

Association of variants m.T16172C and m.T16519C in whole mtDNA sequences with high altitude pulmonary edema in Han Chinese lowlanders

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Abstract

Background: High altitude pulmonary edema (HAPE) is a hypoxia-induced non-cardiogenic pulmonary edema that typically occurred in un-acclimatized lowlanders, which inevitably leads to life-threatening consequences. Apart from multiple factors involved, the genetic factors also play an important role in the pathogenesis of HAPE. So far, researchers put more energy into the nuclear genome and HAPE, and ignored the relationship between the mitochondrion DNA (mtDNA) variants and HAPE susceptibility.

Methods: We recruited a total of 366 individuals including 181 HAPE patients and 185 healthy or non-HAPE populations through two times. The first time, 49 HAPE patients and 58 non-HAPE cases were performed through whole mtDNA sequences to search the mutations and haplogroups, which were associated with the HAPE. The second time, 132 HAPE patients and 127 non-HAPE subjects were collected to apply of verifying these variants and haplogroups of mtDNA with routine PCR method.

Results: We analyzed and summarized the clinical characteristics and sequence data for 49 HAPE patients and 58 non-HAPE cases. We found that a series of routine blood indexes including systolic arterial blood pressure (SBP), heart rate (HR), white blood cell (WBC) and C-reactive protein (CRP) in HAPE group presented higher and displayed the significant differences compared with those in non-HAPE group. Though the average numbers of variants in different regions and groups samples were not statistically significant ($P > 0.05$), the mutation densities of different region in the internal group shown the significant differences. Then we found that two mutations (T16172C and T16519C) associated with the HAPE susceptibility, and the T16172C mutation increased the risk of HAPE, and the T16519C mutation decreased the HAPE rating. Furthermore, the two mutations were demonstrated with 132 HAPE cases and 127 non-HAPE individuals. Unfortunately, all the haplogroups were not associated with the HAPE haplogroups.

Conclusions: We provided the evidence of differences in mtDNA polymorphism frequencies between HAPE and non-HAPE Han Chinese. Genotypes of mtDNA 16172C and 16519C were correlated with HAPE susceptibility, which indicated that the role in mitochondrial genome in the pathogenesis of HAPE.

Background

High altitude pulmonary edema (HAPE) is an acute idiopathic mountain disease which usually occurs quickly in lowlanders when they are exposed to altitude exceeding 2,500 m above sea level. HAPE is considered as a life-threatening non-cardiogenic disease [1]. In some cases, HAPE can develop in the altitude of 1500 m-2500 m for highly susceptible populations [2]. This disease typically occurs 2-5 days after arriving at the altitude and is associated with insidiousness [3]. The mortality rate of untreated HAPE is up to 50%, while the mortality rate of treated HAPE is decreased to 11% [3]. Early symptoms of HAPE include non-productive cough, exertional dyspnea, chest pain, and reduced exercise tolerance. Without treatment, HAPE can progress to dyspnoea at rest [3]. This disease is caused by a variety of risk factors including hypoxic ventilatory response, rapidly ascent, high altitude, tired, lacking sufficient sleeping,

smoking, drinking, heredity, etc. [3-6]. In addition, the patients with the basic disease such as hypertension and diabetes are easier to develop HAPE [7, 8]. Therefore, when studying the relationship between genetics and HAPE, the risk factors need to be eliminated.

So far, the major pathogenic mechanism of HAPE is the imbalance and mutations of the pulmonary endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), which leads to the accumulation of extravascular fluid in the lungs and then lung malfunction [9, 10]. A large number of studies have found that many pathways are participated in the development of HAPE, such as renin angiotensin aldosterone system (RAAS) [11], hypoxia-inducible factor (HIF) pathway [12], the nitric oxide (NO) pathway [13], the mitochondrion DNA (mtDNA) [14, 15], etc. For HAPE patients, the suitable treatments include oxygen suction and lowland transferring, which suggests that HAPE is very sensitive to changes in oxygen/energy metabolism [15-17]. Mitochondria produces ATP through process of [cellular respiration](#), specifically [aerobic respiration](#), which requires oxygen. mtDNA mutations can impair this process [18]. Therefore, the relationship between HAPE and mtDNA is important for understanding the pathogenic mechanism of HAPE.

