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RESEARCH ARTICLE

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TOMM40 and *APOC1* variants differentiate the impacts of the *APOE ε4* allele on Alzheimer's disease risk across sexes, ages, and ancestries

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Abstract

INTRODUCTION: The variability in apolipoprotein E (*APOE*) ε4-attributed susceptibility to Alzheimer's disease (AD) across ancestries, sexes, and ages may stem from the modulating effects of other genetic variants.

METHODS: We examined associations of compound genotypes (CompGs) comprising the ε4-encoding rs429358, *TOMM40* rs2075650, and *APOC1* rs12721046 polymorphisms with AD in White (7181/16,356 AD-affected/unaffected), Hispanic/Latino (2305/2921), and Black American (547/1753) participants across sexes and ages.

RESULTS: The absence and presence of the rs2075650 and/or rs12721046 minor alleles in the ε4-bearing CompGs define lower- and higher-AD-risk profiles, respectively, in White participants. They differentially impact AD risks in men and women of different ancestries, exhibiting an increasing, decreasing, flat, and nonlinear—with lower risks at ages younger than 65/70 years and older than 85 years compared to the ages in between—patterns across ages.

DISCUSSION: The ε4-bearing CompGs have a potential to differentiate biological mechanisms of sex-, age-, and ancestry-specific AD risks and serve as AD biomarkers.

KEYWORDS
Alzheimer's disease risk, *APOE* gene cluster, *APOE* polymorphism, haplotypes, race/ethnic disparities, sex disparities in Alzheimer's disease

Highlights

- Younger White women carrying the lower-risk (LR) CompG are at small risk of AD.
- Black carriers of the LR CompG are at negligible risk of AD at 85 years and older.
- The higher-risk (HR) CompGs confer high AD risk in Whites and Blacks at 70 to 85 years.
- AD risk decreases with age for Hispanic/Lation women carrying the HR CompGs.
- Hispanic/Lation carriers of the LR CompG but not HR CompGs have higher AD risk than Blacks.

1 | BACKGROUND

For decades, the apolipoprotein E (*APOE*) ε4 allele has been considered the strongest single genetic risk factor for late-onset Alzheimer's disease (herein referred to as AD).[1] Given the central role of the ε4 allele in AD, this allele was characterized as a major genetic risk factor for AD.[2] However, the effect of this allele substantially varies across ethnoracial groups, sex, and age. Indeed, the strongest association of the ε4 allele with AD was consistently reported in Non-Hispanic White subjects (NHWs), whereas Hispanic/Latino subjects (H/Ls) and Black American subjects (BAs) carrying the ε4 allele may not be strongly exposed to AD risks,[3–7] despite higher prevalence and incidence of AD in H/Ls and BAs than in NHWs.[8–10] Female carriers of the ε4 allele are reported to have higher AD risk than male carriers.[4,11–14] Age is another factor that is emphasized as a modulator of the ε4-associated AD risks.[4,15–18]

What drives the ε4-associated differences in AD risks across sex/gender, ancestry, and age? An answer to this question is important because the identification of factors modulating the adverse effect of the ε4 allele in these settings is a natural strategy to better understand the mechanism of AD pathogenesis. One approach to address this question is to assume that the effect of the ε4 allele can be modulated by other genetic and nongenetic factors. The influence of other genetic factors can take different forms such as familiar gene-by-gene interactions, joint influences of multiple variants in the forms of haplotypes, combinations of genotypes (herein referred to as compound genotypes [CompGs]), the impacts of local or global ancestry, transcriptional regulation, and so forth.[19–25] Nongenetic factors can modulate the effect of the ε4 allele through well-known gene-by-environment interactions or systemic influences, such as AD exposome.[26] Accordingly, understanding the role of the complex of factors modulating the ε4 allele effect across sex/gender, race/ethnicity, and age is a challenging endeavor that explains why studies examining the ε4-associated differences in AD risks across all these settings are rare.[4,27]

We advance previous research by combining promising approaches examining the joint effects of genetic variants on AD and the differences in these effects in NHWs, H/Ls, and BAs across sexes and ages. This study draws on prior findings indicating that the *APOE* ε4 allele encoded by the rs429358 single nucleotide polymorphism (SNP) confers substantially lower AD risks in NHWs when it does not cluster with minor alleles of *TOMM40* rs2075650 and *APOC1* rs12721046 SNPs.[21,28] This finding points to the role of the ε4-bearing CompGs in predisposition to AD. Here, we examine whether CompGs comprising these three SNPs can differentiate the ε4-associated AD risks among NHWs, H/Ls, and BAs—men and women separately and combined—and across ages. Our main focus was on samples derived from seven population/community-based and AD-centric studies. This encompassed 7181/16,356 AD-affected/unaffected NHWs, 2305/2921 AD-affected/unaffected H/Ls, and 547/1753 AD-affected/unaffected BAs who do not carry the ε2 allele. This work capitalizes on using pooled samples from different studies to improve the characterization of the roles of the ε4-bearing CompGs in AD pathogenesis.

RESEARCH IN CONTEXT

1. **Systematic review**: A literature review conducted on PubMed and Google Scholar identified publications examining the roles of combinations of genotypes and haplotypes in predisposition to Alzheimer's disease (AD) in the *APOE* gene cluster. Most research, however, was focused on populations of European ancestry in age-aggregated samples. These relevant citations are appropriately made.
2. **Interpretation**: This study demonstrates that minor and major alleles of the *TOMM40* rs2075650 and/or *APOC1* rs12721046 polymorphisms differentiate the ε4-allele-attributable AD risks across sexes and ages in populations of White, Hispanic/Latino, and Black American ancestries.
3. **Future directions**: Our findings call for exploring the biological mechanisms underlying the differential impacts of the ε4-bearing polygenic combinations of genotypes on AD risks. These findings also suggest the need to examine such combinations as potential biomarkers of sex-, age-, and ancestry-specific AD pathology.

2 | METHODS

2.1 | Study cohorts and AD phenotype

For the main analysis, subjects come from three longitudinal cohorts and four AD-centric projects (Table 1). The longitudinal cohorts included subjects from the following population/community-based samples: the Framingham Heart Study (FHS), parental (FHS 1) and offspring (FHS 2) cohorts[29]; the Cardiovascular Study (CHS)[30]; and the Health and Retirement Study (HRS).[31] The four AD-focused projects included the National Institute on Aging's (NIA) Late–Onset Alzheimer Disease Family Based Study (LOADFBS)[32]; seven cohorts from the NIA Alzheimer's Disease Centers (ADC1-ADC7), which are part of the Alzheimer's Disease Genetics Consortium (ADGC) initiative[33]; the NIA collaborative study from the Alzheimer's Disease Sequencing Project (ADSP)[34]; and the Hispanic Late–Onset Alzheimer Disease Study (HispLOAD).[32] The analyses were performed in pooled samples of NHWs (from FHS 1, FHS 2, CHS, HRS, LOADFBS, ADGC, and ADSP), H/Ls (from CHS, HRS, LOADFBS, and HispLOAD), and BAs (from CHS, HRS, LOADFBS, and ADSP). Data for NHW, BA, and H/L subjects were analyzed separately. Additionally, data for NHWs from FHS, CHS, and HRS cohorts were pooled in a sample referred to as WFCH, which was used in validation analysis. Overlapping subjects in ADSP that came from FHS, CHS, HRS, ADC, and LOADFBS were excluded.

