Polymorphisms in Estrogen Synthesis Genes and Symptom Clusters During the Menopausal Transition and Early Postmenopause: Observations From the Seattle Midlife Women's Health Study

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Abstract

During the menopausal transition and early postmenopause, participants in the Seattle Midlife Women's Health Study (SMWHS) experienced one of the three symptom severity clusters identified through latent class analysis: severe hot flashes with moderate sleep, mood, cognitive, and pain symptoms (high-severity hot flash); low-severity hot flashes with moderate levels of all other symptom groups (moderate severity); and low levels of all symptom groups (low severity). In an effort to determine whether gene polymorphisms were associated with these symptom severity classes, we tested associations between gene polymorphisms in the estrogen synthesis pathways (cytochrome P450 19 [CYP 19] and 17 beta hydroxysteroid dehydrogenase [17HSD1]) and the three symptom severity clusters. SMWHS participants ($N = 137$) recorded symptoms monthly in diaries and provided buccal smears for genotyping. Multilevel latent class analysis with multinomial regression was used to determine associations between gene polymorphisms and symptom severity clusters. Only the 17HSD1 polymorphisms (*rs615942* and *rs592389*) were associated significantly with the high-severity hot flash cluster versus the low-severity symptom cluster. None of the polymorphisms was associated with the moderate-severity cluster versus the low-severity symptom cluster. Findings of associations of the 17HSD1 polymorphisms with the high-severity hot flash symptom cluster are consistent with those of an association between 17HSD1 polymorphisms and hot flashes in the Study of Women and Health Across the Nation population and our previous findings of associations between these polymorphisms with greater estrone levels.

Keywords

menopause, gene polymorphisms, estrogen synthesis genes, CYP 19, 17HSD1, symptom clusters

Despite recommendations for personalizing therapies by using tools from the study of the human genome, few investigators have studied gene polymorphisms and symptoms women experience during the menopausal transition (MT; Crandall, Crawford, & Gold, 2006; Kardia, Chu, & Sowers, 2006; Woods et al., 2006). Although there is evidence relating estradiol and estrone levels to hot flash severity (Freeman et al., 2007; Woods, Cray, Mitchell, & Herting, 2014), to date researchers have devoted little attention to potential associations between polymorphisms in the estrogen synthesis genes and symptoms. Both the cytochrome P 450 (CYP 19) and 17 beta hydroxysteroid dehydrogenase (17HSD) enzymes play important roles in estrogen synthesis (Gruber, Uschugguel, Schneeberger, & Huber, 2002; see Figure 1). CYP 19 (aromatase) mediates the conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone, respectively), processes that occur in both gonadal and extragonadal tissues (Hosono et al., 2015). The CYP 19 gene, located on 15q21.1, encodes

aromatase, and the rs10046 single-nucleotide polymorphism (SNP) has been related to circulating levels of estradiol (Dumas & Diorio, 2011). The 17HSD1 catalyzes the conversion of estrone to estradiol, and 17HSD2 catalyzes the conversion of estradiol to estrone (Hosono et al., 2015). The 17HSD gene is located on chromosome 17q11-q21, and 17HSD1 SNPs have

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Figure 1. CYP 19 (aromatase) and 17HSD in sex steroid synthesis pathways. CYP 19 (aromatase) mediates the conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone, respectively). CYP 19 = cytochrome P450 19; 17HSD = 17 beta hydroxysteroid dehydrogenase.

been related to age at menopause (Mitchell et al., 2008) and to estradiol trajectories around the time of menopause (Sowers et al., 2011).

Investigators in the Study of Women and Health Across the Nation (SWAN) examined the effects of estrogen synthesis genotypes on hot flashes, linking polymorphisms in *17HSD1* (rs615942, rs592389, and rs2830) in White women to lower odds of reporting hot flashes (Crandall et al., 2006). Our early analyses of gene polymorphisms influential in estrogen synthesis, metabolism, and response, measured in the Seattle Midlife Women's Health Study (SMWHS) cohort, revealed positive associations between *CYP 19 11r (TTTA)_n*, a repeat polymorphism, and severity of hot flashes (Woods et al., 2006).

