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Publication Date

2006-09-01

The American Journal of Medicine (2006) Vol 119 (9A), S94–S102

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THE AMERICAN JOURNAL *of* MEDICINE®

Cognitive Functioning and Sex Steroid Hormone Gene Polymorphisms in Women at Midlife

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ABSTRACT

Single nucleotide polymorphism (SNP) genotype frequencies were examined to determine whether variation in 6 estrogen-related genes was associated with differences in cognitive functioning in women at midlife. DNA from a multiracial/multiethnic sample of 875 African American, Caucasian, Chinese, and Japanese women aged 45 to 56 years participating in the Study of Women's Health Across the Nation (SWAN) was genotyped. Gene markers from the sex steroid hormone pathway were linked to measures of cognitive functioning including the Digit Span Backward Test (DSB), a measure of working memory; the Symbol Digit Modalities Test (SDMT), a measure of perceptual speed; and the East Boston Memory Test (EBMT), a measure of episodic memory. Statistical models were fit using logistic regression and general linear models to estimate the strength of association of estrogen-related polymorphisms with DSB, SDMT, and EBMT scores. On the EBMT, African American women and Caucasian women with *ESR1 rs9340799 GG* genotypes had about 1.5 to 2.0 times greater odds of remembering story elements on the EBMT-immediate recall test. Caucasian women with *ESR1 rs2234693 CC* genotypes had 1.3 to 1.5 times greater odds of remembering story elements on the EBMT-delayed recall test. Chinese women with *17HSD rs615942 GG* genotypes, *17HSD rs592389 TT* genotypes, and *17HSD rs2830 GG* genotypes had about 1.7 times greater odds of remembering story elements on the EBMT-immediate recall test. African American women with *CYP 19 rs936306 CC* genotypes had about 0.25 to 0.40 lower odds of remembering story elements on the EBMT-immediate recall test, whereas Chinese women with *CYP 19 rs936306 CC* genotypes had about 2.3 times greater odds of remembering story elements on both the EBMT-immediate and -delayed recall tests. On the DSB, African American women with *CYP 19 rs749292 GG* genotype had a higher mean score. On the SDMT, Japanese women with *ESR1 rs728524 GG* genotypes had a higher mean score. On the 3 tests of cognitive functioning, there was 1 significant finding for *CYP1A1* and none for the *CYP1B1* or *ESR2* SNPs. We conclude that selected genes involved in estrogen synthesis and metabolism may be associated with performance differences on cognitive function tests. Also, the relevant estrogen-related polymorphisms may vary by race/ethnicity.

KEYWORDS: Cognition; Digit Span Backward Test; East Boston Memory Test; Genetics; Sex steroid hormones; Symbol Digit Modalities Test

The Study of Women's Health Across the Nation (SWAN) and the SWAN Repository were supported by grants from the National Institutes of Health (NIH), Department of Health and Human Services, through the National Institute on Aging, the National Institute of Nursing Research, and the NIH Office of Research on Women's Health (Grant Nos. AG017719, NR004061, AG012505, AG012535, AG012531, AG012539, AG012546, AG012553, AG012554, and AG012495). The Sex Steroid Hormone Genetics Protocol was supported by the SWAN Repository. Work on this report also was supported by Grant No. K25 HL68139-01A1 from the National Heart, Lung, and Blood Institute to Dr. Peter M. Meyer.

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 obtaining access to the resources at www.swanrepository.org.

Reprints are not available.

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0002-9343/$ -see front matter
doi:10.1016/j.amjmed.2006.07.030

As many as 60% of women transitioning through menopause self-report memory problems. A total of 39% of participants aged 40 to 55 years in the Study of Women's Health Across the Nation (SWAN) cross-sectional screening survey reported incidents of "forgetfulness" in the 2 weeks preceding their interviews.[1] However, studies of the menopausal transition using objective tests of cognitive function have been inconsistent in demonstrating impairment. A longitudinal study[1] of cognitive functioning from SWAN, using tests selected for their sensitivity to early cognitive decline or anticipated susceptibility to changes in estrogen levels, showed no decrements in verbal learning and memory in relation to menopausal status, as defined by bleeding criteria.

Whereas early life conditions are related to cognitive development in childhood and cognitive function in adulthood,[2] there is considerable consensus among researchers that general cognitive ability (g) is substantially heritable,[3,4] although the specific proportion of heritability could vary in different cultures.[5] Genetic influence is substantial for specific cognitive abilities but somewhat less than for g,[6] and verbal and spatial abilities have shown greater heritability than have perceptual speed and memory abilities.[7] Furthermore, heritability for cognitive ability actually increases across the lifespan,[5] so that relatively small genetic effects early in life may increase during development and create larger phenotypic effects.