Samples from the third generation FHS 3 cohort[29] and the Coronary Artery Risk Development in Young Adults (CARDIA) study[35] were used to ascertain frequencies of CompGs in younger AD-unaffected NHWs and BAs.

TABLE 1 Basic characteristics of the genotyped participants in the selected studies with no carriers of the ε2 allele.

		AD-unaffected subjects			AD-affected subjects		
Cohort	Ancestry	*N*	Men (%)	Age mean (SD), years	*N*	Men (%)	Age mean (SD), years
Cohorts for the main analysis							
HRS	NHWs	5622	2455 (43.7)	78.9 (8.0)	218	81 (37.2)	85.3 (6.9)
	BAs	765	277 (36.2)	76.8 (8.4)	129	56 (43.4)	77.6 (8.2)
	H/Ls	620	254 (41.0)	76.6 (8.0)	22	8 (36.3)	79.5 (7.2)
CHS	NHWs	2987	1322 (44.3)	83.3 (5.4)	198	70 (35.4)	84.5 (4.8)
	BAs	530	208 (39.2)	81.3 (5.8)	33	10 (30.3)	85.0 (5.7)
	H/Ls	45	19 (42.2)	83.8 (4.9)	4	1 (25.0)	89.6 (4.5)
FHS 1 and 2	NHWs	2667	1191 (44.7)	75.1 (10.7)	257	96 (37.4)	88.9 (7.2)
LOADFBS	NHWs	1359	547 (40.3)	69.1 (11.3)	1379	478 (34.7)	81.8 (7.2)
	BAs	51	13 (25.5)	68.1 (11.7)	75	25 (33.3)	80.9 (8.8)
	H/Ls	167	57 (34.1)	67.6 (11.7)	328	105 (32.0)	81.8 (9.3)
ADGC	NHWs	3022	1420 (47.0)	77.1 (8.81)	4640	2380 (51.3)	79.9 (7.54)
ADSP	NHWs	699	345 (49.4)	79.9 (7.32)	489	210 (42.9)	80.8 (8.30)
	BAs	407	88 (21.6)	69.2 (6.60)	310	74 (23.9)	73.8 (8.59)
	H/Ls	638	184 (28.8)	74.1 (8.6)	664	247 (37.2)	76.2 (7.7)
HispLOAD	H/Ls	1451	459 (31.6)	72.7 (8.82)	1287	461 (35.8)	74.7 (9.42)
Cohorts for characterizing genotype frequencies in younger AD-unaffected subjects							
FHS 3	NHWs	4012	1880 (46.9)	41.4 (8.45)	NA		
CARDIA	NHWs	1180	561 (47.5)	25.7 (3.3)	NA		
	BAs	942	385 (40.9)	39.6 (3.8)	NA		

Note: The HRS, FHS, and CHS are longitudinal population/community-based studies ascertaining AD cases during follow-up. LOADFBS, ADGC, ADSP, and HispLOAD are AD-focused studies funded by NIA.
A pooled sample of NHWs included subjects from the FHS parental (FHS 1) and offspring (FHS 2) cohorts, CHS, HRS, LOADFBS, ADGC, and ADSP.
A pooled sample of H/Ls included subjects from the CHS, HRS, LOADFBS, and HispLOAD.
A pooled sample of BAs included subjects from CHS, HRS, LOADFBS, and ADSP.
Abbreviations: AD, Alzheimer's disease; ADGC, the AD Genetics Consortium sample comprising seven cohorts from the NIA Alzheimer's Disease Centers (ADC1-ADC7); ADSP, NIA AD Sequencing Project; BAs, Black American participants; CARDIA, the Coronary Artery Risk Development in Young Adults; CHS, the Cardiovascular Health Study; FHS 1 and 2, the Framingham Heart Study (FHS) parental and offspring cohorts; FHS 3, the FHS grandchildren cohort; HispLOAD, Columbia University Study of Caribbean Hispanics with Familial and Sporadic Late–Onset Alzheimer's disease; H/Ls, Hispanic/Latino participants; HRS, the Health and Retirement Study; LOADFBS, the National Institute on Aging (NIA) Late-Onset AD Family Based Study; *N*, sample of men and women combined; NA, not available; NHWs, Non-Hispanic White participants; NIA, National Institute on Aging; SD, standard deviation.

AD in LOADFBS, FHS, ADSP, and HispLOAD was ascertained by researchers in each study using diagnoses made according to the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA). A diagnosis of AD in HRS and CHS was drawn from ICD-9:331.0x codes in Medicare service use files. The ADGC cohort included autopsy- and clinically confirmed AD-affected and cognitively normal subjects who were ascertained by the clinical and neuropathology cores of the NIA-funded ADCs according to the NINCDS-ADRDA.

2.2 | Genotypes

We focus on CompGs comprising three SNPs from the *APOE* (rs429358, T/c; upper/lower case denotes here major/minor allele; allele "c" encodes the ε4 allele), *TOMM40* (rs2075650, A/g), and *APOC1* (rs12721046, G/a) genes. This triple was selected because we previously identified that minor alleles of the *TOMM40* and *APOC1* variants differentiated AD risks attributed to the *APOE* ε4 allele in NHWs.[21,28]

For three biallelic SNPs, there are 27 (= 3^3) CompGs. To simplify notations, genotypes were conveniently encoded by dosage (counts) of minor alleles in an SNP, that is, 0, 1, 2. Then, CompGs for SNPs ordered as rs429358, rs2075650, and rs12721046, are denoted as 000, 001, 002, 010, 011, 012, and so forth (Table S1). For example, 012 indicates a CompG comprising no ε4 allele (0$\underline{1}$2), heterozygote of rs2075650 (0$\underline{1}$2), and minor allele homozygote of rs12721046 (01$\underline{2}$).

To select samples with no carriers of the *APOE* ε2 allele, we included the rs7412 (C/t) SNP, whose minor allele encodes the ε2 allele. With no ε2 carriers, subjects having a CompG with a leading 0 are carriers of the ε3ε3 genotype, those with a leading 1 are carriers of the ε3ε4 heterozygote, and those with a leading 2 are carriers of the ε4ε4 homozygote.

2.3 | Analysis

CompG frequencies were assessed in the pooled sample of the older-aged subjects from WFCH, ADGC, LOADFBS, ADSP, and HispLOAD, and in the pooled sample of the younger subjects from FHS_C3 and CARDIA within ethnoracial groups.

AD risk was characterized by the effect (β) and odds ratio (OR) for carriers of individual and aggregated (defined in Section 3.2) CompGs. We used $p < 0.05$ as a significance level. Reference CompGs were explicitly stated. We employed the base R function glm for logistic regression. The models were adjusted for age, sex (except sex-stratified models), and study composition, defined by field centers (CHS), cohorts (FHS and HRS), and seven ADC centers (ADGC). No other adjustments have been made. We did not adjust for potential population clustering because the analyses were conducted within major ancestral groups separately, whereas the analysis of the role of more fine structures within these groups, if any, was beyond the scope of this paper.[36] The models were not adjusted for potential familial clustering because its role was minor at best. In addition, we presented the results of the analyses in independent non-family-based samples to ensure that the family design did not alter our findings.

