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Original Article

Genetic Association Between Epigenetic Aging-Acceleration and the Progression of Mild Cognitive Impairment to Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, and previous studies have shown its association with accelerated aging. In this study, we hypothesized that single nucleotide polymorphisms (SNPs) that contributed to aging acceleration are also associated with the progression from mild cognitive impairment (MCI) to AD. By applying genetic correlation analysis and single-locus survival analysis, we investigated the associations between intrinsic- and extrinsic-epigenetic-age-acceleration (IEAA and EEAA) related SNPs and the progression time from MCI to AD dementia using the data of 767 MCI participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study and 1 373 MCI patients from the National Alzheimer's Coordinating Center (NACC) study. Genetic correlations were found between IEAA/EEAA and AD (positive for IEAA-AD and negative for EEAA-AD). We revealed that 70 IEAA and 81 EEAA SNPs had associations with the progression time from MCI to AD with Bayesian false-discovery probability ≤ 0.8 in the ADNI study, with 22 IEAA SNPs and 16 EEAA SNPs being replicated in the NACC study ($p < .05$). Polygenic risk score (PRS) analysis showed that EEAA PRS but not IEAA PRS was associated with AD progression and the trend of decreasing fusiform gyrus volume in 2 data sets. Risk models incorporating both EAA PRSs did not show any significant improvement in predictive accuracy. Our results revealed multiple genetic variants with pleiotropic effects on both EAA and AD, which suggested shared genetic architecture between epigenetic age acceleration and AD progression.

Keywords: Alzheimer's disease, Epigenetic aging, Mild cognitive impairment, Polygenic risk score, Survival analysis

Aging affects the functions of all organs of the human body and is one of the leading risk factors for many diseases, including Alzheimer's disease (AD) (1,2). Previous studies have revealed the detrimental relationship between accelerated brain aging and AD early-stage neurodegeneration (3,4). Several approaches have been reported to estimate brain age acceleration by using epigenetic data (DNAm age) of brain samples and imaging data (4–8). Epigenetic age acceleration (EAA), which is referred to as the difference between the estimated age by methylation data and the chronological age, is an effective biomarker for the prediction of multiple aging-related phenotypes, including telomere length, cancer, obesity, metabolic syndrome, post-traumatic stress disorder (PTSD), morbidity, and mortality (5,9–13).

There are 2 commonly used EAA measures: Horvath EAA and Hannum EAA (14,15). Horvath EAA is the predicted value of age based on DNA methylation levels of 353 CpG sites reported previously and is conserved across cell types (5). Intrinsic-epigenetic-age-acceleration (IEAA) is a derivative variation of Horvath EAA with adjustment for white blood cell composition and can be used as a biomarker of cell-intrinsic aging (16). In contrast, Hannum EAA is based on the DNA methylation levels at the 71 CpGs identified by Hannum et al. (17), and its variation called extrinsic-epigenetic-age-acceleration (EEAA) as it can track age-related changes in blood cells, which are supposed to be correlated with lifestyle and health-span-related factors (17). Several AD risk factors, including body mass index (BMI), cholesterol ratios, socioeconomic status, high blood pressure, and smoking behavior, were reported to modulate both EAA measures (18). Age-related methylation changes are also observed in AD susceptibility loci and may contribute to late-onset AD pathology (19). However, no significant association was found between AD genetic factors (AD family history, polygenetic risk score, and APOE E4 copies) and EAA (18). These results suggested that EAA may mainly mediate the effects of nongenetic factors involved in AD (20). In 2 recent studies, the Horvath EAA and EEAA were reported to be associated with a faster rate of cognitive decline in men with ages greater than 50 years old (21,22). However, the association between EAA and dementia is still in controversy. One previous study with 52 individuals with baseline aged between 55 and 65 reported a significant positive association between methylation age and dementia risk (23), while a negative correlation was found between 4 types of EAA and dementia risk in another population study with 488 participants aged above 79 (24). One recent study also showed that IEAA was not associated with cognitive impairment overall but only among women who developed coronary heart disease (25). Such inconsistency might be due to small sample size of these studies or the age difference between populations.

Single nucleotide polymorphisms (SNPs) have been proposed to test the causal relationship between complex traits, and multiple genome-wide association studies (GWASs) have been performed to identify genetic variants associated with aging-related changes in brain functions and structures as well as methylation patterns (7,8,14,26–29). Gibson et al. recently conducted a large scale GWAS study on EAA measured in peripheral blood or saliva of 13 493 unrelated individuals of European ancestry, and reported ten genetic variants associated with Horvath-based EAA, and 1 variant associated with the Hannum-based EAA (14). However, currently, there is no study to investigate the genetic correlation between EAA and AD progression, as well as the shared genetic variants of them. In this study, we hypothesized that EAA-related genetic variants would affect the progression from MCI to AD. To test this hypothesis, we investigated the genetic correlation between EAA and AD risk, as well as the associations between EAA-related SNPs and the progression time from mild cognitive impairment (MCI) to AD dementia by using the available GWAS data and clinical data from the AD Neuroimaging Initiative (ADNI), and replicating our findings using data from the National Alzheimer's Coordinating Center (NACC).