There is a role for estrogen in cognitive functioning.[8] Estradiol is synthesized in the brain via steroidogenic enzymes localized in the brain, including cytochrome P450 (CYP), aromatase, 5α-reductase, 3α-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase (17HSD).[9] Estrogen functions as a multipurpose brain messenger that can interact with neurotransmitter systems at critical brain nuclei and facilitate neuronal function via gene expression and transmitter-gated ion channels. Estrogen action is mediated through estrogen receptors (ERs) ERα and ERβ, which are widely distributed throughout the brain and located in regions associated with cognitive activities. Whereas the highest levels of ERα are observed in the amygdala and hippocampal areas, ERβ is expressed predominantly in the hippocampal formation, entorhinal cortex, thalamus, and claustrum.[8] The aromatase gene encodes the CYP enzyme that catalyzes the final stage of the conversion of androgens to estrogens in specific brain areas.

Clinical trials, primarily involving postmenopausal women, have shown that estrogen monotherapy or estrogen-progestin combinations have only inconsistently improved cognitive functioning.[10,11] In the Women's Health Initiative Memory Study (WHIMS), there was an increased risk of developing dementia in the combined treatment group after 4.05 years,[12] and women treated with hormone had slightly less improvement in global cognition (as measured by the Modified Mini-Mental Status Examination [3MS]) compared with those in the placebo group.[13] In the comparison of estrogen alone versus placebo, estrogen alone did not protect against dementia.[14,15] Consequently, estrogen's role in sustaining cognition in women aged >65 years remains unclear.

However, women with certain genetic makeup may respond differently to estrogen. ERα polymorphisms, for example, may modify the effects of exogenous estrogen on cognitive impairment.[16] Diversity in brain sensitivity to estrogenic neurosteroids might explain differences in cognitive symptoms and allelic variants of ERs, which could affect neurotransmitter function differentially, and may help explain variations in this sensitivity. Similarly, genetic variation may influence women's susceptibility to exogenous estrogen's harmful or beneficial effects.

The purpose of this article is to expand the search for risk factors for low cognitive performance associated with the menopausal transition to include genetic factors. The focus will be on single nucleotide polymorphisms (SNPs) regulating sex steroid hormone metabolism and mediating brain estrogen activity. We hypothesized that measured cognitive performance would be associated with SNPs in estrogen-related genes and that these associations would differ among racial/ethnic groups.

SUBJECTS AND METHODS

SWAN is a multicenter, multiracial/multiethnic longitudinal study of women's health across the menopausal transition, whose study design and cohort recruitment have been described elsewhere.[17] Inclusion criteria were (1) an intact uterus and $\geq$1 ovary, (2) $\geq$1 menstrual period in the previous 3 months, (3) no sex steroid hormone use in the previous 3 months, (4) not pregnant, and (5) age 42 to 52 years.

A total of 1,538 women from 6 of the 7 sites participating in SWAN provided DNA for genotyping. Of this total, 1,053 who completed cognitive function testing at the fourth annual followup were included in the analyses and 485 were excluded (246 hormone users and 43 missing information on hormone status, 92 with stroke or missing information on stroke, 73 surgically menopausal women, 31 missing data on all cognitive measures). We then excluded from further analyses 238 women who were taking antidepressant and/or antianxiety medications (n = 166) or who had a Center for Epidemiologic Studies–Depression (CES-D) scale score $\geq$ 16 (n = 49), or both (n = 23), leaving 815 participants. African American women were recruited at 4 SWAN sites (Boston, Chicago, Pittsburgh, and the Detroit area); Chinese women (Oakland) and Japanese women (Los Angeles) were recruited at 1 site each in California; and Caucasian women were recruited at all 6 sites. Racial/ethnic designation was based on self-classification. Each site's institutional review board approved the study, and all women gave written informed consent to participate in both the Core SWAN and the SWAN Genetics Study. This study was conducted under a certificate of confidentiality administered by the SWAN Repository at the University of

Table 1 Common naming conventions and rs numbers for selected single nucleotide polymorphisms (SNPs)

Gene	SNP (rs Number)	Other Conventions
ESR1	*rs9340799*	*ESRA464, XbaI RFLP*
	rs2234693	*ESRA418, PvuII RFLP*
	rs728524	*ESR728524*
17HSD	*rs615942*	*HSD615942*
	rs592389	*HSD592389*
	rs2830	*HSD17B2830*
CYP1A1	*rs1531163*	*CYP1531163, −11781* Promoter
CYP 19	*rs936306*	*CYP196306*
	rs2446405	*CYP196405*
	rs749292	*CYP199292*

Michigan. Identification numbers were encrypted to preserve the individual participants' anonymity.

