Mitochondrial DNA Mutations Associated with Essential Hypertension in Xin Jiang Han Chinese Population

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Abstract

Background: Genetic factors contribute to essential hypertension (EH) etiology, however, causal genes haven't been identified. Mitochondrial dysfunction is common in EH. However, evidence supporting mitochondrial DNA (mtDNA) mutation involved in EH in Chinese Han is lacking. We aimed to characterize relationship between mtDNA mutation and EH in China.

Methods: Totally 216 individuals including 151 EH patients and 65 controls from Chinese Han population in Xinjiang were sequenced for whole mtDNA genome. Novel variations and haplogroups were identified for each mtDNA sequence. Frequencies of gene mutations were compared between cases and controls, and functional changes of mtDNA genes associated with EH were predicted.

Results: Haplogroups of Chinese Han population in Xinjiang were consistent with those in northern China. No association of mitochondrial haplogroup with EH was observed. Nine novel variations and three EH-associated mutations were identified. Variants in mutation m.12361A>G, m15662A>G and m.1598G>A were predicted to affect functions of ND5, CYTB and 12S rRNA, respectively.

Conclusions: Our results have provided a new clue for mitochondrial genetic characteristics in etiology of EH in Chinese Han population in Xinjiang.

Background

Essential hypertension (EH) is a common chronic disease worldwide, accounting for 9.4 million deaths annually [1]. EH, characterized by elevated arterial pressure, is a major risk factor for morbidity and mortality from myocardial infarction, congestive heart failure, stroke and kidney failure [2]. EH results from interaction between environmental and inherited risk factors, which can be caused by a single-gene or multi-genes. Genetic factors contribute to blood pressure variation, ranging from 20 to 50% [3]. EH cases from families with maternal transmission of disease suggest that mitochondrial DNA (mtDNA) mutations might account for EH [4].

Mitochondria are bacterium-sized organelles in all nucleated cells. Mitochondria contain unique genome (mtDNA), transcription, and translation systems. The human mitochondrial genome is a circular 16.569 base pair (bp) extra-nuclear DNA molecule. mtDNA encodes 13 proteins as subunits of oxidative phosphorylation (OXPHOS) system, as well as 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) [5]. Unlike nuclear genome, mitochondria are in proximity of reactive oxygen species (ROS) generation sites and lacking reparative mechanisms. mtDNA is hence vulnerable to a high rate of mutation. When mtDNA mutations arise, cells initially contain a mixture of wild-type and mutated DNA. If mutational mtDNA exceeds threshold, it can cause dysfunction in specific tissue or organ [6].

The most widely studied class of mtDNA point mutations associated with EH affects single tRNA genes. Mutations in 9 of 22 tRNAs genes have been reported in association with cardiomyopathy or EH, such as tRNA^{Met} A4435G [7], tRNA^{Met} /tRNA^{Gln} A4401G [8], tRNA^{Ile} T4291C [9] and A4295G [10]. Besides tRNAs,

mutations in protein coding genes and rRNAs are associated with EH. They may affect OXPHOS and result in failure in mitochondrial protein synthesis, such as *12S rRNA* A1555G [11], *ND1* T3308C [12], *ND5* T12338C [13], and *CYTb* G15059A [14]. To investigate if mitochondrial genetic defects are involved in EH pathogenesis, probe capture and the second-generation sequencing were carried out. Systematic and extended mutational screening of whole mtDNA genome was conducted in a cohort of 216 Han population consisting of EH patients and healthy controls from Xinjiang region, China, in an attempt to discovering relationship of EH with mtDNA mutations.

Methods

Subjects

Patients with EH and healthy controls were enrolled at the Fifth Affiliated Hospital of Xinjiang Medical University from January 2013 to December 2014. All selected patients were diagnosed with EH according to Chinese Guidelines for Prevention and Treatment of Hypertension (2018 Revision) [15]. All participants were Han Chinese, without inclusion of their relatives. Inclusion criteria: (1) older than 18 years old; (2) diagnosed as primary hypertension; (3) without congenital heart diseases. Blood samples were collected from 151 patients with EH and 65 healthy controls. The study was approved by Ethics Committee of the Fifth Affiliated Hospital of Xinjiang Medical University. The written informed consent for clinical evaluation and genetic analysis was obtained from each participant.