mtDNA is an extranuclear double-stranded DNA found only in mitochondria. In most eukaryotes, mtDNA is a circular molecule and is inherited from the mother. The full length of mtDNA is 16,569 base pairs (bp), divided into heavy (H) brand and light (L) brand, including 37 genes encoding 2 rRNA (12S rRNA and 16S rRNA), 22 tRNA, and 13 polypeptide subunits (Complex I, III, IV, and V) that produce mitochondrial ATP and participate in the oxidative phosphorylation together with proteins coded by nuclear genome [19]. In addition to coding region, mtDNA also contains a part of noncoding region called control region or displacement loop (D-loop), which is divided into high variant sequencing-I (HVS-I) and high variant sequencing-II (HVS-II), and is vital to mtDNA replication and regulation [20]. Mitochondrial function can be affected by variations in mtDNA, including polymorphisms, content changes, deletions [21]. These variants play an important role in acclimatizing or adapting to hypoxia [21]. In this study, we mainly focused on the association between mtDNA mutants and HAPE susceptibility. For example, Luo et al. used polymerase chain reaction (PCR) methods instead of mtDNA whole sequences to reveal that genotypes of mtDNA 3397G and 3552A were correlated with HAPE susceptibility in Han Chinese [22]. Some results suggested that mitochondrial haplogroups B and M7 were associated with the inadaptability of hypoxic environments, whereas haplogroups G and M9a1a1c1b may be associated with hypoxic adaptation [23]. Recently, a literature presented that variants G4491A, A4944G and A14002G associated with haplogroup M33a2'3 may be the main reasons for the susceptibility of Indian male lowlanders to HAPE; however, the study only included 20 controls and 15 cases through long PCR and the results were not verified [15].

Even though these results have already established the association between mtDNA mutations and HAPE, there are still some problems worth exploring and studying, such as limited samples, reverification of polymorphisms and haplogroups, and the methods behind them. In this study, we recruited two groups of people. The first group including 51 HAPE cases (2 patients were removed due to poor quality of mtDNA) and 58 non-HAPE persons was studied using whole mtDNA sequences to find the possible

association between mutations and haplogroups. The second group including 132 HAPE patients and 127 non-HAPE patients was used to verify some variants of mtDNA with routine PCR method. We found two mutations (T16172C and T16519C) associated with the HAPE susceptibility using the largest samples so far, and the samples were studied using whole mtDNA sequencing. The T16172C mutation indicated an increased susceptibility to HAPE, and the T16519C mutation could reduce the risk factor for obtaining HAPE.

Methods

Samples collections

A total of 366 subjects were recruited in this study, including 181 HAPE patients and 185 non-HAPE subjects from September 2018 to September 2020. All subjects were divided into 2 groups to explore and validate the pathogenic factors, respectively. The first group contained 51 HAPE cases and 58 healthy people, and all of them were low-altitude Han-Chinese who climbed rapidly to an altitude of 3658 m in Lhasa, Tibet, China, (Table 1 and Additional file 1). In addition, the first group of patients were male and met the following conditions: 1) HAPE patients were diagnosed according to the clinical standard requirements, which mainly included cough, dyspnea at rest, white or pink foamy sputum, central cyanosis, pulmonary crackles, and the presence of flake or cloud infiltrate shadows at unilateral or bilateral pulmonary hilar on chest X-ray [24, 25]. 2) All the subjects were from General Hospital of Tibetan Military Command and had no relationship with each other. 3) These subjects did not have the history of smoking, drinking, altitude sickness, hypertension, diabetes, cardiopulmonary disease, acute or chronic pulmonary infection (e.g. pneumonia), and the remaining mtDNA-related diseases. The second group had 132 HAPE patients and 127 non-HAPE individuals, and also met the above screening criteria (Additional file 1).

DNA extraction, sequencing and haplogroup classification

Blood samples were collected from all the HAPE and non-HAPE subjects and stored at -80°C. Genomic DNA was extracted from peripheral blood using Magbead Blood DNA Kit (CWBio, Beijing, China) according to standard procedure. For each patient, 200 ng genomic DNA was sheared by Biorupter (Diagenode, Belgium) to acquire 150 ~ 200 bp fragments. The ends of DNA fragment were repaired, and Illumina Adaptor was added (Fast Library Prep Kit, iGeneTech, Beijing, China). After sequencing library was constructed, the whole exons were captured with AI-Mito-Cap (iGeneTech, Beijing, China) and sequenced on Illumina platform (Illumina, San Diego, CA), with 150 base paired-end reads. Raw reads were filtered by FastQC to remove low quality reads. Then clean reads were mapped to the reference genome GRCh37 using Bwa. After duplications were removed, SNV and InDel were called and annotated by GATK, Samtools, VarScan. Through the PhyloTree Build 17 standard, using the software development tool MitoTool, the haplogroups of entire mtDNA sequences were constructed according to phylogenetic analysis method [26-28]. When evaluating the mtDNA variants, a series of affecters were considered, including the different variants of the particular branches of each haplogroup, the calescence time of

variation distribution and the location of protein- or RNA-encoded gene based substitutions. All these factors were integrated into phylogenetic analysis to construct Haplogroup.