Procedures

We considered 23 SNPs from 6 estrogen-related genes; these included 6 SNPs from the CYP aromatase enzyme, encoded by *CYP 19* and located on chromosome 15q21.1. Also evaluated were 4 *CYP1A1* and 3 *CYP1B1* SNPs, located on chromosomes 15q22-q24 and 2p21, respectively. A total of 3 SNPs for *17HSD,* located on chromosome 17q11-q22, were examined. Finally, we evaluated 4 *ESR1* and 3 *ESR2* SNPs, located on chromosomes 6q25.1 and 14q23.2, respectively. Ten SNPs were included in the final association analyses: *17HSD rs615942*, *17HSD rs592389*, *17HSD rs2830*, *ESR1 rs9340799*, *ESR1 rs2234693, ESR1 rs728524, CYP 19 rs936306*, *CYP 19 rs749292*, *CYP 19 rs2446405,* and *CYP1A1 rs1531163* (**Table 1**).

Beginning at the fourth annual follow-up examination, SWAN began administering a cognitive function test battery that included the East Boston Memory Test (EBMT), Digit Span Backward Test (DSB), and the Symbol Digit Modalities Test (SDMT). These tests were selected because they are sensitive to cognitive decline and, in the case of EBMT, assess domains of cognitive function considered susceptible to estrogen levels. The EBMT,[18,19] a test of episodic memory, measures the ability to learn and recall contextual verbal information, the domain hypothesized to be most strongly affected by declining estrogen levels. The EBMT 36-word paragraph was read to the participant, who was asked to repeat it immediately (EBMT-immediate) and again after a delay of approximately 10 minutes (EBMT-delayed). The EBMT-immediate and EBMT-delayed conditions were analyzed separately. The DSB and SDMT were administered during the delay. With DSB (working memory), participants repeated backwards as many increasingly longer strings of digits as possible, ranging from 2 to 7 digits, without error. The test was scored according to the *Wechsler Memory Scale–Revised* manual.[20] With SDMT (perceptual speed), participants identified as many symbol-digit matches as possible in 90 seconds.[21] These tests were offered in English, Cantonese, or Japanese.

Environmental and psychosocial covariates found to be related to cognitive symptoms include those described by Meyer and colleagues.[1] Those pertinent to these analyses are shown in **Table 2**.

Data Analyses

We examined SNPs for linkage disequililbrium and Hardy-Weinberg equilibrium (HWE) in R version 2.1.1 (R Foundation for Statistical Computing, Vienna, Austria)[22] using the genetics package version 1.1.3 (Genetics: Population Genetics, G. Warnes and F. Leisch, copyright 2005).

We used 2 different sets of predictors for adjustment in multivariate models. The first set (base model) was common across all analyses and included age, education, self-reported health, and family income. When cell counts in a racial/ethnic group were sparse, neighboring categories were pooled. The second set (fully adjusted model) was specific for each racial/ethnic group and outcome. Candidate predictors were selected based on the literature[1] as well as on menopausal symptoms that could be related to cognitive functioning. Stepwise analyses were used to identify those candidate predictors significant at the 0.05 level in addition to base model predictors. Those variables included in $\geq$1 fully adjusted model were menopausal status (coded as premenopausal, early perimenopausal, late perimenopausal, or postmenopausal), CES-D score (range, 0 to 49), perceived stress (range, 4 to 19), number of days with leaking urine in the past 2 weeks (coded as never, $<$1 day/wk, several days, or daily/almost daily), and the presence/absence of various symptoms in the previous 2 weeks (coded as none, 1 to 5 days, 6 to 8 days, 9 to 13 days, or daily), including mood change, stiffness, hot flashes, irritability, forgetfulness, nervousness, dizziness, vaginal dryness, and cold sweats.

DSB and SDMT were approximately normally distributed and were analyzed using general linear models. Both EBMT outcomes consist of the number of story elements that were successfully remembered out of a total of 12 possible elements. This is like a binomial response of the number of successes out of 12 trials, so EBMT outcomes were analyzed using logistic regression.

