Original Research Article

Aromatase Variants Modify Risk for Alzheimer's Disease in a Multiethnic Female Cohort

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Key Words

Alzheimer's disease · Alzheimer's disease and Hispanics · Estrogen · Genetic polymorphisms in degenerative dementias · Genetic risk factors · *CYP19* gene

Abstract

Background/Aims: Few studies of gene variants that affect estrogen activity investigate their association with risk for Alzheimer's disease (AD) in women of different ethnicities. We investigated the influence of *CYP19* polymorphisms on risk for AD in a multiethnic cohort of women, with individual ethnicity assessed by genetic population ancestry informative markers (AIMs) as well as by self-identified ethnicity. **Methods:** Among 1,686 women participating in the Washington Heights Inwood Columbia Aging Project, association with risk for AD was assessed for 41 single nucleotide polymorphisms (SNPs) on the *CYP19* gene using multivariable logistic regression, adjusting for age, presence of an *APOE* ϵ 4 allele, years of education, and body mass index. **Results:** Risk for AD was associated with 6 SNPs in women of predominantly Caucasian AIMs-defined ancestry. Of these, 2 were also associated with decreased risk of AD in women of admixed/Hispanic AIMs ancestry. Two separate SNPs were found to be protective in women of predominantly African AIMs-based ancestry. **Conclusions:** *CYP19* polymorphisms affect risk for AD in women, and risk alleles vary by AIMs-defined ancestry. These effects are possibly due to linkage disequilibrium patterns or differences in the prevalence of comorbid risk factors mediating the SNP effect on risk for AD by group.

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Introduction

Estrogens are important in maintaining brain function in regions typically affected by Alzheimer's disease (AD) and variations in estrogen exposure over the lifetime may affect cognitive decline associated with AD [1, 2]. However, evaluating the role of hormones and enzymes in aging and cognition is difficult since many hormone levels decline with age. It is likely that polymorphisms in genes encoding the estrogen synthesis pathway contribute to variations in lifetime hormone exposure, including age-related changes in hormone levels. Estrogens including estradiol and estrone are formed locally in the brain from the conversion of androgens by aromatase [3], a cytochrome p450 enzyme encoded by the *CYP19* gene located on chromosome 15q21.2. Several studies [4–6], but not all [7, 8], have found an association between multiple single nucleotide polymorphisms (SNPs) in *CYP19* and AD. However, most studies have been conducted in Caucasian ethnic groups, and few polymorphisms have been assessed in a multiethnic cohort in which the members have all been evaluated in a consistent manner. Examination of SNPs 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 (LD) patterns between ethnicities, or differences in the distribution of comorbid conditions and risk factors for AD by ethnic group. In this study, we examined the relationship between *CYP19* SNPs and the risk of AD in a multiethnic cohort of elderly women from northern Manhattan, with individual ancestry assessed by population ancestry markers as well as by self-identified ethnicity [9]. The aims of this study were to confirm previous findings of *CYP19* polymorphisms which were found to be significantly associated with risk for AD, to identify additional SNPs which confer risk for AD using a denser set of SNPs than in previous studies, and to examine whether *CYP19* variants would affect risk for AD differently in groups of women with different population ancestry. We hypothesized that genetic variants would demonstrate different patterns of association between groups with different population ancestries due to distinctive allele frequencies or LD patterns between ethnic 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 aged 65 years and older, residing in northern Manhattan. The population from which the 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). The sampling strategies and recruitment outcomes of these three cohorts have been described in detail elsewhere [9].

Each subject underwent an in-person interview of health and functional ability followed by a standardized medical assessment and neuropsychological battery [10]. Assessments were conducted at 18- to 24-month intervals over a mean of 6.1 years of follow-up. AD diagnosis was based on NINCDS-ADRDA criteria. 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, SNP Selection and Genotyping

Genomic DNA was extracted from total peripheral blood leukocytes using standard methods. We used a multistep selection process to identify candidate SNPs for genotyping. We first selected SNPs within *CYP19*

Fig. 1. Plot of WHICAP participants by AIMS-defined ancestry vs. 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).

that were previously reported to be associated with an increased incidence or earlier age at onset of AD in any population. We then referenced the International HapMap Project (www.hapmap.org) 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 approximately 3.0 kb between SNPs, which provided good coverage of the gene as viewed on LD maps (online suppl. fig. 1–3; for all online suppl. material, see www.karger.com/doi/10.1159/000343074).

