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Genetic Polymorphisms in Steroid Hormone Metabolizing Enzymes in Human Breast Cancer

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Abstract: Epidemiologic studies indicate that most risk factors for breast cancer are related to reproductive and hormonal factors. The evaluation of associations between breast cancer risk and genetic polymorphisms in enzymes involved in hormone metabolism may be a cost effective manner in which to determine individual breast cancer susceptibility. A number of molecular epidemiologic studies have been conducted to evaluate associations between polymorphic genes involved in steroid hormone metabolism (i.e., *CYP17, CYP19, CYP1A1, CYP1B1, MnSOD, COMT*, and *GST*) that may account for a proportion of enzymatic variability. An evaluation of associations between breast cancer risk and genetic polymorphisms in enzymes involved in hormone metabolism is described in this brief review.

Key Words: Breast cancer, Genetic polymorphism, Steroid hormone metabolism

Breast cancer is the most commonly occurring cancer among women, and the morbidity rate of this disease continues to rise, whereas the mortality rate is declining due to more advanced diagnosis and treatment techniques (1). Fewer breast cancer cases can be explained by rare, highly penetrant genes such as *BRCA1*, *BRCA2* and *TP53*. In principle, common, low penetrance genes could explain the majority of breast cancer cases (2).

Endogenous steroid hormones are important in the development and progression of breast cancer. Steroid hormones exert growth-promoting effects and induce breast cell proliferation by binding to intracellular receptors and regulating gene transcription (3). Several breast cancer risk factors are thought to act by influencing lifetime exposure to steroid hormones. The rate of increase in breast cancer incidence declines after menopause, probably due to lower circulating estrogen and progestrone levels. Age at menarche, age at menopause, postmenopausal obesity, and postmenopausal hormone use are well-established breast cancer risk factors that influence the dose and duration of estrogen and progestrone exposure (4).

Once formed, estrogens are extensively metabolized by a number of oxidative and conjugate reactions that can lead to their deactivation and subsequent elimination (5). Metabolic activation of 17β-estradiol (E2) has been postulated to be a factor in mammary carcinogenesis. E2 is metabolized via two major pathways: formation of catechol estrogens, the 2-OH and 4-OH derivates; and C-16α hydroxylation (Figure). The 2-OH and 4-OH catechol estrogens are oxidized to semiquinones and quinones. The latter are reactive electrophilic metabolites and are capable of forming DNA adducts. Further DNA damage results from quinone-semiquinone redox cycling, generated by enzymatic reduction of catechol estrogen quinones to semiquinones and subsequent auto-oxidation back to quinones. C-16α hydroxylation has also been suggested to be involved in breast carcinogenesis (6).

Genetic polymorphism has been found to be the basis of frequently observed individual variation in activities of drug metabolizing enzymes among human populations. Striking ethnic dissimilarities, as well as inter-individual differences, in genes involved in drug metabolism are well known (7). As observed in drug and chemical metabolism, there is considerable inter-individual genetic variability in the metabolic and biosynthetic pathways in steroidogenesis (5). Many of the enzymes involved in estrogen metabolism are polymorphically distributed within the human population (i.e., *CYP17, CYP19, CYP1A1, CYP1B1, MnSOD, COMT,* and *GST*) (5,8). Inherited alterations in the activity of any of these