Population and Methods

Participants

Patients diagnosed with MCI at baseline or during follow-up were selected from the ADNI study (http://adni.loni.ucla.edu) and NACC, https://naccdata.org, of which the former was used as the discovery data set and the latter as a replication data set (30,31). In ADNI, 916 participants were diagnosed with MCI at baseline or any follow-ups. After merging the genotype data and clinical data, 767 participants remained for further analysis, and 294 participants were diagnosed with dementia with AD as the etiologic diagnosis during the follow-up period. In NACC, the genotyping data and clinical data were available for 1 373 MCI patients, of which 864 participants were diagnosed with AD etiology causes of dementia during the follow-up period.

The ADNI and NACC studies were approved by local institutional review boards, and all participants or participant's guardians provided written informed consent. Additional information about ADNI and NACC studies are available at http://www.adni-info.org and https://www.alz.washington.edu/WEB/study_pop.html, respectively.

Genotyping and Imputation

The GWAS data from the ADNI cohort were genotyped with the platforms of Illumina Human610-Quad, Illumina Human OmniExpress and Illumina Omni 2.5M on 1 674 MCI participants from ADNI 1, GO, and ADNI 2 phases, respectively (Illumina, Inc., San Diego, CA). We used SHAPEIT for phasing and performed imputation with minimac4 on the Michigan imputation server (https://imputationserver.sph.umich.edu) with the HRC reference panel (Version r1.1 2016) consisting of 64 940 haplotypes of predominantly European ancestry (32). For imputation, a set of high-quality SNPs were used: MAF > 0.01; call rate > 95%, Hardy–Weinberg equilibrium test $p > 10^{-6}$; allele frequency difference ≤ 0.20 between the sample data and the reference panel. For the NACC study, we downloaded the GWAS data for the 10 256 participants in NACC AD Centers 1–7, of which the genotyping was performed with the platforms of Human660W-Quad_v1_A, HumanOmniExpress-12v1_A/H, and humanomniexpressexome-8v1-2_a, respectively (https://www.alz.washington.edu/ADGC/GENOtype.html) (31). We then conducted genotyping quality control and imputation with the same procedure as used with the ADNI genotype data.

EAA/AD-Related SNP Selection and Association Analysis

For SNP-heritability analysis, we used the summary statistics of the IGAP AD GWAS (33) (https://www.niagads.org/datasets/ng00075) and 1 methylations aging acceleration GWAS-meta study (https://datashare.is.ed.ac.uk/handle/10283/3427) (14). For association analysis, we selected 275 716 and 277 045 SNPs associated with IEAA and EEAA, respectively, with $p < .05$, minor allele frequency ≥ 0.05, and consistent directions between the 2 studies in the published GWAS-meta study. We then extracted the imputation data of these SNPs for the ADNI data set and performed single-locus analysis for the association between these SNPs and AD progression. Bayesian false-discovery probability (BFDP) was used to control for multiple testing. SNPs with BFDP < 0.8 were chosen to be replicated in the NACC data set.

Statistical Methods and In Silico Functional Annotations

SNP-heritability (defined as the proportion of phenotypic variance explained by SNPs), heritability enrichments, and genetic correlations of IEAA, EEAA, and AD risk were estimated by using the aforementioned GWAS summary statistics with the LDAK model (http://dougspeed.com/) (34). The association between single SNPs

and the progression from MCI to AD was tested by using Cox proportional hazards regression with adjustment for age at baseline, sex, years of education, race, top 3 significant principal components (PCs) from the GWAS data, and the number of allele copies of APOE E2 and E4. The progression time from MCI to AD (in years) was calculated from baseline for patients with MCI at baseline or from the date of the first diagnosis of MCI to AD conversion. The endpoint event was the occurrence of AD. Censoring was based on the date of last visit or date of death. We selected SNPs with independent effects by applying linkage disequilibrium (LD) clumping (paired-wise $r^2 < 0.10$) and survival LASSO regression (regularized Cox regression) for those identified SNPs in the single locus analysis. We then used those independent SNPs and their corresponding effect sizes on EAA from previous GWAS to construct IEAA-PRS and EEAA-PRS with PRSICE-2 (https://www.prsice.info/). The standardized *z*-score of PRS (centering by mean and scaling by standard deviation [*SD*]) was used in the following analysis (35).

