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MITOCHONDRIAL DNA VARIANTS AND PULMONARY FUNCTION IN OLDER PERSONS

Carlos A Vaz Fragoso, MD^{a,b}, Todd M. Manini, PhD^c, John A. Kairalla, PhD^d, Thomas W. Buford, PhD^e, Fang-Chi Hsu, PhD^f, Thomas M. Gill, MD^a, Stephen B. Kritchevsky, PhD^g, Mary M. McDermott, MD^h, Jason L. Sanders, MD, PhDⁱ, Steven R. Cummings, PhD^j, and Gregory J. Tranah, PhD^j

^aYale School of Medicine, Department of Medicine, New Haven, CT

^bVeterans Affairs Connecticut Healthcare System, Department of Medicine, West Haven, CT.

^cUniversity of Florida, Department of Aging and Geriatric Research, Gainesville, FL.

^dUniversity of Florida, Department of Biostatistics, Gainesville, FL.

^eUniversity of Alabama at Birmingham, Department of Medicine, Birmingham, AL.

^fWake Forest School of Medicine, Department of Biostatistical Sciences, Winston-Salem, NC.

^gWake Forest School of Medicine, Sticht Center for Healthy Aging and Alzheimer's Prevention, Winston-Salem, NC.

^hNorthwestern University, Feinberg School of Medicine, Chicago, Illinois.

ⁱMassachusetts General Hospital, Department of Medicine, Boston, MA.

^jCalifornia Pacific Medical Center Research Institute, San Francisco, CA.

Abstract

BACKGROUND.—We provide the first examination of mitochondrial DNA (mtDNA) variants and pulmonary function in older persons.

METHODS.—Cross-sectional associations between mtDNA variants and pulmonary function were evaluated as a combined p-values meta-analysis, using data from two independent cohorts of older persons. The latter included white and black participants, aged 70 years, from the Lifestyle Interventions and Independence for Elders study (LIFE) (N=1,247) and the Health, Aging and

Address correspondence to: Carlos A. Vaz Fragoso, MD; 950 Campbell Ave., West Haven, CT 06516 USA; Phone 203-932-5711; Fax 203-937-4932; carlos.fragoso@yale.edu.

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Body Composition study (Health ABC) (N=731), respectively. Pulmonary function included the forced expiratory volume in one-second as a Z-score (FEV1z) and the maximal inspiratory pressure (MIP) in cm of water.

RESULTS.—In black participants, significant associations were found between mtDNA variants and MIP: m.7146A>G, *COI* (p=3E-5); m.7389T>C, *COI* (p=2E-4); m.15301G>A, *CYB* (p=9E-5); m.16265A>G, *HVI* (p=9E-5); meta-analytical p-values <0.0002. Importantly, these mtDNA variants were unique to black participants and were not present in white participants. Moreover, in black participants, aggregate genetic effects on MIP were observed across mutations in oxidative phosphorylation complex IV (p=0.004), complex V (p=0.0007), and hypervariable (p=0.003) regions. The individual and aggregate variant results were significant after adjustment for multiple comparisons. Otherwise, no significant associations were detected for MIP in whites or for FEV1z in whites or blacks.

CONCLUSIONS.—We have shown that mtDNA variants of African origin are cross-sectionally associated with MIP, a measure of respiratory muscle strength. Thus, our results establish the rationale for longitudinal studies to evaluate whether mtDNA variants of African origin identify those at risk of subsequently developing a respiratory muscle impairment (lower MIP values).

Keywords

mitochondrial DNA; pulmonary function; aging

INTRODUCTION

A respiratory impairment in older persons is most often established by spirometry, in particular the forced expiratory volume in 1-second (FEV1) (1, 2). A decreased FEV1 usually denotes impaired respiratory mechanics (e.g. airflow obstruction) and is associated with adverse health outcomes (1–4). In addition, because age-related sarcopenia and cardiorespiratory disease may decrease the force generating capacity of the diaphragm, a respiratory impairment may be established by a decreased maximal inspiratory pressure (MIP), a measure of respiratory muscle strength (1, 5, 6). A decreased MIP is likewise associated with adverse health outcomes (5–8).

