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Estrogen Receptor β Variants Modify Risk for Alzheimer's Disease in a Multiethnic Female Cohort

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Abstract.
Background: Few studies of gene variants that affect estrogen activity investigate their association with age at onset of Alzheimer's disease (AD) in women of different ethnicities. We investigated the influence of *ESR2* polymorphisms on age at onset of AD in a multiethnic cohort of women.
Objectives: To determine whether gene variants would affect risk for AD differently in women of different population ancestries. Methods: Among 1,686 women participating in the Washington Heights Inwood Columbia Aging Project (WHICAP), association with risk for AD was assessed for 20 *ESR2* single-nucleotide polymorphisms (SNPs) using multivariate logistic regression, adjusting for age at time of study enrollment, presence of an *APOE* ε4 allele, years of education, and body mass index.
Results: Increased risk for AD was associated with four *ESR2* SNPs in women of predominantly Caucasian AIMS-defined ancestry: rs944045, rs1256062, rs10144225, and rs2274705 (OR range 1.6–1.9, empiric *p*-value range 0.002–0.004). A separate SNP (rs10137185) was associated with decreased risk for AD in women who identified themselves as Black (OR 0.6, 95% CI = 0.4–0.9). When vascular risk factors were included in the model, a separate SNP (rs1256059) was associated with increased risk for AD in women of admixed/Hispanic ancestry (OR 1.5, 95% CI = 1.1–2.4).
Conclusions: *ESR2* polymorphisms affect risk for AD in women, and risk alleles vary by AIMs-defined ancestry and self-identified ethnicity. These effects are possibly due to different linkage disequilibrium patterns or differences in comorbid risk factors mediating SNP effect on risk for AD by group.

Keywords: Alzheimer's disease, estrogen receptor 2, estrogen receptors beta, genetic association studies, Hispanic, women

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INTRODUCTION

Estrogens are important in the normal maintenance of brain function in regions typically affected by Alzheimer's disease (AD) and may play a role in the cognitive decline associated with AD [1, 2]. Estrogen benefits brain structure and physiology by promoting human neural stem cell proliferation [3]; promoting the growth and survival of cholinergic neurons [4, 5], increasing cholinergic activity [6]; exerting antioxidant properties [7]; promoting the non-amyloidogenic metabolism of the amyloid-β protein precursor [8]; increasing amyloid-β clearance [9]; and through potentiation of mitochondrial function [10]. Estrogens act by binding to two estrogen receptors, α and β, which differ in protein structure [11] and function [12], and are encoded by two different genes, *ESR1* and *ESR2,* located on chromosome 14q23.2. *In vivo* studies demonstrate that activation of ERβ can regulate hippocampal synaptic plasticity and improve memory, and suggest a role for *ESR2* polymorphisms in risk for AD [13]. Several studies [14–17], but not all [18], have found an association between AD or cognitive impairment and multiple *ESR2* polymorphisms. However, most studies have been conducted in Caucasian ethnic groups, and few polymorphisms have been assessed in a multiethnic cohort. Examination of estrogen gene polymorphisms in multiethnic groups which are evaluated without taking ancestry into account may have several limitations, including a loss of significant association due to different allele frequencies, different linkage disequilibrium patterns between ethnicities, or differences in the distribution of comorbid conditions and risk factors by ethnic group. In this study, we examined the relationship between polymorphisms in *ESR2* and the risk of AD in a multiethnic community of elderly women from northern Manhattan, with individual ancestry assessed both by population ancestry markers as well as by self-identified ethnicity [19]. The aims of this study was to confirm previous findings of *ESR2* polymorphisms which were found to be significantly associated with risk for AD; to identify additional single-nucleotide polymorphisms (SNPs) which confer risk for AD by performing denser genotyping than performed in previous studies; and to examine whether *ESR2* variants affect risk for AD differently in groups of women with different self-identified ethnicity or genetic population ancestry. We hypothesized that *ESR2* variants would demonstrate different patterns of association with AD between populations of differing ancestry due to different allele frequencies or linkage disequilibrium patterns between groups, as well as varying environmental factors.

MATERIALS AND METHODS

Subjects

The study included 1,686 women participating in the Washington Heights Inwood Columbia Aging Project (WHICAP), a prospective study of aging and dementia among Medicare recipients age 65 years and older, residing in northern Manhattan. Thirty women participating in WHICAP were diagnosed as having dementia not related to AD (including vascular dementia, Lewy body dementia, and Parkinson's disease dementia), and were not included in the study analyses. The population from which participants were drawn was comprised of individuals from several different countries of origin representing three broadly self-identified ethnicities (Caribbean Hispanic, African-American, and non-Hispanic White of European ancestry). Subjects were recruited in two waves, one beginning in 1992 and the other in 1999. The sampling strategies and recruitment outcomes of these two cohorts have been described in detail elsewhere [19].

