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## ER $\alpha$ Variants Affect Age at Onset of Alzheimer's Disease in a Multiethnic Female Cohort

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### Abstract

**Background/Aims**—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 *ESRI* polymorphisms on age at onset of AD in a multiethnic cohort of women.

**Methods**—Among 1,436 women participating in the Washington Heights Inwood Columbia Aging Project (WHICAP), association with age at AD onset was assessed for 41 single-nucleotide polymorphisms (SNPs) on the *ESRI* gene using Cox proportional hazard models, adjusting for presence of an *APOE*  $\epsilon$ 4 allele, years of education, and body mass index (BMI).

**Results**—Six SNPs in self-identified White women were protectively associated with delayed age of AD onset in this self-identified group, including the two restriction fragment length polymorphisms (RFLPs) PvuII (rs2234693) and XbaI (rs9340799) (HR range 0.420 – 0.483). Two separate SNPs were found to affect age of AD onset in self-identified Black women.

**Conclusions**—*ESRI* polymorphisms affect age of onset for AD in women, and risk alleles vary by ethnicity. These effects are possibly due to different linkage disequilibrium patterns or

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differences in comorbid environmental or cultural risk factors mediating SNP effect on risk for AD.

### Keywords

AD; age of onset; Alzheimer disease and Hispanics; estrogen receptors; gene polymorphism; genetic association studies in subjects with dementia; female; estrogen receptor alpha; late-onset AD

## 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]. Numerous papers have established that estrogen may have beneficial effects on multiple pathways that affect risk for AD. Estrogens promotes the growth and survival of cholinergic neurons[3,4] increase cholinergic activity[5], have antioxidant properties[6], and promote the nonamyloidogenic metabolism of the amyloid precursor protein[7]. Estrogens also play an important role in 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[8,9]. 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 pathway contribute to variations in lifetime hormone exposure, including age-related changes in hormone levels. Estrogen exerts its action through at least two receptors, estrogen receptor  $\alpha$  (ER $\alpha$ ), encoded by *ESR1* on chromosome 6q25.1, and estrogen receptor  $\beta$  (ER $\beta$ )[2]. The association of polymorphisms in *ESR1* with risk of AD has been investigated in a number of studies, but findings have been inconsistent [10-23]. However, most studies have been conducted in relatively homogeneous ethnic groups, and few polymorphisms have been assessed in a multiethnic cohort. Examination of SNPs in multiracial groups which are evaluated without taking ethnicity 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 for AD by ethnic group. In this study, we examined the relationship between *ESR1* SNPs and the risk of AD in a multiethnic by self-identified ethnicity as well as by genetic population ancestry markers [24]. The aims of this study were to confirm previous findings of *ESR1* 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 *ESR1* variants would affect risk for AD differently in groups of women with different self-identified ethnicity. We hypothesized that genetic variants would demonstrate different patterns of association between groups of different ethnicities due to different allele frequencies or linkage disequilibrium patterns between ethnic groups, as well as varying environmental risk factors.

## Materials and Methods

### Subjects

The initial cohort 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. Each subject underwent an in-person interview of health and functional ability followed by a standardized medical assessment and neuropsychological battery [25]. Assessments were conducted at 18 - 24 month intervals over a mean of 6.1 years of follow-up. 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, n=400; African-American, n=485; and non-Hispanic White of European ancestry, n=551). The sampling strategies and recruitment of these two cohorts have been described in detail elsewhere [24].

AD diagnosis was based on NINCDS - ADLDA criteria. Participants were classified as non-demented if they remained without cognitive or functional decline through their last study assessment (n=1107). Participants were classified as having incident AD if they were non-demented at baseline visit and then were classified as having probable or possible AD by NINCDS - ADLDA criteria at any later study visit (n=329). Participants with incident AD had no other medical or psychiatric conditions that might mimic AD including other neurologic conditions such as Parkinson's disease or stroke. Age at initial diagnosis of AD was used to estimate age at onset of dementia.

### 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 *ESR1* 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](http://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 6.2 kilobase pairs between SNPs, which provided good coverage of the gene as viewed on linkage disequilibrium maps (Supplementary Figures 1-3).

Forty-one *ESR1* SNPs as well as 100 ancestry informative markers (AIMs) were genotyped in a total of 1,436 samples using Illumina GoldenGate custom panels and the Illumina iScan platform. Genotyping was performed according to standard protocols ([www.Illumina.com](http://www.Illumina.com)). The complete list of *ESR1* SNPs that were genotyped, along with their minor allele frequencies (MAF) by self-identified ethnicity, is presented in Table 3. Duplicate

genotyping was performed on ten percent of samples to verify accuracy, and the concordance rate was greater than 97 percent.

### Assessment of ethnicity and genetic population ancestry

Participants were first categorized by self-identified ethnicity as given at time of study enrollment (with self-identified categories including Black of non-Hispanic ancestry, White of non-Hispanic ancestry, and Hispanic), as most previous studies on *ESR1* polymorphisms and AD have used this means of classification. Participants were then re-categorized by genetic population ancestry. To control for potential confounding that can be generated by population stratification, we used a set of 100 unlinked ancestry informative markers (AIMs) from a panel of 650Y Illumina SNPs to assess population structure. The AIMs were selected because they 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[26,27]. To anchor ancestry, we included data from Caucasians (CEPH), Yorubans (YRI) and Mexican/Central Americans from the HapMap project (Figure 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  $\geq 0.6$  AIMs markers consistent with CEPH profile,  $n= 551$ ) versus those who were of predominant African ancestry (defined as  $\geq 0.6$  AIMs markers consistent with YRI profile,  $n= 485$ ). In doing so, individuals previously self-identified as Hispanic were reclassified as being of predominantly Caucasian or predominantly African AIMs-defined 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 ( $n= 400$ ).

### Potential Confounders

Potential confounders included years of education, which was found to be independently associated with risk for AD in our group (Table 1); the presence of at least one *APOE*  $\epsilon 4$  allele, which was included as a potential confounder because of its strong association with risk for AD in previous studies and because the allele frequencies of the  $\epsilon 4$  allele vary substantially by race and ethnicity[28]; and body mass index (BMI), included because of the association of adipose tissue with higher estrogen levels [29-31]. Participants were classified according to the presence or absence of at least one *APOE*  $\epsilon 4$  allele. Height and weight were measured at the initial evaluation to compute BMI. Other potential vascular risk factor confounders, including smoking and history of diabetes mellitus, were not included in the analyses because they were not found to be associated with risk for AD in our group (Table 1).

