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MtDNA Haplogroups M7 and B in Southwestern Han Chinese at Risk for Acute Mountain Sickness

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Abstract

We conducted a case-control study to investigate the association of mitochondrial DNA (mtDNA) haplogroups with acute mountain sickness (AMS) in Han Chinese from southwestern (SW) China. Pearson's chi-square test or Fisher's exact test revealed significant reduction of mtDNA haplogroups D and M9, while a significant increase of haplogroup M7 in AMS subjects compared with non-AMS subjects. The multivariate logistic regression analysis after adjustment for body mass index (BMI), a risk factor of AMS in the present study, showed that both D and M9 were associated with significantly decreased risk of AMS, while M7 was associated with a significantly increased risk of AMS (OR = 0.605, $p = 0.000$; OR = 0.037, $p = 0.001$, and OR = 2.419, $p = 0.001$, respectively). In addition, the further analysis stratified by the AMS severities indicated that haplogroup B was correlated with a 2.41-folds increased risk of developing severe AMS (95% C.I. = 1.288–4.514, $p = 0.006$). Our findings provide the evidence that, in SW Han Chinese, mtDNA haplogroups D and M9 are related to individual tolerance to AMS, while haplogroups M7 and B are risk factors for AMS.

Keywords

acute mountain sickness; mitochondrial DNA; haplogroup; altitude; hypoxia

1. Introduction

The past several decades have seen an increase in mountain travel by skiers, backpackers, climbers, runners, hunters, fishermen and even spiritual seekers. However, upon arrival at high altitude, many people often develop altitude-related illness such as acute mountain sickness (AMS), high altitude pulmonary edema (HAPE), or high altitude cerebral edema (HACE). AMS is the most common and mild form of altitude-related illness caused by

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Conflicts of interest

None of the authors have any conflict of interests.

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hypoxia. The illness typically affects people within 6–12 hrs of arriving at altitudes above 2,500 m, and is characterized by headache plus the presence of at least one of the following symptoms: gastrointestinal symptoms (anorexia, nausea, or vomiting), insomnia, dizziness, and lassitude or fatigue (Bartsch et al., 2004). Although AMS is generally not serious, if it is ignored and ascent continues, it can develop into life-threatening HACE.

As a neurological disorder caused by hypoxia, the underlying pathophysiology of AMS is still unclear. Growing evidence indicates that reactive oxygen species (ROS) contribute to the pathophysiology of AMS. Hypoxia is characterized by lack of oxygen in relation to aerobic ATP requirements and an increased generation of ROS. An excessive load of ROS generated under hypoxic conditions may result in cell injury and dysfunction (Clanton, 2007). ROS are prone to oxidize unsaturated fatty acids in biological membranes, a process called lipid peroxidation, forming a variety of free radicals. Some investigators have linked the development of AMS with the condition of altered blood–brain barrier permeability, possibly related to hypoxia induced free radical formation (Askew, 2002). A field study at high altitude showed the content of malondialdehyde, a final product of lipid peroxidation, in exhaled breath condensate of mountaineers to correlate with Lake Louis score of AMS (Araneda et al., 2005). Furthermore, two recently published studies have shown that hypoxia stimulated cerebral oxidative stress and AMS was associated with altered redox homeostasis (Bailey et al., 2009a and 2009b). Actually, the findings that antioxidant prophylaxis with a cocktail of aqueous and lipid-phase dietary antioxidant vitamins during an ascent to 5,180m attenuated AMS strongly supported the role of ROS in AMS (Bailey and Davies, 2001).