Nursing research has provided evidence that people experience clusters of symptoms that are associated with health problems as well as treatments. Researchers have identified symptom clusters, for example, in relation to cancer and cancer therapy (Dodd, Miaskowski, & Paul, 2001; Miaskowski, Aouizerat, Dodd, & Cooper, 2007; Miaskowski et al., 2017). Current research has focused increasingly on symptom clusters experienced in association with chronic diseases such as heart failure (Jurgens et al., 2009; Moser et al., 2014), HIV/AIDS (Moens, Siegert, Taylor, Namisango, & Harding, 2015; Namisango et al., 2015), renal disease (Amro, Waldum, Dammen, Miaskowski, & Os, 2014; Lee & Jeon, 2009), and chronic obstructive pulmonary disease (Breland et al., 2015; Park & Larson, 2014) among others.

Until recently, studies of symptoms women experience during the MT and early postmenopause have focused on individual symptoms such as hot flashes, depressed mood, or sleep disruption. Using latent class analysis, we found that a community-based sample of midlife women experienced three symptom clusters during this period of reproductive aging: a cluster with high-severity hot flashes and moderate-severity levels of sleep, mood, pain, and cognitive symptoms; a cluster with low-severity hot flashes and moderate levels of all other symptoms; and a cluster of low-intensity symptoms of all types (Cray, Woods, Herting, & Mitchell, 2012). These clusters have been replicated in analyses of data from the Menopausal

Strategies: Finding Lasting Answers to Symptoms and Health clinical trial participants using standardized measures to assess symptoms (Woods et al., 2016).

The National Institute of Nursing Research describes an investigative sequence for advancing symptom science that begins with the identification and description of a symptom cluster (or symptom) that is characterized as a phenotype using biological and clinical data. Progression of research leads to the application of genomic and other methodologies that, in turn, can prompt development of therapeutics (Cashion & Grady, 2015). In an effort to identify biomarkers associated with the symptom clusters we identified in women undergoing MT and early postmenopause, we found that urinary estrone levels were lower and follicle-stimulating hormone (FSH) levels higher among women with the high-severity hot flash cluster of symptoms than women with the other two clusters. In addition, women with the high-severity hot flash symptom cluster had lower epinephrine and higher norepinephrine levels when compared to those with the low-severity symptom cluster (Woods et al., 2014).

Although prior studies have established relationships between estrogen synthesis-related gene polymorphisms and hot flashes (Crandall et al., 2006), there have been no published studies to date of associations between these gene polymorphisms and symptom clusters in women during the MT (including hot flashes, mood, sleep, cognitive, and pain symptoms measured simultaneously), thus limiting the ability to use genotyping results to target care to those most likely to benefit. The purpose of the present set of analyses, part of the larger SMWHS, was to test associations between symptom clusters identified through latent class analysis and polymorphisms in the estrogen synthesis pathway genes including *CYP 19 (rs10046 CT)*, *CYP 19 11 r (TTTA)_n*, and *17HSD1 (rs615942 TG, rs592389 GT, and rs2830 AG)*.

Method

Sample and Design

The data for these analyses are part of a larger longitudinal study of the MT, the SMWHS, described in detail elsewhere (Woods & Mitchell, 2016). In the parent study, women entered the cohort between 1990 and early 1992 when most were not yet in the MT or were in the early stages of the transition to menopause. To be eligible for the parent study, women had to be 35–55 years of age, have had a menstrual period within the previous 12 months, still have at least one ovary and a uterus, not be pregnant or lactating, and be able to read and speak English. Screening all households within selected multiethnic neighborhoods in Seattle (11,222 households) yielded 820 women eligible for the study, 508 of whom agreed to the initial interview. After completing the initial in-person interview administered by a trained registered nurse interviewer, 390 participants continued to provide data annually by questionnaire and menstrual calendar, and 367 of these women provided a health diary (Mitchell, Woods, & Mariella, 2000;

Table 1. Polymerase Chain Reaction Primers and Allele-Specific Probes Used in the Genotyping Assays.

