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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.**

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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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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

95°C/30″, 58°C/30″, 72°C/3′, and final extension for 72°C/5′. The PCR products of first round were diluted (1:50) with water (Milli Q Millipore; Millipore S.A. 67120 Molsheim, France) and used as templates to amplify different regions of *SP-A1* and *SP-A2*.[17] The details of the primers used for amplification are shown in Table 1. Amplification conditions for other PCRs were similar to those given above except the specific annealing temperatures, as follows: 60°C for primer pairs 6/7 and 8/9; 59.8°C for primer pairs 12/13 and 14/15, and 70°C for primer pair 16/17. The PCR conditions for primer pairs 4/5 and 10/11 were 95°C/2′, 5 cycles of 95°C/30″, 50°C/1′, 70°C/1′, 27 cycles of 95°C/30″, 55°C/1′, 70°C/1′, and final extension for 72°C/5′.

Purification of PCR Products and Sequencing

The PCR products were purified and sequenced following a previously described protocol.[11]

Determination of Biochemical Parameters

The thiobarbituric acid-reactive MDA and RBC-reduced GSH levels were measured by standard methods.[18,19] Circulating plasma LDH activity was estimated by opt. kits (LD; Randox Laboratories Ltd; Antrim, UK).

Statistical Analysis

Allele frequencies at variable loci of *SP-A1* and *SP-A2* among different groups of subjects were compared by a 2×2 table, Fisher exact test, and statistical power tools using an online statistical analysis tool (http://home.clara.net/sisa). The odds ratio (OR) and p value were calculated at 95% confidence interval (CI). The biochemical parameters were analyzed for statistical significance using one-way analysis of variance, and pairwise comparisons were made by the Bonferroni multiple range test.

Results

Eight loci in *SP-A1* (C1101T, C1162T, C1193G, C1416T, G1544A, T3138C, T3192C, and T3234C) and eight loci in *SP-A2* (C1382G, T1492C, G1649C, A1660G, C2474T, A2491C, T3018C, and A3265C) were found to be polymorphic in the study groups (Table 2). LAN HAPE patients showed significant differences ($p \leq 0.01$) in the frequencies of three single-nucleotide polymorphisms (SNPs) in *SP-A1* (C1101T: OR, 6.3 [95% CI, 2.8 to 4.3]; T3192C: OR, 8.9 [95% CI, 4.5 to 17.6]; and T3234C: OR, 8.9 [95% CI, 4.5 to 17.6]) and one SNP in *SP-A2* (A3265C: OR, 2.2 [95% CI, 1.2 to 4.1]) in comparison with the LAN control subjects (Table 3) using a 2×2 table analysis. At a threshold for α (type 1 error probability) of 5%, our study will have around 80% power to detect SNP with a prevalence of $\geq 20\%$, resulting in an increase in the relative risk of disease of at least 2.2 to 8.9. At 50% retrospective power of the test, the minimal detectable differences for these statistical analyses are 0.28, 0.46, 0.46, and 0.16, respectively.

The SP-A1 T3192C and T3234C were redundant SNPs, that is, not causing a change in amino acids, whereas nonredundant SNPs, that is, SNPs causing change in amino acids, were SP-A1 C1101T (GCG: alanine and GTG: valine) and SP-A2 A3265C (AAG: lysine and CAG: glutamine). The alleles GCG (SP-A1 1101), TAT (SP- A1 3192), GAT (SP-A1 3234), and CAG (SP-A2 3265) were significantly associated with patients (Fig 1). The frequencies of various genotypes in patients and control subjects with respect to these four SNPs are shown in Figure 2. The mean values of the MDA (34.5% increase), GSH (17.8% decrease), and LDH (40.6% increase) for patients were significantly different from LAN control subjects indicating increased oxidative damage in patients (Table 4).

