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ARTICLE

The role of mitochondrial genome in essential hypertension in a Chinese Han population

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Earlier genetic studies of essential hypertension have focused on nuclear genes or family-based mitochondrial screening in Caucasian and African-American pedigrees. The role of mitochondria in sporadic Chinese hypertensives is unknown. We sequenced mitochondrial genomes in 306 age- and gender-balanced Chinese Han hypertensives and controls. In 153 hypertensives, putative functional changes included 4 changes in *rRNA* genes, 11 changes in *tRNA* genes and 25 amino-acid substitutions. The remaining variants were synonymous changes or non-coding regions. In the 153 controls, 2 base changes in the *tRNA* genes and 13 amino-acid substitutions were found. A8701G in *ATP6* gene (belongs to haplogroup M; $P = 0.0001$) and C8414T in *ATP8* gene (belongs to haplogroup D; $P = 0.01$) were detected significantly different in the cases and controls. Interestingly, the cases were more likely to have two or more amino-acid changes and RNA variants compared with the controls (57.43 *versus* 23.81%, $P = 0.0001$). In addition, several variants we found were highly conserved and/or specifically located at the 3′ end adjacent to the anticodon, which may contribute to the stabilization of structure, and thus lead to the decrease of tRNA metabolism. In conclusion, mitochondrial SNPs (mtSNPs) may affect the course of hypertension in sporadic Chinese hypertensives. Some specific mtSNP within mitochondria may have potential role in the Chinese hypertensives due to their function. Synergetic interaction between mitochondrial mtSNPs and/or haplogroups is needed to be investigated in the future.

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Introduction

Hypertension is a common condition, a risk factor for heart disease, renal failure and stroke, affecting approximately 1 billion individuals worldwide and 160 million in China.[1,2] Thus, understanding the underlying etiology of hypertension has been a major research focus, especially the genetics of hypertension. Indeed, as the completion of the draft sequence of the human genome, geneticists have announced that within 10 years, they expect to determine the significance of the genome as related to essential hypertension.[3] Some progress has been made. For example, for systolic blood pressure alone, 27 nuclear loci have been identified in populations of European and African ancestry.[3] However, to date, no consistent results have been obtained across ethnicity and races supporting the need for genetic studies in diverse populations.[4–8]

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Although the nuclear genome has been studied extensively with respect to hypertension, much less work has been carried out with the mitochondrial genome (mtDNA). Yet, there is evidence to suggest that mitochondria and mtDNA may be important in hypertension. For example, mitochondria produce reactive oxidative species (ROS), and these ROS can cause hypertension.[9–11] With respect to the evidence of mtDNA, a hallmark of involvement of mtDNA is maternal inheritance. Multiple studies have identified strong maternal inheritance of blood pressure, with one study suggesting that over one-third of hypertension could be attributed to mtDNA variation.[12–14] Interestingly, a variant in mitochondrial $tRNA^{Ile}$ has been identified in a single family, which segregated with hypertension and appeared causal.[15] Taken together, this study suggests the importance of looking at mtDNA variation to further our understanding of the underlying etiology of essential hypertension.

Thus, our objective was to determine the relationship between mtDNA variation and essential hypertension. We carried out a systematic and extensive screening of mitochondrial genes at the Institute of Geriatric Cardiology, Chinese People's Liberation Army (PLA) General Hospital and Cincinnati Children's Hospital Medical Center in a Chinese cohort. We focused on this Chinese Han population because of the high morbidity of essential hypertension in Chinese adults (nearly 11.8%)[2] and the limited amount of research on this racial group. We used a population instead of family-based strategy for two reasons: (1) large numbers of hypertensives without family history are not detected in China, and they might be overlooked for lacking of medical knowledge and regular checks and (2) the morbidity of essential hypertension is not totally family-based; 50–60% of hypertensives are sporadically distributed.[2]