The analyses were performed for men and women combined and separately. Additional analysis of age interaction with the CompGs was performed. First, we leveraged a common strategy to fit a model with a CompG by standardized age interaction term. This approach is useful for identifying a linear trend across ages. Because linear relationships may not necessarily hold in the case of age-related traits such as AD, we also evaluated AD risk in age-stratified samples. To make the interaction analysis more interpretable and robust, an age stratification analysis was performed for several cutoffs: 65, 70, 75, 80, and 85 years. To ensure robustness, we used cumulative samples, for example, a cutoff of 65 years stratified the samples into younger than 65 years and 65 years and older groups, a cutoff of 70 years included all subjects aged younger than 70 years and 70 years and older, and so forth. Adjustments were made the same as above.

The differences in the effects between groups defined by race/ethnicity, sex, and age were conveniently quantified using the chi-square statistic as follows[37]:

$$\chi^2 = (\beta_1 - \beta_2)^2 / \left(SE_1^2 + SE_2^2\right),$$

where β_1 and β_2 are the beta coefficients of the effects and SE_1 and SE_2 are their standard errors in two contrasted groups 1 and 2 (eg, men and women).

3 | RESULTS

3.1 | Linkage disequilibrium among SNPs and frequencies of CompGs

The selected rs429358, rs2075650, and rs12721046 SNPs were in weak to moderate linkage disequilibrium (LD) in BAs (r^2: 0.6% to 6.6%), H/Ls (r^2: 17.7% to 20.8%), and NHWs (r^2: 53.1% to 57.9%) (Table S2).

Complete major allele homozygote (000) was the most common CompG in the pooled samples of older cohorts of each ethnoracial group (AD-affected and unaffected subjects combined) with proportions of 52.0% in NHWs, 53.1% in H/Ls, and 42.9% in BAs (Table S1). This CompG was followed by different CompGs in these groups: 111 (21.4%) and 100 (6.5%) in NHWs, 100 (16.4%) and 111 (7.7%) in H/Ls, and 100 (26.9%) and 010 (11.1%) in BAs. The 000 CompG was also the most common within the ε3ε3 genotype in each ethnoracial group (Figure 1 and Table S1). Within the ε3ε4 genotype, minor alleles of rs2075650 and rs12721046 clustered more often with the ε4 allele in NHWs (79.8%) than in H/Ls (47.7%) or BAs (28.4%). The same relationship was observed within the ε4ε4 genotype, 95% in NHWs, 71.6% in H/Ls, and 40.4% in BAs. In young to mid-aged participants not affected by AD, CompG frequencies in NHWs and BAs resembled those in the older populations indicating, a marginal effect of survival selection (Table S1). Nevertheless, the role of the ε4 allele in younger populations, especially of H/L ancestry, requires more thorough analyses.[38,39]

3.2 | CompG-specific risks of AD for men and women combined

None of the ε3ε3-bearing CompGs were significantly associated with AD in either ethnoracial group (Table S3). The vast majority of the ε4-bearing CompGs were significantly associated with AD, except for the 101 CompG in H/Ls and 111 in BAs, although many of them were not common. We, therefore, constructed five aggregated ε4-bearing CompGs. Two of them included one (1XY) or two (2XY) copies of the ε4 allele and at least one minor allele of the rs2075650 or rs12721046 SNP. The other three included one or two copies of the ε4 allele and either (1) did not have minor alleles of rs2075650 and rs12721046 SNPs (100+200), (2) included complete heterozygous or minor allele homozygous genotypes of these two SNPs (111+222), or (3) were CompGs having at least one minor allele of rs2075650 or rs12721046 SNP (1XY+2XY) (Figure 2 and Table S3; refer to Figure 2 caption for detailed information on how aggregated CompGs are encoded).

AD risks in NHWs were consistently larger when the ε4 allele clustered with at least one minor allele of rs2075650 or rs12721046 than with major allele homozygotes of these two SNPs. The largest AD risks were observed for NHWs having two copies of the ε4 allele and minor allele homozygotes of both rs2075650 and rs12721046 SNPs ($\beta = 2.70$, OR = 14.84, $p = 1.53 \times 10^{-114}$ for 222 CompG) or at least one minor allele of those two SNPs ($\beta = 2.56$, OR = 12.87, $p = 1.94 \times 10^{-174}$ for 2XY CompG).

AD risk for the 2XY aggregated CompG in NHWs was significantly larger than that in H/Ls or BAs (Table S3). However, AD risks for NHWs, H/Ls, and BAs carrying two copies of the ε4 allele but no minor alleles of these two SNPs were about the same (Figure 2, 200). There were also no significant differences in the AD risks between NHWs and H/Ls carrying either the 100 or 100+200 CompG. For the other CompGs in Figure 2, AD risks were significantly larger in NHWs than in non-NHWs. AD risks for H/Ls and BAs did not differ significantly for all CompGs, except for 100, for which the risk was larger in H/Ls than in BAs.

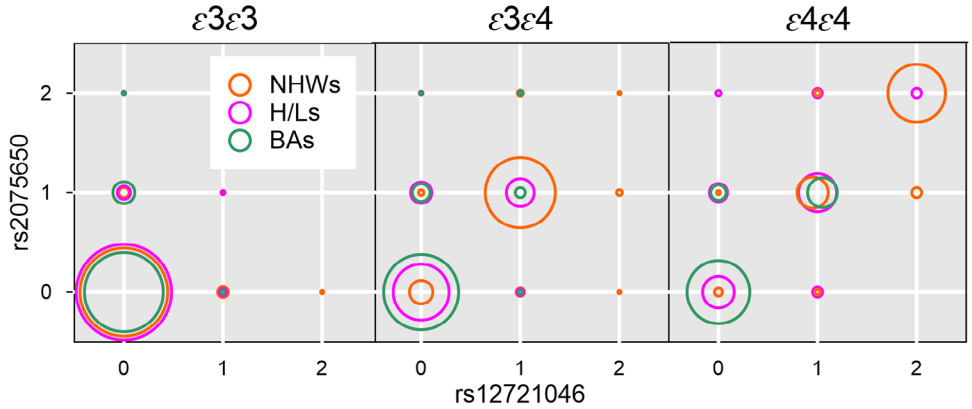


FIGURE 1 Bubble plot of frequencies of compound genotypes in Non-Hispanic White participants (NHWs), Hispanic/Latino participants (H/Ls), and Black American participants (BAs). On the x- and y-axes, the symbols 0, 1, and 2 indicate the dosage of minor alleles for *APOC1* rs12721046 and *TOMM40* rs2075650 SNPs. A larger radius represents a higher proportion of genotypes comprising rs12721046 and rs2075650 SNPs among carriers of the *APOE* ε3ε3, ε3ε4, and ε4ε4 genotypes, individually. Bubbles for NHWs and H/Ls carrying the ε4ε4 homozygote and having rs12721046 and rs2075650 heterozygotes are slightly offset to enhance resolution. Detailed numerical estimates are provided in Table S1. *APOE*, apolipoprotein E; SNP, single nucleotide polymorphism.

