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Association of Genetic Variation in the Mitochondrial Genome with Blood Pressure and Metabolic Traits

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Abstract

Elevated blood pressure (BP) is a major risk factor for cardiovascular disease. Several studies have noted a consistent maternal effect on BP; consequently, mitochondrial DNA (mtDNA) variation has become an additional target of investigation of the missing BP heritability. Analyses of common mtDNA polymorphisms, however, have not found evidence of association with hypertension. To explore associations of relatively rare (frequency < 5%) mtDNA variants with BP, we identified uncommon/rare variants through sequencing the entire mitochondrial genome in 32 unrelated individuals with extreme-high BP in the Framingham Heart Study (FHS) and genotyped 40 mtSNPs in 7,219 FHS participants. The nonsynonymous mtSNP 5913G>A (Asp4Asn) in the cytochrome c oxidase subunit 1 of Complex IV demonstrated significant associations with BP and fasting blood glucose (FBG) levels. Individuals with the rare 5913A allele had, on average, 7 mm Hg higher systolic BP at baseline ($P_{empirical}$ = 0.05) and 17 mg/dL higher mean FBG over 25 years of follow up ($P_{empirical}$ = 0.009). Significant associations with FBG levels were also detected for nonsynonymous mtSNP 3316G>A (Ala4Thr) in the NADH dehydrogenase subunit 1 of Complex I. On average, individuals with rare allele 3316A had 17 and 25 mg/dL higher FBG at baseline ($P_{empirical}$ = 0.01) and over 25 years of follow up ($P_{empirical}$ = 0.007). Our findings provide the first evidence of putative association of variants in the mitochondrial genome with SBP and FBG in the general population. Replication in independent samples, however, is needed to confirm these putative associations.

Keywords

Mitochondrial genome; Association study; Genetics; Hypertension; Diabetes

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Introduction

Hypertension affects about 1 billion people worldwide including 75 million adults in the United States and it is a major risk factor for myocardial infarction, heart failure, and stroke[1]. Although blood pressure (BP) has a substantial genetic component, with a heritability of long-term BP estimated at about 55 percent[2], the associated markers identified in genome-wide association studies (GWAS) of BP and hypertension[3-6] account for only a small fraction of interindividual variation, and the heritability of hypertension remains largely unexplained[3, 7]. To date, most of the reported genetic variants associated with BP in the population were identified in studies of the nuclear genome [3-6, 8-11]; only limited insights have come from investigations of the mitochondrial genome. The two genomes that coexist in each human cell jointly specify the multimeric protein complexes of the major energy-generating oxidative phosphorylation (OXPHOS) pathway [12], and the integrity of both genomes, their communication [13], coordinated expression, and functional compatibilities [14] are required for energy homeostasis. As the OXPHOS pathway mediates an individual's response to factors known to affect BP – such as diet and physical activity – inherited variation in the core OXPHOS subunits coded by the mitochondrial genome may contribute to interindividual BP differences and susceptibility to hypertension.

The human mitochondrial genome is a double-stranded circular molecule of approximately 16.6 kb that encodes 37 genes, all contributing to a single pathway of energy production: 13 protein subunits of the OXPHOS complexes and a set of 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) required for their translation. In contrast to the nuclear genome, the mitochondrial DNA (mtDNA) is present in hundreds to thousands copies per cell, and is inherited exclusively from the mother. The 13 polypeptides are essential OXPHOS subunits that determine the mitochondrial coupling efficiency, thus contributing to the interindividual differences in energy utilization and, consequently, to the wide spectrum of metabolic phenotypes in the population[15]. In addition to energy production, mitochondria play a critical role in other cellular processes[15]. In the last decade, growing evidence has implicated mitochondrial dysfunction in a number of complex diseases, including autism [16] and metabolic syndrome [17].

Indirect evidence of mitochondrial genetic effects on BP and hypertension has come from observations of mother-offspring correlation of BP levels [18-23] and reports of maternal effects on hypertension status and quantitative systolic BP of offspring [24-26]. Although these findings are indicative of a mitochondrial pattern of inheritance, maternal effects may also reflect asymmetric expression of parental alleles due to genetic imprinting [27], or indirect influence of maternal genotype on offspring phenotype [28]. Likewise, mtDNA mutations have been described in families with putative maternal inheritance of abnormal BP phenotypes [29-33], but their causal role in hypertension has not been demonstrated, with a notable exception of the 4291 T>C mutation in the mitochondrial tRNA-isoleucine gene in a carefully characterized large kindred with a cluster of maternally transmitted metabolic defects [34]. To our knowledge, the association of specific mitochondrial mutations with interindividual BP variation in the general population has not been reported. In particular, a comprehensive analysis of common mtDNA variants compiled from publicly available data sets found no evidence of association with hypertension in five study samples[35].