Methods

Patients

We evaluated 153 individuals with hypertension; all participants were (1) Beijing residents who were outpatients or in-patients at the Institute of Geriatric Cardiology, Chinese PLA General Hospital from January 2006 to January 2007; (2) older than 18 years old; (3) diagnosed as primary hypertension;and (4) none of the patients were receiving antihypertensive medication. Exclusion criteria: Participants were diagnosed as (1) secondary hypertension (primary aldosteronism, renal arterial sterosis, aortic coarctation, and so on); (2) congenital heart diseases; and (3) organic valve diseases. All participants were Han Chinese evaluated and underwent a thorough examination, including medical record review, clinical evaluation, echocardiographic scanning, biochemical assay as well as genetic analysis. The protocol was approved by the Ethics Committee of Chinese PLA General Hospital and Institute Review Board of Cincinnati Children's Hospital Medical Center; all participants gave written informed consent. Controls were healthy Beijing residents who accepted annual checkup in Chinese PLA General Hospital from January 2006 to January 2007. None of the control subjects had a family history of hypertension, and all had a systolic BP (SBP) of <130 mmHg and a diastolic BP (DBP) of <85 mmHg.

Patient assessment

Weight and height were measured while the subjects were fasting overnight and wearing only underwear. Blood pressure was measured in triplicate by a single physician who was expert in the evaluation of hypertension, with an appropriate arm cuff and a mercury sphygmomanometer with the subject in sitting position after 5 min rest. The arithmetic mean of the last two measurements was calculated. Korotkoff phase I was taken for SBP and V for DBP. Hypertension was defined as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg on 3 consecutive day according to the Seventh Report of the Joint National Committee.[1] After 12-h fast, 4 ml venous blood was drawn to test biochemical assays, including fasting blood sugar, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, blood urea nitrogen, urea acid and creatinine by automatic biochemistry analyzer (Hitach 7600DDP, Japan), using Roche biochemical reaction kits.

Mitochondrial DNA analysis

DNA was isolated from whole blood using Promega Wizard Genomic DNA Purification Kit (Madison, WI, USA), The hottest spots of cardiovascular diseases[16] were screened using oligodeoxynucleotides 3150–3600, 3796–4654 and 7937–8797 bp, purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer, using the BigDye Terminator Cycle sequencing reaction kit, and analyzed with SeqWeb program GAP(GCG) according to the updated consensus Cambridge sequence.[17]

The primers are as follows: 3243F:5′-AGGACAAGAGAAA TAAGGCCT-3′, 3243R:5′-TAAATAGGAGGCCTAGGTTG-3′, 6F:5′-TGCTCCTTTAACCTCTCCA-3′, 6R:5′-AAGGATTATG GATGCGGTTG-3′, 12F:5′-ACGAGTACACCGACTACGGC-3′ and 12R:5′-TGGGTGGTTGGTGTAAATGA-3′.

Statistical analysis

Comparison of the cases and controls, continuous variables are expressed as mean ± SD. Discrete variables in groups are expressed as frequency. Relations between continuous variables were assessed by *t*-test. And discrete variables were analyzed by Pearson's χ^2-test.

To determine whether the cases and controls differ with respect to mtDNA variation, we used logistic regression to determine the chance of having hypertension for subjects with gene variants *versus* those without adjusting for age,

gender or more variables, including heart rate and body surface area.

All the statistical analyses were carried out using the software Stata 7.0 (StataCorp, College Station, TX, USA) and SAS 9.1 (SAS Instituite, Carey, NC, USA).

Results

Clinical evaluation

Descriptive information for the 153 age- and gender-balanced casesand controls is provided in Table 1. The average age (52.06 ± 4.84 *versus* 50.99 ± 15.96 years, $P = 0.43$), gender (54 *versus* 54%, $P = 1$), body mass index (24.32 ± 2.20 *versus* 24.04 ± 3.03, $P = 0.36$) of the cases is similar to the controls. In the cohort, more hypertensives smoke and drink, and hypertensives have higher low-density lipoprotein and creatinine than normotensives.

Analysis of mitochondrial sequence variants

A list of pathogenic variants was taken from MitoMap (http://www.mitomap.org/) and a list of polymorphisms from mtDB (http://www.genpat.uu.se/mtDB/).[18,19] In the 153 hypertensives, putative functional changes included 4 changes in *rRNA* genes, 11 changes in *tRNA* genes and 25 amino-acid substitutions. The remaining variants were synonymous changes or non-coding regions (Table 2). In the 153 controls, 2 base changes in the *tRNA* genes and 13 amino-acid substitutions were found.

