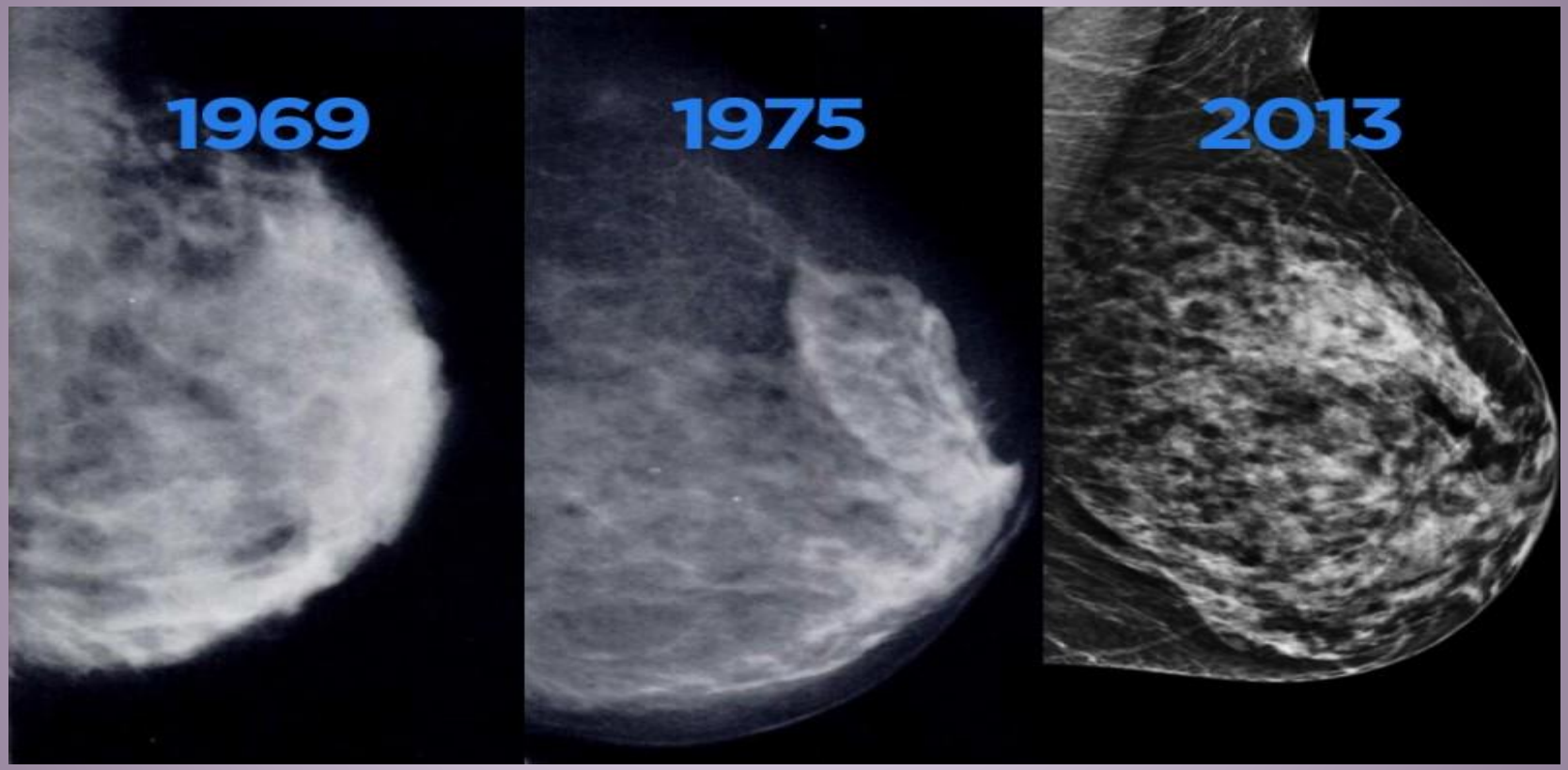
Dr. Jennifer Pearlman
Age Management Medicine Group | AMMG
Tucson, AZ | November 2nd, 2018

Estrogen Metabolism, Epigenetics & Breast Cancer

- ✓ Introduction
- ✓ Estrogen Biosynthesis
- ✓ Estrogenomic Variability
- ✓ Phase 1 Estrogen Metabolism
- ✓ Phase 2 Estrogen Metabolism
- ✓ Estrogen Quinones and Breast Cancer
- ✓ Epigenetics of Estrogen Metabolism
- ✓ Risk Mitigation with Precision Medicine
- ✓ Conclusion & Discussion

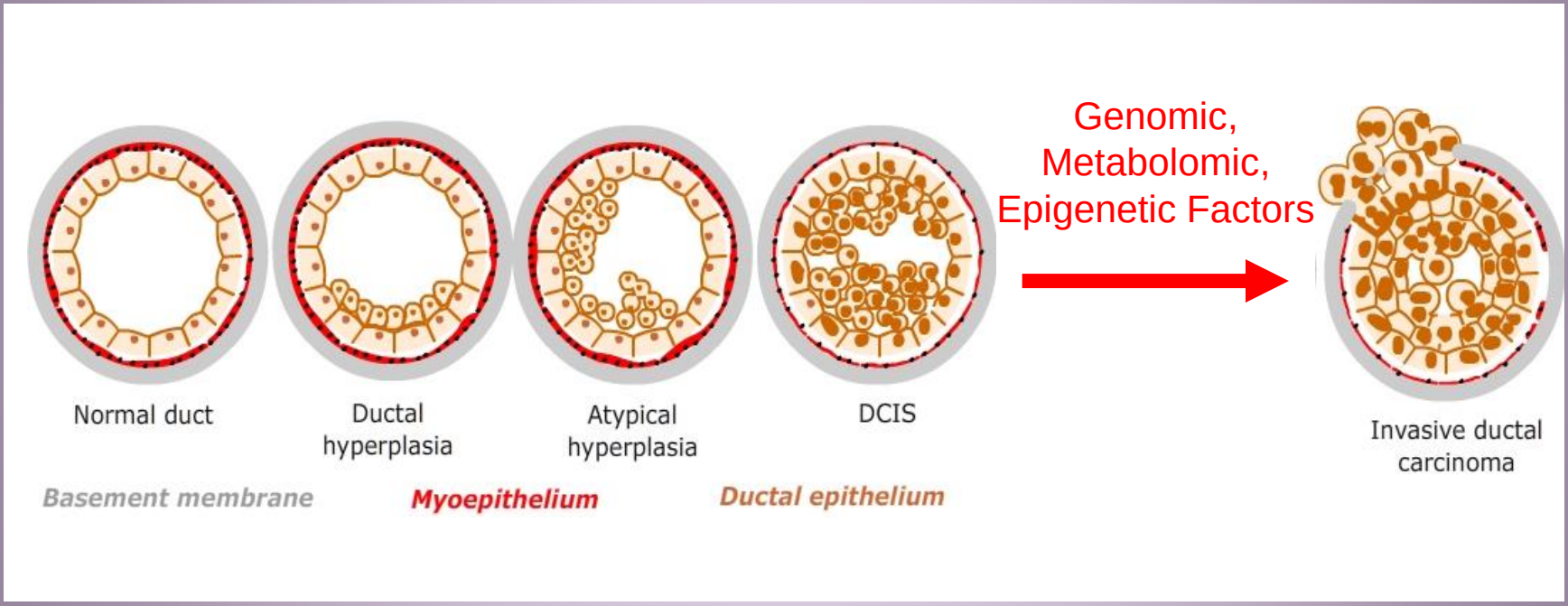

Find The Cure

Estrogen Metabolism and Breast Cancer



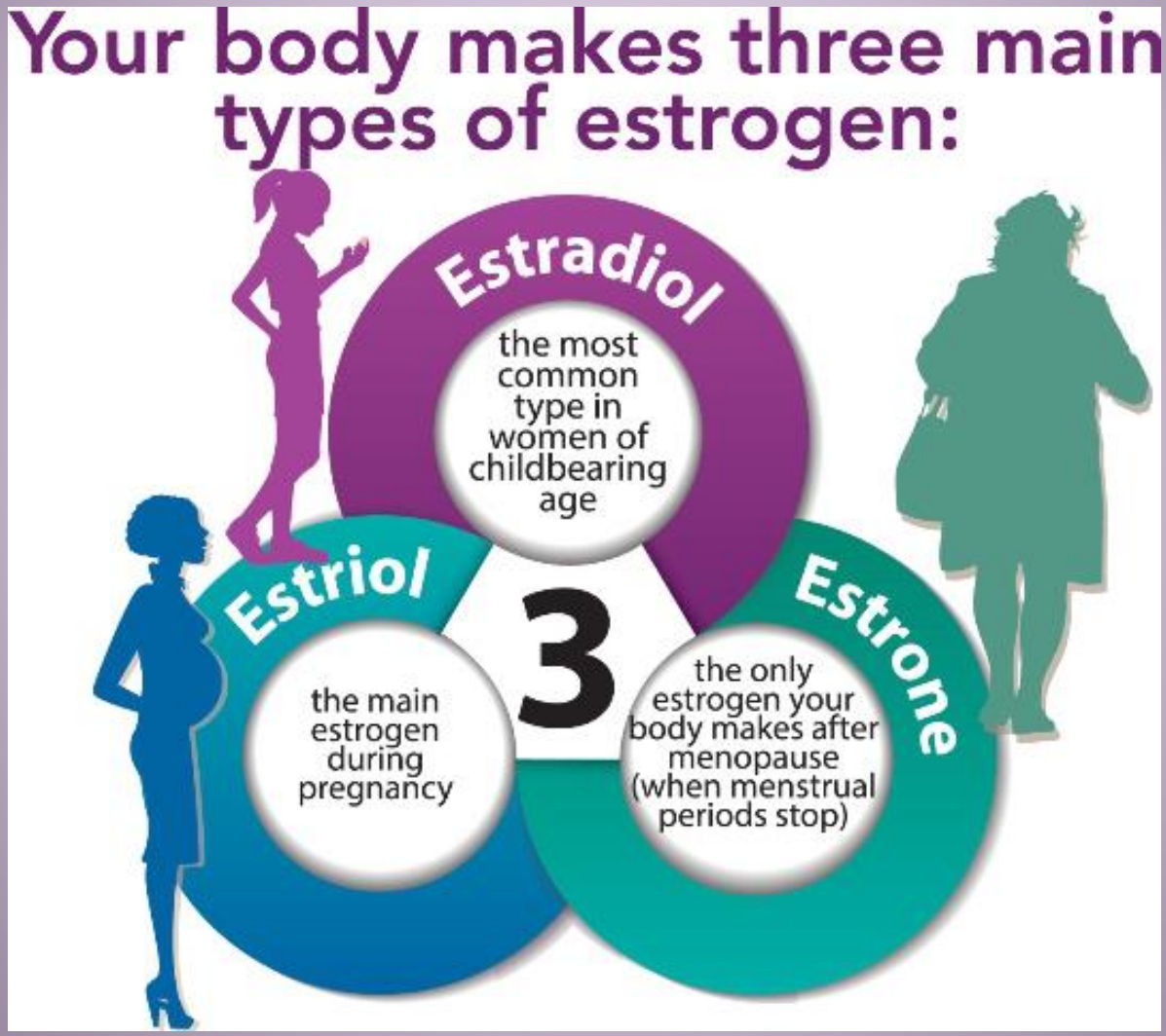
"An ounce of prevention is worth a pound of cure", Benjamin Franklin

