Meta-analysis identifies mitochondrial DNA sequence variants associated with walking speed

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Abstract Declines in walking speed are associated with a variety of poor health outcomes including disability, comorbidity, and mortality. While genetic factors are putative contributors to variability in walking, few genetic loci have been identified for this trait. We

examined the role of mitochondrial genomic variation on walking speed by sequencing the entire mitochondrial DNA (mtDNA). Data were meta-analyzed from 1758 Lifestyle Interventions and Independence for Elders (LIFE) Study and replication data from 730 Health,

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Aging, and Body Composition (HABC) Study participants with baseline walking speed information. Participants were 69+ years old of diverse racial backgrounds (African, European, and other race/ethnic groups) and had a wide range of mean walking speeds [4–6 m (0.78–1.09 m/s) and 400 m (0.83–1.24 m/s)]. Meta-analysis across studies and racial groups showed that m.12705C>T, *ND5* variant was significantly associated ($p < 0.0001$) with walking speed at both short and long distances. Replication and meta-analysis also identified statistically significant walking speed associations ($p < 0.0001$) between the m.5460.G>A, *ND2* and m.309C>CT, *HV2* variants at short and long distances, respectively. All results remained statistically significant after multiple comparisons adjustment for 499 mtDNA variants. The m.12705C>T variant can be traced to the beginnings of human global migration and that cells carrying this variant display altered tRNA expression. Significant pooled effects related to stopping during the long-distance walk test were observed across OXPHOS complexes I ($p = 0.0017$) and III ($p = 0.0048$). These results suggest that mtDNA-encoded variants are associated with differences in walking speed among older adults, potentially identifying those at risk of developing mobility impairments.

Keywords Gait speed · Aging · Mobility · Disability · Energy

Introduction

The number of older persons is expected to double to 86.7 million or to 20.6% of the US population by the year 2050 (Blackwell et al. 2014). This surge in population growth of the aging population has prompted the Institute on Medicine to develop specific literature focused on retooling medical resources for an aging America (Committee on the Future Health Care Workforce for Older Americans 2008) to cope with the 42% or 15.6 million who report having one or more limitations performing daily tasks (e.g., walking 2–3 blocks, transferring from the chair) that are essential for maintaining mobility and independence in the community (Statistics 2008). Walking speed is a powerful marker of mobility function as it is associated with a diverse array of poor health outcomes including disability, comorbidities, and mortality (Newman et al. 2006; Studenski et al. 2011). As such, efforts to understand the

determinates of walking speed decline with age are critical. One domain that has not received significant attention is identifying early genetic risk profiles that predispose individuals to gait speed declines.

Walking is a complex task that requires coordination between multiple, linked organ systems. The cardiorespiratory system provides oxygen to muscles for producing force output that causes propulsion. Upper and lower spinal cord neural circuits initiate and fine-tune the walking pattern and speed to effectively navigate the environment (Takakusaki 2013). Twin studies suggest that genetic factors account for 16–17% of the variance of walking speed in older adults (Ortega-Alonso et al. 2006), although the contribution of genetic factors may increase with age (Carmelli et al. 2000; Ortega-Alonso et al. 2006). Recently, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium found no nuclear genome wide significant signals in 30,000 elderly adults for walk speed measured at distances ranging from 8 to 25 ft (Ben-Avraham et al. 2017). However, the lack of genetic signal may be due to a narrow phenotype definition of walk speed. In addition, the missing heritability may be explained by rare genetic variants or genomic regions not examined as part of the genome-wide study, including variation in the mitochondrial genome.

Mitochondrial oxidative phosphorylation (OXPHOS) supplies 90% of human energetic requirements and is required for all systems involved in ambulation. Energy from OXPHOS is dependent upon the coordinated expression and interaction of genes encoded in the nuclear and mitochondrial DNA (mtDNA). The majority of genes encoded by the mtDNA are crucial for the machinery that converts metabolic energy into ATP, including 37 genes encoding polypeptides, tRNAs, and rRNAs. Collections of rare variants within genes or genomic regions influence phenotypic expression in important ways (Schork et al. 2009). For example, Leber's hereditary optic neuropathy (LHON), Leigh syndrome, and mitochondrial myopathies are rare but have significant effects on vision, hearing, and muscle function. Since human mtDNA has a mutation rate that is 10–20 times higher than that of nuclear DNA (Merriwether et al. 1991) and up to one-third of sequence variants found in the general population may be functionally important (Wallace 2010), it is likely that most of the mtDNA variation that impacts function is rare in frequency and only detectable by direct sequencing. We have previously shown that both common and rare mtDNA variants are important

contributors to numerous diseases and aging-related traits (Katzman et al. 2014; Lam et al. 2012; Tranah 2011; Tranah et al. 2012). In fact, we recently observed that heteroplasmy of m.5703G>A—a mutation with confirmed myopathic phenotypes—was associated with lower walking speed in a relatively small sample of older adults (Tranah et al. 2015). However, there is a paucity of research examining associations between mtDNA sequence variants association and walk speed.

