Estrogen Gene Test:

American Institute for Cancer Research Presentation & Update

Presentation of Ongoing Study to American Institute for Cancer Research & Updated Findings

The following summary is based on a poster presented at The American Institute for Cancer Research November 5-6, 2008 which examined the impact of estrogen metabolism modifiable gene testing and the impact of the mitigation efforts to their breast cancer risk. In 2008, there were 100 women being followed. As of June 2013, over 175 women have been tested and enrolled in risk mitigation. 37 of these women have estrogen positive breast cancer and the others have been identified as high risk based on their mutations and exposure to estrogenic medications. Results to date are that none of 175 have either developed breast cancer or had a recurrence.

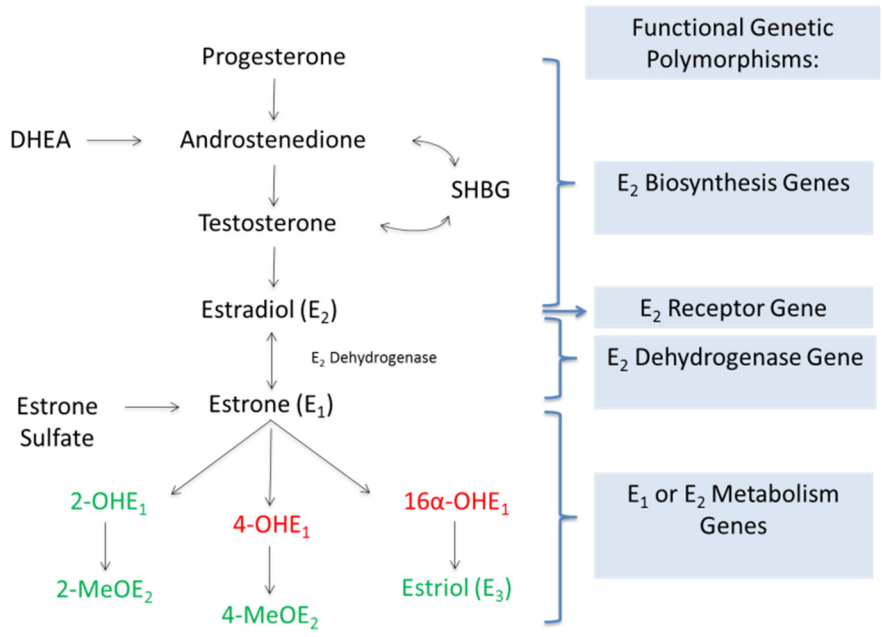
In 2008, of the women with breast cancer, 81% had SNPs on CYP1B1 and COMT and GSTM1 was absent in 82%. The 37 women with estrogen positive breast cancer had had significant exposure to exogenous estrogens through either hormone replacement therapy (HRT) and/or in vitro fertilization (IVF). In addition to their traditional cancer treatment, supplements were used to alter their genetic pathways including DHEA, DIM-PRO, SAM-E, and Glutathione & Anti-oxidants such as NAC, Milk Thistle and Vitamins E & C.

Since the presentation of this work in 2008, new research has been published which correlates with our experience. A study published in the Journal for Gynecological Oncology, June 2012, found that when there were 3 or more gene mutations, risk increased by 2.5 to 13 times depending on the genes involved.

Estrogen Metabolism

The cytochrome P450 enzyme system uses oxygen to modify toxic compounds, drugs or steroid hormones. These enzymes are responsible for metabolizing estrogen (Figure 1). SNPs in genes coding for cytochrome P450 enzymes can increase or decrease the activity of that enzyme. For example, a SNP on CYP1B1 up-regulates the conversion of estrone to 4-OH estrone, an undesirable estrogen metabolite know to induce estrogen sensitive cancer. A SNP on CYP1A1 down regulates estrone's conversion to 2-OH estrone, an estrogen metabolite considered to be protective. SNPs in Phase II detoxification enzymes associated with methylation (Catechol-O-methyl transferase or COMT), 7 genes associated with acetylation (N-acetyl transferase), 3 genes linked to glutathione conjugation (Glutathione s-transferase or GST) and 3 gene locations for superoxide dismutase (SOD). COMT and GST facilitate estrogen metabolite detoxification and therefore SNPs can increase an individual's risk for estrogen-induced cancers.