We performed log transformations for the CSF biomarkers (ABETA, Tau, and PTau). The volumes of 5 regions (ie, Ventricles, Hippocampus [HIPP], Entorhinal, Fusiform gyrus [FUS], and Middle temporal gyrus [MidTemp]) were represented as the percentage relative to the intracerebral volume. The correlations between PRSs and the longitudinal changes of these CSF and imaging biomarkers were investigated with a linear mixed model by including a random intercept and a random slope of time with adjustment for age, baseline, sex, years of education, race, top significant PCs, and the allele copies of APOE E2 and E4. In these analyses, the *survival* and *nlme* packages in R were used. All analyses were conducted with R (version 3.5.1) if not mentioned otherwise.

To elucidate the possible biological functions of SNPs in the final identified regions, we applied the online tool HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), based on the Encyclopedia of DNA elements (ENCODE) data, to perform functional annotation. We also performed in silico methylation quantitative trait loci (mQTL) analysis by using data from the mQTL database (http://www.mqtldb.org/cgi-bin/search.cgi), which includes the mQTL results at 5 different life stages in human blood (36), and expression quantitative trait loci (eQTL) with the data from BRAINEAC (http://www.braineac.org/), which includes the eQTL results of 10 different tissues (n = 130) from UK Brain Expression Consortium (UKBEC)(37).

For comparison, we also downloaded the European-ancestries meta-analysis GWAS summary statistics of 2 other epigenetic clocks (ie, DNAm PhenoAge and GrimAge from https://datashare.ed.ac.uk/handle/10283/3645) and tested the associations of their related SNPs (with $p < .05$ in the original GWAS results) with AD progression (38–40).

Results

Characteristics of the Study Populations

The workflow of the present study is depicted in Figure 1. Distributions of demographic and clinical variables are presented in Supplementary Table 1. In the univariate analysis, we found that age, ABETA, total tau, and ptau levels at baseline, APOE E2, and E4 alleles were all significantly associated with the time to progression to AD among MCI patients from the ADNI cohort (Supplementary Table 1, $p < .05$). The distributions of characteristics of the NACC data set (ie, age, sex, education years, race, and APOE E2/3/4 alleles) can be found in Supplementary Table 2. We found significant

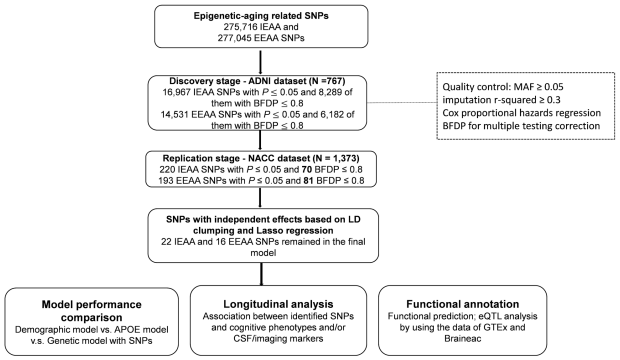

Figure 1. Study workflow. IEAA = intrinsic-epigenetic-age-acceleration; EEAA = extrinsic-epigenetic-age-acceleration; ADNI = the Alzheimer's Disease Neuroimaging Initiative study; NACC = the National Alzheimer's Coordinating Center studies; MAF = minor allele frequency; BFDP = Bayesian false-discovery probability; eQTL = expression quantitative trait locus; GTEx = The Genotype-Tissue Expression (GTEx) project.

associations between race, APOE genotypes and the progression time from MCI to AD. In the ADNI data set, about 91.3% participants are non-Hispanic Whites, and in the NACC data set, there are about 98% non-Hispanic Whites. It should be noted that the CSF biomarkers (ie, ABETA and tau/ptau) are unavailable from the NACC cohorts.

SNP-Heritability and Genetic Correlation of EAA and AD

To test if there exist genetic variants with pleiotropic effects on both EAA and AD, we first performed SNP-heritability and genetic correlation analysis by using their GWAS summary statistics after excluding loci with heritability greater than 1%. We found the estimates of SNP-heritability of AD, IEAA, and EEAA were 16% (*SD* = 1.4%), 26.5% (*SD* = 7.4%), and 26.3% (*SD* = 7.4%; Supplementary Table 3), respectively. Genetic correlation analysis showed that IEAA and AD are positively correlated with an estimate of 0.508 (*SD* = 0.343); while EEAA and AD had a negatively genetic correlation with an estimate of −0.179 (*SD* = 0.357). Enrichment heritability analysis showed that SNPs located in the 5 functional categories (ie, H3K27ac_Hnisz and H3K4me1_Trynka for active enhancers, Recomb_Rate_10kb for recombination hotspots, Nucleotide_Diversity_10kb for nucleotide diversity influenced by positive and negative selection, and CpG_Content_50kb for CpG sites) contributed the most heritability (≥10%) of IEAA, EEAA, and AD (Supplementary Figure 1). We also presented the results of other 8 enriched functional categories (ie, FetalDHS_Trynka [fetal DNase I hypersensitivity sites from the Trynka group], H3K27ac_PGC2 [histone H3K27ac sites from The Psychiatric Genomics Consortium], H3K4me3_Trynka [histone H3K4me3 sites from the Trynka group], Intron_UCSC [intron regions from the UCSC database], Repressed_Hoffman [transcription repressed regions from Hoffman et al. (41)], SuperEnhancer_Hnisz [super-enhancers reported by Hnisz et al. (42)], and TFBS_ENCODE [transcription factor binding sites from ENCODE]) with estimated heritability (≥10%) on either IEAA, EEAA, or AD.

