Association of Polymorphisms in Pulmonary Surfactant Protein A1 and A2 Genes With High-Altitude Pulmonary Edema*

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Study objectives: **A potential pathogenetic cofactor for the development of high-altitude pulmonary edema (HAPE) is an increase in capillary permeability, which could occur as a result of an inflammatory reaction and/or free-radical-mediated injury to the lung. Pulmonary surfactant protein A (SP-A), the most abundant surfactant protein, has potent antioxidant properties and protects unsaturated phospholipids and growing cells from oxidative injury. Single-nucleotide polymorphisms (SNPs) in *SP-A1* and *SP-A2*, genes encoding SP-A, have been associated with susceptibility to respiratory distress syndrome, COPD, and pulmonary infections. In view of the protective role of SP-A against inflammatory reactions and oxidative damage, the two underlying mechanisms in development of HAPE, we examined the association of constitutional susceptibility to HAPE with polymorphisms in *SP-A1* and *SP-A2*.**

Design: **A cross-sectional case-control study.**

Setting: **Blood samples were collected at an altitude (≥ 3,500 m).**

Participants: **Twelve low-altitude native (LAN) subjects with a history of HAPE, 15 healthy LAN sojourners without a history of HAPE (LAN control subjects), and 19 healthy high-altitude natives (HANs) without a history of HAPE (HAN control subjects).**

Measurements: **The SNPs in four exons and intermediate introns of the *SP-A1* and *SP-A2* were screened by polymerase chain reaction and sequencing. Biochemical parameters related to oxidative stress (malondialdehyde and reduced glutathione in RBC) and membrane permeability (circulating levels of lactate dehydrogenase) were measured in plasma.**

Results: **Allele frequencies of three loci in *SP-A1* and one in *SP-A2* were significantly different between LAN HAPE patients (SP-A1 C1101T: C allele, 36.4% and T allele, 63.6%; SP-A1 T3192C: T allele, 61.1% and C allele, 38.9%; SP-A1 T3234C: T allele, 61.1% and C allele, 38.9%; and SP-A2 A3265C: A allele, 21.4% and C allele, 78.6%) and LAN control subjects (SP-A1 C1101T: C allele, 8.3% and T allele, 91.7%; SP-A1 T3192C: T allele, 15% and C allele, 85%; SP-A1 T3234C: T allele, 15% and C allele, 85%; and SP-A2 A3265C: A allele, 37.5% and C allele, 62.5%) [C1101T odds ratio [OR], 6.3 with 95% confidence interval (CI), 2.8 to 14.3; T3192C OR, 8.9 with 95% CI, 4.5 to 17.6; T3234C OR, 8.9 with 95% CI, 4.5 to 17.6; and A3265C OR, 2.2 with 95% CI, 1.2 to 4.1 ($p \leq 0.01$)]. Heterozygous individuals, with respect to SP-A1 C1101T and SP-A2 A3265C, showed less severity in oxidative damage in comparison with homozygous subjects (SP-A1 T1101 and SP-A2 C3265).**

Conclusion: **The polymorphisms in *SP-A1* (C1101T, T3192C, and T3234C) and *SP-A2* (A3265C) might be one of the genetic factors contributing to susceptibility to HAPE.**

(CHEST 2005; 128:1611–1619)

Key words: genetic predisposition; high-altitude pulmonary edema; oxidative damage; single-nucleotide polymorphisms; *SP-A1*; *SP-A2*

Abbreviations: CI = confidence interval; GSH = reduced glutathione; HAN = high-altitude native; HAPE = high-altitude pulmonary edema; LAN = low-altitude native; LDH = lactate dehydrogenase; MDA = malondialdehyde; OR = odds ratio; PCR = polymerase chain reaction; SNP = single-nucleotide polymorphism; SP-A = pulmonary surfactant protein A; SP-A1 C1101T = single-nucleotide polymorphism between C and T alleles at 1101 base pair in *SPA1* gene