We sought to test the hypothesis that uncommon mtDNA variants (frequency < 5%) affect common complex phenotypes in the general population. To facilitate the discovery of infrequent alleles that influence BP, we sequenced the mitochondrial genomes of 32 unrelated individuals with extreme-high BP from the Framingham Heart Study (FHS), and a subset of variants identified in these individuals was genotyped in 7,219 FHS participants and tested for associations with quantitative BP phenotypes. Because increased BP is a

common feature of metabolic syndrome - a cluster of co-occurring CVD risk factors (obesity, hyperglycemia, dyslipidemia, and elevated blood pressure)[36] - we also explored potential associations of these variants with some metabolic phenotypes. We report the results of our analyses of mitochondrial genome variants in relation to BP and several metabolic traits.

Methods

Please refer to the Online Supplement for methods used to identify maternal lines and mitochondrial lineages (haplogroups), conduct mtDNA sequencing and sequence analysis, genotyping and the quality control of the genotype data, and for expanded description of association and permutation analyses of single mtSNPs and haplogroups.

Study Participants

In 1971, children and spouses of children of the original FHS cohort participants were recruited into the Framingham offspring cohort, which consists of 5,124 men and women[37]. The FHS offspring participants have been examined every four to eight years [37, 38] and, unless specified otherwise, common clinical phenotypes from all examinations were available for this investigation. From 2002 to 2005, a third generation cohort of 4,095 individuals was recruited to the FHS[39]. Due to the recent recruitment of the FHS third generation cohort, clinical data from only a single examination were available for this study. All participants gave written informed consent for genetic research. All protocols in this study were approved by the Institutional Review Board of Boston University Medical Center.

Blood Pressure Phenotypes

At each clinic visit, BP was measured twice by a physician, and the two BP measurements were averaged to derive the systolic (SBP) and diastolic BP (DBP) for that examination. If only one BP measurement was obtained, its value was used for that examination. For participants receiving antihypertensive medication, adjusted SBP and DBP were calculated by adding 10 mm Hg and 5 mm Hg to the measured SBP and DBP values, respectively[2]. Other exclusion criteria were described previously [2, 40].

To evaluate genetic associations using both single occasion measures and longitudinal phenotypes, we constructed several phenotypes for SBP and DBP. We defined the “baseline” BP traits as the BP values at examination 1 of the offspring (1971-1975) and the third generation (2002-2005) cohorts. “Contemporary” BP traits (reflecting ascertainment contemporaneously with DNA collection) were measured at examination 7 (1998-2001) of the offspring participants and at examination 1 of the third generation participants. “Long-term averaged” BP was calculated separately for SBP and DBP using mean values across examinations 1-7 of the offspring participants with data from at least 3 clinic examinations. In addition to continuous BP traits, we analyzed hypertension as a categorical phenotype, defined as SBP of at least 140 mmHg or DBP of at least 90 mmHg or current antihypertensive drug treatment at the contemporary BP examination.

Metabolic Phenotypes

Body mass index (BMI) was used as an index of obesity. To calculate BMI, weight (kilograms) at each examination cycle of the offspring cohort and at the initial examination of the third generation cohort, were used in conjunction with height (meters) at the initial examination. In parallel with BP phenotypes, four adiposity phenotypes were utilized in this study. The baseline, contemporary and long-term BMI phenotypes were defined similarly to

those for BP phenotypes. In addition, a participant was defined as obese if his/her BMI was at least 30kg/m^2 for the contemporary BMI measurement.

Four FBG-based traits were used in association analyses. Because fasting status was not uniformly ascertained at the first two examinations of the offspring cohort, the baseline phenotype used FBG at examination 3 of offspring participants and examination 1 of the third generation cohort. The contemporary phenotype consisted of FBG at examination 7 of the offspring and examination 1 of the third generation cohort. In continuous trait analyses of FBG, we excluded individuals receiving drug treatment for diabetes who had a FBG < 126 mg/dL. Diabetes was defined as a FBG of at least 126 mg/dL or currently receiving insulin or hypoglycemic drug treatment for diabetes at the contemporary examination. Long-term FBG was calculated over examinations 3-7 of the offspring cohort for all participants who had at least two FBG values.