Twin studies suggest that genetic factors account for 32%–67% of the variance in pulmonary function and that the contribution of genetic factors to pulmonary function increases with age (9). Several genome-wide association studies of pulmonary function have identified a modest number of loci associated with these traits (10–14). However, the missing heritability in pulmonary function 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. This energy is supplied by a complex OXPHOS system that is dependent upon the coordinated expression and interaction of genes encoded in the nuclear and mitochondrial DNA (mtDNA). The majority of genes encoded by mtDNA are crucial for the machinery that converts metabolic energy into ATP, including 37 genes encoding

polypeptides, tRNAs, and rRNAs. With a mutation rate 10–20 times higher than nuclear DNA (15), mtDNA is most appropriately studied via complete sequencing, as collections of common and rare variants have been shown to influence phenotypes in numerous ways (16). Although common and rare mtDNA variants have been shown to contribute to several diseases and aging-related traits (17–21), there is a paucity of research examining associations between mtDNA variants and pulmonary function.

In the current study, we provide the first examination of mitochondrial genomic variation and pulmonary function in older persons. Our objective was to perform a combined p-values meta-analysis, using data from two independent cohorts of older persons. The latter included white and black participants, aged 70 years, from the Lifestyle Interventions and Independence for Elders study (LIFE) (N=1,247) and the Health, Aging and Body Composition study (Health ABC) (N=731) (22–24). Pulmonary function included the FEV1 as a Z-score (FEV1z) and the MIP in cm of water, both evaluated as continuous variables and with results stratified by white and black race (i.e., race is a predictor of pulmonary function) (1, 2, 25). In secondary analyses, we additionally evaluated FEV1z and MIP as categorical variables (< lower limit of normal). We hypothesized that mtDNA variants are associated with pulmonary function, potentially identifying those at risk of having a respiratory impairment and new therapeutic targets.

METHODS

Study populations

We evaluated two independent, well-characterized cohorts of older persons, specifically the LIFE and Health ABC studies (22–24).

LIFE was a multicenter, single-blinded, parallel randomized trial, having enrolled 1,635 participants at 8 field centers across the U.S. between February 2010 and December 2013. LIFE included persons aged 70–89 years who: (a) were inactive (<20 min/week in the past month performing regular physical activity 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) (26) score ≤ 9 ; (c) could walk 400 m in <15 minutes without sitting, leaning, or the help of another person or walker; (d) had no major cognitive impairment; and (e) could safely participate in a moderate physical activity intervention.

Health ABC enrolled participants from two field centers between 1997 and 1998. Health ABC included 3,075 persons aged 70–79 years who self-reported: (a) no difficulty in walking a distance of 0.4 km or climbing at least 10 stairs; (b) independence in activities of daily living; (c) had plans to live in the area for 3 years; and (d) had no life-threatening illnesses. As per study design, Health ABC enrolled a similar frequency of blacks and whites, and females and males.

The institutional review board at all field sites approved each study protocol and written informed consent was obtained from all study participants. LIFE is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT01072500 and NCT00116194).

Baseline characteristics

Age, self-designated race/ethnicity and sex (fixed set of options), body mass index (BMI, kg/m²), smoking status (never- and ever-smoker), and self-reported, physician diagnosed chronic conditions were recorded at baseline. Ever-smoker included a history of 100 cigarettes smoked.

Pulmonary function

Participants underwent spirometry at baseline, using protocols from the American Thoracic Society (ATS) (2, 8, 27). The spirometric measure of interest was the FEV₁, specifically the highest value recorded during at least two ATS-acceptable spirometric maneuvers (1–4). For comparisons between measured and predicted FEV₁ values, we calculated Z-scores using reference equations from the Global Lung Function Initiative (GLI) (1, 8, 25). A Z-score for FEV₁ (FEV_{1z}) of –1.64 defined the lower limit of normal (LLN) as the 5th percentile of distribution (1, 8, 25). Participants were classified as having a respiratory impairment if FEV_{1z} was <LLN (1, 8, 25).