## 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 group of unaffected individuals using the  $\chi^2$  goodness-of-fit test in Haploview [32]. We used Cox proportional hazards modeling to assess the relationship between *ESR1* genotypes and age at onset of AD, adjusting for presence of at least one *APOE*  $\epsilon 4$  allele, years of education and BMI. To minimize the risk of a false-positive finding from rare variants and multiple testings, we computed empirical p-values by generating the null distribution on the basis of 1000 replicates of analyses. The use of empirical p-values corrects for the fact that multiple analyses were done. Cox modeling was done by stratifying first by self -reported ethnicity and then by AIMS - defined ancestry. We hypothesized that differences in associations between these two sets of analyses might reflect culturally - associated environmental risk factors for AD within groups defined by self-report. Conversely, similarities in significant SNPs between the two analyses would suggest a more direct genetic effect of *ESR1* polymorphisms on risk for AD. The time to event variable was age at onset for participants who developed AD and age at last assessment for participants who remained non-demented throughout the follow-up period.

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 ( $\pm$  6.7) years, and ranged from 65 to 95 years. Mean length of follow-up was 6.1 ( $\pm$ 4.3) years. The majority of women were self - identified as Hispanic (n= 573, 39.9 %) and Black (n=483, 33.6%), while 380 women were self - identified White (26.4%). Among all participants, 329 were classified as having incident AD (22.9%) and 1107 as non-demented. The frequency of incident AD was greater in self – identified Blacks and Hispanics than in Whites (Blacks: 22.4%; Hispanics: 31.6%; Whites: 10.5%). BMI, history of diabetes mellitus, and current smoking status did not differ significantly between individuals with or without AD when all ethnicities were analyzed as a whole; however, there was a significant level of variability (Table 2) among all covariates (BMI, presence of an *APOE*  $\epsilon 4$  allele, and years of education) between the three self-identified ethnicities.

### Analysis of *ESR1* SNPs by self-defined ethnicity

Table 4 presents the locations and minor allele frequencies (MAFs) of *ESR1* SNPs that were found to be associated with age at onset of AD by self-defined ethnicity. It also reports the association between these *ESR1* genotypes and their hazard ratios (HR) for AD, stratified by self-reported ethnicity. After adjusting for BMI, years of education, and the presence of an *APOE*  $\epsilon 4$  allele, women who identified themselves as White and who were heterozygous or homozygous (i.e. carried one or two copies) for the A allele at rs6902771, for the C allele at

rs2853250, for the A allele at rs4870056, for the C allele at rs2234693 (also known as the PvuII restriction site), for the G allele at rs9340799 (also known as the XbaI restriction site), and the A allele at rs9322332 had a roughly two-fold decrease in the hazard ratio for AD (Table 4; HR range 0.420 – 0.483) compared with women carrying no copies of these alleles (Table 4; HR for rs6902771 (AA/AG) = 0.437, empirical p-value = 0.015; HR for rs3853250 (CC/AC) = 0.442, empirical p-value = 0.017; HR for rs4870056 (AA/AG) = 0.420, empirical p-value = 0.011; HR for rs2234693 (CC/CT) = 0.443, empirical p-value = 0.014; HR for rs9340799 (GG/AG) = 0.441, empirical p-value = 0.017; HR for rs9322332 (AA/AC) = 0.483, empirical p-value = 0.033). Additionally, women who identified themselves as Black and who were heterozygous or homozygous (i.e. carried one or two copies) of the C allele at rs2077647 and for the A allele at rs2982684 had an increase and decrease in the hazard ratio for AD, respectively, compared with women carrying no copies of these alleles (HR for rs2077647 (CC/CT) = 0.629, 95% CI, 0.414 – 0.955, empirical p-value = 0.030; HR for rs2982684 (AA/AC) = 1.745, 95% CI, 1.152 – 2.644, empirical p-value = 0.009) (Table 4). Survival curves for these SNPs in self-identified Whites and Blacks are shown in Figures 2 and 3. These SNPs were not significantly associated with increased or decreased hazard rates for AD among women identifying themselves as Hispanic (Table 4).

### Haplotype Analysis

Pairwise linkage disequilibrium (LD) was conducted between the six significant *ESR1* SNPs in self-identified Whites, as implemented in the Haploview program using the  $D'$  value[32] (Supplementary Table 1). The possibility of multi-locus association at adjacent variants was supported by the strong pairwise LD between SNP loci in this block, as well as the strong hazard rate findings at adjacent SNPs in this block (Supplementary Figure 1, also Table 4). In contrast, the two SNPs associated with increased or decreased likelihood of AD among African-Americans were not in strong LD (Supplementary Figure 3), indicating that they do not likely act by interaction with one another but perhaps in relation to separate, unrelated markers. We performed a “sliding window” haplotype analysis in the significant region in self-identified Whites, each analysis including two to four consecutive SNPs. While numerous haplotypes constructed from these *ESR1* SNPs were found to be significantly associated with decreased risk for dementia (Supplementary Table 1), the most robust associations in self-identified Whites were haplotype C-A-C-G at SNPs 12 - 15 (rs3853250 through rs9340799, O.R. 0.656, p=0.009), which also contains the significant haplotypes A-C-G at SNPs 13 – 15 (rs4870056 through rs9340799, O.R. 0.656, p=0.009) and C-G at SNPs 14 – 15 (rs2234693 through rs9340799, O.R. 0.658, p=0.009).

### Analysis of *ESR1* SNPs by AIMS-defined Ancestry

We then repeated the analyses within strata defined by AIMS-defined predominant genetic ancestry. Using this categorization, one unique SNP was found to be associated with increased hazard ratio (decreased age of onset of AD) among women of predominantly Caucasian genetic ancestry (rs9322332, HR AA/AG = 1.862, 95% CI= 1.030 – 3.366, empirical p-value = 0.040) (Table 5). Additionally, rs2982684, which had previously been found to be associated with decreased age at onset of among self-identified Blacks, was also found to be associated with decreased age at onset among women of predominantly African

genetic ancestry (rs2982684, HR AA/AC= 1.528, empirical p-value = 0.31) (Table 5). Following recategorization using AIMs-defined genetic ancestry, the six SNPs which previously had been found to be associated with delayed age at onset of AD among self-defined Whites (rs6902771, rs3853250, rs4870056, rs2234693, rs9340799, and rs9322332) were not found to be significant among women of predominantly Caucasian AIMs-defined ancestry (Supplementary Table 2). Likewise, rs2077647, which had been found to be associated with delayed age at onset among self-defined Blacks, was not associated with age at onset among women of predominantly African AIMs-defined ancestry (Supplementary Table 2).

