HLA DRB 1 Alleles Distribution and Chronic Mountain Sickness Susceptibility in Tibet

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SUMMARY

A total of 16 HLA-DRB1 alleles were found, with 39 different HLA-DRB1 genotypes. The most common alleles in the chronic mountain sickness (CMS) group were DRB1*0403, DRB1*0901, and DRB1 *1201, with frequencies of 34.7%, 12%, and 12%, respectively. Within the control group, DRB1*0405?DRB1*0901, DRB1*1201, and DRB1*0403 were the most common observed alleles, with a frequency of 7.9%, 9.5%, 9.5%, and 6.3%, respectively. The frequency of DRB1*0403 in the CMS group was significantly higher than that of the control group ($p<0.001$). The mean hemoglobin (Hb) in the CMS group was also higher than that of the control group as well with 25.6±1.4g/dl and 17.8±1.4g/dl respectively. The level of oxygen saturation (SaO_2) in the CMS group and the control group was 82.6+3.2% and 88.1+2.1% respectively ($p<0.001$). The results suggest that HLA-DBR1*0403 appears to be associated with a maladaptation to high altitude causing CMS.

The immunogenic susceptibility present in patients with CMS has never been reported within the Tibetan population. So in this paper, the human leukocyte antigen (HLA) class II gene DRB1 was chosen for an association study of chronic mountain sickness (CMS). Forty-seven patients with CMS and 63 healthy people, as the control, were genotyped for HLA-DRB1. Both CMS and control subjects were of Tibetan ethnicity, permanently living at an altitude of 4500m in the Qinghai-Tibetan plateau.

People who are longtime residents at above 2500m are at risk of developing chronic mountain sickness (CMS, Monge's disease). CMS is characterized by excessive erythrocytosis (EE), marked pulmonary hypertension (HAPH) and severe hypoxemia, and the clinical picture of CMS gradually disappears after descending to low altitude and reappears upon returning to high altitude. The prevalence of CMS is greater in males than in females, rises with increasing altitude equally in both sexes, and is lower in Tibetan natives compared with Han Chinese[1] or Peruvians[2]. Increasing age, nocturnal oxygen desaturation, and obesity have proven to be additional risk factors in the development of this syndrome. High altitude hypoxia is an etiologic key to the development of this disease, but the exact mechanisms underlying the pathogenesis are not fully understood. Our recent data[3] indicated that the excessive chronic hypoxemia in CMS sub-jects were significantly associated with an increase in some of the natriuretic peptides and angiogenic cytokines, which may play an important role in the pathogenesis and clinical expression of CMS.

The human leukocyte antigen (HLA) system includes a great number of various representatives of genes and products of expression. Genes of the HLA system are disposed on a short shoulder of the chromosome 6, which contains approximately 4 millions pairs of bases that correspond to 0.1% of the human genome and form more than 200 gene-clusters. A great deal of study data has shown that HLA had a higher association with immunologic diseases[4,5]. Striking genetic association has been reported between certain HLA alleles and susceptibility to some non-immunological diseases, especially on associations of high alti-tude pulmonary edema (HAPE) with HLA-DR6 and DQ4[6], and of pulmonary hypertension with HLA-DR6[7] has been reported. In addition, Praditpornsilpa[8] reports an association of HLA-DRB1*09 and DQB1*0309 with anti-HuEPO associated red cell apla-sia. Pulmonary hypertension and excessive erythrocytosis are an important pathophysiological trait of CMS. We hypothesized that individual susceptibility is also associated with HLA in CMS, and propose to examine whether immunogenetic susceptibility is present in CMS-susceptible subjects. The current investigation is the first report about the genetic back-ground of CMS on the Tibetan plateau.

