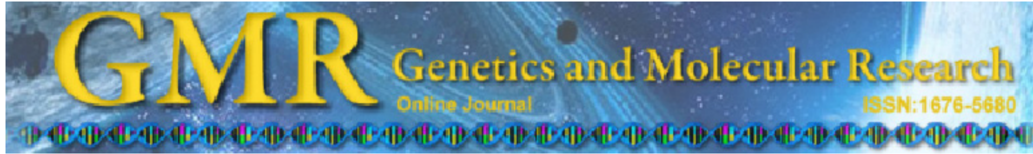

Mitochondrial haplogroup D4 confers resistance and haplogroup B is a genetic risk factor for high-altitude pulmonary edema among Han Chinese

Y.J. Luo[1,2,3]*, W.X. Gao[1,3]*, S.Z. Li[4], X.W. Huang[4], Y. Chen[4], F.Y. Liu[1,3], Q.Y. Huang[1,3] and Y.Q. Gao[1,3]

[1]Department of Pathophysiology and High Altitude Physiology, College of High Altitude Military Medicine, Chongqing, China
[2]Department of High Altitude Disease, College of High Altitude Military Medicine, Chongqing, China
[3]Key Laboratory of High Altitude Medicine, Third Military Medical University, Ministry of Education, Chongqing, China
[4]Department of Mountain Sickness, General Hospital of Tibet Military Command, Lhasa, Tibet, China

*These authors contributed equally for this study.
Corresponding author: Y.Q. Gao
E-mail: gaoy66@yahoo.com

Genet. Mol. Res. 11 (4): 3658-3667 (2012)
Received September 2, 2011
Accepted September 21, 2012
Published October 9, 2012
DOI http://dx.doi.org/10.4238/2012.October.9.1

ABSTRACT. High-altitude pulmonary edema (HAPE) is a life-threatening condition caused by acute exposure to high altitude. Accumulating evidence suggests that genetic factors play an important role in the etiology of HAPE. However, conclusions from association studies have been hindered by limited sample size due to the rareness of this disease. It is known that mitochondria are critical for hypoxic adaptation, and mitochondrial malfunction can be an important factor in HAPE development. Therefore, we tested the hypothesis that

 ©FUNPEC-RP www.funpecrp.com.br

mitochondrial DNA haplotypes and polymorphisms affect HAPE susceptibility. We recruited 204 HAPE patients and 174 healthy controls in Tibet (3658 m above sea level), all Han Chinese, constituting the largest sample size of all HAPE vulnerability studies. Among mtDNA haplogroups, we found that haplogroup D4 is associated with resistance to HAPE, while haplogroup B is a genetic risk factor for this condition. Haplogroup D4 (tagged by 3010A) may enhance the stability of 16S rRNA, resulting in reduced oxidative stress and protection against HAPE. Within haplogroup B, subhaplogroup B4c (tagged by 15436A and 1119C) was associated with increased risk for HAPE, while subhaplogroup B4b may protect against HAPE. We indicate that there are differences in HAPE susceptibility among mtDNA haplogroups. We conclude that mitochondria are involved in adverse reactions to acute hypoxic exposure; our finding of differences in susceptibility as a function of mitochondrial DNA haplotype may shed light on the pathogenesis of other disorders associated with hypoxia, such as chronic obstructive pulmonary disease.

Key words: HAPE; Mitochondrial DNA; mtSNP; Haplogroup; Haplotype; Hypoxia

INTRODUCTION

High-altitude pulmonary edema (HAPE) is a life-threatening condition that occurs in predisposed but otherwise healthy individuals after acute exposure to altitudes above 3000 m (Peacock, 1995). The altitude, cold temperature, physical exertion, speed, and mode of ascent are the most important factors affecting HAPE. Although the risk of recurrence is significant but unpredictable, HAPE-prone and HAPE-resistant individuals are known to be present in the population, which suggests a constitutional and possibly genetic component in its etiology (Mortimer et al., 2004). Despite intensive work to unravel the molecular mechanisms underlying HAPE, the pathogenesis of this condition has yet to be elucidated. Furthermore, the rareness of the complication (0.1 to 0.4%; Basnyat and Murdoch, 2003) and resultant limitations on sample size have hampered the progress of genetic studies on HAPE.