Table 1—*Details of Primers Used for PCR Amplification

Oligo (No.)	Location	S/AS	Nt Position	Sequence
SP-A1FF (1)	Intron A	S	469–488	5′ ACTCCATGACTGACCACCTT 3′
SP-A1,2FR (2)	Intron E	AS	3845–3865	5′ TGCCACAGAGACCTCAGAGT 3′
SP-A2FF (3)	Intron 1	S	472–491	5′ ATCACTGACTGTGAGAGGGT 3′
SP-A1E2,3F (4)	Intron A	S	995–1014	5′ GATGGGGTCACGGCCATCCC 3′
SP-A1E2,3R (5)	Intron C	AS	1962–1991	5′TGCCTCGTCCGCATTTCACCCTTCAGA CAGC 3′
SP-A1E4F (6)	Intron C	S	2340–2361	5′ TCGTGGCAGCAAGTGGGAGTCT 3′
SP-A1E4R (7)	Intron D	AS	2654–2677	5′ TCGGGGAAGTGAATTGTGTCTGCT 3′
SP-A1E5F (8)	Intron D	S	2896–2919	5′ TGAGGGAGGTGGCTTAGAGACAAA 3′
SP-A1E5R (9)	3′ UTR	AS	3364–3385	5′ GATGGAGGCCGACAAGGAGAGC 3′
SP-A2E3,4F (10)	Exon 3	S	1078–1097	5′ GCTGTGCCCTCTGGCCCTTA 3′
SP-A2E3,4R (11)	Intron 4	AS	1980–2009	5′TGCCTCGTCCGCATTCACCCTTCAGAC TGC 3′
SP-A2E5F (12)	Intron 4	S	2385–2406	5′ ACTGGCCTGCCCCTCCTTCTGT 3′
SP-A2E5R (13)	Intron 5	AS	2577–2600	5′ GCACGCTTGTTTGTCATCTCTATT 3′
SP-A2E6F (14)	Intron 5	S	2908–1931	5′ TGAGGGAGGTGGCTTAGAGACAAA 3′
SP-A2E6R (15)	Exon 6	AS	3386–3404	5′ CCTGGGGATGGAAACTGAA 3′
SP-A2SSF (16)	Exon 4	S	1602–1631	5′TGCCTCGTCCGCATTCACCCTTCAGAC TGC 3′
SP-A2SSR (17)	Intron 4	AS	1980–2009	5′TGCCTGGAGCCCCTGGTGTCCCTGGAGAGC 3′

*Oligo = oligonucleotide; UTR = untranslated region; Nt = nucleotide; S = sense; AS = antisense; SP-A1FF = name given to the primer based on the details that it is forward primer to amplify *SP-A1* full gene.

Table 2—*Nature of Polymorphisms Observed in SP-A1 and SP-A2 in the Present Study

Gene	Location	Nucleotides		Amino Acid	
		Position	Change	Position	Change
SP-A1	Signal peptide	1101	C/T	19th	Val to Ala
SP-A1	Exon 2	1162	C/T	39th	His to His
SP-A1	Exon 2	1193	C/G	50th	Leu to Val
SP-A1	Intron B	1416	C/T		
SP-A1	Exon 3	1544	G/A		
SP-A1	Exon 5	3138	T/C	184th	Tyr to Tyr
SP-A1	Exon 5	3192	T/C	202nd	Asp to Asp
SP-A1	Exon 5	3234	T/C	216th	Pro to Pro
SP-A2	Intron 3	1382	C/G		
SP-A2	Intron 3	1492	T/C		
SP-A2	Exon 4	1649	G/C	91st	Ala to Pro
SP-A2	Exon 4	1660	A/G	94th	Arg to Arg
SP-A2	Exon 5	2474	C/T	114th	Phe to Phe
SP-A2	Exon 5	2491	C/A	120th	Gln to Pro
SP-A2	Exon 6	3018	T/C	140th	Ser to Ser
SP-A2	Exon 6	3265	A/C	223th	Gln to Lys

*Nucleotide positions of *SP-A1* and *SP-A2* are according to the gene sequences reported in Entrez Nucleotide (National Center for Biotechnology Information) [Accession No. M 30838 and M 68519, respectively].