Polymorphism	Forward and Reverse PCR Primers	Wild-Type and Variant Allele Probes ^a
HSDBI rs615942 (87 bp)	(FP)5'gCgTgATgAAATCAAgAACCTCAAA-3' (RP)5'TgCAgCCgggCATgAg-3'	5'-VIC-CCCCAgCTCAgCCC-MGBNFQ-3' 5'-FAM-CCCCAgTACAgCCC-MGBNFQ-3'
HSDBI rs2830 (92 bp)	(FP)5'gCgTgATgAAATCAAgAACCTCAAA-3' (RP)5'TgCAgCCgggCATgAg-3'	5'-VIC-CCCCAgTCCAgCCC-MGBNFQ-3' 5'-FAM-CCCCAgTACAgCCC-MGBNFQ-3'
HSDBI rs592389 (65 bp)	(FP)5'gCACCTAACTCCTCCTgCAgATg-3' (RP)5'gTCCATATTTAAAgCCAACACAgT-3'	5'-VIC-CCCCTTgggTATCCA-MGBNFQ-3' 5'-FAM-CCCCTTggTTATCCA-MGBNFQ-3'
CYP 19 3'-UTR (rs10046)	(FP) 5'-cccaagaaactcagacaggtct-3' (RP) 5'-caaggtggatgattgatgtgaac-3'	5'-VIC-CCCCTTggTTATCCA-MGBNFQ-3' 5'-FAM-cagtaccactactgga-3'
CYP19 (TTTA; rs2389)	(FP)5'CTCTggAAAACAACCTgACCCCT-3' (RP)5'ggTTACAgTgAgCCAAGTCgT-3'	5'-VIC-tcagtacctactctggagc-3' NA NA

Note. FAM = 6-carboxy-fluorescein; FP = forward primer; PCR = polymerase chain reaction; RP = reverse primer; VIC = Applied Biosystems, Inc., trademark.

^aNucleotide differences between the wild-type and the variant allele probes are indicated in bold-faced, italicized letters.

Woods & Mitchell, 2016). At the end of 5–7 years in the study (1997), 243 women were still eligible for the study and agreed to continue data collection. Of these women, 174 provided a buccal cell smear in 2000–2002 (Mitchell et al., 2008; Woods et al., 2014). The sample for the present study ($N = 137$) is a subset of the 174 women who provided buccal cell samples.

To be eligible for the present study, in addition to meeting the original eligibility criteria, the 137 participants had to meet the Stages of Reproductive Aging Workshop + 10 criteria for late reproductive stage, early or late MT stage, or early postmenopause, as well as have kept a diary that provided data for the symptoms that serve as our outcome variables (Harlow et al., 2012; Mitchell et al., 2000). Exclusion criteria for staging included any data collection occasions with estrogen, progestin, androgen, or selective estrogen receptor modulator use; pregnancy or up to 3 months postpartum; lactation; hysterectomy; bilateral oophorectomy; and chemotherapy, radiation, or endometrial ablation. We also excluded from the analyses any women who did not keep a diary since it served as the source of the outcome variables (symptom clusters). This sample provided a total of 4,511 data points.