The most prominent pathophysiologic aspects of HAPE are inhomogeneous hypoxic pulmonary vasoconstriction, exaggerated capillary hypertension, and consequent reduced fluid clearance from the alveolar space (Bartsch et al., 2005; Sartori et al., 2007). Accumulating evidence suggests that the excessive pulmonary response in HAPE-prone individuals is due to reduced nitric oxide (NO) bioavailability under hypoxic conditions (Dehnert et al., 2007). Consistent with this premise, single nucleotide polymorphisms (SNPs) in NO synthase 3, which is involved in NO synthesis, have been associated with HAPE susceptibility (Droma et al., 2002; Ahsan et al., 2006). In addition, the renin-angiotensin-aldosterone cascade may also play an important role in the pathogenesis of HAPE; SNPs in angiotensin-converting enzyme (Hotta et al., 2004) and have been positively correlated with risk for HAPE. Furthermore, synergistic effects of genetic polymorphisms in angiotensin-converting enzyme and aldosterone synthase CYP11B2 confer a high predisposition to HAPE in Han Chinese (Qi et al., 2008).

Genetic variations in additional genes implicated in hypoxia-induced responses (Mortimer et al., 2004), including those of pulmonary surfactant proteins A1 and A2 (Saxena et al., 2005) and the Hsp70 family (Qi et al., 2009), have also been proposed to be associated with HAPE susceptibility. However, SNPs of tyrosine hydroxylase (Hanaoka et al., 2003) and vascular endothelial growth factor (Hanaoka et al., 2009) were not associated with HAPE susceptibility. Therefore, HAPE susceptibility is heterogeneous, and interactions between genetic and environmental factors result in the condition; however, the genetic factors in the majority of HAPE cases are yet to be elucidated.

Hypoxia is thought to be the most potent factor in the progression of HAPE. Mitochondria are important players in oxygen sensing and may elicit essential adaptive responses to hypoxia tolerance. Indeed, the two most notable responses to oxygen deprivation - down-regulation of energy turnover and up-regulation of ATP production efficiency - are both primarily modulated by mitochondria (Hochachka, 1986). The mitochondrial electron transport chain has also been implicated in hypoxia signaling (Kwast et al., 1999).

After exposure to reduced concentrations of oxygen, mammalian cells experience oxidative stress that leads to the increased production of reactive oxygen species (ROS), toxic byproducts of energy metabolism. Mitochondrially generated ROS can trigger hypoxia-induced transcription by stabilizing hypoxia-inducible factor 1α (Chandel et al., 1998). Furthermore, under hypoxic conditions, mitochondria produce elevated levels of NO, which can induce the expression of nuclear genes associated with hypoxia (Castello et al., 2006). Cytochrome C oxidase is a mitochondrial enzyme that generates NO, a well-documented factor for HAPE development. Additionally, when an oxygen shortage first occurs, ROS originating in the mitochondria lead to reduced Na^+-K^+-ATPase activity in alveolar epithelial cells, a phenomenon also observed in HAPE (Dada et al., 2003). Finally, it has been shown that high altitude-induced ROS and the overwhelmingly high oxidative stress that results may up-regulate nuclear factor kappa B and cause increased transvascular leakage in the lungs of mice (Sarada et al., 2008).

All of these observations indicate the significant role of mitochondria and the electron transport chain in the etiology of HAPE (Wallace, 2005). Interestingly, it is known that pathogenic mutations in mtDNA are directly associated with degenerative diseases, and mtDNA polymorphisms can also affect disease susceptibility. For example, mitochondrial haplogroup N9b is protective against myocardial infarction in Japanese men (Nishigaki et al., 2007). Thus, mtDNA haplotypes may also have significant impacts on vulnerability to HAPE. To test this hypothesis, we performed an association study on HAPE and mtDNA haplogroups. We found that mitochondrial haplogroup D4 confers resistance to HAPE, whereas haplogroup B is a genetic risk factor for this condition in Han Chinese.