Although the hypothesis that some individuals are particularly susceptible to AMS is supported by clinical epidemiological data, there is little genetic evidence, thus far, that supports innate, inherited factors as a cause (Rupert et al., 2006). Human mitochondrial DNA (mtDNA) is a circular molecule of about 16.6 kb, encoding 37 genes that are essential for respiratory function of mitochondrion (Wallace et al., 1987 and 2010). Evidence from metabolic studies and mitochondrial genetics suggested that mtDNA haplogroup may be related to AMS. Prior metabolic studies in rodents and humans exposed to hypobaric hypoxia noted changes in mitochondrial oxidative phosphorylation (OXPHOS) function, ROS production, and activation of the mitochondrial K^+ channel and of the mitochondrial permeability transition pore (mtPTP) (Gvozdjáková et al., 1992; Tan et al., 2002; Magalhães et al., 2005; Gonzales et al., 2005; Zhu et al., 2006). Additionally, some mtDNA haplogroups were found to be associated with human susceptibility to metabolic and degenerative diseases such as neurodegenerative diseases, diabetes, metabolic syndrome, as well as to influence the risk of cancer and longevity; and mitochondrial ROS production was considered to make an important contribution to these diseases and longevity (Wallace et al., 2005). Furthermore, the common and “non-pathological” mtDNA haplotypes were found to determine differences in OXPHOS performance and ROS production both in mice and Human (Moreno-Loshuertos et al., 2006; Marcuello et al., 2009; Martínez-Redondo et al., 2010).

Given that ROS, most of which are byproducts of OXPHOS of mitochondria, may play roles in the pathogenesis of AMS and mtDNA haplogroups contribute to the differences in OXPHOS performance, ROS production and maximal oxygen consumption, we hypothesize that mtDNA variants may be involved in the development of AMS. To test this hypothesis, we conducted a case-control study to investigate the role of mtDNA haplogroups in AMS in a Han Chinese population from southwestern (SW) China.

2. Material and Methods

2.1. Subjects

In December 2008, 1,234 new male army recruits who were born and raised at an altitude lower than 1,000 m and who had no previous exposure to high altitude environments were recruited for the study. All participants were maternally unrelated Han Chinese from SW China including three provinces and one municipality (Sichuan province (SC), Yunnan province (YN), Guizhou province (GZ), and Chongqing city (CQ)). Before transferred to altitude, all the new soldiers gathered at a military camp in a city of SW China (altitude of 500 m) for a week. During their staying at 500 m, a short physical examination was conducted, and those with apparent respiratory tract infection and/or a fever of higher than 38 °C were excluded from ascending to altitude. Only those who passed the physical examination underwent acute ascent to altitude of 3,700 m about 2 hrs by air. After arriving at the altitude, all subjects had the same work and rest regime, and were forbidden to exercise vigorously. Alcohol was forbidden but water was unrestricted. The weather at altitude during the investigation period was stable, with ambient temperatures of −7 °C at night and 10 °C in the day, with weak winds and cloudless days.

2.2. Assessment of AMS and sampling

All data were collected in the camps at 3,700 m within 48 hrs of arrival. After explaining the purpose and procedures of the study, all participants gave written informed consent. AMS was assessed by the Lake Louise Self-Assessment Score (AMS-LL) (<http://www.high-altitude-medicine.com/AMS-worksheet.html>). This commonly used assessment includes five symptoms: headache, gastrointestinal distress, fatigue or weakness (or both), dizziness or lightheadedness, and difficulty sleeping. Each symptom is scored 0 (not present) through 3 (severe or incapacitating) for a combined minimal score of 0 and a maximal score of 15. Our criteria for AMS were 1) headache; 2) plus at least one other symptom; and 3) a total score of 3 or more. AMS was defined as mild if the score was 3 to 4, moderate if the score was 5 to 6, and severe if the score was more than 7. This self-reported system also included some personal and historical questions, such as height and weight for subsequent calculation of body mass index (BMI), and altitude of residence. Blood samples were drawn into Na-EDTA tubes from all 1,234 subjects and stored at −70 °C for genomic DNA extraction.