Additional continuous phenotypes included serum K^+ and Mg^{2+} levels measured at examination 2 of the offspring cohort[41], and fasting insulin levels measured at examination cycles 7 and 1 of the offspring and third generation cohorts, respectively[42]. Detailed information about these traits is described in the Online Supplement.

Association Analyses and Permutation Tests

Association analysis between an independent variable (e.g. a mtSNP or haplogroup) and a phenotype was performed using a linear mixed effects (LME) model for a continuous phenotype and the generalized estimation equation (GEE) model for a disease phenotype to properly account for residual correlation among the related individuals. All association analyses were conducted using the R software. Permutation testing was carried out to account for multiple testing, correlations among traits, and to minimize potential false findings from mtSNPs with low minor allele frequencies.

Results

Expanded results are provided in the Online Supplement

Characteristics of the Study Participants—Table 1 summarizes the characteristics of the study participants at the initial examinations of the two study cohorts. At their initial clinic visits, the third generation cohort individuals were, on average, about five years older than the offspring cohort participants (40.2 versus 34.9), had higher BMI (26.9 kg/m^2 versus 24.9 kg/m^2), and a higher prevalence of obesity. Although the third generation and offspring cohorts had about equal proportions of hypertensive individuals (~16%), a higher proportion were treated for hypertension in the third generation cohort (8.2% versus 2.2%). Supplementary Table S1 summarizes the contemporary BP and metabolic phenotypes of the two cohorts. Except for DBP, which was similar in both cohorts, all other trait values were higher in the offspring than in the third generation cohort due to the age difference between the two cohorts at the contemporary examination. By design, only the offspring cohort participants contributed to the long-term measurements (Supplementary Table S2). Moderate to high correlation was observed across traits within several phenotype categories (e.g. BP/hypertension, BMI/obesity, and FBG/diabetes). The correlation (measured by the Pearson correlation coefficient) ranged from 0.4 to 0.8 for BP phenotypes, 0.6 to 0.9 for BMI phenotypes, and 0.5 to 0.8 for FBG phenotypes (Supplementary Figure S1).

Association Analyses—Table 2 summarizes the information for the 40 mtSNPs used in association analysis. These mtSNPs were matched with the positions in reference of the Revised Cambridge Reference Sequence (rCRS, GenBank # NC_012920)[43]. Four of the 40 mtSNPs genotyped in 7,219 FHS individuals were excluded from association analyses due

to very low minor allele frequencies (Table 2). The association results that remained significant after adjustment for multiple testing ($P_{empirical} \leq 0.05$) are presented in Table 3. Additional association results with observed p-values ≤ 0.05 are provided in Supplementary Tables S3, S4, and S6-8.

The association between mtSNP 5913G>A and baseline SBP remained significant after correction for multiple testing (Table 3). Individuals harboring the rare allele 5913A had, on average, 7 mm Hg higher SBP at baseline than those with the common G allele. Additional permutation analyses across continuous phenotypes at three time periods (baseline, contemporary, and long-term averaged) demonstrated significant associations of 5913A ($P_{empirical\_sum} = 0.005$) across the three SBP phenotypes (Supplementary Table S5).

For FBG traits, several associations remained significant after permutation analyses. The variant 3316G>A ($MAF_A = 0.4\%$) showed associations with baseline ($P_{empirical} = 0.01$) and long-term average ($P_{empirical} = 0.007$) FBG. Individuals with the rare allele 3316A had, on average, 17 and 25 mg/dL higher FBG at baseline and over 25 years of follow up, respectively. In addition, the variant 5913G>A demonstrated association with contemporary ($P_{empirical} = 0.03$) and long-term average ($P_{empirical} = 0.009$) FBG. Individuals with the rare allele 5913A had 13 mg/dL and 17 mg/dL higher FBG, respectively, at the contemporary examination and during long-term follow up than those with the common allele. This variant also demonstrated a significant summary p-value ($P_{empirical\_sum} = 0.007$) across the three FBG traits (Supplementary Table S5).

After correction for multiple testing, no association remained significant for BMI related phenotypes (Supplementary Table S6), insulin levels (Supplementary Table S7), or K^+ or Mg^{2+} levels (data not shown). No association with phenotypes remained significant between common European haplogroups (Supplementary Methods) after permutation analysis (Supplementary Table S8).