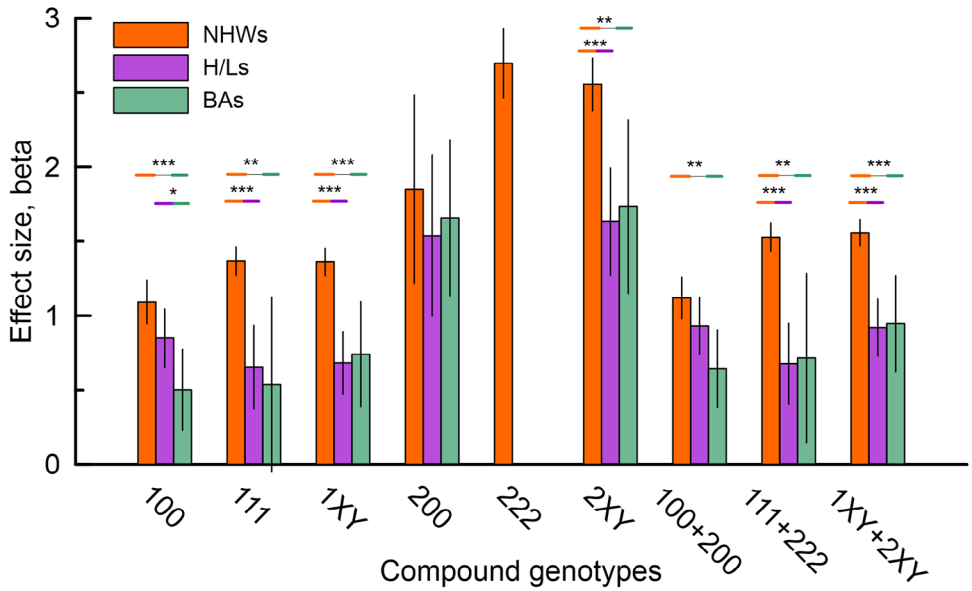


FIGURE 2 Associations of compound genotypes (CompGs) with Alzheimer's disease (AD) within different ethnoracial groups. Orange, purple, and green bars display the effect sizes of the associations of CompGs shown on the x-axis with AD in pooled samples of Non-Hispanic White participants (NHWs), Hispanic/Latino participants (H/Ls), and Black American participants (BAs), respectively. The sample composition is provided in Table 1 footnotes. CompGs are encoded by the dosage of minor alleles (0, 1, or 2) in each SNP ordered as rs429358 (*APOE*), rs2075650 (*TOMM40*), and rs12721046 (*APOC1*). The reference is the complete major allele homozygous genotype for three SNP (000). The symbols "X" and "Y" indicate aggregated CompGs; these symbols take values of 0, 1, or 2 but not simultaneously 0. The symbols 100+200, 111+222, and 1XY+2XY denote aggregated CompGs, which include one (leading 1) or two (leading 2) copies of the ε4 allele. Vertical lines depict the 95% confidence intervals. Statistical significance levels are indicated for differences between ethnoracial groups. Detailed numerical estimates are available in Table S3. *APOE*, apolipoprotein E; SNP, single nucleotide polymorphism. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

3.3 | The excess of the AD risks for selected CompGs for men and women combined

To quantify the effects of the rs2075650 and rs12721046 variants on the connections between the ε4 allele and AD, we evaluated the increased risks (ie, the excess) for individuals with CompGs that contained the minor alleles of the rs2075650 and/or rs12721046 SNPs compared to CompGs that did not include these minor alleles. The excess was evaluated in carriers of each CompG with one (111 and 1XY), two (222 and 2XY), and one or two (111+222 and 1XY+2XY) copies of the ε4 allele within each ethnoracial group (Tables S4 and S5, and Figure 3).

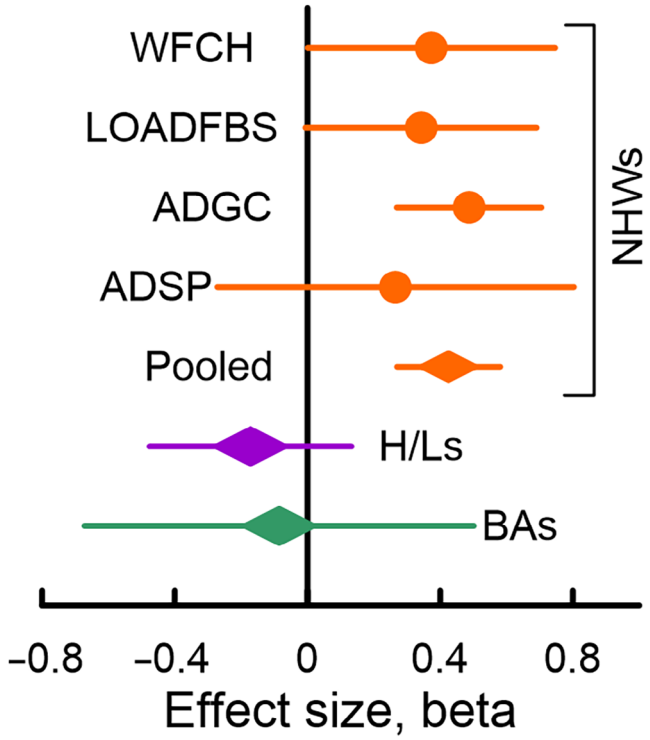


FIGURE 3 The excess of the risks of Alzheimer's disease (AD) within different ethnoracial groups. The AD risk excess is evaluated in Non-Hispanic White participants (NHWs; orange), Hispanic/Latino participants (H/Ls, purple), and Black American participants (BAs; green) carrying the higher-risk 111+222 compound genotype (CompG) compared to carriers of the lower-risk 100+200 CompG. This figure shows that NHWs, but not H/Ls or BAs, carrying one or two copies of the ε4 allele are at significantly higher AD risk when the ε4 allele clusters with minor alleles of rs2075650 and rs12721046 (111+222 CompG) than with major allele homozygotes for both these SNPs (100+200 CompG). Figure 2 caption provides more details on the encoding of CompGs. WFCH denotes the pooled sample of NHWs from FHS parental and offspring cohorts, CHS, and HRS. "Pooled" denotes the pooled sample of NHWs from WFCH, LOADFBS, ADGC, and ADSP cohorts. The composition of the pooled samples of H/Ls and BAs is provided in Table 1 footnotes. Horizontal lines show the 95% confidence intervals. Detailed numerical estimates are provided in Table S4 for NHWs and Table S5 for H/Ls and BAs. ADGC, Alzheimer's Disease Genetics Consortium; ADSP, Alzheimer's Disease Sequencing Project; CHS, Cardiovascular Study; FHS, Framingham Heart Study; HRS, Health and Retirement Study; LOADFBS, Late–Onset Alzheimer Disease Family Based Study; SNP, single nucleotide polymorphism.

NHWs are at significantly higher AD risk when the ε4 allele clusters with minor alleles of rs2075650 and/or rs12721046. The largest excess was for the 222 CompG contrasted with the 200 CompG ($\beta = 1.017$, OR = 2.76, $p = 8.95 \times 10^{-3}$). The most significant excess of 58% was for the 1XY+2XY CompG relative to the 100+200 CompG ($\beta = 0.458$, OR = 1.58, $p = 2.29 \times 10^{-9}$), which was about the same as that for the 111+222 CompG, ($\beta = 0.425$, OR = 1.53, $p = 8.16 \times 10^{-8}$). To emphasize the differences in the risks, the ε4-bearing CompGs with at least one minor allele of the rs2075650 or rs12721046 SNPs are referenced hereafter as the higher-risk CompGs, whereas those without these minor alleles are called the lower-risk CompGs.