For each racial/ethnic group we identified all SNPs (1) that were in HWE, (2) that had subjects in all 3 genotype categories, (3) that were significantly associated with 1 of the outcomes at the 0.05 level after adjustment for the base model, and (4) in which the observed affects were monotone (i.e., the estimated effect for the heterogeneous SNP fell between the 2 homogeneous SNPs). In each case, we tested whether the SNP remained significant in the fully adjusted model. Confidence intervals (CIs) were constructed using a bootstrap resampling procedure holding the SNP percentages fixed and also allowing them to vary.[23] Because these are exploratory analyses with a previously untested set of SNPs, we did not correct for multiple comparisons[24]; in-

Table 2 Baseline characteristics of Study of Women's Health Across the Nation (SWAN) participants with no reported antidepressant or antianxiety medication use and Center for Epidemiologic Studies–Depression (CES-D) scale score < 16 with DNA genotyping*†

Characteristic	All Women	African American Women	Caucasian Women	Chinese Women	Japanese Women
Age, yr (mean ± SD)	49.8 ± 2.6	49.7 ± 2.7	49.7 ± 2.5	49.7 ± 2.3	50.4 ± 2.5
CES-D (mean ± SD)	5.2 ± 4.3	5.6 ± 4.4	5.1 ± 4.2	5.0 ± 4.4	5.2 ± 4.3
Perceived stress (mean ± SD)	6.9 ± 2.3	6.9 ± 2.5	6.6 ± 2.2	7.1 ± 2.1	7.8 ± 2.4
Participants, n (%)	815 (100.0)	196 (100.0)	426 (100.0)	96 (100.0)	97 (100.0)
Menopausal status, n (%)					
Premenopausal	84 (10.3)	24 (12.2)	45 (10.6)	8 (8.3)	7 (7.2)
Early perimenopausal	468 (57.4)	89 (45.4)	253 (59.4)	66 (68.8)	60 (61.9)
Late perimenopausal	108 (13.3)	34 (17.3)	58 (13.6)	11 (11.5)	5 (5.2)
Postmenopausal	155 (19.0)	49 (25.0)	70 (16.4)	11 (11.5)	25 (25.8)
Site, n (%)					
Boston, MA	117 (14.4)	35 (17.9)	82 (19.2)	0 (0.0)	0 (0.0)
Chicago, IL	86 (10.6)	33 (16.8)	53 (12.4)	0 (0.0)	0 (0.0)
Michigan	158 (19.4)	89 (45.4)	69 (16.2)	0 (0.0)	0 (0.0)
Pittsburgh, PA	129 (15.8)	39 (19.9)	90 (21.1)	0 (0.0)	0 (0.0)
Oakland, CA	158 (19.4)	0 (0.0)	62 (14.6)	96 (100.0)	0 (0.0)
Los Angeles, CA	167 (20.5)	0 (0.0)	70 (16.4)	0 (0.0)	97 (100.0)
Marital status, n (%)‡					
Married	547 (67.1)	90 (45.9)	312 (73.2)	65 (67.7)	80 (82.5)
Unpartnered	268 (32.9)	106 (54.1)	114 (26.8)	31 (32.3)	17 (17.5)
Education, n (%)					
High school or less	142 (17.5)	54 (28.0)	54 (12.7)	18 (18.8)	16 (16.5)
More than high school	669 (82.5)	139 (72.0)	371 (87.3)	78 (81.2)	81 (83.5)
Annual income, in US$, n (%)					
<$10,000	19 (2.4)	14 (7.6)	5 (1.2)	0 (0.0)	0 (0.0)
$10,000–$19,999	33 (4.2)	19 (10.3)	14 (3.3)	0 (0.0)	0 (0.0)
$20,000–$34,999	89 (11.3)	37 (20.0)	41 (9.8)	7 (7.4)	4 (4.4)
$35,000–$49,999	109 (13.8)	27 (14.6)	63 (15.0)	12 (12.8)	7 (7.7)
$50,000–$74,999	193 (24.5)	55 (29.7)	99 (23.6)	14 (14.9)	25 (27.5)
$75,000–$99,999	126 (16.0)	18 (9.7)	69 (16.5)	16 (17.0)	23 (25.3)
≥$100,000	220 (27.9)	15 (8.1)	128 (30.5)	45 (47.9)	32 (35.2)
Perceived health, n (%)					
Excellent/very good	556 (68.7)	102 (52.3)	331 (78.3)	55 (57.9)	68 (70.8)
Good	189 (23.4)	69 (35.4)	78 (18.4)	25 (26.3)	17 (17.7)
Fair/poor	64 (7.9)	24 (12.3)	14 (3.3)	15 (15.8)	11 (11.5)
Vasomotor symptoms, n (%)					
Not at all	405 (50.2)	66 (34.7)	219 (51.8)	60 (62.5)	60 (61.9)
1–5 days	267 (33.1)	69 (36.3)	146 (34.5)	26 (27.1)	26 (26.8)
6–8 days	47 (5.8)	17 (8.9)	20 (4.7)	6 (6.2)	4 (4.1)
9–13 days	33 (4.1)	14 (7.4)	14 (3.3)	2 (2.1)	3 (3.1)
Every day	54 (6.7)	24 (12.6)	24 (5.7)	2 (2.1)	4 (4.1)

*Columns may not sum to 100% owing to rounding.