MATERIAL AND METHODS

Samples

For the correlation study, we recruited 204 patients with HAPE (45.6% from Sichuan Province, 34.30% from Chongqing Province, 15.20% from Guizhou Province, and 4.9% from other provinces) and 174 healthy controls (46.6% from Sichuan Province, 33.90%

from Chongqing Province, 14.40% from Guizhou Province, and 5.2% from other provinces) in Lhasa, Tibet (3658 m) between December 2005 and April 2006. Patients with HAPE developed the disease after flying from lowland areas (Chongqing or Chengdu, both near 300 m) to high altitude (Lhasa, 3658 m) within 7 days. Samples were collected at the General Hospital of Tibet Military Command, Lhasa. HAPE diagnosis was based on standard criteria (Hultgren and Marticoremna, 1978), including cough, dyspnea, cyanosis at rest, absence of infection, and the presence of pulmonary rales. Chest X-rays of HAPE patients showed patchy shadows (Figure S1A) and those of HAPE patients after recovery showed that the water had been reabsorbed (Figure S1B). Control subjects comprised 174 individuals that were age-, gender-, and nationality-matched with the HAPE patients (Tabl e 1). All HAPE and control subjects were permitted to rest after ascending to high altitude. Controls were unrelated to each other and had no acute mountain sickness after flying from the lowlands to high altitude within 7 days. Those with reported mtDNA-related diseases (such as diabetes, Parkinson's disease, Alzheimer's disease) in their medical or family histories were excluded. This study was approved by the Ethics Committee of the Third Military Medical University in China. Written consent was obtained from all subjects in accordance with the guidelines Declaration of Helsinki. Our study population represented the largest sample size of any HAPE susceptibility study.

Table 1. Characteristics of the subjects.

	HAPE	Control
Total	204	174
Male	174	150
Female	30	24
Age (years)	32.01 ± 10.70	31.80 ± 11.08
Nationality	Han	Han
Mode of ascent	By the plane	By the plane

HAPE = high-altitude pulmonary edema.

Mitochondrial DNA SNPs (mtSNP) genotyping

Fourteen polymorphic sites that define the major haplogroups in Han Chinese were selected for mtDNA haplotyping (Figure 1A). For the mtSNPs in the study, two (A10398G, C10400T) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP; Yao et al., 2002), and the other single-nucleotide variations and a 9-bp deletion were characterized using the PCR-ligase detection reaction (PCR-LDR). The PCR-LDR experiments were designed as described elsewhere (Barany, 1991; Nigou et al., 1998). This technology relies on the high fidelity of *Taq* DNA ligase to discriminate known single-base variations and offers a quick and automated platform for high-throughput genotyping. All PCR primers and the probes for LDR are shown in Tables S1 and S2, respectively. Each set of LDR probes was composed of one common probe and two allele-specific probes that differed in length by 3 nucleotides. PCRs were carried out in an ABI 9600 (Applied Biosystems, USA) using the following conditions: 94°C for 15 min; 35 cycles at 95°C for 30 s, 50°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. The ligation reactions were carried out with *Taq* DNA ligase (New England Biolabs, USA) for 40 cycles at 94°C for 30

s and 50°C for 2 min. The LDR profile was then analyzed using an ABI PRISM 3730 DNA Sequencer (Applied Biosystems). Performance of PCR-LDR and PCR-RFLP was assessed through direct sequencing of 100 samples; all SNPs detected with PCR-LDR and PCR-RFLP were confirmed by sequencing, indicating that these methods are reliable. Our report is the first to adapt the highly specific PCR-LDR system to large-scale mtDNA haplotyping (Luo et al., 2010).

Complete mitochondrial genome sequencing and analysis

The entire mtDNA genome from each subject in haplogroup B was amplified in eight overlapping fragments and sequenced with 44 primers (Tables S3 and S4). Reactions (50-µL volume) were performed using 50 ng template DNA, 0.1 mM deoxynucleotide triphosphates (Takara, Dalian), and 10 pmol each of the forward and reverse primers. Reactions were undertaken using 2.5 U *Taq* DNA polymerase enzyme (Takara) in Taq buffer as supplied, supplemented with 1.5 mM $MgCl_2$. PCR was undertaken using a thermal cycler system (ABI, USA). Program parameters were one cycle of 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 56°C for 30 s, and 72°C for 3 min. After a final incubation at 72°C for 4 min, the products were cooled to 4°C. PCR products were assayed using gel electrophoresis on a 1% agarose gel stained with ethidium bromide. The 62 complete mitochondrial genome sequences were analyzed using MitoMaster (Brandon et al., 2009) to identify polymorphisms that differed from the revised Cambridge Reference Sequence. The mtDNA subhaplogroups within haplogroup B were assigned according to previous publications (Kivisild et al., 2002; Yao et al., 2002; Kong et al., 2006).