Materials and Methods

Subjects: The research protocol was approved by the human subject protection committee at the Medical College of Qinghai University. Informed consent was obtained from each subject. Forty-seven Tibetans with CMS (mean age 43.5+9.8yr) were selected from local hospital and sixty-three healthy Tibetans (mean age 39.7+14.1yr) were selected by healthy physical examination in this study. All subjects were born and lived their entire life at Qumalai County (4500m) and Zhiduo County (4500m), Qinghai province. This is one of the highest permanently inhabited areas of the Qinghai province. The climate in this region is characterized not only by a low atmospheric pressure (baro-metric pressure 430 mmHg), but also by a number of complex and interlinked environmental stresses. The mean annual ambient temperature is -0.2-2.5°C and the minimum temperature is -36°C. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2) of the subjects. None of the participants

had a history of cardiovascular or respiratory abnormalities and none of the participants had any history of respiratory or cardiovascular disease, such as chronic obstructive pulmonary disease, pulmonary infection, asthma, shunt, valvular disease, congenital heart disease, or hypertensive heart disease.

Assessment of CMS: Each subject completed a CMS self-report questionnaire. The evaluation tool used was the Qinghai CMS score[9], established during the VI World Congress on Mountain Medicine and High Altitude Physiology in 2004. The questionnaire criteria included headache, dizziness, breathlessness, palpitations, sleep disturbance, cyanosis, tinnitus, paresthesias, and venous dilatation. Each criterion was graded on a scale from 0 to 3, with 0 representing no symptoms; 1, mild symptom; 2, moderate symptoms; and 3, severe symptoms. Hemoglobin (Hb) concentration was graded differently for males and females. For males: <21g/dl, a score of 0 is assigned, while for Hb of >21g/dl, a score of 3 is given. For females, the corresponding Hb values are <19g/dl and >19g/dl, score3. According to the sum of points given for symptoms and Hb, the severity of CMS is defined as: absent for a score 0 to 5; mild for a score 6 to 10; mod-erate for a score 11 to 14; and severe if the score is >15.

Pulmonary function test: All participants underwent pulmonary function testing using a standard electric spirometer controlled by a microcomputer (Ransfer Screen II: Jaeger; Wuirzburg, Germany) Lung volumes, maximal flow-volume loop, and maximal voluntary ventilation (MVV) were recorded and analyzed by a Jaeger system. Pulmonary function testing was performed according to the guidelines of the American Thoracic Society. The spirometer was calibrated daily with a 1 liter syringe before testing began. Oxygen saturation (SaO2) was measured by pulse oximetry (Ohmeda 3700 Pulse Oximeter, Datex-Ohmeda, Boulder, Colorado). Hemoglobin (Hb) and Hematocrit (Hct) was determined by a blood cell analyzer. All indices of the above -mentioned were measured in the local hospital at 4500m.

HLA Typing: Venous blood samples were obtained from all participants and transferred into a tube with EDTA. Lymphocytes and leukocytes were isolated from the peripheral blood by use of a density gradient centrifugation technique, and genomic DNA was extracted by the protease-K method, and the DNA was amplified by the polymerase chain reaction (PCR) (Specific Primers include seven forward primers and a reverse primer: *(see table below)*

Statistical Analysis: Data are expressed as means + standard deviation (SD). Comparisons of various parameters between the two groups were made by the Fisher protected least significant difference test at the 95% significant level. A student's independent two sample *t*-test was used in comparing physiological data (Height, Hb, Hct, BMI, BP, VC, FEV1, FEF 25%-75%, MVV, HR, and SaO2) of the CMS and control groups. The frequencies of HLA class II alleles (DRB1) were determined by direct counting for the patient and control group. The chi-squared test was used to compare the expected value of genotype with its observed value in order to confirm whether it satisfies the Hardy-Weinberg law. Difference of loci positive rates or negative rates between two groups were esti-mated from 2?2 tables by chi-squared analysis, only used the Fisher exact or Yates correction test when the expected value was less than one or five. Relative risk (RR) was calculated and evaluated by Woolf formula. A *P* value <.05 was held to represent significance.