Each subject underwent an in-person interview of general health and functional ability followed by

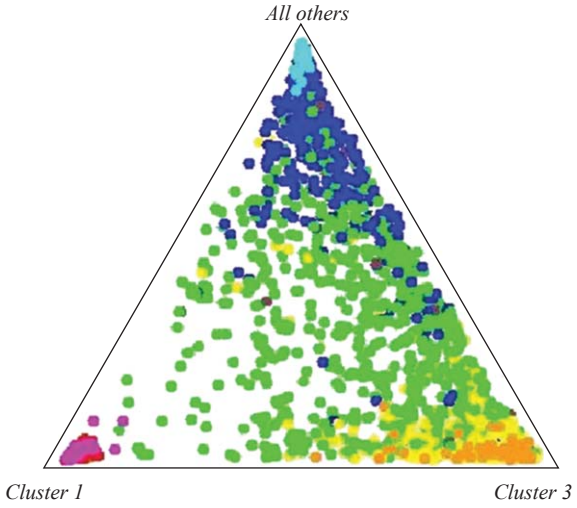

Fig. 1. Plot of WHICAP participants by AIMs-defined ancestry versus HapMap populations. *WHICAP participants:* Yellow, Predominantly Caucasian AIMs-defined ancestry; Green: Admixed/Hispanic AIMs-defined ancestry; Blue: Predominantly African AIMs-defined ancestry; *HapMap populations:* Light Brown: Ancestrally homogenous Caucasian population (CEPH); Light Blue: Ancestrally homogenous Yoruban Black population (YRI); Red: Ancestrally homogenous East Asian population (CHJA).

a standardized assessment, including medical and medication history, physical and neurological examination, and a neuropsychological battery that included measures of memory, orientation, language, abstract reasoning, and visuospatial ability [20]. Height and weight were measured at each visit to compute body mass index (BMI). Assessments were conducted at 18–24 month intervals over a mean of 6.1 years of follow-up. AD diagnosis was based on NINCDS-ADRDA criteria. It was established by consensus at a conference of physicians, neurologists, neuropsychologists,and psychiatrists, using all available information gathered from the initial and follow-up assessments and medical records. We used a conservative definition of AD in our analyses, excluding definitions of mild cognitive impairment or isolated low neuropsychological scores in order to obtain the most robust phenotype.

Standard protocol approvals, registrations, and patient consents

This study was reviewed and approved by the Columbia University institutional review board, and written informed consent was previously obtained from all subjects.

DNA isolation and genotyping

Genomic DNA was extracted from total peripheral blood leukocytes using standard methods. Genotyping was carried out blind to the participant's dementia status and any other identifying characteristics. We used a multistep selection process to identify candidate SNPs for genotyping. We first selected SNPs within *ESR2* that were previously reported to be associated with an increased incidence or earlier age of onset of AD in any population. We then referenced the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) to select tagging SNPs in both Caucasian and African populations. To provide sufficient coverage of the gene, we selected SNPs to maintain a pairwise r^2 threshold of 0.8 in SNPs with a minimum minor allele frequency of 0.2. We obtained an average intermarker distance of 3.9 kilobase pairs between SNPs, which provided good coverage of the gene as viewed on linkage disequilibrium maps (Supplementary Figures 1–3).

Twenty *ESR2* SNPs as well as 100 ancestry informative markers (AIMs) were genotyped in a total of 1,686 samples using Illumina GoldenGate custom panels and the Illumina Iscan platform. Genotyping was performed according to standard protocols (http://www.illumina.com/). Briefly, following extension and amplification steps, array products were hybridized to the array matrix for individual SNP genotype readout. After hybridization, the Iscan Reader was used to analyze fluorescence signal on the Sentrix Array Matrix, which was in turn analyzed using Genomestudio software (Illumina Inc.) for automated genotype clustering and calling. Duplicate genotyping was performed on 10% of samples to verify accuracy, and the concordance rate was greater than 98%.