Because mitochondria perform diverse functions in different tissues related to walking, specific mutations in mtDNA may lead to abnormalities in walking measures—namely the speed of walking. In the current study, we present results from the first comprehensive examination of mitochondrial genomic variation and walking speed. Identifying mitochondrial genetic variants that influence walking speed may advance risk assessment and lead to a screening approach for identifying those most at risk of developing mobility impairments or those who would benefit from existing pharmacologic and behavioral treatments that target the mitochondria. Identifying potentially pathologic mtDNA variants and modifiable targets may lead to development of novel pharmacologic agents or new clinical strategies for preventing or treating mobility impairment.

Methods

Population

This study used data from two older adult samples. The Lifestyle Interventions and Independence for Elders (LIFE) served as the identification cohort and the Health, Aging, and Body Composition (HABC) served as the replication cohort. Participants in the LIFE study were considered to have moderate to low physical function as part of the eligibility criteria while the HABC participants were high functioning. This approach expands the variability required to assess important variations in walk speed and allows a strong premise for replication of the findings across diverse samples.

Design, recruitment, baseline characteristics, and main outcomes of the LIFE and HABC studies are published and described elsewhere (Fielding et al. 2011; Visser et al. 2002). Participants in the LIFE study originated from two samples—381 from the LIFE Pilot and 1376 from the main LIFE study. These studies were combined into one sample because both were

multicenter, single-blinded, parallel randomized trials, and the eligibility criteria were identical. The LIFE Pilot study enrolled participants across four field centers between April 2004 and February 2005. The LIFE study enrolled participants in eight field centers across the USA between February 2010 and December 2013. The Health ABC study enrolled participants from two field centers between 1997 and 1998.

The eligibility criteria for the LIFE study consisted of men and women aged 70–89 years who (a) were inactive (reporting < 20 min/week in the past month performing regular structured exercise and < 125 min/week of moderate physical activity); (b) were at high risk for mobility disability based on lower extremity functional limitations measured by the Short Physical Performance Battery (SPPB) (Guralnik et al. 1995) score ≤ 9 out of 12; (c) could walk 400 m in ≤ 15 min without sitting, leaning, or the help of another person or walker; (d) had no major cognitive impairment; and (e) could safely participate in the intervention as determined by medical history, physical exam, and resting ECG. For HABC, the eligibility criteria included self-reporting no difficulty in walking a distance of 0.4 km or climbing at least 10 stairs, independently performing activities of daily living, plans to live in the area for the next 3 years, and no evidence of life-threatening illnesses. The sample was approximately balanced for sex, (53% women) and 48% of participants were black.

The institutional review board at all field sites approved each study protocol, and written informed consent was obtained from all study participants. Both LIFE studies were additionally monitored by a data and safety monitoring board appointed by the National Institute on Aging. LIFE and LIFE Pilot is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) with the identifiers NCT01072500 and NCT00116194, respectively.

Walking outcomes

There are a variety of ways to ascertain walking speed in older adults. The two most common methods are walking over short distance (4–6 m) or a long distance (e.g., 400 m) performed at a usual or comfortable pace. Additionally, some older adults are unable to complete a long-distance walk test or they need to rest to complete the test. This analysis examined these two types of walk speed. First, short-distance walking speed was measured over 4 m in the LIFE studies and 6 m in the Health ABC study. For the LIFE study, the instructions were to “walk

to the other end of the course at your usual speed, just as if you were walking down the street to go to the store.” The instructions for the Health ABC participants were to “walk at a comfortable pace.” All studies provided a deceleration period during the walk test where timing of the test stopped a few steps prior to the participant ending their walk.

A long-distance walk test was also assessed in the cohorts. In the LIFE studies, participants performed a 400-m walk test that served as the primary outcome of the trials. Participants were asked to walk 400 m at their usual pace, without over exerting, on a 20-m course for 10 laps (40 m per lap). Participants were required to complete the test within 15 min without sitting and without the help of another person or walker. Participants in the Health ABC study were asked walk 400 m over 10 laps as quickly as possible, at a pace they could maintain over the 10 laps. Walking speed was calculated as the time to complete 400 m or the maximal distance completed. The differences in walk instructions between LIFE and Health ABC studies could be cause for concern and a major reason for why analyses were conducted separately and then meta-analyzed.

A second variable was extracted from 400 m walk test. Participants in both LIFE and Health ABC studies were permitted to stop for fatigue or other health-related symptoms and then continue the test. In the LIFE study, participants who stopped were asked to stand in place but were not permitted to sit in a chair or lean against a wall. Participants were permitted to continue the test if the rest period was shorter than 1 min. All participants in the LIFE studies were required to complete 400 m at baseline to be eligible for the study (but were allowed to stop as above). For the Health ABC study, when a participant stopped, the test was stopped. Those who had to stop during the long-distance walk test were labeled mobility impaired defined as those who stopped during the test regardless of whether they completed the test.