## Discussion

Six SNPs in *ESR1* were associated with an over two-fold decreased risk of AD among self-identified White women. Self-identified Whites with at least one minor allele at rs6902771, rs3853250, rs4870056, rs2234693, rs9340799, or rs9322332, had less than a one-half hazard ratio of AD compared with women without these risk alleles, indicating that these SNPs were associated with a delayed age of AD onset. These six intronic SNPs cover an 8.9 kb region with high linkage disequilibrium (minimum pairwise  $D' > 0.8$ ), with rs2234693 (also known as restriction fragment length polymorphism (RFLP) *PvuII*) and rs9340799 (RFLP *XbaI*), located with 46 bp of one another. Haplotype analysis reveals that two- to four-SNP combinations of risk alleles among these six SNPs are all significantly associated with decreased risk for AD, with the most significant haplotype (C-A-C-G) including rs3853250, rs4870056, rs2234693, and rs9340799. When women were recategorized according to predominant AIMs-defined genetic ancestry, these six SNPs were not associated with AD age at onset among women of predominantly Caucasian AIMs-defined ancestry; however one unique SNP, rs6909023, was associated with an increased hazard ratio (decreased age at onset) of AD (Table 5). The discrepancy between SNPs found to be significant by self-defined ethnicity versus AIMs-defined predominant genetic ancestry confirms our hypothesis that SNPs' effect on AD age at onset may be affected by environmental and/or cultural factors that affect women who define themselves as White, as opposed to women of predominant Caucasian genetic ancestry, as will be discussed in more detail below.

Among self-identified Blacks, women with at least one minor allele at rs2077647 had a significantly decreased hazard ratio (delayed age at onset) for AD; while women with at least one minor allele at rs2982684 had a significantly increased hazard ratio (earlier age at onset) for AD. When women were recategorized according to predominant AIMs-defined genetic ancestry, rs2982684 continued to be associated with decreased age at onset for AD among women of predominantly African AIMs-defined ancestry (Table 5), while rs2077647 was no longer significant. The persistent association of rs2982684 with decreased AD age at onset despite ethnic group recategorization indicates that this SNP may have a more direct genetic effect on AD among women of self-identified Black ethnicity or predominantly African genetic ancestry.

Among women of Hispanic self-defined ethnicity and women of Admixed AIMs-defined genetic ancestry, no significant findings were demonstrated. This may be due to the fact that

women in these groups demonstrate significant heterogeneity in predominant genetic ancestry as well as environmental risk factors, including DM, smoking, and education.

Differential association of polymorphisms in a susceptibility gene for AD in groups of different self-identified ethnicities may occur for several reasons. First, differences in LD patterns between ethnic groups may contribute to discrepancies in genotype associations (Supplementary Figures 1-3). In our group, SNPs which were protective against AD in self-defined White women were located within a defined LD block (Supplementary Figure 1, *Block 2*). Notably, this block was less cohesive in self-defined Hispanics (Supplementary Figure 2, *Blocks 2-3*) and self-defined Blacks (Supplementary Figure 3, *Blocks 3-4*). This suggests that different LD patterns between *ESR1* 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 self-defined or genetic ancestries may play a significant role in phenotypic expression of the variants. This may explain why the six SNPs which were found to be protective in self-defined Whites were not found to be associated with age at AD onset among women of predominantly Caucasian genetic ancestry. As seen in Supplementary Table 3, when ethnic group stratification was changed from self-defined ethnicity to predominantly AIMS-defined genetic ancestry, the recategorization had the greatest effect on sample group when comparing self-identified Whites versus individuals of predominantly Caucasian AIMS-based ancestry, and self-identified Black versus women of predominantly African AIMS-based ancestry. Specifically, the shift in classification of race revealed that many individuals who identified themselves as Hispanic actually had genetic markers that were predominantly Caucasian or African. These individuals also had significantly higher rates of diabetes mellitus and smoking, and fewer years of education (Supplementary Table 3) than individuals of predominantly African AIMS who identified themselves as Black or participants of predominantly Caucasian AIMS who identified themselves as White. The potential mediation of SNP effect by vascular risk factors is reinforced by the different results in p-values for Cox analyses performed with and without the inclusion of vascular covariates (Supplementary Figure 4). For example, the inclusion of several vascular risk factors in the Cox analyses (including history of diabetes and current smoking) attenuated the significance of SNPs in self-identified White women (Supplementary Figure 4). These discrepancies indicate that environmental and biological covariate risk factors may affect the association of different alleles with age at onset for AD in this group.

While it is possible that discrepancies in SNPs found to be significantly associated with age at onset of AD may be due to different MAFs between ethnicities, this was not the case in our study group. Comparisons of SNPs found to be associated with age at AD onset demonstrate no significant difference in MAF between self-identified ethnicities (Tables 3 and 4) or between AIMS-defined predominant genetic ancestries (Table 5).

The association of polymorphisms in *ESR1* with risk of AD has been investigated in a number of cohorts of Asian and European men and women, but findings have been inconsistent [10-18,20-23,33] *ESR1* is more than 140 kb long and has eight exons and five



domains[34]. The strongest associations within *ESRI* have been found for the two RFLPs, PvuII (-397 T/C; rs2234693) and XbaI (-351 A/G; rs9340799), located 46 bp apart in the first intron, which have been associated with risk of developing cognitive impairment and AD [10,16,18,23]. These RFLPs have traditionally been recorded in PvuII as PP versus pp (where “P” is associated with the “T” allele, and “p” is associated with the “C” allele) and in XbaI as XX versus xx (where “X” is associated with the “A” allele and “x” is associated with the “G” allele). Capital “P” and “X” indicate the absence of the restriction site for each endonuclease, while lower case letters “p” and “x” indicate the presence of the restriction site. In our study, the C allele of rs2234693 (or “p”) and the G allele of rs9340799 (or “x”) were found to be protective, or associated with delayed age at AD onset; conversely the TT genotype of PvuII and the AA genotype of XbaI were found to be associated with earlier age at AD onset.