Measures

Symptoms. A 3-day health diary included 47 symptoms that women rated for severity over the previous 24 hr on a 5-point Likert-type scale (0 = *not present*, 4 = *extreme*; Mitchell & Woods, 1996). Women completed the diary over 3 consecutive days each month through 2000 and then completed the diary 4 times per year (quarterly) thereafter. If they were still having periods, women filled out the diary on menstrual cycle days 5, 6, and 7; if not, they completed it on any consistent 3 days each month or each quarter. For analysis, we averaged symptom ratings over the 3-day period (Cray, Woods, Herting, et al., 2012). We determined the five symptom groups for these analyses from a previous principal components analysis of the following 15 individual symptoms: early awakening, awakening at night, and difficulty falling to sleep (sleep); joint pain, backache, and headache (pain); depressed, mood

changes, crying, and irritable (mood); problem concentrating and forgetful (cognitive); nervous, panic, and tension (tension; Cray, Woods, & Mitchell, 2012). We used hot flash as a single symptom.

Symptom clusters that we included in the multilevel latent class analysis (MLCA) were composite variables created by combining individual symptoms into the sleep, pain, mood, cognitive, and tension groups along with hot flashes as a single item. Using the above symptoms in the MLCA yielded three latent classes, which we term *symptom clusters* in this report. For each woman, we estimated a latent class for each measurement occasion.

Gene polymorphisms. All genotyping was performed in the Center for Ecogenetics and Environmental Health Functional Genomics Laboratory at the University of Washington. DNA from buccal swabs was analyzed for the following genotypes for this study: estrogen-synthesizing gene polymorphisms *CYP 19 3'-UTR* (rs10046 [C>T]), *CYP 19 11r* (TTTA)*n* (rs2389), *17HSDB1* (rs2830 [A>G]), *rs592389* [G>T], and *rs615942* [T>G]). Applied Biosystems, Inc. (Hercules, CA), designed the polymerase chain reaction (PCR) primers and the TaqMan probes for these genotyping assays using the software File Builder 3.0. The assays for the *CYP19* (TTTA)*n* alleles were previously described, and the particular PCR primer sequences used are described in detail in other publications (Cray, Woods, Herting, et al., 2012; Woods et al., 2007). The sequences of the primers and probes used for each genotyping assay are listed in Table 1.

Data Analysis

We used *t* tests to compare descriptive statistics characterizing the women who met the inclusion criteria for the present analyses with those characterizing the women who did not. To identify empirically latent classes of menopausal symptoms based on individual diary observations, we used MLCA with the five symptom groups (sleep, pain, mood, cognitive, and tension groups) identified from a previous principal components analysis and hot flashes as an individual symptom (Cray,

Table 2. Sample Characteristics at Start of Study (1990–1991) of Women From the Seattle Midlife Women's Health Study by Eligibility for the Analyses of Symptom Clusters and Genetic Polymorphisms.

Characteristic	Eligible Women (<i>n</i> = 140) Mean (SD)	Ineligible Women (<i>n</i> = 368) Mean (SD)	<i>p</i> Value ^a
Age (years)	40.9 (3.9)	42.0 (4.8)	.006
Years of education	16.1 (2.9)	15.5 (2.9)	.03
Family income (\$)	39,800 (15,000)	35,800 (16,400)	.008
BMI (kg/m ²)	25.2 (5.7)	26.3 (6.5)	.07
Exercise (min/week)	179 (305.7)	186 (331.1)	.83
Currently employed, yes	<i>n</i> (%) 120 (85.7)	<i>n</i> (%) 318 (86.4)	<i>p</i> value ^b .84
Race/ethnicity			<.001
African American	4 (2.9)	54 (14.7)	
Asian/Pacific Islander	12 (8.6)	31 (8.4)	
Caucasian	123 (87.8)	268 (72.8)	
Other (Hispanic, mixed)	1 (0.7)	15 (4.1)	
Marital status			.13
Married/partnered	105 (75.0)	243 (66.0)	
Divorced/widowed/ not partnered	26 (18.6)	99 (26.9)	
Never married/ partnered	9 (6.4)	26 (7.1)	

Note. BMI = body mass index. ^aIndependent *t* test. ^b χ^2 test.

