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# Recruitments of Sherpa highlanders and non-Sherpa lowlanders

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ABSTRACT

In order to carry out the research project of genetic adaptation to high-altitude hypoxia in Sherpa highlanders, we recruited Sherpa highlanders in Namche Bazaar village at a high altitude of 3,440 meters (m) above sea level and non-Sherpa lowlanders in Kathmandu city at 1,300 m in Nepal. Venous blood was sampled to obtain plasma and extract DNA in each subject. The concentrations of factors in plasma were measured. The single-nucleotide polymorphisms (SNPs) in the hypoxia-associated genes were genotyped.

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1 Recruiting Sherpa highlanders in Namche Bazaar village (3,440 m) in the Solu-Khumbu region in Nepal.

2 The Sherpas in Namche Bazaar village voluntarily participated in this investigation.

3 The Sherpa clan was identified with the Sherpa surname and confirmed by a senior native Sherpa.

4 All Sherpas were born and permanently resided in Namche Bazaar.

5 They were unrelated to each other in the first and second degree and had no history of intermarriage with other ethnic groups.

6 They did not leave their residences within three months of the sample collections.

7 Information regarding demographics, health status, altitude of residence, occupation, and mountaineering history was obtained during an interview.



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8 Symptoms of chronic mountain sickness (CMS), such as headache, dizziness, dyspnea, sleep disturbances, physical and mental fatigue, and cyanosis were specifically evaluated during the interviews.

9 A percutaneous arterial oxygen saturation (SpO2) and pulse rate were measured using a pulse oximeter (Pulsox-3, Minolta, Osaka, Japan) with a probe connecting to a finger.

10 Venous blood samples were taken and placed in tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for plasma sampling and DNA extraction.

11 The plasma was separated from the whole blood by centrifuging for 10 minutes at 3,000 rpm using a portable centrifuge at the site. The pelleted leukocytes were used for DNA extraction.

12 The plasma samples and the precipitation were stored in a freezer at < −20 °C until measurements of factors in plasma and DNA extraction.

13 Recruiting Non-Sherpa lowlanders in Kathmandu (1,300 m) in Nepal.

14 The non-Sherpa Nepalese were identified by their languages and social status (caste groups) with the help of native Nepalese.

15 They did not travel to high altitudes within three months of the sample collections.

16 The protocol of recruitments and sample collections for the non-Sherpa lowlanders was followed as that for the Sherpa highlanders in Namche Bazaar.

17 All the samples were transported in frozen status from Namche Bazaar to Kathmandu by porters, and then to Japan by air cargo.

18 The experiments were undertaken in our laboratory at Shinshu University, Matsumoto (600



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m), Japan.

19 The concentrations of factors in plasma were measured using the quantitative sandwich ELISA kit following the manufacturer's instructions.

20 The genomic DNA was extracted from venous blood leukocytes by phenol extraction of sodium dodecyl sulfate-lysed and proteinase K-treated cells.

21 Allele discrimination was performed using the TaqMan® SNP Genotyping Assay with the Applied Biosystems 7500 Fast Real-time PCR System (Applied Biosystems Inc. Foster City, CA, USA) following the manufacturer's instructions.

22 After thermal cycling, genotype data were automatically acquired and analyzed using sequence detection software (SDS v1.3.1; Applied Biosystems, Inc.).



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