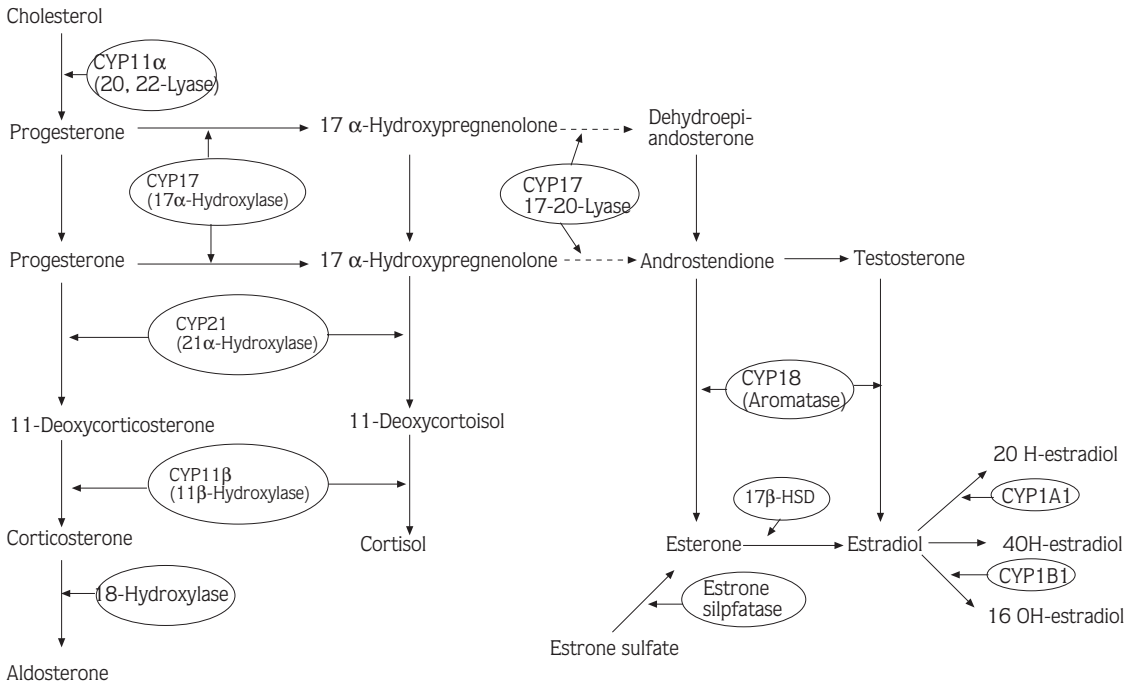

Figure. The estradiol synthesis metabolic pathway (8)

enzymes hold the potential to define differences in breast cancer risk associated with estrogen carcinogenesis. However, it is evident that no single genotype can be linked to all breast cancers. Known genetic polymorphisms in steroid hormone metabolizing enzymes in human breast cancer are shown in the Table.

The cytochrome P450 enzyme *P45017* has both 17α-hydroxylase and 17,20-lyase activities and catalyzes two distinct steps in steroid hormone production. In steroidogenesis, a gene encoding the steroidogenic enzyme 17α-hydroxylase converts pregnenolone and progesterone to 17-hydroxypregnenolone and 17-

Table. Known Genetic Polymorphisms in Steroid Hormone Metabolizing Enzymes in Human Breast Cancer (5,8)

Enzyme	Role in Estrogen Metabolism	Allelic variants
CYP17	17-α hydroxylase / C17-20 lyase, catalyzes rate-limiting step in ovarian and adrenal biosynthesis pathways for androstenedione	Two variants, enhanced promoter activity
CYP19	Aromatase / estrogen synthetase, converts testosterone and androstenedione to E2 and E1, respectively	Fourteen variants, may alter splice site and ability of converting activity
CYP1A1	2-hydroxylase, generates 2-OH CE	Four variants, changed activity
CYP1B1	4-hydroxylase, generates 4-OH CE	Seven variants, changed activity
MnSOD	Manganese superoxide dismutase, converts 2 superoxide radicals to H_2O_2 and O_2	Two variants, altered protein trafficking
COMT	Methyltransferase, methylates and inactivates CE	Two variants, decreased methylation activity
GST	Glutathione sulfotransferases, decreases oxidative stress generated during estrogen metabolism	GSTM1, deletion of the entire gene, the null allele GSTT1, deletion of the entire gene, the null allele GSTP1, two variants, reduced activity

hydroxyprogesterone, respectively. In women, *P450c17α* is primarily expressed in ovarian theca cells and adrenal cortex. *CYP17* contains a single-base polymorphism that creates a SP1-type (CCACC box) promoter and also generates alleles correlating with different promoter activity (9). Rare mutations in the coding region of *CYP17* have recently been associated with breast cancer risk. Studies suggest that the A2 allele of *CYP17* elevates endogenous hormone levels, but is not a strong independent risk factor for breast cancer (10).

The aromatase enzyme catalyzes the conversion of androgens to estrogens in the estrogen biosynthesis pathway. Because increased exposure to estrogen is considered to be a risk factor for breast cancer, polymorphic human aromatase gene (*CYP19*) is a plausible candidate for low penetrance breast cancer susceptibility (5). The polymorphic repeat (TTTA) in intron 5, TCT insertion / deletion in intron 4, and a substitution in intron 6 of the *CYP19* gene create 14 alleles in which to change aromatase activity. Although there have been numerous reports of different TTTA repeat alleles being associated with variations in breast cancer risk, the *CYP19* gene has no major role in common breast cancer incidence (11).

In the breast, *CYP1A1* and *CYP1B1* are responsible for the hydroxylation of estrogens to the 2-hydroxy estrogen (2-OH HE) and 4-OH HEs. In turn, *CYP1B1* exceeds *CYP1A1* in its catalytic efficiency as E2 hydroxylase and differs from *CYP1A1* in its principal site of catalysis (12). HEs are an important means of eliminating estrogen. Oxidation occurs via major pathways, one of which involves C-2 of estradiol, resulting in the formation of the 2-HE and 4-HE, whereas the other involves C-16, resulting in the formation of 16α-HE. These products are able to bind to DNA, creating adducts and subsequently causing gene mutations. Thus, increased formation of 4-HE and 16α-HE has been associated with an elevated risk of breast cancer (9). To date, at least four polymorphisms have been described in the human *CYP1A1* gene. Two of these, *m1* (a base subsitution in noncoding region) and *m2* (a point mutation in codon 462 of exon 7, leading to a amino acid subsitution) are associated with increased breast cancer risk (13,14).