Table 3—*Comparison of Allele Frequencies at Variable Loci of SP-A1 and SP-A2 in Study Groups

Locus	LAN Ctrl, % Allele Freq (No.)	LAN HAPE, % Allele Freq (No.)	HAN Ctrl, % Allele Freq (No.)	p Value	
				LAN Ctrl vs LAN HAPE	LAN Ctrl vs HAN Ctrl
C1101T					
T allele	91.7 (22)	63.6 (14)	80.0 (16)	< 0.001† (0.021)‡	< 0.018†
C allele	8.3 (2)	36.4 (8)	20.0 (4)	6.3 (2.8–14.3)§	2.8 (1.2–6.5)§
C1193G					
G allele	46.7 (14)	33.3 (8)	8.3 (2)	0.053 (0.338)‡	< 0.001†
C allele	53.3 (16)	66.7 (16)	91.7 (22)	1.8 (0.9–3.1)§	9.7 (4.3–22.0)§
C1416T					
C allele	92.9 (26)	91.7 (22)	79.2 (19)	0.750 (0.807)‡	< 0.005†
T allele	7.1 (2)	8.3 (2)	20.8 (5)	1.2 (0.4–3.4)§	3.4 (1.4–8.5)§
T3138C					
T allele	90.0 (18)	100.0 (10)	77.8 (14)	0.001 (0.322)‡	< 0.018†
C allele	10.0 (2)	0.0 (0)	22.2 (4)	0	2.6 (1.1–5.7)§
T3192C					
C allele	85.0 (17)	38.9 (7)	62.5 (10)	< 0.001† (0.003)‡	< 0.001†
T allele	15.0 (3)	61.1 (11)	37.5 (6)	8.9 (4.5–17.6)§	3.4 (1.7–6.7)§
T3234C					
C allele	85.0 (17)	38.9 (7)	62.5 (10)	< 0.001† (0.003)‡	< 0.001†
T allele	15.0 (3)	61.1 (11)	37.5 (6)	8.9 (4.5–17.6)§	3.4 (1.7–6.7)§
G1649C					
G allele	40.0 (12)	29.2 (7)	20.8 (5)	0.108 (0.486)‡	< 0.003†
C allele	60.0 (18)	70.8 (17)	79.2 (19)	1.6 (0.9–2.9)§	2.5 (1.4–4.8)§
C2474T					
C allele	93.7 (15)	100.0 (22)	83.3 (20)	0.011 (0.211)‡	< 0.02†
T allele	6.3 (1)	0.0 (0)	16.7 (4)	0	2.9 (1.1–7.8)§
A2491C					
A allele	96.7 (29)	100.0 (22)	83.3 (20)	0.067 (0.712)‡	< 0.001†
C allele	3.3 (1)	0.0 (0)	16.7 (4)	0	5.9 (1.7–19.8)§
A3265C					
A allele	37.5 (9)	21.4 (3)	36.4 (8)	< 0.012† (0.175)‡	0.872 (0.881)‡
C allele	62.5 (15)	78.6 (11)	63.6 (14)	2.2 (1.2–4.1)§	1.0 (0.6–1.9)§

*Values in parentheses are No., unless otherwise indicated. Ctrl = control; Freq = frequency.
†Value is statistically significant according to 2 × 2 table.
‡Value in parentheses is OR.
§Value in parentheses is 95% CI.

