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Relations of Mitochondrial Genetic Variants to Measures of Vascular Function

Jessica L. Fetterman^{*}, Chunyu Liu^{†,‡}, Gary F. Mitchell[‡], Ramachandran S. Vasan^{*,§,||}, Emelia J. Benjamin^{*,§,||}, Joseph A. Vita[^], Naomi M. Hamburg^{*}, and Daniel Levy^{†,‡}

^{*}Cardiovascular Section, Boston University School of Medicine, Boston, MA

[†]National Heart, Lung, and Blood Institute and Boston University's Framingham Heart Study, Framingham, MA

[#]Population Sciences Branch and Division of Intramural Research, National Heart, Lung, and Blood Institute of the National Institutes of Health, Bethesda, MA

[‡]Cardiovascular Engineering, Inc., Norwood, MA

[§]Preventive Medicine Section, Boston University School of Medicine, Boston, MA

^{||}Department of Epidemiology, Boston University School of Public Health, Boston, MA

Abstract

Mitochondrial genetic variation with resultant alterations in oxidative phosphorylation may influence vascular function and contribute to cardiovascular disease susceptibility. We assessed relations of peptide-encoding variants in the mitochondrial genome with measures of vascular function in Framingham Heart Study participants. Of 258 variants assessed, 40 were predicted to have functional consequences by bioinformatics programs. A maternal pattern of heritability was estimated to contribute to the variability of aortic stiffness. A putative association with a microvascular function measure was identified that requires replication. The methods we have developed can be applied to assess the relations of mitochondrial genetic variation to other phenotypes.

Keywords

Mitochondrial genetics; vascular function

Corresponding author: Jessica L. Fetterman, PhD, Boston University School of Medicine, Evans Building, Room 748, Boston, MA 02118, 617-414-1572, 617-414-1563 (FAX), jefetter@bu.edu.

[^]Deceased

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Disclosures

The other authors report no conflicts.

1. Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in the United States and its burden may further increase as a result of the obesity epidemic [1]. Endothelial function is central to maintaining vascular homeostasis and changes in endothelial function serve as early measures of cardiovascular injury [2–4]. Additionally, endothelial dysfunction and increased vascular stiffness are associated with increased risk of cardiovascular disease outcomes [2–4]. Studies have demonstrated that mitochondrial abnormalities contribute to endothelial dysfunction and may precede the development of atherosclerotic plaques [5–9].

The mitochondrion has long been appreciated for its role as the energy powerhouse of the cell, but more recent studies have underscored mitochondrial involvement in additional roles including the regulation of cell death and proliferation, heme biosynthesis, activation of reduction-oxidation sensitive signaling pathways, calcium homeostasis, heat generation, and steroid hormone synthesis [10]. Mitochondria contain their own genome of approximately 16.6 kb of DNA in humans, and each mitochondrion contains 5–10 copies. The mitochondrial genome encodes 13 protein subunits of the oxidative phosphorylation chain that are essential for ATP generation (Figure 1). The mitochondrial genome also encodes its own translation machinery (22 tRNAs and 2 rRNAs) necessary for mitochondrial protein biosynthesis [10]. The mitochondrial genome is unique in that it is maternally inherited, does not undergo recombination events, and displays distinct regional variation that reflects maternal ethnic origins, thereby offering potential insights into the pathogenesis of ethnicity-related variation in susceptibility to cardiometabolic diseases [10–13].

Analysis of the mitochondrial genome is underrepresented in genetic studies despite the fact the mitochondrion plays an essential role in bioenergetic pathways that are closely linked to pathologic states. A number of deleterious mutations in the mitochondrial genome result in several rare but severe mitochondrial diseases. Such diseases reflect a broad spectrum of phenotypes that often include cardiovascular pathology [10, 14, 15]. The mitochondrial genome shows significant diversity and some of the variants are thought to induce small changes in mitochondrial function that may contribute to an individual's susceptibility to cardiometabolic diseases. In fact, a previous study found several mitochondrial genetic variants that were associated with systolic blood pressure and fasting blood glucose [16]. However, the contribution of mitochondrial genetic variation to alterations in vascular function has not been explored in a community-based sample. We investigated the associations of mitochondrial variants with measures of vascular function in Framingham Heart Study (FHS) participants. We assessed the association of maternal inheritance and the association of both common and rare mitochondrial variants, grouped by gene, with measures of vascular function. Finally, we identified mitochondrial variants that are predicted to have deleterious effects on the function of the coded protein and explored whether such variants were associated with measures of vascular function. These complementary approaches provide novel methodology for relating mitochondrial variants to vascular phenotypes and can promote our understanding of the role of mitochondrial genetics in human disease.