Statistical analysis

The data were analyzed with the SPSS 12.0 software (SPSS, USA); P values, odds ratios (OR), and 95% confidence interval (95%CI) were calculated, and the two-tailed Fisher test was used to assess the significance of the correlation between mtDNA haplogroups, subhaplogroup B, and risk for HAPE.

RESULTS

Haplogroup D and HAPE

Figure 1A shows the significantly lower prevalence of haplogroup D4 (HAPE = 14.2%; control = 22.9%; P = 0.033, OR = 0.555, 95%CI = 0.327-0.942) observed in HAPE patients. MtDNA haplogroup D4 is a subhaplogroup of haplogroup D. However, the incidence of haplogroup D (HAPE = 16.7%; control = 23.6%; P = 0.012, OR = 0.649, 95%CI = 0.390-1.078) itself did not appear to correlate with susceptibility to HAPE in this study (Table 2). Haplogroup B is defined by a 9-bp deletion located in the intergenic region between cytochrome c oxidase subunit 2 and transfer RNA-lysine. In this study, a notably elevated frequency of haplogroup B (HAPE = 20.6%; control = 11.0%; P = 0.013, OR = 2.118, 95%CI = 1.194-3.756) was found in HAPE patients (see Figure 1A).

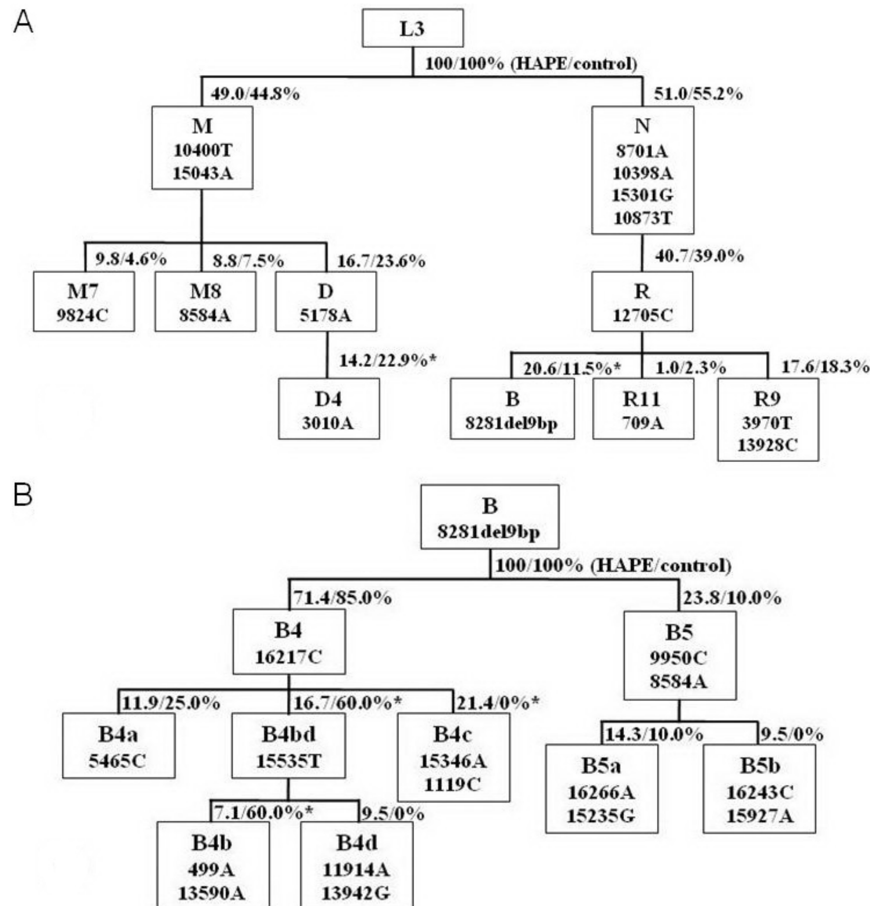

Figure 1. mtSNPs defining the major haplogroups in Hans (**A**) and subhaplogroups within haplogroup B (**B**). Percentages of subjects for high-altitude pulmonary edema (HAPE) and controls are indicated for each haplogroup or subhaplogroup.*P < 0.05.