Breast Cancer Transformation



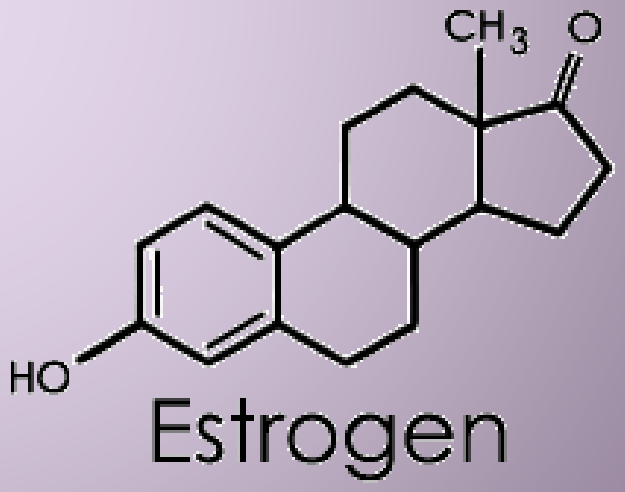
Transformation of normal breast tissue to ductal carcinoma in situ to invasive ductal carcinoma.

Lifetime Estrogen Burden

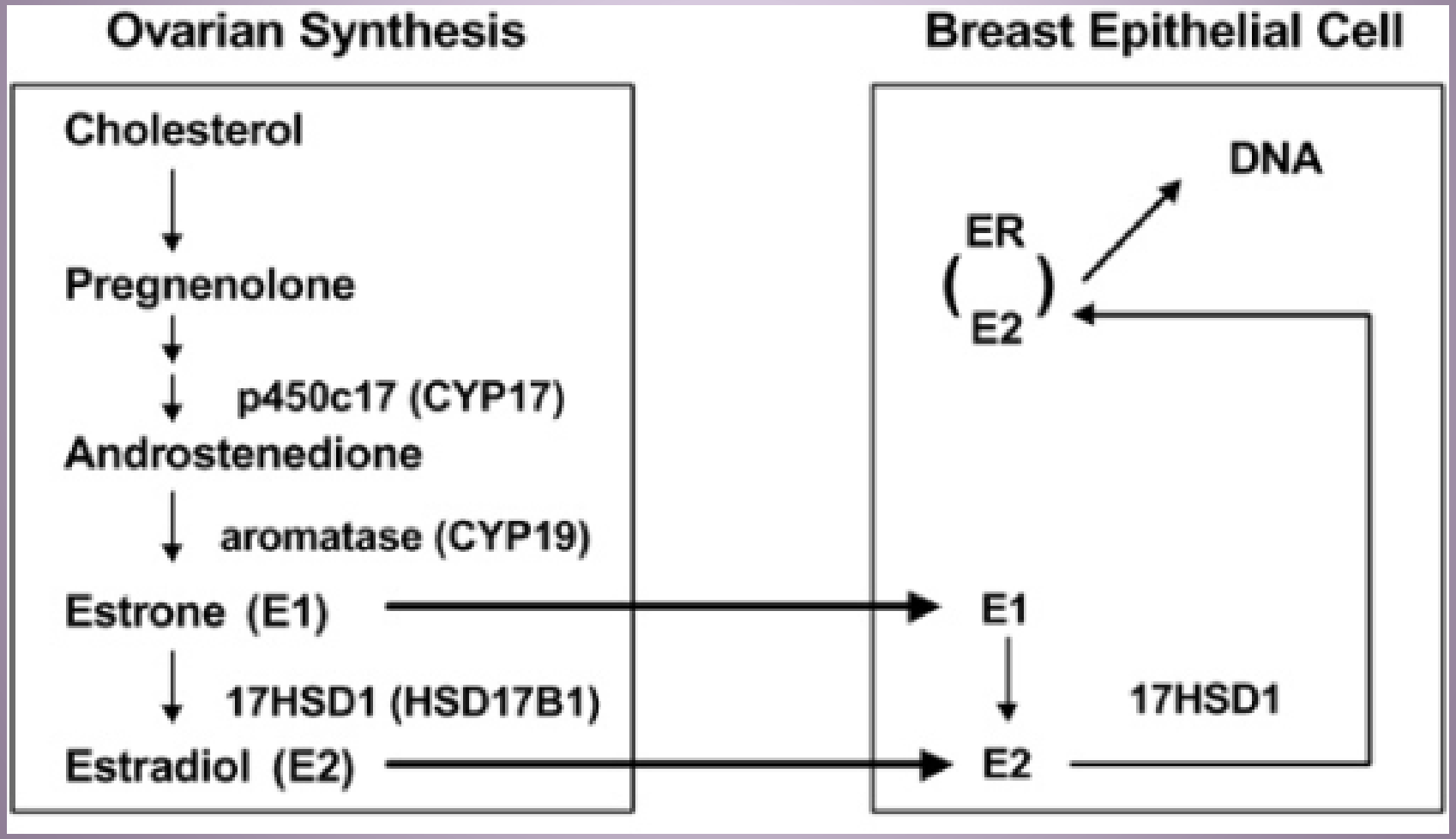


Estrogen

Genomic variability in Estrogen Biosynthesis and Metabolism combined with Epigenetic influence shape Breast Cancer risk



Estrogen Biosynthesis:
Tissue Specificity




Estrogenomic Variability

Estrogen Biosynthesis: Estrogenomic Variability

*Hydroxylation: CYP17A1 (17a-Hydroxylase) catalyzes

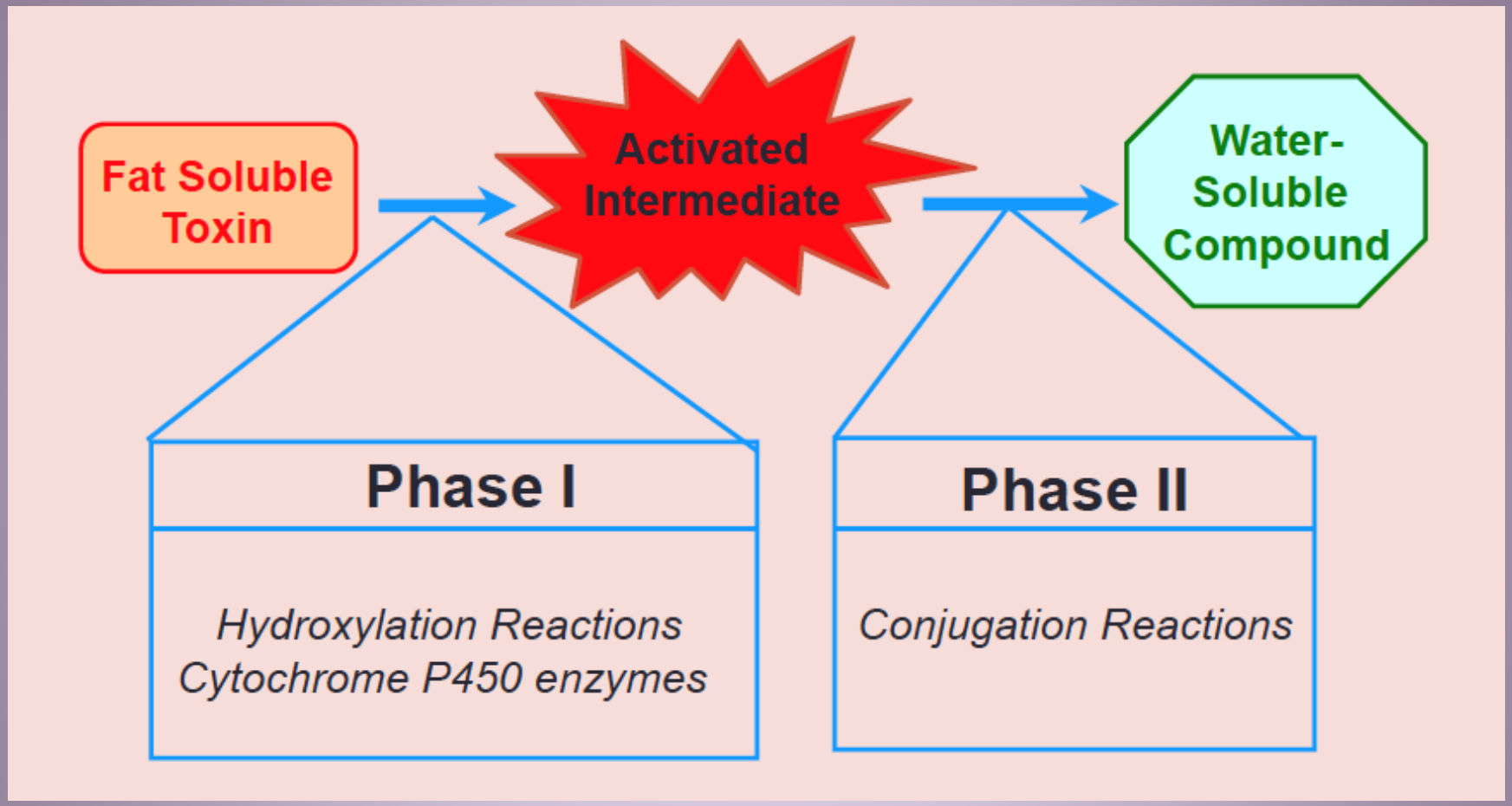
- ✓ Pregnenolone→DHEA* (major, ovary)
- ✓ Progesterone→Androstenedione (minor)

rs743572 SNP G/G (A/A) increased activity associated with inc PCOS, infertility, endometriosis, breast cancer (HT x10yrs)