Forty-one *CYP19* 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 (www.illumina.com). Duplicate genotyping was performed on 10% of samples to verify accuracy, and the concordance rate was greater than 98%.

Assessment of Genetic Population Ancestry

To evaluate population stratification, we used a set of 100 unlinked AIMs to classify population ancestry. We selected the 100 unlinked SNPs from a panel of 650Y Illumina SNPs using a subset of subjects that had previously also had GWAS data collected. The AIMs were selected because they have allele frequencies that are significantly different among the three ethnoracial 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 [11, 12]. To anchor ancestry, we included data from Caucasians (CEPH), Yorubans (YRI) and Mexican/Central Americans from the HapMap project (fig. 1). Our self-identified White population closely aligned with the Caucasian (CEPH) samples in the HapMap dataset and our self-identified Black population clustered around the Yoruban (YRI) samples. As expected, Caribbean Hispanics clearly showed admixture of Caucasian (CEPH) and Yoruban (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 ≥ 0.6 AIMS markers consistent with CEPH profile, $n = 632$) versus those who were of predominant African ancestry (defined as ≥ 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 AIMS-defined ancestry (if their AIMS index scores were ≥ 0.6 CEPH or YRI, respectively), or admixed/Hispanic if they did not have one predominant genetic ancestry ($n = 473$). Comparison of populations as defined by AIMS-defined ancestry versus self-identified ethnicity is illustrated in online supplementary table 2.

Table 1. Population Characteristics

	Nondemented	Alzheimer's disease
Sample size	1,175	511
Age at time of enrollment*	75.7 ± 6.0	79.9 ± 7.2
Self-identified ethnicity*		
White	358 (84.6)	65 (15.3)
Hispanic	415 (61.8)	257 (38.2)
Black	389 (67.8)	185 (32.2)
Other	13 (76.5)	4 (23.5)
BMI	28.2 ± 7.9	27.1 ± 5.8
Years of education*	10.4 ± 4.6	7.1 ± 4.7
Diabetes	180 (15.3)	104 (20.3)
Smoking	97 (8.3)	44 (8.7)
At least one copy APOE ε4*	284 (24.2)	156 (30.5)

Values are presented as number (with percentage in parentheses) or means ± SD.

* $p < 0.05$.

Potential Confounders

Potential confounders included age at time of study enrollment, presence of an *APOE* ε4 allele, years of education, and body mass index (BMI). Supplementary analyses (online suppl. fig. 4–6) were also performed to evaluate the potential effects of vascular risk factor covariates on significant SNPs, and included history of diabetes mellitus and current smoking. Participants were classified according to the presence or absence of at least one *APOE* ε4 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 [13]. SNPs were then evaluated in genotypic association analyses to further characterize their relationship to AD, stratifying first by AIMS-defined ancestry and then by self-reported ethnicity. We hypothesized that differences in associations between these two sets of analyses might reflect culturally associated environmental risk factors for AD. Conversely, similarities in significant SNPs between the two analyses would demonstrate a more direct genetic effect of *CYP19* polymorphisms on risk for AD. We used multivariable logistic regression to estimate the likelihood of AD by SNP genotype, adjusting for age, presence of at least one *APOE* ε4 allele, and BMI. To provide the most robust model for observing an effect of the minor allele, 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 minor allele.

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 (range 65–95 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 as White (25.1%). Among all the participants, 511 were classified as possible or probable AD (29.5%) and 1,175 as

Table 2. Covariate characteristics by self-identified ethnicity and AIMS-defined ancestry

	Self-identified ethnicity			AIMS-defined ancestry		
	White	Hispanic	Black	predominantly Caucasian	admixed/Hispanic	predominantly African
Number of individuals	423	672	574	632	473	581
At least one copy of <i>APOE</i> $\epsilon 4^*$	93 (22.0)	167 (24.9)	190 (33.1)	148 (23.4)	117 (24.7)	189 (32.5)
Diabetes mellitus*	31 (7.3)	136 (20.2)	105 (18.3)	64 (10.1)	104 (22.0)	109 (18.8)
Smoking*	22 (5.2)	39 (5.8)	71 (12.4)	27 (4.3)	41 (8.7)	65 (11.2)

Results are expressed as number (with percentage in parentheses) of individuals with ethnicity/AIMS-defined ancestry.