Table 2. Association between mtDNA haplogroup and susceptibility to high-altitude pulmonary edema (HAPE) in Hans.

Haplogroup	HAPE (N = 204)	Control (N = 174)	HAPE *vs* Control		
			OR	95%CI	P
M	49.0% (100/204)	44.8% (78/174)	1.183	0.789-1.776	0.469
M7	9.8% (20/204)	4.6% (8/174)	2.255	0.968-5.258	0.075
M8	8.8% (18/204)	7.5% (13/174)	1.199	0.570-2.522	0.709
D	16.7% (34/204)	23.6% (41/174)	0.649	0.390-1.078	0.120
D4	14.2% (29/204)	22.9% (40/174)	0.555	0.327-0.942	0.033*
N	51.0% (104/204)	55.2% (96/174)	0.845	0.563-1.268	0.469
R	40.7% (83/204)	39.0% (68/174)	1.069	0.707-1.616	0.753
B	20.6% (42/204)	11.5% (20/174)	2.118	1.194-3.756	0.013*
R11	1.0% (2/204)	2.3% (4/174)	0.421	0.076-2.326	0.420
R9	17.6% (36/204)	18.3% (32/174)	0.951	0.562-1.069	0.894

OR = odds ratio. 95%CI = 95% confidence interval. *P < 0.05.

Subhaplogroup B and HAPE

To understand better how haplogroup B increases vulnerability to HAPE and to identify mtSNPs correlated with risk for HAPE, we fully characterized the mitochondrial genome sequences of the 42 HAPE patients and 20 controls in the haplogroup B group. The distribution of HAPE patients and controls within haplogroup B revealed a preponderance of controls in subhaplogroup B4b (HAPE = 7.1%; control = 60%; P < 0.01, OR = 0.055, 95%CI = 1.364-infinity; Figure 1B and Table 3). Within haplogroup B, subhaplogroup B4c further raises vulnerability for HAPE (HAPE = 21.4%; control = 0%; P = 0.02, OR = 0.555, 95%CI = 0.327-0.942; see Figure 1B and Table 3). Subhaplogroup B4c was tagged with mtSNPs G15346A and T1119C. Accordingly, the positive correlation between mtSNPs typifying G15346A (HAPE = 21.4%; control = 0%; P = 0.02), T1119C (HAPE = 21.4%; control = 0%; P = 0.02), and HAPE is statistically significant (see Figure 1B and Table 4).

Table 3. Number of high-altitude pulmonary edema (HAPE) and control subjects in different subhaplogroups with haplogroup B.

	HAPE (N = 42)	Control (N = 20)	P
B unclassified	2	1	0.55
B4 unclassified	9	0	0.02*
B4a	5	5	0.35
B4bd	7	12	<0.01*
B4b	3	12	<0.01*
B4d	4	0	0.38
B4c	9	0	0.02*
B5	10	2	0.35
B5a	6	2	0.95
B5b	4	0	0.38

*P < 0.05.

Table 4. mtSNPs of which incidences are significantly different between high-altitude pulmonary edema (HAPE) and control subjects.

	rCRS	SNP	HAPE (42)	Control (20)	Locus	mtDB (2704)	P (Fisher/chi-square)	Note
150	C	T	9	0	HVS2	247	0.02	
499	G	A	5	10		38	<0.01	B4b tag
709	G	A	20	3	12S rDNA	444	0.03	B5 tag; G-C → A C in a stem
827	A	G	7	10	12S rDNA	54	<0.01	B4bd tag
1119	T	C	9	0	12S rDNA	26	0.02	B4c tag; in a loop; increase risk for HAPE
2952	T	A	3	6	16S rDNA	none	0.05	A-U → C U in a stem
13590	G	A	6	10	ND5	110	<0.01	B4b tag; syn
15346	G	A	9	0	CytB	31	0.02	B4c tag; syn
15535	C	T	7	10	CytB	48	<0.01	B4bd tag; syn
16140	T	C	16	2	HVS1	51	0.05	B5 tag
16362	T	C	0	3		444	0.05	

rCRS = revised Cambridge Reference Sequence. SNP = single nucleotide polymorphism.