2.3. MtDNA analysis

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit according to the manufacturer's recommendation (QIAGEN, Maryland, USA). To determine mtDNA haplotypes, the entire mtDNA sample was amplified into 22 overlapping PCR fragments. Each was digested with 14 restriction endonucleases (*Alu* I, *Ava* II, *Bam*HI, *Dde* I, *Hae* II, *Hae* III, *Hha* I, *Hinc* II, *Hinf* I, *Hpa* I, *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I) (Torroni et al., 1994; Starikovskaya et al., 2005). For the mtDNA 9-bp deletion in the COII/tRNA^{lys} intergenic region, the primer pair L8215/H8292 was used. The primer pairs L9794/H10164 were used for haplogroup M7 (9820+*Hinf* I) and L3084/H3459 for M9 (3391+ *Hae* III) (Yao et al., 2002). In each run, a negative control was included to avoid artificial contamination caused by potential sample crossover. Restrictive fragment length polymorphism (RFLP) analysis was supplemented by sequencing of HVSI (from position 16024 to 16383, relative to the revised Cambridge Reference Sequence, rCRS) (Andrews et al., 1999). Sequencing reactions were performed using 1.5 µl of PCR product purified with ethanol/NaAc (3 M, pH 5.2)/160 nM primer/Big Dye Terminator dideoxy nucleotide terminators (Big Dye Terminator V3.1 Cycle Sequencing Kit, ABI, Branchburg, NJ, USA). Electrophoresis and base "calling" were performed using an ABI Prism 3100XL Genetic Analyzer. In addition, selected mtDNAs representing the major lineages were completely

sequenced. Trace files were analyzed using the SeqScape Software (v.2.5, ABI) and compared with the rCRS. The mtDNA haplotypes based on the RFLPs/HVS I sequences were classified into haplogroups according to the phylogenetic analyses of mtDNAs and the Mitomap-Phylogeny (Mishmar et al., 2003; Derbeneva et al., 2002a and 2002b; Yao et al., 2002 and 2004; Saillard et al., 2000).

2.4. Data analyses

All statistical analyses were performed using the Statistical Package for Social Science 13 for Windows (SPSS Inc, Chicago, IL). The difference in mtDNA haplogroup frequency was assessed by Pearson's chi-square test or Fisher's exact test, and the chi-square test for linear-by-linear association. Multivariate logistic regression analysis was used to analyze the association between mtDNA haplogroups and AMS after adjustment for BMI, a risk factor of AMS in this study. The *p* values, odds ratios (ORs), and 95% confidence intervals (95% CIs) were calculated. For multiple comparisons of haplogroups, the Bonferroni correction was used (required significance level = 0.05/number of comparisons). This changed the significance levels from < 0.05 to < 0.0033 for Table 4 (0.05/15 = 0.0033), and < 0.01 for Tables 5 and 6 (0.05/5 = 0.01). Quantitative clinical data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD) and compared between cases and controls using unpaired student's *t*-test.

3. Results

3.1. Subject characteristics

The study enrolled 1,234 unrelated male Han Chinese (aged from 17 to 22 years) from SW China, none of whom developed HAPE or HACE. Among the 1,234 subjects, 499 (40.44%) suffered from AMS at 3,700 m. No significant differences in age, weight, or height were found between the AMS and non-AMS population. However, the AMS population had a significantly higher BMI than the non-AMS population did ($p < 0.001$). No significant difference in AMS prevalence was found among subjects from the four regions of SW China ($p = 0.397$). The demographic characteristics of the subjects are summarized in Table 1 and the AMS prevalence by region in SW China is shown in Table 2.

3.2. Distribution of subjects based on AMS- LL score and symptoms of AMS

As shown in Fig. 1 and Table 3, at the altitude of 3,700 m, 700 of 1,234 subjects (56.73%) had an AMS-LL score less than 2 (including 269 subjects who had no symptoms of AMS). 35 subjects who had an AMS-LL score more than 3 but no headache were classified as non-AMS, including 12 subjects with score 3, 7 with score 4, 10 with score 5, and 6 with score 6. Consequently, in the present study, we recruited 499 AMS and 735 non-AMS subjects. The most common symptoms of AMS were fatigue and/or weakness (57.05%), followed by dizziness/lightheadedness (48.70%), difficulty sleeping (48.14%), headache (42.45%), and gastrointestinal symptoms (21.80%).