By using the R package "TwoSampleMR," we performed Mendelian randomization analysis to test the causal associations between EAA and AD by using 1002 and 1046 independent SNPs (pair-wise $r^2 < 0.1$) associated with IEAA and EEAA with $p \leq .05$. The results of 3 methods (ie, MR Egger, weighted median, and

inverse variance weighted) were shown in Supplementary Table 4. It is interesting that the estimators with the methods of MR Egger and weighted median showed that IEAA has a positive effect while EEAA has a negative effect on the risk of late-onset AD. Such difference was consistent with the results of genetic correlation analysis with LDAK heritability model. However, all effects were minor and nonsignificant.

Survival Analysis of EAA-Related SNPs and the Progression Time of MCI to AD

We investigated the association between EAA-related SNPs and the progression time from MCI to AD by applying the Cox proportional hazard model. In the ADNI data set, we found 16 967 IEAA SNPs with $p \leq .05$ and 8 289 of them with BFDP ≤ 0.8, and 14 531 EEAA SNPs with $p \leq .05$ and 6 182 of them with BFDP ≤ 0.8. No SNP survived false discovery rate (FDR) correction (FDR ≤ 0.2). The overall association results of IEAA SNPs and EEAA SNPs were shown in Supplementary Figure 2A and B. We also performed an independent replication by using the NACC data set to replicate the associations identified in the ADNI data set. With BFDP < 0.8 for multiple testing corrections, we found that 70 IEAA SNPs, and 81 EEAA SNPs were replicated in the NACC data set (Supplementary Tables 4 and 5). We also extracted the GWAS data of 319 740 and 325 325 SNPs associated with 2 other epigenetic clocks (PhenoAge and GrimAge, respectively) with $p < .05$, and investigated their associations with AD progression time. We found 138 PhenoAge SNPs and 37 GrimAge SNPs passing multiple testing corrections (BFDP < 0.8) in both the ADNI and NACC data sets (Supplementary Tables 6 and 7).

By applying LD clumping and survival lasso regression analysis (Supplementary Figure 3A and B), we selected 22 IEAA SNPs and 16 EEAA SNPs with independent effects on AD progression. Two SNPs (ie, rs13172823 and rs79608085) overlapped between the 2 SNP sets. The association results of each one of those SNPs in both ADNI and NACC data sets are shown in Table 1. We also presented the independent effects of those SNPs with the adjustment for other SNPs in multivariable regression model (Supplementary Tables 8 and 9).

Survival Analysis of PRS and the Progression Time From MCI to AD

To test the combined effect of these identified SNPs (22 IEAA SNPs and 16 EEAA SNPs), we constructed IEAA PRS and EEAA PRS by using SNPs with independent effects, and tested their associations with the progression from MCI to AD. The effect sizes of the SNPs in the PRS were from the previous EAA GWAS and shown in Supplementary Table 10. As presented in Table 2, we found IEAA aging PRS contributed to a shorter progression time of AD from MCI in the ADNI data set ($p = .048$). However, this association was not replicated in the NACC data set ($p = .699$). The association between EEAA PRS and shorter AD progression time presented consistent effects in both the ADNI and NACC data set ($p = .036$ and .003, respectively). We then performed subgroup analysis by sex and APOE E4 status and found the EEAA PRS was associated with AD progression time in both subgroups of males and those without APOE E4 alleles in the ADNI and NACC data sets (Supplementary Table 11).

We also calculated the PhenoAge and GrimAge PRSs by using the identified SNPs in this study and their effect sizes from previous GWAS. We found only the PhenoAge PRS showed a significant association with AD progression time in the ADNI data set ($p = .002$; Supplementary Table 12). However, this association cannot be replicated in the NACC data set. No significance was found for the GrimAge PRS in both the ADNI and NACC data sets.

We evaluated the predictive accuracy of different models by using the Harrell's C-statistic (Table 3). As shown, the model including both number of APOE E2 and E4 alleles had a significantly increased C-statistic compared with the model that included only demographic variables in the ADNI data (0.636 vs 0.555, $p = 5.72E-07$). After adding IEAA and EEAA PRSs to the APOE model, there was a slight increase in the Harrell's C-statistic (0.641 and 0.639, respectively). However, models incorporating both EAA PRSs did not show any significant improvement in the predictive accuracy ($p = .216$ and .409 for the C-statistic comparison between IEAA model and APOE model, EEAA model and APOE model, respectively). Similar results were observed in the NACC data set.