* $p \leq 0.001$.

nondemented. The frequency of AD was greater in self-identified Blacks and Hispanics than in Whites (Blacks: 32.2%, Hispanics: 38.2%, Whites: 15.3%). Mean years of education differed significantly between those with and without AD (7.1 vs. 10.4 years). BMI, history of diabetes mellitus, and current smoking status did not differ significantly between individuals with or without AD. However, there were statistically significant differences between self-identified groups as well as between AIMS-defined populations in the prevalence of vascular risk factors, including diabetes mellitus and current smoking (table 2). The proportion of women with at least one copy of the *APOE* $\epsilon 4$ allele was highest in Blacks, followed by Hispanics and then Whites (table 2).

Genotypic Associations

For ease of discussion, we will use the numbered order of SNPs to refer to each SNP (table 3). Among women of predominantly Caucasian AIMS-defined ancestry, 2 SNPs (SNPs 10 and 15 – rs4775935 and rs727479, respectively), located at the 5' end of the *CYP19* gene, were found to be associated with decreased risk for AD, adjusting for age, BMI, and presence of an *APOE* $\epsilon 4$ allele. Four SNPs (27, 28, 37 and 39 – rs17647719, rs1902586, rs10163138, and rs7168331), clustered at the 3' end of the gene, were found to be associated with increased risk for AD. Odds ratios (ORs) varied between 0.6 and 0.7 among protective SNPs (table 3), and ranged from 1.7 to 2.6 among SNPs associated with increased risk for AD. Among women of admixed/Hispanic AIMS-defined ancestry, SNPs 10 and 15 were also found to be protective (OR 0.6 and 0.7, respectively). Two different SNPs (SNPs 38 and 41 – rs6493495 and rs11070843) were associated with decreased risk for AD in women of predominantly African AIMS-defined ancestry, both with ORs of 0.7 (table 3). To minimize the risk of false-positive findings from multiple testing, we computed empirical p values by generating the null distribution on the basis of 1,000 replicate data sets. As shown in table 3, calculation of empirical p values slightly attenuated the degree of significance for some genotypes (including SNP 15 among women of admixed/Hispanic AIMS-defined ancestry and SNP 38 among women of predominantly African AIMS-defined ancestry); however, most remained significant.

We then repeated the analyses within strata defined by self-identified ethnicity to take into account the potential role of cultural/environmental risk factors within ethnorracial groups (online suppl. table 1). Among self-identified Whites, SNPs 10 and 15 remained significant while ORs for the remaining 4 SNPs that were significant among women of predominantly Caucasian AIMS-defined ancestry became attenuated and were no longer significant. However, 4 additional SNPs flanking the region – specifically, SNPs 5, 16, 18 and 19 – were

