RESEARCH ARTICLE

Association of Mitochondrial DNA Copy Number With Brain MRI Markers and Cognitive Function

A Meta-analysis of Community-Based Cohorts

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Abstract

Background and Objectives

Previous studies suggest that lower mitochondrial DNA (mtDNA) copy number (CN) is associated with neurodegenerative diseases. However, whether mtDNA CN in whole blood is related to endophenotypes of Alzheimer disease (AD) and AD-related dementia (AD/ADRD) needs further investigation. We assessed the association of mtDNA CN with cognitive function and MRI measures in community-based samples of middle-aged to older adults.

Methods

We included dementia-free participants from 9 diverse community-based cohorts with whole-genome sequencing in the Trans-Omics for Precision Medicine (TOPMed) program. Circulating mtDNA CN was estimated as twice the ratio of the average coverage of mtDNA to nuclear DNA. Brain MRI markers included total brain, hippocampal, and white matter hyperintensity volumes. General cognitive function was derived from distinct cognitive domains. We performed cohort-specific association analyses of mtDNA CN with AD/ADRD endophenotypes assessed within ±5 years (i.e., cross-sectional analyses) or

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Coinvestigators are listed at links.lww.com/WNL/C692.

Glossary

AD = Alzheimer disease; **ADRD** = AD-related dementia; **ARIC** = Atherosclerosis Risk in Communities; **CARDIA** = Coronary Artery Risk Development in Young Adults; **CHS** = Cardiovascular Health Study; **CN** = copy number; **FHS** = Framingham Heart Study; **GeneSTAR** = Genetic Study of Atherosclerosis Risk; **GENOA** = Genetic Epidemiology Network of Arteriopathy; **GOBS** = Genetics of Brain Structure and Function Study; **GWAS** = genome-wide association study; **HCHS/SOL** = Hispanic Community Health Study/Study of Latinos; **IVW** = inverse-variance weighted; **LD** = linkage disequilibrium; **MESA** = Multi-Ethnic Study of Atherosclerosis; **MR** = mendelian randomization; **mtDNA** = mitochondrial DNA; **qPCR** = real-time PCR; **SNV** = single nucleotide variation; **TOPMed** = Trans-Omics for Precision Medicine; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing; **WMH** = white matter hyperintensity.

5–20 years after blood draw (i.e., prospective analyses) adjusting for potential confounders. We further explored associations stratified by sex and age (<60 vs ≥60 years). Fixed-effects or sample size-weighted meta-analyses were performed to combine results. Finally, we performed mendelian randomization (MR) analyses to assess causality.

Results

We included up to 19,152 participants (mean age 59 years, 57% women). Higher mtDNA CN was cross-sectionally associated with better general cognitive function (β = 0.04; 95% CI 0.02–0.06) independent of age, sex, batch effects, race/ethnicity, time between blood draw and cognitive evaluation, cohort-specific variables, and education. Additional adjustment for blood cell counts or cardiometabolic traits led to slightly attenuated results. We observed similar significant associations with cognition in prospective analyses, although of reduced magnitude. We found no significant associations between mtDNA CN and brain MRI measures in meta-analyses. MR analyses did not reveal a causal relation between mtDNA CN in blood and cognition.

Discussion

Higher mtDNA CN in blood is associated with better current and future general cognitive function in large and diverse communities across the United States. Although MR analyses did not support a causal role, additional research is needed to assess causality. Circulating mtDNA CN could serve nevertheless as a biomarker of current and future cognitive function in the community.

Dementia is a major cause of disability and dependence worldwide.[1] Alzheimer disease (AD), the most common cause of dementia, accounts for 60%–80% of cases, and an estimated 6.2 million Americans aged 65 years and older were currently living with AD in 2021.[2] The pathologic processes contributing to AD and AD-related dementias (AD/ADRDs) begin several decades before the onset of symptoms,[3] complicating the development of effective treatments. Currently, there is a lack of disease-modifying therapies for the disease, and its characterization relies on expensive or invasive testing. Therefore, the identification of useful biomarkers for risk prediction continues to be a high priority for both early disease detection and understanding disease etiology.

The brain is the most energy-demanding organ in the body in a resting state, and normal neuronal functions rely almost exclusively on the cellular energy generated from glucose under aerobic conditions in the mitochondria.[4] Mitochondria are the powerhouse of most human cells, with glucose serving as the main substrate for producing the cellular energy except in red blood cells. Mitochondria have their own DNA, a 16.6-kb double-stranded DNA (mtDNA) molecule encoding 13 key oxidative phosphorylation proteins, 22 transfer RNAs, and 2 ribosomal RNAs.[5] The number of mtDNA copies in the cell, or mtDNA copy number (mtDNA CN), correlates with the number of mitochondria and therefore the cellular energy-generating capacity and metabolic status.[6] Because of their high-energy demand, neurons and synapses may contain several tens of thousands of copies of mtDNA.[7] Whereas direct assessment of mtDNA within the brain is not possible, mtDNA measured from whole blood provides a global estimate of mitochondrial function that can provide insight into brain mtDNA status.

Neuroimaging studies suggest the presence of defects in glucose metabolism in the brain of persons at risk of cognitive decline and AD/ADRD long before the onset of clinical symptoms.[8-10] Therefore, it is biologically plausible that global changes in mitochondria as measured by mtDNA CN could be associated with preclinical changes in people who later develop AD. A few studies have investigated the link between mtDNA CN and neurologic disorders including dementia. A significant reduction of mtDNA CN has been reported in patients with Parkinson disease in both peripheral blood and tissue from the midbrain (substantia nigra pars compacta).[11] Another study based on Danish cohort surveys found a correlation between higher whole-blood mtDNA CN, derived from real-time PCR (qPCR), and higher cognitive composite scores in 870 adults aged 75 years or older.[12] A recent study in the UK Biobank reported that higher whole-blood mtDNA CN, derived from whole-exome sequencing (WES), was related to a lower prevalence and incidence of a

composite of neurodegenerative diseases including non-AD dementias, AD, and Parkinson disease.[13] Finally, a recent study reported up to 14% lower mtDNA CN in brain tissue from patients with AD vs controls and associations with tau and TAR DNA-binding protein 43 pathology.[14] However, little information is available on the association of mtDNA CN derived from whole-genome sequencing (WGS) with dementia endophenotypes preceding disease onset in a diverse sample. Importantly, WGS provides the most accurate method to estimate mtDNA CN compared with WES or qPCR.[15]

In this work, we aimed to investigate the association between WGS mtDNA CN measured in whole blood and AD endophenotypes in diverse and well-characterized community-based cohorts from the United States as part of the TransOmics for Precision Medicine (TOPMed) program.[16] We leveraged the full extent of available data by exploring associations with temporally recent and more distant endophenotypes including general cognitive function and MRI markers of atrophy, specifically total brain and hippocampal volumes, and cerebral small vessel disease, specifically white matter hyperintensity (WMH) volume. Finally, we characterized these associations stratified by age and sex strata, 2 biologically relevant factors that affect mtDNA CN and the onset of AD/ADRD.