PCR conditions

We directly sequenced the PCR products to genotype the A263G, T310N, 310-311insC, T16172C, and T16519C polymorphisms in the remaining individuals. The primers (forward: 5'-CAGCCACCATGAATATTGTACG-3', reverse: 5'-GTTAGGCTGGTGTAGGGTTC-3') were designed using the Primer 5.0 software (PREMIER Biosoft International, Palo Alto, CA, USA). The reactions were performed in 50 μ L volume containing 50 ng template DNA, 5 mM dNTP (Takara, Dalian, China), 10 pmol forward primers, 10 pmol reverse primers, 2.5 U Taq DNA polymerase enzyme (Takara) in Taq buffer, and 75 mM $MgCl_2$. Then PCR amplification was performed under the following conditions: a pre-denaturation cycle at 94 °C for 4 min; 45 cycles at 94 °C for 20 s, 56 °C for 30 s, 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. The obtained products were cooled to 4°C. The PCR products were directly sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). The sequenced results were assigned with the standard mitochondrial reference genome called Revised Cambridge reference sequence (rCRS).

Ethical statement

All samples collected in this study had written informed consent from patients. All clinical and experimental steps were approved by the Ethical Committee of the Seventh Medical Center of PLA General Hospital and the Ethical Committee of the General Hospital of Tibetan Military Command. Meanwhile, these contents conducted in accordance with the Helsinki Declaration guideline.

Statistical analyses

All the statistical analyses of data were performed using SPSS 17.0. Pearson χ^2 test was used to assess the significance of the differences in haplogroup and SNP frequencies between HAPE and non-HAPE groups. Quantitative data were expressed as mean \pm standard deviation (mean \pm SD) and the unpaired student's t-tests were used to compare the case group and control group. The P-values, odds ratios (ORs), and 95% confidence intervals (95% CI) were calculated. A two-sided P-value < 0.05 was considered statistically significant. All P-values were adjusted by Bonferroni correction.

Results

Clinical characteristics of all the individuals

Firstly, we collected 109 individuals, including 51 HAPE patients and 58 non-HAPE people. Among these cases, 2 HAPE cases were removed because of the poor-quality DNA levels. Then, a total of 49 HAPE patients and 58 non-HAPE individuals were further analyzed. They were male Han Chinese without the history of smoking, drinking and other diseases associated with mtDNA variants. They ascended into the

same altitude and suffered similar environments. The detailed clinical information is listed in Table 1 and Additional file 1.

As shown in Table 1, the age distribution was consistent between the HAPE patients and non-HAPE individuals. Except for red blood cell (RBC), all the routine blood indexes such as systolic arterial blood pressure (SBP), white blood cell (WBC) and C-reactive protein (CRP) in HAPE cases were significantly different from non-HAPE ($p < 0.05$), which was probably related to the adaption of high environments [1, 29, 30].

Data summary of sequencing and quality control

The mtDNA of 107 human blood samples was isolated and sequenced using Illumina platform to study the base changes in HAPE and non-HAPE groups. Nearly 92% of total data generated a Q > 30 Phred score, and the mean sequence depth of 8400x was observed after removing the duplications, which accounted for 24.6% on average. For all the samples, the whole mitochondrial genome region was covered. All the variants of HAPE and non-HAPE patients are listed in Additional file 2 and file 3. After processing these variants, the detailed and summarized results are shown in Fig. 1 and Fig. 2.

Compared to non-HAPE group, the mean numbers of variants in HAPE patients were higher; however, the difference between non-HAPE and HAPE groups was not significant ($P = 0.799$) (Fig. 1a). In HAPE group, a total of 1945 mutations were distributed in non-coding region (602, 30.95%), tRNA region (20, 1.03%), rRNA region (255, 13.11%), complex I (527, 27.10%), complex III (237, 12.19%), complex IV (173, 8.89%), and complex V (131, 6.74%) (Fig. 1b). On the contrary, there were 2284 mutations in non-HAPE individuals, which were located in non-coding region (767, 33.58%), tRNA region (18, 0.79%), rRNA region (299, 13.09%), complex I (581, 25.44%), complex III (260, 11.38%), complex IV (214, 9.37%), and complex V (145, 6.35%) (Fig. 1b). Unfortunately, the two randomly corresponding regions did not show significant differences (Additional file 4). Then, we analyzed the numbers of variants of coding and non-coding regions in HAPE and non-HAPE groups. The results indicated that the differences between HAPE and non-HAPE groups were not significant (Fig. 1c). However, the average variants between coding region and non-coding region in HAPE patients were significantly different, the similar findings were found in non-HAPE group (Fig. 1c), which may be related to the length of coding regions and non-coding regions in the sequence.

To further explore the variants' distributions characteristics of mitochondrial genome in HAPE and non-HAPE patients, we used the mutation densities (mutation number in a region/region bp length) to describe the variants distributions in coding regions and non-coding regions. The results indicated that the mutation densities in coding regions or non-coding regions were consistent between HAPE and non-HAPE groups (Fig. 2a). However, within the same group (HAPE or non-HAPE group), the mutation densities in the non-coding regions were higher than those in the coding regions (Fig. 2a). This suggested the sequences of non-coding regions were inclined to mutations. In all the variants, the top three molecular consequences in both HAPE group and non-HAPE group were 44.06% upstream gene, 31.36% synonymous and 23.55% missense variants (Fig. 2b), and there was no significant difference between

both groups (Additional file 5). Interestingly, a few of positions in the mitochondrial genome had very high mutation rates (variants number/samples number), such as 73th position (1.0), 2706th position (1.0), 8860th position (1.0) in HAPE patients (Fig. 2c and Additional file 6). On the contrary, these positions in non-HAPE group also showed the similar results and the difference between HAPE and non-HAPE groups was not significant (Fig. 2c and Additional file 6). The result suggested that the mitochondrial genome had some high frequency mutation sites that were not associated with HAPE.

