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Endogenous Estradiol and Its Association with Estrogen Receptor Gene Polymorphisms

MaryFran R. Sowers, PhD,[a,b] Mary L. Jannausch, MS,[a] Daniel S. McConnell, PhD,[a] Sharon R. Kardia, PhD,[a] John F. Randolph, Jr., MD[b]

[a]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA; and [b]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

ABSTRACT

We evaluated potential associations between single nucleotide polymorphism (SNP) variants of the estrogen receptor genes *ESR1* and *ESR2* and circulating estradiol (E_2) concentrations in women of 4 races/ethnicities. The study population was drawn from participants in the Study of Women's Health Across the Nation (SWAN). A total of 1,538 African American, Caucasian, Chinese, and Japanese women from SWAN participated in the Sex Steroid Hormone Genetics Protocol by providing blood for sex steroid hormone analyses and consenting to lymphocyte transformation from which DNA was extracted and genotyped. We evaluated 4 *ESR1* SNPs (*ESR1 rs9340799*, *ESR1 rs2234693*, *ESR1 rs728524*, and *ESR1 rs3798577)*, and 3 *ESR2* SNPs (*ESR2 rs1255998*, *ESR2 rs1256030*, and *ESR2 rs1256065*). Mean E_2 level was 196.0 ± 4.0 pmol/L in women who were premenopausal and perimenopausal (with blood drawn on days 2 through 5 of the menstrual cycle follicular phase); however, mean E_2 levels in Chinese and Japanese women were lower (155.7 ± 10.6 pmol/L and 170.0 ± 10.3 pmol/L, respectively) than in African American (196.4 ± 8.1 pmol/L, $P < 0.05$) or Caucasian women (210.7 ± 5.9 pmol/L, $P < 0.002$). The *ESR1 rs3798577 CC* genotype was associated with lower circulating E_2 concentrations in African American women ($P < 0.07$) and explained about 1% of the variation in circulating E_2 concentrations. In Japanese women, the *GC* genotype of *ESR2 rs1255998* was associated with significantly lower circulating E_2 concentrations that explained about 4% of the variation. Circulating E_2 concentrations were not strongly or consistently associated with selected polymorphisms for the estrogen receptor genes. The 2 strongest associations explained <4% of the total variation in the circulating E_2 concentrations.

KEYWORDS: Estradiol; Estrogen metabolism; Estrogen receptor; Genetics

17β-Estradiol (E_2) is the most potent estrogen of the 3 major endogenous estrogens, which also include estrone (E_1) and estriol (E_3). It is a pivotal hormone in female reproductive physiology, and its withdrawal around the midlife has been widely associated with health events, including bone loss and vasomotor menopausal symptoms.[1,2] Estrogen signaling has a key regulatory role in the proliferation and differentiation of estrogen-responsive cells.[3]

The primary source of E_2 in women before menopause is the ovary. However, there is additional contribution from nonovarian sources, including adipose tissue, where the enzyme aromatase converts the androgens androstenedione and testosterone to estrone and estradiol, respectively.[4] Before menopause, the relative amount of estrogen produced in the adipose tissue compared with in the ovary is small,

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This report is based on samples from the SWAN DNA Repository. Scientists interested in developing studies based on this resource can find a description of the SWAN Core Repository and DNA Repository and information on how to obtain access to the resources at www.swanrepository.org.

Requests for reprints should be addressed to MaryFran R. Sowers, PhD, 339 East Liberty, Suite 10, School of Public Health, University of Michigan, Ann Arbor, Michigan 48104.

E-mail address: mfsowers@umich.edu.

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but the nonovarian contribution may be increasingly important after menopause.

Two functional estrogen receptors, ERα and ERβ, have been described to date in humans[5–7] and are encoded by the genes *ESR1* and *ESR2*, respectively. Unliganded ERα is predominantly located in the nucleus and is found as a monomer or dimer.[8–10] On binding with a ligand, ERα undergoes a conformational change to a dimer.[11] ERα associates with E_2-responsive genes, leading to the development of estrogen response elements. With ERα activation, complexes are recruited to initiate transcription activity or to modify local chromatin structure.[12–14]

Estrogen receptors are usually considered homodimers, but heterodimers of both ERα and ERβ have been described.[15,16] ERβ can inhibit the transcriptional activity of ERα through formation of these heterodimers, suggesting the possibility of a gene-by-gene interaction.[15–18] In the absence of ERα, ERβ can replace some of its activity.