Assessment of population ancestry

To evaluate population stratification, we used a set of 100 unlinked SNPs to classify population ancestry. These unlinked SNPs from a panel of 650Y Illumina SNPs have allele frequencies that are significantly different among three ethno-racial groups: non-Hispanic Whites, non-Hispanic African, and individuals of Mexican/Central American ancestry. To assess population stratification, we performed population structure analysis as implemented in the STRUCTURE program [21, 22]. To anchor ancestry, we included data from Caucasian (CEPH), Yoruban (YRI), and Mexican/ Central Americans from the HapMap project (Fig. 1). Our self-identified White population closely aligned with the CEPH samples in the HapMap dataset and our self-identified Black population clustered around the YRI samples. As expected, Caribbean Hispanics clearly showed admixture of CEPH and YRI genetic population ancestry, and the range of admixture varied widely.

We then classified participants into groups who were of predominant Caucasian ancestry as defined by the AIMs index (defined as $\geq$0.6 AIMs markers consistent with CEPH profile, $n = 632$) versus those who were of predominant African ancestry (defined as $\geq$0.6 AIMs markers consistent with YRI profile, $n = 581$). In doing so, individuals previously self-identified as Hispanic were reclassified as being of predominant Caucasian or African ancestry (if their AIMs index scores were $\geq$0.6 CEPH or YRI, respectively), or admixed/ Hispanic if they did not have one predominant genetic ancestry ($n = 473$). While the AIMs-defined classification largely agreed with classification by self-reported ethnicity, there was some disparity as shown in Table 2.

The present population stratification approach based on 100 SNPs provides similar results when compared with the genome wide principle component approach that uses all available SNPs ($n \sim 650{,}000$). Although the classification is not as distinct as using all 650,000 SNPs, we were able to well differentiate three genetic

Table 1
Population characteristics

Characteristic	Non Demented	Incident AD
Sample Size	1175	511
Age at time of enrollment (mean ± S.D.)*	75.7 (6.0)	79.9 (7.2)
Body mass index (mean ± S.D.)	28.2 (7.9)	27.1 (5.8)
Years of education (mean ± S.D.)*	10.5 (4.6)	7.5 (4.7)
Ever diagnosed with diabetes mellitus (*n*, %)	174 (15.9)	60 (18.2)
Current smokers (*n*, %)	96 (8.6)	31 (9.4)
At least one copy *APOE* ε4 (*n*, %)*	284 (24.2)	156 (30.5)
Self-identified ethnicity (*n*, %)*		
White	340 (89.5)	40 (10.5)
Hispanic	392 (68.4)	181 (31.6)
Black	375 (77.6)	108 (22.4)

*$p < 0.05$.

Table 2
Self-identified ethnicity versus AIMs-defined ancestry

		Self-identified ethnicity				
		White	Hispanic	Black	Other	Total
AIMs-defined ancestry *(n, % of self-identified ethnicity)*	Predominantly Caucasian	397 (93.9%)	218 (32.3%)	13 (2.3%)	4 (23.5%)	632
	Admixed/Hispanic	23 (5.4%)	376 (55.5%)	68 (11.8%)	6 (35.3%)	473
	Predominantly African	3 (0.7%)	84 (12.4%)	487 (84.8%)	7 (41.1%)	581
	Total	423	672	574	17	1,686

population ancestry groups. This analysis provided relatively comparable results as our earlier analysis based on larger samples, which was able to determine population ancestry [23].

Potential confounders

Potential confounders included age at time of study enrollment, presence of an *APOE* ε4 allele, and years of education, all of which were found to be independently associated with risk for AD in our group. BMI was also included as a confounder because of the association of adipose tissue with higher estrogen levels [24–26]. History of diabetes mellitus and current smoking status did not differ significantly between individuals with or without AD. However, there were notable differences between self-identified African-Americans, Hispanics, and Whites in prevalence of vascular risk factors including diabetes mellitus (AA:18.8%; HIS: 20.5%; WH: 7.3%) and current smoking (AA:14.0%; HIS: 6.5%; WH: 5.8%); as a result, these confounders were included in secondary analyses examining the potential mediating effects of vascular risk factors on risk for AD. History of coronary artery disease or hypertension was not statistically significant between patients with or without AD, and did not vary by ethnicity. *APOE* genotyping was carried out as described in a previous study [27] by PCR/RFLP analysis or by PreventionGenetics (http://www.preventiongenetics.com). A dominant model was used to categorize *APOE* status; that is, participants with at least one copy of an *APOE* ε4 allele were considered to have a risk allele. Height and weight were measured at the initial evaluation to compute BMI. History of diabetes mellitus was defined as self-reported current or past history of treated or untreated diabetes.