Association of mtDNA variants with HAPE susceptibility

In this study, a total of 1945 mutations and 2284 mutations were observed in HAPE and non-HAPE groups, respectively, which were located in 483 variants' sites and 530 variants' sites (combined with 742 variants' sites), respectively (Additional file 6). There were 14 different and typical mutations in Table 2, including 5 variants sites (A263G, T310N, (310-311) insC, T16172C and T16519C) with significant differences between both groups, 6 variants sites (A8459G, C8684T, C14067T, T14470C and A16164G) only found in HAPE group, and 3 variants sites (C194T, C6960T and G16390A) only found in non-HAPE group (Table 2). After Bonferroni correction, only 4 mutations (T310N, (310-311) insC, T16172C and T16519C) were statistically significant between HAPE and non-HAPE groups ($P < 0.05$), and they were located in the D-loop of mtDNA non-coding regions. Since 310th site was short repeats sequences C bases, this site was removed in the further analysis. Our results showed that the frequencies of T16172C in HAPE group (20.41%) were higher than those in the non-HAPE group (5.17%, p -adjust = 0.035, OR=4.701, and 95%CI = 1.214 – 18.204). In contrast, the frequencies of T16519C in HAPE individuals (34.69%) were significantly lower than those in non-HAPE individuals (67.24%, p -adjust = 0.002, OR=0.259, and 95%CI = 0.116 – 0.578) (Table 2). The two mutations were previously reported in other studies, but not in high-altitude diseases [31-33].

Haplogroup comparison between HAPE and non-HAPE patients

A total of 107 complete mtDNA sequences were analyzed using MitoTool software according to the PhyloTree Build 17 criteria (GenBank J01415.2) [26-28]. We found that all the mtDNA sequences were mapped to the single initial haplogroup L3, which originated from Africa and composed of two macrohaplogroups, M and N (Table 3). All the 107 subjects belonged to 16 haplogroups. No differences were observed in the haplogroups between the HAPE and non-HAPE groups ($P > 0.05$, Table 3). These haplogroups were further divided into next level haplogroups, which were not statistically significant ($P > 0.05$, Additional file 7).

Validation of two mutations (T16172C and T16519C)

In order to validate the association between two mutations (T16172C and T16519C) and HAPE susceptibility, we collected samples from 259 people, including 132 HAPE patients and 127 non-HAPE individuals according to the above screening criteria (Additional file 1). The basic clinical information is listed in Additional file 8. The PCR results of these variants are shown in Table 4. The frequencies of T16172C in HAPE patients (44, 33.33%) were higher than those in non-HAPE individuals (27, 21.26%, p -

adjust = 0.042, OR=1.852, and 95%CI = 1.060 – 3.236). The frequencies of the mutation T16519C in HAPE group (52, 39.39%) were lower than the non-HAPE group (67, 52.76%, p-adjust = 0.042, OR=0.582, and 95%CI = 0.355–0.953). The above results indicated that the T16172C mutation increased the HAPE susceptibility and the T16519C mutant decreased the impact from HAPE.

Discussion

HAPE is a severe acute disease caused by high-altitude hypoxia. Since the pathogenic mechanism is the comprehensive result of genetic diversity and environmental triggers, the incidence is variable and cannot be predicted. Some individuals with genetic variants are more likely to suffer from the mountain disease than others [34, 35]. Mitochondria are major ATP energy production centers in eukaryotic cells, and its function can be affected by the mtDNA variants [18]. HAPE also has major impact on the energy generating OXPHOS system of mitochondria [34]. Several studies have found that the mutations of mtDNA are located in coding region and associated with the HAPE susceptibility; however, these results could not be verified by another samples [22, 36]. In this study, we found two mutations (T16172C, T16519C) located in D-loop of non-coding region were associated with HAPE susceptibility in Han Chinese, and validated the conclusion using a different group of patients. The frequencies of T16172C mutation in HAPE group were higher than those in non-HAPE group. In contrast, the 16519C mutations tended to reduce the HAPE susceptibility. Two mutations were also reported previously in the other diseases, but not in high-altitude diseases [31-33].