Results

General characteristics: Forty-seven patients with CMS and sixty-three healthy subjects were recruited for this study. The two groups were similar in terms of age and height but the CMS group had a higher BMI, lower SaO_2, and higher hemoglobin and hematocrit than the control group. There were no significant dif-ferences in vital capacity (VC), forced expiratory vol-ume in 1 second (FEV1), forced expiratory flow dur-ing the first half of the forced vital capacity (FEF 25%-75%) or maximal voluntary ventilation values (MVV) between the two groups. The general characteristics of both groups are listed in Table 1. In this study, both groups of subjects were Tibetan natives.

Polymorphistic distribution of HLA-DRB 1: A total of 39 HLA-DRB1 alleles in the present study were found, in which 16 alleles were clearly detected. The frequencies for the HLA-DRB1 in patients with CMS and control subjects are shown in Table 2. The most common alleles in the CMS group were DRB1*0403, DRB1*0901, and DRB1 *1201, with a frequency of 34.7%, 12%, and 12%, respectively. In the control group, DRB1*0405, DRB1*0901, DRB1*1201, and DRB1*0403 were observed with a frequency of 7.9%, 9.5%, 9.5%, and 6.3%, respectively. In the control group, the frequency of HLA-DRB1*0701, HLADRB1* 0803, HLA-DRB1*1105 and HLADRB1* 02(6.3%) were kept within the region of 4 to 10%, the frequency of the rest of the alleles were lower than 4%, but HLA-DRB1*1301/1302, HLADRB1* 1001, HLA-DRB1*010101, HLA-DRB1*0401, HLA-DRB1*1202, HLA-DRB1*02, and HLADRB1*1105 were not found in the CMS group; HLADRB1* 080402 and HLA-DRB*1115 were not detected in either group.

	Sequence	*length*
forward primers	DRB1-1F 5′-CCACAGCACGTTTCTTGGAGTACTCTA-3′	27 base
	DRB1-2 F 5′-CCAGTTTCTTGTGGCAGCTTAAGT T-3′,	25 base
	DRB1-3 F 5′-TCGTTCCTGTGGCAGGGTAAGTATA-3′	25 base
	DRB1-4 F 5′-AGCCGTTTCTTGAAGCAGGATAAGTT-3′	26 base
	DRB1-5 F 5′ -CCAAGCACGTTTCTTGGAGGAGG-3′	23 base
	DRB1-6 F 5′-TCGTTCCTGTGGCAGCCTAAG A-3′	22 base
	DRB1-7 F 5′-AGCCGTTTCTTGGAGCAGGTTAAA C-3′	25 base
reverse primer	DRB1-R 5′-CTGTTACCTCGCCACTGCAC-3′	20 base

Reactive conditions: 96°C 2min; 96°C 30 seconds, 60°C 30 seconds, 70? 1min, 35 cycles, and extend 10min at 72°C. Finally, the DNA was then collected, and the purity quotient of DNA was improved by agarose gel electrophoresis (voltage 220 V, 20 min). DNA purity production was determined by ABI-3730XL (Applied Biosystems, Foster City, CA, USA). (Cycle sequence kit:BigDye@ Terminator v3.1 was offered by Applied Biosystems. (include a forward primer and a reverse primer):

	Sequence	*length*
forward primer	DRB1-F 5′-TTGCAATT CTTCAATGGGAC-3′	20 base
reverse primer	DRB1-R 5′ -ACCACCCG GTAGTTGTGTC-3′	19 base

Reactive condition: 96°C 2min; 96°C 10s, 50°C 5s, 60°C 4min, 25 cycles, and heat preservation at 72°C). Sequences of HLA-DRB1 alleles were measured and analyzed by MatchTools**TM** and MT Navigator**TM** software (Applied Biosystems).

A comparison of the frequency distribution of CMS and control subjects is shown in table 2. Patients with CMS had a significantly higher frequency of the HLA-DRB1*0403 allele ($p<0.001$) when compared with control subjects. CMS susceptibility was closely associated with the frequency of the HLA allele, and the relative risk (RR) of DRB1*0403 in the CMS group was significantly higher. Furthermore, HLA-DRB1*0403 and HLA-DRB1*0301 had higher risk for the susceptibility of CMS via evaluation by the Woolf formula.