The excess of the AD risks in NHW carriers of the higher-risk CompGs was consistently observed in each independent sample regardless of the family based or non-family-based design of the studies (Figure 3 and Table S4). No significant excess of AD risk for these CompGs was identified in H/Ls or BAs (Figure 3 and Table S5).

3.4 | Associations of CompGs with AD within each sex and ethnoracial group

To ensure the robustness of the estimates in smaller samples of each sex and ethnoracial group, our downstream analyses focused on carriers of the aggregated lower-risk 100+200 CompG and higher-risk 111+222 and 1XY+2XY CompGs.

Table 2 shows that AD risks in men are consistently smaller than in women for either CompG and for either ethnoracial group, except for nearly the same AD risks in NHW men and women carrying the lower-risk 100+200 CompG. However, none of the differences in the AD risks between sexes attained statistical significance.

Table 2 also shows that substantially, and significantly, larger AD risks were observed for NHWs than for H/Ls in each sex for carriers of the higher-risk CompGs, for example, $\beta = 1.591$ ($p = 2.32 \times 10^{-137}$) for NHW women versus $\beta = 0.813$ ($p = 3.97 \times 10^{-6}$) for H/L women carrying the 111+222 CompG ($p_{W\text{-}HL} = 3.24 \times 10^{-5}$ for the difference in the effects). For carriers of the lower-risk CompG, the AD risks were also larger in NHWs than H/Ls for each sex, but the differences in the effects were much smaller and not significant. NHW men and women had substantially larger AD risks than the same-sex BAs for each CompG, although the differences in the effects did not attain significance for the 111+222 CompG likely due to a smaller sample of BAs than NHWs. No significant differences in the AD risks between H/Ls and BAs were identified for each sex.

We further show that there were no differences in the significant excesses of the AD risks for the higher-risk CompGs over the lower-risk CompG between men and women, for example, $\beta = 0.421$ ($p = 3.57 \times 10^{-5}$) for women versus $\beta = 0.384$ ($p = 2.72 \times 10^{-3}$) for men carrying the 111+222 CompG ($p_{F\text{-}M} = 0.823$ for the difference in the effects between men and women) (Table S6). Neither significant excess within sex nor differences in the excesses between sexes were observed for H/Ls or BAs.

3.5 | Interactions between CompGs and age

Next, we examined whether age modulated the associations of the lower- and higher-risk CompGs with AD. Leveraging conventional analysis by fitting a model with a CompG-by-age interaction term (Table S7), we identified a significant interaction between 100+200 CompG and age in NHWs ($\beta = 0.186$, $p = 1.24 \times 10^{-2}$) and between 111+222 and age in H/Ls ($\beta = -0.362$, $p = 4.95 \times 10^{-3}$). In NHWs, this effect was strong and significant in women ($\beta = 0.243$, $p = 1.19 \times 10^{-2}$), but

TABLE 2 Associations of the aggregated compound genotypes with Alzheimer's disease (AD) within the same sex and ethnoracial group.

	Women							Men							χ^2 test
CompG	N_{AD}	N_{noAD}	β	SE	OR	*p-value*	χ^2 test	N_{AD}	N_{noAD}	β	SE	OR	*p-value*	χ^2 test	$p_{F\text{-}M}$
Non-Hispanic White participants (NHWs)															
000	1310	5696	Reference				$p_{W\text{-}HL}$	1061	4446	Reference				$p_{W\text{-}HL}$	
100+200	411	546	1.173	0.094	3.23	5.03E-36	2.63E-01	293	343	1.113	0.116	3.04	6.02E-22	8.58E-02	6.89E-01
111+222	1602	1618	1.591	0.064	4.91	2.32E-137	3.24E-05	1298	1218	1.469	0.073	4.35	2.15E-89	1.81E-05	2.12E-01
1XY+2XY	2101	2014	1.639	0.060	5.15	4.52E-166	1.37E-06	1681	1507	1.470	0.069	4.35	4.01E-102	2.45E-04	6.31E-02
Hispanic/Latino participants (H/Ls)															
000	420	913	Reference				$p_{HL\text{-}B}$	260	489	Reference				$p_{HL\text{-}B}$	
100+200	295	209	1.005	0.118	2.73	2.15E-17	2.46E-01	121	89	0.756	0.173	2.13	1.28E-05	1.99E-01	2.35E-01
111+222	88	107	0.813	0.176	2.25	3.97E-06	7.31E-01	50	76	0.451	0.226	1.57	4.60E-02		2.07E-01
1XY+2XY	263	231	0.983	0.122	2.67	9.75E-16	5.40E-01	153	136	0.818	0.164	2.27	5.66E-07	4.35E-01	4.21E-01
Black American participants (BAs)															
000	106	539	Reference				$p_{B\text{-}W}$	58	277	Reference				$p_{B\text{-}W}$	
100+200	152	307	0.773	0.162	2.17	1.87E-06	3.23E-02	60	177	0.380	0.236	1.46	1.07E-01	5.26E-03	1.71E-01
111+222	20	32	0.944	0.339	2.57	5.33E-03	6.07E-02								
1XY+2XY	89	134	1.125	0.198	3.08	1.36E-08	1.30E-02	31	58	0.545	0.309	1.73	7.75E-02	3.48E-03	1.14E-01

Note: CompGs encoded by the dosage of minor alleles (0, 1, or 2) in each SNP ordered as rs429358 (*APOE*), rs2075650 (*TOMM40*), and rs12721046 (*APOC1*). Refer to Figure 2 caption for detailed information on the encoding of CompGs. N_{AD} and N_{noAD} show the number of AD-affected and unaffected subjects, respectively; *p*-values from the χ^2 test $p_{W\text{-}HL}$, $p_{HL\text{-}B}$, $p_{B\text{-}W}$, and $p_{F\text{-}M}$ characterize the significance of the differences in the associations of CompGs with AD between NHWs and H/Ls, H/Ls and BAs, BAs and NHWs, and women and men, respectively.

Per HRS requirements, the values for cells with counts ≤10 subjects were suppressed.

Abbreviations: AD, Alzheimer's disease; BAs, Black American participants; CompG, compound genotype; H/Ls, Hispanic/Latino participants; NHWs, Non-Hispanic White participants; OR, odds ratio; SE, standard error.

weak and highly nonsignificant in men ($\beta = 0.049$, $p = 0.689$). The same relationship between sexes, but an opposite relationship for 111+222-by-age interactions was observed in H/Ls, that is, $\beta = -0.413$, $p = 1.21 \times 10^{-2}$ in women and $\beta = -0.249$, $p = 2.36 \times 10^{-1}$ in men. No significant interactions were identified in BAs, either in men and women combined or separately.