Table 3. ORs for AD by *CYP19* SNPs, stratified by AIMS-defined ancestry

SNP No.	SNP	Position	Dis- tance bp	Minor Allele	Predominantly Caucasian				Admixed/Hispanic				Predominantly African						
					MAF	OR	95% CI	p value	emp. p value	MAF	OR	95% CI	p value	emp. p value	MAF	OR	95% CI	p value	emp. p value
1	rs4646	49290136		A	0.3	1.2	0.9–1.8	0.526	0.525	0.3	0.6	0.5–1.0	0.073	0.080	0.3	1.1	0.8–1.8	0.301	0.301
2	rs10046	49290278	142	G	0.4	1.0	0.7–1.6	0.692	0.696	0.6	0.5	0.4–1.1	0.108	0.115	0.8	1.2	0.4–2.4	0.905	0.855
3	rs700519	49295260	4,982	A	0.1	0.9	0.5–1.9	0.770	0.776	0.1	1.1	0.6–1.7	0.904	0.903	0.2	0.9	0.6–1.3	0.628	0.646
4	rs1143704	49297994	2,734	T	0.4	0.8	0.7–1.3	0.599	0.593	0.4	1.0	0.6–1.5	0.855	0.087	0.2	0.7	0.5–1.1	0.152	0.913
5	rs6493488	49301214	3,220	C	0.4	0.9	0.5–1.1	0.177	0.177	0.6	0.9	0.5–1.4	0.479	0.478	0.7	0.8	0.4–1.8	0.440	0.676
6	rs16964201	49302643	1,429	A	0.1	0.9	0.5–1.8	0.799	0.807	0.2	1.2	0.7–1.7	0.785	0.790	0.3	1.1	0.7–1.4	0.849	0.826
7	rs2899472	49303347	704	A	0.3	1.1	0.7–1.4	0.794	0.780	0.2	1.0	0.6–1.4	0.623	0.633	0.1	0.6	0.4–1.3	0.269	0.243
8	rs8023263	49304889	1,542	C	0.5	0.9	0.6–1.5	0.776	0.824	0.4	0.8	0.4–1.2	0.225	0.229	0.3	1.0	0.5–2.2	0.940	0.945
9	rs4775934	49306061	1,172	G	0.1	1.0	0.6–1.9	0.894	0.897	0.2	0.9	0.6–1.4	0.776	0.802	0.4	0.8	0.6–1.3	0.551	0.534
10	rs4775935	49306568	507	A	0.3	0.7	0.4–0.9	0.036	0.041	0.3	0.5	0.4–0.9	0.032	0.034	0.3	1.2	0.7–1.6	0.681	0.695
11	rs1065778	49307498	930	G	0.5	1.3	0.7–1.7	0.627	0.624	0.3	1.1	0.7–1.5	0.880	0.873	0.2	0.7	0.5–1.1	0.147	0.144
12	rs12594287	49311199	3,701	A	0.1	1.4	0.9–2.5	0.085	0.082	0.2	0.8	0.6–1.4	0.644	0.626	0.2	0.9	0.6–1.3	0.643	0.645
13	rs700518	49316404	5,205	G	0.5	1.2	0.7–1.8	0.572	0.589	0.3	1.0	0.7–1.5	0.880	0.869	0.2	0.7	0.5–1.1	0.200	0.198
14	rs2414096	49317071	667	A	0.5	1.1	0.7–1.7	0.737	0.742	0.3	1.2	0.7–1.6	0.713	0.720	0.2	0.9	0.5–1.1	0.149	0.151
15	rs727479	49321589	4,518	C	0.3	0.7	0.4–0.9	0.019	0.014	0.3	0.7	0.4–0.9	0.047	0.066	0.2	1.3	0.8–1.8	0.284	0.298
16	rs10459592	49323433	1,844	C	0.4	0.7	0.5–1.0	0.080	0.081	0.5	0.8	0.5–1.5	0.712	0.724	0.6	1.0	0.6–1.9	0.915	0.903
17	rs767199	49327679	4,246	A	0.5	1.1	0.7–1.6	0.795	0.800	0.3	1.2	0.7–1.7	0.627	0.615	0.1	0.9	0.5–1.3	0.373	0.384
18	rs12911554	49330049	2,370	G	0.4	0.9	0.5–1.2	0.202	0.203	0.5	0.8	0.4–1.1	0.093	0.109	0.5	1.0	0.7–1.6	0.919	0.902
19	rs7172156	49333590	3,541	A	0.4	0.8	0.5–1.1	0.165	0.159	0.4	1.1	0.7–1.5	0.913	0.907	0.3	0.8	0.6–1.3	0.556	0.559