3.3. Distribution of mtDNA haplogroups in AMS and non-AMS populations

All 1,234 subjects were classified into 15 common Asian mitochondrial haplogroups by RFLP analysis plus HVS I sequencing. The overall distribution of mtDNA haplogroups in both AMS and non-AMS population are shown in Table 4. Pearson's chi-square test or Fisher's exact test revealed that the distributions of mtDNA haplogroups D, M7, and M9 in AMS subjects were significantly different from those of non-AMS subjects ($p = 0.000$, 0.003, and 0.000, respectively). Multivariate logistic regression analysis after adjustment for BMI revealed that, on the basis of a *p* value of < 0.05, haplogroups A, D, and M9 were associated with significantly decreased risk of AMS (OR = 0.592, 95%CI = 0.371–0.945, *p*

= 0.028; OR = 0.605, 95% C.I = 0.460–0.795, $p = 0.000$ and OR = 0.037, 95% C.I = 0.005–0.277, $p = 0.001$, respectively), whereas haplogroup M7 was associated with a significantly increased risk of AMS (OR = 2.419, 95% C.I = 1.459–4.010, $p = 0.001$). When the Bonferroni correction was applied, haplogroup A did not reach the adjusted p level of < 0.0033 .

Comparisons of the regional distribution of mtDNA haplogroups among subjects with and without AMS are shown in Table 5. Because of a limited number of subjects from GZ ($n = 187$), YN ($n = 145$) and CQ ($n = 283$), subjects from GZ, YN, and CQ were pooled to improve statistical power. When the Bonferroni correction was applied, the significant p -value was set as < 0.01 . Consequently, multivariate logistic regression analysis with adjustment for BMI demonstrated that in the SC region, haplogroup M9 was significantly correlated with resistance against AMS (OR = 0.067, 95% C.I = 0.08–0.540, $p = 0.010$), while subjects classified as haplogroup M7 had a 3.57-fold risk of developing AMS compared with non-carriers (OR = 3.574, 95% C.I = 1.464–8.729, $p = 0.005$). In the YN+GZ+CQ region, haplogroup D was found to be associated with a significantly decreased risk of AMS (OR = 0.587, 95% C.I = 0.398–0.868, $p = 0.008$).

The subsequent linear-by-linear association test, stratified by AMS-LL rating, revealed a decreasing trend for mtDNA haplogroups D and M9 and an increasing trend for haplogroup B from non-AMS to severe AMS ($p = 0.000$, 0.000 , and 0.002 , respectively). Haplogroup B reached its highest frequency in severe AMS subjects (35.42%), and haplogroup M7 was mainly prevalent in mild AMS subjects (10%). Further multivariate logistic regression analysis demonstrated that haplogroup M7 was associated with a 3-fold increased risk of developing mild AMS, and haplogroup B was associated with a 2.4-fold increased risk of developing severe AMS (BMI adjusted OR = 3.033, 95% C.I = 1.770–5.198, $p = 0.000$ and BMI adjusted OR = 2.411, 95% C.I = 1.288–4.514, $p = 0.006$, respectively). (Table 6).

4. Discussion

Neurovascular headache, hallmark of AMS, is now considered to be caused by the interaction of free radicals and the trigeminovascular system (Bailey et al., 2009c). Since most intracellular ROS are byproducts of OXPHOS of mitochondria, and mtDNA haplogroups are found to determine the differences in OXPHOS performance, ROS production and maximal oxygen consumption, we recruited 1,234 male soldiers from SW China based on a strict case-control matching design to investigate the association between different mtDNA haplogroups and the risk of AMS. Consistent with Dean et al. (1990), we found that, at 3,700 m, the incidence of AMS was 40.44%. The prevalence of AMS was not significantly different among subjects from the four regions of SW China. The mtDNA haplogrouping analysis in this cohort of SW Han Chinese revealed that both haplogroups D and M9 were associated with decreased risk of AMS, while M7 was associated with an increased risk of AMS. In addition, the protective effect of M9 and the risk effect of M7 on AMS were found only in the SC region; and haplogroup D was found to be a protective factor of AMS in the YN+GZ+CQ region. Further analysis stratified by severities of AMS showed that haplogroup M7 was related to increased risk of mild-AMS subjects, while haplogroup B was related to increased risk of severe-AMS subjects.