*Aromatization: CYP19A1 (Aromatase) converts Androgens to Estrogens (E1/E2)

- ✓ Androstenedione→Estrone
- ✓ Testosterone→Estradiol (major, adipose tissue)

rs10046 SNP T/T allele associated with increased aromatase activity, higher levels of circulating estrogen in preMP women, POF, PostMP BC. C/C allele associated with lower levels estrogen in postMP women, dec PostMP BC.



BiPhasic Estrogen Metabolism

Estrogen Metabolism

Biotransformation of Estrogens in biphasic detoxification

Phase I

Estrogen Hydroxylation

- ✓ Cytochrome p450
- ✓ REDOX
- ✓ CYP1A1 (2-OHE)
- ✓ CYP3A4 (16a-OHE)
- ✓ CYP1B1 (4-OHE)

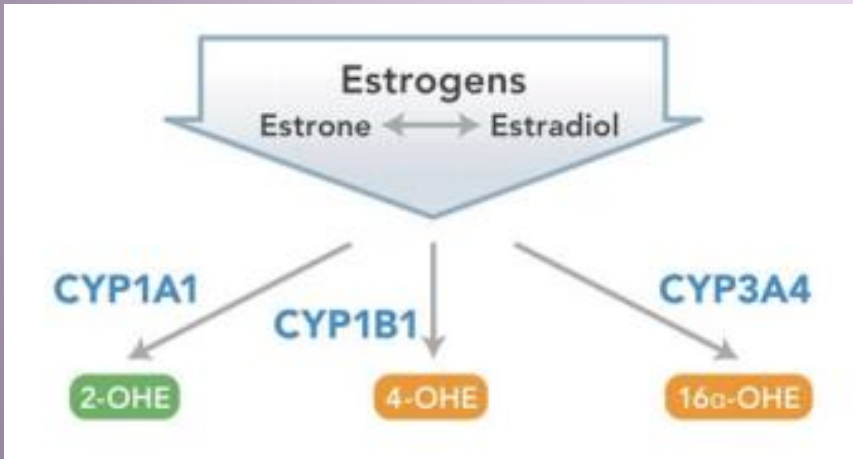
Phase II

Estrogen Conjugation

- ✓ Methylation (COMT)
- ✓ Glutathionization (GST)
- ✓ Glucuronidation (UGT)
- ✓ Sulfation (SOD2)
- ✓ Acetylation

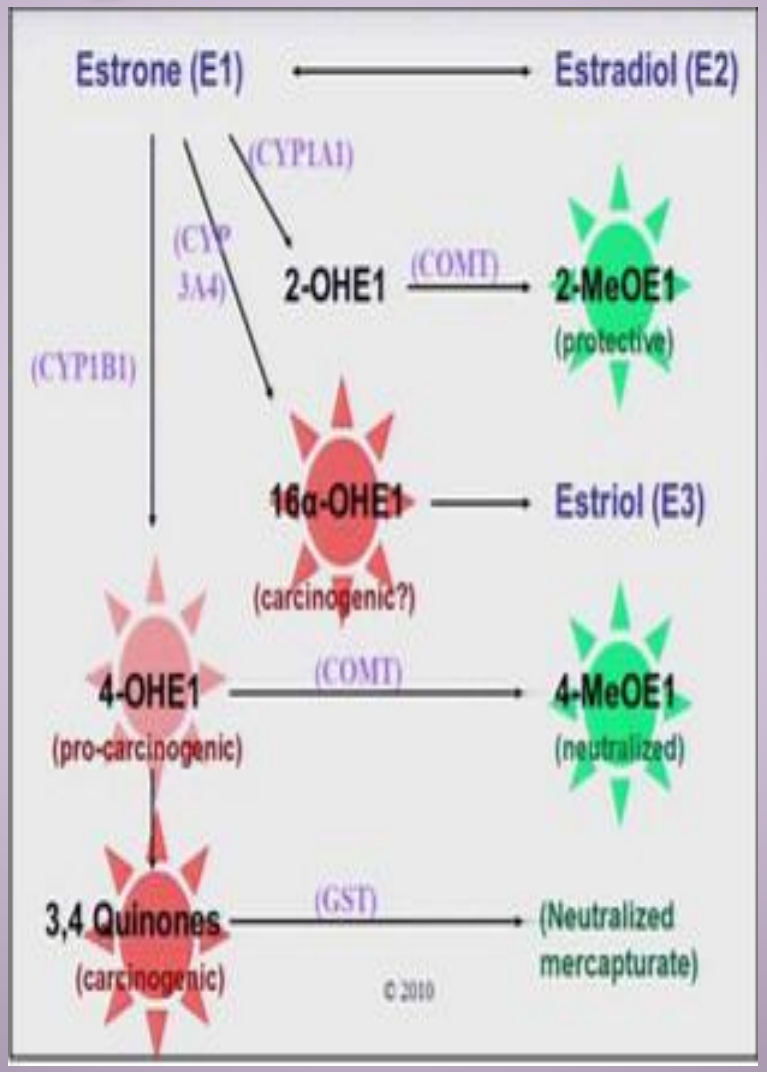
Phase I: Estrogen Hydroxylation

Phase 1 involves the hydroxylation of Estrone and Estradiol via the cytochrome P450 system through REDOX reactions. Catechol estrogen intermediates have variable estrogenic potential.



- CYP1A1 (2-OHE)
++ CYP3A4 (16a-OHE)
+++ CYP1B1 (4-OHE)

Genomic Variation of Estrogen Metabolism



Estrogen Metabolism Ratio (EMR): Predictive Value of the 2:16 EMR

- ✓ CYP1A1: 2-OH Estrogens are inert, exert negative feedback, activates genotoxic polyaromatic hydrocarbons (smoke)

- ✓ CYP3A4: 16a-OH Estrogens are proliferative, estrogenic, activates ER, mutagenic, carcinogenic. Induced by GCC, smoking, EtOH, OC, SJW and inhibited by grapefruit

SNP rs2740574 G/G increased activity associated with early menarche, BC, EC, PC in men.

Martínez-Ramírez, O.C., Pérez-Morales, R., Castro, C., Flores-Díaz, A., Soto-Cruz, K.E., Astorga-Ramos, A., Gonsebatt, M.E., Casas, L., Valdés-Flores, M., and Rubio, J. (2013). Polymorphisms of catechol estrogens metabolism pathway genes and breast cancer risk in Mexican women. The Breast *22*, 335–343.
Ghisari, M., Eiberg, H., Long, M., and Bonefeld-Jørgensen, E.C. (2014). Polymorphisms in phase I and phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environ. Health Glob. Access Sci. Source *13*, 19.
Sowers, M.R., Wilson, A.L., Kardia, S.R., Chu, J., and McConnell, D.S. (2006). CYP1A1 and CYP1B1 Polymorphisms and Their Association with Estradiol and Estrogen Metabolites in Women Who Are Premenopausal and Perimenopausal. Am. J. Med. *119*, S44–S51.

EMR and Breast Cancer Risk

Estrogen metabolite ratio: Is the 2-hydroxyestrone to 16α-hydroxyestrone ratio predictive for breast cancer?

This article was published in the following Dove Press journal:
International Journal of Women's Health
8 February 2011
Number of times this article has been viewed