2. Methods

2.1. Study Participants

The Original FHS cohort consists of 5209 individuals recruited in 1948 and participants have been examined every two years [17]. The children and spouses of the children of the Original FHS cohort were recruited in to the FHS Offspring cohort in 1971 and consists of 5124 individuals who have been examined every 4–8 years [18]. The Third Generation cohort was recruited in 2002–2005 and consists of 4095 children of the Offspring cohort [19]. Vascular phenotypes were assessed in subsets of the Offspring and Third generation cohorts as outlined in Table 1. All participants gave written informed consent for genetic research and assessment of vascular traits, and all protocols were approved by the Institutional Review Board of Boston University Medical Center.

2.2. Vascular Phenotypes

Noninvasive Measures of Endothelial Function—Conduit arterial endothelial function was assessed using flow-mediated vasodilation as previously described [20]. Hyperemic flow serves as the stimulus for vasodilation and is an indicator of microvascular function. A Toshiba SSH-140A ultrasound system was used to determine brachial artery diameter at baseline and for one minute following 5 minutes of forearm cuff occlusion. Flow-mediated dilation data were assessed with commercially available software (Brachial Analyzer version 3.2.3, Medical Imaging Applications). Hyperemic flow was assessed using semi-automated signal averaging (Cardiovascular Engineering).

Peripheral Arterial Tonometry (PAT)—Fasting digital PAT was assessed with the Endo-PAT2000 device (Itamar Medical, Caesarea, Israel) placed on the tip of each index finger. Pulse volume changes in the finger were measured following application of pressure to the pulp of the fingertip with a pneumatic plethysmograph in the PAT device. Inflation pressure was set electronically to 10 mmHg below diastolic blood pressure or 70 mm Hg, depending on which was lower. Baseline pulse amplitude was assessed in each finger for 2 minutes and 20 seconds after which arterial flow was interrupted for 5 minutes with a cuff placed on a proximal forearm (Hokanson AG101, D.E. Hokanson Inc, Bellevue, WA). Arterial flow was interrupted with an occlusion pressure set to 200 mm Hg or 60 mm Hg plus systolic blood pressure, whichever was higher. A computerized, automated algorithm was used to analyze the average pulse amplitude for each 30 second interval following forearm cuff deflation up to 4 minutes.

Carotid-Femoral Pulse Wave Velocity—Arterial stiffness was measured by determining carotid-femoral pulse wave velocity as previously described [4]. Arterial tonometry was determined in the supine position after 5 minutes of rest. Simultaneous ECG recordings were obtained from carotid and femoral arteries with a commercially available tonometer (SPT-301, Millar Instruments, Houston, TX). The time delay between the foot of the carotid and femoral waveforms was measured and the carotid-femoral transit distance was estimated by measuring the distance from the suprasternal notch to the carotid and femoral sites, which was corrected for the parallel transmission along the brachiocephalic and carotid arteries and around the aortic arch.

2.3. Genotyping and Quality Control for Mitochondrial Variants

Genotyping was performed on FHS DNA samples using a) the Illumina Infinium Human Exome Chip v1.0, b) an Illumina custom genotyping array, and c) the Illumina Human Omni 2.5 microarray [16]. To ensure genotyping quality for the Exome Chip v1.0 variants, the raw genotyping data were jointly called with other study samples by the Human Genetics Center of the University of Texas Health Science Center at Houston [21]. Initial quality control procedures were applied to genotyping data in joint calling. Because mitochondrial DNA is maternally inherited, all individuals in the same maternal lineage should have the same genotype in the absence of de novo mutations. Therefore, additional quality control was performed by comparing all genotypes for individuals in the same maternal lineages. If an individual's genotype differed from other individuals in the same maternal lineage, his or her genotype was set to missing. Few individuals displayed heteroplasmy and did so for only a few variants; consequently, we had no statistical power to analyze heteroplasmy and therefore, heteroplasmic genotypes were set to missing before statistical analysis. Variants were removed for genotype call rates less than 95%, and individual samples were removed for call rates across the mitochondrial genome of less than 95%.