Woods, & Mitchell, 2012). Traditional latent class analysis is a statistical method used to identify subgroups of related cases that are not directly identifiable based on categorical or continuous observed variables. The traditional method assumes independence, whereas MLCA allows for the nested structure of the diary data, that is, data are nested within days of the diary and also within stages of the MT and early postmenopause (Cray, Woods, Herting, et al., 2012; Muthén & Muthén, 2000). All models tested in these analyses used monthly symptom reports as the unit of analysis and accounted for the clustering of symptom reports within individual women. Each of the gene polymorphisms was included as a covariate to assess its relationship to class membership. Multinomial logistic regression was used to estimate odds ratios (ORs) and confidence intervals. Our preliminary analyses indicated there were no significant associations between age, education, body mass index (BMI), or smoking and symptom clusters. Because there were no significant associations between race/ethnicity (Asian/Pacific Islander, African American, and White) and genotypes for any of the polymorphisms that were significantly related to the symptom clusters analyzed in this study, we did not include race in the models tested.

Results

As shown in Table 2, women whose data were available for analysis and were eligible for inclusion were midlife women with a mean age of 40.9 (*SD* = 3.9) years and 16.1 (*SD* = 2.9)

Figure 2. Symptom clusters during the menopausal transition and early postmenopause identified by latent class analysis. Mean symptom severity levels for symptom groups within clusters.

years of education at the beginning of the study. Most (85.7%) were employed and 75% were married or partnered. The distribution of self-identified race/ethnicity was 87.8% White, 8.6% African American, and 2.9% Asian/Pacific Islander. The majority of eligible women were nonsmokers (86.4%), and they had a mean BMI of 25.2 kg/m² and spent a mean of 179-min weekly performing physical exercise. These women were similar to women who were not included in the analyses with respect to marital status, BMI, and exercise activity level, but they differed significantly (*p* < .05) in age, years of education, family income, and race/ethnicity. Eligible women were slightly younger, had completed more formal education, had higher family incomes, and were more likely to be White than those who were ineligible.

The results of latent class analysis of the symptoms confirmed three symptom clusters, as identified in our earlier research: Cluster 1—high-severity hot flash with moderate levels of sleep, mood, pain, and cognitive symptoms; Cluster 2—low-severity hot flashes and moderate levels of all other symptoms; and Cluster 3—low severity of all symptoms (see Figure 2). Cluster 1 accounted for 12% of the observations, Cluster 2 accounted for 16%, and Cluster 3 accounted for 72%.

Using the all low-severity symptom cluster (Cluster 3) as referent, we found that having variants *17HSD1 rs615942* (*T*>*G*) and *17HSD1 rs592389* (*G*>*T*) significantly decreased the likelihood of having the high-severity hot flash symptom cluster and increased the likelihood of having the low-severity symptom cluster (*OR* = 6.836, *p* < .001 and *OR* = 10.00, *p* < .001, respectively). There were no significant relationships between *17HSD1 rs2830* polymorphisms and any of the symptoms clusters, nor were any of the *17HSD1* variants associated with the moderate-severity symptom cluster with low-severity hot flashes. In addition, there were no significant relationships between the *CYP 19 rs10046* and repeat polymorphisms tested and any of the symptom clusters (see results of these analyses in Table 3).

Discussion

Two *17HSD1* variants, *rs615942* and *rs592389*, were associated with lower likelihood of having the high-severity hot flash

Table 3. Effects of Polymorphisms in Estrogen Synthesizing Genes on Latent Class Symptom Cluster Membership.