MtDNA haplogroup D, one of the most characteristic mtDNA lineages found in Northeast Asian populations is defined by a specific variation at C5178A. This variation, resulting in a Leu→Met substitution at amino acid 237 (L237M) in the mitochondrial NADH dehydrogenase subunit 2 (ND2), is reported to be related to human longevity (Tanaka et al., 1998; Cai et al., 2009), anti-atherosclerotic effects in diabetic patients, and protection against myocardial infarction (Takagi et al., 2004). The L237M replacement in the ND2

subunit might have a protective effect against oxidative damage to mitochondria since methionine residues constitute an important anti-oxidant defense mechanism (Takagi et al., 2004). Thus, the protective effect of mtDNA haplogroup D against oxidative damage to mitochondria may help individuals with haplogroup D to be protected against AMS.

Haplogroup M9 is defined by a specific variant at mtDNA T3394C, leading to a missense mutation in a complex I gene. The T3394C mutation is in the evolutionarily conserved region of ND1 and replaces a hydrophilic neutral tyrosine with hydrophilic basic histidine at amino acid 237 (Y30H), which may change the predicted secondary structure and thus the function of ND1 (Liu et al., 2007). Of note, in this study, of 499 AMS sufferers, only one belonged to mtDNA haplogroup M9 with the HVS I motif 16223-16234-16316, but lacking the T3394C mutation. The possible mechanism of M9 tolerant to AMS might be related to natural selection of hypobaric hypoxia, since M9 reaches its greatest frequency (20.3%) in Tibetan populations (Qin et al., 2010).

MtDNA haplogroup M7, mainly found in East Asia, is defined by a specific variation at np T9824C leading to a synonymous mutation in the cytochrome c oxidase subunit III gene. By studying the penetrance of Leber's hereditary optic neuropathy (LHON) in 1,859 individuals from 182 Chinese families (including one from Cambodia), mtDNA haplogroup M7b1'2, a subhaplogroup of M7, was found to increase the penetrance of LHON (Ji et al., 2007). In our study, haplogroup M7 was found to be a risk factor only for mild AMS; the underlying mechanism is worthwhile to investigate further.

Haplogroup B is identified by a 9-bp deletion in the COII/tRNA^{Lys} intergenic region of mtDNA plus the HVS I motif 16189–16519 and has not been strongly influenced by cold selection (Ruiz-Pesini et al., 2004). In the present study, Pearson's chi-square test revealed no difference of mtDNA haplogroup B prevalence between the AMS and non-AMS populations. However, the linear-by-linear association analysis showed haplogroup B increased with AMS symptom exacerbating. Multivariate logistic regression analysis after adjustment for BMI demonstrated that haplogroup B was associated with a 2.4-fold increased risk of developing severe AMS.

MtDNA variants were found to be selected by climate and some mtDNA haplogroups were enriched in specific environments (Ehinger et al. 2002; Mishmar et al., 2003; Fontanillas et al., 2005). In our study, the possible reason for the reduction of haplogroup D and enrichment of haplogroup B in the AMS cohort may be that mtDNA haplogroups selected by cold climates may help human beings to reduce hypothermia, which is often confounded with AMS in hypoxia and cold altitude conditions (Basnyat and Murdoch, 2003).

Our study has certain limitations. First, the Lake Louise scoring system applied in this study for diagnosing AMS is a self-reported census system. It is difficult to differentiate some malaise and diseases like exhaustion, dehydration, and migraine from AMS. Second, because of lower frequency of haplogroup M9 and relatively small numbers of subjects with severe AMS and subjects from YN and GZ in this study, subgroup analysis would produce less effective conclusion. Nonetheless, because of strict case-control, and a large and relatively homogeneous population related to environmental exposure, we are confident that the participants in this study represent SW Han Chinese and their haplogroup frequency, allowing reliable estimated risk for AMS.