Other measures

The main baseline descriptive measures included self-reported demographic characteristics including race and ethnicity reported according to NIH requirements, medical and hospitalization history, body mass, and height. Participants self-designated their race and ethnicity from a fixed set of options that were used to categorize individual's as white, black, and other (Asian, Native Hawaiian/Pacific Islander, Native American/Alaskan Native, and other).

Mitochondrial DNA sequencing

A total of 1758 LIFE Study-LIFE Pilot participants who consented to genomic studies were included. For Health ABC, a random sample of 788 participants who consented to genomic studies, stratified by race and gender, were identified for mtDNA sequencing. DNA was extracted from buffy coat using whole blood collected from Health ABC study participants at baseline and from LIFE study participants collected at the baseline or 12-month visit. Complete mtDNA sequencing was performed using the next-generation sequencing Illumina MiSeq platform, which has been validated for this purpose (Li et al. 2010; Tang and Huang 2010). Briefly, the entire mitochondrial genome is first amplified in two long-range PCR reactions (9.1 kb and 11.2 kb) spanning the entire human mtDNA genome. The size distribution of each PCR amplicon is then verified using an Agilent Technologies 2100 Bioanalyzer. The two PCR amplicons were then pooled at equi-molar concentrations leading to unbiased representation across the entire mitochondrial genome prior to simultaneous fragmentation and tagging with adapter sequences as specified by Illumina Nextera XT DNA sample Preparation kit. Prior to sequencing, each sample is amplified using a limited cycle PCR program that adds unique indexes to each sample for a total of 384 indexes per sequencing run. In preparation for cluster generation and sequencing, equal volumes of bead-based normalized library are combined, diluted in hybridization buffer, and heat denatured prior to MiSeq sequencing. After sequencing and alignment to the Cambridge reference sequence (NC_12920), sequence processing is accomplished with the MiSeq Reporter and the mtDNA Analysis Tool for variant calling. Multiplex-sequencing of 384 barcoded samples yielded an average depth of coverage > 250× and a minimum of 100× per individual.

Data analyses

Walking speed was compared between the major and minor alleles for individual mtDNA variants in both LIFE and Health ABC studies using the generalized linear model. Unconditional logistic regression was used to obtain odds ratios (ORs) as estimates of relative risks (hereafter called risk) and 95% confidence intervals (CIs) for risk of mobility impairment and individual mtDNA variants. Traditional confounders were not

included in models because, to be eligible, the variable needs to influence both the primary predictor (mtDNA variant) and the mobility outcome. No variables collected, except for race (which is stratified in this analysis), are known to be associated with a specific variant and mobility outcome. To ensure adequate sample size to test associations, allele thresholds were set to 1% minor allele frequency (MAF) for white participants and 2% MAF for black and other participants. All analyses were stratified by race (white, black, and other) and adjusted for age and sex using custom R scripts and SAS version 9.4 (SAS Institute Inc., Cary, NC).

For genetic association analyses, we followed recommendations pervasive in the literature regarding replication and meta-analysis (Cantor et al. 2010; Evangelou et al. 2014; Gogele et al. 2012; Kraft et al. 2009; Nguyen et al. 2009; Skol et al. 2007; Zeggini and Ioannidis 2009). Associations were first conducted in the LIFE study cohort and then replicated in the HABC cohort. Then, associations were meta-analyzed across the LIFE and Health ABC studies using METAL (Willer et al. 2010) with a fixed effects model. Meta-analysis reduces the probability of false-negatives and the winner's curse phenomenon (Higgins et al. 2003; Ioannidis et al. 2007) and importantly provides an opportunity to evaluate the heterogeneity of genetic effect across the studies. We also leveraged sequence-based analysis with the Sequence Kernel Association Test (SKAT) (Wu et al.) method that collapses and tests the collective effects of common and rare variants. The SKAT method is designed for resequencing data where rare mutations are observed directly and generally has higher power than alternative methods for genetic burden analyses (Wu et al. 2010). This test was developed based on random effect models, which assumes a common distribution for the genetic effects of variants at different sites and tests for the null hypothesis that the distribution has zero variation. We applied the SKAT approach in association tests for the continuous walking speed and dichotomous mobility impairment outcome and computed statistical significance for each test using 10,000 independent simulations. For the SKAT testing, we considered a “hierarchy” of annotations within seven mtDNA regions that will allow us to refine the influence of collections of rare and common variants, including each of the four individual OXPHOS complexes (I, III, IV, and V), two rRNAs, all tRNAs combined, and combined HV regions.