The previous study found the 16172 (rs2853817) and 16519 (rs3937033) mutations showed preliminary association with mitochondrial function without changing primary sequences of protein [37]. The site of T16172C mutation was found in the hypervariable region of mtDNA. So far, little is known about the T16172C mutation and other diseases including mountain sickness, except for one paper that demonstrated the relationship between the T16172C and ataxia telangiectasia for twins [33]. The site of mtDNA 16519 has been reported to have the highest world-wide mutation rates [37], and have the association with some diseases. Navaglia et al. found the T allele of mtDNA 16519 SNP correlated with shorter life expectancy in pancreatic cancer [31]. The relationship between 16519 site and other cancers such as breast cancer and familial nasopharyngeal carcinoma has also been demonstrated [38, 39]. Liao et al. reported that the T16519C mutation showed susceptible tendency to type-2 diabetes mellitus (T2DM) in Chinese Han population [40]. The significant differences were also found in the change rate of VO (2 max) and citrate synthase activity as a result of training between the two groups at 16519. Thus, the authors proposed that mtDNA polymorphisms in the control region might result in individual differences in endurance capacity or trainability [41]. In addition, mtDNA polymorphisms might also cause other common disorders such as migraine headache or psychiatric disorders such as schizophrenia [32, 42, 43]. So far, the T16172C and T16519C variants in mtDNA were first reported to be associated with the HAPE of mountain sickness. Although both the 16172 and 16519 variants are located in the D-loop region of mtDNA, these variants may act by changing the transcription levels of mitochondrial proteins, which are related to oxidative phosphorylation, and the changes in this process

may lead to β -cell failure or ATP production lack [44, 45]. These phenomena need to be further verified in future research.

In addition to variant genotypes of mtDNA, the mitochondrial haplogroup classification in HAPE and non-HAPE individuals was performed using MitoTool. The mtDNA is a well-known genetic marker because it has the characteristics of high mutation rate, maternal inheritance, high copy number and lack of recombination, which makes it different from the nuclear genome [36]. On this basis, mtDNA haplogroups such as the non-recombining region of Y-chromosome are usually considered as the main measures for investigating of human evolution and origin [46, 47]. Mitochondrial DNA haplogroups are characteristic cluster of tightly linked mtDNA polymorphism that forms continent-specific genotypes [46]. Studies have also shown that certain mitochondrial haplogroups are predisposed to high-altitude diseases while others can prevent mountain sickness. For example, the studies on the mitochondrial genome have shown that there is a significant difference in the frequency of the mitochondrial 3010G–3970C haplotype between high- and low-altitude populations. This haplotype is believed to be associated with the improved adaptability of the Tibetan population to the low-oxygen environment [48]. Other studies have also shown that the mitochondrial haplogroups are associated with high-altitude adaptation, such as haplogroups B, M7, M9a1a1c1b, B4b, D4, etc. [23, 36, 49]. These mtDNA haplogroups were also observed in our samples; however, we found that they were not significantly different between HAPE and non-HAPE groups (Additional file 7). This phenomenon was inconsistent with the results in the previous study that mtDNA haplogroup M33a2'3 may be linked with the HAPE susceptibility of 20 controls and 15 Indian male lowlander cases [15].

Though we have found two mutations (T16172C, T16519C) associated with the HAPE susceptibility, our study still has limitations, including a relatively small sample size and lack of functional experiments to identify the effects of the genetic variants on gene structure/function. Therefore, our findings need to be validated in a larger population and the functional significance of the important genetic variants should be further investigated.

Conclusions

In this study, we established the associations between mtDNA mutations and HAPE susceptibility. First, 49 HAPE patients and 58 non-HAPE individuals were studied to find the possible mutations and haplogroups related to HAPE using whole mtDNA sequences and MitoTool software. The two mutations, i.e., T16172C (rs2853817) and T16519C (rs3937033) were located in non-coding regions of mtDNA D-loop. The T16172C mutation increased the risk of HAPE, while the variant of T16519C tended to be resistant to HAPE. Furthermore, these two mutations were verified with 132 HAPE and 127 non-HAPE patients. Meanwhile, we found that a few of routine blood indexes, such as SBP, HR, WBC and CRP, showed significant differences between HAPE group and non-HAPE group. We also found that haplogroups of mtDNA did not play a role in the HAPE susceptibility. In conclusion, these findings enrich the association between mtDNA and HAPE, and can provide a new perspective to reveal the genetic mechanism of HAPE.

List Of Abbreviations

base pairs bp confidence intervals CI C-reactive protein CRP displacement loop D-loop endothelial cells ECs heavy H High altitude pulmonary edema HAPE hypoxia-inducible factor HIF heart rate HR high variant sequencing-I HVS-I high variant sequencing-II HVS-II light L mitochondrion DNA mtDNA nitric oxide NO odds ratios ORs polymerase chain reaction PCR renin angiotensin aldosterone system RAAS red blood cell RBC Revised Cambridge reference sequence rCRS systolic arterial blood pressure SBP type-2 diabetes mellitus T2DM vascular smooth muscle cells VSMCs white blood cell WBC.

Declarations

Ethics approval and consent to participate

All clinical and experimental steps were approved by the Ethical Committee of the Seventh Medical Center of PLA General Hospital and the Ethical Committee of the General Hospital of Tibetan Military Command. All samples were obtained with written informed consent from populations including HAPE and non-HAPE persons.