The MIP was also evaluated at baseline, based on the performance of up to five MIP trials (5, 8, 27). For comparisons between measured and predicted MIP values, we used reference equations from the Multi-Ethnic Study of Atherosclerosis (MESA) to establish the LLN (5th percentile of distribution) (5). Participants were classified as having a respiratory impairment (i.e., respiratory muscle weakness) if their highest MIP in cm of water was <LLN (5, 8).

Mitochondrial DNA sequencing

MtDNA sequence variants were evaluated in 1,377 LIFE participants and 788 Health ABC participants. DNA was extracted from buffy coat using whole blood collected from Health ABC participants at baseline and from LIFE participants at baseline or 12-month visit.

Complete mtDNA sequencing was performed using the next-generation sequencing Illumina MiSeq platform. 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 using TaKaRa LA (“long and accurate”) Taq DNA Polymerase. TaKaRa LA Taq DNA Polymerase combines Taq DNA polymerase and a DNA-proofreading polymerase, with 3'→5' exonuclease activity to enable long and accurate PCR amplification of DNA targets. The size distribution of each PCR amplicon is then verified using an Agilent Technologies 2100 Bioanalyzer. Using the Nextera XT DNA sample Preparation kit, designed specifically for human mtDNA, the PCR amplicons were simultaneously fragmented and tagged with adapter sequences. 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. For variant calling we specified a Q30 quality score (Q-score) which has a 0.1% error rate and an Analytic Threshold of 0.1 for making base calls.

Multiplex-sequencing of 384 barcoded samples yielded an average depth of coverage >250× and a minimum of 100× per individual.

Statistical analysis

Genetic associations followed established norms for performing a meta-analysis (28–34). This included evaluating genetic associations, first in the LIFE cohort and then replicated in the Health ABC cohort, followed thereafter by a combined p-values meta-analysis. The meta-analysis evaluates the heterogeneity of genetic effect across studies and reduces the probability of false-negatives and winner's curse phenomenon (35, 36).

Using a generalized linear model and allele thresholds set to 4% carrier frequency (CF), the continuous measures of FEV1z and MIP were first compared between major and minor alleles for individual mtDNA variants in both LIFE and Health ABC. Next, using unconditional logistic regression models, the odds ratios (ORs) and 95% confidence intervals (CIs) of having a respiratory impairment, defined categorically by FEV1z<LLN and MIP<LLN, respectively, were additionally calculated for individual mtDNA variants in both LIFE and Health ABC. Thereafter, combined p-values meta-analyses of FEV1z and MIP results were performed using METAL (37) with a fixed effects model using the inverse-variance weighting method. Because fixed effects meta-analysis assumes that true effect-size is constant across studies the degree of between-study heterogeneity was determined by Cochran's Q-test and I² index. All analyses were stratified by white and black race and adjusted for age, sex, and smoking status, using custom R scripts and SAS version 9.4 (SAS Institute Inc, Cary, NC).

The Sequence Kernel Association Test (SKAT) (38) was also applied, and is designed for resequencing data where rare mutations are observed directly. The SKAT method has higher power than alternative methods for genetic burden analyses (aggregate effects across mutations) (38) and has previously been used for mtDNA sequence analysis (39, 40). The SKAT 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. Specifically, in the current study, SKAT was applied in association tests involving continuous FEV1z and MIP measures and computed the statistical significance for each test, using 10,000 independent simulations. For SKAT testing, a 'hierarchy' of annotations within 7 mtDNA regions was considered in order to refine the influence of collections of rare and common variants, including: each of four OXPHOS complexes (I, III, IV, and V); two rRNAs; all tRNAs combined; and combined hypervariable (HV) regions

Notably, the above associations were examined for all variants in both white and black participants from LIFE and Health ABC. To account for population stratification, all analyses were adjusted for 6 eigenvectors of mitochondrial genetic ancestry derived from principal component analysis (PCA). In prior mtDNA sequencing work, the first 6 eigenvectors accounted for >70% of the variance in the mtDNA sequence dataset (41).