For the current analysis, associations were examined for all variants in both the white and black participants

from the LIFE and Health ABC Studies. Due to limited sample size in the other race category, we used this group as an additional validation for significant associations identified in black and white participants from both studies. To avoid false positives due to population stratification, all analyses were adjusted for six eigenvectors of mitochondrial genetic ancestry derived from principal component analysis (PCA) (Price et al. 2006). In our previous mtDNA sequencing work, the first six eigenvectors have accounted for 71% of the variance in the mtDNA sequence dataset (Tranah et al. 2011). Mitochondrial PCA has been shown to outperform haplogroup-stratified or adjusted association analyses with no loss in power for the detection of true associations (Biffi et al. 2010). Several in silico methods were employed to examine mtDNA nucleotide conservation (GERP (Cooper et al. 2005) and PhyloP (Pollard et al. 2010)) for all significant variants and to predict the potential functional impact of non-synonymous (NS) substitutions on amino acid protein sequences (Sorting Tolerant From Intolerant' [SIFT] (Ng and Henikoff 2006), MutPred (Li and Leal 2009), and PolyPhen2 (Adzhubei et al. 2010)). All values are presented as means $\pm$ standard error for continuous measurements or sample number and percentages for categorical measurements.

Results

Participant characteristics

MtDNA sequence variants were evaluated in 1758 and 788 (730 had least one walk test) participants in the LIFE and Health ABC studies, respectively (Table 1). Participants in the LIFE studies were older, had a higher BMI, and generally had more chronic diseases. Among the 1758 LIFE participants with complete sequence data, 1757 had completed the 4 m walk test and 1758 completed the 400 m walk test. Among 788 Health ABC studies with complete sequence data, 659 had completed the 6 m walk test and 577 completed the 400 m walk test (with 730 completing at least one of the two distances reported in Table 1). Regarding the primary phenotype, the average short-distance walking speed in LIFE participants was 0.31 m/s slower than Health ABC participants. As expected, participants in the Health ABC study were more likely to be black and gender balanced than participants in the LIFE study.

Table 1 Baseline characteristics of LIFE and Health ABC cohorts

	LIFE (<i>N</i> = 1758)*	Health ABC (<i>N</i> = 730)*
Age, years, mean (SD)	78.4 (5.1)	74.0 (2.9)
White, <i>n</i> (%)	1346 (76.6)	381 (52.2)
Black race, <i>n</i> (%)	291 (16.6)	349 (47.8)
Other race, <i>n</i> (%)	121 (6.9)	–
Women, <i>n</i> (%)	1169 (66.4)	384 (52.6)
Body mass index, kg/m ² , mean (SD)	30.2 (5.9)	27.3 (4.6)
Short distance usual walk speed, m/s, mean (SD)**	0.78 (0.16)	1.09 (0.26)
Walking speed ≥ 1.0 m/s, <i>n</i> (%)	151 (8.6)	432 (65.6)
0.8 ≤ Walking speed < 1.0 m/s, <i>n</i> (%)	580 (33.0)	149 (22.6)
0.6 ≤ Walking speed < 0.80 m/s, <i>n</i> (%)	809 (46.0)	65 (9.9)
Walking speed < 0.60 m/s, <i>n</i> (%)	217 (12.4)	13 (2.0)
Long distance meter walk speed, mean (SD)***	0.83 (0.17)	1.24 (0.21)
Stopped during long-distance walk test, <i>n</i> (%)	288 (16.4)	50 (6.8)
Prevalent health conditions, <i>n</i> (%)		
Hypertension	1170 (66.6)	318 (43.6)
Diabetes	343 (19.5)	111 (15.2)
Cardiovascular disease****	240 (13.7)	84 (11.5)
Cancer	367 (20.9)	100 (13.7)
Chronic pulmonary disease	253 (14.4)	44 (6.0)
Osteoarthritis with pain	279 (15.9)	23 (3.2)
Depressive symptoms, CESD, mean (SD)	8.2 (7.5)	4.6 (5.0)
Morbid obesity (BMI ≥ 35)	347 (19.7)	45 (6.2)

*Participants with complete sequence data. Baseline characteristics are summarized for 1758 LIFE and 730 Health ABC participants with sequence data and a completed short or long walking test assessment

**Usual walking speed measure on a 6-m course in the Health ABC study and a 4-m course in the LIFE study

***Walking speed performed on a 400-m course in both Health ABC study LIFE studies

****Myocardial infarction, stroke, or heart attack

Sequencing and short-distance walk speed

Sequencing of ~16.5 kb of mtDNA from 1758 LIFE Study-LIFE Pilot and 788 Health ABC Study participants yielded a cumulative total of 3364 variants including 89 common variants (MAF, > 5%), 459 low frequency variants (MAF, 1–5%), and 2816 rare variants (MAF, < 1%). In the analysis, we examined SNPs ≥ 1% among white

and ≥ 2% among black and other racial/ethnic participants. After accounting for variants in complete LD, we performed a total of 499 independent tests. Associations with walking speed and mobility impairment were tested with a Bonferroni adjusted $p = 0.0001$ for significance that accounts for multiple comparisons. Associations between mtDNA variants and short-distance (Table 2) and long-distance (Table 3) walks are reported. Race-specific results are based on self-report of white, black, or other ancestry. For the short-distance walk, we identified three common mtDNA variants associated with reduced walking speed among white participants from both the LIFE and Health ABC studies (Table 2). Among black participants from both studies, we identified 11 common mtDNA variants associated with either reduced or increased walking speed for the short-distance walk (Table 2). Among these variants, two complex I loci were significantly associated with reduced walking speed in both white and black populations, including m.12705C>T, *ND5* and m.5460.G>A, *ND2*. The meta-analysis involving all LIFE Study (black, white, and other) and Health ABC Study (black and white) participants yielded highly significant associations ($p < 0.0001$) for both variants (Fig. 1a). Figure 2a illustrates short-distance walking speeds for each allele at the m.12705 and m.5460 positions for Health ABC and LIFE studies in whites, blacks, and other races/ethnicities.