Mitochondrial DNA extraction and sequencing

Total DNA was extracted from blood using DNA Blood Mini Kit 250 (QIAGEN Translational Medicine Co., Ltd., German) according to manufacturer's instruction. DNA was interrupted into 150-200 bp fragments with Covaris S220 and then purified. Agilent SureSelect kit was used to capture mtDNA fragments and Illumina HiSeq 2500 to generate 2 × 150 bp paired-end reads for sequencing.

Mutation and haplogroups classification of mitochondrial genome

The sequencing data were assembled and mapped against revised Cambridge Reference Sequence (rCRS) of human mtDNA (GenBank accession no.NC_012920.1). SNV/INDEL calling was performed using Genome Analysis Toolkit (version 2.5.2), producing files in VCF format [16]. Then, VCF files were subjected to annotate using ANNOVAR [17]. All sites were compared with previously reported mutations or polymorphisms listed in MITOMAP database (<http://www.gen.emory.edu/mitomap.html>) and mtDB (<http://www.mtddb.igp.uu.se/>) to confirm new SNV/INDEL. Haplogroups and corresponding sub-haplogroups of mtDNA were determined according to mtDNA tree 17 by software Mitotool and Haplogrep2 [18-20].

Statistical analysis

Plink software was used to calculate genotype frequencies of SNV sites in EH patients and controls [21]. Differences in locus frequencies were compared between two groups by chi-square tests. Haplogroup frequencies for EH patients and controls were calculated. P-values were generated by two-tailed Fisher's Exact Tests or Chi-square tests from R package (version 3.6.3). We applied GCTA program to analyze PCA and used software R to cluster PC1 and PC2 [22].

Comparison and structure prediction of mitochondrial genome in EH patients and health controls

DnaSP5 was used to calculate nucleic acid differential values (Pi) for whole length of mtDNA, protein-coding genes and rRNA genes [23]. SWISS-MODEL was used to predict protein 3D structures with a mutation p.T9A in *MT-ND5* and p.I206V in *MT-CYTB* [24]. Structural stability caused by single nucleotide site mutation is calculated by SVM algorithm [25]. Structural changes of 12S RNA were predicted by RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) and the minimum free energy was calculated [26].

Results

Novel variations

A total of 1137 variations were observed in 216 individuals. Compared with MITOMAP and mtDB databases, we identified 9 novel variations in mtDNA (Table1). Six variations were harbored in EH patients, and two variations in healthy controls. G9986C mutation in *COIII* gene was identified in both EH patients and healthy controls. Exception for a mutation site on 12S RNA gene, all new mutation sites were located on coding genes. Notably, two new mutation sites were identified in *CYTB* gene.

Comparisons of haplogroups between EH patients and controls

From 216 subjects, whole mtDNA genome sequences were analyzed using Mitotool and Haplogrep2 according to mtDNA tree 17. mtDNA sequences pertained to haplogroups of M and N (Table 2). 15 haplogroups (D, M8, M*, G, M7, M9, B, R9, A, N9, R*, R0, N1, JT and X) were constructed. Haplogroups D, M8, R9 and B accounted for about 70% (67.5%) of all populations. Each haplogroup (M7, M9, N9, R*, R0, N1, JT, X) accounted for less than 5%, all haplogroups for less than 15%. There were no significant differences among all haplogroups between cases and controls.

To further explore association between mtDNA and EH, principal component analysis (PCA) was performed. 216 subjects were classified into 5 clusters (Fig. 1). The components of haplogroups in each cluster were described in supplementary Table1. The first and second principal components (PC1 and PC2) accounted for 10.07% and 7.72%, respectively. Cluster 1 and cluster 3 were mainly consisted of haplogroups D and R9. Cluster 4 and cluster 5 were mainly consisted of haplogroup B, and cluster 5 was consisted completely of EH patients.