2.4. Mitochondrial Variant Identification

All mitochondrial variant positions and gene locations were confirmed by aligning the probes to the revised Cambridge Reference sequence (rCRS; GenBank #NC_012920) in Geneious 6.1.5 [22]. Missense mutations were determined by replacement of the nucleotide in the rCRS and translated to the amino acid sequence using the vertebrate mitochondrial translation table in Geneious.

2.5. Protein Pathogenicity Scoring for Mitochondrial Variants

PolyPhen-2—A pathogenicity score was calculated utilizing PolyPhen-2 for mitochondrial variants that resulted in a nonsynonymous mutation [23, 24]. PolyPhen-2 calculates the pathogenicity score based on a number of criteria including sequence, phylogenetic, and structural features. A score resulting in the classification of 'probably damaging' is indicative of a pathogenicity score with low confidence. UniProtKB accession numbers were used to identify the proteins (ND1, P03886; ND2, P03891; ND3, P03897; ND4, P03897; ND4L, P03901; ND5, P03915; ND6, P03923; CYB, P00156; COX1, P00395; COX2, P00403; COX3, P00414; ATP6, P00846; ATP8, P03928). All scoring was performed utilizing the HumDiv dataset, the preferred data set for evaluating rare alleles potentially involved in complex phenotypes. The HumDiv dataset treats mildly deleterious variants as having an effect on protein function, which is likely to be the case for mitochondrial DNA variants. PolyPhen-2 has a false positive rate of 20% and true positive rates of 92% using the HumDiv dataset [24].

PredictSNP—PredictSNP utilizes the six best performing bioinformatic software programs (MAPP, nsSNPAnalyzer, PANTHER, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT, and SNAP) to generate a consensus classifier for nonsynonymous variants [25]. Each individual prediction tool has been developed utilizing different training sets, machine learning methods, and principles and hence, can be combined to generate a consensus score for

missense mutations. PredictSNP has been shown to have lower false positive rates and higher true positive rates compared to any of the individual methods alone [25]. All known previously described pathogenic mitochondrial genetic variants were predicted to have a functional impact on protein function using Poly Phen-2 and PredictSNP (Supplemental Table 1).

2.6. Statistical Analysis

Association analysis was performed using the individual vascular traits (see above) as the dependent measure and the mitochondrial variant as the exposure variable, adjusting for age, sex, and body mass index in regression models. To account for nuclear and mitochondrial inheritance, the familial and maternal correlation matrices were included as random effects in all association analyses. To evaluate aggregation effects of low-frequency and rare coding variants on a vascular phenotype, we performed gene-based tests adjusting for the same set of covariates including age, sex, body mass index. Both the standard burden test [26, 27] and the sequence kernel association test (SKAT) [28] were performed at minor allele frequencies (MAFs) 0.01 (T1) and 0.05 (T5) levels for gene-based test. We assessed normality of the seven outcome phenotypes. Carotid-femoral pulse wave velocity and baseline brachial artery flow were right skewed and therefore were log-transformed for association analyses [29, 30]. The other five phenotypes were approximately normally distributed and were used without transformation in the association analyses. The software R package seqMeta was used for all statistical analyses [31]. To account for multiple testing, $P < 0.05/n$ was used to establish significance, where n is the number of variants or the number of genes at single variant level or gene level, respectively. We did not report associations found to be significant if the variant was present in fewer than 10 individuals.