Long-distance walk speed

In the LIFE studies, significant associations were identified for seven common mtDNA variants among white participants and 12 among black participants for both studies (Table 3). Of these, m.12605C>T, *ND5* and m.309C>T, *HV2* were associated with walking speed among black and white participants. The meta-analysis involving all LIFE Study and Health ABC Study participants yielded highly significant associations for both variants, m.12705C>T, *ND5* ($p = 0.0001$) and m.309C>T, *HV2* ($p < 0.0001$) (Fig. 1b). Figure 2b illustrates long-distance walking speeds for alleles for m.12705 and m.309 positions for Health ABC and LIFE studies in whites, blacks and other races/ethnicities.

Mobility impairment

In the LIFE and HABC studies, 288 out of 1758 (16.4%) and 50 out of 577 (8.6%) participants stopped (i.e., mobility impaired) during the 400 m walk test, respectively.

Table 2 Meta-analytic variant associations with short-distance walking speed (LIFE studies—4 m; Health ABC—6 m)

	LIFE studies (<i>n</i> = 1757)			Health ABC (<i>n</i> = 659)			Meta-analysis <i>p</i> value
Race and variant	Beta (SE)	<i>p</i>	MAF	Beta (SE)	<i>p</i>	MAF	
White							
m.73A>G, HV2	− 0.020 (0.01)	0.019	0.52	− 0.085 (0.03)	0.0056	0.21	0.002
m.12705C>T, ND5	− 0.054 (0.022)	0.014	0.04	− 0.056 (0.032)	0.080	0.12	0.02
m.5460G>A, ND2	− 0.041 (0.020)	0.042	0.05	− 0.081 (0.04)	0.043	0.11	0.02
Black							
m.5603C>T, tRNA-try	0.093 (0.038)	0.014	0.06	0.149 (0.039)	0.00015	0.13	0.0001
m.316G>A, HV2	− 0.058 (0.028)	0.037	0.11	0.132 (0.042)	0.0016	0.12	0.0006
m.5460G>A, ND2	− 0.074 (0.030)	0.014	0.08	− 0.103 (0.038)	0.0078	0.15	0.002
m.16362T>C, HV1	0.048 (0.023)	0.038	0.16	0.104 (0.047)	0.03	0.09	0.003
m.8206G>A, COII	− 0.050 (0.019)	0.009	0.27	0.094 (0.044)	0.033	0.1	0.007
m.12519T>C, ND5	0.081 (0.032)	0.01	0.08	− 0.053 (0.026)	0.044	0.37	0.008
m.5442T>C, ND2	0.059 (0.035)	0.091	0.07	0.094 (0.041)	0.023	0.12	0.01
m.12705C>T, ND5	− 0.095 (0.026)	0.0003	0.88	− 0.448 (0.229)	0.05	0.997	0.01
m.5046G>A, ND2	0.078 (0.031)	0.013	0.08	− 0.097 (0.042)	0.022	0.11	0.01
m.3450C>T, ND1	0.078 (0.033)	0.018	0.07	0.105 (0.057)	0.067	0.06	0.02
m.12810A>G, ND5	− 0.061 (0.032)	0.06	0.08	− 0.071 (0.037)	0.055	0.15	0.02
Other*							
m.12705C>T, ND5	− 0.17 (0.029)	< 0.0001	0.68				
m.5460G>A, ND2	0.022 (0.056)	0.69	0.12				

*Other race used for replication of significant findings and meta-analysis

Associations between mtDNA variants and risk of mobility impairment during the long-distance walk test were examined. Variants with significant meta-analytic results are displayed in Fig. 3. A single variant, m.6260G>A, *COI*, was associated with increased risk of mobility impairment among white participants from the LIFE (odds ratio [OR] = 1.4, 95% confidence interval [CI] = 1.1–4.2, $p = 0.034$) and HABC (OR = 2.1, 95% CI = 1.9–8.1, $p = 0.005$) studies (meta-analysis $p = 0.002$). Among black participants, the m.16256C>T, HV1 was associated with increased risk of mobility impairment in the LIFE (OR = 2.6, 95% CI = 1.4–13.7, $p = 0.026$) and HABC (OR = 1.4, 95% CI = 1.2–3.9, $p = 0.024$) studies (meta-analysis $p = 0.005$). None of the previously identified variants were associated with walking speed (m.12705C>T, ND5; m.5460.G>A, ND2; and m.309C>T, HV2) or the risk of mobility impairment. Significant pooled effects ($p = 0.007$ due to multiple test correction for seven mtDNA regions) on mobility impairment were observed across OXPHOS complex I ($p = 0.0017$) and III ($p = 0.0048$) regions. No pooled effects were observed for walking speed.