Methods

Study Design

The TOPMed Program, an initiative supported by the National Heart, Lung and Blood Institute, aims to understand risk factors for heart, lung, blood, and sleep disorders by measuring WGS and multiomic data in well-characterized cohorts.[16] In this work, we include data from 9 TOPMed cohorts that have previously collected MRI and/or cognitive outcomes: the Atherosclerosis Risk in Communities (ARIC) study, the Coronary Artery Risk Development in Young Adults (CARDIA) study, the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), the Genetic Study of Atherosclerosis Risk (GeneSTAR), the Genetic Epidemiology Network of Arteriopathy (GENOA) study, the Genetics of Brain Structure and Function Study (GOBS), the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), and the Multi-Ethnic Study of Atherosclerosis (MESA). The design of each cohort is briefly described in eAppendix 1 (links.lww.com/WNL/C693); extensive details have been published elsewhere.

Figure 1 presents the overall design of the study. As whole-blood mtDNA CN changes over time,[17] we created 2 periods of analysis based on the timing of the blood draw used to derive mtDNA CN and the timing of measurement of the neurologic outcomes. The first period, used for cross-sectional analyses, includes MRI and cognitive outcomes assessed within 5 years before or after the blood draw. The second period, used for prospective analyses, considers outcomes assessed more than 5 and up to 20 years after the blood draw.

We excluded participants with prevalent dementia and stroke and, if the information was available, those with incidental MRI findings impeding an accurate estimation of brain volumes (i.e., tumor and multiple sclerosis). We also excluded participants outside the predefined time windows of analysis as described above. Details on the number of participants excluded at each stage are presented in eTables 1 and 2 (links.lww.com/WNL/C693).

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent for genetic studies was provided by all study participants. The protocols for WGS were approved by the institutional review boards of the respective participating institutions (eAppendix 1, links.lww.com/WNL/C693).

Estimation of mtDNA CN

WGS was performed with DNA extracted from whole blood by the TOPMed sequencing centers. Coverage was defined as the number of reads mapped to a given nucleotide in the reconstructed sequence.[18] The average coverage for the whole genome was ~39-fold across samples. The average mtDNA CN per cell was estimated as twice the ratio of mtDNA average coverage to the nuclear DNA average coverage. The TOPMed Informatics Research Center jointly estimated mtDNA CN across participants in TOPMed cohorts using the fastMitoCalc program included in the mitoAnalyzer software.[18] More details of WGS are included in eAppendix 1 (links.lww.com/WNL/C693).

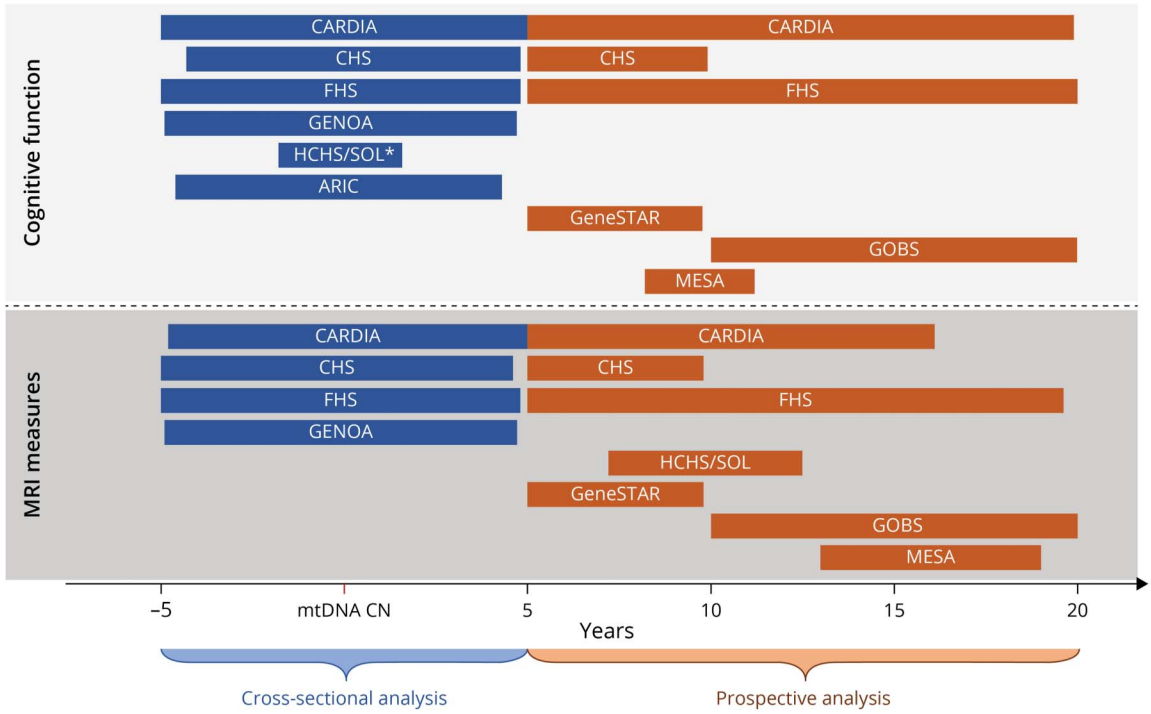
General Cognitive Function

Because each cohort tested participants using different neuropsychological batteries, we opted to study a previously harmonized measure of general cognitive function.[19] Briefly, each cohort selected at least 3 cognitive tests, whenever possible assessing distinct cognitive domains. After verification that all the tests had the same directionality (i.e., higher scores representing better cognitive performance), individual cohorts applied principal component analysis and derived a measure of general cognitive function from the first unrotated principal component. The scores were later standardized (mean = 0 and SD = 1). eTable 3 (links.lww.com/WNL/C693) details the cognitive tests used to derive the phenotype in each cohort.