Discussion

In recent years, the conventional view of mitochondria as static power plants that burn the dietary calories for energy and heat production and house metabolic pathways has been revised and expanded. It is now appreciated that mitochondria form dynamic cellular networks that integrate external and internal signals to regulate numerous cell processes in addition to energy production and thermogenesis, including calcium homeostasis, cellular redox state, reactive oxygen species (ROS), and apoptosis [15]. Epidemiologic studies have demonstrated that mitochondrial disorders are common[44, 45], and the imbalance between energy intake and expenditure is now recognized as an important factor in diverse pathologies, including common multifactorial disorders [16, 17].

The primary goal of this study was to identify mitochondrial genetic determinants of BP. We also explored the effect of mtDNA variants on several metabolic traits. To our knowledge, this is the largest family-based study of mitochondrial genome variation in relation to BP and metabolic phenotypes conducted to date.

Results of our investigation provide support for the hypothesis that rare/infrequent alleles in the mitochondrial genome modulate complex quantitative phenotypes. In particular, we identified an association between mitochondrial variant 5913G>A (rs201617272) in the cytochrome c oxidase subunit 1 (MT-CO1) and BP levels. Individuals with a rare 5913A allele had, on average, 7 mm Hg higher baseline SBP than those with a common 5913G allele. Remarkably, this variant has also demonstrated association with FBG levels; individuals with 5913A had, on average, 17 mg/dL higher mean FBG over 25 years of follow up. Associations with FBG levels were also detected for SNP 3316G>A (rs2853516)

in NADH dehydrogenase subunit 1 (MT-ND1). Individuals with the rare allele 3316A demonstrated much higher FBG levels than those carrying the common 3316G allele, especially in long-term analyses, suggesting its life-long effects.

The OXPHOS pathway is at the core of mitochondrial function and dysfunction. Both 3316G>A in the MT-ND1 subunit of Complex I and 5913G>A in the MT-CO1 of Complex IV are rare nonsynonymous mutations and may affect the functional properties of the respective OXPHOS complexes of ATP synthesis. While overtly pathogenic mutations result in severe and frequently fatal neuromuscular and metabolic diseases[46], milder mutations may have subtle phenotypic consequences. Mitochondrial SNP 3316A replaces the non-polar alanine at position #4 of the MT-ND1 with a polar threonine, and SNP 5913A substitutes the negatively charged aspartic acid at position #4 of the MT-CO1 with a neutral asparagine. Neither change involves the highly conserved site or the known functional domain, and the predicted pathogenicity scores for 3316G>A and 5913G>A are 0.463 and 0.197, respectively [47, 48]. Therefore, both missense mutations are likely to have subtle effects on protein function. These score values, however, are based on the programs designed primarily to quantify the severity of mutations in single-gene diseases[49] and may not necessarily apply to late-onset multifactorial disorders[49] Interpretation of predictive scores is further obscured due to potential interactions of mitochondrial subunits with each other and with nuclear proteins. Moreover, the same mtDNA variant may be either deleterious or beneficial depending on the haplogroup and the environmental context [50]. These properties are not integrated in the prediction scores, necessitating “bench” experimentation to ascertain the effects of a mutation on the OXPHOS function.

To our knowledge, no disease association has been previously reported for SNP 5913G>A. This variant defines a rare subclade K1b of the haplogroup K[51], and in our sample, 41 individuals from 16 maternal lines who carried this variant belonged, in fact, to haplogroup K. However, a search of public databases revealed that 5913A also resides on other haplogroup backgrounds {Pereira, #1609;Rubino, 2011 #1608}. It would be interesting to see if the effect of 5913A on the OXPHOS function is modulated by the haplogroup background. The 3316A variant occurred in multiple haplogroups in our study sample. Sixteen of the 24 individuals who had the rare allele belonged to four haplogroups (unambiguous assignment of the remaining eight individuals to one of nine common European haplogroups was not possible). Previously, 3316G>A was reported in isolated cases of cardiomyopathy [53] and in diabetic patients with left ventricular hypertrophy[54].

Our findings suggest that rare variants in the mitochondrial genome may play an important role in the development of hypertension and diabetes. Replication of our results in independent study samples is warranted, although very large sample sizes are required to identify associations with such low frequency (0.4% - 0.6%) variants. Inadequate sample sizes and the focus on common mtDNA polymorphisms and major haplogroups may explain the failure of earlier population-based association studies to identify an association of mtDNA variants with BP and metabolic traits [35, 55, 56]. Advances in sequencing may facilitate the identification of rare mitochondrial variants in additional large study cohorts and allow replication of our findings in the near future.