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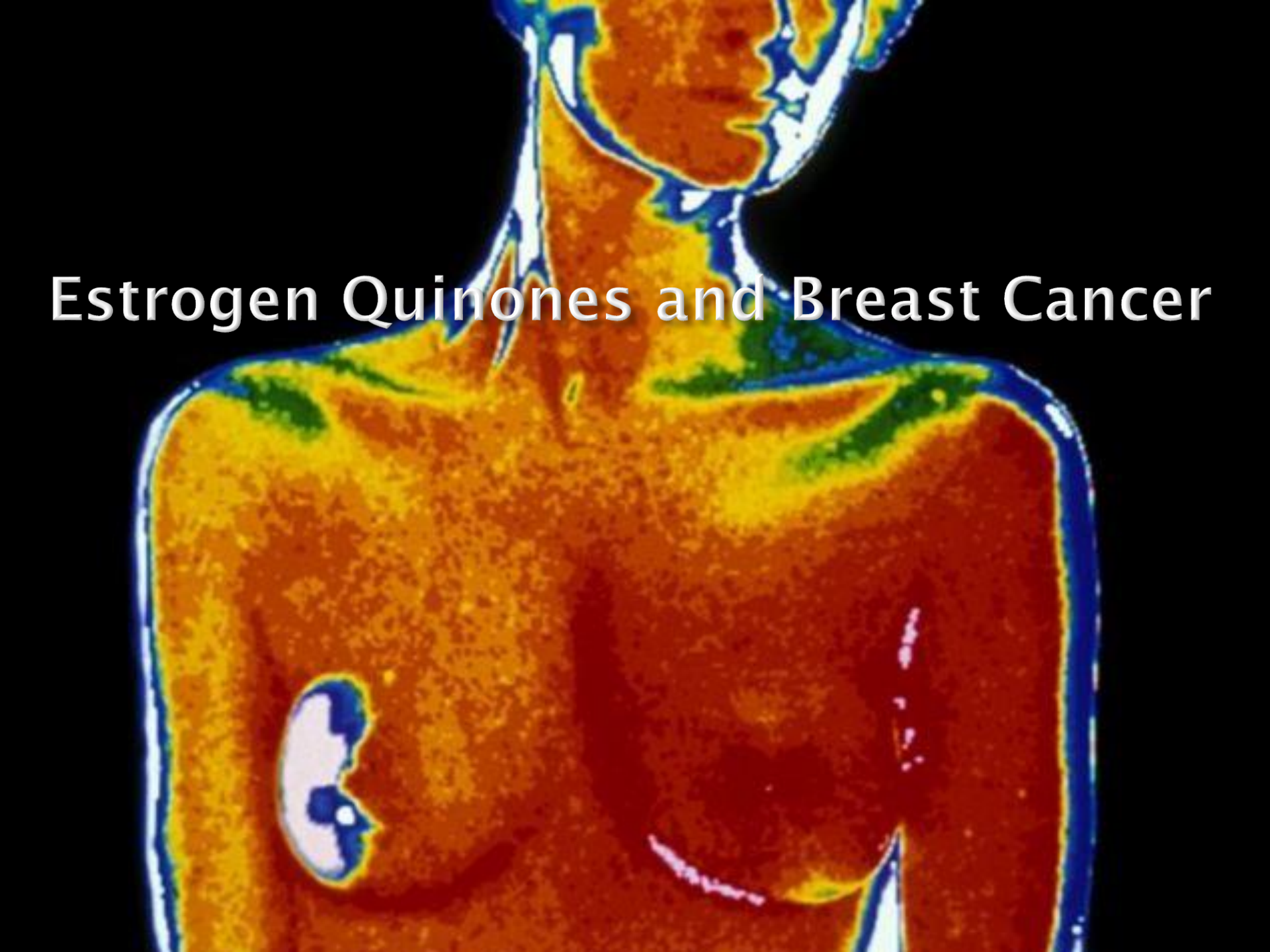
Abstract: Experimental studies have shown that two main estrogen metabolites hydroxylated by CYP1A1 and CYP1B1 in the breast differentially affect breast cell proliferation and carcinogenesis. Although 16α-hydroxyestrone (16αOHE1) exerts estrogenic activity through covalent estrogen receptor (ER) binding, 2-hydroxyestrone (2OHE1) presumably has antiestrogenic capabilities. The ratio of 2OHE1 to 16αOHE1 represents the relative dominance of one pathway over the other and is believed to be modifiable by diet. It was hypothesized that women with or at high risk of breast cancer have a lower estrogen metabolite ratio (EMR) compared with women without breast cancer. We conducted a systematic review on the EMR as a predictor for breast cancer. A total of nine studies (six prospective and three retrospective) matched our inclusion criteria, comprising 682 premenopausal cases (1027 controls) and 1189 postmenopausal cases (1888 controls). For the highest compared with the lowest quantile of urinary EMR, nonsignificant associations suggested at best a weak protective effect in premenopausal but not in postmenopausal breast cancer (range of odds ratios: 0.50–0.75 for premenopausal and 0.71–1.31 for postmenopausal). Circulating serum/plasma EMR was not associated with breast cancer risk. Associations were inconclusive for receptor subtypes of breast cancer. Uncontrolled factors known to be involved in breast carcinogenesis, such as 4-hydroxyestrone (4OHE1) concentration, may have confounded results for EMR. Results of the prospective studies do not support the hypothesis that EMR can be used as a predictive marker for breast cancer risk. Future research should concentrate on profiles of estrogen metabolites, including 4OHE1, to gain a more complete picture of the relative importance of single metabolites for breast cancer.

Keywords: estrogen metabolite ratio, 2-hydroxyestrone, 16α-hydroxyestrone, breast cancer, predictive marker, review

The 2:16 Estrogen Metabolism Ratio predicts BC in premenopausal women.

Estrogen Quinones and Breast Cancer



4-OH Estrogens & Estrogen Quinones

- ✓ CYP1B1 expressed in breast tissue
- ✓ Hydroxylates to 4-OH Estrogens
- ✓ Proliferative, Estrogenic
- ✓ Forms Estrogen-3,4-Quinones
- ✓ Activated by CEE, benzopyrene, polyaromatic HC, dioxins, , industrial cleaners (PFAS, ScotchGaurd)
- ✓ Activating SNP G/G associated with ER+ BC

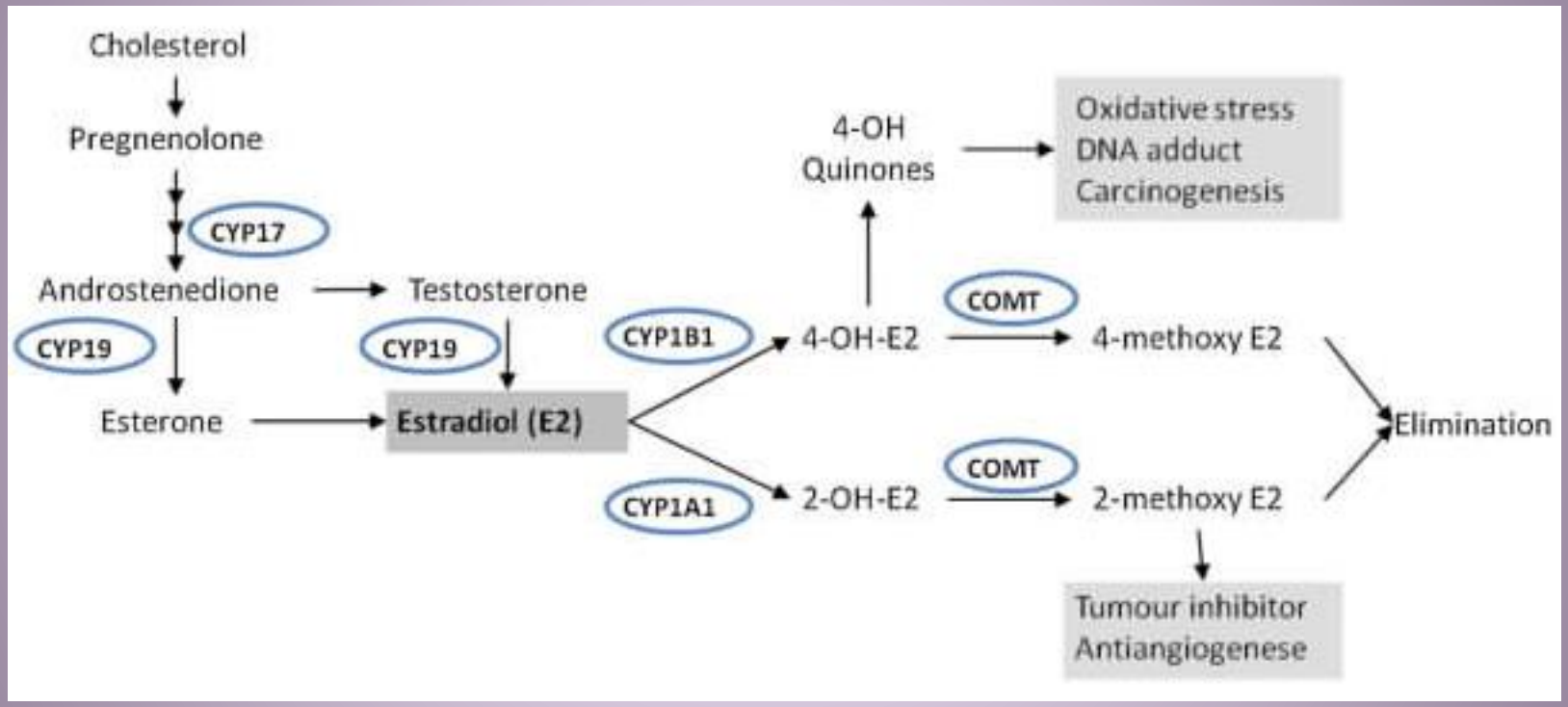
SNP rs1056836 G/G 3x increased activity, C allele more common in SEA women, induced by toxins, high BMI, CEE

Kocaba;, N., Aygun, Neslihan, S., Cholerton, S., Daly, A., and Karakaya, A. (2002). Cytochrome P450 CYP1B1 and catechol O -methyltransferase (COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. Arch. Toxicol. *76*, 643–649.
Kato, I., Cichon, M., Yee, C.L., Land, S., and Korczak, J.F. (2009). African American-preponderant single nucleotide polymorphisms (SNPs) and risk of breast cancer. Cancer Epidemiol. *33*, 24–30.