Association between mtDNA variants and EH

18 variants were significantly different (χ^2 or Fisher's exact test) in mtDNA whole sequences between EH patients and controls (Table3). One variant in 12SRNA, 14 variants in encoding gene and 3 variants in D-loop region were identified. In EH patients, frequencies of 6 variants in mtDNA were significantly higher than controls, including 1598G>A in 12SRNA gene, 8829C>T in ATPase6 gene, 12361A>G in ND5 gene, 15223C>T and 15662A>G in CYTB gene and 16243T>C in D-loop region. Remarkably, variants of 12361A>G in ND5 gene and 15662A>G in CYTB gene can cause nonsynonymous mutations in encoded proteins.

Structures of different variations of mtDNA between EH patients and controls

To explore potential effects of variations on encoded proteins in EH patients, 3D structure simulation prediction was performed using SWISS-MODEL to assess variants p.T9A in ND5 gene and p.I206V in CYTB gene.

The ND5 A12361G transition converts threonine (Thr) to alanine (Ala) in the 9th amino acid. The CYTB A15662G transition converts isoleucine (Ile) to valine (Val) in the 306th amino acid. When ND5 9th amino acid was mutated from Thr to Ala or CYTB 306th amino acid mutated from Ile to Val, significant changes in amino acid residues or a decrease in H-bond number indicated a more unstable structure (Fig.2). Meanwhile, we predicted changes of protein free energy induced by single amino acid mutation through SVM algorithm. A23361G and A15662G mutation caused reduced protein stability in ND5 (delta G=-0.57) and CYTB (delta G=-0.37), respectively.

Besides, we predicted structure changes caused by a variant m.1598 G > A in 12S RNA genes through RNA-fold server. As shown in Fig.3, the secondary structure changed obviously in response to G1598 A base transition. Meanwhile, the minimum free energy increased from -223.50 kcal/mol to -222.70 kcal/mol suggested decreased structure stability upon mutation.

Analysis of nucleotide variability

We calculated average nucleic acid variability value (Pi) of whole mtDNA genome sequence in EH patients and healthy controls. The Pi value of EH or healthy control was 0.00231 and 0.00222, respectively. Particularly, Pi values of 12 coding genes were calculated (Supplementary Table 2). Average nucleic acid variability values of ND5 and CYTB gene in EH patients were higher than healthy controls (Supplementary Table 2). Nucleic acid variability values of ND5 and CYTB gene in EH patients and healthy controls were estimated through curves of 100 bp/Window and 10bp/Step in Fig .4. The Pi values of EH patients were higher than healthy controls in regions indicated that this partial genome was under greater pressure of choice.

Discussion

Xinjiang, located in the northwest of China, is a multi-ethnic community, with Uygur (45.8%) and Han (40.1%) as the majority [27]. It is a vast area, with great differences in climate factors, geographical environment and living habits compared with other regions. Different from other areas, Han population in Xinjiang is gradually immigrating from all over the country. Gene frequency distribution probably has changed in order to adapt to unique geographical environment of Xinjiang in the past few hundred years. However, due to regional and economic constraints, genomic research on EH patients in Xinjiang is very limited, especially on potential role of mtDNA in EH pathogenesis. In this study, we compared mtDNA genome between 151 EH patients and 65 healthy controls, expected to discover evidence of mtDNA gene mutations related to pathogenesis of EH.

Based on mtDNA sequence, 15 haplogroups were identified in Han population in Xinjiang, mainly including D (20.8%), M8 (12%) in branches of M and R9 (16.2%), and B (18.5%) in branches of N, accounting for 67.5% of the total population. In terms of global distribution, haplotypes D, M8, B and R9 are mainly distributed in East Asia and Oceania [28-30]. In Europe, basically all mitochondrial haplogroups can be classified into N1, N2, X, R0, JT and U [31]. The European-related haplogroups of Han populations in Xinjiang were N1 (0.92%), X (0.46%), R0 (1.39%) and J10 (0.46%), respectively, totally accounting for 3.23%. In conclusion, Han population in Xinjiang is similar to northern East Asia (mainly for haplogroups with high frequency in the North).