Several studies have similarly found an association between the T-allele of PvuII and A-allele of XbaI and increased risk for AD. One study of elderly Japanese men and women (205 cases versus 92 controls), found a marginal association between the “X” polymorphism (A-allele) of XbaI and risk for AD, ( $p=0.036$ ) but no significant association between the PvuII “P” polymorphism (T-allele) and risk for AD ( $p=0.99$ )[15]. A second study of elderly Japanese found that frequencies of the “P” polymorphism (T-allele) of PvuII and “X” polymorphism (A-allele) of the XbaI RFLPs were significantly higher in the AD group than in the control group (49.4% P allele in AD cases v. 36.3% p allele in controls,  $p < 0.01$ ; 29.1% X allele in AD cases v. 16.7% x allele in controls,  $p < 0.01$ ) [33]. This finding was replicated by the same research group in two other studies of elderly Japanese which included both men and women [11,18]. A study of elderly Han Chinese men and women also found overrepresentation of “P” or “X” polymorphisms (T- and A-alleles, respectively) among AD patients compared with healthy controls (“P” allele:  $p=0.023$ , OR= 2.94, 95% CI 1.13- 7.1; “X” allele:  $p=0.046$ , OR = 2.28, 95% CI 1.003- 5.17)[14]. This trend was more distinct in elderly Han Chinese women than men (“P” allele:  $p= 0.016$ , OR=3.68, 95% CI 1.22- 11.08; “X” allele:  $p= 0.029$ , OR= 29.5, 95% CI 1.10- 7.94)[14]. A study of elderly Italian men and women found the *ESRI* “PP” (TT) and “XX”(AA) genotypes to be associated with an increased risk for AD only in men (OR= 3.6, 95% CI 1.2- 10.9)[22]. The lowest *APOE* concentrations were observed in men carrying “PP” (TT) or “XX” (AA) genotypes ( $p=0.006$ ), and in men carrying “PP” (TT) and/or “XX” (AA) genotypes together with the *APOE*  $\epsilon 4$  allele ( $p=0.003$ ). In women with AD, *ESRI* “PP” (TT) and “XX” (AA) genotypes were also associated with lower MMSE values ( $p=0.0007$ )[22]. Finally, a separate study of elderly Italian men and women that combined the alleles of the PvuII and XbaI polymorphisms demonstrated that the “PPXX” (TTAA) haplotype was significantly more frequent in patients with AD than in controls[10]. The risk for AD increased seven-fold in individuals homozygous for the *APOE*  $\epsilon 4$  allele with the “PPXX” (TTAA) *ESRI* genotype[10].

Conversely, other studies have demonstrated an association between the “p” polymorphism (C-allele) of PvuII and “x” polymorphism (A-allele) of XbaI and increased risk for AD[16,17,20,23] or cognitive impairment[23], all in primarily homogenous Caucasian populations in Finland[16], Italy[17,20], and the United States[23]. Finally, other studies

have found no association between XbaI I [12,21,35] or PvuII[12,21] genotypes and risk for AD. Conflicting results between our data and the data reported within previous studies may be due to several factors. First, study designs differ in sample size. Second, a difference in ethnicities between samples is a well-known confounding factor in genetic studies. It is also possible that the increased risk of AD of cognitive impairment with ER $\alpha$  polymorphisms is due to linkage disequilibrium with nearby genes that may in turn cause increased risk for developing AD. Finally, the age at onset of disease and clinical manifestations of disease may be modulated by environmental or cultural factors. It is possible that various risk factors lead to differential rates of progression.

A number of hypotheses for the functional significance of these polymorphisms have been reported in the literature. Given their location, 397 and 351 bp upstream from the start of exon 2, possible functional mechanisms include changed *ESR1* expression via altered binding of transcription factors and influence on alternative splicing of the *ESR1* gene. Both these mechanisms can be a result of either of these polymorphisms or through linkage disequilibrium with a truly functional, but so far unknown, sequence variation elsewhere in the *ESR1* gene. To support the former hypothesis, Maruyama *et al.*[12] showed that the intronic region of the *ESR1* gene carrying the PvuII and XbaI polymorphisms demonstrates a weak enhancer activity. Such activity appears to be influenced by genotype, as the enhancer activity of the G-allele appeared to higher than the A-allele. Additionally, according to Herrington *et al.* [36], the T-allele of the PvuII RFLP eliminates a functional binding site for the transcription factor B-myb. This implies that the presence of this allele may result in lower *ESR1* transcription or may alter stability or structure of the ER $\alpha$  transcript and the subsequent ER $\alpha$  protein. This is supported by one study which demonstrated that the PvuII T-allele was associated with decreased plasma estradiol (E<sub>2</sub>) levels in an allele-dose-dependent manner in postmenopausal women [37], and findings in other studies that the PvuII polymorphism T-allele is associated with conditions affected by lower levels of E<sub>2</sub> including increased risk of osteoporosis [38,39] and myocardial infarction [40]. The fact that the XbaI polymorphism A-allele is also associated with lower E<sub>2</sub> levels may be due to linkage disequilibrium with the PvuII SNP or another functional polymorphism, or to functional significance of the XbaI polymorphism itself.

