

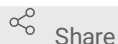


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Recruitments of Sherpa highlanders and non-Sherpa lowlanders

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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.

- 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

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.).