Perspective

Results of our investigation have yielded further clues into the genetic causes of common complex disorders. Our findings also suggest that the mitochondrial genome may harbor additional rate variants that contribute to the risk of hypertension and metabolic disorders in the population. Drastic decreases in the cost of sequencing now make it possible to sequence

the entire mitochondrial genome in hundreds of individuals at a relatively low cost. Such efforts are likely to identify additional rare causal mtDNA variants.

The associations we observed involve the rare nonsynonymous mutations. The actual effect of the 5913A and 3316A variants on OXPHOS remains to be determined by functional studies with the mutant MT-CO1 and MT-ND1 proteins. The analysis of intergenic interactions within the mitochondrial genome and between the mitochondrial and nuclear loci is another important venue of inquiry into mechanisms involved in the development of hypertension and metabolic syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

We present the first evidence of associations of mtDNA variants with SBP and FBG in the general population. In contrast to earlier population-based association studies of common mtDNA polymorphisms (frequency>5%) and major mitochondrial haplogroups with BP and metabolic traits, the focus of our investigation was on uncommon/rare variants (frequency<1%) identified through sequencing the entire mitochondrial genome in 32 unrelated individuals with extreme-high BP.

If confirmed in other populations, our findings may have clinical implications for individuals harboring causal mtDNA variants. Our results also suggest that extensive resequencing efforts directed at the mitochondrial genome will likely reveal additional variants modulating BP and metabolic phenotypes.

Table 1
Characteristics of the Study Participants at the Baseline Examination

Phenotypes/Covariates	Offspring Cohort (examination cycle 1: 1971-1975)		Third Generation Cohort (examination cycle 1: 2002-2005)	
	Mean (SD)	N	Mean (SD)	N
Age (yrs)	34.9 (9.7)	3201	40.2 (8.8)	4011
Body mass index (kg/m2)	24.9 (4.2)	3201	26.9 (5.6)	4011
Systolic blood pressure (mmHg) (measured)	120.1 (14.4)	2936	116.8 (14.2)	3817
Systolic blood pressure (mmHg) (treatment adjusted)	120.4 (14.8)	2936	117.6 (15.1)	3817
Diastolic blood pressure (mmHg) (measured)	77.8 (10.0)	2936	75.3 (9.6)	3817
Diastolic blood pressure (mmHg) (treatment adjusted)	77.9 (10.2)	2936	75.7 (10.0)	3817
*Fasting blood glucose (mg/dL)	93.0 (18.7)	2628	95.1 (18.5)	3981
Hypertension (%)	15.9	2937	16.3	3817
Hypertension treatment (%)	2.2	2937	8.2	3817
Obesity (%)	10.4	3201	23.0	4011
Diabetes (%)	2.4	2632	3.1	4011
Diabetes treatment (%)	1.0	2632	1.8	4011
†K^+	47.0 (5.1)	3708	NA	NA
†Mg^{2+}	187.9 (16.0)	3691	NA	NA

*In the offspring cohort, we reported the initial FBG from examination 3 (1984-1987) because the fasting was not uniformly ascertained at the first two examinations. At the examination 3, the average age and BMI (standard deviation, N) were 48.05 (9.70, 2632), and 26.02 (4.52, 2626), respectively.

†The K^+ and Mg^{2+} were measured only at examination 2 (1979-1983) for the offspring cohort.

NA, Not Applicable.