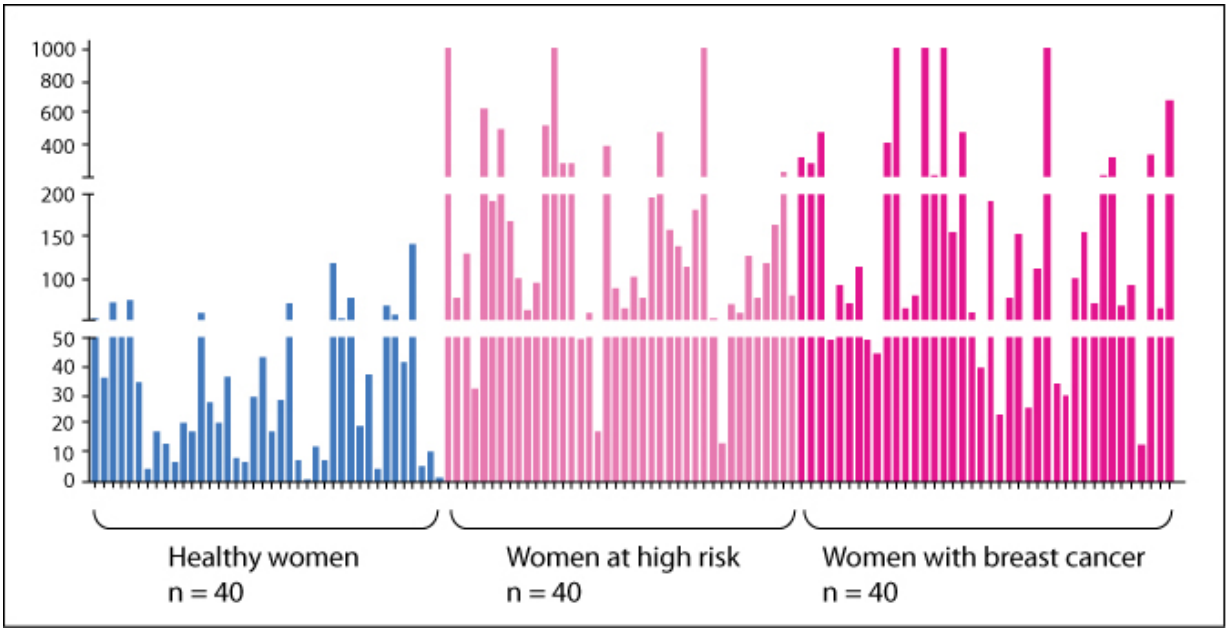
Figure 1. Estrogen Biosynthesis and Metabolism



Carcinogenic metabolites are shown in red and anticarcinogenic or neutral metabolites are shown in green.

If the breast cancer tumor is ER+, we use functional genomic testing and nutrigenomic interventions to understand the genomic influence on estrogen metabolites (2-OH estrone, 4-OH estrone, 16- alpha-OH estrone, 2-methoxy estrone, 4-methoxyestrone, and 3,4 estrone quinone) and how best to either up regulate or down regulate gene expression. The goal is to alter the balance of estrogen metabolism away from harmful metabolites and towards more favorable metabolites. The 4-OHE1 metabolite is particularly toxic. Women with the highest amount of 4-catechol estrogen–DNA adducts also have the highest risk for or rates of breast cancer (Figure 2).

Figure 2 – Estrogen-DNA Adducts and Breast Cancer



Ratio of depurinating estrogen-DNA adducts to estrogen metabolites/conjugates. 4-OHE2-DNA adducts are responsible for >98% of the ratio. Ratios are much higher in women with breast cancer or who are at high risk for developing breast cancer. **Adapted from Cavalieri and Rogan, 2010.[5]**

Materials and Methods

Study Participants

Each woman completed an 18-page medical history questionnaire prior to any genomic, saliva, or urine testing. Responses to the questionnaire provided a detailed family, emotional, and mental health history along with a history of dietary and lifestyle choices and current signs and symptoms. In order to be included in this study, women must be in good health overall. For women diagnosed with breast cancer, the tumor was documented as ER+ and the disease was generally well-controlled with medications. Women without breast cancer who participated in this study were considered to be at high risk of developing breast cancer by results of genetic estrogen metabolism testing.

Genetic Estrogen Metabolism Profile

Single nucleotide polymorphisms (SNPs) were identified among eight cytochrome P450 genes responsible for the following enzymes: CYP1A1, CYP1B1, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Multiple SNP locations were evaluated for CYP1A1, CYP1B1, CYP2C9, CYP2C19 and CYP3A4. Briefly, a 10 ml blood sample was collected in an EDTA test tube and sent via overnight carrier to the testing laboratory where the samples were centrifuged and prepared for genomic analysis using Third Wave Invader DNA assay. This assay detects polymorphisms in a patient's DNA sample using a solution hybridization method in which two oligonucleotides hybridize in tandem with specific DNA sequences. Subsequent Cleavase and hybridization reactions result in generation of fluorescent signal. The biplex format of the assay enables simultaneous detection of all variants in a single reaction tube. The sensitivity and specificity of this assay is 99.7 %.