We further tested the correlation between EAA PRS and the longitudinal changes of cognitive abilities (ie, MMSE, MOCA, CDRSB, FAQ, and ADAS11/13/Q4), and with biomarkers from CSF and imaging. As shown in Supplementary Table 13, we found significant correlations between IEAA PRS and the ABETA level in CSF, EEAA PRS, and the volume changes of the FUS ($p = .028$ and .032, respectively). However, no significant association was found for other cognitive phenotypes and biomarkers.

We also investigated the associations of SNPs from IEAA and EEAA with the cognitive phenotype (represented by CDR-SB), and found that 41 IEAA SNPs (Supplementary Table 14) and 163 EEAA SNPs (Supplementary Table 15) showed consistent correlations in both the ADNI and NACC studies.

Functional Annotation of the Identified SNPs

Functional annotations from HaploReg for these identified SNPs (ie, 22 IEAA SNPs and 16 EEAA SNPs) with independent effects are summarized in Supplementary Table 16. Of the 38 SNPs, there were 33 SNPs with potential effects on the promoter or enhancer activities, changing the binding activities of transcription factors, or with significant eQTL evidence. We also retrieved the cis meQTL results of blood samples from the mQTL database, and the eQTL results with multiple brain tissues from the BRAINEAC database for these SNPs. We found 5 EEAA SNPs (ie, rs7011999, rs4293193, rs112837743, rs2590959, and rs45473297) had a significant association with the methylation changes of 9 genes in blood tissues collected in middle age (Supplementary Table 17). Except for 2 genes, *CEBA2T3* and *SPAG4*, decreased methylation levels of the other 7 genes (ie, *ERICH1-AS1, FAM66C, CDT1, SCAND1, CPNE1, RBM12*, and *APP*) were observed to be correlated with the variant allele of these SNPs. The eQTL results showed that 11 SNPs had significant correlations with mRNA expression levels across 10 normal brain tissues (Supplementary Table 18). For example, 3 SNPs (ie, rs4972565, rs62193947, and rs2590959) showed a significant correlation with the mRNA expression of *CDCA7, MPP4/ NOP58*, and *ERGIC3/CPNE1/RBM12* in the tissues of the HIPP, thalamus (THAL), temporal cortex (TCTX), and intralobular white matter (WHMT), respectively.

Discussion

In this study, we tested the genetic correlation between EAA and the progression from MCI to AD. By using the GWAS summary statistics of AD and EAA, we found genetic variants in 5 functional categories contributed to the heritability of both IEAA, EEAA, and

Table 1. 22 IEAA and 16 EEAA SNPs Associated with AD Progression in Both ADNI and NACC Studies