Table 2
Description of the 40 mtSNPs Genotyped in 7,219 FHS Individuals

* Mitochondrial SNPs (Amino Acid Change)	† MAF	Map Locus
150C>T	T(0.0377)	D-loop
217T>C	C(0.0132)	D-loop
228G>A	A(0.0256)	D-loop
‡456C>T	T(0.043)	D-loop
462C>T	T(0.0709)	D-loop
499G>A	A(0.0257)	D-loop
§1719G>A	A(0.0659)	MT-RNR2
3316G>A (Ala4Thr)	A(0.0036)	MT-ND1
3505A>G (Thr67Ala)	G(0.022)	MT-ND1
4216T>C (Tyr304His)	C(0.1782)	MT-ND1
4336T>C	C(0.0167)	MT-TQ
§4580G>A	A(0.0302)	MT-ND2
4917A>G (Asn150Asp)	G(0.0926)	MT-ND2
5046G>A (Val193Ile)	A(0.0118)	MT-ND2
5460G>A (Ala331Thr)	A(0.0556)	MT-ND2
5913G>A (Asp4Asn)	A(0.0059)	MT-CO1
6663A>G (Ile254Val)	G(0.0022)	MT-CO1
§7028T>C	C(0.4387)	MT-CO1
§8251G>A	A(0.0643)	MT-CO2
‡8255G>C (Val224Leu)	C(4e-04)	MT-CO2
8393C>T (Pro10Ser)	T(0.0117)	MT-ATP8
‡8411A>C (Met16Leu)	C(3e-04)	MT-ATP8
8857G>A (Gly111Ser)	A(0.0019)	MT-ATP6
§9055G>A (Ala177Thr)	A(0.0898)	MT-ATP6
9804G>A (Ala200Thr)	A(0.0045)	MT-CO3
10084T>C (Ile9Thr)	C(0.0041)	MT-ND3
§10398A>G (Thr114Ala)	G(0.2116)	MT-ND3
10463T>C	C(0.0936)	MT-TR
10506A>G (Thr13Ala)	G(0.004)	MT-ND4L
§12308A>G	G(0.2257)	MT-TL2
12820G>A (Ala162Thr)	A(0.0014)	MT-ND5
§13368G>A	A(0.088)	MT-ND5
§13708G>A	A(0.1123)	MT-ND5
13934C>T (Thr533Met)	T(0.0188)	MT-ND5
13966A>G (Thr544Ala)	G(0.0185)	MT-ND5
14798T>C (Phe18Leu)	C(0.1406)	MT-CYTB
15452C>A (Leu236Ile)	A(0.1779)	MT-CYTB

* Mitochondrial SNPs (Amino Acid Change)	† MAF	Map Locus
15693T>C (Met316Thr)	C(0.023)	MT-CYTB
‡15889T>C	C(5e-04)	MT-TT
15928G>A	A(0.0876)	MT-TT

*The position of a mitochondrial variant within the Revised Cambridge Reference Sequence (rCRS, GenBank # NC_012920)[43] and its common>minor alleles. For a nonsynonymous mtSNP, the changed amino acid and its position within the protein is shown in parentheses. All nucleotide changes are indicated as L-strand substitutions.

†MAF and minor allele frequency were calculated using the entire sample.

‡mtSNPs were excluded from statistical analysis due to low call rate (< 95%, mt456) or having fewer than 10 variants (mt8255, mt8411, and mt15889) in the entire sample.

§The mtSNPs that define the nine major European mtDNA haplogroups.

Abbreviations: MT, mitochondrially encoded; MT-RNR2, 16S ribosomal RNA; MT-ND1, MT-ND2, MT-ND3, MT-ND4L, MT-ND5, are the NADH dehydrogenase subunits 1, 2, 3, 4L, and 5, respectively; MT-CO1, MT-CO2, MT-CO3 are the Cytochrome c oxidase subunits 1, 2, and 3; MT-ATP6 and MT-ATP8, ATPase subunits 6 and 8; MT-CYTB, Cytochrome b; tRNA, transfer RNA; MT-TL2, tRNA leucine 2; MT-TQ, tRNA glutamine; MT-TR, tRNA arginine; MT-TT, tRNA threonine. D-loop, DNA-displacement loop - the non-coding region between the nucleotides 16024-576 in a circular mtDNA molecule.

Table 3

Association Results for Blood Pressure and Fasting Blood Glucose Phenotypes for mtSNPs with Observed P-values <0.05

Phenotype		mtSNP	Sample size	* Beta (SE)	Effect allele	† $P_{empirical}$
Systolic BP	Baseline	5913G>A	6742	7.24 (2.38)	A	0.05
Fasting blood	Baseline	3316G>A	6593	17.26 (3.92)	A	0.01
glucose	Contemporary	5913G>A	6780	13.0 (3.47)	A	0.03
	Long-term Average	3316G>A	3082	24.81 (5.77)	A	0.007

*Beta estimate and standard error (SE) estimated from linear mixed effects (LME)

†P-value obtained from comparing the observed p-value with pooled smallest permutation p-values of all mtSNPs for a trait after 10,000 permutations of the haplotypes. BP=blood pressure.