Estrogen Quinones and Breast Cancer

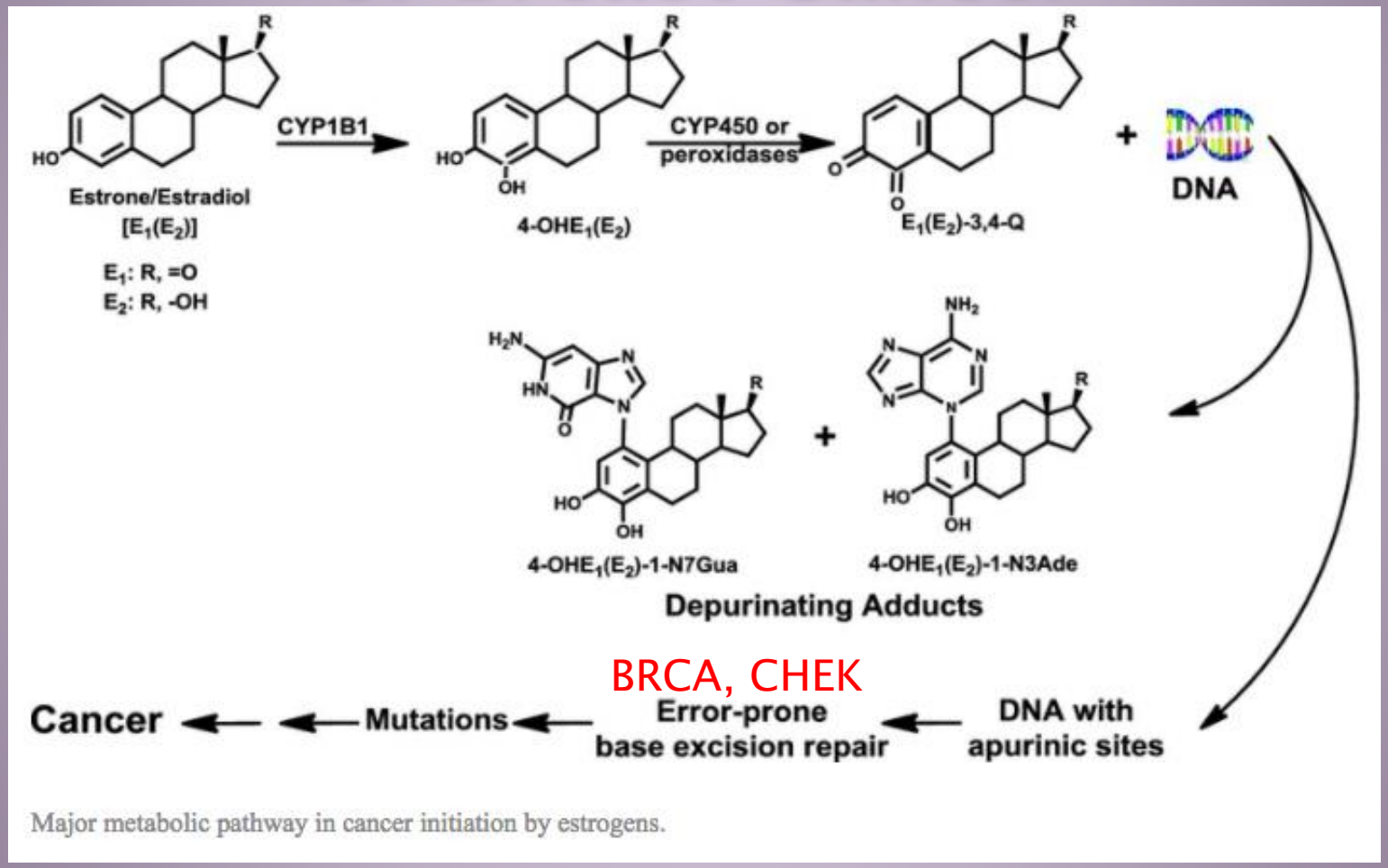
- ✓ Estrogen-3,4-Quinones are highly carcinogenic
- ✓ Potentiated by oxidative damage and EDC exposure
- ✓ Genotoxic: DNA breaks/adducts
- ✓ Mutagenic: depurinate DNA
- ✓ Carcinogenic: high levels in BC and HR breast tissue
- ✓ Induction & Propagation of BC
- ✓ Increased risk hormone responsive BC

4-OH Estrogens and Breast Cancer



4-hydroxy Estrogens and Breast Cancer Risk

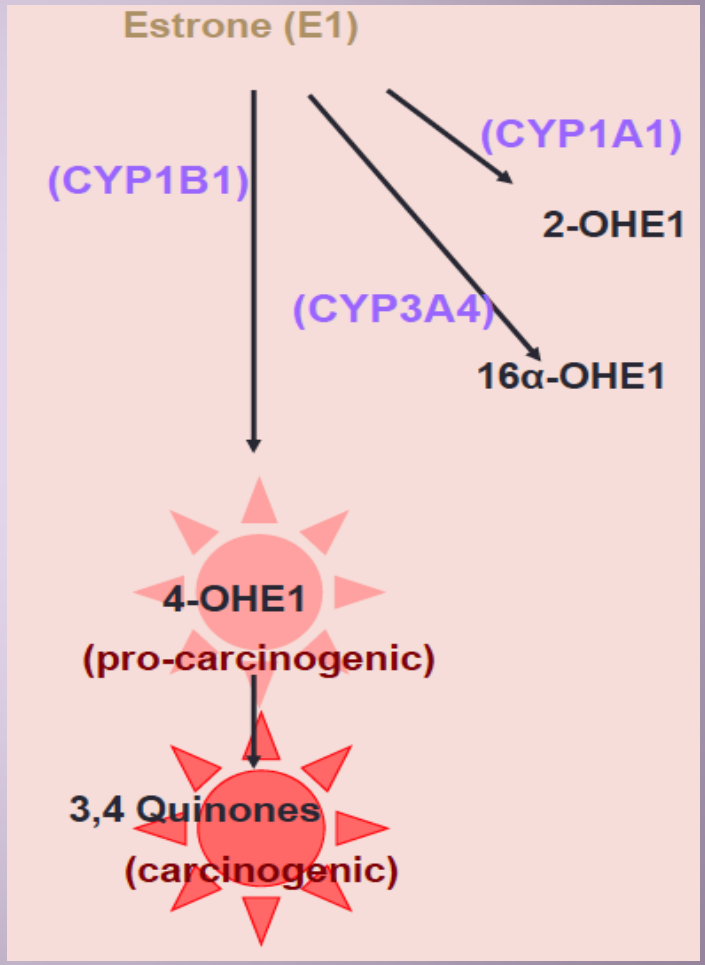
Estrogen-DNA adducts induction of Breast Cancer



BRCA1 functions as a whole genome repair system. When impaired by SNPs or with aging the carcinogencity of estrogen quinones may be augmented.

Epigenetics and 4-OH Estrone Carcinogenecity

- ✓ Genetic SNP (3x)
- ✓ Obesity
- ✓ CEE
- ✓ Smoking
- ✓ Benzopyrene
- ✓ Polyaromatic HC
- ✓ Dioxin Herbicides
- ✓ Industrial Cleaners (PFAS/ScotchGaurd)



Estrogen-DNA adducts and Breast Cancer

Depurinating estrogen-DNA adducts, generators of cancer initiation: their minimization leads to cancer prevention

Ercole L. Cavalieri[1,2*] and Eleanor G. Rogan[1,2]