						ADNI					NACC		
SNP	Gene	Chr	Pos (hg19)	A1*	A2*	beta†	se†	p^2	FDR	BFDP	beta†	se†	p^2
IEAA SNPs													
rs76670701	*PTGFRN*	1	117494279	G	A	0.380	0.118	.001	0.743	0.242	0.191	0.075	.011
rs57789409	*LAX1*	1	203728206	C	T	−0.255	0.103	.013	0.743	0.716	−0.181	0.072	.011
rs1441837	*LOC107984934/LINC01648*	1	30172441	T	C	−0.397	0.167	.017	0.743	0.699	−0.327	0.121	.007
rs10904575	*LARP4B*	10	890252	G	A	0.203	0.076	.007	0.743	0.666	0.137	0.052	.008
rs61905163	*SORL1*	11	121266600	G	C	0.452	0.162	.005	0.743	0.470	0.281	0.107	.008
rs7968623	*RP11-439H13.2/RASSF3*	12	64934166	G	C	0.289	0.119	.015	0.743	0.719	0.186	0.078	.017
rs7229512	*BRUNOL4/LOC388474*	18	36353977	A	G	0.393	0.186	.034	0.767	0.792	0.259	0.113	.022
rs62193947	*KIAA2012*	2	202945280	G	A	−0.408	0.140	.004	0.743	0.404	−0.220	0.094	.019
rs13407491	*LINC01800*	2	65095571	A	C	0.500	0.154	.001	0.743	0.202	0.217	0.091	.017
rs6772965	*NLGN1*	3	173945890	A	C	0.195	0.076	.010	0.743	0.725	0.150	0.049	.002
rs12509085	*FSTL5*	4	163048511	T	G	−0.347	0.137	.011	0.743	0.649	−0.233	0.091	.010
rs621356	*CTD-2247C11.2*	5	4931128	T	G	−1.633	0.662	.014	0.743	0.768	−1.104	0.371	.003
rs13172823	*KIAA0947*	5	5599662	C	G	−0.237	0.103	.021	0.743	0.794	−0.193	0.070	.006
rs9480937	*CD164*	6	109679832	G	A	0.431	0.201	.032	0.760	0.776	0.259	0.114	.023
rs77702925	*TBXT*	6	166630734	A	C	0.387	0.170	.023	0.743	0.745	0.354	0.114	.002
rs3892832	*RREB1*	6	7142488	G	A	−0.377	0.126	.003	0.743	0.361	−0.290	0.093	.002
rs10237105	*SHH*	7	155644686	T	A	−0.380	0.154	.013	0.743	0.664	−0.247	0.103	.017
rs10093080	*RNF19A/ANKRD46*	8	101509461	C	G	−0.251	0.097	.010	0.743	0.674	−0.179	0.064	.005
rs79608085	*SGCZ*	8	14681337	A	G	−0.361	0.126	.004	0.743	0.460	−0.197	0.083	.018
rs28874035	*SH2D4A*	8	19236870	T	A	0.298	0.130	.022	0.743	0.768	0.230	0.081	.004
rs2654014	*DLGAP2*	8	934539	G	A	−0.295	0.081	2.56E-04	0.743	0.083	−0.150	0.055	.007
rs11138749	*LOC107987084/LOC105376103*	9	83195753	A	T	−0.413	0.164	.012	0.743	0.637	−0.242	0.097	.012
EEAA SNPs													
rs147449969	*ATP2B4*	1	203720905	C	T	−0.476	0.129	2.14E-04	0.898	0.057	−0.207	0.085	.015
rs75269594	*PAK1*	11	77096603	G	A	−0.415	0.157	.008	0.941	0.565	−0.375	0.117	.001
rs4293193	*ZNF705A*	12	8307358	G	A	−0.215	0.079	.006	0.941	0.619	−0.160	0.051	.002
rs187776	*ALDH1A2*	15	58646011	G	C	−0.289	0.088	.001	0.941	0.224	−0.148	0.057	.009
rs112837743	*GALNS*	16	88902931	G	C	−0.328	0.144	.022	0.941	0.758	−0.352	0.091	1.02E-04
rs4972565	*CDCA7*	2	174314089	G	A	−0.316	0.126	.012	0.941	0.672	−0.243	0.080	.002
rs72853313	*LOC107985792/KLHL29*	2	23215521	C	T	0.404	0.174	.020	0.941	0.720	0.303	0.114	.008
rs2590959	*CNBD2*	20	34615776	T	C	−0.251	0.093	.007	0.941	0.615	−0.159	0.063	.011
rs45473297	*APP*	21	27541906	A	G	−0.247	0.085	.004	0.941	0.501	−0.143	0.056	.010
rs11924725	*EPHB1*	3	134505041	G	A	−0.363	0.158	.022	0.941	0.744	−0.293	0.099	.003
rs7707210	*LINC02039/GRAMD2B*	5	125334086	A	G	−0.380	0.145	.009	0.941	0.590	−0.357	0.112	.001
rs13172823	*KIAA0947*	5	5599662	C	G	−0.237	0.103	.021	0.941	0.794	−0.193	0.070	.006
rs4959195	*LINC01600/MYLK4*	6	2596934	C	T	−0.218	0.092	.017	0.941	0.780	−0.253	0.060	2.32E-05
rs17162828	*MGC4859/LOC105375149*	7	10299150	A	G	−0.270	0.076	4.13E-04	0.898	0.127	−0.142	0.056	.011
rs7011999	*DLGAP2*	8	1008446	A	G	−0.227	0.080	.005	0.941	0.553	−0.167	0.053	.001
rs79608085	*SGCZ*	8	14681337	A	G	−0.361	0.126	.004	0.941	0.460	−0.197	0.083	.018

Notes: IEAA = intrinsic-epigenetic-age-acceleration; EEAA = extrinsic-epigenetic-age-acceleration; AD = Alzheimer's disease; ADNI = the Alzheimer's Disease Neuroimaging Initiative study; NACC = the National Alzheimer's Coordinating Center studies; chr = chromosome; pos = position; BFDP = Bayesian false-discovery probability.

*A1 = effect allele, A2 = reference allele.

†Adjusted for age, sex, education, race, the copy numbers of APOE E2 and E4, and top significant principal components.

Table 2. Association Between PRSs and the Progression of MCI to AD in Both ADNI and NACC Data Set

	ADNI			NACC		
Variable	beta*	se*	*p**	beta*	se*	*p**
IEAA-PRS	0.123	0.062	.048	0.013	0.034	.699
EEAA-PRS	0.134	0.064	.036	0.11	0.037	.003

Notes: PRS = polygenic risk score; MCI = mild cognitive impairment; AD = Alzheimer's disease; IEAA = intrinsic-epigenetic-age-acceleration; EEAA = extrinsic-epigenetic-age-acceleration; ADNI = the Alzheimer's Disease Neuroimaging Initiative study; NACC = the National Alzheimer's Coordinating Center studies.