Overall, our findings confirm previous studies' findings of a strong association between *ESR1* polymorphisms and age at onset 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 multi-ethnic 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. This allowed us to perform haplotype analysis among the six most significant SNPs in self-identified White women, which identified haplotype C-A-C-G at SNPs 12 - 15 (rs3853250 through rs9340799) containing the RFLPs PvuII and XbaI as significantly decreasing risk for AD, (O.R. 0.656, p=0.009). Moreover, our studies clearly 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 vary significantly between the models within one ethnic group and among 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 (CNVs). 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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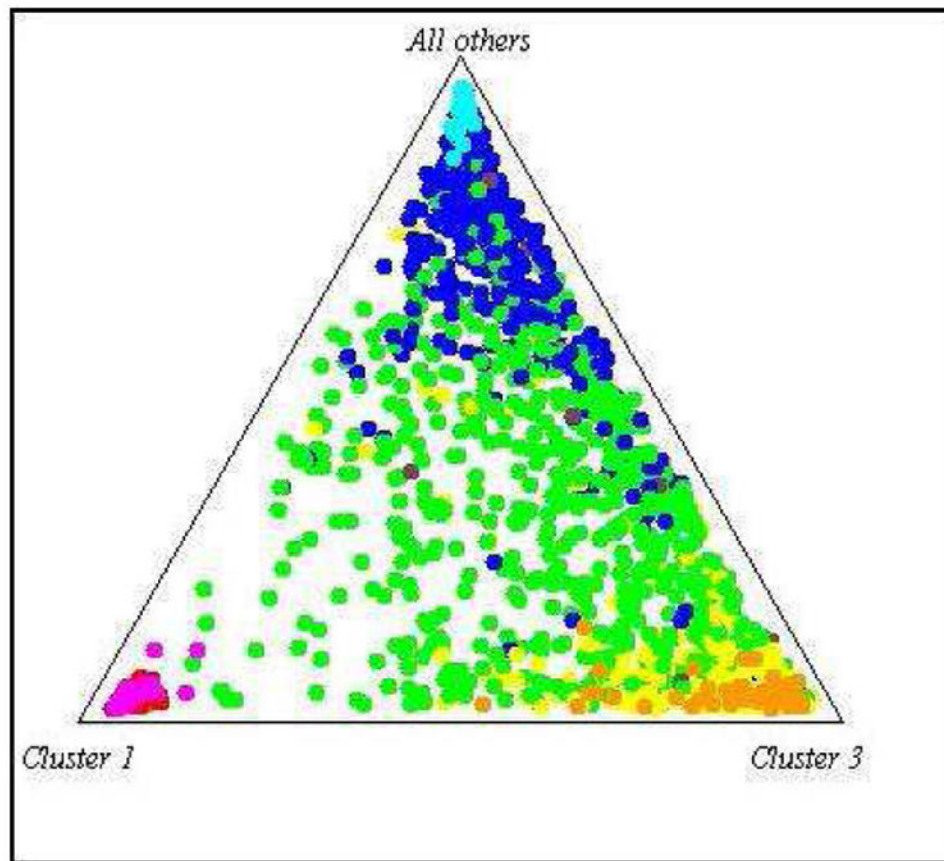
## References

1. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *Journal of Comparative Neurology*. 1997; 388:507–525. [PubMed: 9388012]
2. McEwen BS. Invited review: Estrogens effects on the brain: Multiple sites and molecular mechanisms. *J Appl Physiol*. 2001; 91:2785–2801. [PubMed: 11717247]
3. Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem*. 1996; 66:1836–1844. [PubMed: 8780008]
4. Toran-Allerand CD, Miranda RC, Bentham WD, Sohrabji F, Brown TJ, Hochberg RB, MacLusky NJ. Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. *Proc Natl Acad Sci U S A*. 1992; 89:4668–4672. [PubMed: 1316615]
5. Luine VN. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp Neurol*. 1985; 89:484–490. [PubMed: 2990988]
6. Behl C, Widmann M, Trapp T, Holsboer F. 17-beta estradiol protects neurons from oxidative stress-induced cell death in vitro. *Biochem Biophys Res Commun*. 1995; 216:473–482. [PubMed: 7488136]

7. Jaffe AB, Toran-Allerand CD, Greengard P, Gandy SE. Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J Biol Chem.* 1994; 269:13065–13068. [PubMed: 8175728]
8. Gerhard M, Ganz P. How do we explain the clinical benefits of estrogen? From bedside to bench. *Circulation.* 1995; 92:5–8. [PubMed: 7788916]
9. Simoncini T, Mannella P, Genazzani AR. Rapid estrogen actions in the cardiovascular system. *Ann N Y Acad Sci.* 2006; 1089:424–430. [PubMed: 17261785]
10. Brandi ML, Becherini L, Gennari L, Racchi M, Bianchetti A, Nacmias B, Sorbi S, Mecocci P, Senin U, Govoni S. Association of the estrogen receptor alpha gene polymorphisms with sporadic Alzheimer's disease. *Biochemical and biophysical research communications.* 1999; 265:335–338. [PubMed: 10558867]
11. Ji Y, Urakami K, Wada-Isoe K, Adachi Y, Nakashima K. Estrogen receptor gene polymorphisms in patients with Alzheimer's disease, vascular dementia and alcohol-associated dementia. *Dementia and Geriatric Cognitive Disorders.* 2000; 11:119–122. [PubMed: 10765041]
12. Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T, Arai H, Yasuda M, Tanaka C, Emson PC, Nakamura S, Kawakami H. Lack of an association of estrogen receptor alpha gene polymorphisms and transcriptional activity with Alzheimer disease. *Archives of Neurology.* 2000; 57:236–240. [PubMed: 10681083]
13. Lambert JC, Harris JM, Mann D, Lemmon H, Coates J, Cumming A, St-Clair D, Lendon C. Are the estrogen receptors involved in Alzheimer's disease? *Neurosci Lett.* 2001; 306:193–197. [PubMed: 11406328]
14. Lin GF, Ma QW, Zhang DS, Zha YL, Lou KJ, Shen JH. Polymorphism of alpha-estrogen receptor and aryl hydrocarbon receptor genes in dementia patients in Shanghai suburb. *Acta Pharmacologica Sinica.* 2003; 24:651–656. [PubMed: 12852830]
15. Usui C, Shibata N, Ohnuma T, Higashi S, Ohkubo T, Ueki A, Nagao M, Arai H. No genetic association between the myeloperoxidase gene -463 polymorphism and estrogen receptor-alpha gene polymorphisms and Japanese sporadic Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders.* 2006; 21:296–299. [PubMed: 16484808]
16. Mattila KM, Axelman K, Rinne JO, Blomberg M, Lehtimäki T, Laippala P, Roytta M, Viitanen M, Wahlund L, Winblad B, Lannfelt L. Interaction between estrogen receptor 1 and the epsilon 4 allele of apolipoprotein E increases the risk of familial Alzheimer's disease in women. *Neuroscience Letters.* 2000; 282:45–48. [PubMed: 10713392]
17. Porrello E, Monti MC, Sinforiani E, Cairati M, Guaita A, Montomoli C, Govoni S, Racchi M. Estrogen receptor alpha and APOE epsilon 4 polymorphisms interact to increase risk for sporadic AD in Italian females. *European journal of Neurology : the Official Journal of the European Federation of Neurological Societies.* 2006; 13:639–644. [PubMed: 16796589]
18. Isoe-Wada K, Maeda M, Yong J, Adachi Y, Harada H, Urakami K, Nakashima K. Positive association between an estrogen receptor gene polymorphism and Parkinson's disease with dementia. *European Journal of Neurology : the Official Journal of the European Federation of Neurological Societies.* 1999; 6:431–435. [PubMed: 10362895]
19. Isoe K, Urakami K, Adachi Y, Nakashima K. Genetic association of estrogen receptor gene polymorphisms with Alzheimer's disease. *Alzheimer's Research.* 1997; 3:195–197.
20. Monastero R, Cefalu AB, Camarda C, Noto D, Camarda LK, Caldarella R, Imbornone E, Averna MR, Camarda R. Association of estrogen receptor alpha gene with Alzheimer's disease: A case-control study. *J Alzheimers Dis.* 2006; 9:273–278. [PubMed: 16914837]
21. Ma SL, Tang NL, Tam CW, Lui VW, Lau ES, Zhang YP, Chiu HF, Lam LC. Polymorphisms of the estrogen receptor alpha (*ESR1*) gene and the risk of Alzheimer's disease in a southern Chinese community. *Int Psychogeriatr.* 2009; 21:977–986. [PubMed: 19586561]
22. Corbo RM, Gambina G, Ruggeri M, Scacchi R. Association of estrogen receptor alpha (*ESR1*) PvuII and XbaI polymorphisms with sporadic Alzheimer's disease and their effect on apolipoprotein E concentrations. *Dementia and Geriatric Cognitive Disorders.* 2006; 22:67–72. [PubMed: 16699281]