†Category total numbers (N) for all characteristics do not sum to participant totals for column owing to missing data. The percentages are calculated after excluding participants with missing data.

‡"Married" was defined as legally married or living as married. "Unpartnered" was defined as single, never married; separated; widowed; or divorced.

stead, replication should be the confirmatory criterion. Analyses were conducted using R version 2.1.1.[22]

RESULTS

The baseline characteristics for the 815 women not taking antidepressant or antianxiety medication and having CES-D scores <16 are shown in Table 2 as combined and stratifed by race/ethnicity. **Table 3** shows the mean (SD) scores on the 4 cognitive function tests. Caucasian women had the highest mean scores on both EBMTs and the DSB. Japanese women had the highest mean score on the SDMT and second highest on the EBMTs. The Chinese women were second highest on the SDMT. On all tests, the African American women had the lowest mean scores (tied with the Chinese group on the EBMT-immediate recall test). Significant findings are presented below and in **Table 4** and **Table 5**.

EBMT and 17HSD *Genotypes.* Chinese women with the *17HSD rs615942 GG* genotype, the *17HSD rs592389 TT* genotype, and the *17HSD rs2830 GG* genotype all had higher odds of remembering story elements on the EBMT-immediate recall test compared with Chinese women with the *17HSD rs615942 TT* genotype (odds ratio [OR], 1.72;

Table 3 Baseline cognitive function test scores for Study of Women's Health Across the Nation (SWAN) participants with no reported antidepressant or antianxiety medication use and Center for Epidemiologic Studies–Depression (CES-D) scale score < 16 with DNA genotyping

Characteristic	All Women	African American Women	Caucasian Women	Chinese Women	Japanese Women
Participants, n (%)	815 (100)	196 (24.0)	426 (52.3)	96 (11.8)	97 (11.9)
EBMT-immediate, mean ± SD	10.2 (1.7)	9.7 (1.9)	10.6 (1.5)	9.7 (1.8)	10.0 (1.6)
EBMT-delayed, mean ± SD	10.1 (1.8)	9.4 (2.1)	10.5 (1.5)	9.7 (1.7)	10.0 (1.5)
SDMT, mean ± SD	56.6 (10.8)	49.4 (11.2)	58.4 (9.5)	59.0 (10.3)	61.2 (8.6)
DSB, mean ± SD	6.8 (2.3)	5.6 (2.1)	7.4 (2.4)	6.5 (1.8)	6.5 (2.0)

DSB = Digit Span Backward Test; EBMT = East Boston Memory Test; SDMT = Symbol Digit Modalities Test.

95% CI, 1.09 to 2.70], the *17HSD rs592389 GG* genotype (OR, 1.73; 95% CI, 1.10 to 2.72] or the *17HSD rs2830 AA* genotype (OR, 1.72; 95% CI, 1.10 to 2.69], respectively. These 3 genotypes also had higher odds of remembering story elements on the EBMT-immediate recall test compared with the *17HSD rs615942 GT* genotype (OR, 1.68; 95% CI, 1.10 to 2.56), the *17HSD rs592389 GT* genotype (OR, 1.73; 95% CI, 1.12 to 2.67) or the *17HSD rs2830 GA* genotype (OR, 1.70; 95% CI, 1.11 to 2.60), respectively. These associations did not extend to the EBMT-delayed recall test (Table 4).

EBMT and ER *Genes.* Caucasian women and African American women with the *ESR1 9340799 GG* genotype had higher odds of remembering story elements on the EBMT-immediate recall test compared with Caucasian women and African American women, respectively, with the *AA* genotype (OR, 1.68, 95% CI, 1.15 to 2.44 [Caucasian women]; OR, 1.96, 95% CI 1.18 to 3.25 [African American women]) or the *GA* genotype (OR, 1.49, 95% CI, 1.02 to 2.16 [Caucasian women]; OR, 1.93, 95% CI 1.15 to 3.26 [African American women]) (Table 4). This *ESR* polymorphism was not statistically significantly associated with performance on the delayed recall test. Only Caucasian women with the *ESR1 rs2234693 CC* genotype had higher odds of remembering story elements on the EBMT-delayed recall test, but not on the EBMT-immediate recall test, compared with those with the *TT* genotype (OR, 1.46; 95% CI 1.12 to 1.91) or the *CT* genotype (OR, 1.32; 95% CI 1.02 to 1.69) (Table 4). For the *ESR2* polymorphisms, no genotype comparisons were significantly associated with EBMT results.