Consent for publication

Consent for publication of all the individuals were obtained for the HAPE patients and non-HAPE persons including clinical data and mtDNA sequences results. And all authors have read and approved the submission of the manuscript.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing financial interests.

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Authors' contributions

Yan Wang and Zhichun Feng conceived the project, supervised the study, and provided guidance in bioinformatics analysis. Yan Wang and Fujun Peng analyzed the data and wrote the manuscript drafting. Xuewen Huang and Huiling Han collected the samples, selected the samples, and analyzed the clinical information. Yanan Gu and Xin Liu performed PCR validation for mutations. Yan Wang, Xuewen Huang

and Zhichun Feng revised the manuscript for scholarly publication. All authors reviewed and approved the final manuscript as submitted.

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Tables

Table 1 Basic clinical characteristics of all the subjects

	HAPE (n=49)	non-HAPE (n=58)	P-value¶
Gender	Male	Male	
Race	Han	Han	
Smoking	No	No	
Drinking	No	No	
Age	27.6±6.7	25.4±5.9	0.082
SBP (mmHg, <140)	127.0±19.7	118.5±11.3	<0.0001**
DBP (mmHg, <90)	85.2±14.5	80.7±10.4	<0.0001**
HR (bpm, 60-100)	106.1±17.5	90.7±14.4	<0.0001**
WBC (×10 ⁹ /L, 3.5-9.5)	11.0±3.7	8.1±3.6	<0.0001**
RBC (×10 ¹² /L, 4.4-6.0)	5.4±0.7	5.4±0.5	0.073
HCT (% , 49-57)	48.2±4.7	50.3±4.3	<0.0001**
Hb (g/L, 144-175)	157.0±16.7	168.3±10.1	<0.0001**
CRP (mg/L, 0-10)	50.9±37.4	37.6±34.6	<0.0001**

SBP, systolic arterial blood pressure; DBP, diastolic arterial blood pressure;

HR, heart rate; WBC, white blood cell; RBC, red blood cell; HCT, hematocrit;

Hb, hemoglobin concentration; CRP, C-reactive protein.

Data are presented as mean±SD. ¶, t-test; *, P-value < 0.05; **, P-value < 0.01.

Table 2 Comparison of variant frequencies between HAPE and non-HAPE groups

Site	Genotype	Gene	Mutation	Protein change	HAPE (n=49)	non-HAPE (n=58)	HAPE vs. non-HAPE			
							P-value	P-adjust	OR	95%CI
C194T	T	TRNF	c.-383C>T		0 (0%)	3 (5.17%)	0.106	0.304	1.055	0.993-1.120
A263G	G	TRNF	c.-314A>G		45 (91.84%)	58 (100%)	0.027*	0.088	0.918	0.845-0.998
T310N	N	TRNF	c.-267T>N		27 (55.10%)	44 (75.86%)	0.024*	0.039*	0.390	0.171-0.890
(310-311) insC	insC	TRNF	c.-267_-266insC		22 (44.90%)	14 (24.14%)	0.024*	0.039*	2.561	1.124-5.836
G6179A	A	COX1	c.276G>A	p. Met92Ile	3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
C6960T	T	COX1	c.1057C>T	p. Leu353Leu	0 (0%)	3 (5.17%)	0.106	0.304	1.055	0.993-1.120
A8459G	G	ATP8	c.94A>G	p. Asn32Asp	3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
C8684T	T	ATP6	c.158C>T	p. Thr53Ile	3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
C14067T	T	ND5	c.1731C>T	p. Thr577Thr	3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
T14470C	C	ND6	c.204A>G	p. Gly68Gly	3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
A16164G	G	ND6	c.-1491T>C		3 (6.12%)	0 (0%)	0.056	0.186	0.939	0.874-1.008
T16172C	C	ND6	c.-1499A>G		10 (20.41%)	3 (5.17%)	0.016*	0.035*	4.701	1.214-18.204
G16390A	A	ND6	c.-1717C>T		0 (0%)	3 (5.17%)	0.106	0.304	1.055	0.993-1.120
T16519C	C	ND6	c.-1846A>G		17 (34.69%)	39 (67.24%)	0.001**	0.002**	0.259	0.116-0.578

Note: P-value, Person χ^2 test; P-adjust, adjust Person's value; OR, odds ratio; CI, confidence interval.

*, P-value < 0.05; **, P-value < 0.01.