To estimate heritability attributed to the mitochondrial genome, we first constructed maternal lineage based on pedigree structure [32]. Briefly, for a woman founder, she and all of her daughters and sons belong to the same maternal lineage. All daughters from the woman founder pass down the same maternal lineage to all grandchildren, whereas sons from the woman founder do not pass down the same maternal lineage. A marry-in man belongs to a singleton lineage. We performed linear mixed effect (LME) models to estimate heritability of vascular traits potentially due to the mitochondrial genome [33]. Briefly, we assumed that the total variance in a vascular phenotype can be broken down into the nuclear component, the mitochondrial component, and random error. We estimated the nuclear and the mitochondrial components by including the kinship correlation matrix and maternal correlation matrix in the LME model using the `lmekin` R function (<http://cran.r-project.org/src/contrib/Archive/kinship/>) [34]. The heritability due to the mitochondrial DNA is the proportion of the contribution of the mitochondrial component to the total phenotypic variance. Because the variance component method was more sensitive to normality, we used rank based inverse normal transformation for carotid-femoral pulse wave velocity and baseline brachial artery flow in heritability analysis [35]. The other five phenotypes were used directly in heritability analysis.

3. Results

3.1. Characteristics of the Study Participants

The clinical characteristics and vascular measures of the study participants are summarized in Table 1.

3.2. Characterization of Mitochondrial Variants

We assessed 258 variants within peptide coding regions of the mitochondrial genomes of up to 3,374 FHS Offspring and Third generation cohort participants (Figure 1). The potential functional impact of each variant was determined using PolyPhen-2 and PredictSNP, both of which utilize algorithms taking into account phylogenetic, structural, and biochemical changes by amino acid replacement. Of the 258 mitochondrial genetic variants genotyped in the FHS participants, 40 variants were identified as having potential functional consequences by one or both programs (Table 2) whereas 218 variants were predicted to have no functional consequence (Supplemental Table 2).

3.3. Association Analyses

A pattern of mitochondrial heritability was estimated to contribute to 4.6% of the interindividual variability of carotid-femoral pulse wave velocity, a measure of vascular stiffness. The other vascular measures showed no statistically significant maternal inheritance (Table 3). Age, sex, and BMI explained varying proportions of the variation in the vascular phenotypes (Supplemental Table 3).

The 13966A>G (G, MAF=0.02) variant within NADH dehydrogenase 5 (MT-*ND5*) was associated with PAT ratio, a measure of microvascular function ($p=4\times 10^{-5}$), after adjusting for multiple testing. The 13966A>G explained 0.4% of variation in PAT ratio. Individuals with the rare 13966G allele had a 0.09 lower PAT ratio than those with the 13966A allele. Additional associations that had unadjusted P values <0.05 are provide in Supplemental Table 4. After multiple testing correction, no mitochondrial variants remained significantly associated with baseline brachial artery diameter or flow, brachial artery flow-mediated dilation, hyperemic flow velocity, or baseline pulse amplitude. After adjusting for multiple testing, no significant associations with any of the vascular measures were observed when the mitochondrial genetic variants were grouped by gene and analyzed using a gene-based test. Gene level results with unadjusted P values <0.05 are provided in Supplemental Table 5.

4. Discussion

We evaluated the relations of mitochondrial genetic variants with vascular measures in Framingham Heart Study participants. We identified a mitochondrial genetic variant (A13966G) that was associated with PAT ratio. We did not observe significant associations of the variants with other vascular phenotypes when the variants were grouped by gene. We provide the annotation and predicted functional impacts of the peptide-encoding mitochondrial genetic variants and describe methods for exploring the association of mitochondrial genetic variants with phenotypes that can be applied to other traits.

Mitochondrial oxidative phosphorylation with limited oxidant production is essential for maintaining vascular function [6, 8, 9, 36, 37]. Within the vasculature, disruption of oxidative phosphorylation results in reduced ATP production and elevated oxidant production, which promotes oxidative damage, inflammation, and a loss of nitric oxide signaling. More recently, studies have suggested that pathologic conditions result in a switch from predominately nitric oxide-mediated vasodilation to mitochondrial oxidant-mediated vasodilation [37]. Whereas this switch to mitochondrial oxidant-mediated dilation may help preserve vascular tone, the elevation in oxidants induces oxidative damage and a loss in the anti-atherogenic properties of nitric oxide.