Mutations and polymorphisms have both been identified in the *CYP1B1* gene. Six polymorphisms of the gene have been described, of which four result in amino acid substitutions. Two of these amino acid substitutions have been described in exon 3, which encodes the heme-binding domain: codon 432 Val→Leu and codon 453 Asn→Ser; and the other two in codon 48 Arg→Gly and 119 Ala→Ser in exon 2 (15). Polymorphisms are inherited alterations in the activity of *CYP1B1* that hold the potential to define differences in estrogen metabolism and, thereby, possibly explain inter-individual differences in breast cancer risk associated with estrogen-mediated carcinogenesis (16).

Superoxide dismutase (Mn, Cu, ZnSOD) catalyzes the dismutation of two superoxide radicals, producing hydrogen peroxide and oxygen, because ROS, including those generated by estrogens and their metabolites, may be involved in breast carcinogenesis and because *MnSOD* is a major enzyme involved in the scavenging of free radicals (5). An amino acid exchange at the 9 position of *MnSOD* in the signal peptide sequence apparently alters the structure of the enzyme, affecting its ability to enter the mitochondrion. *MnSOD* alanine allele could be related to breast cancer risk by having an altered capacity to reduce oxidative stress (17).

Several Phase II enzymes either inactivate CEs or protect against estrogen carcinogenesis by detoxifying products of oxidative damage that may arise on redox cycling of CEs. Genetic variants of each of these enzymes involved in CE metabolism have been identified, some with proven or suspected change in function. Catechol-*O*-methyltransferase (*COMT*) is one of several phase II enzymes responsible for the detoxification of CEs, including 2-CE and 4-CE by *O*-methylation and is polymorphic in the human population with 22% of a Turkish population being homozygous for a low activity allele of the enzyme (18,19). The level of COMT activity is controlled by a DNA exonic polymorphism at position 108 and 158 of the Soluble (*S-COMT*) and Membrane-bound (*MB-COMT*) form of the enzyme, respectively. Reduced COMT activity might increase the risk of breast cancer, secondary to accumulation of CE, which causes oxidative DNA damage. In addition, 2-CE and 4-CE may be oxidized to CE quinones, which react with DNA to form adduct. These adducts, especially CE-3,4-quinones derived from 4-CE, can cause depurination leaving apurine sites, which is the major type of genetic damage leading to mutation and genomic deletion during tumorigenesis (20, 21). Some findings suggest that the allele encoding low activity *COMT* may be an important

contributor to the development of breast cancer, and also, has recently been associated with the clinical stage and extent of metastasis of breast cancer (22).

The glutathione-dependent peroxidases (Glutathione S-transferase) are involved in detoxification of products of oxidative damage, by catalyzing conjugation of glutathione with ROS. Genetic polymorphisms are known to affect enzyme activity in *GSTM1, GSTT1, GSTP1* isoenzymes. Both *GSTM1* and *GSTT1* enzyme activities are absent from approximately 50% and 30% of Caucasians, respectively, (23) and absent from 20% of Turks for *GSTT1* due to homozygous deletions of the genes (24). An amino acid exchange at the 105 position of *GSTP1* has caused reduced activity. Because all *GST* enzymes are present in human breast tissue, it is plausible that a lack of these isozymes could increase breast cancer risk. However, the outcomes of epidemiological studies on *GST* genotypes and breast cancer have been inconsistent (12,13, 23).

The study of the relationship among human genetic polymorphisms, cancer susceptibility, toxicity, and environmental exposure is a new and exciting area of research. These person-to-person differences, which are, in part, attributed to allelic variability or gene polymorphisms, might define subpopulations of women with higher lifetime exposures to hormone-dependent growth promotion or to cellular damage from particular estrogens and estrogen metabolites. Such variation could explain a portion of the cancer susceptibility associated with reproductive events and hormone exposure. Currently, the evaluation of associations between breast cancer risk and genetic polymorphisms in enzyme involved in hormone metabolism may be a cost effective manner in which to evaluate metabolic variability (5).

The studies of polymorphisms in steroid hormone metabolizing enzymes and breast cancer risk, and those of gene-environment interactions, have yielded conflicting results. Therefore, there is undoubtedly a need for elucidating the basis of breast cancer, identifying etiologic factors and clarifying the genetic polymorphisms in steroid hormone metabolizing genes. Molecular epidemiological studies have increasingly important implications for breast cancer risk assessment and the prevention, early diagnosis and intervention of the disease.

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