DISCUSSION

Our analysis revealed a significantly lower prevalence of haplogroup D4 and a notably elevated frequency of haplogroup B in HAPE patients. mtDNA haplogroup D4 is a subhaplogroup of haplogroup D. However, the incidence of haplogroup D in itself appeared

to have no correlation with susceptibility to HAPE in this study (see Table 2), suggesting that the mtSNP characteristic of haplogroup D4 could have some functional effects that confer resistance to HAPE. Haplogroup D4 is tagged by 3010A (see Figure 1A), located at the root of stem 48 (position 1340) in domain V of the 16S ribosomal RNA (rRNA) gene, which contributes to the functional architecture of the peptidyl-transferase center of the human mitochondrial ribosome (Burk et al., 2002). A transition from guanine to adenine at this position converts a wobble guanine:uracil pair into a classical Watson-Crick adenine:uracil pair and hence increases the stability of the secondary structure. This substitution has arisen independently in several mtDNA haplogroups, including J1, D4, and H1. Interestingly, 3010A has been proposed to contribute to the protective effects of haplogroup H1 against ischemic stroke (Rosa et al., 2008). This mtSNP is also enriched in Japanese centenarians (Alexe et al., 2007), and subgroup D4a is a marker for extreme longevity (Bilal et al., 2008). All of these observations indicate that 3010A may enhance the stability of the 16S rRNA gene and be associated with reduced oxidative stress.

Haplogroup B is defined by a 9-bp deletion located in the intergenic region between cytochrome C oxidase subunit 2 and transfer RNA-lysine. It is the only haplogroup with a distribution that encompasses both sides of the Pacific Ocean. It is believed to have arisen in Asia about 50,000 years ago, and the migration of humans belonging to this haplogroup to the Americas is generally thought to have been from Siberia (Schurr and Wallace, 2002). To understand better how haplogroup B increases vulnerability to HAPE and to identify mtSNPs correlated with the risk for HAPE, we fully characterized the mitochondrial genome sequences of the 42 HAPE patients (and 20 controls in this lineage) using PCR and direct sequencing. The 62 complete mitochondrial genome sequences were analyzed using MitoMaster (Brandon et al., 2009) to retrieve polymorphisms differing from the revised Cambridge Reference Sequence (Andrews et al., 1999). The mtDNA subhaplogroups within haplogroup B were assigned according to previous publications (Kivisild et al., 2002; Yao et al., 2002; Kong et al., 2006).

The distribution of HAPE patients and controls within haplogroup B revealed a preponderance of controls in subhaplogroup B4b and a significantly higher incidence of HAPE in B4c patients (see Figure 1B and Table 3). Accordingly, the positive correlation between mtSNPs typifying subhaplogroup B4c (15346A and 1119C) and HAPE was statistically significant (see Table 4). mtSNP 15346A is a synonymous substitution in cytochrome b and is therefore not expected to have functional impacts on the mitochondria. mtSNP 1119C is located in loop 28 of the predicted secondary structure of the 12S rRNA gene, and this position is conserved among humans, bovines, and mice. Thus, this transition may modulate the stability and function of the 12S rRNA, impair mitochondrial function at high altitude, and consequently lead to a higher risk of HAPE. This mtSNP has also been associated with susceptibility to obesity (Guo et al., 2005). The total incidence of B4b subjects within haplogroup B (15/62 = 24.2%) is close to that found in the normal Han population [17.7% (Yao et al., 2002); 15.4% (Wen et al., 2004)]. However, only 7.1% of HAPE patients belong to this lineage, whereas the B4b subhaplogroup represents an extraordinary 60% of the healthy controls, suggesting that the mtSNPs characteristic of this lineage (see Figure 1B and Table 4) may have protective effects against HAPE.

mtDNA 13590A is a synonymous variation in ND5, and 499A is situated between the two promoters for H-strand (545-567) and L-strand (392-445) transcription in the D-loop. Nevertheless, how these two mtSNPs confer resistance to HAPE remains to be elucidated.

 ©FUNPEC-RP www.funpecrp.com.br

The effects of these haplogroup tags on mitochondrial function could be tested with cybrid technology; however, finding a control that differs by only one nucleotide from the subject would be difficult.