MRI Measures

The MRI markers included total brain, hippocampal, and WMH volumes. Image acquisition and segmentation procedures varied by cohort and are summarized in eTable 4 (links.lww.com/WNL/C693). Briefly, structural measures of total brain and hippocampal volumes were segmented from T1-weighted images and derived using automated segmentation protocols.[20,21] In most cohorts, WMH volumes were

Figure 1 Study Design and Data Contribution by Cohort



The x-axis is the time interval in years between the blood draw and cognition/MRI measures. *Blood draw and cognitive function assessment were conducted at the same time in the HCHS/SOL. ARIC = Atherosclerosis Risk in Communities study; CARDIA = Coronary Artery Risk Development in Young Adults study; CHS = Cardiovascular Health Study; CN = copy number; FHS = Framingham Heart Study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GENOA = Genetic Epidemiology Network of Arteriopathy Study; GOBS = Genetics of Brain Structure and Function Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; mtDNA = mitochondrial DNA.

segmented using T2/fluid-attenuated inversion recovery and quantified using fully automated methods.[22] The GOBS used T1-weighted sequences to derive white matter hypointensities volumes, an alternative way to measure these lesions using FreeSurfer (hereafter grouped as WMH), and the CHS used a validated, semiquantitative visual rating scale accounting for head size to derive WMH burden in an ordinal scale of 0–9. Total intracranial volume was segmented from T1-weighted sequences and derived from different automated methods.[23,24] To account for differences in head size, brain volumes are expressed as a percentage of total intracranial volume.

Covariates

Several covariates measured close to the time of the blood draw were considered for adjustment, including age at MRI/cognitive evaluation, sex, race/ethnicity, educational level, time interval between blood draw and MRI/cognitive evaluation, cohort-specific variables, blood cell counts, and cardiometabolic traits. Cohort-specific variables, such as site or family relationships, were adjusted appropriately based on the study design of each cohort. Because previous studies have reported associations of white blood cell count, blood differential count, and platelet count with mtDNA CN,[25,26] we adjusted for these blood cell counts in secondary models if this information was available in the cohort as described in eTable 5 (links.lww.com/WNL/C693). Cardiometabolic traits included obesity, hypertension, diabetes, and hyperlipidemia assessed at the time of the blood draw. We defined obesity as body mass index ≥30 kg/m^2. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or any use of antihypertensive medications. Diabetes was defined as fasting blood glucose ≥126 mg/dL or any current use of medications used to treat diabetes. Hyperlipidemia was defined as fasting total cholesterol ≥200 mg/dL, triglyceride ≥150 mg/dL, or use of any lipid-lowering medications.

Statistical Analyses

We regressed mtDNA CN on age, age squared, and blood collection year reflecting batch effects to obtain residuals,[17] which were later standardized (mean = 0, SD = 1). Participants exceeding 5 SD units of mtDNA CN residuals were deemed as outliers and excluded from analyses. Standardized mtDNA CN residuals were used as the independent variable in all statistical analyses. WMH volumes were log transformed to normalize their skewed distribution.

We performed cohort-specific analyses to assess the associations between mtDNA CN and neurologic outcomes. Our primary model (model 1) was adjusted for age at MRI/cognitive evaluation, sex, the time difference between blood draw and MRI/cognitive evaluation, and race/ethnicity and/or any cohort-specific covariates if applicable. Secondary

models were additionally adjusted for blood cell counts when available (model 2) and cardiometabolic traits (model 3). Furthermore, we conducted a sensitivity analysis adjusting for *APOE4* status (i.e., presence of at least 1 e4 allele vs none) in addition to model 1 covariates (model 4). When modeling general cognitive function, we additionally adjusted for educational level in all models.

We hypothesized that there was a common effect of mtDNA CN in all studies. Therefore, we performed fixed-effects inverse-variance meta-analyses to combine cohort-specific association results. Because WMH was assessed both volumetrically and using a rating scale, we applied an optimally weighted *Z*-test method to combine *p* values of WMH across all cohorts.[27]

Stratified Analyses

Because the mtDNA CN level differs by sex and declines rapidly after 60–65 years of age,[16] we performed stratified analyses by sex and age strata (<60 and ≥60 years) using the same strategy described above. In age-specific analyses, standardized mtDNA CN residuals were obtained by regressing mtDNA CN on sex and blood collection year. In sex-specific analyses, we regressed mtDNA CN on age at blood draw, age squared, and blood collection year. Residuals were then standardized in each cohort. In addition, considering the diversity of the sample, we also performed race/ethnicity stratified analyses. Meta-analyses with fixed-effects inverse-variance method were performed to combine results from stratified analyses.

Mendelian Randomization Analyses

To investigate whether mtDNA CN had a causal effect on AD/ADRD outcomes with significant findings, we performed 2-sample mendelian randomization (MR) analyses with the TwoSampleMR R package.[28] The instrumental variables were independent single nucleotide variations (SNVs [formerly SNPs]) with linkage disequilibrium (LD) $r^2 < 0.001$ from a recent large genome-wide association study (GWAS) of mtDNA CN.[29] SNVs were pruned using the PLINK clumping method with an LD clumping window of 10,000 kb. This clumping method interacts with OpenGWAS API, which houses LD reference panels for the 5 superpopulations in the 1000 Genomes reference panel.[28] The general cognitive function–associated SNVs were obtained from a previous GWAS.[19] After LD pruning and removing SNVs that were palindromic and known to be associated with AD (rs7412), 76 SNVs served as instrumental variables in the MR analyses. The causal effect of each SNV was estimated by the Wald method.[30,31] The inverse-variance weighted (IVW) method under a fixed-effects model was used to combine the causal effect estimates for each SNV.[32,33] To assess for potential horizontal pleiotropy and outliers, we also applied other more robust MR methods, such as MR-Egger, MR-PRESSO, and weighted median. Finally, we used a power calculation procedure designed for the general 2-sample MR approach to investigate the power of our MR methods.[34]

Data Availability

Deidentified data included in this manuscript can be requested by qualified investigators through dbGaP using TOPMed and study-specific accession numbers ncbi.nlm.nih.gov/gap/advanced_search/?TERM=topmed.[16]