Different mitochondrial haplogroups were significantly related to blood pressure and cardiovascular diseases. In northern Europe, blood pressure level of haplogroup J is 5 mm lower than that of non-haplogroup J per 100 mm Hg [32]. In Spain, for patients with hypertrophic cardiomyopathy (HCM), haplogroup T was related to development of HCM [33]. While in patients with severe aortic stenosis, haplogroup H was more common, which may be a risk factor for aortic stenosis, however, haplogroups UK and H were to the opposite [34]. The existing studies were mainly from Europe, however, correlation between hypertension and mitochondrial haplogroups has not been reported in Xinjiang Han population. There was no significant correlation between haplogroup and EH in Xinjiang Han people. However, haplogroup B5 in EH patients (8.6%) was higher than that in healthy controls (4.6%). It was not excluded that increasing sample size in the future may lead to a significant correlation between haplogroup B5 and hypertension.

Compared with nuclear DNA, mitochondrial DNA had a higher mutation rate due to its lack of histone protection and exposure to oxygen free radicals produced by oxidative phosphorylation. The mutation rate of mitochondrial genome was estimated to be 1.7×10^{-8} mutation/nucleotide/year, except for control region, which was ten times faster than nuclear genome [35]. Considering the high mutation rate of mtDNA, we identified 9 new variants which were not recorded in MITOMAP and mtDB databases. Some mtSNP in mitochondrial genome may have potential effects on Chinese EH. Mitochondrial dysfunction may lead to EH due to reduced production of energy, increased ROS production, oxidative stress and consequent cardiovascular and renal damage.

We compared mutation frequencies of mitochondrial genomic locus in EH patients and healthy individuals. Genotype frequencies of 6 single nucleotide variant (SNP) sites were significantly higher in EH patients than healthy controls. For example, ND5 A12361G and CYTB A15662C in coding genes could induce amino acid alteration. It was reported that ND5 A12361G was related to nonalcoholic fatty liver [36]. The CYTB G15662A mutation could lead to dysfunction of oxidative respiratory chain complex and damage to multiple systems [37]. We simulated and analyzed protein structure before-and-after mutation of ND5 A12361G and CYTB G15662A, respectively. Their corresponding amino acid residues and connected hydrogen bonds were significantly changed (Fig. 2). Besides, structure of mitochondrially encoded rRNA is critical for assembly of mitochondrial ribosome [38]. G1598A in EH significantly changed the secondary structure of 12S RNA, which may affect RNA modifications, protein synthesis as well as ribosome assembly (Fig. 3). Change of signal nucleotide site of 12S RNA A1555G could affect the secondary structure, resulting in myocardial damage and abnormal hearing function [12, 39]. Therefore, these mutations may be pathogenic factors of EH in Xinjiang Han populations. How mitochondrial mtSNPs induce EH needs to be investigated in further studies.

Conclusions

This study, for the first time, correlates mitochondrial genome with EH in Han nationality in Xinjiang, and investigates potential role of mitochondria in EH. Han population in Xinjiang is similar to that in northern East Asia, although a haplogroup prone to hypertension has yet to be identified. Moreover, mutations of ND5 A12361G, CYTB A15662C and 12S RNA G1598A are closely related to hypertension in Xinjiang Han population.

Abbreviations

EH: essential hypertension; mt DNA: mitochondrial deoxyribonucleic acid; tRNAs: transfer ribose nucleic acids; ND5: NADH dehydrogenase subunit-5 gene; CYTB: Cytochrome B; rRNA: ribosomal RNA; NADH: nicotinamide-adenine dinucleotide; SNV: single nucleotide variant; INDEL: insertion-deletion; rCRS: Cambridge Reference Sequence; SVM: support vector machine; COIII: cytochrome oxidase III; PCA: principal component analysis; HCM: hypertrophic cardiomyopathy; ROS: reactive oxygen species

Declarations

Ethics approval and consent to participate

The study was approved by Ethics Committee of the Fifth Affiliated Hospital of Xinjiang Medical University. The written informed consent for clinical evaluation and genetic analysis was obtained from each participant.

Consent for publication

Written informed consent to publish this information was obtained from study participants.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

JWX and GML designed the experiments. JWX, WPP and SGL collected the blood samples and extracted DNA from the blood samples. LQ, LRH and JXD analyzed the raw data. JWX, GML and LQ participated in manuscript writing and editing. GML was responsible for reviewing the data. All authors read and approved the final manuscript.