Salivary Female Hormone Profile

Eleven saliva samples (3 ml each) were collected in individual plastic vials between 7 am and 9 am during a 30-day period. All samples were frozen until the final sample was collected then shipped in a refrigerated Styrofoam box via an overnight carrier to the laboratory. Free estradiol and progesterone levels for each vial were determined using RIA. Free testosterone was determined only on the last vial collected using RIA.

Urinary Estrogen Metabolite Profile

Prior to nutrigenomic intervention and then 3 months following the intervention, urine was collected during a 24 hr period in two, 1 liter plastic containers with ascorbic acid preservative. After homogenization, a 120 ml aliquot was transferred to a cup with screw lid, then shipped overnight to the laboratory. Estradiol, estrone (E1), estriol, 2-OHE1, 4-OH E1, 16α-OHE1, 2-methoxy E1 and 4-methoxyE1 were determined by GC-MS.

Single Nucleotide Polymorphism Testing

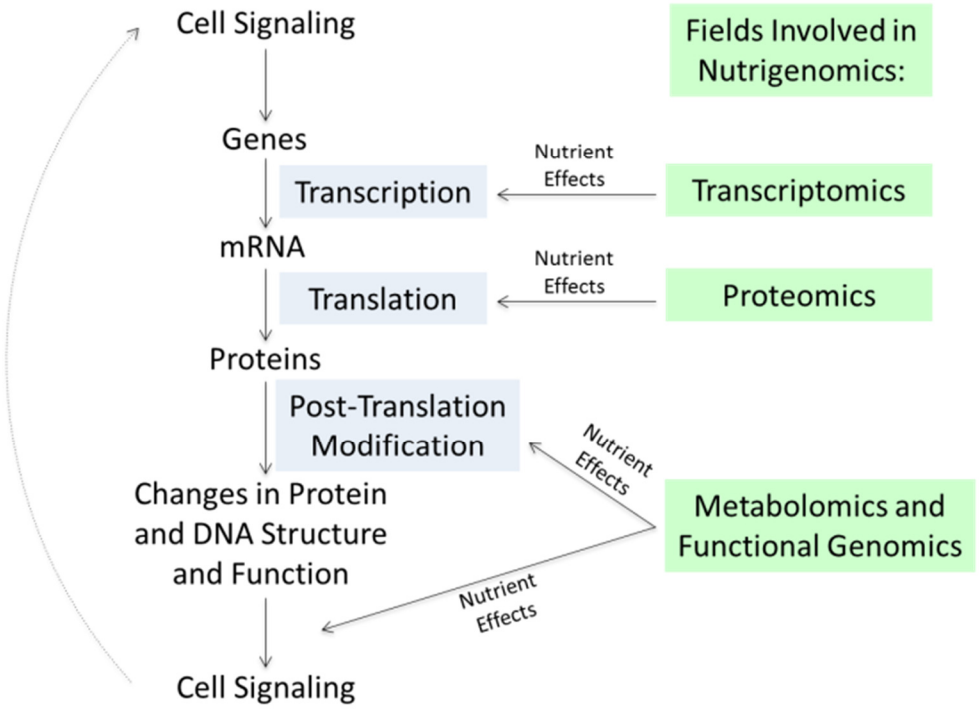
The Estrogen Gene Test, performed on a sample of saliva, identifies single nucleotide polymorphisms, or SNPs, in 6 genes involved in estrogen metabolism (Table 1).These SNPs and their encoded enzyme variants determine circulating levels of estrogen and estrogen metabolites.[6] A woman's particular profile of SNPs predicts a modifiable component of her overall risk of developing breast cancer.

Table 1. SNPs Detected in The Estrogen Gene Test

Gene	Result of SNP	What Does the Gene do?
CYP1A1	Slow metabolizer	Fast Metabolizers quickly convert estrone, a potentially carcinogenic estrogen, to a more desirable estrogen metabolite (2-OHE1).
CYP1B1	Rapid metabolizer	Fast Metabolizers, favor the conversion from estrone to a potentially carcinogenic estrogen (4-OHE1).
CYP3A4	Rapid metabolizer	Fast metabolizers quickly convert estrones to a potentially carcinogenic estrogen. (16-AlphaHydroxy estrone)
COMT	Slow metabolizer	If you have reduced enzyme activity, you clear toxic estrogen metabolites less efficiently.
GSTP1	Slow metabolizer	If you have reduced or NO activity, you very poorly convert toxic estrogens to more desirable by-products.
GSTM1	Absent enzyme	
GSTT1	Absent enzyme	
MnSOD	More available in mitochondria	Reduced MnSOD enzyme activity increases risk for cancer, especially if antioxidants are low