*Adjusted for age, sex, education, race, the copy numbers of APOE E2 and E4, and top significant principal components.

Table 3. Performance Evaluation of Models Without/With AD PRS Constructed With the Identified SNPs

	IEAA PRS	
Model	Harrell's C (95% CI)	*p*
ADNI data set		
Demographic model*	0.555 (0.521-0.590)	
APOE model†	0.636 (0.600-0.671)	5.72E-07‖
IEAA-PRS model‡	0.641 (0.606-0.676)	0.216¶
EEAA-PRS model§	0.639 (0.604-0.675)	0.409¶
NACC		
Demographic model*	0.524 (0.502 - 0.546)	
APOE model†	0.575 (0.554-0.597)	3.52E-04‖
IEAA-PRS model‡	0.575 (0.553-0.596)	0.435¶
EEAA-PRS model§	0.578 (0.557-0.600)	0.519¶

Notes: AD = Alzheimer's disease; PRS = polygenic risk score; IEAA = intrinsic-epigenetic-age-acceleration; EEAA = extrinsic-epigenetic-age-acceleration; ADNI = the Alzheimer's Disease Neuroimaging Initiative study; NACC = the National Alzheimer's Coordinating Center studies; CI = confidence interval.

*Demographic model including age, sex, education, and race.

†APOE model including age, sex, education, race, the copy numbers of APOE E2, and E4.

‡Genetic model including age, sex, education, race, the copy numbers of APOE E2/E4, and IEAA-PRS.

§Genetic model including age, sex, education, race, the copy numbers of APOE E2/E4, and EEAA-PRS.

‖Results of APOE model versus Demographic model.

¶Results of PRS model versus APOE model.

AD. In further survival analysis, we identified 22 IEAA SNPs and 16 EEAA SNPs that had significant independent effects on the progression time from MCI to AD in both the ADNI cohort and the NACC study after adjusting for demographic and clinical variables. Further PRS analysis revealed a combined effect of those SNPs on AD progression, longitudinal changes of ABETA in CSF, and the volume changes of the FUS. Functional annotation showed multiple SNPs with potential functions is regulation of mRNA expression. Our findings revealed multiple genetic variants with pleiotropic effects on EAA and AD, and suggested shared genetic architecture between these traits.

Previous studies have reported links between age acceleration and AD pathogenesis (ie, plaque, amyloid load, and cognitive decline) as well as AD-related environmental risk factors (ie, BMI, cholesterol level, blood pressure, and smoking behavior) (6,18). However, the underlying biological mechanisms remained unclear. In this study, we found the genetic correlations with AD risk are positive for IEAA and negative for EEAA. This finding reflects the 2 types of EAAs measure different aspects of aging. IEAA is a measure of biological aging that is independent of proportions of naive or senescent cytotoxic T cells, whereas EEAA captures the aging-related functional decline of the immune system (16). Previous studies have reported that the peripheral and central immune systems are dysregulated in AD (43). However, currently, there is no agreed-upon immune marker profile in the periphery for the severity of AD. This is partly due to the inconsistent evidence suggesting that some peripheral inflammatory markers peak in the early symptomatic stages of AD and decline in later stages.

In this study, we also identified a number of SNPs with potential functions associated with both EAA and the progression from MCI to AD. Of them, 5 EEAA SNPs also showed significant associations with the methylation levels of corresponding genes. One SNP rs45473297 is located in the second intron of a well-known AD-related gene—amyloid precursor protein (*APP*). The later encodes the amyloid-β precursor protein, which can be cleaved into amyloid β (Aβ) peptide, a major component of amyloid plaques (44). The aggregation of the Aβ peptide in the brain's parenchyma is the key event of AD pathology (44). One study had revealed abnormal CpG methylation and increased expression of the APP gene in AD brains (45). Tohgi et al. reported that hypo-methylation of the promoter region of *APP* might result in Aβ deposition in the cerebral cortex of the human brain (46), although this was not validated in another study with 6 familial AD patients (47). In this study, we found the variant allele A of SNP rs45473297 was associated with late-onset time of AD. In addition, the variant allele was also correlated with an increased methylation level of *APP* (probe ID: cg14414154) in the AD prefrontal cortex (http://mostafavilab.stat.ubc.ca/xqtl/snp_query/) and decreased mRNA level in brain substantia nigra and spinal cord in the GTEx database (https://www.gtexportal.org/home/snp/rs45473297). Such findings were consistent with the previously reported functions of APP on AD development. Significant eQTL results of this SNP were also found in several other brain tissues (ie, cerebellar cortex, substantia nigra, and THAL) with the UK Biobank data (http://www.braineac.org/). These results provided biological support for our association findings, which implied that the variant allele of this SNP may slow the progression from MCI to AD by altering the methylation and expression levels of *APP*. It should be noted that this SNP presented opposite effects in the blood cells, in which the variant allele A was correlated with decreased methylation level (probe ID: cg01286133) and increased mRNA expression level. Such discrepancy may be due to tissue-specificity of gene expression and will need to be investigated by future functional studies with large sample size.