*EBMT and Aromatase (*CYP 19 *Genotypes).* African American women with the *CYP 19 rs936306 CC* genotype had lower odds of remembering story elements on the EBMT-immediate recall test compared with those with the *TT* genotype (OR, 0.60; 95% CI, 0.43 to 0.84); those with the *CC* genotype also had marginally but nonsignificantly lower odds of remembering story elements than did those with the *CT* genotype (OR, 0.76; 95% CI, 0.56 to 1.03). There were no statistically significant associations with the EBMT-delayed recall test (Table 4). On the other hand, Chinese women with the *CYP 19 rs936306 CC* genotype had higher odds of remembering story elements on both the EBMT-immediate (OR, 2.26; 95% CI, 1.28 to 3.97) and EBMT-delayed (OR, 2.33; 95% CI, 1.34 to 4.06) recall tests compared with those with the *TT* genotype (Table 4). Those with the *CT* genotype had marginally but nonsignificantly higher odds of remembering story elements compared with those with the *TT* genotype on both EBMT tests (OR, 1.73; 95% CI, 0.97 to 3.08 [EBMT-immediate]; OR, 1.75, 95% CI, 0.99 to 3.07 [EBMT-delayed]).

EBMT and CYP1A1 *Genotypes.* African American women with the *CYP1A1 rs1531163 GG* genotype had higher odds of remembering story elements on the EBMT-delayed recall test, but not on the EBMT-immediate recall test compared with those with both the *GA* genotype (OR, 1.81; 95% CI, 1.02 to 3.19) and the *AA* genotype (OR, 2.59; 95% CI, 1.46 to 4.60). Those with the *GA* genotype also had higher odds of remembering story elements on the EBMT-delayed recall test than did those with the *AA* genotype (OR, 1.44; 95% CI, 1.01 to 2.05) (Table 4). There were no statistically significant associations with the EBMT-immediate recall test.

EBMT and CYP1B1 *Genotypes.* There were no statistically significant associations with the *CYP1B1* polymorphisms and EBMT in any racial/ethnic group.

*DSB and Aromatase (*CYP 19 *Genotypes).* African American women with the *CYP 19 rs749292 GG* genotype (difference = 0.96; 95% CI, 0.12 to 1.80) had statistically significantly higher mean DSB scores compared with those with the *AA* genotype. Those with the *GA* genotype had only a marginally nonsignificant difference compared with women with the *AA* genotype (difference = 0.72; 95% CI, −0.01 to 1.46) (Table 5).

DSB and 17HSD *or* CYP1A1 *or* CYP1B1 *Genotypes or* ER *Genes.* There were no statistically significant associations with DSB testing in any of the racial/ethnic groups for *17HSD*, *CYP1A1*, *CYP1B1*, or *ESR1* or *ESR2* polymorphisms.

SDMT and Sex Steroid Hormone–Related SNPs. Japanese women with the *ESR1 rs728524 GG* genotype had a

Table 4 Associations of the East Boston Memory Test (EBMT)* with estrogen-related polymorphisms