Lastly, the *in silico* PhyloP (42) method examined mtDNA nucleotide conservation, as none of the subsequent associated variants conferred nonsynonymous 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 wherein positive scores reflect evolutionary conservation across taxa (slower rates than expected) and negative scores reflect accelerated evolution (faster than expected).

RESULTS

Table 1 reports characteristics of the LIFE and Health ABC study samples. On average, LIFE participants were older, had greater representation of whites and females, higher proportion of morbid obesity and health conditions, more depressive symptoms, but lower proportion of ever-smokers. Of the 1,377 LIFE participants with complete mtDNA sequencing, 1,149 had FEV1z values and 1,169 had MIP values. Of the 788 Health ABC participants with complete mtDNA sequencing, 731 had FEV1z values and 695 had MIP values. On average, LIFE participants had a lower FEV1z and MIP, and higher proportion of FEV1z<LLN and MIP<LLN.

The sequencing of ~16,5kb of mtDNA from LIFE and Health ABC participants yielded a total of 3,364 variants including: 89 common variants (CF, >5%); 459 low frequency variants (CF, 1%–5%); and 2,816 rare variants (CF, <1%). Among white participants, a total of 103 independent mtDNA variants with CF 4% were examined for associations with pulmonary function, resulting in a Bonferroni adjusted $p=0.0005$ for significance that accounted for multiple comparisons. Among black participants, a total of 216 independent mtDNA variants with CF 4% were examined for associations with pulmonary function, resulting in a Bonferroni adjusted $p=0.0002$ for significance that accounted for multiple comparisons.

Table 2 reports meta-analytic variant associations with FEV1z as a continuous measure. Three mtDNA variants were nominally associated with FEV1z in white participants and ten mtDNA variants were nominally associated with FEV1z in black participants. None of these variants were statistically significant after Bonferroni adjustment for multiple comparisons.

Table 3 reports meta-analytic variant associations with MIP as a continuous measure. Among white participants from both study samples, one common mtDNA variant (m. 16296C>T, HV1) was nominally associated with FEV1z, but not statistically significant after adjustment for multiple comparisons. Among black participants from both study samples, twelve common mtDNA variants were associated with a decreased or increased MIP, of which four were statistically significant after adjustment for multiple comparisons (Table 3, Figures 1 and 2): m.7146A>G, *COI* ($p=3E-5$); m.7389T>C, *COI* ($p=2E-4$); m. 15301G>A, *CYB* ($p=9E-5$); and m.16265A>G, *HVI* ($p=9E-5$).

Table 4 reports meta-analytic variant associations with FEV1z<LLN and MIP<LLN, respectively. A nominally reduced risk of having MIP<LLN was observed for the m. 5460G>A variant in white participants from both study samples, as well as in black participants from Health ABC. This association was not statistically significant after adjustment for multiple comparisons. Of note, the number of black LIFE participants who

had MIP<LLN was limited (n=37) and none carried the variant m.5460A allele. In addition, no associations with FEV1z<LLN reached statistical significance in white or black participants. There was no evidence of between-study heterogeneity for each within-race analysis (Q test) 0.05, I^2 <50%).

Using SKAT, statistically significant aggregate effects on MIP (p=0.007 due to multiple test correction for 7 mtDNA regions) were observed across OXPHOS complex IV (p=0.004), complex V (p=0.0007), and HV (p=0.003) regions in blacks from LIFE and Health ABC. No aggregate genetic effects were observed for MIP in whites, nor for FEV1z in blacks or whites.