Table 3 Distribution of haplogroups in HAPE and non-HAPE groups

Macrohaplogroup	Haplogroup	HAPE		Non-HAPE		HAPE vs. non-HAPE			
		(n=49)	%	(n=58)	%	P-value	P-adjust	OR	95% CI
M	C	3	6.82%	3	5.17%	0.831	1.000	1.196	0.230-6.210
	D	12	27.27%	10	17.24%	0.355	0.494	1.557	0.607-3.995
	G	4	9.09%	2	3.45%	0.291	0.526	2.489	0.436-14.210
	M21	0	0.00%	1	1.72%	0.356	1.000	1.018	0.983-1.053
	M7	5	11.36%	11	18.97%	0.205	0.320	0.486	0.156-1.509
	M8a	3	6.82%	0	0.00%	0.056	0.186	0.939	0.874-1.008
	M9	2	4.55%	1	1.72%	0.462	0.882	2.426	0.213-27.588
	Z	0	0.00%	1	1.72%	0.356	1.000	1.018	0.983-1.053
N	A	5	11.36%	3	5.17%	0.324	0.537	2.083	0.472-9.200
	B	7	15.91%	15	25.86%	0.140	0.216	0.478	0.177-1.289
	F	4	9.09%	5	8.62%	0.932	1.000	0.942	0.239-3.721
	H	0	0.00%	1	1.72%	0.356	1.000	1.018	0.983-1.053
	N9a	2	4.55%	2	3.45%	0.863	1.000	1.191	0.162-8.786
	R11	0	0.00%	1	1.72%	0.356	1.000	1.018	0.983-1.053
	R9b	0	0.00%	1	1.72%	0.356	1.000	1.018	0.983-1.053
	Y	1	2.27%	0	0.00%	0.274	0.932	0.980	0.941-1.020

Note: P-value, Person χ^2 test; P-adjust, adjust Person's value; OR, odds ratio; CI, confidence interval.

Table 4 Validation of association between mt16172C, mt16519C genotypes and HAPE susceptibility

Position	Genotype	HAPE (n=132)	non-HAPE (n=127)	P-value	P-adjust	OR	95% CI
16172	C	44 (33.33%)	27 (21.26%)	0.029	0.042	1.852	1.060-3.236
	T	88 (66.67%)	100 (78.74%)				
16519	C	52 (39.39%)	67 (52.76%)	0.031	0.042	0.582	0.355-0.953
	T	80 (60.61%)	60 (47.24%)				

Note: P-value, Person χ^2 test; P-adjust, adjust Person's value; OR, odds ratio; CI, confidence interval.