3.6 | Differential effects of CompGs on AD risks across ages in men and women combined

In line with the significant interaction of the lower-risk 100+200 CompG with age in NHWs (see Section 3.5), our age-stratified analysis further confirmed significantly smaller AD risks for this lower-risk CompG at younger ages than at older ages, for example, $\beta = 0.640$, $p = 3.99 \times 10^{-3}$ versus $\beta = 1.200$, $p = 3.52 \times 10^{-53}$ for ages younger than 70 (<70) years and 70 years and older (70+), respectively, with $p = 1.74 \times 10^{-2}$ for the difference in the effects (Figure 4A and Table S8). These risks increased with age, but the estimates show their potential decrease at ages older than 85 years.

Similar to the pattern observed in NHWs, H/L carriers of the lower-risk 100+200 CompG had a reduced AD risk at younger ages compared to older ages. This difference was significant ($p = 4.09 \times 10^{-2}$) at the age cutoff of 65 years (Figure 4G and Table S8). In contrast, for BAs having 100+200 CompG, we observed larger AD risks at younger ages (eg, $\beta = 0.922$, $p = 3.45 \times 10^{-2}$ for < 65 years), which gradually decreased and became negligible at 85+ years ($\beta = 0.013$, $p = 9.76 \times 10^{-1}$) (Figure 4N and Table S8). AD risks for NHWs carrying the lower-risk 100+200 CompG did not differ significantly from those for H/Ls at any age cutoff. However, they were significantly larger than those for BAs at around 65+ years, for example, $\beta = 1.152$, $p = 6.15 \times 10^{-54}$ for NHWs versus $\beta = 0.608$, $p = 2.70 \times 10^{-5}$ for BAs ($p = 8.36 \times 10^{-4}$ for the difference in the effects). The AD risks for H/Ls were also typically significantly larger than those for BAs for 65+ years.

Our age-stratified analysis showed an apparent nonlinear relationship in the associations of the higher-risk 111+222 CompG with AD across ages in NHWs as evidenced by significantly smaller AD risks at younger (<65 years) and older (85+ years) years than at the age cutoffs in between, that is, at 65 to 85 years (Figure 4D). This nonlinear pattern was even more pronounced for the higher-risk 1XY+2XY CompG (Figure S1A). These patterns clarify why there were no significant interactions between the higher-risk CompGs and age (see Section 3.5), as conventional interaction analysis is not suited to capture nonlinearity.

Aligned with the significant linear interaction between the higher-risk 111+222 CompG and age in H/Ls (see Section 3.5), Figure 4K and Table S8 show that AD risks are higher at younger ages, but they gradually decrease until at least 80 years (relatively small sample at ages of 85+ years prevents robust characterization of the AD risks at those ages). Interestingly, for carriers of the higher-risk 1XY+2XY CompG in BAs, we observe a similar nonlinear pattern as in NHWs, but not in H/Ls, although the differences in the effects between the older and younger groups did not attain significance at any specific age cutoff (Figure 4O and Table S8).

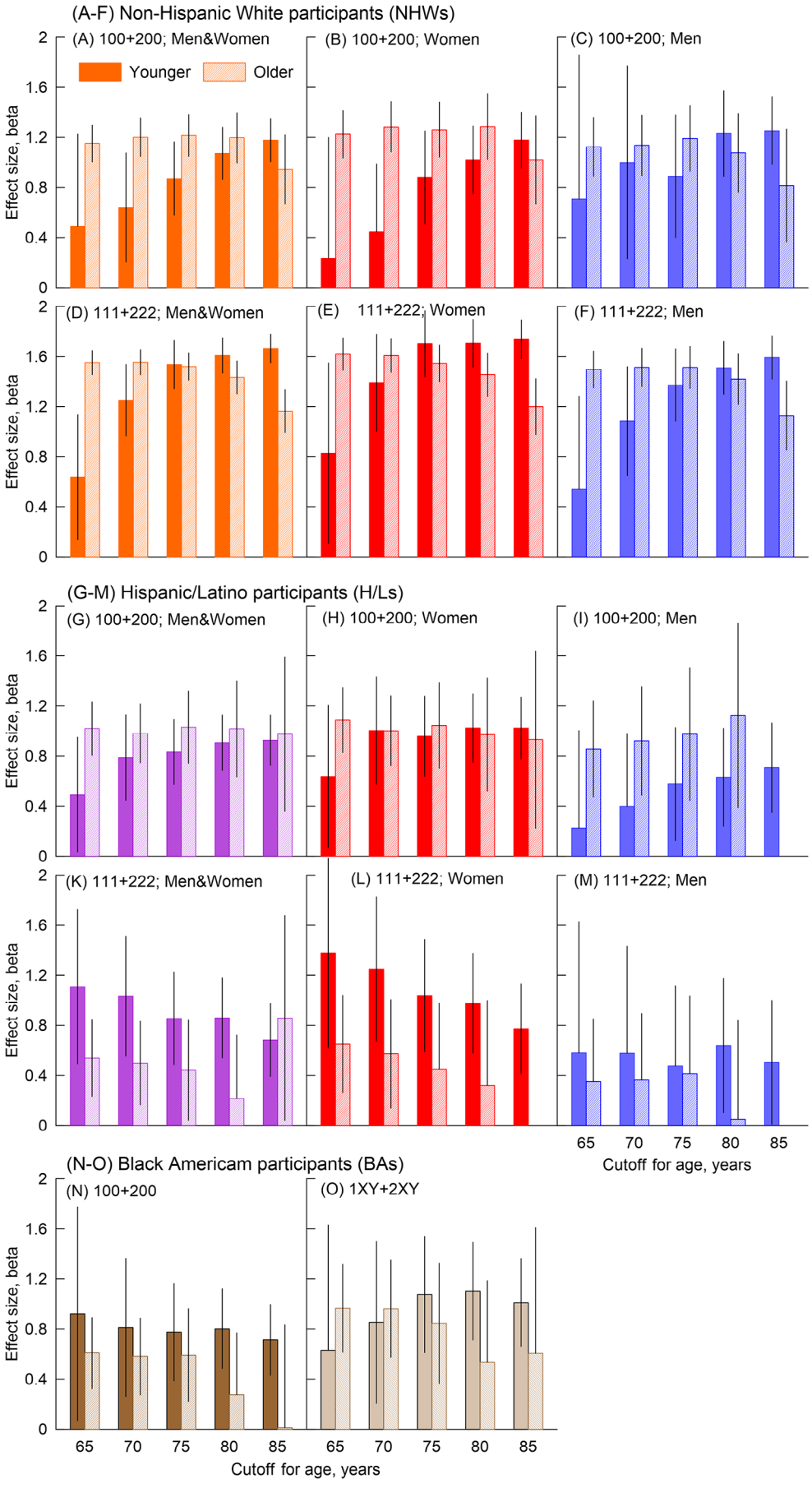
AD risks for carriers of the higher-risk CompGs in NHWs and H/Ls are comparable and do not differ significantly at ages younger than 70 years. They, however, become larger in NHWs than in H/Ls at older ages due to a steep downward trend in H/Ls (Figures 4D,K, S1A, S1D; and Table S8). AD risks for carriers of the higher-risk 1XY+2XY CompGs in NHWs and BAs are also comparable at younger ages (<65 years), while the difference in the risks is larger and significant at ages 65 to 85 years (Table S8). No significant differences in AD risks were identified between H/Ls and BAs at any specific age cutoff.