In silico prediction methods focused on nucleotide conservation generated via phyloP, as none of the associated variants conferred nonsynonymous substitutions. Results showed that three of the MIP-associated variants were not highly conserved among multiple vertebrate taxa, i.e., these variants exhibited negative phyloP scores: m.7146A>G = -0.40, m.15301G>A = -14.9, and m.16265A>G = -1.3. A single variant associated with MIP exhibited a positive phyloP score, m.7389T>C = 0.07, consistent with modest conservation at this site.

DISCUSSION

In a combined p-values meta-analysis of two independent cohorts of older persons, our results suggest that mtDNA variants are associated with MIP, a measure of respiratory muscle strength. Specifically, in black participants, four loci were associated with MIP as a continuous variable: m.7146A>G, *COI*, m.7389T>C, *COI*, m.15301G>A, *CYB*, and m.16265A>G, *HVI*. Importantly, these mtDNA variants were unique to black participants, and not present in white participants. Otherwise, associations with MIP as a continuous variable were not statistically significant in whites, and associations with FEV1z as a continuous or categorical variable were not statistically significant in whites or blacks.

Our MIP results potentially identify populations that differ in respiratory muscle strength (MIP), specific to mtDNA variants. For example, as shown in Figure 3, m.7146A>G *COI* and m.15301G>A, *CYB* identify members of specific branches within sub-Saharan African superhaplogroup L. In particular, the m.7146A>G *COI* mutation, which was associated with higher MIP values (increased respiratory muscle strength), encodes the split between basal lineages of the human mtDNA tree (haplogroups L0 [112–188 kya], L1 [108–174 kya] and the more recently derived haplogroups L2–L6 [<68–11 kya]). Conversely, the m.15301G>A, *CYB* mutation, which was associated with lower MIP values (decreased respiratory muscle strength), encodes the split between haplogroups L5 and L2, 3, 4, 6.

We also note that the m.7389T>C, *COI* and m.16265A>G, *HVI* variants, which were associated with higher MIP values (increased respiratory muscle strength), are found in multiple sub-lineages of superhaplogroup L (L0, L1, and L3). Our observation that m.7389T>C and m.16265A>G are associated with MIP, regardless of genomic or phylogenetic background, supports a role for these variants in mitochondrial function. There was minimal evidence for evolutionary conservation at these associated sites and no previously identified

associations with mitochondrial diseases (43). Nevertheless, mitochondrial variants occurring in one of the OXPHOS complexes (m.7389T>C, *COI*) may lead to impairment of the respiratory chain (electron transport chain [ETC]), affecting reactive oxygen species (ROS) production, ATP generation efficiency, or apoptosis (44–46). In addition, HV1 sequence variation (m.16265A>G) may be involved in regulating mtDNA copy number (47), which may impact the capacity of the muscle to generate force.

Significant pooled effects on MIP were observed in black participants across OXPHOS complexes IV and V, and the HV region. The 13 mtDNA encoded OXPHOS genes are essential to mitochondrial energy production (48), and collective genetic variation within complexes IV and V may be specifically related to impairment of the respiratory chain (ETC), ROS production, or ATP generation (49–51). Defects in complex IV are associated with Leigh Syndrome, hypertrophic cardiomyopathy, and myopathy (49). Defects in complex V are associated with ATP synthase deficiency and it has been proposed that mutations in ATP6 and ATP8 are associated with reduced complex V assembly and impaired ATP synthase function (50, 51).

Prior research provides strong evidence linking mitochondrial function to pulmonary function and health (52–57). For example, diaphragmatic fatigue related to mitochondrial dysfunction may contribute directly to decreased diaphragmatic contractility and age-related declines in exercise capacity, oxygen consumption, and carbon dioxide production (54, 55). To date, however, a large, multi-ethnic examination of the role of mtDNA variation in pulmonary function in the elderly has not been conducted. The current work identified statistically significant associations of mtDNA variants with MIP that are unique to black participants. Overall, African populations represent the most ancient human group and exhibit the largest extent of mtDNA variation when compared with Asian- and European-populations. In the current study, we identified over twice as many common mtDNA variants in black participants, as compared with white participants. Thus, surveying the more polymorphic mitochondrial African genome may have yielded more informative loci related to the role of mitochondrial variation in respiratory muscle strength.