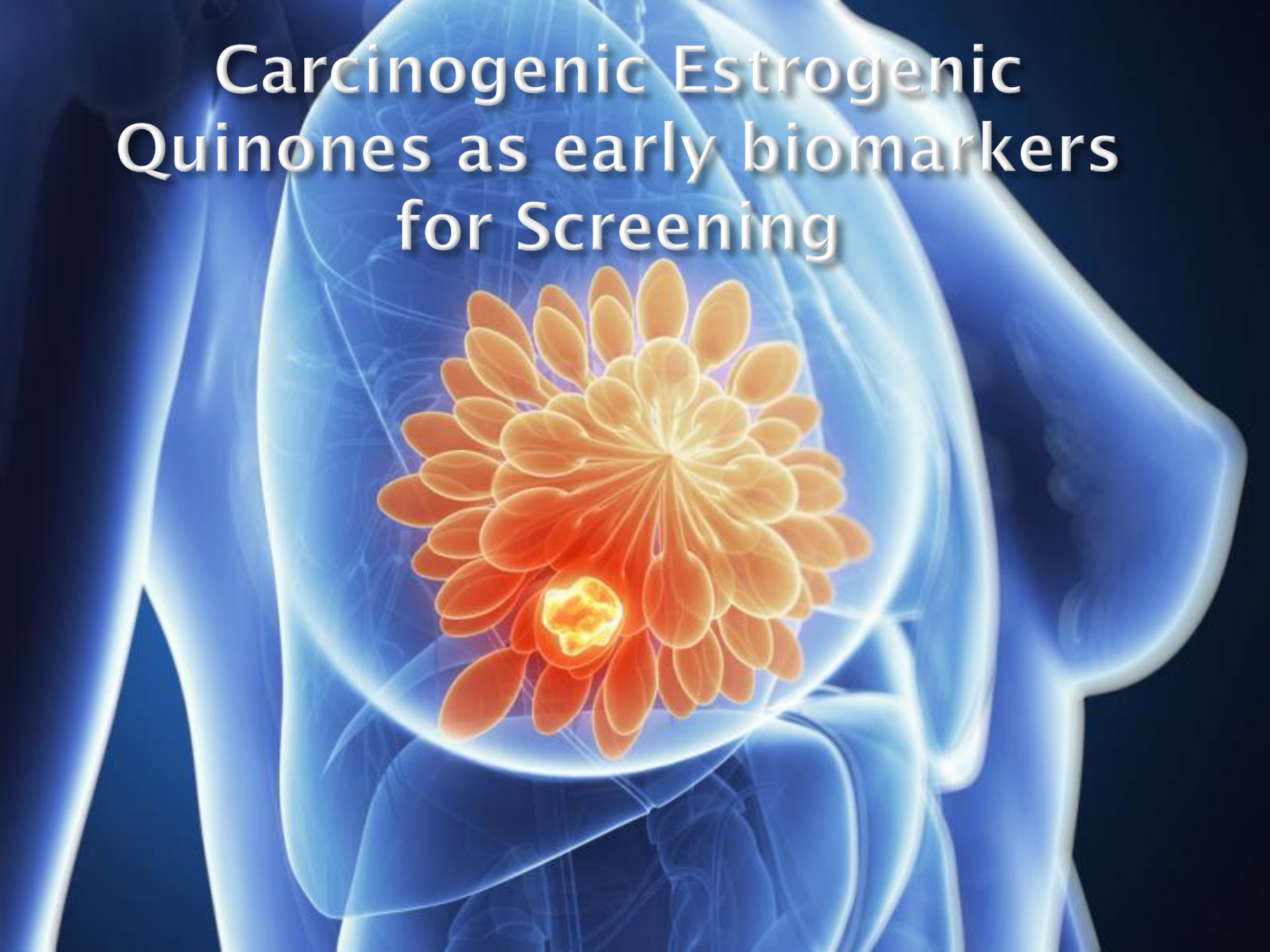
Abstract

Estrogens can initiate cancer by reacting with DNA. Specific metabolites of endogenous estrogens, the catechol estrogen-3,4-quinones, react with DNA to form depurinating estrogen-DNA adducts. Loss of these adducts leaves apurinic sites in the DNA, generating mutations that can lead to the initiation of cancer. A variety of endogenous and exogenous factors can disrupt estrogen homeostasis, which is the normal balance between estrogen activating and protective enzymes. In fact, if estrogen metabolism becomes unbalanced and generates excessive catechol estrogen 3,4-quinones, formation of depurinating estrogen-DNA adducts increases and the risk of initiating cancer is greater. The levels of depurinating estrogen-DNA adducts are high in women diagnosed with breast cancer and those at high risk for the disease. High levels of depurinating estrogen-DNA adducts before the presence of breast cancer indicates that adduct formation is a critical factor in breast cancer initiation. Women with thyroid or ovarian cancer also have high levels of estrogen-DNA adducts, as do men with prostate cancer or non-Hodgkin lymphoma. Depurinating estrogen-DNA adducts are initiators of many prevalent types of human cancer. These findings and other discoveries led to the recognition that reducing the levels of estrogen-DNA adducts could prevent the initiation of human cancer. The dietary supplements *N*-acetylcysteine and resveratrol inhibit formation of estrogen-DNA adducts in cultured human breast cells and in wo
risk of developing various pr

Keywords: Estrogen metab
genotoxicity, Estrogen carcin

High levels of Estrogen-DNA adducts before the presence of breast cancer indicates that adduct formation is a critical factor in breast cancer initiation.

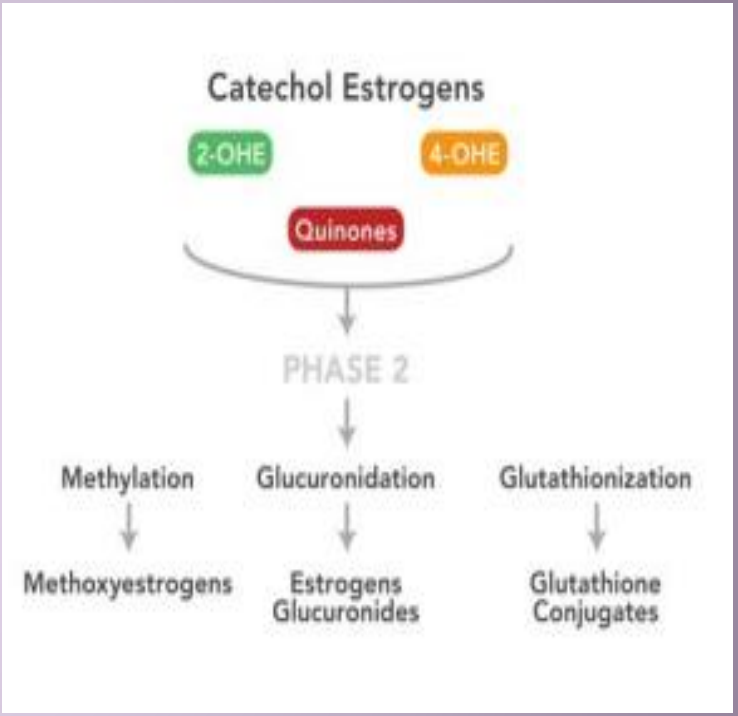
Carcinogenic Estrogenic Quinones as early biomarkers for Screening



Phase II: Estrogen Conjugation

Phase II involves the inactivation of catechol estrogens (CE) formed in Phase I CYP P450 hydroxylation. Conjugation increases solubility, hydrophilicity of CEs and enables renal and biliary excretion

- ✓ Methylation (COMT)
- ✓ Glutathionization (GST)
- ✓ Glucuronidation (UGT)
- ✓ Anti-oxidation (SOD2)



COMT and Methylation

- ✓ COMT major metabolizer of CE as well as catecholamines, NT, xenobiotics, Rx
- ✓ Methylated 2-OH Estrogens are anti-estrogenic, protective, anti-proliferative (ER)
- ✓ Lower levels of methylation of 4-OHE associated with increased risk BC

SNP rs4680 A/A lower level activity, less methylation, strongly influenced by lifestyle factors to increase risk BC, Obesity, Folate deficiency potentiate risks and genotoxicity

Naushad, S.M., Reddy, C.A., Rupasree, Y., Pavani, A., Digumarti, R.R., Gottumukkala, S.R., Kuppusamy, P., and Kutala, V.K. (2011). Cross-Talk Between One-Carbon Metabolism and Xenobiotic Metabolism: Implications on Oxidative DNA Damage and Susceptibility to Breast Cancer. Cell Biochem. Biophys. *61*, 715–723.
Wan, G.-X., Cao, Y.-W., Li, W.-Q., Li, Y.-C., and Li, F. (2014). The Catechol-O-Methyltransferase Val158Met Polymorphism Contributes to the Risk of Breast Cancer in the Chinese Population: An Updated Meta-Analysis. J. Breast Cancer *17*, 149.

Increased 4-OH Metabolism and lower level of 4-OH Methylation predicts BC in Postmenopausal Women

Estrogen Metabolism and Risk of Breast Cancer in Postmenopausal Women

Barbara J. Fuhrman, Catherine Schairer, Mitchell H. Gail, Jennifer Boyd-Morin, Xia Xu, Laura Y. Sue, Saundra S. Buys, Claudine Isaacs, Larry K. Keefer, Timothy D. Veenstra, Christine D. Berg, Robert N. Hoover, Regina G. Ziegler

Manuscript received January 19, 2011; revised July 16, 2011; accepted December 2, 2011.

Correspondence to: Barbara J. Fuhrman, PhD, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Blvd, Rm 5100, Bethesda, MD 20892 (e-mail:fuhrmanb@mail.nih.gov).

Background Estrogens are recognized causal factors in breast cancer. Interindividual variation in estrogen metabolism may also influence the risk of breast cancer and could provide clues to mechanisms of breast carcinogenesis. Long-standing hypotheses about how estrogen metabolism might influence breast cancer have not been adequately evaluated in epidemiological studies because of the lack of accurate, reproducible, and high-throughput assays for estrogen metabolites.

Methods We conducted a prospective case–control study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). Participants included 277 women who developed invasive breast cancer (case subjects) and 423 matched control subjects; at PLCO baseline, all subjects were aged 55–74 years, postmenopausal and not using hormone therapy, and provided a blood sample. Liquid chromatography–tandem mass spectrometry was used to measure serum concentrations of 15 estrogens and estrogen metabolites, in unconjugated and conjugated forms, including the parent estrogens, estrone and estradiol, and estrogen metabolites in pathways defined by irreversible hydroxylation at the C-2, C-4, or C-16 positions of the steroid ring. We calculated hazard ratios (HRs) approximating risk in highest vs lowest deciles of individual estrogens and estrogen metabolites, estrogens and estrogen metabolites grouped by metabolic pathways, and metabolic pathway ratios using multivariable Cox proportional hazards models. All statistical tests were two-sided.

Results Nearly all estrogens, estrogen metabolites, and metabolic pathway groups were associated with an increased risk of breast cancer; the serum concentration of unconjugated estradiol was strongly associated with the risk of breast cancer (HR = 2.07, 95% confidence interval [CI] = 1.19 to 3.62). No estrogen, estrogen metabolite, or metabolic pathway group remained statistically significantly associated with the risk of breast cancer after adjusting for unconjugated estradiol. The ratio of the 2-hydroxylation pathway to parent estrogens (HR = 0.66, 95% CI = 0.51 to 0.87) and the ratio of 4-hydroxylation pathway catechols to 4-hydroxylation pathway methylated catechols (HR = 1.34, 95% CI = 1.04 to 1.72) were statistically significantly associated with the risk of breast cancer and remained so after adjustment for unconjugated estradiol.

Conclusions More extensive 2-hydroxylation of parent estrogens is associated with lower risk, and less extensive methylation of potentially genotoxic 4-hydroxylation pathway catechols is associated with higher risk of postmenopausal breast cancer.

J Natl Cancer Inst 2012;104:326–339

Accessory Methylation Pathways and BC Risk

*Polymorphisms in genes encoding enzymes of folate metabolism confer BC risk due to their role in DNA methylation, synthesis and repair.