20	rs2008691	49335352	1,762	G	0.2	1.3	0.8–1.8	0.402	0.414	0.3	0.9	0.5–1.2	0.212	0.206	0.4	1.2	0.7–1.4	0.841	0.837
21	rs11856927	49335997	645	C	0.4	1.4	0.8–1.9	0.280	0.297	0.4	1.0	0.6–1.5	0.840	0.839	0.5	0.6	0.4–1.0	0.053	0.059
22	rs1008805	49336891	894	G	0.4	0.8	0.5–1.2	0.236	0.203	0.3	1.2	0.7–1.7	0.626	0.637	0.2	1.0	0.7–1.5	0.829	0.801
23	rs6493494	49337127	236	A	0.4	1.4	0.9–2.1	0.164	0.174	0.3	1.2	0.8–1.9	0.246	0.263	0.3	1.0	0.7–1.4	0.771	0.767
24	rs10519299	49338638	1,511	C	0.5	1.6	0.9–2.3	0.092	0.092	0.4	1.3	0.8–1.9	0.333	0.352	0.3	0.9	0.6–1.3	0.693	0.693
25	rs12050767	49344549	5,911	G	0.5	1.5	0.9–2.3	0.096	0.109	0.4	1.1	0.7–1.9	0.497	0.505	0.4	0.8	0.6–1.3	0.472	0.472
26	rs749292	49346023	1,474	A	0.5	1.4	0.9–2.3	0.093	0.089	0.4	1.0	0.7–1.7	0.651	0.680	0.5	0.9	0.6–1.4	0.592	0.620
27	rs17647719	49355496	9,473	G	0.1	1.8	1.1–2.8	0.037	0.038	0.2	1.0	0.7–1.5	0.904	0.896	0.3	1.0	0.7–1.5	0.854	0.869
28	rs1902586	49358145	2,649	A	0.1	1.7	1.1–2.8	0.033	0.026	0.3	1.3	0.8–1.8	0.337	0.313	0.5	0.8	0.6–1.4	0.659	0.647
29	rs16953058	49359552	1,407	A	0.1	1.6	0.7–4.0	0.230	0.249	0.1	0.8	0.5–1.4	0.544	0.531	0.1	0.9	0.6–1.4	0.723	0.734
30	rs936306	49366890	7,338	A	0.2	1.4	0.9–2.0	0.166	0.168	0.4	0.9	0.6–1.4	0.725	0.723	0.5	0.7	0.5–1.2	0.295	0.310
31	rs12594203	49370853	3,963	T	0.1	1.5	0.9–2.4	0.158	0.175	0.2	1.2	0.8–1.9	0.263	0.242	0.4	0.8	0.6–1.2	0.355	0.356
32	rs2470176	49371231	378	G	0.2	1.4	0.9–2.0	0.159	0.157	0.6	1.1	0.7–1.5	0.936	0.934	0.6	0.7	0.5–1.3	0.305	0.322
33	rs2470152	49382264	11,033	A	0.5	0.7	0.5–1.2	0.253	0.230	0.5	0.6	0.4–1.1	0.127	0.128	0.5	0.9	0.6–1.5	0.800	0.832
34	rs3751592	49393870	11,606	G	0.3	1.3	0.9–2.0	0.098	0.095	0.4	1.2	0.9–1.9	0.232	0.229	0.3	1.4	0.9–1.8	0.207	0.210
35	rs1004984	49400821	6,951	A	0.4	1.4	0.9–2.1	0.113	0.099	0.5	1.0	0.7–1.6	0.873	0.880	0.5	0.8	0.5–1.1	0.192	0.193
36	rs1902585	49401198	377	G	0.5	0.9	0.5–1.1	0.184	0.181	0.3	1.1	0.7–1.6	0.842	0.850	0.1	1.1	0.8–1.8	0.419	0.424
37	rs10163138	49401416	218	A	0.1	2.6	1.1–6.3	0.044	0.026	0.1	0.8	0.5–1.4	0.568	0.554	0.3	1.1	0.8–1.6	0.587	0.606
38	rs6493495	49405594	4,178	G	0.1	1.3	0.8–1.9	0.317	0.305	0.2	1.1	0.7–1.6	0.781	0.796	0.3	0.7	0.4–0.9	0.042	0.053
39	rs7168331	49408275	2,681	G	0.4	1.5	1.1–2.5	0.028	0.025	0.5	1.0	0.6–1.6	0.913	0.912	0.6	0.8	0.6–1.4	0.646	0.615
40	rs1870049	49412515	4,240	G	0.2	1.2	1.0–2.2	0.083	0.072	0.4	1.2	0.7–1.6	0.736	0.706	0.4	0.9	0.6–1.3	0.510	0.545
41	rs11070843	49413843	1,328	G	0.1	1.3	0.8–2.0	0.251	0.233	0.2	1.1	0.7–1.7	0.632	0.629	0.2	0.6	0.4–0.9	0.027	0.038