Nucleotide conservation

We focused in silico prediction methods on the assessment of nucleotide conservation generated via GERP (Cooper et al. 2005) and phyloP (Pollard et al. 2010) as none of the associated variants conferred NS substitutions. In brief, phyloP performs multiple alignments of 100 vertebrate species and calculates metrics of evolutionary conservation for each variant under a null hypothesis of neutral evolution. The absolute values of phyloP scores represent $-\log p$ values with positive scores reflecting evolutionary conservation across taxa (i.e., slower rates than expected) and negative scores reflecting accelerated evolution (i.e., faster than expected). Of the five associated variants, four exhibited negative phyloP scores implying that these variants are not highly conserved among multiple vertebrate taxa: m.12705C>T = -4.98 , m.5460.G>A = -1.10 , m.309C>CT = -0.61 , and m.16256C>T = -2.48 . A single variant associated with mobility impairment exhibited a positive phyloP score, m.6260G>A = 1.0, implying modest conservation at this site. GERP identifies

Table 3 Meta-analytic variant associations with long-distance walking speed (LIFE and Health ABC studies—400 m)

	LIFE studies (<i>n</i> = 1758)			Health ABC (<i>n</i> = 577)			Meta-analysis <i>p</i> value
Race and variant	Beta (SE)	<i>p</i>	MAF	Beta (SE)	<i>p</i>	MAF	
White							
m.12705C>T, ND5	− 0.074 (0.023)	0.001	0.04	− 0.01 (0.027)	0.07	0.12	0.002
m.309C>CT, HV2	0.035 (0.009)	0.0001	0.44	0.052 (0.021)	0.014	0.37	0.003
m.16362T>C, HV1	0.036 (0.015)	0.012	0.1	0.069 (0.033)	0.039	0.11	0.005
m.489T>C, HV3	− 0.03 (0.016)	0.058	0.09	− 0.073 (0.037)	0.047	0.09	0.007
m.150C>T, HV2	− 0.032 (0.016)	0.044	0.1	0.212 (0.091)	0.02	0.02	0.008
m.14167C>T, ND6	0.029 (0.013)	0.032	0.13	− 0.118 (0.049)	0.018	0.04	0.01
m.9540T>C, COIII	− 0.018 (0.01)	0.069	0.11	− 0.107 (0.056)	0.057	0.04	0.02
Black							
m.309C>CT, HV2	0.05 (0.018)	0.007	0.34	0.052 (0.021)	0.014	0.37	0.001
m.189A>G, HV2	0.038 (0.018)	0.038	0.36	0.181 (0.068)	0.009	0.02	0.006
m.247G>A, HV2	0.05 (0.021)	0.016	0.24	0.065 (0.036)	0.076	0.18	0.009
m.2789C>T, 16S	− 0.058 (0.023)	0.011	0.18	0.052 (0.028)	0.06	0.16	0.01
m.5036A>G, ND2	0.084 (0.032)	0.01	0.08	0.078 (0.039)	0.046	0.08	0.01
m.7771A>G, COII	− 0.054 (0.023)	0.018	0.18	− 0.2 (0.105)	0.057	0.01	0.01
m.16189T>C, HV1	0.044 (0.018)	0.015	0.5	− 0.069 (0.037)	0.062	0.08	0.02
m.8206G>A, COII	− 0.046 (0.02)	0.02	0.27	0.074 (0.041)	0.071	0.07	0.02
m.15301G>A, CYB	− 0.045 (0.019)	0.017	0.67	0.057 (0.032)	0.074	0.13	0.03
m.12705C>T, ND5	− 0.047 (0.027)	0.078	0.88	− 0.01 (0.027)	0.01	0.98	0.03
m.8027G>A, COII	0.058 (0.027)	0.029	0.13	0.036 (0.022)	0.1	0.3	0.04
m.7146A>G, COI	0.041 (0.02)	0.036	0.29	0.037 (0.023)	0.104	0.3	0.05
Other*							
m.12705C>T, ND5	− 0.084 (0.04)	0.023	0.68				
m.309C>CT, HV2	0.034 (0.042)	0.42	0.36				

*Other race used for replication of significant findings and meta-analysis

constrained elements in multiple sequence alignments from 29 mammalian species. Substitution deficits represent substitutions that would have occurred if the element were neutral DNA are quantified. All five associated variants exhibited highly negative GERP scores further indicating that these variants are not highly conserved among multiple vertebrate taxa.