CYP1A1, Cytochrome P450 1A1; CYP1B1, Cytochrome P450 1B1; CYP3A4, Cytochrome P450 3A4; COMT, Catechol-O-methyltransferases; GST, Glutathione-S-transferases; MnSOD, Manganese superoxide dismutase PAH, polycyclic aromatic hydrocarbons; SAM-E, S-adenosyl methionine; NAC, N-acetylcysteine; DIM, diindolylmethane complex; DHEA, dehydroepiandrosterone

Figure 3. Therapeutic Applications of Nutrigenomics



Targeted Interventional Approach

Information from genetic and hormonal assays was used to devise nutrigenomic interventions aimed at modulating high-risk genes and metabolic pathways (Figure 3). Nutrigenomic interventions target the transcription of genes to mRNA, the translation of mRNA to proteins, and the post-translation modification of proteins and DNA into structurally and functionally distinct forms; the end result is a regulation of cellular functions with subsequent modulation of disease or physiologic states.

The supplement form of diindolylmethane (DIM), known as DIM-Pro, has been shown to upregulate the metabolism of E_1 and E_2 into the anticarcinogenic 2-OHE_1 and 2-OHE_2 metabolites, respectively.[7] S-adenosylmethionine (SAM) supplements, known as SAM-E, are also able to increase the detoxification of carcinogenic E_1 and E_2 metabolites into their stable and excretable 2-methoxy and 4-methoxy counterparts.[8] SAM-E is also postulated to enhance the enzymatic degradation of catecholamines, which are secreted in response to stress and could be implicated in the association between stress and breast carcinogenesis.

Results

As of April 2013, 175 women have enrolled in the study, 37 of whom had breast cancer at the start of the study and the remaining 138 women were at high risk of the disease. Over the study period no women enrolled in the study developed breast cancer or had a recurrence of breast cancer. All 37 women had significant exposure to exogenous estrogens through either hormone replacement therapy (HRT) and/or in vitro fertilization (IVF). In addition to their traditional cancer treatment, supplements were used to alter the pathway including DHEA, DIM-PRO, SAM-E, Glutathione, NAC, Milk Thistle and Vitamins E & C.

Conclusions

1. By evaluating an individual's genomic information, a clinician can better understand the underlying genetic predisposition(s) associated with the disease process and then tailor specific prevention and/or pharmacological regimens specific to that individual.
2. While the genetics of the tumor are important to identify the proper pharmacological treatment, it is equally important to include the patient's pharmacogenomics to improve treatment outcomes.
3. Functional genomic testing and evaluating a woman's steroid levels can be useful strategies in breast cancer prevention particularly in women taking birth control, HRT, bio-identical hormones and who have high circulating levels of estrogen (peri-menopause).
4. Nutrigenomic interventions specific to that patient's functional genomics (phase I and phase II detoxification pathways) and circulating levels of estradiol (salivary) and estrone metabolites (urine) can be used to decrease the recurrence of ER+ breast cancer and prevent the occurrence of breast cancer.
5. The Salivary Female Hormone Profile and Urinary Estrogen Metabolites provide "snap shots" of current circulating levels of steroids (estradiol, progesterone and testosterone) and levels of specific estrogen metabolites (4-OH estrone, 16-alpha-OH estrone, and 3,4-estrone quinone).
6. These "snap shots" are useful tools to measure the effectiveness of nutrigenomic interventions.

References

1. Fisher B, Dignam J, Bryant J, et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *J Natl Cancer Inst.* Nov 6 1996;88(21):1529-1542.

2. Johnson MD, Zuo H, Lee KH, et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat.* May 2004;85(2):151-159.

3. Lim YC, Li L, Desta Z, et al. Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells. *J Pharmacol Exp Ther.* Aug 2006;318(2):503-512.

4. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. *Clin Pharmacol Ther.* May 2011;89(5):718-725.

5. Cavalieri EL, Rogan EG. Depurinating estrogen-DNA adducts in the etiology and prevention of breast and other human cancers. *Future Oncol.* Jan 2010;6(1):75-91.

6. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett.* Sep 28 2005;227(2):115-124.

7. Bradlow HL. Review. Indole-3-carbinol as a chemoprotective agent in breast and prostate cancer. *In Vivo.* Jul-Aug 2008;22(4):441-445.

8. Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. *Curr Drug Metab.* Jun 2002;3(3):321-349.