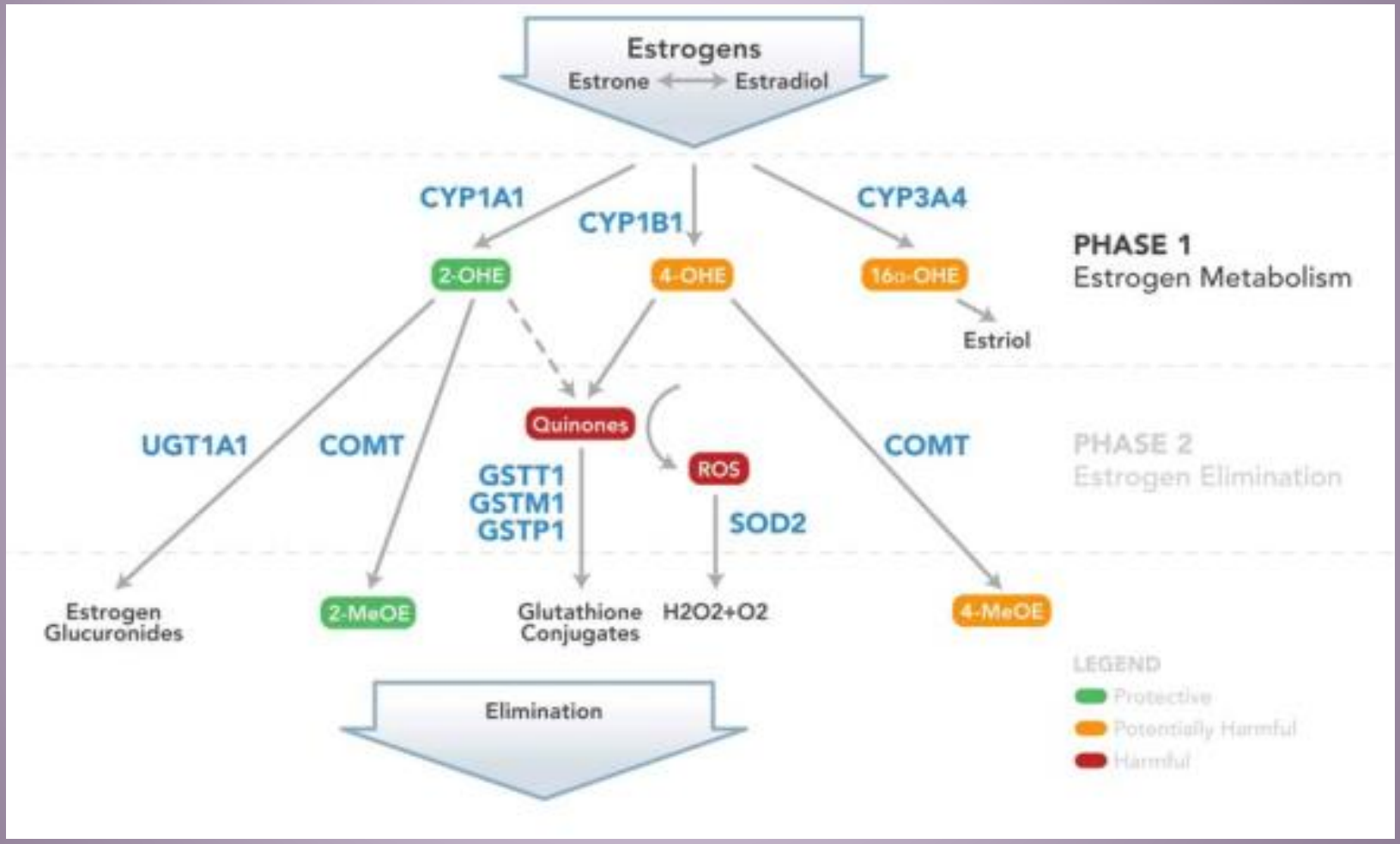
*MTHFR polymorphisms are associated with breast cancer risk when co-existent with methionine synthase (MTR) polymorphisms in the heterozygous state."

Anticancer Res. 2012 Nov;32(11):4805-11

Glutathionization

- GST conjugates reduced glutathione to quinones and semiquinone by-products of CE metabolism. GSTT1 is dominant form subject to CNVs. GSTT1 inactivates heavy metals, pharmaceuticals, xenobiotics, and ROS
- GSTT1: null poor detoxification, single is most common and CNVs of 2 or higher associated with increased inactivation of CE
- GSTM1, GSTP1 accessory pathways, subject to SNP variations

Imbalanced Estrogen Metabolism

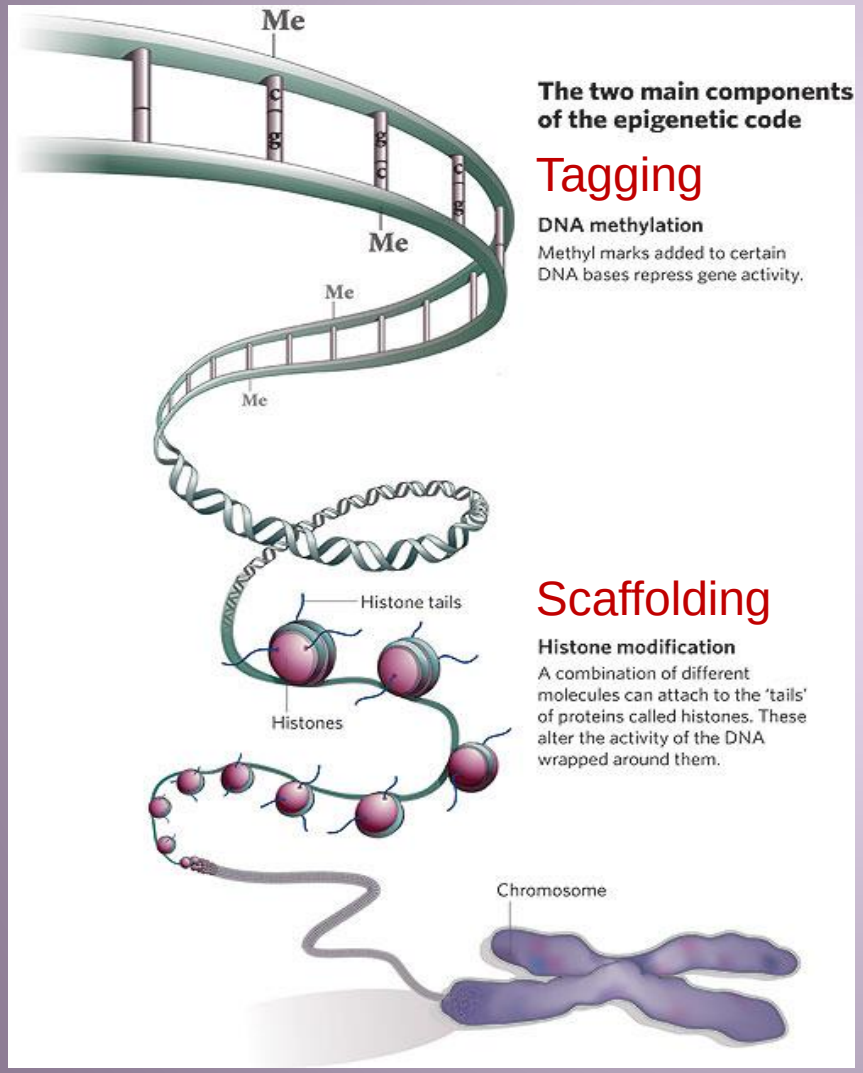


Estrogen and Breast Cancer

- ✓ Lifetime Estrogen Burden: early menarche, late MP
- ✓ Endogenous Estrogen Levels: 2-3x inc BC
- ✓ Circulating Primary Hormones: SHBG, DHEAS
- ✓ Genetic Variations in Estrogen Biosynthesis (Aromatase) and Receptor binding (ERb)
- ✓ Genetic Variations in Estrogen Metabolism: CYP1B1, CYP1A1, COMT, GST
- ✓ Methylation Status: COMT, MTHFR, MTR, Hg, Mg
- ✓ Circulating Estrogen Metabolites: 16-OHE_1, 4-OHE
- ✓ Urinary 2:16 Ratio: dec risk BC OR 0.74
- ✓ Estrogen DNA adducts: predicts BC risk

Your DNA Is Not Your Destiny

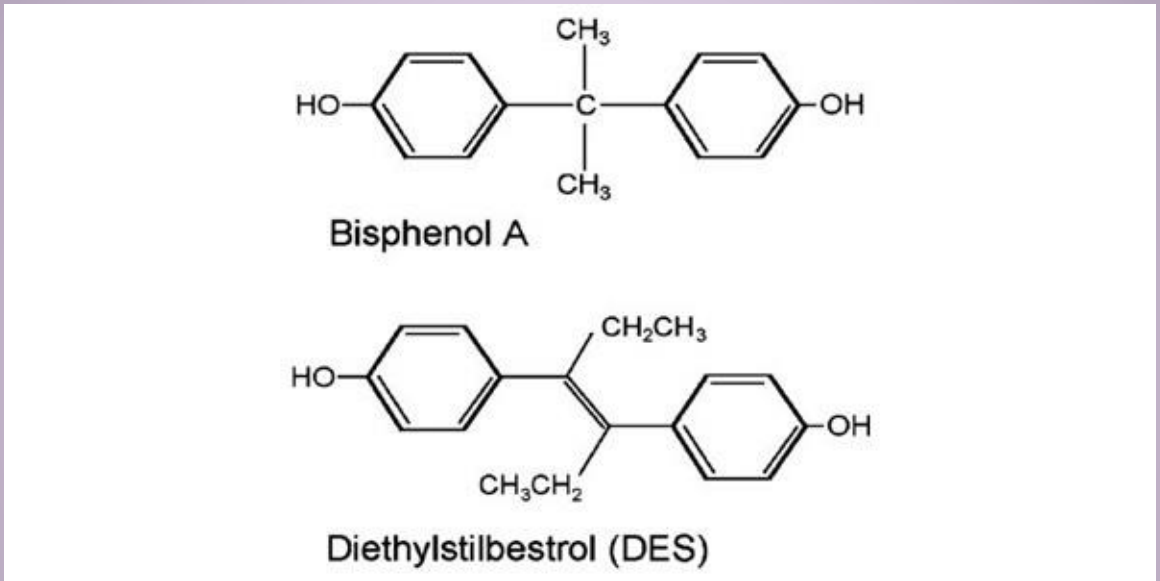
The Epigenetic Code



Environmental Xenoestrogen Exposure and Breast Cancer Risk

Ghisari M, et al. Polymorphism in Phase I and II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environmental Health. 2014. March. 13:19.

Endocrine Disrupting Chemicals: BPA and DES



BPA and DES are EDCs that are associated with increased breast cancer risk through direct and indirect effects; with estrogen receptor modulation and imbalancing of estrogen metabolism as well as epigenetic effects in estrogen biosynthesis and metabolism.

BPA's carcinogenicity and impact on Estrogen Metabolism

IUBMB Life. 2010 Oct;62(10):746-51. doi: 10.1002/iub.376.