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.

Analyses are adjusted for age, BMI, years of education, and presence of at least one copy of an APOE ε4 allele.

MAF = Minor allele frequency; emp. p value = to minimize the risk of false-positive findings from multiple testing, we computed empirical p values by generating the null distribution on the basis of 1,000 replicate data sets.

found to be significant. Among self-identified Hispanics, 2 significant SNPs (10 and 16) were found in close proximity to those in the AIMS-defined ancestry groups for Hispanics and Whites. Among self-identified Blacks, 2 protective SNPs (SNPs 38 and 31) were no longer found to be significantly associated with risk for AD.

Haplotype Analysis

Genotypic analyses demonstrated that SNPs associated with risk for AD clustered in several distinct regions of high LD (online suppl. fig. 1–3). Strong pairwise LD between SNP loci in these blocks supported the possibility of multilocus association at adjacent variants. We performed ‘sliding window’ haplotype analysis within these regions as implemented in the Haploview program using the D' value [13], with each haplotype including 2–4 consecutive SNPs. While numerous haplotypes constructed from these *CYP19* SNPs were found to be significantly associated with increased or decreased risk for dementia, the most robust associations in women of predominantly Caucasian AIMS-based ancestry were haplotype AA at SNPs 10–11 (OR 0.4, $p = 0.001$) and haplotype AAC at SNPs 37–38–39 (OR 3.17, $p = 0.04$; data not shown). Among women of admixed/Hispanic AIMS-defined ancestry, the most significant haplotype was GCAG at SNPs 14–15–16–17, which was protective (OR 0.6, $p = 0.008$). Among women of predominantly African AIMS-defined ancestry, only one protective haplotype, GG at SNPs 40–41 was found to be significant (OR 0.72, $p = 0.04$; data not shown).

Discussion

Among 1,686 community-dwelling elderly women in a multiethnic cohort, risk for developing AD was associated with 6 SNPs in women of predominantly Caucasian AIMS-defined ancestry (rs4775935, rs727479, rs17647719, rs1902586, rs10163138 and rs7168331). Of these, 2 SNPs (rs4775935 and rs727479) were associated with decreased risk of AD in women of admixed/Hispanic AIMS ancestry. Additionally, 2 different SNPs (rs6493495 and rs11070843) were found to be protective in women of predominantly African AIMS-based ancestry. Use of empirical p values slightly attenuated the degree of significance for rs727479 among women of admixed/Hispanic AIMS-defined ancestry and rs6493495 among women of predominantly African AIMS-defined ancestry; however the directionality and magnitude of effect remained the same.

Numerous papers have established that estrogen may have beneficial effects on multiple pathways that affect risk for AD. Estrogens promote the growth and survival of cholinergic neurons [14, 15], increase cholinergic activity [16], have antioxidant properties [17], and promote the nonamyloidogenic metabolism of the amyloid precursor protein [18]. Estrogens also play an important role in the regulation of the vascular endothelium where they activate rapid vasodilatation, exert anti-inflammatory effects, stimulate endothelial growth and migration, and protect the vessels from atherosclerotic degeneration by elevating nitric oxide and prostaglandin levels [19, 20]. Aromatase is a potentially important factor in these processes as it controls estrogen biosynthesis and is expressed in regions of the brain affected by AD [21–24]. Previous studies have demonstrated that common *CYP19* polymorphisms are associated with estradiol and androgen serum levels in premenopausal and postmenopausal women [25–28]. As a result, *CYP19* gene variants could potentially affect risk for AD by reducing or increasing the rate of conversion of androgens into estrogens, resulting in altered protection against neuronal injury or degeneration through multiple mechanisms.

Differential association of polymorphisms in a susceptibility gene for AD in groups of disparate population ancestries may occur for several reasons. First, differences in LD patterns between ethnic groups may contribute to discrepancies in genotype associations

(online suppl. fig. 1–3). In our cohort, SNPs which were protective against AD in women of predominantly Caucasian AIMS-defined ancestry were located at the 5' end of the gene (online suppl. fig. 1, block 2), and those which increased risk for AD in this population clustered in two LD blocks at the 3' end of the gene (online suppl. fig. 1, blocks 5–6). Notably, these LD blocks which remained cohesive in women of predominantly Caucasian ancestry were represented by smaller sets of SNPs in women of admixed/Hispanic AIMS-defined ancestry (online suppl. fig. 2). LD blocks in this region were even more fragmented among women of predominantly African AIMS-defined ancestry (online suppl. fig. 3). This suggests that different LD patterns between *CYP19* alleles and alleles of as yet unidentified loci for susceptibility to AD between populations of different genetic ancestries may contribute to the observed variability in genotypic association.