Model	b	SE	Wald(1)	OR	95% CI
Cluster 1 vs. Cluster 3					
Intercept					
<i>17HSD1</i>	−9.066	3.922	−2.311	N/A	N/A
<i>rs615942</i>	1.922	0.541	3.550**	6.836	[2.805, 16.659]
<i>rs2830</i>	−0.592	0.580	−1.021	0.553	[0.213, 1.436]
<i>rs592389</i>	2.303	0.488	4.722**	10.000	[4.484, 22.305]
<i>CYP 19</i>	0.138	0.800	0.173	1.148	[0.308, 4.282]
<i>rs10046</i>					
<i>CYP19 7r</i>	−0.371	0.953	−0.389	0.690	[0.144, 3.311]
<i>CYP19 7r-3</i>	0.272	0.821	0.332	1.313	[0.340, 5.065]
<i>CYP19 11r</i>	0.039	0.480	0.080	1.039	[0.472, 2.288]
Cluster 2 vs. Cluster 3					
Intercept					
<i>17HSD1</i>	−7.540	4.687	−1.609	NA	NA
<i>rs615942</i>	1.481	1.061	1.396	4.397	[0.768, 25.168]
<i>rs2830</i>	−0.285	0.559	−0.510	0.752	[0.300, 1.886]
<i>rs592389</i>	1.779	1.101	1.615	5.921	[0.968, 36.233]
<i>CYP 19</i>	−0.187	0.812	−0.231	0.829	[0.218, 3.153]
<i>rs10046</i>					
<i>CYP19 7r</i>	0.299	0.865	0.345	1.348	[0.325, 5.596]
<i>CYP19 7r-3</i>	−0.763	0.830	−0.919	0.466	[0.119, 1.826]
<i>CYP19 11r</i>	0.282	0.338	0.834	1.325	[0.760, 2.310]

Note. Polymorphisms of interest (ones with a significant effect on class membership) are coded as follows: *17HSD1 rs615942*: 1 = T/T, 2 = T/G, 3 = G/G; *17HSD1 rs2830*: 1 = A/A, 2 = A/G, 3 = G/G; *17HSD1 rs592389*: 1 = G/G, 2 = G/T, 3 = T/T; *CYP 19 rs10046*: 1 = CC, 2 = CT, 3 = TT; *CYP19 7r*: 0 = no repeats, 1 = 1 repeat, 2 = 2 repeats, and so on. Cluster 1 = high-severity hot flashes, moderate-severity all other symptoms, $n = 544$ observations (12%); Cluster 2 = low-severity hot flashes, moderate-severity all other symptoms hot flash group, $n = 707$ observations (16%); Cluster 3 = all-low-severity symptoms, used as the referent, $n = 3,260$ observations (72%).

[†] $p < .01$. ** $p < .05$. *** $p < .001$.

symptom cluster (and with an increased likelihood of having the low-severity symptom cluster). None of the other polymorphisms, including *CYP 19 rs10046*, the *CYP 19* repeat polymorphisms, and the *17HSD1 rs2830* polymorphism, was associated with the three symptom clusters studied.

In addition to the association between these *17HSD1* polymorphisms and a reduced likelihood of experiencing the symptom cluster with severe hot flashes, our unpublished data from the SMWHS cohort indicate that having the *17HSD1 rs592389* polymorphism was associated with urinary estrone levels ($p < .05$), such that women with the GT genotype had higher estrone levels than women with the GG or TT genotypes. These findings are consistent with earlier analyses of the SMWHS endocrine data, in which we found that women experiencing the high-severity hot flash symptom cluster had lower urinary estrone levels (Woods et al., 2014). Our earlier analyses also showed a marginal association between *17HSD1 rs615942* and higher levels of urinary estrone ($p = .06$), but we found no evidence for significant associations of *17HSD1 rs2830* with urinary estrone levels. Thus, our data suggest that the *17HSD1* polymorphisms *rs615942* and *rs592389* are associated with both higher levels of urinary

estrone and a lower likelihood of having the high-severity hot flash symptom cluster and that having higher levels of urinary estrone is also associated with a lower likelihood of having the high-severity hot flash symptom cluster. Further work is needed to establish examine the relationship between the *17HSD1* polymorphisms and estrogen levels as well as symptom clusters in a larger population.