In summary, using a strict case-control matching design, we analyzed a large population for association of mtDNA haplogroups with individual risk of AMS. Our findings provide genetic evidence that mtDNA haplogroups M7 and B are risk factors for AMS in SW Han Chinese, while haplogroups D and M9 are related to individual tolerance to AMS.

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Fig. 1.
Distribution of AMS-LL scores among 1,234 subjects

Table 1

Subject characteristics

	AMS (<i>n</i> = 499)	Non-AMS (<i>n</i> = 735)	<i>p</i> value
Age (yr)	18.47 ± 1.24	18.34 ± 1.45	0.06
Weight (kg)	60.21 ± 11.53	59.15 ± 10.48	0.10
Height (cm)	168.85 ± 3.34	169.15 ± 4.93	0.25
BMI (kg/m ²)	21.03 ± 0.85	20.08 ± 1.66	< 0.001
Altitude of permanent residence > 1,000m	No	No	–
History of prior AMS	No	No	–
Altitude exposure of (> 1,500 m) in the previous 3 months	No	No	–
Drug intake before and after ascent to the altitude	No	No	–
Rate of ascent	2 hrs from sea level to the altitude by air	2 hrs from sea level to the altitude by air	–
Altitude reached (m)	3,700	3,700	–

^a All subjects were male. BMI: body mass index.

Table 2

Prevalence of AMS in four regions of SW China

Region	AMS	Non-AMS	Total
SC	241 (38.93%)	378 (61.07%)	619
CQ	126(44.52%)	157 (55.48%)	283
GZ	77(41.18%)	110(58.82%)	187
YN	55(37.93%)	90(62.07%)	145
Total	499(40.44%)	735(59.56%)	1,234

SC, Sichuan province; YN, Yunnan province; GZ, Guizhou province; CQ, Chongqing city

Table 3

Summary of AMS-Louis Lake Self-Assessment in 1,234 individuals at 3,700 m

Symptoms	Number of cases, n (%)			
	Never	Seldom/mild	Often/moderate	Regularly/severe
Headache	710(57.54)	435(35.25)	77(6.24)	12(0.97)
Gastrointestinal symptoms	965(78.20)	232(18.80)	31(2.51)	6(0.49)
Fatigue and/or weakness	530(42.95)	607(49.19)	95(7.70)	2(0.16)
Dizziness/lightheadedness	633(51.30)	527(42.71)	69(5.59)	5(0.41)
Difficulty sleeping	640(51.86)	322(26.09)	264(21.39)	8(0.65)

Table 4

Distribution of mtDNA haplogroups in AMS and non-AMS Han populations

mtDNA haplogroups	AMS (n = 499)	Non-AMS (n = 735)	p value (χ^2)	Adjusted p value ^a	OR (95% C.I.) ^a
A	30 (6.01%)	69 (9.39%)	0.032	0.028	0.592 (0.371–0.945)
B	112 (22.44%)	132 (17.96%)	0.052	0.067	1.322 (0.981–1.782)
F	91 (18.24%)	105 (14.29%)	0.062	0.108	1.305 (0.943–1.805)
Y1	3 (0.60%)	3 (0.41%)	0.691	0.980	0.979 (0.189–5.072)
N9a	11 (2.20%)	12 (1.63%)	0.466	0.400	1.450 (0.610–3.447)
R3	9 (1.80%)	10 (1.36%)	0.535	0.668	1.232 (0.474–3.201)
D	117 (23.45%)	242 (32.93%)	0.000	0.000	0.605 (0.460–0.795)
G	44 (8.82%)	46 (6.26%)	0.090	0.169	1.379 (0.872–2.181)
M2	2 (0.40%)	3 (0.41%)	1.000	0.908	1.116 (0.172–7.246)
M7	41 (8.22%)	31 (4.22%)	0.003	0.001	2.419 (1.459–4.010)
M8(M8a+C+Z)	35 (7.01%)	42 (5.71%)	0.354	0.143	1.445 (0.883–2.364)
M9	1 (0.20%)	32 (4.35%)	0.000	0.001	0.037 (0.005–0.277)
M13a	3 (0.60%)	4 (0.53%)	1.000	0.771	1.268 (0.255–6.299)
M11	0 (0%)	2 (0.27%)	NA	NA	NA
M	0 (0%)	2 (0.27%)	NA	NA	NA