We note the lack of association between mitochondrial variants and FEV1z in black and white participants, suggesting that mitochondrial function may not have a prominent role regarding reductions in pulmonary function that are attributed to impairments in respiratory mechanics (e.g. airflow obstruction). This conclusion is premature, however, because our LIFE and Health ABC study samples included only a small number of participants with FEV1z <LLN (n=186 and 89, respectively). Hence, future work should include larger study samples with FEV1z <LLN, including stratification by spirometry-confirmed chronic obstructive pulmonary disease (COPD). As discussed earlier, prior research suggests that a link may exist between mitochondrial dysfunction and respiratory diseases (56, 57).

This study had several strengths, including: complete mtDNA sequencing, allowing for an unbiased assessment of mitochondrial genomic variation; two independent, well-characterized cohorts of older persons, having participants from multiple ancestries; and assessments of both FEV1 and MIP. Limitations are acknowledged, including: small sample sizes, limiting statistical power when evaluating the LIFE and Health ABC cohorts

separately and when evaluating the effect of individual rare variants; and the mtDNA variants identified in this study may not be causally related to MIP (i.e., based on cross-sectional associations). Thus, replication cohorts are needed, including those with a substantially greater representation of older persons having FEV1 and MIP <LLN, respectively.

In conclusion, using data from two independent cohorts of older persons, our results suggest that mtDNA variants of African origin are associated with MIP, potentially identifying those at risk of developing a respiratory muscle impairment. Future larger studies are needed, however, to further evaluate associations between mtDNA variants and pulmonary function, especially regarding MIP.

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Highlights

- Combined p-values meta-analysis of two separate cohorts of older persons
- Respiratory muscle strength assessed by the maximal inspiratory pressure (MIP)
- mtDNA variants of African origin were associated with MIP
- mtDNA variants of African origin: m.7146A>G, m.7389T>C, m.15301G>A, m.16265A>G
- No statistically significant associations with MIP in white participants

Figure 1.

Solar plot representing mtDNA variant associations with MIP, expressed as a continuous measure (cm of water), in black participants from the LIFE and Health ABC study samples. Each dot represents a mtDNA variant association with MIP, color coded by mitochondrial gene. The blue circle within the solar plot represents a p-value of 0.05. Abbreviation: MIP, maximal inspiratory pressure (cm of water).

Figure 2.

Average MIP values for the m.7146A>G, m.7389T>C, m.15301G>A, and m.16265A>G positions in black participants from the LIFE and Health ABC study samples. Open squares represent the reference allele according to the Cambridge reference sequence (NC_12920), while open circles represent the substituted base (i.e., mtDNA variant). The line crossing the open squares and circles represent standard error bars for the respective average MIP values. Abbreviation: MIP, maximal inspiratory pressure (cm of water).

*m.7389T>C, m.16265A>G found in multiple sub-lineages of haplogroups L0, L1, and L3

Figure 3.

Relationships of major haplogroups within sub-Saharan African superhaplogroup L. Variations m.7146A>G *COI* and m.15301G>A, *CYB* identify members of specific branches within superhaplogroup L.

Abbreviation: mtMRCA, mitochondrial most recent common ancestor.

Table 1.

Baseline characteristics of study samples.