People, who rapidly ascend to high altitude and who are unable to acclimatize, suffer from high-altitude pulmonary edema (HAPE).[1] The pathophysiology of HAPE, a multifactorial and life-threatening disease, is complex, and reinductees are more susceptible to the disease.[2,3] A potential pathogenetic cofactor for the development of HAPE is an increase in capillary permeability, which could occur as a result of an inflammatory reaction and/or free-radical-mediated injury to the lung. A protective role for alveolar surfactant in HAPE was identified very early.[4] Droma et al[5] observed that the pulmonary surfactant in HAPE subjects not only lined the alveolar surface but was also patchily distributed within alveoli. Pulmonary surfactant protein A (SP-A), the most abundant surfactant protein, has a regulatory role in pulmonary surfactant secretion and recycling.[6] Bridges et al[7] have shown that SP-A has potent antioxidant properties and protects unsaturated phospholipids and growing cells from oxidative injury. Polymorphisms in *SP-A1* and *SP-A2*, genes encoding lung surfactant protein A, have been associated with susceptibility to respiratory distress syndrome, COPD, and pulmonary infections.[8–11] SP-A gene variants on ozone exposure produced significantly different amounts of proinflammatory cytokines interleukin 8 and tumor necrosis factor α.[12]

Genetic susceptibility has been suggested in the development of HAPE, and positive associations have been reported[13,14] with HLA-DR6, HLA-DQ4, and endothelial nitric oxide synthase genes. In view of the protective role of SP-A against inflammatory reactions and oxidative damage, the two underlying mechanisms in the development of HAPE, we examined the association of polymorphisms in *SP-A1* and *SP-A2* genes with HAPE for the first time. Because the high-altitude native (HAN) population is proposed to be relatively resistant to HAPE, the HAN controls were also studied to determine whether the alleles that are determining susceptibility to HAPE in low-altitude native (LAN) populations are in low frequency in the HAN population. Biochemical parameters related to oxidative stress, such as circulating malondialdehyde (MDA), an indicator of free-radical-induced lipid peroxidation, RBC-reduced glutathione (GSH), an indicator of antioxidant levels, and lactate dehydrogenase (LDH), an indicator of altered cell membrane permeability, are reported to participate in the course of HAPE.[15] The effect of variant genotypes on the oxidative damage was evaluated by a comparative analysis of these biochemical parameters.

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Ms. Shweta Saxena is a recipient of Senior Research Fellowship from Council of Scientific and Industrial Research. This study was supported by the Council of Scientific and Industrial Research, the Institute of Genomics and Integrative Biology, and the Defense Institute of Physiology and Allied Sciences.
Manuscript received November 10, 2003; revision accepted February 10, 2005.

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Materials and Methods

Subjects

The study population consisted of 46 age-matched male volunteers. All of the subjects were unrelated natives of India. Written informed consent was obtained from each subject after a full explanation of the study, which was approved by the human ethics committee of the Defense Institute of Physiology and Allied Sciences, Defense Research and Development Organization. LAN sojourners who suffered from HAPE at high altitude were brought to the hospital (at 3,500 m) immediately after the onset of symptoms. The blood samples were drawn from the HAPE patients within 2 to 3 days of occurrence of the symptoms. At the time of collection of the samples, the HAPE patients were still ill and under treatment in the hospital. The mean (± SE) age, height, and weight of volunteers were 28.8 ± 1.5 years, 170 ± 0.7 cm, and 59.8 ± 0.9 kg, respectively. The subjects were categorized in three groups, as described below.

The first group was composed of 15 healthy LAN control subjects, that is, those who did not suffer either from HAPE on exposure (≥ 1 month) to a high altitude (≥ 3,500 m) or any medical problems related to altitude or cardiopulmonary disorders. The second group consisted of 12 LAN subjects who suffered from HAPE at high altitude (≥ 3,500 m). All of these subjects showed presence of most of the clinical features, including shortness of breath, vomiting, progressive dyspnea, chest pain, fever, frothy pink expectoration, cyanosis of lips, and weakness at the onset of the disorder. The radiograph findings confirmed the pulmonary infiltrates. All of the patients recovered promptly with treatment. The effective treatment for the HAPE patients was bed rest, supplemental oxygen, and diuretics. The third group consisted of 19 HAN control subjects (≥ 3,500 m) who never suffered from HAPE.

Genomic DNA Isolation

Genomic DNA was extracted from peripheral blood mononuclear cells following a previously described protocol.[16] Purified genomic DNA samples were used as templates in the polymerase chain reaction (PCR) amplification of various regions of *SP-A1* and *SP-A2*.

PCR

For specific amplification and economical use of DNA samples, two rounds of PCR amplification were carried out for *SP-A1* and *SP-A2*. The first round of PCR amplified the complete *SP-A1* and *SP-A2* genes separately (primer 1/2 for *SP-A1* and primer 3/2 for *SP-A2*). The PCR conditions were 95°C/2′, 33 cycles of