23. Yaffe K, Lui LY, Grady D, Stone K, Morin P. Estrogen receptor 1 polymorphisms and risk of cognitive impairment in older women. *Biological Psychiatry*. 2002; 51:677–682. [PubMed: 11955468]
24. Tang MX, Cross P, Andrews H, Jacobs DM, Small S, Bell K, Merchant C, Lantigua R, Costa R, Stern Y, Mayeux R. Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. *Neurology*. 2001; 56:49–56. [PubMed: 11148235]
25. Stern Y, Andrews H, Pittman J, Sano M, Tatemichi T, Lantigua R, Mayeux R. Diagnosis of dementia in a heterogeneous population. Development of a neuropsychological paradigm-based diagnosis of dementia and quantified correction for the effects of education. *Arch Neurol*. 1992; 49:453–460. [PubMed: 1580806]
26. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*. 2000; 155:945–959. [PubMed: 10835412]
27. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*. 2003; 164:1567–1587. [PubMed: 12930761]
28. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. ApoE and Alzheimer disease meta analysis consortium. *JAMA : the Journal of the American Medical Association*. 1997; 278:1349–1356. [PubMed: 9343467]
29. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. *American Journal of Epidemiology*. 1989; 129:1120–1131. [PubMed: 2729251]
30. Meldrum DR, Davidson BJ, Tataryn IV, Judd HL. Changes in circulating steroids with aging in postmenopausal women. *Obstetrics and Gynecology*. 1981; 57:624–628. [PubMed: 7219911]
31. Judd HL, Shamonki IM, Frumar AM, Lagasse LD. Origin of serum estradiol in postmenopausal women. *Obstetrics and Gynecology*. 1982; 59:680–686. [PubMed: 7078905]
32. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. 1990; 31:545–548. [PubMed: 2341813]
33. Isoe K, Urakami K, Wakutani Y, Yong J, Adachi Y, Nakashima K. Screening for mutation of the presenilin 2 gene in Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders*. 1997; 8:60. [PubMed: 8997554]
34. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human oestrogen receptor gene. *The EMBO Journal*. 1988; 7:3385–3388. [PubMed: 3145193]
35. Prince JA, Feuk L, Sawyer SL, Gottfries J, Ricksten A, Nagga K, Bogdanovic N, Blennow K, Brookes AJ. Lack of replication of association findings in complex disease: An analysis of 15 polymorphisms in prior candidate genes for sporadic Alzheimer's disease. *European Journal of Human Genetics : EJHG*. 2001; 9:437–444. [PubMed: 11436125]
36. Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Meyers DA, Bleecker ER. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation*. 2002; 105:1879–1882. [PubMed: 11997270]
37. Schuit SC, de Jong FH, Stolk L, Koek WN, van Meurs JB, Schoofs MW, Zillikens MC, Hofman A, van Leeuwen JP, Pols HA, Uitterlinden AG. Estrogen receptor alpha gene polymorphisms are associated with estradiol levels in postmenopausal women. *Eur J Endocrinol*. 2005; 153:327–334. [PubMed: 16061840]
38. van Meurs JB, Schuit SC, Weel AE, van der Klift M, Bergink AP, Arp PP, Colin EM, Fang Y, Hofman A, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet*. 2003; 12:1745–1754. [PubMed: 12837697]
39. Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM,

- Schuit SC, Sherlock RE, Uitterlinden AG. Differential genetic effects of *ESR1* gene polymorphisms on osteoporosis outcomes. *JAMA*. 2004; 292:2105–2114. [PubMed: 15523071]
40. Schuit SC, Oei HH, Witteman JC, Geurts van Kessel CH, van Meurs JB, Nijhuis RL, van Leeuwen JP, de Jong FH, Zillikens MC, Hofman A, Pols HA, Uitterlinden AG. Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. *JAMA*. 2004; 291:2969–2977. [PubMed: 15213208]



**Figure 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)

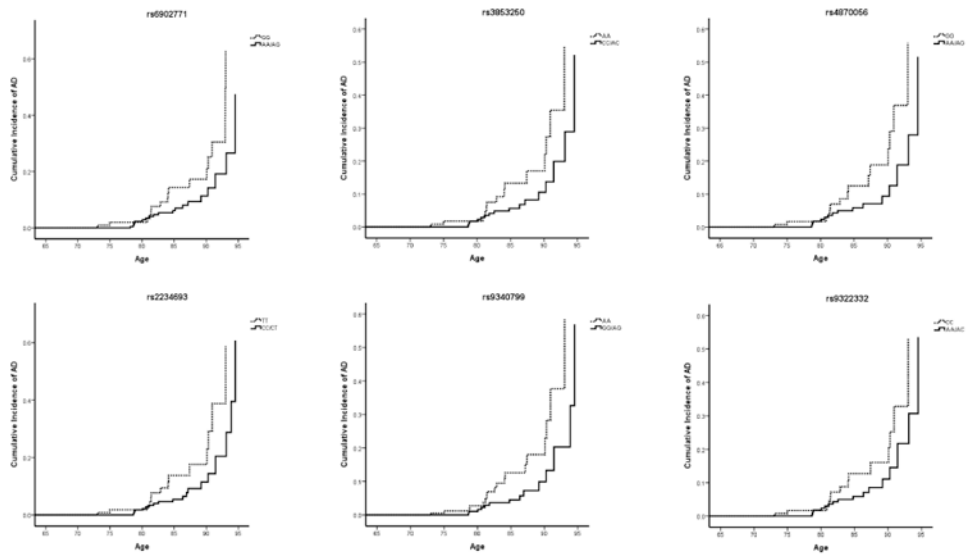


Figure 2. Survival curves, *ESRI* SNPs associated with incident AD in self-identified Whites