Mitochondrial genetic variants have been reported to be associated with both macrovascular and microvascular disease in European and Asian populations. Mitochondrial haplogroup T has been found to be more prevalent among patients with coronary artery disease in a Middle European case-control study [38]. This same study also found mitochondrial haplogroup T to have a higher prevalence of the vascular complication diabetic retinopathy among individuals with diabetes [38]. Recently, a study showed that cultured human umbilical vein endothelial cells from donors with a European mitochondrial haplogroup differed bioenergetically from cells with an African mitochondrial haplogroup. The investigators reported that endothelial cells from individuals with an African mitochondrial haplogroup produced more oxidants and sustained higher levels of mitochondrial DNA damage than endothelial cells from individuals with a European mitochondrial haplogroup [39]. Hence, mitochondrial genetics may contribute to vascular function in people but the specific variants have yet to be identified.

We observed a putative association of one mitochondrial genetic variant with a measure of microvascular function. The A13966G variant is found within the NADH dehydrogenase subunit 5 and results in the replacement of a polar threonine with a non-polar alanine at amino acid position 544. The NADH dehydrogenase subunit 5 is the core catalytic subunit of complex I of the electron transport chain and consequently, this change from a polar amino acid to a non-polar amino acid may impact the folding of the subunit, its interaction with the other subunits in complex I, or its activity. How the mitochondrial variants associated with vascular traits mediate their potential impact on vascular function will need to be elucidated in future studies.

Genome sequencing microarrays allow researchers to assess hundreds to thousands of genetic variants at one time aiding in the identification of variants associated with complex diseases. However, microarray genomic technology advances result in massive amounts of data and hence, methods for narrowing down the number of null hypotheses tested to increase the statistical power is becoming increasingly important. Several bioinformatic programs have been created to predict the potential effects of nonsynonymous mutations on protein function utilizing either phylogenetic analyses alone or in combination with predictions of the physiochemical properties of the amino acid replacement [23–25]. Bioinformatic and statistical models that combine prediction scores from multiple platforms to estimate the chance of a nonsynonymous mutation causing activity changes within a protein can be utilized to guide which variants are most likely to yield an association with disease traits, and hence limit the numbers of null hypotheses tested. However, this

methodology has not been applied to the analysis of variants within the mitochondrial genome.

The strengths of our study include that we examined a community-based sample with rigorous ascertainment of several vascular phenotypes. Further, we outline methods applicable for potentially enhancing our understanding of how mitochondrial genetic variation impacts complex diseases. Our study has several limitations. The functional prediction scoring of the nonsynonymous variants by bioinformatic databases is largely based on known information on particular peptides. Information on the oxidative phosphorylation proteins is limited as the crystal structures and spatial positioning of the amino acid residues are still being elucidated. Hence, the bioinformatic predictions may underestimate the functional effects of the mitochondrial variants, which may explain why the variants associated with vascular phenotypes were not predicted to have functional effects by the bioinformatics programs. We also did not account for the interaction of the mitochondrial genetic variants with nuclear genetic variants in the oxidative phosphorylation peptides and such interaction likely determines the function of the oxidative phosphorylation proteins. Finally, many of the mitochondrial variants evaluated were present at very low frequencies and obtaining a large enough sample to achieve adequate statistical power to detect small differences in traits may be challenging. The associations identified in this study will be followed up for validity as replication studies become available. Our study was restricted to adults of European ancestry and our findings may not be generalizable to individuals who are younger or of other racial/ethnic descent. Our study was observational and cross-sectional and hence we cannot establish causal relations. We adjusted for age- sex- and body mass index, and we cannot exclude residual confounding. In addition, the effect sizes and the proportion of phenotypic variation explained by genetic variants were small, and may not be clinically significant. Future work will be needed to replicate these associations in independent samples.