Results

Characteristics of Study Participants

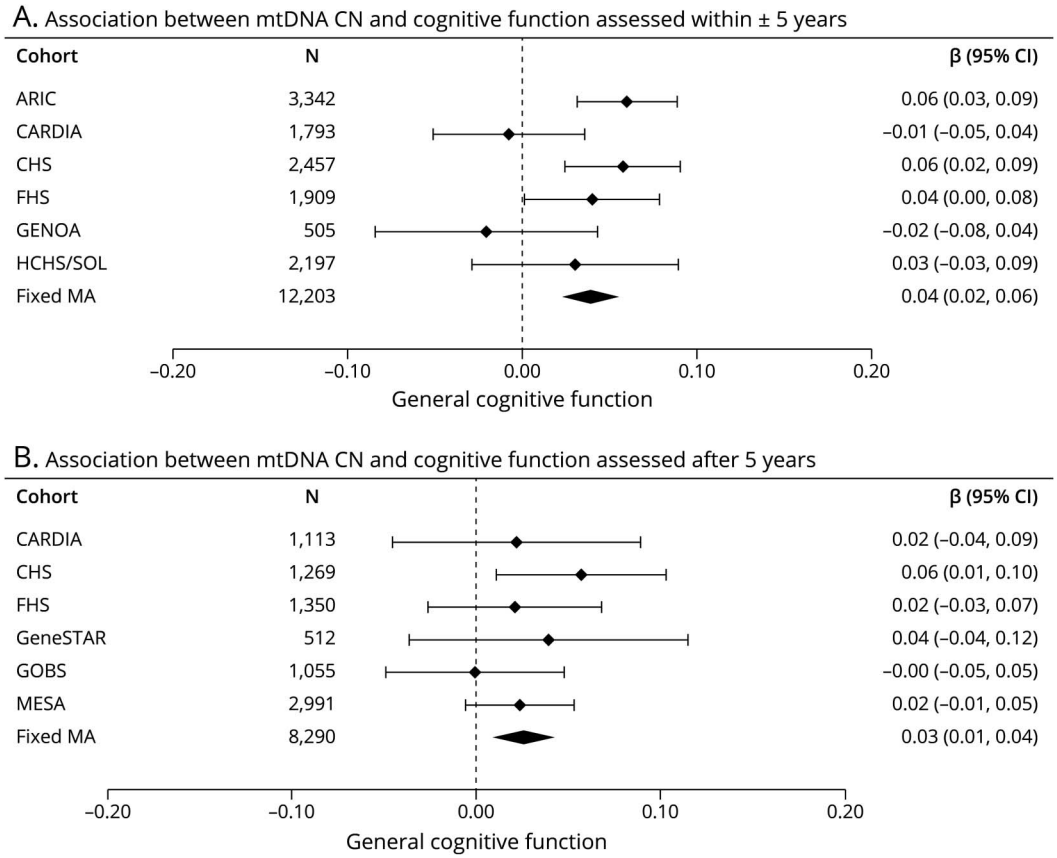
Depending on the analysis, we included a different number of cohorts with available data (Figure 1). An extended description of population characteristics is included in eTables 6 and 7 (links.lww.com/WNL/C693). Overall, the study included up to 19,152 participants from 9 cohorts contributing to cross-sectional and prospective analyses, including 11,208 non-Hispanic White, 3,653 Black, 4,147 Hispanic, and 144 Chinese Americans. On average, 57% of participants were women, and the mean age at blood draw was 59 years (range: 20–96 years, 7,523 participants over 60 years of age). As expected, mtDNA CN was lower in older participants and in men than in women. The average general cognitive function ranged from 0.07 to 0.10 across 8 cohorts. The average total brain volume ranged from 951 to 1,152 cm^3 across 9 cohorts, and that of hippocampal volume ranged from 3.3 to 10.5 cm^3 across 9 cohorts. The median values of WMH volume assessed quantitatively ranged from 0.7 to 7.9 cm^3 across 7 cohorts.

Association of mtDNA CN With General Cognitive Function

Primary cross-sectional meta-analyses showed that each SD unit increase in mtDNA CN was significantly associated with better general cognitive function (β = 0.039; 95% CI 0.023–0.055; n = 12,203; Figure 2A). Adjusting for blood cell counts in addition to the covariates in the primary model, we found that the association between mtDNA CN and general cognitive function was maintained but slightly attenuated (β = 0.035; 95% CI 0.015–0.055; n = 9,146). Adjusting for diabetes, hypertension, hyperlipidemia, and obesity in addition to the covariates in the primary model also led to a slightly attenuated result (β = 0.035; 95% CI 0.019–0.052; n = 11,462; eTable 8, links.lww.com/WNL/C693).

In the prospective analyses, we investigated the association of the baseline (i.e., at the time of blood draw) mtDNA CN with general cognitive function assessed within a window of 5–20 years after blood draw for WGS in a total of 8,290 participants. We found that each SD unit increase in mtDNA CN was significantly associated with better prospective general cognitive function (β = 0.026; 95% CI 0.007–0.045; n = 8,290; Figure 2B; eTable 9, links.lww.com/WNL/C693). Five of the 6 cohorts showed consistent directionality for the prospective association between baseline mtDNA CN with prospective general cognitive function. Additional adjustment for *APOE4* genotype in sensitivity analyses revealed virtually unchanged results compared with the primary model in both

Figure 2 Forest Plots of the Association of mtDNA CN With General Cognitive Function per Cohort and Meta-analysis Results



Covariates included age, sex, batch effect, self-reported race/ethnicity, the time between blood draw and cognitive evaluation, cohort-specific variables, and education. β is the estimated difference in standardized general cognitive function score per SD unit increment in mtDNA CN. (A) Cross-sectional analyses. (B) Prospective analyses. ARIC = Atherosclerosis Risk in Communities study; CARDIA = Coronary Artery Risk Development in Young Adults study; CHS = Cardiovascular Health Study; CN = copy number; FHS = Framingham Heart Study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GENOA = Genetic Epidemiology Network of Arteriopathy Study; GOBS = Genetics of Brain Structure and Function Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; mtDNA = mitochondrial DNA.