3.7 | Sex-specific impacts of CompGs on AD risks across ages in NHWs and H/Ls

For the lower-risk 100+200 CompG, our age-stratified analysis shows that AD risks for younger NHW women are small and nonsignificant ($\beta = 0.235$, $p = 0.633$ at < 65 years), but they increase and become significant at older ages (Figure 4B and Table S8). For NHW men, AD risks do not differ significantly between younger and older ages (Figure 4C). While there is a significant interaction between the lower-risk CompG and age for NHW women (eg, $p_{\text{Fyng-Fold}} = 4.37 \times 10^{-3}$ for the 70-year age cutoff), no significant interaction is observed for men (eg, $p_{\text{Myng-Mold}} = 0.741$ for the same cutoff) (Table S8). For H/L women, AD risks are significant and nearly the same for all ages (Figure 4H). For H/L men, AD risks are small and nonsignificant at younger ages ($\beta = 0.227$, $p = 0.566$ at < 65 years), but they increase and become significant at older ages (Figure 4I). No significant interactions between the lower-risk CompG and age were observed for them (Table S8). There are no significant differences in AD risks between NHW and H/L women. For men, the risks are significantly smaller in H/Ls than in NHWs at ages younger than 80 years—$\beta = 0.631$ ($p = 1.51 \times 10^{-3}$) and $\beta = 1.231$ ($p = 1.54 \times 10^{-12}$), respectively ($p = 2.34 \times 10^{-2}$ for the difference in the effects)—but they are nearly the same at 80+ years (Table S8).

The nonlinear relationship between each of the two higher-risk CompGs and AD with age holds for each sex in NHWs, as evidenced by significantly smaller AD risks at younger (<65 years) and older (85+ years) ages compared to the age cutoffs in between (Figures 4E,F; S1B; S1C; and Table S8).

At younger ages, H/L women carrying the higher-risk 111+222 CompG exhibit a high and significant AD risk, which decreases with age, although its interaction with age did not attain statistical significance (Figure 4L and Table S8). This downward age trend in H/L women explains the significantly smaller AD risks for them compared to NHW women at older ages (Figure 4L,E and Table S8). AD risks for H/L men carrying the higher-risk 111+222 CompG remain relatively flat across age, and they are significantly smaller than those for NHW men aged 65+ years (Figure 4F,M and Table S8). For H/Ls, the age pattern for the higher-risk 1XY+2XY CompG resembles that for the higher-risk 111+222 CompG, although the AD risks for H/L carriers of the 1XY+2XY CompG are larger and mostly significant.


(A-F) Non-Hispanic White participants (NHWs)
(A) 100+200; Men&Women
Younger
Older
(B) 100+200; Women
(C) 100+200; Men
(D) 111+222; Men&Women
(E) 111+222; Women
(F) 111+222; Men
(G-M) Hispanic/Latino participants (H/Ls)
(G) 100+200; Men&Women
(H) 100+200; Women
(I) 100+200; Men
(K) 111+222; Men&Women
(L) 111+222; Women
(M) 111+222; Men
(N-O) Black Americam participants (BAs)
(N) 100+200
(O) 1XY+2XY
Effect size, beta
0
0.4
0.8
1.2
1.6
2
65
70
75
80
85
Cutoff for age, years

No significant differences in AD risks between men and women are observed for either CompG in NHWs or H/Ls (Table S8). No age-stratified analyses were conducted in BA men and women separately due to insufficient samples.

4 | DISCUSSION

We examined associations of CompGs comprising the *APOE* ε4 allele and *TOMM40* rs2075650 and *APOC1* rs12721046 variants with AD across NHWs, H/Ls, BAs, men, women, and ages.

4.1 | Associations of the ε4-bearing CompGs with AD in the sex- and age-aggregated samples

We show that all ε4-bearing CompGs in NHWs and their vast majority in H/Ls and BAs are significantly associated with AD (Section 3.2). AD risks in NHWs are consistently higher for the ε4 allele carriers who have at least one minor allele of rs2075650 and/or rs12721046 (higher-risk CompGs) than their major allele homozygotes (lower-risk CompGs) (Section 3.3). These differences underscore a highly significant excess in AD risks for the higher-risk CompGs compared to the lower-risk CompGs in NHWs, for example, 59% ($p = 1.50 \times 10^{-9}$) for the 1XY+2XY CompG. This analysis did not show a difference in the AD risks for the ε4-bearing CompGs in H/Ls or BAs (Figure 3). These results highlight minor alleles of the *TOMM40* and *APOC1* variants as modulators of the ε4-associated AD risks in NHWs.

In line with previous research,[3–7] we show that AD risks are generally significantly larger in NHWs than in non-NHWs. Our analysis further clarifies that this elevated risk in NHWs is more prominent for carriers of the higher-risk than lower-risk CompGs (Figure 2). The AD risks for H/Ls and BAs were generally comparable for each CompG, except for a higher risk among H/Ls than BAs carrying the lower-risk 100 CompG.

Differences in the ε4-associated AD risks across ancestries are important for identifying modulators of the detrimental effects of the ε4 allele.[25,40] A study reported an AD-protective variant on chromosome 19q13.31 that could ameliorate the impact of the ε4 allele on AD risk in populations of African ancestries.[41] Our findings in NHWs highlight another perspective, that the ε4-AD association might be heightened by minor alleles of the *TOMM40* and *APOC1* variants. This suggests that the adverse effect of the ε4 allele can be mitigated by buffering its interactions with the *TOMM40* and *APOC1* genes. The latter highlights the importance of studying *TOMM40*- and *APOC1*-related mechanisms of AD pathogenesis.[42–46]

4.2 | The role of sex in the associations of the ε4-bearing CompGs with AD in the age-aggregated samples

In line with prior studies,[4,11–14,47] we found that the AD risks are consistently, although not significantly, smaller in men than in women regardless of ethnoracial groups and regardless of carrying the lower- or higher-risk CompGs, except for nearly the same AD risks in NHW men and women carrying the lower-risk 100+200 CompG (Table 2). These findings suggest potential sex-specific roles of the lower- and higher-risk CompGs in NHWs, as further exemplified by the results of the age-stratified analysis (see below). These results may explain why differences in the ε4-specific risks of AD between sexes might not be observed.[48]

The less severe impact of the lower-risk CompG in NHWs mainly drives the lack of significant differences in AD risk between same-sex NHWs and H/Ls, with a smaller difference in women (Table 2). Nonetheless, both BA men and women face lower AD risks compared to same-sex NHWs for each CompG. Sex-specific AD risks do not differ significantly between H/Ls and BAs.