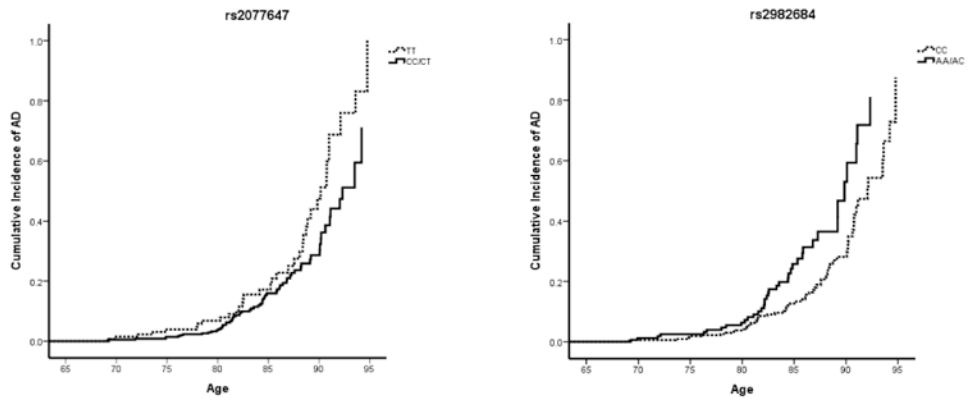


Figure 3. Survival curves, *ESRI* SNPs associated with incident AD in self-identified Blacks

**Table 1**  
**Population Characteristics**

Characteristic	Non Demented	Incident Alzheimer's Disease
Sample Size	1107	329
Age at time of enrollment (mean $\pm$ S.D.)*	75.1 (5.8)	77.4 (6.3)
Body Mass Index (mean $\pm$ S.D.)	28.1 (5.7)	27.8 (5.8)
Years of education (mean $\pm$ 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 <i>APOE</i> $\epsilon$ 4 (n, %)	285 (26.0)	91 (28.9)
Self-identified Ethnicity (n, %)*		
<i>White</i>	340 (89.5)	40 (10.5)
<i>Hispanic</i>	392 (68.4)	181 (31.6)
<i>Black</i>	375 (77.6)	108 (22.4)

\*  
p 0.05

Table 2

## Characteristics by self-identified ethnicity

Characteristic	Self-identified White	Self-identified Hispanic	Self-identified Black
Sample size (n)	380	573	483
Age at time of enrollment (mean $\pm$ S.D.) *	77.9 (7.2)	75.3 (6.9)	77.3 (6.8)
Body Mass Index (mean $\pm$ S.D.) *	26.2 (5.1)	28.2 (6.3)	28.3 (5.1)
Years of Education (mean $\pm$ S.D.) *	12.4 (3.8)	6.0 (4.3)	11.0 (3.8)
At least one copy of <i>APOE</i> $\epsilon$ 4 (n, %) *	83 (21.8)	137 (23.9)	186 (32.3)

\* P &lt; 0.05

Table 3

**ESR1 SNPs investigated**

SNP #	SNP	Position	Distance (bp)	Minor Allele	MAF Self-identified White	MAF Self-identified Hispanic	MAF Self-identified Black
1	rs2077647	152171020		C	0.5	0.5	0.5
2	rs17847065	152171427	407	A	0.0	0.0	0.0
3	rs11155814	152193127	21700	G	0.1	0.3	0.5
4	rs7761133	152193806	679	G	0.2	0.4	0.6
5	rs17081749	152193868	62	C	0.1	0.3	0.4
6	rs6903763	152195099	679	A	0.1	0.1	0.2
7	rs6909023	152195640	541	A	0.1	0.1	0.2
8	rs6937568	152195907	267	G	0.0	0.1	0.2
9	rs827421	152199065	3158	A	0.5	0.5	0.4
10	rs17081777	152199272	207	G	0.0	0.1	0.1
11	rs6902771	152199824	552	A	0.5	0.5	0.5
12	rs3853250	152201843	2019	C	0.5	0.5	0.5
13	rs4870056	152204170	2327	A	0.5	0.5	0.5
14	rs2234693	152205278	1108	C	0.5	0.5	0.5
15	rs9340799	152205324	46	G	0.4	0.3	0.3
16	rs9322332	152208744	3420	A	0.5	0.4	0.4
17	rs3936674	152209254	510	A	0.3	0.2	0.1
18	rs712221	152222184	12930	T	0.5	0.5	0.4
19	rs1709183	152235939	13755	G	0.2	0.3	0.4
20	rs11155819	152241302	5363	G	0.3	0.3	0.1
21	rs9340835	152241874	572	A	0.3	0.3	0.3
22	rs9322335	152242072	198	A	0.2	0.3	0.3
23	rs9322338	152242692	620	A	0.1	0.2	0.5
24	rs6557170	152245047	2355	A	0.2	0.2	0.1
25	rs9478251	152245222	175	C	0.0	0.2	0.3
26	rs2347923	152269364	24142	C	0.3	0.4	0.4

SNP #	SNP	Position	Distance (bp)	Minor Allele	MAF Self-identified White	MAF Self-identified Hispanic	MAF Self-identified Black
27	rs2347867	152271793	2429	G	0.3	0.5	0.6
28	rs6557171	152276536	4743	A	0.3	0.4	0.4
29	rs4870062	152279561	3025	C	0.3	0.4	0.5
30	rs4583998	152302611	23050	A	0.3	0.5	0.5
31	rs9371564	152304056	1445	A	0.3	0.5	0.6
32	rs3020377	152314341	10285	A	0.6	0.5	0.3
33	rs1884051	152325222	10881	G	0.4	0.4	0.5
34	rs985695	152328648	3426	A	0.2	0.3	0.4
35	rs726281	152344521	15873	G	0.2	0.5	0.7
<b>36</b>	<b>rs2982684</b>	<b>152348147</b>	<b>3626</b>	<b>A</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>
37	rs12212176	152351950	3803	A	0.2	0.1	0.1
38	rs7757956	152359083	7133	A	0.1	0.2	0.2
39	rs9340954	152362115	3032	C	0.3	0.4	0.6
40	rs722208	152364828	2713	G	0.3	0.4	0.6
41	rs2207396	152424325	59497	A	0.2	0.2	0.2