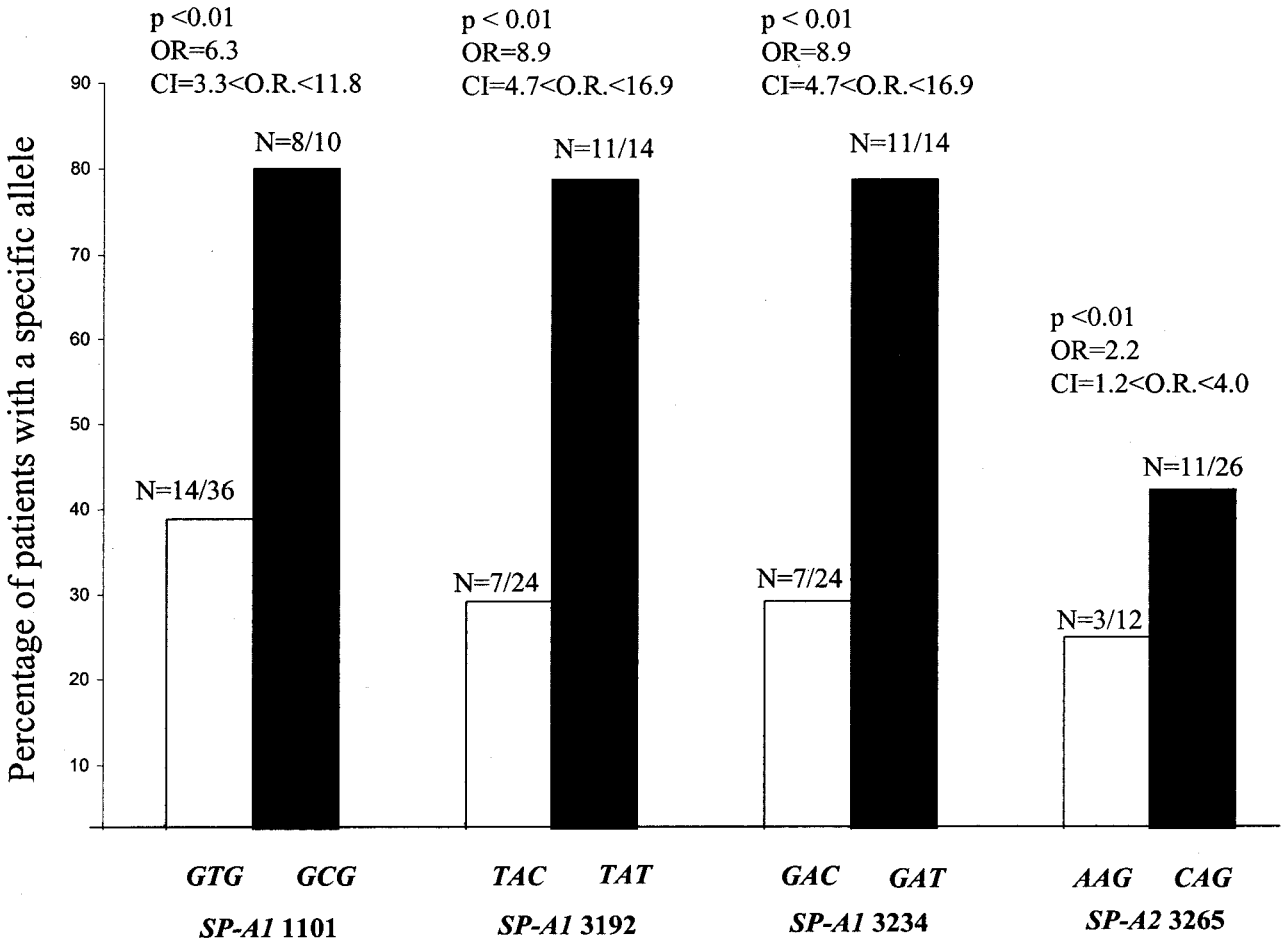

FIGURE 1. Percentage of patients from the total subjects (□, LAN controls and ■, HAPE patients) with a specific allele at SP-A1 C1101T, SP-A1 C3192T, SP-A1 C3234T, and SP-A2 A3265C. Statistically significant association of GCG, TAT, GAT, and CAG alleles was observed with patients in comparison with control subjects. OR and p values are shown above the bars. N = number of alleles.