	EBMT-Immediate			EBMT-Delayed		
SNP/Racial–Ethnic Group	OR† (95% CI)	Wald Test *P*-value	Likelihood Ratio Test *P*-value	OR† (95% CI)	Wald Test *P*-value	Likelihood Ratio Test *P*-value
17HSD rs615942/Chinese (N = 93)			0.024‡			
GG (n = 28) vs. *TT* (n = 23)	1.72 (1.09–2.70)	0.019§				
GT (n = 42) vs. *TT* (n = 23)	1.02 (0.69–1.53)	0.908				
GG (n = 28) vs. *GT* (n = 42)	1.68 (1.10–2.56)‖	0.016§				
17HSD rs592389/Chinese (N = 91)			0.020‡			
TT (n = 28) vs. *GG* (n = 23)	1.73 (1.10–2.72)‖	0.018§				
GT (n = 40) vs. *GG* (n = 23)	1.00 (0.66–1.51)	0.997				
TT (n = 28) vs. *GT* (n = 40)	1.73 (1.12–2.67)‖	0.013§				
17HSD rs2830/Chinese (N = 93)			0.021‡			
GG (n = 29) vs. *AA* (n = 25)	1.72 (1.10–2.69)	0.017§				
GA (n = 39) vs. *AA* (n = 25)	1.01 (0.68–1.52)	0.945				
GG (n = 29) vs. *GA* (n = 39)	1.70 (1.11–2.60)‖	0.015§				
ESR1 rs9340799/African American (N = 174)			0.020‡			
GG (n = 16) vs. *AA* (n = 85)	1.96 (1.18–3.25)	0.009§				
GA (n = 73) vs. *AA* (n = 85)	1.01 (0.79–1.31)	0.914				
GG (n = 16) vs. *GA* (n = 73)	1.93 (1.15–3.26)	0.013§				
ESR1 rs9340799/Caucasian (N = 409)			0.017‡			
GG (n = 39) vs. *AA* (n = 170)	1.68 (1.15–2.44)	0.007§				
GA (n = 200) vs. *AA* (n = 170)	1.13 (0.93–1.36)	0.210				
GG (n = 39) vs. *GA* (n = 200)	1.49 (1.02–2.16)‖	0.037§				
ESR1 rs2234693/Caucasian (N = 408)						0.017‡
CC (n = 80) vs. *TT* (n = 123)				1.46 (1.12–1.91)	0.005§	
CT (n = 205) vs. *TT* (n = 123)				1.11 (0.91–1.35)	0.293	
CC (n = 80) vs. *CT* (n = 205)				1.32 (1.02–1.69)‖	0.032§	
CYP1A1 rs1531163/African American (N = 104)						0.002‡
GG (n = 15) vs. *AA* (n = 47)				2.59 (1.46–4.60)	0.001§	
GA (n = 42) vs. *AA* (n = 47)				1.44 (1.01–2.05)‖	0.045§	
GG (n = 15) vs. *GA* (n = 42)				1.81 (1.02–3.19)‖	0.042§	
CYP 19 rs936306/African American (N = 173)			0.013‡			
CC (n = 38) vs. *TT* (n = 49)	0.60 (0.43–0.84)	0.003§				
CT (n = 86) vs. *TT* (n = 49)	0.79 (0.60–1.05)	0.101				
CC (n = 38) vs. *CT* (n = 86)	0.76 (0.56–1.03)	0.076				
CYP 19 rs936306/Chinese (N = 93)			0.017‡			0.010‡
CC (n = 48) vs. *TT* (n = 10)	2.26 (1.28–3.97)‖	0.005§		2.33 (1.34–4.06)	0.003§	
CT (n = 35) vs. *TT* (n = 10)	1.73 (0.97–3.08)	0.062		1.75 (0.99–3.07)	0.053	
CC (n = 48) vs. *CT* (n = 35)	1.31 (0.89–1.90)	0.167		1.33 (0.92–1.94)	0.131	

CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.

*EBMT-immediate recall and EBMT-delayed recall tests.

†OR (second genotype listed in each pair is the referent) adjusted for age, education, self-reported health, and family income, plus:
For all *17HSD* (immediate) and for *CYP 19 rs936306*/Chinese women/immediate and delayed:mood change, stiffness.
For *ESR1 rs9340799* and *CYP 19 rs936306*/African American women/immediate: hot flashes, irritability, stiffness.
For *CYP1A1 rs1531163*/African American women/delayed: hot flashes, days with leaking.
For *ESR1 rs9340799*/Caucasian women/immediate: menopausal status, nervous, forgetful.
For *ESR1 rs2234693*/Caucasian women/delayed: nervous, dizzy, stiff, menopausal status.

‡*P*-value for Likelihood Ratio Test (comparing the model including genotype, age, education, self-reported health, family income, and additional covariates, with the model including only age, education, self-reported health, family income, and additional covariates).

§*P*-value for Wald Test (pairwise comparisons between genotypes) <0.05.

‖Bootstrap CIs for OR include 1 (i.e., nonsignificant, $P>0.05$).

statistically significantly higher mean SDMT score compared with those with the *AA* genotype (difference = 11.90; 95% CI, 2.44 to 21.37). The estimated difference is larger than a standard deviation for this racial/ethnic group. Japanese women with the *GA* genotype also had a higher mean score than did those with the

Table 5 Associations of the Digit Span Backward Test (DSB) and Symbol Digit Modalities Test (SDMT) with estrogen-related polymorphisms

	DSB			SDMT		
SNP/Racial-Ethnic Group	Difference* (95% CI)	Coefficient *P* Value	F-Test *P* Value	Difference* (95% CI)	Coefficient *P* Value	F-Test *P* Value
ESR1 rs728524/Japanese (N = 91)						<0.018†
GG (n = 3) vs. *AA* (n = 67)				11.90 (2.44–21.37)	0.016‡	
GA (n = 21) vs. *AA* (n = 67)				4.13 (0.08–8.19)§	0.049‡	
GG (n = 3) vs. *GA* (n = 21)				7.77 (−1.81–17.35)	0.116	
CYP 19 rs749292/African American (N = 171)			0.063†			
GG (n = 45) vs. *AA* (n = 42)	0.96 (0.12–1.80)	0.027‡				
GA (n = 84) vs. *AA* (n = 42)	0.72 (−0.01–1.46)	0.056				
GG (n = 45) vs. *GA* (n = 84)	0.24 (−0.50–0.97)	0.527				

*Difference score (second genotype listed in each pair is the referent) adjusted for age, education, self-reported health, and family income, plus:
For *ESR1 rs728524*/Japanese women/SDMT: no additional covariates.
For *CYP 19 rs749292*/African American women/DSB: nervous.