Figures

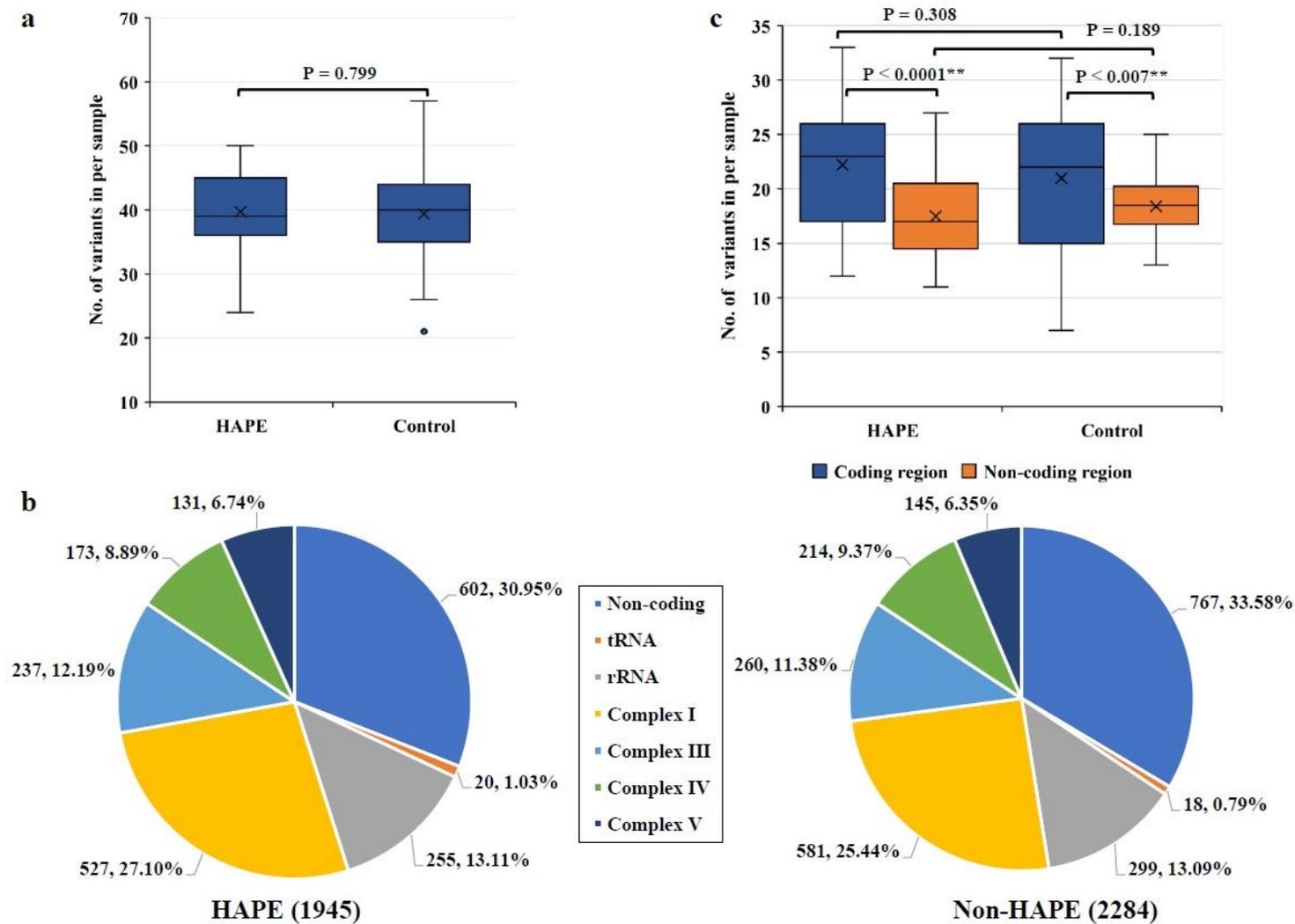


Figure 1

Distribution of variants in HAPE and non HAPE groups.

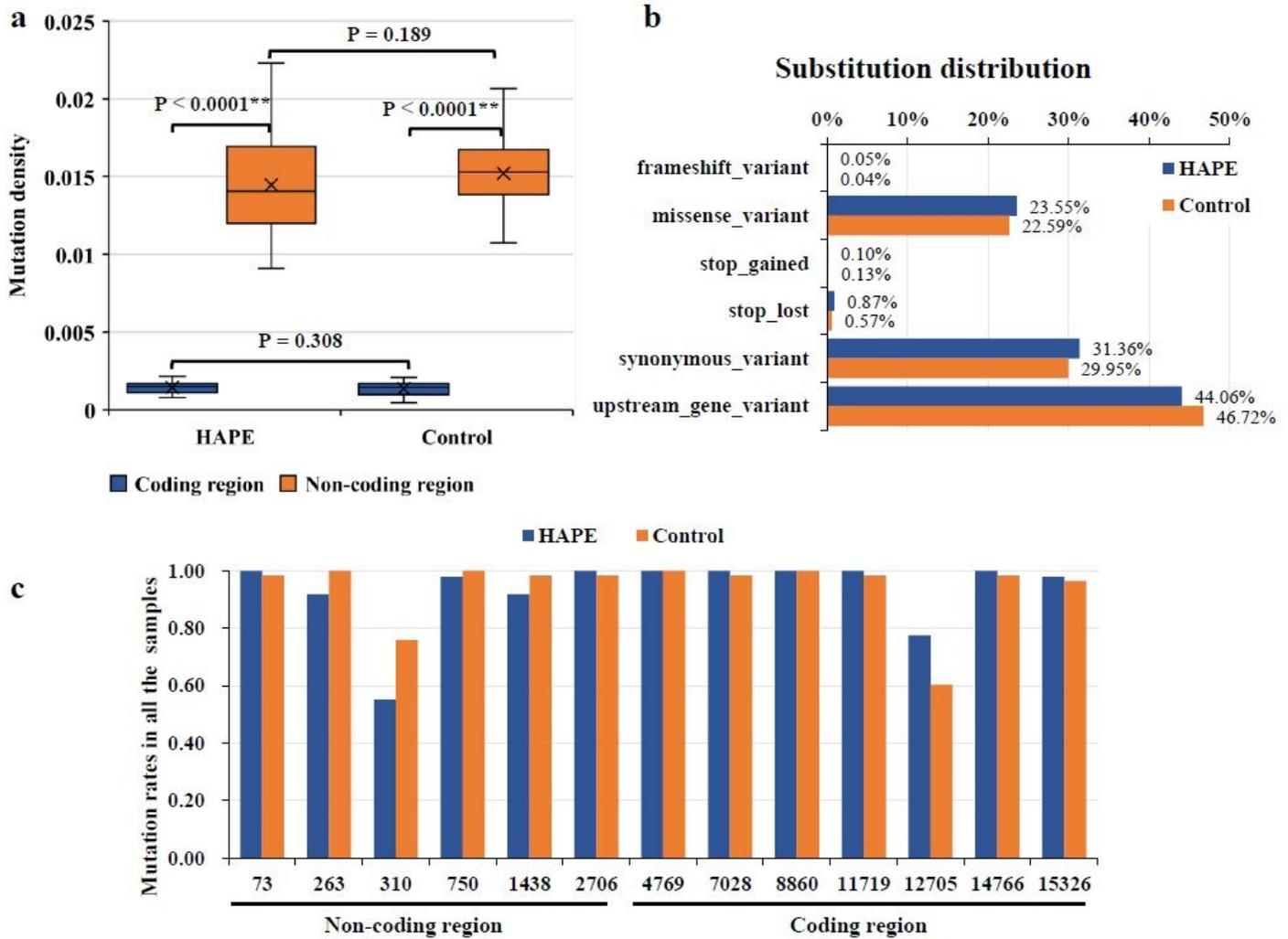


Figure 2

Gene distributions in mitochondrial genome of HAPE and non HAPE groups.

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