Discussion

Common genetic factors influencing walking speed have not been identified despite a recent effort to identify loci in over 30,000 elderly adults (Ben-Avraham et al. 2017). In the current study, we present results from the first comprehensive examination of mitochondrial genomic variation in walking speed and provide

evidence that specific mtDNA variants are associated with walking speed. We examined associations within the LIFE and Health ABC studies and performed a meta-analysis approach to capture the range of walking speeds. However, it should be noted that most associations were uniquely significant within each cohort. Importantly, the associations were observed with walk speed over short and long distances, as well as risk of mobility impairment. Involving older participants from two well-characterized studies that included multiple races, we identified three loci associated with walking speed (m.12705C>T, ND5; m.5460.G>A, ND2; and m.309C>T, HV2). Importantly, the magnitude of walk speed differences between the alleles ranged between 0.04 and 0.45 m/s, which generally exceeded a clinically meaningful difference of 0.03 m/s noted in the literature (Perera et al. 2006). Lastly, two loci demonstrated higher

Fig. 1 Solar plots representing mtDNA variant associations with **a** short distance (LIFE studies—4 m; Health ABC—6 m) and **b** long distance (LIFE and Health ABC studies—400 m) walk speeds.

odds of mobility impairment (m.6260G>A, *COI*, and m.16256C>T, *HV1*).

Of the identified mtDNA variants, m.12705C>T, *ND5* exhibited the most consistent pattern of association across studies, race, and walking distance. Overall, the m.12705T allele was associated with reduced walking speed when compared with the m.12705C allele. The m.12705C>T, *ND5* variant is diagnostic for macrohaplogroup R, daughter clade to superhaplogroup N, and a rooting ancestor for most of the Caucasian population and for the Asian population harboring haplogroups B and F. This fundamental mtDNA variant is notable for its presence at the early stages of modern human evolution and can be traced back through phylogenetics to the beginnings of human global migration. The m.12705T variant is present in all African and some Asian and European mitochondrial lineages. The m.12705C allele is present in most recently derived European lineages as well as some Asian lineages. Distinct mitochondrial gene expression patterns among 454 lymphoblast cell lines from the 1000 Genomes Project have been associated with specific mtDNA variants and haplogroups, including m.12705C>T (Cohen et al. 2016). Higher expression of tRNA glycine was observed in cells carrying the m.12705C allele when compared with cells carrying m.12705T, possibly explaining, in part, walking speed differences observed in the current study. For example, altered tRNA expression may

Each dot represents a mtDNA variant association with walking speed color coded by mitochondrial gene. The blue circle represents a *p* value of 0.05 (color figure online)

affect mitochondrial function by affecting the rate or efficiency of mitochondrial biogenesis (increase in mitochondrial number and/or mass). Previous genetic association studies suggest that mtDNA haplogroup R (carriers of the m.12705C allele) predicts survival advantage in severe sepsis (Yang et al. 2008), recovery from septic encephalopathy (Yang et al. 2011), and sperm motility (Feng et al. 2013). Additional mechanistic work is needed to identify the specific causal pathways that allow carriers of the m.12705C allele to have higher walk speed.

The analysis of mtDNA is often complicated by recurrent mutation at the same mtDNA site across divergent haplogroups, which can sometimes hide diagnostic specificity of a particular variant. That the same mutations have been observed repeatedly on different mtDNA backgrounds (e.g., haplogroups) has been cited as evidence of convergent adaptive evolution of particular mtDNA mutations (Wallace 2010). Significant differences in the frequency of non-synonymous mutations among haplogroups also suggest that some mutations may be non-neutral within specific phylogenetic lineages but neutral within others (Moilanen et al. 2003). For example, the m.5460G>A, *ND2*; m.6260G>A, *COI*; and m.16256C>T, *HV1* variants are found in multiple lineages from superhaplogroups L, M, and N. The m.309C>T, *HV2* occurs in a poly-C area that is highly variable (differing even among relatives), thus providing little phylogenetic value (van

Fig. 2 Average walk speed for the **a** short-distance walk test for m.12705 and m.5460 positions and **b** average walk speed for the long-distance walk test for m.12605C>T, and m.309C>T positions. Values are expressed as means (SEM) in Health ABC and LIFE studies in whites (W), blacks (B), and other races/ethnicities (O) (only in LIFE studies)

Fig. 3 Odds of mobility impairment—defined as stopping during the long-distance walk test—according to variants that were significant in meta-analyses. m.6260G>A in whites and m.16256C>T in blacks was associated increased odds of mobility impairment in both Health ABC (HABC) and LIFE studies

Oven and Kayser 2009). In general, there was minimal evidence for evolutionary conservation at any of the associated sites. Unlike the previously described m.12705C>T mutation which occurred at a crucial point during global dispersal, these mutations arose multiple times across all major human lineages (African, Asian, and European). The observation that these variants (m.5460G>A, m.6260G>A, m.309C>T, and m.16256C>T) impact walking speed and mobility regardless of genomic or phylogenetic background suggests that they directly impact mitochondrial function. For example, mitochondrial variants occurring in one of the OXPHOS complexes (m.5460G>A, *ND2*; m.6260G>A, *COI*) can lead to impairment of the respiratory chain and affect ROS production, ATP generation efficiency, or apoptosis (Langston and Ballard 1983; Ramsay and Singer 1986). While it is not clear how the m.16256C>T, HV1 m.309C>T, HV2 variants are associated with walking speed and risk of mobility impairment during the long-distance walk, it is possible that this variation is involved in regulating mtDNA copy number (Suissa et al. 2009). The functions of the HV2 region include priming site for mtDNA replication, the heavy strand origin encoding 12 of the 13 OXPHOS genes, three conserved sequence blocks, and two transcription factor binding sites (MITOMAP 2011). In a previous study, the HV2 sequence variation was found to increase both mtDNA transcription and copy number (Suissa et al. 2009). This may also explain why the m.309C>T allele was associated with a long distance, but not a short-distance walk. The latter is more dependent on glycolytic metabolism.