Statistical analyses

Prior to association analysis, we assessed whether each SNP was in Hardy Weinberg equilibrium. This analysis was performed separately within each self-identified ethnicity as well as within each AIMs-defined population of unaffected individuals using the χ^2 goodness-of-fit test in Haploview [28]. SNPs were then evaluated in genotypic association analyses to further characterize their relationship to AD. Analyses were first conducted stratifying by models of AIMs-defined ancestry, and then by self-reported ethnicity. We used multivariate logistic regression to estimate likelihood of AD by SNP genotype, adjusting for age at time of study enrollment, presence of at least one copy of an *APOE* ε4 allele, years of education, and BMI. To minimize the risk of a false-positive finding from rare variants and repeated analyses, we computed empirical *p*-values by generating the null distribution on the basis of 1000 replicates of analyses. The first regression model did not include vascular risk factor

covariates because these variables and their effects on risk for AD could potentially be confounded by social, health, or economic factors associated with ethnicity. A second regression model then included additional vascular risk factors, including history of diabetes mellitus and current smoking, to observe the potential modifying effect of these covariates on SNPs which were significantly associated with risk for AD. Particular attention was given to whether inclusion of these vascular risk factors affected the relationships between significant SNPs and risk for AD among self-identified ethnicity or AIMs-defined ancestry groups. SNPs were analyzed using a dominant model, in which participants homozygous for the common allele were used as the reference group and the risk group included participants who were heterozygous or homozygous for the rare allele, in order to provide the most robust model for observing an effect of the minor allele. We hypothesized that differences in associations between these two sets of analyses might reflect culturally-defined environmental risk factors for AD; conversely, similarities in significant SNPs between the two analyses would demonstrate a more direct genetic effect of *ESR2* polymorphisms on risk for AD.

RESULTS

Demographic characteristics

Table 1 presents the demographic characteristics of our cohort. The mean age of the participants at baseline was 77.0 (±6.7) years, and ranged from 65 to 97 years. Mean length of follow-up was 6.1 (±4.3) years. The majority of women were self-identified as Hispanic ($n = 672$, 39.9%) and Black ($n = 574$, 34.0%), while 423 women were self-identified White (25.1%), and 17 women were classified as "other". Among all participants, 511 were classified as possible or probable AD (29.5%) and 1,175 as nondemented. Compared with women who remained free of dementia throughout the follow-up period, women with dementia were older at baseline (79.9 versus 75.7 years), had fewer years of education, and were more like to have at least one *APOE* ε4 allele. The prevalence of AD was elevated in self-identified African-Americans and Hispanics compared with Whites (AA: 32.2%; HIS:38.2%; WH:15.3%). BMI, history of diabetes mellitus, and current smoking status did not differ significantly between individuals with or without AD. However, there were notable differences between self-identified African-Americans, Hispanics, and Whites in prevalence of vascular risk factors including diabetes mellitus (AA:18.8%; HIS: 20.5%; WH: 7.3%) and current smoking (AA:14.0%; HIS: 6.5%; WH: 5.8%). History of coronary artery disease and hypertension was not statistically significant between patients with or without AD.

Genotypic associations

For ease of discussion, we will use the order of SNPs to refer to each SNP. Among women of predominantly Caucasian AIMs-defined ancestry, four SNPs (SNPs 3, 5, 7, 10: rs944045, rs1256062, rs10144225, rs2274705, respectively), were found to be associated with increased risk for AD, with odds ratios (OR) ranging from 1.6–1.9 (Table 3a). SNPs that were found to be associated with risk for AD in the predominantly Caucasian AIMs-defined population were not found to be significant in women of predominantly African or admixed/ Hispanic AIMs-defined ancestry.

We then repeated the analyses within strata defined by self-identified ethnicity (Table 3b). Of the four SNPs that were significant in the predominantly Caucasian AIMs population, three (SNPs 5, 7, 10) continued to demonstrate increased risk for AD in self-reported Whites, with similar or slightly stronger strength of association (OR 1.9–2.1), as found when individuals were clustered by AIMs. One additional SNP (SNP 20, rs10137185) was also found to be significantly associated with a reduced risk for AD in the self-identified Black population (OR 0.6, 95% confidence interval (CI) = 0.4–0.9).

The addition of vascular covariates (history of diabetes mellitus and current smoking) to the AIMs-defined (Supplementary Table 1) and self-identified ethnicity (Supplementary Table 2) models revealed one additional SNP to be significant among self-identified Hispanics as well as among individuals with admixed/ Hispanic AIMs-defined ancestry (rs1256059; OR 1.5, 95% CI = 1.1–2.4); otherwise, the SNPs which were found to be significant in our first models remained the same, with similar odds ratios.