Mitochondrial genetic variation may contribute to cardiovascular disease susceptibility. We have now extended our previous work that identified the association of several mitochondrial genetic variants with systolic blood pressure and fasting glucose levels to measures of vascular function [16]. Our work suggests an association of mitochondrial genetic variation with select measures of vascular function, but it warrants replication in additional studies that have assessment of both vascular function and variation in the mitochondrial genome. Herein, we propose a method for the analysis of mitochondrial genetic data that may be applied to other genomic platforms and phenotypes to enhance our understanding of the mitochondrial genetic contribution to complex diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Gary Mitchell is owner of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular stiffness.

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Highlights

- Mitochondrial genetic variation may influence vascular function and susceptibility to cardiovascular disease.
- We assessed the relations of peptide-encoding variants in the mitochondrial genome with measures of vascular function in Framingham Heart Study participants.
- We identified mitochondrial genetic variants predicted to have functional effects using bioinformatics programs.
- We identified a putative association with a measure of microvascular function.
- The methods we developed can be applied to assess the relations of mitochondrial genetic variation to other phenotypes.

Figure 1. Annotation of Mitochondrial Genetic Variants. The mitochondrial variants assessed within the FHS cohorts were mapped to the Revised Cambridge Reference Sequence (blue blocks = gene, purple block = D-loop, yellow = non-functional variant, red = functional variant, green = variant associated with vascular measures).

Table 1

Characteristics of the Study Participants

<u>Variable</u>	<u>Offspring Cohort</u>		<u>Generation 3 Cohort</u>	
	N	Mean (%)	N	Mean (%)
Women, N (%)		1476 (46)		1898 (47)
Age, years	3197	60±9	4050	40±8
Body mass index, kg/m ²	3197	28.1±5.2	4050	26.9±5.5
Baseline brachial artery diameter, mm	2308	4.3±0.9	3893	4.1±0.8
Baseline brachial velocity, cm/s	2307	8.2±4.8	3894	7.4±4.3
Hyperemic flow velocity, cm/s	2307	50.6±21.4	3894	62±18.4
Flow-mediated dilation, %	2308	2.8±2.8	3852	5.9±3.7
ln baseline pulse amplitude	2331	5.7 ±0.9	1975	5.6±0.9
Peripheral arterial tonometry ratio	2331	0.7±0.4	1975	0.7±0.4
Carotid-femoral pulse wave velocity, m/s	2553	10.6±3.8	3875	7.0±1.4

The measurements were measured at the seventh examination cycle (1998–2001) for Offspring cohort and at the first examination cycle for the Third Generation (2002–2005).

SD, standard deviation. Ln, natural logarithm.

Table 2

Functional Mitochondrial DNA Variants

dbSNPID	Variant	Gene	FHS MAF	Substitution	PolyPhen-2 Prediction	Predict SNP Prediction	Described Phenotypes *
rs41460449	T3394C	ND1	0.009582	Y30H	Benign	Deleterious	LHON; Diabetes; Carnitine palmitoyltransferase deficiency; High altitude adaptation, Acute leukemia
rs200453691	C3571T	ND1	0	L89F	Probably Damaging	Neutral	Oncocytoma
rs199684756	C3746T	ND1	0	A147L	Possibly Damaging	Deleterious	
rs28358274	A4824G	ND2	0.002417	T119A	Possibly Damaging	Neutral	
rs2853489	A11172G	ND3	0	N10D	Probably damaging	Neutral	Hypertensive end-stage renal disease
rs28358284	C11177T	ND4	0	N138S	Probably Damaging	Neutral	
rs201803443	T11204C	ND4	0.00616	P140S	Probably Damaging	Neutral	
rs200145866	T11253C	ND4	0.01232	F149L	Benign	Deleterious	
rs200911567	T11984C	ND4	0	I165T	Benign	Deleterious	LHON; Parkinson's disease
rs201922401	C12542T	ND5	0	Y409H	Probably Damaging	Neutral	
rs28359179	T13789C	ND5	0.000614	A69V	Possibly Damaging	Neutral	
rs28359181	C13880A	ND5	0.0006845	Y485H	Probably Damaging	Neutral	
rs200713907	C13924T	ND5	0	S515Y	Possibly Damaging	Neutral	
rs202081448	G13958C	ND5	0.0006854	P530S	Probably Damaging	Deleterious	
rs28359185	T14000A	ND5	0	G541A	Possibly Damaging	Neutral	
rs200933339	T14180C	ND6	0	L555Q	Probably Damaging	Neutral	
rs2853504	A14793G	CYB	0.04061	Y165C	Probably Damaging	Neutral	
rs200786872	T14979C	CYB	0.000602	H16R	Benign	Deleterious	
rs202045169	A15038G	CYB	0.0006845	I78T	Benign	Deleterious	
rs201943501	G15077A	CYB	0.0006845	I98V	Possibly Damaging	Neutral	
rs201194402	G15119A	CYB	0.0006863	E111K	Probably Damaging	Deleterious	Hearing Impairment
rs28357687	T15204C	CYB	0	A125T	Benign	Deleterious	
rs2853506	A15218G	CYB	0.03562	I153T	Benign	Deleterious	
rs41518645	G15257A	CYB	0.0006845	T158A	Possibly Damaging	Neutral	
rs200521299	C15402T	CYB	0	D171N	Benign	Deleterious	LHON; Haplogroup J2 marker, possible helper mutation
rs201262114	G6261A	COX1	0.008264	T219I	Possibly Damaging	Deleterious	
				A120T	Probably Damaging	Neutral	Prostate Cancer; LHON