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Tables

Table 1

Novel variations detected in EH cases and controls

Position	Gene	Nucleotide variation	Amino acid variation	Number found in EH patients	Number found in controls
1525	12S	C-T	-	1	0
3320	ND1	A-G	p.N5S	1	0
4694	ND2	C-G	-	1	0
8549	ATPase8	C-T	p.H62Y	0	1
8876	ATPase6	T-C	p.F117S	1	0
9986	COIII	G-C	-	1	1
14248	ND6	C-A	-	1	0
15503	Cytb	C-T	p.P253S	0	1
15767	Cytb	C-G	p.Q341E	1	0

Table 2

Distribution and comparison of haplogroup frequencies between EH cases and controls

Macrohaplogroup	Haplogroup	EH (n=151)		Control (n=65)		Total (n=216)		P-value ^a
		N	%	N	%	N	%	
M	D	31	20.5	14	21.5	45	20.8	1.0
	M8	18	11.9	8	12.3	26	12.0	1.0
	M*	11	7.3	3	4.6	14	6.48	0.56
	G	8	5.3	3	4.6	11	5.09	1.0
	M7	7	4.6	3	4.6	10	4.63	1.0
	M9	2	1.3	0	0	2	0.93	1.0
N	B	28	18.5	12	18.5	40	18.5	1.0
	B4	15	9.93	9	13.8	24	11.1	0.55
	B5	13	8.6	3	4.6	16	7.4	0.40
	R9	26	17.2	9	13.8	35	16.2	0.68
	A	11	7.3	6	9.2	17	7.87	0.83
	N9	3	2.0	3	4.6	6	2.78	0.37
	R*	2	1.3	1	1.5	3	1.39	1
	R0	2	1.3	1	1.5	3	1.39	1
	N1	1	0.67	1	1.5	2	0.92	0.51
	JT	1	0.67	0	0	1	0.46	1
	X	0	0	1	1.5	1	0.46	1

P value was calculated by χ^2 test or Fisher's exact test.

Table 3

Distribution and comparison of significant variation loci on mitochondrial genome between EH patients and Controls.

Position	Gene	Nucleotide variation	Amino acid variation	Frequency in EH patients (%)	Frequency in controls (%)	P
1598	12SRNA	G-A	-	7.285	0	0.025
4117	ND1	T-C	-	0	3.077	0.030
6040	CO1	A-G	p. N46S	0	3.077	0.030
6221	CO1	T-C	-	0	3.077	0.030
6413	CO1	T-C	-	0	4.615	0.007
6960	CO1	C-T	-	0.662	4.615	0.048
8829	ATPase6	C-T	-	7.285	0	0.025
9055	ATPase6	G-A	p. A177T	0	3.077	0.030
10265	ND3	T-C	-	0	3.077	0.030
10742	ND4L	T-G	p.H91Q	0	3.077	0.030
12361	ND5	A-G	p.T9A	7.285	0	0.025
13620	ND5	T-C	-	0	3.077	0.030
14133	ND5	A-G	-	0	3.077	0.030
15223	CYTB	C-T	-	7.285	0	0.025
15662	CYTB	A-G	p. I306V	6.623	0	0.033
16224	D-loop	T-C	-	0	3.077	0.030
16227	D-loop	A-G	-	0.662	4.615	0.048
16243	D-loop	T-C	-	6.623	0	0.033

Figures

Figure 1

PCA based on mitochondrial whole-genome variations in EH patients and controls. PC1 and PC2 accounted for 10.07% and 7.72%, respectively. Red triangle indicates EH case. Black crossover indicates control. Purple oval represents cluster 1. Yellow oval represents cluster 2. Green oval represents cluster 3. Black oval represents cluster 4. Blue oval represents cluster 5.

Figure 2

Partial 3D structure of MT-ND5 or MT-CYTB protein. (a) and (b) ribbon diagrams of ND5 and ND5-T9A. (c) and (d) ribbon diagrams of CYTB and CYTB-I306V. Green dotted line: H-bonds

Figure 3

Secondary structure of 12S RNA. (A) Secondary structure of 12S RNA with m.1598 G. (B) Secondary structure with m.1598 G > A.

Figure 4

Sliding window of alignment for ND5 and CYTB in HE and healthy controls. The green and red curve represented EH patients and healthy controls, respectively.

Supplementary Files

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