The frequency of the GCG and GNG genotypes with respect to nonredundant SNP SP-A1 C1101T was more, whereas that of GTG was less in patients in comparison with control subjects (Fig 2, *top left*, *A*). The single HAPE patient with the GCG genotype showed increased oxidative damage in comparison with the GTG patients, exhibited by increased MDA (10.6%) and decreased GSH (15.3%) [Fig 3]. However, the LDH value showed a decrease of 87.7%, indicating less tissue injury in comparison with GTG patients. The GNG patients showed decreased oxidative damage, suggesting heterozygote advantage (MDA value was lower by 10.1%, GSH value was higher by 29%, and LDH value was lower by 44% in comparison with GTG patients) [Fig 3]. A correlation of biochemical parameters with the redundant SNPs SP-A1 T3192C and SP-A1 T3234C could not be established, although these two SNPs appear to be always coexisting.

As observed with nonredundant SNP SP-A1 C1101T, the NAG patients, with respect to nonredundant SNP SP-A2 A3265C, showed decreased oxidative damage as indicated by a 15.9% decrease in the MDA level, a 15.6% increase in the GSH level, and a 24.0% decrease in the LDH level in comparison with the CAG patients (Fig 3). Interestingly, the patients heterozygous at SP-A1 C1101T were also heterozygous at SP-A2 A3265C. Also, the mean value of MDA was 17.8% lower, the mean GSH level was 24% higher, and the mean LDH level was 11.6% lower in these double heterozygous patients (at both SP-A1 C1101T and SP-A2 A3265C loci) in comparison with patients who were homozygous for either allele at both these loci.

In the HAN control subjects, the mean values of MDA were lower and the mean values of GSH were higher in comparison with the LAN control subjects. Significant genetic differences were observed at six of eight variable loci in *SP-A1* (C1101T, C1193G, C1416T, T3138C, T3192C, and T3234C) and three