Association between mtDNA varianats and hypertension

Comparison of the frequency of variants in the 153 balanced cases and controls is presented in Table 2. Two single base pair changes were significantly ($P = 0.01$ and $P = 0.0001$,) different in the hypertensive patients and the controls: A8701G in *ATP6* gene (threonine to alanine, belongs haplogroup M), and C8414T (LEUCINE to phenylalanine, belongs to haplogroup D) in *ATP8* gene after adjusting age and gender. The results were consistent after adjusting more variables, including heart rate and body surface area. Frequency and type of the rest sequence variants did not differ significantly between cases and controls. The results remained unchanged when all the statistically significant variables in Table 1 were adjusted for. Interestingly, comparing 1 variant with ≥ 2 variants, the hypertensives harbored more variants (≥ 2) than the controls when amino-acid changes and RNAs variants were considered (57.43 *versus* 23.81%, $P = 0.0001$), as seen in Table 3.

Discussion

This is the first population-based report of a systematic screen for mitochondrial variants and their effect on Chinese Han essential hypertension. In our Chinese population, we found a substantial number of mitochondrial variants. Importantly, two amino-acid changes, A8701G in *ATP6* gene (threonine to alanine) C8414T in

Table 1 Clinical manifestations of the hypertensives and the controls

	Hypertensives (n = *153*)	*Controls* (n = *153*)	P-*value*
Women, *n* (%)	83 (54)	83 (54)	1.00
Age, years			
At testing	52.06 ± 4.84	50.99 ± 15.96	0.43
At onset	46.46 ± 6.87	...	
Body mass index, kg/m^2	24.32 ± 2.20	24.04 ± 3.03	0.36
Body surface area, m^2	1.70 ± 0.12	1.70 ± 0.19	0.78
Systolic blood pressure, mm Hg	136.4 ± 17.54	120.56 ± 12.03	0.0001[a]
Diastolic blood pressure, mm Hg	85.45 ± 11.10	75.06 ± 7.81	0.0001[a]
Heart rate, b.p.m.	71.56 ± 7.79	74.59 ± 10.77	0.005[a]
Coronary heart disease, *n* (%)	21 (14)	18 (12)	0.61
Cerebrovascular disease, *n* (%)	4 (3)	2 (1)	0.41
Diabetes, *n* (%)	26 (17)	28 (18)	0.77
Gout, *n* (%)	17 (11)	8 (5)	0.06
Hyperlipidemia, *n* (%)	94 (61)	82 (54)	0.17
Renal diseases, *n* (%)	14 (9)	12 (8)	0.68
Alcohol, *n* (%)	35 (23)	12 (8)	0.0002[a]
Smoking, *n* (%)	30 (20)	15 (10)	0.02[a]
Fasting blood glucose, mmol/l	5.44 ± 1.18	5.37 ± 1.31	0.65
Total cholesterol, mmol/l	4.96 ± 0.91	4.84 ± 1.10	0.32
Triglyceride, mmol/l	1.63 ± 1.00	1.73 ± 0.91	0.35
High-density lipoprotein, mmol/l	1.35 ± 0.30	1.34 ± 0.31	0.66
Low-density lipoprotein, mmol/l	2.81 ± 0.74	2.57 ± 0.82	0.007[a]
Blood urea nitrogen, mmol/l	5.38 ± 1.83	5.07 ± 1.50	0.11
Urea acid, μmmol/l	323.89 ± 69.58	280.20 ± 80.50	0.0001[a]
Creatinine, μmmol/l	77.36 ± 29.01	70.67 ± 46.87	0.13

[a]Represents statistically significant.