Pairwise linkage disequilibrium (LD) was conducted between all 20 ESR2 SNPs in each of the three AIMs-based groups as implemented in the Haploview program using the D' value [28] (Supplementary Figures 1–3). SNPs which significantly influenced risk for AD in individuals of predominantly Caucasian AIMs-defined ancestry clustered in one large LD block, although SNP 3 was separated from SNPs 5,7, and 10 by approximately 10.7 kilobases (Supplementary Figure 1). The physical separation of these two groups indicates that they may be separately identified with

Table 3a
Odds ratios for AD by *ESR2* SNPs, stratified by AIMs-defined ancestry

				Predominantly Caucasian				Admixed/ Hispanic				Predominantly African			
SNP #	SNP	Position	Minor Allele	MAF	*OR*	*95% CI*	*emp. p-value*	MAF	*OR*	*95% CI*	*emp. p-value*	MAF	*OR*	*95% CI*	*emp. p-value*
1	rs1152577	63767238	A	0.2	1.0	0.7–1.4	0.880	0.3	1.4	0.9–2.0	0.142	0.2	1.1	0.7–1.7	0.562
2	rs1256065	63768435	C	0.3	1.0	0.7–1.6	0.788	0.3	1.4	0.9–2.1	0.104	0.3	1.1	0.8–1.8	0.519
3	rs944045	63771199	G	*0.3*	*1.6*	*1.1–2.4*	*0.004*	0.4	1.0	0.6–1.4	0.847	0.4	0.8	0.5–1.2	0.250
4	rs1256063	63771720	T	0.4	1.4	0.7–2.6	0.300	0.3	0.8	0.4–1.8	0.668	0.3	0.4	0.1–1.5	0.208
5	rs1256062	63773071	G	*0.2*	*1.9*	*1.2–2.8*	*0.002*	0.2	0.9	0.6–1.4	0.658	0.2	0.9	0.6–1.3	0.484
6	rs1256061	63773346	A	0.3	0.7	0.5–1.0	0.051	0.3	0.8	0.6–1.3	0.414	0.3	1.0	0.7–1.4	0.894
7	rs10144225	63774497	G	*0.3*	*1.9*	*1.2–2.8*	*0.003*	0.3	0.9	0.6–1.3	0.472	0.3	0.8	0.5–1.2	0.228
8	rs1256059	63780170	A	0.4	1.0	0.7–1.5	0.974	0.4	1.4	0.9–2.1	0.096	0.3	1.1	0.7–1.7	0.603
9	rs17766755	63785526	A	0.3	0.7	0.5–1.1	0.096	0.3	1.0	0.7–1.6	0.814	0.3	0.9	0.6–1.3	0.537
10	rs2274705	63786382	G	*0.2*	*1.6*	*1.1–2.4*	*0.002*	0.2	0.9	0.6–1.3	0.602	0.2	0.8	0.6–1.2	0.319
11	rs12435857	63793278	A	0.3	0.8	0.5–1.2	0.271	0.3	0.9	0.6–1.3	0.527	0.3	0.9	0.6–1.3	0.631
12	rs1256049	63793804	A	0.4	1.3	0.6–2.8	0.456	0.4	0.8	0.4–1.4	0.382	0.4	1.3	0.8–2.2	0.275
13	rs1256044	63803780	C	0.4	1.0	0.7–1.5	0.858	0.4	1.3	0.9–1.9	0.206	0.4	1.2	0.8–1.8	0.478
14	rs1256043	63803785	T	0.3	1.1	0.7–1.6	0.782	0.3	1.3	0.9–1.9	0.216	0.2	1.1	0.7–1.6	0.727
15	rs10148269	63806677	A	0.3	1.0	0.7–1.6	0.830	0.3	1.3	0.9–1.9	0.233	0.3	1.1	0.7–1.7	0.606
16	rs1256039	63808482	C	0.4	1.0	0.7–1.5	0.871	0.4	1.3	0.9–1.9	0.217	0.4	1.1	0.7–1.6	0.746
17	rs1256037	63813054	C	0.3	1.0	0.7–1.5	0.927	0.3	1.4	0.9–2.1	0.144	0.3	1.1	0.7–1.6	0.802
18	rs1541060	63816519	T	0.2	1.0	0.1–1.9	0.999	0.2	1.5	0.3–8.7	0.628	0.2	2.1	0.6–7.6	0.268
19	rs1271572	63831670	T	0.3	1.0	0.7–1.4	0.890	0.3	1.3	0.9–2.0	0.171	0.3	1.0	0.7–1.6	0.933
20	rs10137185	63845529	T	0.3	1.5	1.0–2.3	0.062	0.3	1.1	0.7–1.7	0.653	0.3	0.7	0.5–1.0	0.072

ORs were computed using a dominant model in which individuals with at least 1 copy of the minor allele were coded as having a risk allele. Adjusted for years of education, body mass index, and presence of at least one *APOE* ε4 allele. MAF, Minor allele frequency; Emp. *p*-value, empirical *p*-value.