Data from the national SWAN study provide an important source of comparison with our findings. In that study, White women with heterozygous genotypes for *17HSD1* SNPs (*rs592389* GT, *rs2830* AG, and *rs615942* TG) were less likely to report vasomotor symptoms than were women with homozygous genotypes (Crandall et al., 2006). SWAN investigators also found that FSH levels might interact with the *17HSD1* gene polymorphisms, such that women with lower levels of FSH have less-severe hot flashes in the presence of these polymorphisms (Crandall et al., 2006; Kardia et al., 2006). Women in the late reproductive stages and the early MT stage have lower levels of FSH than do women in the late MT stage or early postmenopause (Randolph et al., 2011; Smith-DiJulio, Percival, Woods, Tao, & Mitchell, 2007). Thus, it is possible that the association of the *17HSD1* polymorphisms and symptom clusters in which hot flashes are most prominent may be apparent even among women who are in the late reproductive stage or the early MT stage and who have not yet progressed to the late MT stage or early postmenopause, a time when they are more likely to be experiencing both high FSH levels and a higher prevalence of vasomotor symptoms. The SWAN investigators concluded that the *17HSD1* polymorphisms might influence symptoms early in the course of the MT (Crandall et al., 2006).

Another possible explanation of the association of *17HSD1* polymorphisms with hot flashes and symptom clusters in which hot flashes are prominent involves variability of estrogen levels. In Freeman et al.'s (2007) study of hormonal correlates of hot flashes, investigators found that variability in estradiol levels, not the level per se, was linked to the experience of hot flashes. From their findings in the SWAN study, Crandall, Crawford, and Gold (2006) suggested that decreasing variability in estradiol levels may have been responsible for the reduced likelihood of reporting vasomotor symptoms among women with the *17HSD1* polymorphisms they studied (*rs615942*, *rs592389*, and *rs2830*). Given the role of *17HSD1* in catalyzing conversion of estrone to estradiol, the gene polymorphism may minimize variability of estrogen levels during the MT. During the late MT stage and early postmenopause, estradiol levels drop and estrone becomes a primary source of estrogen (Randolph et al., 2011). Sowers et al. (2011) found that *17HSD1* polymorphisms were associated with patterns of slower estradiol decline among nonobese women in the SWAN population. The role of *17HSD1* in metabolizing estradiol from estrone thus may be important in stabilizing estradiol levels. In addition, the dramatic increase in the influence of androgens such as androstenediol during the MT, which is significantly related to bioactive estrogen concentrations especially among women with lower levels of

estradiol, suggests complex adaptive mechanisms that minimize the variability of estrogenic bioactivity (Lasley et al., 2012). These mechanisms warrant further study in relation to symptoms and symptom clusters.

It is also possible that *17HSD1* polymorphisms are a marker for timing of the MT stages and final menstrual period. Findings from the SWAN study indicated that women with the *17HSD1* polymorphism *rs592389* were less likely than those without it to exhibit no evidence of luteal activity, an indicator of ovarian aging and progression to the final menstrual period (Kardia et al., 2006). Women with this polymorphism were also less likely to have a cycle length of <24 or >31 days (Sowers, Jannausch, McConnell, Kardia, & Randolph, 2006), suggesting that they may have been in either the late reproductive or early MT stage but were not yet in the late MT stage (Harlow et al., 2007). Earlier findings from the SMWHS indicated that women with the *17HSD1* polymorphism *rs2830* (AG variant) were older at the final menstrual period than those with AA or GG variants ($p = .03$). In addition, there was a trend for women with the *rs592389* (GT) and *rs615942* (CA) to be older at the final menstrual period ($p = .09$, $p = .07$, respectively). These results suggest a possible role of *17HSD1* polymorphisms in the timing of reproductive aging (Mitchell et al., 2008).