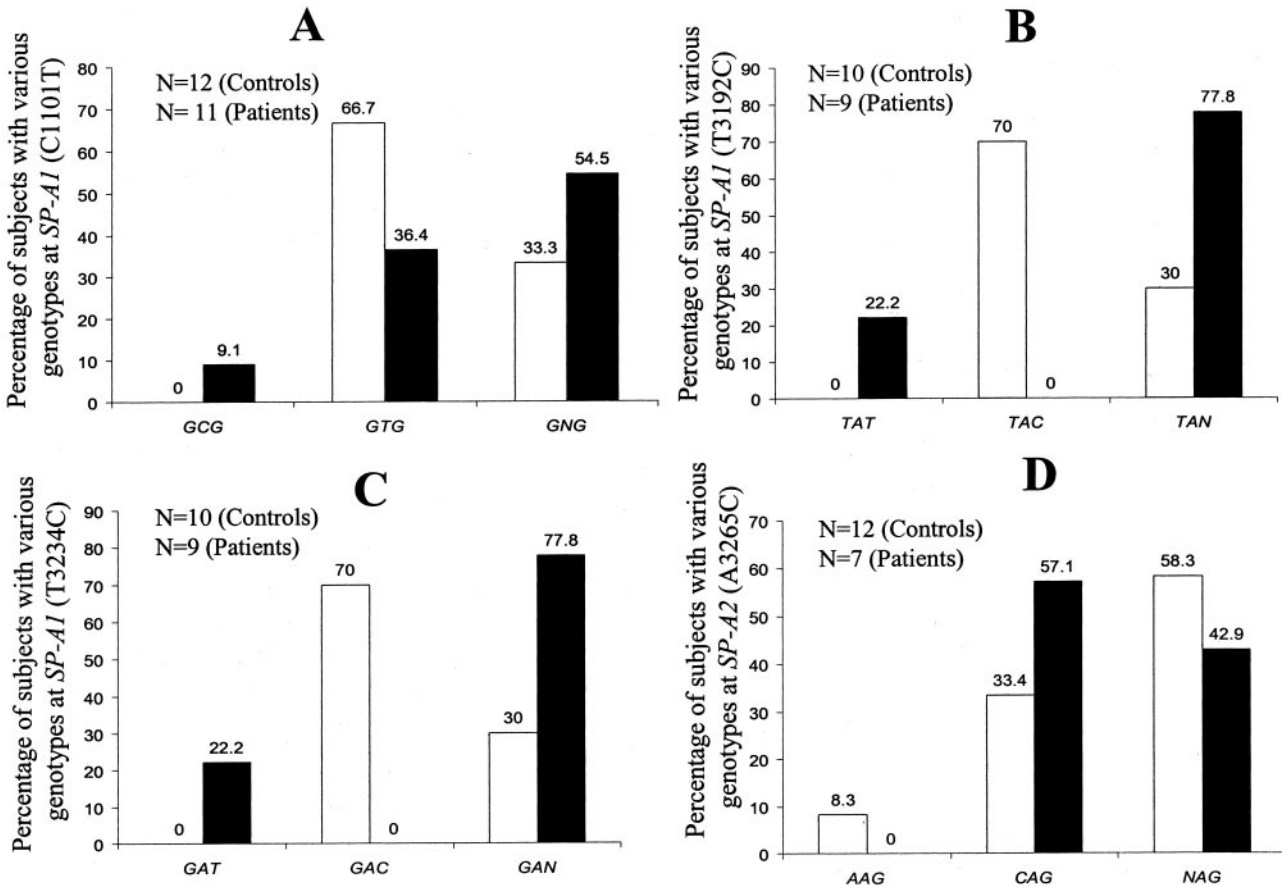

FIGURE 2. Percentage of subjects (□, LAN control subjects and ■, HAPE patients) with various genotypes at polymorphisms observed in *SP-A1* and *SP-A2*. Distribution of genotypes in LAN control subjects and HAPE patients with respect to SP-A1 C1101T (*top left*, *A*), SP-A1 T3192C (*top right*, *B*), SP-A1 T3234C (*bottom left*, *C*), and SP-A2 A3265C (*bottom right*, *D*).

of eight variable loci in *SP-A2* (G1649C, C2474T, and A2491C) [Tables 3, 4].

DISCUSSION

The present study investigated the association of SNPs in *SP-A1* and *SP-A2* with HAPE in Indian LAN subjects on sojourn to high altitude. A total of 16 variable loci (eight each in *SP-A1* and *SP-A2*) were observed in subjects. Of these 16 SNPs, 5 SNPs in *SP-A1* (C1101T, G1544A, T3138C, T3192C, and T3234C) and 3 SNPs in *SP-A2* (C2491A, T3018C, and A3265C) are being reported for the first time.

Three exonic SNPs in *SP-A1* (C1101T, T3192C, and T3234C) and one in *SP-A2* (A3265C) showed statistically significant association with HAPE in LAN sojourners. The SNP SP-A1 C1101T is a nonredundant SNP present in the signal peptide region causing a change in amino acid from valine (GTG) to alanine (GCG). Because the signal peptide

Table 4—*LDH, MDA, and GSH Levels in HAN Control Subjects, LAN Control Subjects, and LAN HAPE Groups**

Parameters	HAN (Control Subjects) (n = 19)	LAN (Control Subjects) (n = 15)	LAN (HAPE) (n = 12)
LDH, U/L	169.0 ± 7.7	160.1 ± 6.0	225.1 ± 18.2†
MDA, μmol/L	4.5 ± 0.2	5.6 ± 0.2‡	7.6 ± 0.3†
GSH, mg %	37.2 ± 1.5	32.5 ± 1.1‡	26.7 ± 1.4†

*Values given as the mean ± SE.
†Values are significantly different between LAN HAPE and LAN controls at p values < 0.05.
‡Values are significantly different between HAN controls and LAN controls at p values < 0.05.