Table 3b
Odds ratios for AD by *ESR2* SNPs, stratified by self-identified ethnicity

				White				Hispanic				Black			
SNP #	SNP	Position	Minor Allele	MAF	*OR*	*95% CI*	*emp. p-value*	MAF	*OR*	*95% CI*	*emp. p-value*	MAF	*OR*	*95% CI*	*emp. p-value*
1	rs1152577	63767238	A	0.2	0.9	0.5–1.5	0.608	0.3	1.3	0.9–1.8	0.152	0.2	1.0	0.6–1.5	0.838
2	rs1256065	63768435	C	0.3	0.9	0.5–1.7	0.808	0.3	1.3	0.9–1.8	0.122	0.3	0.9	0.6–1.4	0.669
3	rs944045	63771199	G	0.3	1.8	1.0–3.4	0.060	0.4	0.9	0.7–1.3	0.743	0.4	0.9	0.6–1.4	0.664
4	rs1256063	63771720	T	0.4	1.2	0.5–3.0	0.714	0.3	1.1	0.6–2.1	0.683	0.3	0.4	0.1–1.3	0.132
5	rs1256062	63773071	G	*0.2*	*2.1*	*1.2–3.7*	*0.002*	0.2	1.1	0.8–1.5	0.698	0.2	0.9	0.6–1.3	0.494
6	rs1256061	63773346	A	0.3	0.7	0.4–1.3	0.264	0.3	0.9	0.7–1.3	0.54	0.3	1.0	0.7–1.4	0.834
7	rs10144225	63774497	G	*0.3*	*2.1*	*1.2–3.8*	*0.001*	0.3	1.0	0.7–1.4	0.948	0.3	0.8	0.6–1.3	0.39
8	rs1256059	63780170	A	0.4	0.9	0.5–1.5	0.607	0.4	1.3	0.9–1.8	0.114	0.3	1.0	0.7–1.5	0.988
9	rs17766755	63785526	A	0.3	0.7	0.4–1.2	0.212	0.3	1.0	0.7–1.3	0.782	0.3	1.1	0.8–1.6	0.63
10	rs2274705	63786382	G	*0.2*	*1.9*	*1.0–3.3*	*0.003*	0.2	1.0	0.7–1.4	0.849	0.2	0.9	0.6–1.3	0.57
11	rs12435857	63793278	A	0.3	0.7	0.4–1.3	0.324	0.3	0.9	0.7–1.3	0.662	0.3	1.0	0.7–1.4	0.848
12	rs1256049	63793804	A	0.4	1.6	0.6–4.6	0.384	0.4	0.9	0.6–1.5	0.709	0.4	1.1	0.7–1.9	0.636
13	rs1256044	63803780	C	0.4	1.0	0.5–1.7	0.903	0.4	1.2	0.9–1.7	0.21	0.4	1.0	0.7–1.5	0.991
14	rs1256043	63803785	T	0.3	0.9	0.5–1.7	0.811	0.3	1.2	0.9–1.7	0.196	0.2	0.9	0.6–1.4	0.56
15	rs10148269	63806677	A	0.3	1.0	0.5–1.7	0.888	0.3	1.2	0.9–1.7	0.191	0.3	0.9	0.6–1.4	0.699
16	rs1256039	63808482	C	0.4	0.9	0.5–1.6	0.753	0.4	1.2	0.9–1.7	0.248	0.4	0.9	0.6–1.4	0.571
17	rs1256037	63813054	C	0.3	1.0	0.5–1.7	0.865	0.3	1.2	0.9–1.7	0.206	0.3	0.9	0.6–1.4	0.598
18	rs1541060	63816519	T	0.2	1.0	0.1–1.9	0.999	0.2	1.9	0.4–8.8	0.42	0.2	1.7	0.4–6.9	0.442
19	rs1271572	63831670	T	0.3	0.9	0.5–1.7	0.818	0.3	1.2	0.9–1.6	0.308	0.3	0.9	0.6–1.4	0.668
20	rs10137185	63845529	T	0.3	1.8	0.9–3.6	0.083	0.3	1.1	0.8–1.6	0.456	*0.3*	*0.6*	*0.4–0.9*	*0.002*

ORs were computed using a dominant model in which individuals with at least 1 copy of the minor allele were coded as having a risk allele. Adjusted for years of education, body mass index, and presence of at least one *APOE* ε4 allele. MAF, Minor allele frequency; Emp. *p*-value, empirical *p*-value.

alleles of different as yet unidentified loci for susceptibility to AD. Additionally, the LD pattern of the predominantly Caucasian AIMs-defined population differed significantly from those of the admixed and predominantly African AIMs-defined populations, both of which demonstrated that the SNP 1-2-3 LD block was distinct from the SNP 7–19 LD block (Supplementary Figures 2–3).