Is bisphenol A a weak carcinogen like the natural estrogens and diethylstilbestrol?

Cavalieri EL[1], Rogan EG.

Author information

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Abstract

Bisphenol A (BPA) displays weal
estrone (E(1)), estradiol (E(2)) a
evidence supports the hypothesis
DNA to cause mutations that can
the 4-catechol estrogens, 4-OHE
DNA to form predominantly the d
constitutes the predominant path
hydroxylation of one of its symme
would lead to reaction with DNA
The resulting apurinic sites in the
catechol of BPA may also alter e
methoxylation of 4-OHE(1)(E(2))
increase formation of E(1)(E(2))
exposure to BPA could increase the risk of developing cancer by direct and/or indirect mechanisms. Knowledge of these

"BPA displays weak estrogenic properties and could be a weak carcinogen by a mechanism similar to that of estrone (E(1)), estradiol (E(2)) and the synthetic estrogen diethylstilbestrol, a human carcinogen…..

The catechol of BPA may alter expression of estrogen activating and deactivating enzymes, and/or compete with methoxylation of 4-OHE(1)(E(2)) by COMT, thereby unbalancing the metabolism of estrogens to increase formation of E(1)(E(2))-3,4-Q and the depurinating estrogen-DNA adducts leading to cancer initiation.

Thus, exposure to BPA could increase the risk of developing cancer by direct and/or indirect mechanisms".

Epigenetics 8:3, 237–245; March 2013; © 2013 Landes Bioscience

Epigenetic regulation of estrogen signaling in breast cancer

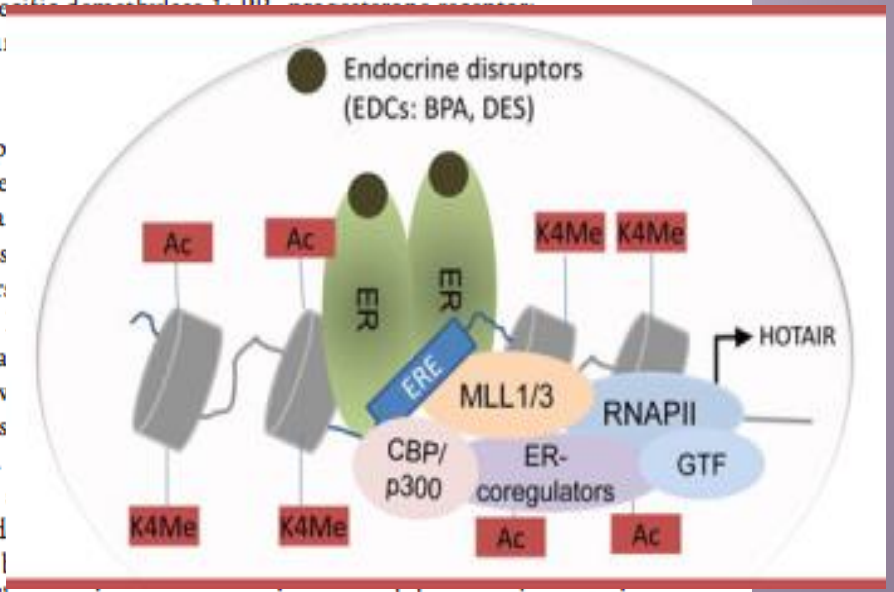
Eric Hervouet,[1,*] Pierre-François Cartron,[2] Michèle Jouvenot[1] and Régis Delage-Mourroux[1]

[1]Université de Franche-Comté; Laboratoire de Biochimie; EA3922; Expression Génique et Pathologies du Système Nerveux Central; SFRIBCT FED 4234; UFR Sciences et Techniques; Besançon, France; [2]Centre de recherche en Cancérologie Nantes-Angers; INSERM; U892 Equipe Aspects mécanistiques et physiopathologiques de l'activité des protéines de la famille de Bcl-2; Equipe labellisée Ligue Nationale Contre le Cancer; Nantes, France

Keywords: epigenetic, estrogen, DNA methylation, histones, breast cancer

Abbreviations: BC, breast cancer; BpA, bisphenol A; Dnmt, DNA methyl transferase; ER, estrogen receptor; ER⁻, estrogen receptor negative; ER⁺, estrogen receptor positive; ESR1, gene encoding ERα; ESR2, gene encoding ERβ; EZH2, enhancer of zeste homolog 2; HMT, histone methylase; HDM, histone demethylase; HAT, histone acetylase; HDAC, histone deacetylase; JMJD, Jumonji domain-containing protein; LSD1, lysine spe… TF, transcription factor; TSG, tu…

Estrogen signaling is mediated by ERα and ERβ in hormone dependent breast cancer (BC). Over the last decade the implication of epigenetic pathways in BC tumorigenesis has emerged: cancer-related epigenetic modifications are implicated in both gene expression regulation and chromosomal instability. In this review, the epigenetic-mediated estrogen signaling, controlling both ER level and ER-targeted gene expression in BC, are discussed: (1) ER silencing is frequently observed in BC and is often associated with epigenetic regulations while chemical epigenetic modulators restore ER expression and increase response to treatment; (2) ER-targeted gene expression is tightly regulated by co-recruitment of ER and both co-activators/co-repressors including HATs, HDACs, HMTs, Dnmts and Polycomb proteins.



recep… seque… of ta… binds… favor… with… the a… follo… genes… ERα… such… ER-d… ated…

Changes in gene expression caused by genetic mutations,

Precision Medicine

A Functional Approach to optimize Estrogen BioSynthesis and Metabolism.

Functional Approach to Optimize Estrogen Biosynthesis

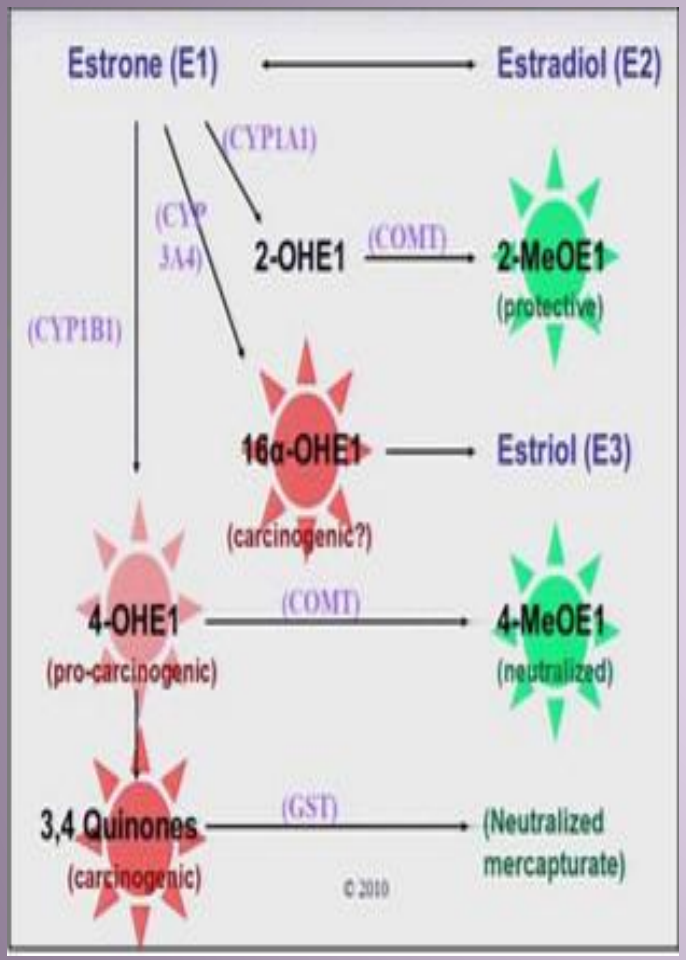
- ✓ **17-hydroxylase** (CYP17A1): ETOH, insulin, E2, PCOS, smoking, azoles
- ✓ **Aromatase** (CYP19A1) Induction: ETOH, BMI, IR, zinc deficiency, BPA, TCC
- ✓ **Aromatase Inhibition**: isoflavones, flax, EGC, vitC, chrysin, flavinoids/stinging nettles, azole Rx, metformin

Functional Approach to Optimize Estrogen Ph1 Detoxification

- ✓ **CYP1A1**: crucifers, I3C, DIM, flax, kudzo, soy, caffeine, rosemary, exercise, T4
- ✓ **CYP3A4**: pesticides, smoking, Rx, OCP, CEE, HoT4, *grapefruit inhibits
- ✓ **CYP1B1**: Polycyclic aromatic hydrocarbons, smoking, dioxin, TCC, PCBs, PFAS **grapefruit, hops inhibit*

Functional Approach to Optimize Ph2 Estrogen Detoxification & Elimination

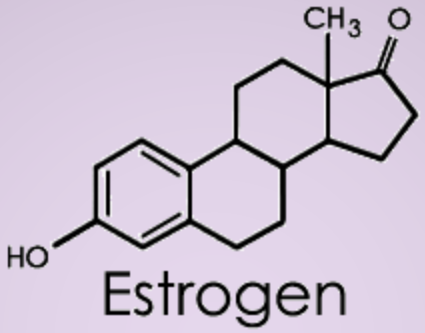
- **COMT**: Hg, HC, Def MTHF, smoking, ROS inhibits. +*Mag, Methyl donors, B vit support*
- **SOD:** smoking, ROS inhibits. +*Vit E, A, C, selenium, curcumin, NAC, EGCG, polyphenols, lycopene, ALA support.*
- **GST**: +*curcumin, glutathione, NAC, selenium*
- **UGT**: dysbiosis, constipation block. +*fibre, flax, lignans, biliary secretagogues*



Genomic and Epigenetic variables in Estrogen
Biosynthesis, Metabolism, Detoxification and Elimination
have vast implications on reproductive aging, risk of disease and
breast cancer.

The Era of Precision Medicine

Thank you!



Estrogen

Questions?

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