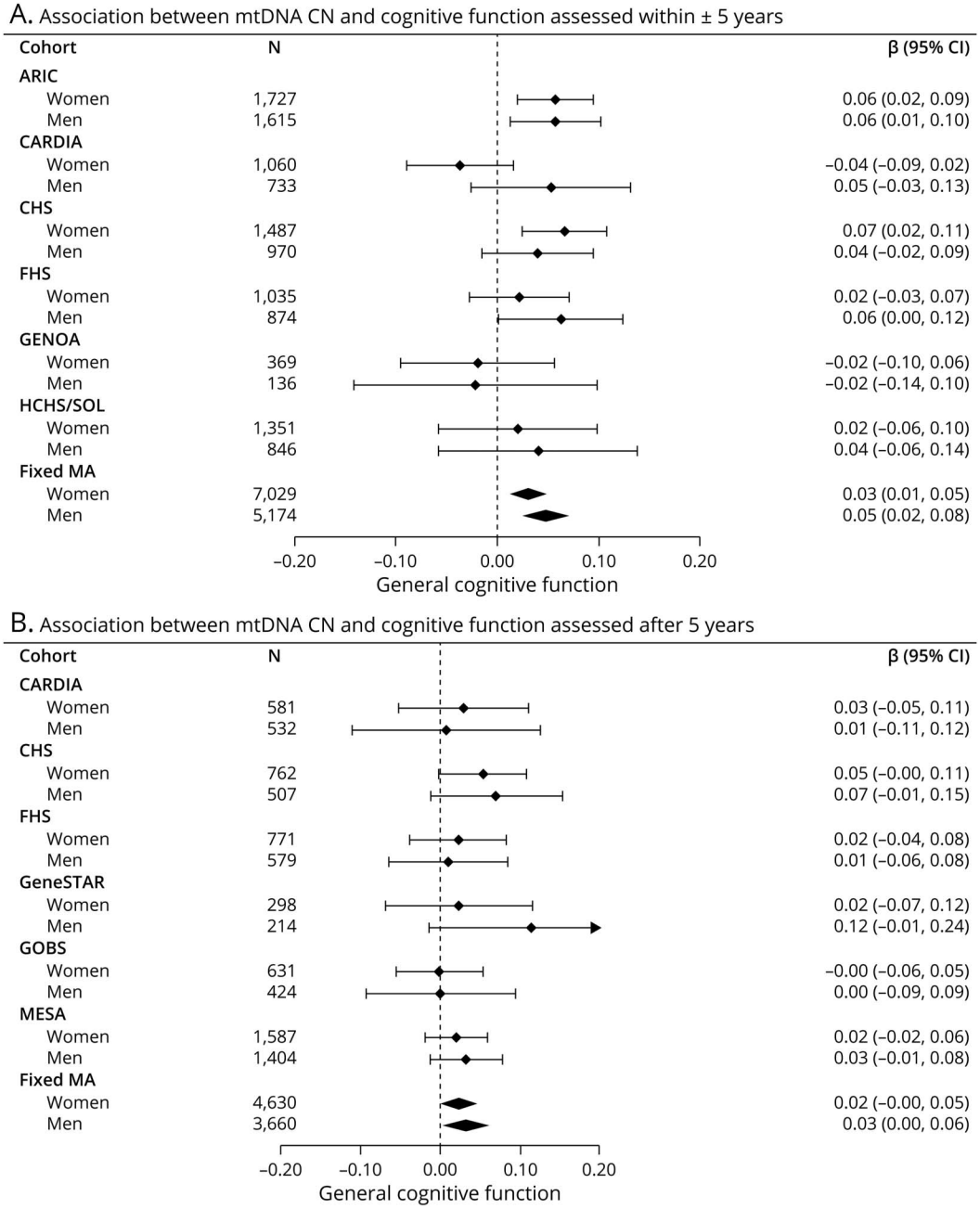
cross-sectional and prospective analyses (eTable 10, links.lww.com/WNL/C693).

Previous studies reported that women had higher mtDNA CN levels than men on average.[17,25] Therefore, we performed a sensitivity analysis to investigate whether the association of mtDNA CN with cognitive function was different between men and women. In cross-sectional analyses, we found significant associations of mtDNA CN with general cognitive function in both women and men (Figure 3A). Although the effect estimate for the association of mtDNA CN with cognitive function seemed slightly larger in men (β = 0.049; 95% CI 0.022–0.075; n = 5,174) than in women (β = 0.031; 95% CI 0.011–0.052; n = 7,029) after adjusting for covariates, results were not statistically different between sex (*p* for interaction = 0.427).

We have previously shown that mtDNA CN in blood significantly declines after 60–65 years.[17] Therefore, we performed association analyses of mtDNA CN and cognitive function in age-stratified analyses (<60 years and ≥60 years) based on their age at blood draw. In cross-sectional analyses, we observed that the effect estimate for the association of mtDNA CN with cognitive function seemed slightly larger in older participants (i.e., age at blood draw ≥60 years) (β = 0.05; 95% CI 0.027–0.071; n = 5,923) than in younger participants (i.e., age at blood draw <60 years) (β = 0.024; 95% CI 0.0009–0.047; n = 6,280) after adjusting for covariates (Figure 4A). However, the difference between age groups was not statistically significant (*p* for interaction = 0.595). Prospective stratified analyses by sex and age gave similar but attenuated results compared with the nonstratified analyses (Figures 3B and 4B).

In race/ethnicity stratified analyses, the association between mtDNA CN and cognitive function remained significant in non-Hispanic Whites (eTables 8 and 9, links.lww.com/WNL/C693). Primary cross-sectional meta-analyses revealed that each SD unit increase in mtDNA CN was significantly associated with better general cognitive function among

Figure 3 Forest Plots of the Sex-Specific Association of mtDNA CN With General Cognitive Function per Cohort and Meta-analysis Results