*HLA-DRB1*0403 and concentration of Hb:* Out of the 47 patients with CMS, 17 were DRB1*0403-positive and 32 subjects were negative. Those DRB1*0403 positive patients had significantly higher concentra-tion of Hb (26.9+1.2 g/dl) and larger Hct (75.1 + 4.6%) when compared to those negative patients (Hb:25.0+1.2g/dl; Hct: 64.1+6.7%) ($P<0.001$). DRB1*0403-positive subjects also had a higher CMS-score (14.1 + 5.3) than that of negative subjects (6.1 + 2.8). There was no significant difference in pulmonary functions between the subgroups.

Discussion

The important findings of this study are that the patients with CMS had a significantly higher frequency of the HLA-DRB1*0403 than that of the control subjects. HLA-DRB1*0403-positive patients with CMS showed a significantly higher Hb, Haematocrit, and CMS-score when compared with those of the negative patients, and the relative risk (RR) of HLA-DRB1*0403 by evaluation of Woolf equation in the CMS group was 8.36. But we find that HLA-DRB1*0403 frequency is lower in healthy Tibetan subjects on Qinghai plateau, and same results occurred in healthy Tibetan subjects on Tibet plateau[10,11]. Our data suggest that a significant increase in HLA-DBR1*0403 may reflect a mal-adaptation to high altitude, and thus immunogenic susceptibility may underlie the development of CMS. This is the first report about the genetic back-ground of CMS in Tibetan plateau.

CMS is a multi-factorial disease caused by mal-adaptation to chronic exposure to a high altitude hypoxic environment. The prevalence of the disease is significantly increased at high elevations and is lower in high altitude natives than in newcomers. With the current migration of a great number of sea level residents to high mountain areas,

a large number of subjects are at risk for developing CMS in the Qinghai -Tibetan plateau. Contributing factors to the development of CMS have been found to include hypoventilation, sleep disorders, and obesity[12,13,14], the immunogenic factors contributing to CMS is undefined. We found that the subjects with CMS, who have a higher body weight, have significantly lower SaO_2 and a higher CMS score. The body mass index (BMI) of both groups correlated negatively with SaO_2 suggesting that heavier subjects at high altitude are more hypox-emic than thinner subjects. Because of the limited sample size, we have not analyzed the relationship with BMI and the incidence of CMS in female and male, respectively. Hence we can not be certain of an association of BMI with CMS. Here we only suggest that people with higher body weight, lower SaO_2, and higher CMS score has a likely correlation with positive-selection of HLA-DRB1*0403 on Qinghai-Tibet plateau.

Pulmonary arterial hypertension is another important pathological character of CMS. Although we did not present data of pulmonary arterial hypertension in this study, severe hypoxemia and excessive erythrocytosis is one of the major elements that result in the development of hypoxic pulmonary arterial hypertension. Many studies have found that HLA class ? type alleles are closely associated with pulmonary hypertension[6, 7,15] and heart disorders[4, 5, 16,17]. These results suggest that susceptibility to CMS is associated with HLA class II type alleles, notably with HLA-DRB1*0403.

Although the increased frequency of HLA-DRB1*0403 in subjects with CMS is demonstrated, and the DRB1*0403-positive subjects have higher concen-trations of Hb and CMS-score than that of DRB1*0403-negative subjects in the CMS group, it is still unclear whether HLA-DRB1*0403 causes CMS directly or indirectly.

The limitations of our study include its small size and limited pulmonary hemodynamic data. Nonetheless, the associations between HLA -DRB1*0403 and the presence and complications of CMS are compelling, and suggest a new avenue of investigation for high altitude medicine. Although we still cannot determine with certainty what role was played by HLA-DRB1*0403 in the pathophysiology of
CMS, the HLA class II type alleles especially for -DRB1*0403 is quite strikingly different in patients with CMS compared to controls. Future work will be required to determine whether the immunogenic factor contributes to individual susceptibility to CMS.