Table 2 Summary of mtDNA sequence analyses in the 153 hypertensives and 153 controls

Position	*Amino-acid change*	*Gene*	*Replacement*	*Conservation (H/B/M/X**)*	*Previously reported*	*EH*	*Controls*	P-*value*	P′-*value*
(A)									
3316	Ala to Thr	ND1	G to A	A/I/M/I	Yes	1	1	—	
3349	Ile to Val	ND1	A to G	I/L/I/L	No	1	0	—	
3398	Met to Thr	ND1	T to C	M/M/M/M	Yes	2	0	—	
3434	Tyr to Cys	ND1	A to G	Y/Y/Y/Y	Yes	1	2	—	
3497	Ala to Val	ND1	C to T	A/A/L/S	No	2	0	—	
3523	Thr to Ala	ND1	A to G	T/L/I/I	No	1	0	—	
3866	Ile to Thr	ND1	T to C	I/I/I/I	Yes	1	0	—	
4027	Ile to Val	ND1	A to G	I/L/I/L	No	2	0	—	
4048	Asp to Asn	ND1	G to A	D/N/Y/—	Yes	7	4	0.35	0.34
4125	Ile to Met	ND1	A to G	I/I/I/V	No	1	0	—	
4136	Tyr to Cys	ND1	A to G	Y/Y/Y/Y	Yes	1	1	—	
4216	Tyr to His	ND1	T to C	Y/H/H/H	Yes	2	1	—	
4485	Gln to Lys	ND2	C to A	Q/F/L/F	No	1	0	—	
4491	Val to Ile	ND2	G to A	V/I/I/V	Yes	1	4	—	
4554	Thr to Ala	ND2	A to G	T/V/M/A	No	1	0	—	
8108	Ile to Val	CO2	A to G	I/I/I/I	Yes	1	1	—	
8414	Leu to Phe	ATP8	C to T	L/F/M/W	Yes	30	14	0.01*	0.01*
8454	Asn to Ser	ATP8	A to G	N/K/S/K	No	0	1	—	
8459	Asn to Asp	ATP8	A to G	N/N/T/K	No	2	0	—	
8466	His to Arg	ATP8	A to G	H/Y/P/F	No	1	0	—	
8547	Leu to Pro	ATP8	T to C	L/P/P/—	No	2	0	—	
8584	Ala to Thr	ATP6	G to A	A/V/V/I	Yes	15	22	0.31	0.23
8659	Thr to Ala	ATP6	A to G	T/L/F/L	No	1	0	—	
8674	Ile to Val	ATP6	A to G	I/L/V/L	No	0	1	—	
8684	Thr to Ile	ATP6	C to T	T/V/I/F	No	3	1	—	
8701	Thr to Ala	ATP6	A to G	T/S/L/Q	Yes	75	46	0.0001*	0.0001*
8720	Gly to Ala	ATP6	G to C	G/G/G/G	No	1	0	—	
(B)									
Position	*RNAs*		*Replacement*	*Conservation (H/B/M/X**)*	*Previously reported*	*EH*	*Controls*	*P-value*	*P′-value*
3168	16SrRNA		C to CC	c/t/c/t	No	1	0	—	
3172	16SrRNA		C to CC	c/g/a/t	Yes	1	0	—	
3173	16SrRNA		G to A	g/a/c/a	No	1	0	—	
3203	16SrRNA		A to G	a/a/a/a	No	2	0	—	
3206	16SrRNA		C to T	c/a/t/a	Yes	6	4	0.39	0.55
3277	$tRNA^{Leu(UUR)}$		G to A	g/t/c/a	Yes	1	0	—	
3290	$tRNA^{Leu(UUR)}$		T to C/T	t/a/a/a	No	1	0	—	
4263	I		A to G	A/A/A/A	No	1	0	—	
4316	I		A to TA	A/A/A/A	Yes	1	0	—	
4343	Q		A to G	A/A/A/—	Yes	1	0	—	
4363	Q		T to C	T/T/T/—	No	1	0	—	
4386	Q		T to C	T/T/A/—	Yes	5	2	0.22	0.25
4410	M		C to A	C/C/C/C	No	1	0	—	
8311	K		T to C	T/T/G/—	No	1	0	—	
8343	K		A to G	A/C/C/—	Yes	1	1	—	
8347	K		A to G	A/A/T/—	Yes	1	0	—	

EH, essential hypertension; — represents the variants were too rare to be analyzed; *represents statistically significant. Two point mutations A8701G in *ATP6* gene (threonine to alanine) ($P=0.0001$), C8414T (leucine to phenylalanine) in *ATP8* gene ($P=0.01$) were statistically different after adjusting age, gender, heart rate and body surface area (*P*-value), the results were consistent after adjusting for age and gender only (*P*′-value).
**H/B/M/X means human/bovine/mouse/xenopus.