• **Bolded** and *italicized* SNPs are those found to be significant in any self-identified ethnicity or AIMS-defined genetic ancestry

**Table 4**  
**ESR1 SNPs associated with incident AD, by self-identified ethnicity**

SNP #	Self-identified White				Self-identified Hispanic				Self-identified Black						
	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value
1	rs2077647		(152171020);	MAF (C): 0.5		rs2077647		(152171020);	MAF (C): 0.5		rs2077647		(152171020);	MAF (C): 0.5	
	CC/CT	279	33	1.761 (0.680 - 4.556)	0.244	CC/CT	146	282	1.183 (0.834 - 1.680)	0.346	CC/CT	358	286	0.629 (0.414 - 0.955)	0.030
	TT	97	6	ref		TT	418	103	ref		TT	124	88	ref	
11	rs6902771		(152199824);	MAF (A): 0.5		rs6902771		(152199824);	MAF (A): 0.5		rs6902771		(152199824);	MAF (A): 0.5	
	AA/AG	272	21	0.437 (0.224 - 0.852)	0.015	AA/AG	399	128	1.072 (0.763 - 1.505)	0.690	AA/AG	352	85	1.361 (0.831 - 2.227)	0.220
	GG	94	17	ref		GG	158	47	ref		GG	114	22	ref	
12	rs3853250		(152201843);	MAF (C): 0.5		rs3853250		(152201843);	MAF (C): 0.5		rs3853250		(152201843);	MAF (C): 0.5	
	CC/AC	267	21	0.442 (0.226 - 0.862)	0.017	CC/AC	411	131	1.076 (0.762 - 1.518)	0.678	CC/AC	368	87	1.276 (0.762 - 2.137)	0.354
	AA	99	17	ref		AA	146	45	ref		AA	97	20	ref	
13	rs4870056		(152204170);	MAF (A): 0.5		rs4870056		(152204170);	MAF (A): 0.5		rs4870056		(152204170);	MAF (A): 0.5	
	AA/AG	262	20	0.420 (0.215 - 0.819)	0.011	AA/AG	401	130	1.136 (0.807 - 1.599)	0.464	AA/AG	340	82	1.156 (0.722 - 1.850)	0.545
	GG	104	18	ref		GG	157	46	ref		GG	124	25	ref	
14	rs2234693		(152205278);	MAF (C): 0.5		rs2234693		(152205278);	MAF (C): 0.5		rs2234693		(152205278);	MAF (C): 0.5	
	CC/CT	275	23	0.443 (0.231 - 0.851)	0.014	CC/CT	413	129	1.037 (0.740 - 1.452)	0.834	CC/CT	381	88	1.275 (0.761 - 2.135)	0.356
	TT	100	17	ref		TT	151	48	ref		TT	98	20	ref	
15	rs9340799		(152205324);	MAF (G): 0.4		rs9340799		(152205324);	MAF (G): 0.3		rs9340799		(152205324);	MAF (G): 0.3	
	GG/AG	233	18	0.441 (0.226 - 0.863)	0.017	GG/AG	309	98	0.919 (0.680 - 1.243)	0.585	GG/AG	241	60	1.171 (0.786 - 1.745)	0.437
	AA	143	21	ref		AA	254	79	ref		AA	239	48	ref	
16	rs9322332		(152208744);	MAF (A): 0.5		rs9322332		(152208744);	MAF (A): 0.4		rs9322332		(152208744);	MAF (A): 0.4	
	AA/AC	257	21	0.483 (0.248 - 0.943)	0.033	AA/AC	379	121	1.025 (0.737 - 1.427)	0.881	AA/AC	146	77	1.399 (0.891 - 2.196)	0.145
	CC	108	17	ref		CC	175	52	ref		CC	317	30	ref	
36	rs2982684		(152348147);	MAF (A): 0.1		rs2982684		(152348147);	MAF (A): 0.1		rs2982684		(152348147);	MAF (A): 0.2	

SNP #	Self-identified White				Self-identified Hispanic				Self-identified Black						
	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value
	AA/AC	61	6	1.120 (0.461 - 2.725)	0.802	AA/AC	134	38	0.748 (0.519 - 1.078)	0.119	AA/AC	159	41	1.745 (1.152 - 2.644)	0.009
	CC	305	32	ref		CC	424	138	ref		CC	306	66	ref	

• Adjusted for years of education, body mass index (BMI), and presence of at least one *APOE* ε4 allele

• MAF: Minor allele frequency

• Emp. p-value: empirical p-value

**Table 5**  
**ESR1 SNPs associated with incident AD, by AIMs-defined predominant genetic ancestry**

SNP #	Predominantly Caucasian AIMs-defined ancestry				Admixed AIMs-defined ancestry				Predominantly African AIMs-defined ancestry						
	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value	SNP	N	AD	HR (95% CI)	emp. p-value
7	rs6909023	rs6909023 (152195640); MAF (A): 0.1				rs6909023 (152195640); MAF (A): 0.1				rs6909023 (152195640); MAF (A): 0.2					
	AA/AG	48	4	1.862 (1.030 – 3.366)	0.040	AA/AG	166	36	0.264 (0.470 – 1.230)	0.264	AA/AG	103	37	1.135 (0.760 – 1.696)	0.536
	GG	318	34	ref		GG	300	71	ref		GG	453	137	ref	
36	rs2982684	rs2982684 (152348147); MAF (A): 0.1				rs2982684 (152348147); MAF (A): 0.2				rs2982684 (152348147); MAF (A): 0.2					
	AA/AC	159	17	0.720 (0.383 – 1.354)	0.308	AA/AC	159	41	0.772 (0.491 – 1.211)	0.260	AA/AC	134	38	1.528 (1.040 – 2.244)	0.031
	CC	206	21	ref		CC	306	66	ref		CC	424	138	ref	

- Adjusted for years of education, body mass index (BMI), and presence of at least one APOE ε4 allele
- MAF: Minor allele frequency
- Emp. p-value: empirical p-value