This study is the first effort to link mtDNA haplogroups and HAPE. Thus, it can provide guidance for the prediction of genetic risks for HAPE, enable targeted prevention for at-risk populations, and thereby contribute to the primary prevention of the condition. Furthermore, investigating the role of mitochondria in acute hypoxic exposure can improve our understanding of other disorders associated with chronic hypoxia. Hypoxia-induced signaling may underlie the pathogenesis of many diseases, including chronic obstructive pulmonary disease (COPD), a major cause of morbidity and mortality in the United States (Yoshida et al., 2007). In COPD, progressive limited airflow and impaired alveolar capillary networks result in reduced oxygen transport and alveolar hypoxia. Approximately 60% of COPD patients display mild chronic hypoxia-induced pulmonary hypertension. The evidence that mtDNA haplogroups can affect susceptibility to HAPE (possibly through modifying hypoxia-induced signaling) presented in this study may translate into a better understanding of hypoxia-induced diseases, such as COPD.

ACKNOWLEDGMENTS

The authors thank all patients and laboratory workers for their enormous contributions to this collaborative study. Research supported by the 973 Project of China (#2012CB518200), the National Key Technology R & D Program of China (#2009BAI85B01), and the National Natural Science Foundation of China (#30900715).

Supplementary material

REFERENCES

Ahsan A, Mohd G, Norboo T, Baig MA, et al. (2006). Heterozygotes of NOS3 polymorphisms contribute to reduced nitrogen oxides in high-altitude pulmonary edema. *Chest* 130: 1511-1519.

Alexe G, Fuku N, Bilal E, Ueno H, et al. (2007). Enrichment of longevity phenotype in mtDNA haplogroups D4b2b, D4a, and D5 in the Japanese population. *Hum. Genet.* 121: 347-356.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, et al. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.* 23: 147.

Barany F (1991). Genetic disease detection and DNA amplification using cloned thermostable ligase. *Proc. Natl. Acad. Sci. U. S. A.* 88: 189-193.

Bartsch P, Mairbaurl H, Maggiorini M and Swenson ER (2005). Physiological aspects of high-altitude pulmonary edema. *J. Appl. Physiol.* 98: 1101-1110.

Basnyat B and Murdoch DR (2003). High-altitude illness. *Lancet* 361: 1967-1974.

Bilal E, Rabadan R, Alexe G, Fuku N, et al. (2008). Mitochondrial DNA haplogroup D4a is a marker for extreme longevity in Japan. *PLoS One* 3: e2421.

Brandon MC, Ruiz-Pesini E, Mishmar D, Procaccio V, et al. (2009). MITOMASTER: a bioinformatics tool for the analysis of mitochondrial DNA sequences. *Hum. Mutat.* 30: 1-6.

Burk A, Douzery EJP and Springer MS (2002). The secondary structure of mammalian mitochondrial 16S rRNA molecules: refinements based on a comparative phylogenetic approach. *J. Mamm. Evol.* 9: 225-252.

Castello PR, David PS, McClure T, Crook Z, et al. (2006). Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab.* 3: 277-287.

Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, et al. (1998). Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc. Natl. Acad. Sci. U. S. A.* 95: 11715-11720.

 ©FUNPEC-RP www.funpecrp.com.br

Dada LA, Chandel NS, Ridge KM, Pedemonte C, et al. (2003). Hypoxia-induced endocytosis of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC-zeta. *J. Clin. Invest.* 111: 1057-1064.

Dehnert C, Berger MM, Mairbaurl H and Bartsch P (2007). High altitude pulmonary edema: a pressure-induced leak. *Respir. Physiol. Neurobiol.* 158: 266-273.

Droma Y, Hanaoka M, Ota M, Katsuyama Y, et al. (2002). Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. *Circulation* 106: 826-830.

Guo LJ, Oshida Y, Fuku N, Takeyasu T, et al. (2005). Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity. *Mitochondrion* 5: 15-33.

Hanaoka M, Droma Y, Hotta J, Matsuzawa Y, et al. (2003). Polymorphisms of the tyrosine hydroxylase gene in subjects susceptible to high-altitude pulmonary edema. *Chest* 123: 54-58.

Hanaoka M, Droma Y, Ota M, Ito M, et al. (2009). Polymorphisms of human vascular endothelial growth factor gene in high-altitude pulmonary oedema susceptible subjects. *Respirology* 14: 46-52.