Characteristics	LIFE (N = 1,247)	Health ABC (N = 731)
Age in years, mean (SD)	78.8 (5.2)	74.0 (2.9)
Race/Ethnicity		
White, n (%)	955 (76.6)	374 (51.2)
Black, n (%)	210 (16.8)	357 (48.8)
Other, n (%)	82 (6.6)	-
Female, n (%)	830 (66.6)	383 (52.4)
BMI, kg/m ² , mean (SD)	30.3 (6.0)	27.6 (4.8)
Morbid obesity (BMI ≥ 35)	254 (20.4)	52 (7.1)
Smoking status *		
Never, n (%)	638 (52.0)	315 (43.1)
Ever, n (%)	589 (48.0)	416 (56.9)
Health conditions, n (%) †		
Hypertension	838 (67.2)	321 (43.9)
Diabetes	256 (20.5)	120 (16.4)
Cardiovascular disease ‡	167 (13.4)	83 (11.4)
Cancer	261 (20.9)	94 (12.9)
Chronic pulmonary disease §	174 (14.0)	42 (5.7)
Osteoarthritis with pain	192 (15.4)	25 (3.4)
Depressive symptoms, mean CES-D score (SD)	8.4 (7.8)	4.6 (5.0)
FEV1z, mean (SD)		
Overall	-0.64 (1.04)	-0.35 (1.12)
White	-0.70 (1.03)	-0.42 (1.09)
Black	-0.42 (1.04)	-0.27 (1.14)
Other	-0.50 (1.01)	-
FEV1z < -1.64 (< LLN), n (%)	186 (16.2)	89 (12.2)
MIP (cm of water), mean (SD)		
Overall	59.6 (22.8)	76.3 (28.0)
White	59.7 (22.8)	79.4 (26.8)
Black	59.1 (23.3)	73.1 (28.8)
Other	58.8 (20.7)	-
MIP (cm of water) < LLN, n (%)	167 (14.3)	57 (8.2)

Abbreviations: BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression Scale; FEV1z, forced expiratory volume in 1 second, expressed as Z-score; LLN, lower limit of normal; MIP, maximal inspiratory pressure; SD, standard deviation.

* Based on history of smoking 100 or more cigarettes.

† Self-reported, physician diagnosed.

[‡]Includes myocardial infarction, stroke, or heart attack.

[§]Includes asthma or chronic obstructive pulmonary disease (chronic bronchitis or emphysema).

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Table 2.

Meta-analytic variant associations with FEV1z expressed as a continuous measure and stratified by white and black ancestry.

Race and mtDNA variant	LIFE (n=1149)		Health ABC (n=731)		Meta-Analysis p-value [‡]		
	Beta (SE) *	p-value	CF	Beta (SE) *		p-value	CF
WHITE							
m.207G>A, HV2	-0.03 (0.13)	0.024	0.07	-0.32 (0.30)	0.059	0.04	0.017
m.497C>T, HV3	0.23 (0.14)	0.085	0.08	0.44 (0.17)	0.020	0.13	0.012
m.16093T>C, HV1	-0.27 (0.15)	0.069	0.06	-0.32 (0.14)	0.025	0.21	0.013
BLACK							
m.2245A>C, 16S [‡]	0.77 (0.31)	0.016	0.05	0.76 (0.32)	0.01	0.04	0.001
m.3796A>G, ND1 [‡]	-0.91 (0.36)	0.012	0.05	-0.34 (0.22)	0.007	0.08	0.001
m.709G>A, 12S [§]	-0.46 (0.23)	0.050	0.11	-0.32 (0.14)	0.007	0.27	0.002
m.15244A>G, CYB [‡]	0.86 (0.34)	0.011	0.05	0.71 (0.19)	0.034	0.12	0.005
m.10321T>C, ND3 [‡]	-0.64 (0.27)	0.019	0.08	-0.32 (0.2)	0.059	0.11	0.009
m.3516C>A, ND1 [‡]	0.67 (0.33)	0.045	0.05	0.80 (0.18)	0.047	0.12	0.009
m.151C>T, HV2 [‡]	-0.64 (0.27)	0.020	0.10	-0.57 (0.18)	0.075	0.13	0.012

Abbreviations: CF, carrier frequency; FEV1z, forced expiratory volume in 1 second, expressed as Z-score; SE, standard error.

* Slope of change in FEV1z, wherein negative values denote a decrease in FEV1z and positive values denote an increase in FEV1z.