dbSNPID	Variant	Gene	FHS MAF	Substitution	PolyPhen-2 Prediction	Predict SNP Prediction	Described Phenotypes*
rs202216551	G6267A	COX1	0.001372	A122T	Benign	Deleterious	Prostate Cancer
	G8255C	COX2	0.0004	V224L	Probably Damaging	Neutral	
rs199999390	T903C	COX3	0	F233L	Probably Damaging	Deleterious	
	G8616T	ATP6	0.006053	L30F	Probably Damaging	Deleterious	
rs200811540	T8654C	ATP6	0	I43T	Possibly Damaging	Neutral	
rs200329150	A8923G	ATP6	0	T133A	Probably Damaging	Deleterious	
	G9055A	ATP6	0.0898	A177T	Possibly Damaging	Neutral	Parkinson's disease protective factor
rs201559119	T9098C	ATP6	0	I191T	Probably Damaging	Neutral	Predisposition to anti-retroviral mitochondrial toxicity
	C8393T	ATP8	0.0117	P10S	Probably Damaging	Neutral	Reversible brain pseudoatrophy
rs200715932	A8411C	ATP8	0.00137	M16L	Benign	Deleterious	Severe mitochondrial disorder
rs28358884	C8414T	ATP8	0.0006845	L17F	Probably Damaging	Neutral	Longevity
rs199616772	C8417T	ATP8	0	L18F	Probably Damaging	Neutral	
rs1116906	A8460G	ATP8	0.0006854	N32S	Probably Damaging	Neutral	

* Obtained from MITOMAP[40].

LHON = Leber's Hereditary Optic Neuropathy, ND = NADH dehydrogenase, CYB = Cytochrome b, COX= Cytochrome c oxidase, ATP= ATP Synthase

Table 3

Estimated Maternal Inheritance of Vascular Phenotypes

Phenotypes	Inheritance due to			p-value
	nDNA	nDNA+mtDNA	mtDNA	
	$h^2_{N(\%)}$	$h'^2_{N(\%)}$	$h'^2_{Mito(\%)}$	
Baseline brachial artery diameter	34	29.9	2.9	0.1
Flow-mediated dilation, %	14	11.7	1.2	-
Baseline brachial artery flow, cm/s	19.5	19.5	0	-
Hyperemic flow velocity, cm/s	6.1	4.7	0.7	-
Carotid-femoral pulse wave velocity, m/s	11.2	3.1	4.6	<0.001
Baseline pulse amplitude	29	24.7	2.8	0.1
Peripheral arterial tonometry ratio	22.8	22.8	0	-

nDNA = nuclear DNA, mtDNA = mitochondrial DNA. Both baseline brachial artery flow and carotid-femoral pulse wave velocity were transformed by rank based normalization in heritability analysis.