Second, it is also possible that differences in environmental or biological risk factors among women of different genetic ancestry may play a significant role in phenotypic expression of the variants. For example, the inclusion of vascular risk factors (including history of diabetes and current smoking) attenuated the significance of SNPs in women of admixed/Hispanic (online suppl. fig. 5) or predominantly African AIMS-based ancestry (online suppl. fig. 6). In contrast, the inclusion of these vascular risk factor covariates increased the significance of several SNPs in women of predominantly Caucasian AIMS-defined ancestry, notably in SNPs located at the 3' end of the gene (online suppl. fig. 4). The potential mediation of SNP effect by vascular risk factors is reinforced by the differential results seen in the logistic regression analyses stratified by self-identified ethnicity versus AIMS-defined ancestry. As seen in table 2, the change in stratification had the greatest effect on sample group characteristics and size when comparing self-identified Whites versus individuals of predominantly Caucasian AIMS-based ancestry, and self-identified Blacks versus women of predominantly African AIMS-based ancestry. Specifically, the shift in classification of race revealed that many individuals who had genetic markers that were predominantly Caucasian or African actually identified themselves as Hispanic. These individuals also had significantly higher rates of diabetes mellitus (online suppl. fig. 7) than individuals of predominantly African AIMS who identified themselves as Black or participants of predominantly Caucasian AIMS who identified themselves as White. Notably, when these individuals were included in the AIMS-based logistic regression models for participants of predominantly Caucasian or African ancestry, several SNPs at the 3' end of the gene became significant (table 3), again highlighting the potential mediation of SNP effect by vascular risk factors in this part of *CYP19*. These discrepancies indicate that environmental and biological covariate risk factors may exert different modulating effects with alleles in groups of different predominant genetic ancestries, or even within different parts of the gene.

Several previous studies have investigated the role of *CYP19* polymorphisms and AD. A study of a Finnish population found 3 SNPs (rs767199, rs727479 and 1065778) to be associated with increased risk for AD, as well as haplotype A1 (CACTTTGTT) [5]. Other SNPs including rs2899472, rs1008805, rs727479, rs1143704, rs1065778, rs10046 and rs4646 have also been found to be associated with risk for AD or age at onset (some in *APOE* ϵ 4 carriers only) [4, 6] in primarily Caucasian populations. In other studies, an interaction between rs1062033 (located in the 5'-UTR region) and the genes for butyrylcholinesterase [29] and interleukin-10 [30] in increasing risk for AD was proposed. In addition, rs2899472 was found to be associated with CSF A β _{1–42} levels in normal subjects in a GWAS investigation [31].

We examined the majority of the SNPs cited in the above studies. While rs727479 was protective among self-defined Whites in our group, we did not find any other SNPs to be significant. Although many of these previously evaluated SNPs are clustered at the 5' end of the gene, in LD blocks 1 and 2 by our map (online suppl. fig. 1), many of the SNPs which we found to be significant in our participants of predominantly Caucasian AIMS-defined ancestry

clustered at the 3' end, in LD blocks 5–7. However, as previously noted, most previous studies were conducted in self-identified White participants who may have had different associated risk factors from our Caucasian population, which also included individuals who identified themselves as Hispanic.

Overall, our findings confirm the findings of previous studies of a strong association between *CYP19* polymorphisms and risk for Alzheimer's disease among women. We also extend these studies through denser genotyping, rather than relying on imputation which can introduce false positives in multiethnic cohorts. From this effort, we identified additional SNPs that are associated with AD risk, and characterized how these SNPs vary among individuals of different AIMS-defined ancestries in the presence of cardiovascular risk factors. Moreover, our study illustrates the importance of controlling for population stratification as well as for environmental risk factors in association studies, as SNPs which we found to be associated with AD varied significantly between ethnic groups.

We note that 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 or may otherwise influence the expression of critical genetic markers. Further studies may characterize other genetic mechanisms that may contribute to AD, including methylation and copy number variations. For example, SNP 27 (rs17647719) is located in a region associated with methylation, and SNP 28 (rs1902586) is located 2.6 kb from this region. Future studies with denser genotyping to achieve high resolution in all ethnic groups, along with gene expression studies, may further provide biological insights. Additional insight may also be gained through future studies conducting similar analyses in men.

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