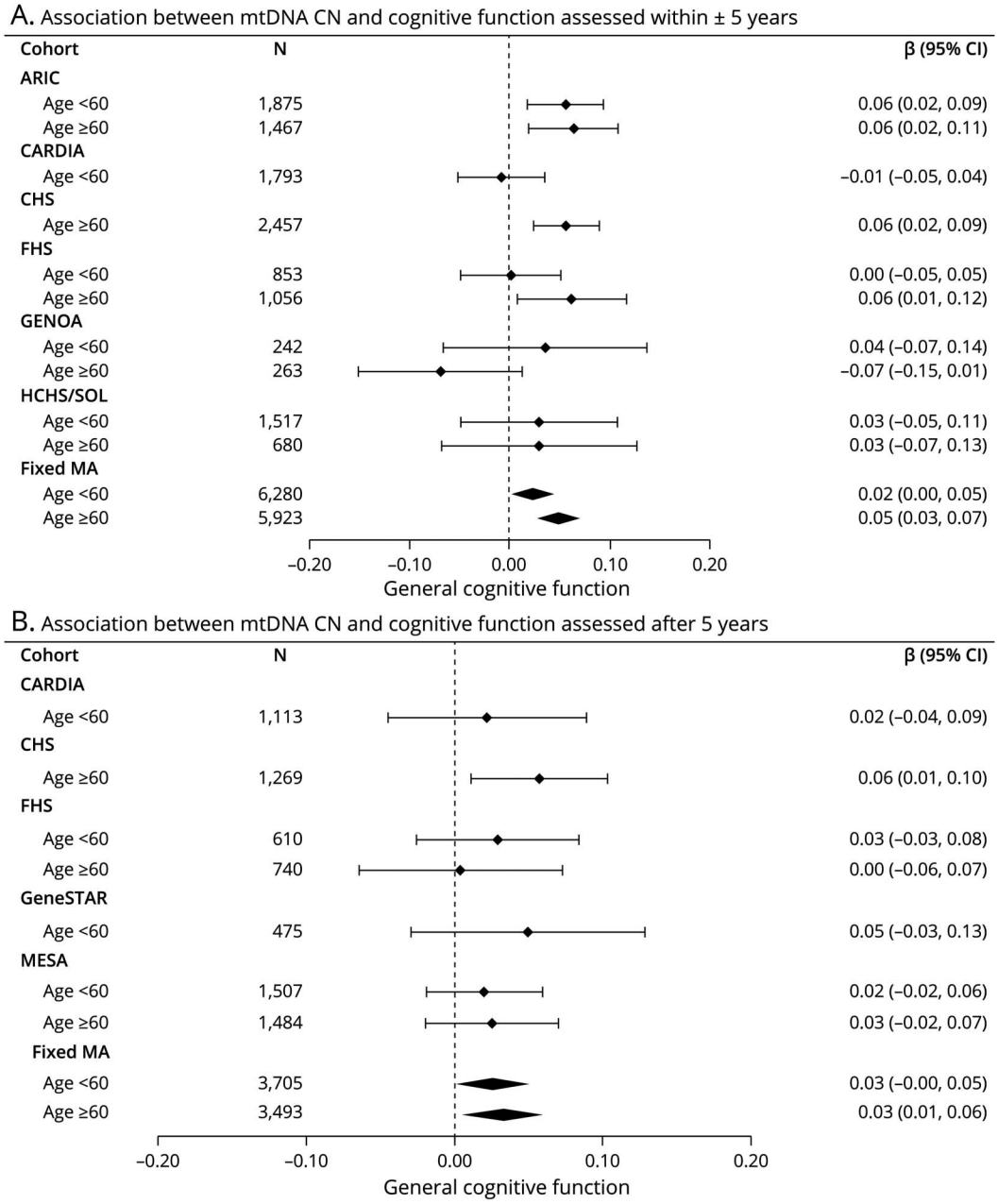


Covariates included age, batch effect, self-reported race/ethnicity, the time between blood draw and cognitive evaluation, cohort-specific variables, and education. β is the estimated difference in standardized general cognitive function score per SD unit increment in mtDNA CN. (A) Cross-sectional analyses. (B) Prospective analyses. ARIC = Atherosclerosis Risk in Communities study; CARDIA = Coronary Artery Risk Development in Young Adults study; CHS = Cardiovascular Health Study; CN = copy number; FHS = Framingham Heart Study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GENOA = Genetic Epidemiology Network of Arteriopathy Study; GOBS = Genetics of Brain Structure and Function Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; mtDNA = mitochondrial DNA.

non-Hispanic White (β = 0.057; 95% CI 0.038–0.075; n = 8,068; Figure 5A). However, the sample sizes of different race/ethnicity were smaller in comparison to the larger group of non-Hispanic White.

MR analyses suggested that mtDNA CN in blood had no significant causal effect on general cognitive function as assessed by IVW (causal estimate = −0.0158; 95% CI −0.0569 to 0.0975) or using 4 other MR methods (eTable 11 and eFigure 1, links.lww.com/WNL/C693). However, the power of MR was low due to limited variance of mtDNA CN explained by top SNVs (power of IVW method = 0.23; eTable 11). Furthermore, MR-Egger regression results indicated no significant directional pleiotropy for the effect of

Figure 4 Forest Plots of the Age-Specific Association of mtDNA CN With General Cognitive Function per Cohort and Meta-analysis Results