^a ORs, 95% C.I.s and adjusted p values were calculated after adjustment for BMI. Bonferroni corrected $p < 0.05/15$.

Table 5
Distribution of mtDNA haplogroups in subjects with and without AMS according to the regions of SW China

MtDNA haplogroup	SC, <i>n</i> (%)		YN+GZ+CQ, <i>n</i> (%)							
	AMS (<i>n</i> = 241)	Non-AMS (<i>n</i> = 378)	<i>p</i> value(χ^2)	Adjusted <i>p</i> value ^a	OR (95%CI) ^a	AMS (<i>n</i> = 258)	Non-AMS (<i>n</i> = 357)	<i>p</i> value(χ^2)	Adjusted <i>p</i> value ^a	OR (95%CI) ^a
A	15 (6.22)	33 (8.73)	0.256	0.526	0.804(0.410–1.577)	15 (5.81)	36 (10.08)	0.058	0.021	0.463(0.241–0.888)
B	61 (25.31)	73 (19.31)	0.077	0.175	1.329(0.881–2.005)	51 (19.77)	59 (16.53)	0.301	0.195	1.335(0.863–2.066)
D	61 (25.31)	131 (34.66)	0.014	0.020	0.634(0.432–0.931)	56 (21.71)	111 (31.09)	0.010	0.008	0.587(0.398–0.868)
M7	15 (6.22)	9 (2.38)	0.016	0.005	3.574(1.464–8.729)	26 (10.08)	22 (6.16)	0.074	0.033	1.955(1.054–3.625)
M9	1 (0.41)	17 (4.50)	0.003	0.010	0.067 (0.08–0.540)	0 (0)	15 (4.20)	0.001	NA	NA

^a ORs, 95%CI and adjusted p values were calculated after adjustment for BMI. Bonferroni corrected $p < 0.05/5$.

Table 6

Distribution of mtDNA haplogroups in 1,234 subjects by AMS severity

MtDNA haplogroups	Non-AMS (n = 735), n (%)	Mild-AMS (n = 307)			Moderate-AMS (n = 143)			Severe-AMS (n = 49)			p trend ^a
		n (%)	p value	OR (95% C.I.)	n (%)	p value	OR (95% C.I.)	n (%)	p value	OR (95% C.I.)	
A	69 (9.39)	18 (5.86)	0.133	0.652 (0.374–1.139)	8 (5.63)	0.157	0.574 (0.266–1.239)	4 (8.33)	0.933	1.447 (1.200–1.745)	0.097
B	132 (17.96)	58 (18.89)	0.802	1.047 (0.732–1.496)	37 (26.06)	0.023	1.603 (1.068–2.405)	17 (35.42)	0.006	2.411 (1.288–4.514)	0.002
D	242 (32.93)	80 (26.06)	0.020	0.691 (0.506–0.942)	29 (20.42)	0.002	0.492 (0.314–0.771)	8 (16.67)	0.014	0.377 (0.173–0.824)	0.000
M7	31 (4.22)	31 (10.00)	0.000	3.033 (1.770–5.198)	8 (5.63)	0.202	1.711 (0.750–3.904)	2 (4.17)	0.849	1.155 (0.264–5.054)	0.157
M9	32 (4.35)	1 (0.33)	0.007	0.063 (0.008–0.470)	0	NA	NA	0	NA	NA	0.000

ORs, 95% C.I.s and *p* values were calculated after adjustment for BMI.

^a *p* trend was calculated by the chi-square test for linear-by-linear association. Bonferroni corrected *p* < 0.05/5.