4.3 | The roles of sex and age in the associations of the ε4-bearing CompGs with AD

Previous studies reported that age can modulate the impact of the ε4 allele on AD risks.[4,15–18] For example, a meta-analysis of 40 studies identified a smaller magnitude of the effect of the ε4 allele on AD risk after the age of around 70 years in NHWs, men and women combined and separately.[4] The authors also suggested a smaller impact of the ε4 allele on AD at older ages in a small sample of H/Ls (261 cases and 267 controls). Subsequent studies, primarily conducted in NHWs, generally indicated smaller effects of the ε4 allele on AD risk at older ages, although the results were not conclusive.[15]

Conventional interaction analysis (Section 3.5) reveals distinct impacts of the lower- and higher-risk CompGs on AD risks, and that

FIGURE 4 Associations of compound genotypes (CompGs) with Alzheimer's disease (AD) across ages for NHWs, H/Ls, and BAs. AD associations for the lower-risk 100+200 CompG for (A–C) NHWs, (G–I) H/Ls, and (N) BAs. AD associations for the higher-risk 111+222 CompG for (D–F) NHWs, (K–M) H/Ls, and (O) BAs. The reference is the complete major allele homozygous genotype for three SNPs, that is, 000. Figure 2 caption provides more details on the encoding of CompGs. The estimates for men and women combined are shown by (A,D) orange for NHWs, (G,K) purple for H/Ls, and (N,O) brown for BAs. The red color indicates associations in (B,E) NHW and (H,L) H/L women. The blue color indicates associations in (C,F) NHW and (I,M) H/L men. The x-axis displays age cutoffs, which were used to stratify the samples into two groups: "younger" (defined as those below the age cutoff, eg, <65 years; depicted with intense color) and "older" (defined as those at or above the age cutoff, eg, 65+ years; depicted with a lighter color). To ensure robustness, we employed cumulative samples, which means that all subjects younger than a given age cutoffe and all subjects at or above that age cutoff were included. The composition of the pooled samples of NHWs, H/Ls, and BAs is given in Table 1 footnotes. Vertical lines depict the 95% confidence intervals (CIs). The negative direction for CIs is not shown for nonsignificant estimates. Detailed numerical estimates are available in Table S8. BAs, Black American participants; H/Ls, Hispanic/Latino participants; NHWs, Non-Hispanic White participants; SNP, single nucleotide polymorphism.

these impacts differ in NHWs and H/Ls. Notably, in NHWs, this analysis yielded seemingly conflicting results with previous studies, indicating that the association of the lower-risk CompG with AD increases with age. This increase is attributed to women but not to men. Conversely, no significant interactions were identified between the higher-risk CompGs and age in NHWs, irrespective of sex. In contrast, in H/Ls, no significant interactions were identified between the lower-risk CompGs and age, regardless of sex. However, the associations of the higher-risk CompGs with AD decreased with age, primarily driven by the decrease in women. No significant interactions were observed in BAs.

Our age-stratified analysis (Sections 3.6 and 3.7) uncovered more intricate relationships between the lower- and higher-risk CompGs and AD across ages that could not be identified through conventional interaction analysis alone.

For NHWs, carriers of the lower-risk CompG exhibit reduced AD risks at younger ages, which increase afterward but may decrease at ages older than 85 years. Women benefit more than men as they have small and nonsignificant AD risks at ages younger than around 65 to 70 years. For the higher-risk CompGs, we uncovered nonlinear age patterns of AD risks. These patterns are characterized by significantly smaller AD risks both in men and women aged <65 and 85+ years compared to the ages between 65 and 85 years.

For H/Ls, the age pattern for the lower-risk CompG resembles an upward trend in NHWs. Notably, in contrast to NHWs, this trend is characteristic of H/L men, whereas for H/L women the age pattern is flat. The age-specific AD risks do not differ significantly in H/L and NHW women, but they are significantly smaller in H/L men than in NHW men at ages younger than 80 years and become nearly the same at 80+ years. For the higher-risk CompGs, AD risks for H/Ls are larger at younger ages and decrease afterward, becoming small and nonsignificant at 80 years. This decrease is driven by a decline in women. The risks for H/L men seem to be not sensitive to age. The results suggest that the higher-risk CompGs predominantly contribute to significantly smaller AD risks in H/Ls compared to NHWs, driven by lower AD risks for H/L men and women, primarily at older ages.

For BAs, AD risks for carriers of the lower-risk CompG are larger at younger ages and become negligible at 85+ years, resembling a downward trend in H/Ls carrying the higher-risk CompGs. For BA carriers of the higher-risk CompG, the AD-risk age pattern resembles that in NHWs. AD risks for BAs are significantly (except one cutoff) smaller than those for NHWs at 65+ years irrespective of CompGs. However, CompGs differentiate AD risks between BAs and H/Ls at 65+ years. Specifically, the risks are smaller (mostly significantly) for BAs than H/Ls carrying the lower-risk CompG, but they are similar for those carrying the higher-risk CompGs.

4.4 | Implications

The differential associations of the lower- and higher-risk ε4-bearing CompGs with amyloid beta 42 and tau biomarkers of AD pathology,[49] along with our present findings of the varying impacts of these CompGs on AD risks, suggest them as potential biomarkers of sex-, age-, and ancestry-specific AD pathology. These insights strengthen previous findings about the utility of the *APOE* ε4 and *TOMM40*-polyT variants as AD biomarkers.[43,50]

4.5 | Limitations

First, the samples of H/Ls and BAs are notably smaller compared to NHWs, particularly at 85+ years. This reflects a common challenge in research involving diverse ancestral groups. Second, because the data available to us did not include young to mid-aged H/Ls, we were unable to clarify whether the frequencies of CompGs are affected by survival selection. Third, while AD diagnosis in most studies followed the NINCDS-ADRDA criteria, AD cases in the CHS and HRS were identified through Medicare service use files. However, the limitation of a nonuniform definition of AD across studies is mitigated by the relatively small fraction of AD cases from CHS and HRS. Fourth, further analyses are required to explore the roles of the lower- and higher-risk CompGs as potential AD biomarkers, and their molecular and cellular mechanisms across sexes, ages, and different ancestries.

4.6 | Summary and conclusions

The absence and presence of the *TOMM40* rs2075650 and/or *APOC1* rs12721046 minor alleles in the ε4-bearing CompGs define lower- and higher-risk profiles for AD, respectively, in NHWs. These CompGs differentially impact AD risks across sexes, ages, and ancestries. For example, for NHW women carrying the lower-risk CompG, we observe an upward trend in AD risk with age starting from a small nonsignificant risk at ages younger than 65/70 years, while for H/L women, the AD-risk age pattern is flat. In contrast, for H/L women carrying the higher-risk CompGs, we observe a downward trend in AD risk with age, while in NHW women, AD risks change in a nonlinear manner with the risks being smaller at ages younger than 65/70 years and older than about 85 years compared to the ages in between.

This study presents compelling evidence that the absence and presence of the minor alleles of *TOMM40* rs2075650 and *APOC1* rs12721046 SNPs strongly modulate the influence of the ε4 allele on AD risk in a sex-, age-, and ancestry-dependent manner. These findings call for exploring the biological mechanisms underscoring the impacts of the lower- and higher-risk ε4-bearing CompGs on AD risks.

AUTHOR CONTRIBUTIONS

Alexander M. Kulminski conceived and designed the experiment and wrote the paper, Ethan Jain-Washburn and Ian Philipp prepared data, performed statistical analyses, and contributed to drafting the manuscript, Yury Loika and Elena Loiko prepared data, Irina Culminskaya prepared data and wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Author disclosures are available in the supporting information.

CONSENT STATEMENT

This study focuses on secondary analysis of existing data and does not entail the collection of data directly from human subjects. Access to the data was obtained upon approval by the Duke Health Institutional Review Board (IRB), and all analyses were conducted in accordance with the guidelines stipulated by the IRB.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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