Understanding the complexity of the relationships between the *17HSD1* polymorphisms and symptoms studied in both the SMWHS and SWAN study requires further analysis of the interaction of these polymorphisms with progression through the MT stages and early postmenopause as well as further study of their functional effects on endocrine levels. The *17HSD1* gene encodes the bidirectional enzyme that converts estrone to estradiol, the more biologically active form of estrogen. Evidence of *17HSD* and *CYP 19* immunoexpression in cytoplasm of the stromal cells of ovaries, epithelium cells coating ovaries, vascular endothelial cells, and epithelial inclusion cysts in ovaries from postmenopausal women, especially within 1–5 years after the final menses and declining during years 5–10 and beyond 10 years postmenopause, suggests a role for these genes in regulating estrogen levels as part of the adaptation to declining estradiol levels (Brodowska et al., 2014).

Limitations of using the data from the SMWHS include the modest number of women who contributed specimens for genotyping and the limited racial/ethnic diversity in the available data. The SWAN study has provided data on sex steroid hormone pathway genes in women of diverse races/ethnicities, but to date, there have been no efforts to examine relationships of these genes to symptom clusters. In addition to the SWAN data from women in the United States, global efforts to examine these relationships in larger and more geographically diverse populations are needed. Moreover, further analysis of the SWAN genotyping data with measures of symptom clusters obtained from women as they reached the late MT stage and early postmenopause would be a valuable contribution to the endeavor to understand the effects of polymorphisms in estrogen-synthesizing genes on symptoms and symptom clusters.

In an era of personalized health care, nurses and advanced practitioners will increasingly be asked for their counsel regarding the implications of gene polymorphisms such as those linked to estrogen-synthesizing functions and a variety of health outcomes. For women experiencing symptoms and symptom clusters including hot flashes during the MT and early postmenopause, it will be important to consider the possible effects of these polymorphisms on estrogen production and related health outcomes. For example, the *17HSD1* gene polymorphisms examined in the SMWHS and SWAN studies have been associated with higher endogenous estrogen levels, thus presenting a challenge for health-care providers to prescribe safe levels of estrogen therapies for women with these polymorphisms. In addition, *17HSD1* polymorphisms have been associated with diabetes mellitus (Lo, Zhao, Scuteri, Brockwell, & Sowers, 2006), mammographic density (Dumas & Diorio, 2010), reproductive diseases, including endometriosis (Hu et al., 2012; Tschuiya et al., 2005), and potentially to reproductive system cancers such as breast and endometrial cancer (Gaudet et al., 2008; Setiawan, Hankinson, Colditz, Hunter, & De Vivo, 2004). In order to address these issues, further research about the functional effects of these polymorphisms and symptoms and symptom clusters is needed, including the likely influence of multiple polymorphisms in the estrogen synthesis, metabolism, and receptor genes on symptoms and symptom clusters and additional health outcomes that women experience during the MT and early postmenopause.

In summary, two *17HSD1* polymorphisms (*rs615942* and *rs592389*) were associated with a lower likelihood of having a symptom cluster that included high-severity hot flashes. These polymorphisms (*rs592389* GT and *rs615942* TG) were also associated higher estrone levels during the MT, though the association for the *rs615942* TG variant was marginal. These findings from the SMWHS are consistent with the results of the SWAN study for White women and suggest the need for further investigation of the functional effects of these polymorphisms in conjunction with other estrogen synthesizing, metabolizing, and receptor gene polymorphisms.

Taken together with work delineating symptom clusters women experience during the MT and early postmenopause and evidence for effects of estrogen on a symptom cluster characterized by severe hot flashes, the present findings contribute to symptom science related to women in this stage of life. Further work on phenotypic characterization and biomarker discovery, as delineated by Cashion and Grady (2015), along with clinical application in intervention trials of symptom management, is warranted to promote the well-being of midlife women.

Authors Contribution

Nancy Fugate Woods contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Lori A. Cray contributed to conception, design, analysis, and interpretation; drafted

the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Ellen Sullivan Mitchell contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Fred Farrin contributed to acquisition; drafted the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Jerald Herting contributed to conception and design; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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