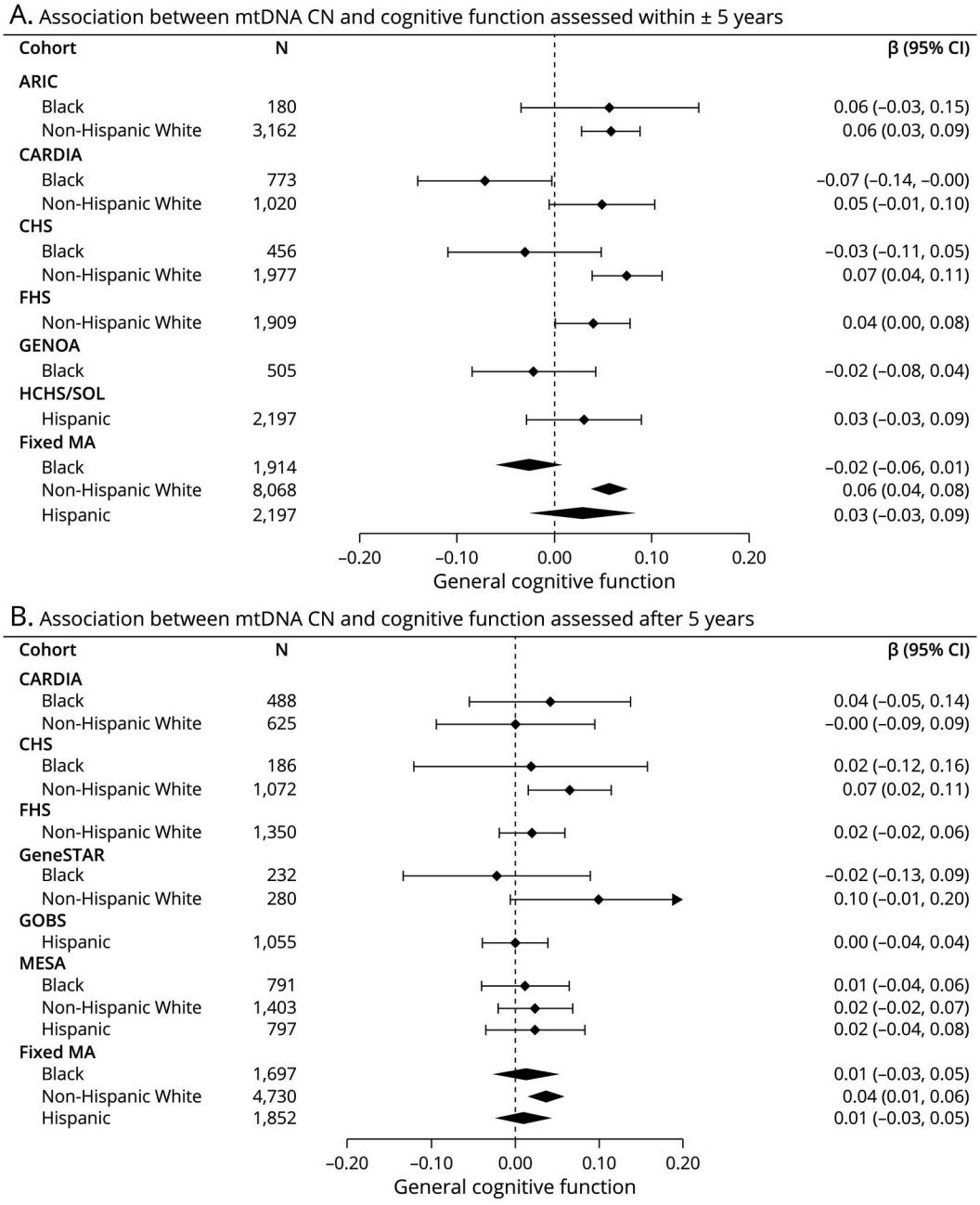
Covariates included age, sex, batch effect, self-reported race/ethnicity, the time between blood draw and cognitive evaluation, cohort-specific variables, and education. β is the estimated difference in standardized general cognitive function score per SD unit increment in mtDNA CN. (A) Cross-sectional analyses. (B) Prospective analyses. ARIC = Atherosclerosis Risk in Communities study; CARDIA = Coronary Artery Risk Development in Young Adults study; CHS = Cardiovascular Health Study; CN = copy number; FHS = Framingham Heart Study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GENOA = Genetic Epidemiology Network of Arteriopathy Study; GOBS = Genetics of Brain Structure and Function Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; mtDNA = mitochondrial DNA.

mtDNA CN on general cognitive function ($p > 0.1$). A sensitivity analysis using mtDNA CN GWAS results from another study[40] did not reveal a significant casual effect of mtDNA CN on general cognitive function using this set of SNVs (eTable 12). In addition, we did not observe a significant genetic correlation between mtDNA CN and cognitive function using LD score regression in exploratory analyses (genetic correlation coefficient = −0.0025; $p > 0.1$).

Association of mtDNA CN With Brain MRI Markers and Meta-analysis

In the primary cross-sectional analyses of mtDNA CN with brain markers, we found that that every SD unit increase in mtDNA CN was significantly associated with a 0.11% (β = 0.11; 95% CI 0.019–0.201; n = 2,825) increase in total brain volume relative to head size. The association between mtDNA CN and total brain volume became nonsignificant when cell count variables or

Figure 5 Forest Plots of the Race/Ethnicity-Specific Association of mtDNA CN With General Cognitive Function per Cohort and Meta-analysis Results



Covariates included age, sex, batch effect, the time between blood draw and cognitive evaluation, cohort-specific variables, and education. β is the estimated difference in standardized general cognitive function score per SD unit increment in mtDNA CN. (A) Cross-sectional analyses. (B) Prospective analyses. ARIC = Atherosclerosis Risk in Communities study; CARDIA = Coronary Artery Risk Development in Young Adults study; CHS = Cardiovascular Health Study; CN = copy number; FHS = Framingham Heart Study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GENOA = Genetic Epidemiology Network of Arteriopathy Study; GOBS = Genetics of Brain Structure and Function Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; mtDNA = mitochondrial DNA.

metabolic traits were further adjusted for in secondary models. We did not observe significant associations of mtDNA CN with hippocampal volume or WMH in the primary models (eTable 8, links.lww.com/WNL/C693). Results from prospective analyses showed no associations between baseline mtDNA CN and MRI measures assessed after 5 years in meta-analyses (eTable 9, links.lww.com/WNL/C693).

In sex-specific analyses, we observed that 1 SD increase in mtDNA CN was cross-sectionally associated with a 0.18% (β = 0.18; 95% CI 0.065–0.296; n = 1,645) greater total brain volume in women, although results did not reach significance in men. We did not observe significant associations between the MRI markers and mtDNA CN in age-stratified analyses (eTables 8 and 9, links.lww.com/WNL/C693).

In race/ethnicity stratified analyses, the primary model suggested cross-sectional associations between mtDNA CN and total brain volume in non-Hispanic White (β = 0.102; 95% CI 0.006–0.198; n = 2,132; eTable 8, links.lww.com/WNL/C693). Furthermore, prospective analyses revealed significant associations between mtDNA CN and total brain volume in Hispanics (eTable 9). However, these associations were no longer significant after adjustment for blood cell counts or metabolic traits.

Discussion

In this study, we found that higher mtDNA CN in whole blood was associated with better general cognitive function in both cross-sectional (up to 12,385 participants) and prospective (up to 8,290 participants) analyses after adjusting for potential confounders, including demographics, blood cell counts, and cardiometabolic traits. Although MR analyses did not support a causal relation between higher mtDNA CN in blood and better cognition, results should be interpreted with caution due to the limited power of this analysis. We did not observe significant associations between mtDNA CN and MRI markers. Taken together, our findings suggest that mtDNA CN may serve as a biomarker of current and future general cognitive function in community-based samples.