The 2 estrogen receptors are encoded by different genes on different chromosomes. The gene for ERα (*ESR1*) is located on chromosome 6q25.1, spans $>$ 140 kilobases (kb),[19] and consists of 8 exons separated by 7 intronic regions.[20–22] The gene for ERβ (*ESR2*) is located on chromosome 14q23.2, spans 40 kb, and also consists of 8 exons.[23] *ESR2* has a similar structure to *ESR1* and they have substantial shared homology in the DNA-binding and ligand-binding regions.[24]

A number of studies have demonstrated that selected variations in the estrogen receptor genes may have functional importance. The polymorphic *ESR1*, frequently identified by the restriction endonucleases *Pvu*II and *Xba*I,[22,25] has been related to expression of the estrogen receptor in breast cancer.[20,26] In some studies, the *Pvu*II and *Xba*I-defined polymorphisms (*ESR1 rs2234693* and *ESR1 rs9340799*, respectively) have also been shown to be predictive of bone mineral density levels that are associated with osteoporosis.[27,28] Using transient transfection assays, Fan and colleagues[29] demonstrated that *BRCA1*, the gene associated with breast cancer, inhibits signaling by the ligand-activated ERα through the estrogen-responsive enhancer element and blocks the C-terminal transcriptional activation function of ERα. These results suggest that wild-type *BRCA1* protein may function, in part, to suppress estrogen-dependent mammary epithelial proliferation by inhibiting ERα-mediated transcriptional pathways related to cell proliferation. Li and associates[30] showed that the ER46 isoform of ERα was more efficient in modulating membrane-initiated estrogen actions, such as activation of endothelial nitric oxide synthase, than full-length ER66. Conversely, ER66 more efficiently mediated estrogen response element reporter–gene transactivation than did ER46.

The circumstances under which estrogen receptors are associated with circulating E_2 concentrations are uncertain. In humans, it is not known how the gene for the estrogen receptor alters the binding affinity or dissociation equilibrium of the receptor with E_2. By studying serum levels of E_2, it may be possible to determine whether there are differences in E_2 uptake into tissue due to the receptor genotypes. Thus, 2 main study questions arose: (1) Are the circulating levels of E_2 concentrations directly related to gene markers for ERα and ERβ? (2) Is there an interaction between *ESR1* and *ESR2* that is reflected in differences in E_2 concentrations, and does this interaction vary in the 4 racial/ethnic groups?

METHODS

Study Population

The data are from participants in the Study of Women's Health Across the Nation (SWAN), a multisite, multiethnic longitudinal study characterizing sex steroid hormone patterns and health status as women approach and traverse the menopause. The study design and recruitment have been described elsewhere.[31] Briefly, eligible women were identified in a cross-sectional survey of 16,602 women, 40 to 55 years of age, conducted in 7 geographically dispersed research programs. Each of the 7 sites recruited a Caucasian and a specific multiethnic sample (African American, Chinese, Japanese, and Hispanic women) for longitudinal study. The longitudinal cohort ultimately included 3,302 women who, at baseline, were 42 to 52 years of age, premenopausal or in their early perimenopausal years, and without exogenous hormone use.

The SWAN Genetics Study, which includes the Sex Steroid Hormone Protocol being reported here (n = 1,538), was conducted under the auspices of each site's internal review board as well as an internal review board for the SWAN Repository at the University of Michigan, which also administered a certificate of confidentiality. The SWAN site in New Jersey did not participate in the SWAN Genetics Study; therefore, there is no representation for a Hispanic subgroup in this study.

Genotyping

Buccal cells in saliva and whole blood were collected from participants who were also offered the opportunity to consent to use a part of the whole blood for cell transformation. Data reported here are based on DNA that was extracted from immortalized lymphocytes and then purified and diluted in preparation for genotyping by TaqMan (Roche Molecular Systems, Inc., Pleasanton, CA) technology on an ABI 7900HT sequence detection system. Four *ESR1* single nucleotide polymorphisms (SNPs)—*ESR1 rs9340799*, *ESR1 rs2234693*, *ESR1 rs728524,* and *ESR1 rs3798577*—as well as 3 *ESR2* SNPs—*ESR2 rs1255998*, *ESR2 rs1256030,* and *ESR2 rs1256065*—were genotyped for this investigation. In this article, we will be referring to each SNP by its rs number (**Table 1**), as identified by dbSNP, the database of single nucleotide polymorphism names.[32]

Assays

This report is based on hormones from the baseline examination. Blood was drawn between days 2 and 5 of the