Significant pooled effects on mobility impairment were observed across OXPHOS complexes I and III. The 13 mtDNA-encoded OXPHOS genes are essential to mitochondrial energy production (Wallace 2010), and collective at complexes I and III specifically may be related to impairment of the respiratory chain (ETC) or ROS production (as these are the sites of ROS production in the ETC) (Brand 2010). Complex I is a large multisubunit, membrane-bound protein which serves as the major entry point for most electrons into the ETC. In eukaryotes, the mitochondrial genome encodes the seven most hydrophobic subunits of complex I (ND1-ND6 and ND4L) (Efremov and Sazanov 2011) and inhibition of complex I leads to increased generation of ROS, decreased ATP levels, and induction of apoptosis (Langston and Ballard 1983). Complex III is the

ETC enzyme responsible for transferring electrons to cytochrome c through the cytochrome b mediated Q cycle. The resulting reduced cytochrome c and then transports the electrons downstream to complex IV. If the mutations identified by sequencing lead to dysfunction in cytochrome b, the result may be a backup of electrons in the upstream OXPHOS components resulting in ROS production and insufficient ATP supply (Tarnopolsky et al. 2004).

Since the 13 mtDNA-encoded OXPHOS genes are essential to mitochondrial energy production (Wallace et al. 2010), the coding region variation identified in this study might be related to ROS production at OXPHOS complexes I (related to m.12705C>T, *ND5* or m.5460.G>A, *ND2*), ATP generation efficiency through the collective impairment of the respiratory chain (Brand 2010), or through apoptosis (Li 2003). Individual and collective variations in the HV1–2 regions (related to m.309C>T, HV2) may affect mitochondrial function by affecting the rate or efficiency of mitochondrial biogenesis (increase in mitochondrial number and/or mass). An important aspect of mitochondrial biogenesis is rate of turnover, which is thought to decline with age (Donati et al. 2001). Impaired ability to turnover may allow for defective mitochondria to accumulate, especially in older, postmitotic cells leading to impaired respiratory capacity (de Grey 1997). It is known that mitochondrial biogenesis is affected by several natural compounds (Lai et al. 2017), behavioral interventions (Civitarese et al. 2007; Guarente 2008; Menshikova et al. 2006), and pharmacologic agents (Bar-Am et al. 2009; Weinreb et al. 2008; Youdim and Buccafusco 2005) that target the mitochondria and have been shown to induce mitochondrial biogenesis and increase ETC activity. Mitochondria-targeted peptides and lipophilic cations may also enhance in vivo delivery of antioxidants or other putative therapeutics (Leitao-Rocha et al. 2015). While these interventions have demonstrated efficacy on biogenesis and ETC activity, few studies have examined their associations with mobility in older adults. Variant associations discovered in this work may explain the variability in response to interventions designed to increase ETC activity and/or biogenesis.

This study had several strengths, including complete mtDNA sequencing, allowing for an unbiased assessment of mitochondrial genomic variation; two well-characterized cohorts, including participants from multiple ancestries; assessment of walking speed at short and long distances; a large range in habitual walk speeds that

represents the range observed in the general population; and evaluation of the inability to continuously walk a long distance (i.e., mobility impairment). Some weaknesses are also acknowledged, including limited power to detect an effect of individual rare variants. It is possible that the mtDNA variants identified in this study may not be causally related to the walking speed; thus, additional replication cohorts beyond the LIFE and Health ABC studies are needed to support the current findings.

In summary, replication and meta-analysis identified several mtDNA variants that are associated with variation in walking speed. Some of these associations were consistent across racial groups and over short and long walking distances. These results may help to uncover specific mitochondrial pathways that explain differences walking speed and maintenance of mobility in older adults, potentially identifying those at risk of developing mobility impairments. Identifying mitochondrial genetic variants that are associated with walking speed and mobility impairment also generates new hypotheses about additional molecular targets (e.g., complexes I and III) or mechanisms (e.g., mitochondrial protein synthesis and assembly) that may be involved in human mobility.

Author contributions TMM and GJT were responsible for the study concept, obtained funding, and created initial draft of manuscript and results; GJT and JAK: statistical analyses; MP: PI of the LIFE study and obtained funding for the LIFE study. Authors MMM, CAF, RAF, FH, NJ, SK, TBH, ABN, SRC, ACK, MP, and AJS were responsible for data collection, critical revisions of content, and approval of final version for publication; GJT and JAK had full access to all of the data (including statistical reports and tables) and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest The authors declare that they have no conflict of interest.

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