Haplotype analysis

The possibility of multi-locus association at adjacent variants was supported by the strong pairwise LD between SNP loci in several blocks. Because the associated SNPs in AIMs-defined populations clustered around 15 kilobases within intron 1 and alternate exon 3, we performed a "sliding window" haplotype analysis, with each window including two to four consecutive SNPs spanning the region containing the associated SNPs. While numerous haplotypes constructed from these *ESR2* SNPs were found to be significantly associated with increased or decreased risk for AD (Supplementary Table 3), the most robust associations among the predominantly Caucasian AIMs population were haplotype C-A at SNPs 4-5, which was associated with reduced risk for AD (OR 0.58, $p = 0.0009$), and haplotype C-G-G at SNPs 6-7-8, which was associated with increased risk for AD (OR 1.64, $p = 0.007$). Among individuals with admixed/ Hispanic AIMs-defined ancestry, the strongest haplotype association was haplotype C-A-C-A at SNPs 2-3-4-5, which was associated with an increased risk for AD (OR 1.49, $p = 0.015$).

DISCUSSION

Among 1,686 community-dwelling elderly women in a multi-ethnic cohort, increased risk for developing AD was associated with four intronic *ESR2* SNPs within a 15 kilobase region in women of predominantly Caucasian AIMs-defined ancestry: SNPs 3, 5, 7, and 10 (rs944045, rs1256062, rs10144225, and rs2274705). Three of these SNPs were also associated with increased risk for AD in women who identified themselves as White. However, these SNPs were not significant in women of admixed/Hispanic or predominantly African AIMs-defined ancestry. One additional SNP (SNP 20: rs10137185) was found to be associated with decreased risk for AD in women who identified themselves as Black, and several SNPs flanking this SNP also suggest weak association with AD (SNPs 17 and 19 in self-identified Hispanics, and SNP 18 in self-identified Blacks). Finally, SNP 8 (rs1256059) was found to be significant in women who identified themselves as Hispanic, as well as in women of admixed/Hispanic AIMs-defined ancestry once vascular risk covariates were included in the models.

Involvement of polymorphisms of a gene in susceptibility to AD in groups of one population ancestry, but not in another, may occur for several reasons. First, differences in LD among populations from different genetic ancestries, as observed in Supplementary Figures 1–3, may contribute to discrepancies in AIMs-restricted genotype associations. Notably, different LD patterns between these three populations occurred in the region of the *ESR2* gene where significant SNPs clustered in the predominantly Caucasian AIMs population (SNPs 3, 5, 7, 10). As a result, the differences in observed associations may be due to different LD patterns between *ESR2* allele and alleles of as yet unidentified loci for susceptibility to AD among these different populations. While it is also possible that environmental interactions or biological risk factors which vary by population or genetic ancestry may play a significant role in phenotypic expression of the variants, the inclusion of several vascular risk factors which were demonstrated to be associated with risk for AD in our group (including history of diabetes and current smoking) did not significantly influence which SNPs were found to be significant, or the size of the effect estimate. The notable exception is that rs1256059, which was of borderline significance in models that did not include vascular covariates, became significantly associated with risk for AD once these variables were included. Therefore, in the case of this SNP, environmental risk factors may in fact influence its effect on risk for AD.

There are a limited number of previous studies that have investigated the role of *ESR2* SNPs and AD in women. Our results are consistent with those reported in prior investigations. A previous evaluation of *ESR2* polymorphisms in women with Down syndrome revealed four SNPs (rs4986938, rs17766755, rs4365213, and rs12435857) to be associated with increased risk for AD [29]. The Health ABC trial performed the most comprehensive evaluation of *ESR2* polymorphisms in community-dwelling elderly individuals prior to this study, examining four *ESR2* SNPs in a cohort of individuals self-identified as Blacks and Whites [17]. Among women, two *ESR2* SNPs (rs1256065, rs1256030) were associated with an increased likelihood of developing cognitive impairment, defined as decline over five or more points on the Modified Mini-Mental Status Examination over four

years. Among men, two SNPs (rs1255998, rs1256030) were also associated with likelihood of developing cognitive impairment. Neither allelic nor genotypic association was stratified by race. Further adjustment for race in the regression models attenuated some results and strengthened others.