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References:

1 Anand, I. S. and T. Wu. Syndromes of subacute mountain sickness. *High Alt Med Biol* 2004; 5: 156-70.
2 Bronson PG, Ramsay PP, Thomson G, Barcellos LF. Analysis of maternal-offspring HLA compatibility, parent-of-origin and non-inherited maternal effects for the classical HLA loci in type 1 diabetes. *Diabetes Obes Metab* 2009; 11: 74-83.
3 Rili G, Vivian YM, James LJ, Guan J, Yinzhong Y, Shufen H, et al. B-type Natriuretic Peptide, Vascular Endothelial Growth Factor, Endothelin-1 and Nitric Oxide Synthase in Chronic Mountain Sickness. *Am J Physiol* 2011; 300: H1427-H1433.
4 Stanevicha V, Eglite J, Sochnevs A, Gardovska D, Zavadska D, Shantere R.HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. *Arthritis research & therapy* 2003; 5: R340-46.
5 Palikhe A, Lokki ML, Saikku P, Leinonen M, Paldanius M, Seppanen M, et al. Association of Chlamydia pneumoniae infection with HLA-B*35 in patients with coronary artery disease. *Clin Vaccine Immunol* 2008; 15: 55-59.
6 Hanaoka M, Kubo K, Yamazaki Y, Miyahara T, Matsuzawa Y, Kobayashi T, et al. Association of high-altitude pulmonary edema with the major histocompatibility complex. *Circulation* 1998; 97: 1124-28.
7 RaychaudhuriB, Bonfield TL, Malur A, Hague K, Kavuru MS, Arroliga AC, et al. Circulating monocytes from patients with primary pulmonary hypertension are hyporesponsive. *Clin Immunol* 2002; 104: 191-98.
8 Praditpornsilpal K, **Kupatawintu P, Mongkonsritagoon W, Supasyndh O, Jootar S, Intarakumthornchai T,** et al. The association of anti-r-HuEpo-associated pure red cell aplasia with HLA-DRB1*09-DQB1*0309. ***Nephrol Dial Transplant*** 2009; 24:1545-49.

9 Leon-Velarde F, Maggiorini M, Reeves JT, Aldashev A, Asmus I, Bernardi L,et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol* 2005; 6: 147-57.
10 Kang LL, Gao F, Zhang HB, Yuan DY, Zhao FC, Li SB. Determination of HLA-DRB1 gene polymorphism in Luoba ethnic group of Tibet. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2005; 30:135-39.
11 Zhang HB, Gao F, Kang LL, Li SB. Determination of HLA-A, B, and DRB1 gene polymorphism in Monba ethnic group of Xizang Autonomous Region, China. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2005; 22: 344-46.
12 Weil J V. Sleep at high altitude. *High Alt Med Biol* 2004; 5: 180-89.
13 Zubieta-Calleja GR, Paulev PE, Zubieta-Calleja L, Zubieta-Calleja N, Zubieta-Castillo G. Hypoventilation in chronic mountain sickness: a mechanism to preserve energy. *J Physiol Pharmacol* 2006; **57:** 425-30.
14 Ye JP, Gao ZG, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am J Physiol Endo & Meta* 2007; **293:** E1118-28.
15 Yoon SH, Oh HB, Kim HK, Hong SC, Oh YM, Lee DS. Association of HLA class II genes with idiopathic pulmonary arterial hypertension in Koreans. *Lung* 2007; 185: 145-49.
16 Gonzalez-Gay MA, Gonzalez-Juanatey C, Lopez-Diaz MJ, Pineiro A, Garcia-Porrua C, Miranda-Filloy JA, et al. HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. *Arthritis Rheum* 2007; 57: 125-32.
17 Chou HT, Chen CH, Chen JY, Chang KC. Association of HLA DRB1-DQA1-DQB1 haplotypes with rheumatic heart disease in Taiwan. *Int J Cardiol* 2008; 128: 434-35.