ATP8 gene (leucine to phenylalanine) were different between cases and controls. These variants belong to haplogroup D[20] (C8414T) and M[21] (A8701G).These results provide evidence that mtDNA variants may affect the course of hypertension in Chinese populations.

In mtDNA, ancestral variants that define groups of types (haplogroups). Haplogroup, population-specific mtDNA lineages, was first initiated to trace back origins of different races and reconstruct ancient migration of women.[22] Recently, the mtDNA lineages were reported more prone to specific symptoms of diseases, including atherothrombotic cerebral infarction,[23] type-2 diabetes[24] and obesity.[24] Meanwhile, haplogroups were found to protect individuals against myocardial infarction and attain longevity.[21,25] In particular, mtSNP A8701G were identified altering mitochondrial matrix pH and intracellular calcium dynamics and suspected to be involved in pathogenesis of diseases.[26] The base pair change C to T at position 8414 in *ATP8* gene,

Table 3 Variants' frequency per patient in the 153 hypertensives and 153 controls

	1 Variant	*≥2 Variants*
Hypertensives	43/101 (42.57%)	58/101 (57.43%)
Controls	48/63 (76.19%)	15/63 (23.81%)
P-value	0.0001[a]	

[a]Represents statistically significant, the above form indicates the frequency of amino-acid substitutions or RNA variants per patient; the result was the same even if all non-coding regions and synonymous changes were included.

part of the larger ATP synthase protein, may reduce the production of ROS and have the potential role to affect the course of hypertension.

In the cohort of hypertensives, there were several variants that occurred too infrequently to have sufficient statistical power, but that have biologic plausibility. T4363C localized at the 3′ end adjacent to the anticodon, which contributed to the stabilization of structure, and thus lead to the decrease of tRNA metabolism. A4263G at the initial part of $tRNA^{Ile}$ may influence the transcription of tRNA herein affect the steady level of protein synthesis. And some other novel RNAs variants (C3168CC, G3173A, A3203G, T3290C, A4263G, T4363C, C4410A and T8311C) in 16s RNA, $tRNA^{Leu(UUR)}$, $tRNA^{Ile}$, $tRNA^{Met}$, $tRNA^{Gln}$ and $tRNA^{Lys}$ were only found in hypertensives, but not controls. In coding regions, even though a novel point variant G8720C in *ATP6* gene, with high conservation among species, was not significant in epidemiological perspective, they may affect the function of protein synthesis and need to be further investigated in the future.

One of the most surprising findings from the analyses using hypertension as the outcomes was that the hypertensive group was more likely to have ≥2 mitochondrial variants than the controls, as seen in Table 3. This novel finding would suggest that there may be a threshold effect with some of these mtDNA variants such that often variability at a single locus will not be sufficient to increase hypertension risk. This is consistent with work in the nuclear genome where it has been proposed that essential hypertension is controlled by multiple genetic loci, each with a relatively weak effect in the population at large;[27–29] and compatible with synergistic pathogenic mutations of diseases that LHON was caused by 'primary mutation' and three mtSNPs called 'secondary mutations' in 11778,14484 and 3460 positions.[30] Otherwise, continent-specific mtDNA variants were prone to different diseases as described earlier.[23,24,31] Thus, investigations on the synergistic interaction between mtSNPs and/or specific mtDNA lineages have been advocated by geneticists to date.[22]

In conclusion, mtSNPs in haplogroups M and D may affect the course of hypertension in sporadic Chinese hypertensives. Some specific mtSNP within mitochondria may have potential role in Chinese hypertensives due to their function. Synergetic interaction between mitochondrial mtSNPs and/or haplogroups is needed to be investigated in the future. This was the first step we marched to investigate the mitochondrial role in Chinese hypertensives. One potential limitation was the small sample size. The conclusion could have been made stronger if the number of people enrolled in the study had been greater.

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