†*P* value for F-Test (comparing the model including genotype, age, education, self-reported health, family income, and additional covariates, with the model including only age, education, self-reported health, family income, and additional covariates).

‡*P* value for a test of 0 difference Wald Test (pairwise comparisons between genotypes) <0.05.

§Bootstrap CIs for difference score do not include 0 (i.e., statistically significant, *P* <0.05).

AA genotype (difference = 4.13; 95% CI, 0.08 to 8.09), a <0.5 SD unit difference (Table 5).

There were no statistically significant associations with SDMT testing in any of the racial/ethnic groups for *17HSD*, *CYP 19*, or *CYP1A1* or *CYP1B1* polymorphisms.

DISCUSSION

These data presented provide preliminary evidence that a number of estrogen-related polymorphisms, particularly from *ESR1*, *17HSD*, and *CYP 19*, were associated with differences in cognitive performance among 4 racial/ethnic groups of women at midlife. However, the magnitude of most of the model-based estimates of associations (ORs, 1.3 to 2.3) and mean differences (<0.8 SD units) were not large, and certain ORs comparing the heterozygous genotype to the homozygous genotype were not significant, based on bootstrap-derived CIs.

Almost all of our significant findings involved the EBMT (a test of episodic memory). Declines in cognitive function are best detected using instruments that measure new learning (perceptual speed and episodic/working memory) as demonstrated by studies relating age-related cognitive decline to apolipoprotein E (*APOE*) genotypes. The episodic and working memory systems are associated with conscious awareness (explicit memory) and recognition/recall of previously presented information (declarative memory).[25,26]

ERs exist throughout the brain but are especially prevalent in the hippocampus,[27] suggesting a role for estrogen in episodic memory.[28] *ESR1* (ERα) messenger RNA (mRNA), in particular, dominates in the amygdala and hypothalamus, with the highest ERα levels in the amygdala-hippocampal area.[8] The presence of ER mRNA in the entorhinal cortex, origin of the perforant pathway to the hippocampus, and the temporal cortex further supports a role for *ESR1* in cognition and declarative memory.[8] Thus, our findings with polymorphisms representing the *ESR1* family and EBMT performance are consistent with expectations based on the biology of memory.

Our results can be compared with those reported by Yaffe and associates,[16] who reported that the *ESR1* polymorphisms *Pvu*II and *Xba*I were associated with greater decline in cognitive scores assessed with the 3MS administered to women of European ancestry. However, this study[16] and 3 other studies[29–31] examining the same associations reported different effects of the polymorphisms. Yaffe and associates[16] noted that 1 study included only Japanese women[30] and suggested that ethnic differences may account for different effects of the polymorphisms. Moreover, in a study of Japanese patients with Alzheimer disease, Maruyama and coworkers[32] found no association between ERα gene polymorphisms.

17HSD mRNA expression also has been detected in temporal lobe and hippocampal areas.[33,34] Expression of this enzyme in subcortical white matter suggests that glial cells might play a role in biosynthesis and deactivation of sex steroids in the brain.[9] Glial cells are involved in myelin formation, which reaches a maximum in midlife and declines in older age[35,36] and is related to the high processing speeds that underlie cognitive functioning.[37,38] Thus, sex steroids, 17HSD enzymatic activities, and myelin function and/or formation may be correlated, and variation in levels of cognitive function may be associated with different *17HSD* genotypes.

Aromatase, a product of the *CYP 19* gene, catalyzes the conversion of androgen to estrogen and is active in both temporal and frontal brain areas, but especially in the temporal regions.[39,40] Its activity in these areas of the brain leads to local production of estrogen that may affect cognitive functioning.

There are limitations to our preliminary findings. We explored race/ethnicity-specific relations in these associations between polymorphism genotype and cognitive function test performance. Although we assessed the 4 racial/ethnic groups separately, we cannot rule out covert population stratification within the racial/ethnic groups. We also cannot exclude the importance of genes for which we did not observe associations. Another consideration is that although we examined 23 polymorphisms in 4 racial/ethnic groups, we decided to minimize type II error and not correct for multiple testing. Some of our results may be considered "borderline" but our statistical approach was conservative. Moreover, these associations are consistent with the neuroanatomy of memory and the role of estrogenic neurosteroids in influencing neuronal function via their effects on gene expression.

In summary, we have presented evidence to support an association between estrogen-related genetic polymorphisms and performance on cognitive function tests that measure new learning in a multiracial/multiethnic cohort of midlife women. These novel results must be replicated. Further study is needed to understand the effects of estrogen on cognitive performance and the related biological pathways and mechanisms.

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