We also found 1 EEAA SNP rs112837743, had a significant association with reduced time of AD progression and decreased methylation level of *CDT1*. *CDT1* is the DNA replication licensing gene, and the Cdt1 protein is a key factor in genome stability by linking cell cycle progression to DNA damage response (48). In response to DNA damage, Cdt1 accumulates to the sites of damage and is subsequently degraded, which is necessary to ensure proper cell cycle regulation, and involves in the regulation of AD pathogenesis mediated by CDT2 (49).

Another EEAA SNP, rs147449969, is located in *ALDH1A2*, a member of the aldehyde dehydrogenase (ALDH) super family. *ALDH1A1/2/3* plays an important role in the regulation of retinoic acid signaling by catalyzing the oxidation of retinal to retinoic acid (50). Dysregulation of retinoic acid results in oxidative

stress, neuroinflammation, and neurodegeneration leading to AD (51). In addition, we also found that the variant allele of 1 IEAA SNP rs62193947 is associated with decreased risk of AD progression. This SNP also had a significant correlation with the mRNA expression of multiple genes, that is, *BMPR2, CASP8, MPP4, NOP58, SNORD11, STRADB*, and *WDR12*. Of them, *CASP8* mediates extrinsic apoptosis and suppresses necroptosis involving programmed cell death, which is a major contributor to the AD neurodegenerative process (52–54). These shared genes and related pathways may contribute to the underlying mechanisms of AD progression and brain aging acceleration.

The FUS of the brain plays an important role in facial recognition (55). In AD, amyloid plaques and loss of synapses often co-occur in the FUS and within the ventral visual network regions (56). FUS is believed to decrease in size with increasing age. However, the correlation between aging and the volume changes of the FUS are inconsistent in published studies. Two studies reported significant negative correlations between age and volume changes of FUS, indicating volume decreases over time (57,58), while another study did not find such correlation in both healthy individuals and in patients with chronic schizophrenia (59). In this study, we found EEAA PRS had a significant association with the decreased volume of FUS, which provided indirect evidence for the correlation between aging and the volume change of the FUS.

There are several limitations of this study. First, although we have revealed significant associations between EAA-related SNPs and AD progression, and provided in silico functional evidence for those identified SNPs, the underlying molecular and cellular mechanisms are still unclear and need to be investigated in future functional studies. Second, the results of this study may not be generalized to other populations because they were mainly based on the data from European populations, and LD structure may differ by ancestry. However, future studies are warranted about the role of EAA SNPs on AD progression in individuals from diverse ancestries. Third, only a small proportion of EAA-related SNPs were found to be associated with the progression of AD from MCI, and a PRS based on these SNP did not show substantial contributions in the AD prediction model integrated with demographic variables and APOE status. Such limitation may be due to that IEAA and EEAA only have relatively small effect sizes on age-related outcomes. Levine et al. and Lu et al. reported 2 other measures of EAA (ie, DNAm PhenoAge and AgeAccelGrim) with strong associations with age-related conditions (39,40). These 2 types of EAA may provide a more accurate prediction of dementia than IEAA and EEAA as the former integrates additional information from plasma biomarkers and smoking. In this study, we investigated the association of genetic variants correlated with the 2 types of EAA with AD progression time from MCI and identified multiple genetic variants in the single locus analysis. However, no significance was found for the associations of both PRSs and AD progression. Such results suggested that these identified SNPs do not contribute substantially to the effect of these epigenetic aging on AD progression. In the future, more genetic variants with causal effects on EAA may be identified with the application of different statistical learning methods and used for EAA PRS calculation to improve the performance of AD risk prediction model. By using a 2-phase study design, we revealed that EEAA PRS but not IEAA PRS was associated with AD progression. Our results are consistent with the findings of previous studies using epigenetic data (22,25) and provide additional evidence for the correlation of EEAA markers and AD progression in MCI patients.

In summary, in the present study, we identified a genetic correlation between IEAA, EEAA, and the progression time from MCI to AD. These findings support shared genetic susceptibility between epigenetic age acceleration and AD progression. Future replication of these findings is warranted, and additional functional studies are necessary to reveal the biological framework underlying the observed associations.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

Author Contributions

Conception or design of the work: H.L. and S.L.; Acquisition and analysis of data: H.L. and S.L.; Preparation of manuscript: H.L.; Interpretation of data: All authors; Critical revision of the work for important intellectual content: All authors; Final approval of the version to be published: All authors.

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