[‡] Adjusted for age, sex, and smoking status. Bonferroni adjusted significance thresholds: White, p=0.0005; and Black, p=0.0002.

[‡] Variant not present in other races.

[§] Variant present but not significant in other races.

Table 3.

Meta-analytic variant associations with MIP expressed as a continuous measure and stratified by white and black ancestry.

Race and mtDNA variant	LIFE (n=1169)		Health ABC (n=695)			Meta-Analysis	
	Beta (SE) *	p-value	CF	Beta (SE) *	p-value	CF	p-value [‡]
WHITE							
m.16296C>T, HV1 [‡]	8.8 (3.8)	0.02	0.04	5.2 (2.4)	0.006	0.11	0.001
BLACK							
m.7146A>G, COI [‡]	10.9 (3.6)	0.002	0.29	13.2 (2.7)	0.002	0.19	0.00003
m.16265A>G, HV1 [‡]	14.8 (5.8)	0.012	0.09	28.4 (7.8)	0.007	0.11	0.00009
m.7389T>C, COI [‡]	11.1 (4.2)	0.009	0.21	6.5 (1.9)	0.004	0.27	0.0002
m.15301G>A, CYB [‡]	-8.1 (3.5)	0.024	0.67	-14.7 (2.2)	0.001	0.83	0.00009
m.151C>T, HV2 [‡]	15 (6.1)	0.015	0.10	13.4 (2.4)	0.004	0.13	0.0003
m.3666G>A, NDI [‡]	10 (4.2)	0.017	0.22	7.7 (1.9)	0.005	0.27	0.0005
m.2885T>C, 16S rRNA [‡]	8.5 (3.7)	0.024	0.30	7.2 (1.8)	0.049	0.34	0.005
m.247G>A, HV2 [‡]	9.8 (3.9)	0.013	0.24	7.5 (1.8)	0.03	0.31	0.003
m.16129G>A, HV1 [§]	10.3 (4)	0.011	0.24	6.7 (1.7)	0.031	0.52	0.005
m.2394A>C, 16S rRNA [‡]	15.9 (6.8)	0.021	0.08	13.3 (6.4)	0.03	0.02	0.005
m.15940T>C, tRNA-thr [‡]	-13.1 (6)	0.03	0.11	-8.2 (4)	0.061	0.14	0.007
m.7256C>T, COI [‡]	6.7 (3.4)	0.054	0.55	9.7 (1.8)	0.017	0.7	0.007

Abbreviations: CF, carrier frequency; MIP, maximal inspiratory pressure (cm of water); SE, standard error.

^{*} Slope of change in MIP, wherein negative values denote a decrease in MIP and positive values denote an increase in MIP.

[‡] Adjusted for age, sex, and smoking status. Bonferroni adjusted significance thresholds: White, p=0.0005; and Black, p=0.0002. **Bold area** within Table 3 includes variants that achieved the Bonferroni adjusted significance threshold.

[§] Variant not present in other races.

[§] Variant present but not significant in other races.

Table 4.

mtDNA variant associations with MIP < LLN, stratified by white and black ancestry.

Race and mtDNA variant	LIFE			Health ABC			Meta-Analysis [*]
	CF	Odds Ratio (95%CI) of having MIP<LLN	p-value	CF	Odds Ratio (95%CI) of having MIP<LLN	p-value	p-value [†]
WHITE							
m.5460G>A	0.08	0.90 (0.85–0.95)	0.04	0.11	0.93 (0.89–0.97)	0.03	0.001
BLACK							
m.5460G>A	No cases carried the variant m.5460A allele			0.15	0.78 (0.67–0.89)	0.01	Not Applicable

Abbreviations: CF, carrier frequency; MIP, maximal inspiratory pressure (cm of water); SE, standard error.

^{*} Meta-analysis includes only white participants from the LIFE and Health ABC studies.

[†] Adjusted for age, sex, and smoking status. Bonferroni adjusted significance thresholds: White, $p=0.0005$; and Black, $p=0.0002$.