Hochachka PW (1986). Defense strategies against hypoxia and hypothermia. *Science* 231: 234-241.

Hotta J, Hanaoka M, Droma Y, Katsuyama Y, et al. (2004). Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects. *Chest* 126: 825-830.

Hultgren HN and Marticorena EA (1978). High altitude pulmonary edema. Epidemiologic observations in Peru. *Chest* 74: 372-376.

Kivisild T, Tolk HV, Parik J, Wang Y, et al. (2002). The emerging limbs and twigs of the East Asian mtDNA tree. *Mol. Biol. Evol.* 19: 1737-1751.

Kong QP, Bandelt HJ, Sun C, Yao YG, et al. (2006). Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations. *Hum. Mol. Genet.* 15: 2076-2086.

Kwast KE, Burke PV, Staahl BT and Poyton RO (1999). Oxygen sensing in yeast: evidence for the involvement of the respiratory chain in regulating the transcription of a subset of hypoxic genes. *Proc. Natl. Acad. Sci. U. S. A.* 96: 5446-5451.

Luo Y, Tang S, Gao W, Chen L, et al. (2010). Genotyping mitochondrial DNA single nucleotide polymorphisms by PCR ligase detection reactions. *Clin. Chem. Lab. Med.* 48: 475-483.

Mortimer H, Patel S and Peacock AJ (2004). The genetic basis of high-altitude pulmonary oedema. *Pharmacol. Ther.* 101: 183-192.

Nigou M, Parfait B, Clauser E and Olivier JL (1998). Detection and quantification of the A3243G mutation of mitochondrial DNA by ligation detection reaction. *Mol. Cell Probes* 12: 273-282.

Nishigaki Y, Yamada Y, Fuku N, Matsuo H, et al. (2007). Mitochondrial haplogroup N9b is protective against myocardial infarction in Japanese males. *Hum. Genet.* 120: 827-836.

Peacock AJ (1995). High altitude pulmonary oedema: who gets it and why? *Eur. Respir. J.* 8: 1819-1821.

Qi Y, Niu W, Zhu T, Zhou W, et al. (2008). Synergistic effect of the genetic polymorphisms of the renin-angiotensin-aldosterone system on high-altitude pulmonary edema: a study from Qinghai-Tibet altitude. *Eur. J. Epidemiol.* 23: 143-152.

Qi Y, Niu WQ, Zhu TC, Liu JL, et al. (2009). Genetic interaction of Hsp70 family genes polymorphisms with high-altitude pulmonary edema among Chinese railway constructors at altitudes exceeding 4000 meters. *Clin. Chim. Acta* 405: 17-22.

Rosa A, Fonseca BV, Krug T, Manso H, et al. (2008). Mitochondrial haplogroup H1 is protective for ischemic stroke in Portuguese patients. *BMC Med. Genet.* 9: 57.

Sarada S, Himadri P, Mishra C, Geetali P, et al. (2008). Role of oxidative stress and NFkB in hypoxia-induced pulmonary edema. *Exp. Biol. Med.* 233: 1088-1098.

Sartori C, Allemann Y and Scherrer U (2007). Pathogenesis of pulmonary edema: learning from high-altitude pulmonary edema. *Respir. Physiol. Neurobiol.* 159: 338-349.

Saxena S, Kumar R, Madan T, Gupta V, et al. (2005). Association of polymorphisms in pulmonary surfactant protein A1 and A2 genes with high-altitude pulmonary edema. *Chest* 128: 1611-1619.

Schurr TG and Wallace DC (2002). Mitochondrial DNA diversity in Southeast Asian populations. *Hum. Biol.* 74: 431-452.

Wallace DC (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39: 359-407.

Wen B, Li H, Lu D, Song X, et al. (2004). Genetic evidence supports demic diffusion of Han culture. *Nature* 431: 302-305.

Yao YG, Kong QP, Bandelt HJ, Kivisild T, et al. (2002). Phylogeographic differentiation of mitochondrial DNA in Han Chinese. *Am. J. Hum. Genet.* 70: 635-651.

Yoshida T and Tuder RM (2007). Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. *Physiol. Rev.* 87: 1047-1082.

 ©FUNPEC-RP www.funpecrp.com.br