Additional studies have been performed in Caucasian populations. Luckhaus et al. [15] conducted an association study of haplotypes arising from three exonic single nucleotide polymorphisms in the 3' untranslated region of *ESR2* (rs4986938, rs1255998, and rs1255953). The study was conducted in an elderly population with all subjects of German or Austrian ancestry, presumably Caucasian. Allelic association of the three individual markers demonstrated no significant associations; however, two three-site haplotypes resulting from these individual polymorphisms were significantly associated with increased incidence of AD. We cannot compare our results with theirs directly because our SNP coverage is not focused on the 3' untranslated region of the gene. Pirskanen et al. [16] investigated the association of five intronic SNPs in *ESR2* in a Finnish population and found that variation in rs1271573 and rs1256043 (SNP 14 in our model) were associated with a significant increase in the risk of AD in women. Additionally, the haplotype containing these two *ESR2* gene risk alleles also increased risk of AD.

The SNPs that we evaluated are located in close proximity to the previously evaluated SNPs (all of the SNPs included in our study are located within 10 kb from those previously evaluated), and are in high LD with those included in these studies. While rs1256065 was found to be associated with increased risk for cognitive impairment among women of Black and White ethnicities in the Health ABC study [17] and rs1256043 was found to be associated with increased risk for AD in a population of Finnish women (presumably Caucasian) [16], these SNPs were not found to be significant in any of our populations, either by self-reported ethnicity or by AIMs-defined ancestry.

Compared with previous studies, our study has several strengths. We assessed the association between polymorphisms and risk of AD in a multiethnic cohort in which ancestry was evaluated through the use of AIMs in addition to self-report. The large number of SNPs that we examined allowed us to evaluate the *ESR2* gene in more detail than was previously possible. Future studies could be improved by analysis of additional polymorphisms, particularly in populations of predominantly African genetic ancestry. These genetically older populations with reduced LD will necessitate denser genotyping to detect SNPs which are associated with AD susceptibility loci.

Although the specific mechanisms by which *ESR2* may affect risk for AD are unclear, there is evidence in the literature to suggest that *ESR2* is linked to AD pathophysiology. In cell lines, ERβ has been demonstrated to be required in estrogen-mediated neuroprotection against AD-related amyloid-β [30] and glutamate toxicity [31, 32]. Additionally, estrogen has been demonstrated to exert anti-inflammatory effects on microglia in the central nervous system through interactions specifically with ERβ [33]. In mouse models, ERβ has been reported to be necessary for neuronal survival [34] and has been linked with hippocampal synaptic plasticity including increased dendritic branching [13]. ERβ are present in the human hippocampus and entorhinal and temporal cortex [35], and immunohistochemistry studies of human brain bank samples has demonstrated increased ERβ immunoreactivity in cellular and extracellular localization in the hippocampi of AD cases compared with normal controls, indicating a role for *ESR2*-mediated effects in AD pathology [36]. *ESR2* polymorphisms also have been associated with susceptibility to diverse hormone-dependent diseases including endometriosis [37, 38], osteoporosis and bone density [39–43], and cancers of the breast [44] and ovaries [45]. As a result, polymorphisms in *ESR2* could account for the increased vulnerability to develop AD through their effects on estrogen-mediated processes. Additionally, ESR2 may also affect risk for AD through its modulation of risk factors such as hypertension [41, 46, 47], the metabolic syndrome [48], obesity [49], or cholesterol levels [50, 51].

Our results support and supplement findings from prior studies that suggest that variants in *ESR2* modify the risk for AD. Additionally, they further extend previous work to suggest that the effects of *ESR2* genetic variants on risk for AD differ by predominant genetic population ancestry or self-identified ethnicity. Most SNPs examined were intronic and therefore may not be the critical location of the pathological variants, but may serve as markers for the critical region. Alternately, they may otherwise influence the expression of critical genetic markers which influence gene transcription or expression or alter ERβ activity. Analysis of additional polymorphisms influencing estrogen biosynthetic pathways and estrogen receptor activity, with additional and denser SNP coverage and correlative studies on mechanism of action will be useful to determine the contribution of estrogen gene variants to cognitive aging and risk for AD. Additional insight

may also be gained through future studies conducting similar analyses in men.

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SUPPLEMENTARY MATERIAL

Supplementary tables and figures are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-130551.

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