It has been shown that mitochondrial dysfunction is associated with aging.[35] Previous studies reported that mtDNA CN in peripheral blood declined with age and may serve as a biomarker of aging and age-related morbidity.[12,36] Hence, mtDNA CN may play an important role in linking mitochondrial biology and age-related neurodegenerative diseases. Results from additional studies suggest a significant decrease of mtDNA CN in the brains of patients with AD,[14] those with the C9ORF72 gene expansion common in frontotemporal dementia and amyotrophic lateral sclerosis,[37] and/or Creutzfeldt Jacob disease.[38] Another study observed a lower mtDNA content level in CSF in patients with AD.[39] Related to our results, a recent study reported significant associations between higher levels of mtDNA CN and lower rates of both prevalent and incident neurodegenerative disease.[13] However, little is known about the association of mtDNA CN measured in whole blood with general cognitive function and MRI markers of aging, key endophenotypes of AD/ADRD. Our epidemiological study contributes data from a large sample (n = 19,152) to investigate the relationship of WGS-derived mtDNA CN in whole blood with these key endophenotypes of general cognitive function and MRI biomarkers of aging and further assess potential causal effects. The finding that higher levels of mtDNA CN in whole blood were related to better general cognitive function, especially among those aged ≥60 years, indicate that mtDNA CN in whole blood may be an informative blood biomarker in studying cognitive decline and neurodegenerative diseases.

Despite consistent associations between mtDNA CN and general cognitive function in cross-sectional and prospective analyses, our MR analyses suggested that mtDNA CN in blood does not have a causal role in cognitive function and instead may be a bystander in this association. These results contrast with a recent study reporting a causal association between low mtDNA CN and a higher AD risk following MR analyses.[40] To eliminate the effect of using different GWAS samples for mtDNA CN, we conducted a sensitivity MR analysis using mtDNA CN GWAS from another GWAS[40] However, we still observed no significant causal effect of mtDNA CN on general cognitive function. This discrepancy might be explained by different selection processes for instruments and different outcomes that we tested on. Furthermore, the instrument may not be ideal, as the top hits derived from the GWAS only explained ~1%–2% variance in mtDNA CN; thus, the power of MR analyses was insufficient to detect a causal effect and validate the observed effect from the primary analyses (eTable 11, links.lww.com/WNL/C693).

Of interest, the study by Klein et al.[14] reported that a reduction of mtDNA CN in the dorsolateral prefrontal cortex was strongly related to the presence of tau pathology and antemortem cognitive decline. Tau pathology is strongly related to cognitive function[41]; thus, mtDNA CN could partly reflect tau burden in the brain. Experimental models provide evidence for the negative effect of phosphorylated tau on mitochondrial bioenergetics, fission and fusion processes, turnover, and axonal transport.[42] More broadly, mitochondrial dysfunction has been related to increased production of reactive oxygen species and amplification of oxidative stress that contributes to accelerated aging and dementia.[43] Thus, there may be other aspects of mitochondrial function, reflected in mtDNA CN, which could have a direct role in abnormal brain aging. Further studies are warranted to elucidate this dynamic.

Our study has several strengths. The large sample consisting of men and women of diverse race/ethnicity backgrounds (non-Hispanic White, Black/African American, Hispanic, and American Chinese) across a wide age range from 21 to 96 years enabled us to investigate the association of mtDNA CN with the key endophenotypes of AD/ADRD and explore age and sex groups. To minimize heterogeneity and confounding, we followed consistent procedures in quality control and statistical analyses across all cohorts. We also adjusted for potential confounders and known batch effects in a series of models to understand whether cell counts or cardiovascular risk factors confounded the relationship of mtDNA CN in blood with cognitive function and MRI markers of brain aging. However, we also acknowledge several limitations. First, mtDNA CN was estimated from whole blood rather than brain tissue or CSF; potential differences in neuronal vs somatic mitochondrial health could affect study outcomes. Second, phenotype data were collected by individual cohorts, leading to heterogeneity. Although we implemented consistent procedures to harmonize the traits, we observed considerable heterogeneity in meta-analyses including MRI outcomes (eTables 8 and 9, links.lww.com/WNL/C693). The observed heterogeneity may be partially explained by different distributions of age, sex, and race/ethnicity across

study cohorts. The different algorithms to derive MRI markers in each cohort likely also partially contributed to the observed heterogeneity. For example, WMH was measured with a visual rating scale in the CHS, whereas other cohorts used several different quantitative methods to derive WMH volumes. Moreover, the sample with available MRI outcomes was considerably smaller than that of cognitive function. Therefore, we may have lacked power to detect associations. In addition, each cohort had a different set of cognitive tests as part of their neuropsychological battery. However, we used a robust method to derive general cognitive function successfully used in prior research. Third, we were unable to include other relevant imaging modalities such as 18-F-fluorodeoxyglucose PET to investigate associations between blood mtDNA CN and cerebral metabolism due to limited data across the cohorts. Future investigations in this area are warranted. Fourth, mtDNA CN was measured at 1 time point. The association of change in mtDNA CN with cognitive decline and incident dementia remains to be investigated in future studies. Fifth, we did not assess associations between blood mtDNA CN and other relevant neurologic presentations, such as markers of nigrostriatal degeneration. Future investigations in suitable clinical samples would be important to expand on markers of Parkinson disease.

In conclusion, we observed that higher mtDNA CN in blood is associated with current and future better general cognitive function in a large sample from diverse community-based samples across the United States. Although MR did not provide evidence supporting a causal effect of blood mtDNA CN on cognition, circulating mtDNA CN may serve as an informative biomarker for brain aging and age-related disease. Future analyses with a stronger instrumental variable and improved power are needed to provide evidence about causality. Additional research to assess the effect of mtDNA heteroplasmic variations derived from blood and brain tissue may further help our understanding of the potential underlying mechanism supporting cognitive function.

Acknowledgment

Detailed acknowledgments for each cohort are included in the supplementary material (eAppendix 2, links.lww.com/WNL/C692). In brief, the authors thank the staff and participants of the Atherosclerosis Risk in Communities study, the Coronary Artery Risk Development in Young Adults study, the Cardiovascular Health Study, the Framingham Heart Study, the Genetic Study of Atherosclerosis Risk, the Genetic Epidemiology Network of Arteriopathy, the Genetics of Brain Structure and Function Study, the Hispanic Community Health Study/Study of Latinos, and the Multi-Ethnic Study of Atherosclerosis. The authors gratefully acknowledge the studies and participants who provided biological samples and data for TOPMed.

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Disclosure

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Appendix 1 *(continued)*

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