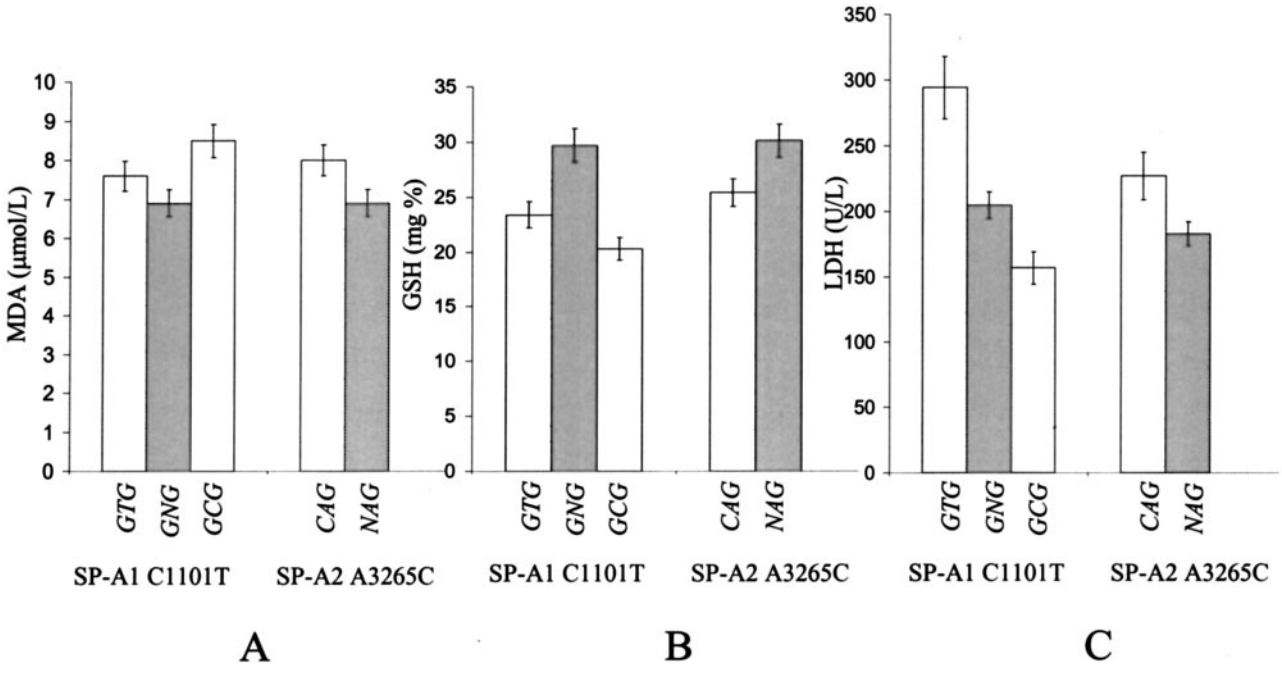

FIGURE 3. Levels of biochemical parameters in patients (□, LAN control subjects and ■, HAPE patients) with various genotypes at SP-A1 C1101T (*GTG*, homozygous for T allele; GNG, heterozygous for C/T alleles; and GCG, homozygous for C allele) and SP-A2 A3265C (CAG, homozygous for C allele; and NAG, heterozygous for A/C alleles). *Left*, *A*: levels of MDA. *Middle*, *B*: levels of GSH. *Right*, *C*: levels of LDH.

is important for correct protein targeting, C1101T may adversely affect the normal protein targeting.

The only patient who was found homozygous for the GCG allele showed increased oxidative damage as compared with GTG patients. The clinical history of this patient revealed that he had suffered five episodes of HAPE on reinduction to a high altitude during the last 10 years. Although no conclusive statement can be drawn with a single patient, the recurrence of the HAPE and the severity of biochemical parameters suggest a possible role for the GCG allele in predisposing subjects to HAPE. The absence of GCG homozygotes in the control subjects and the small number of GCG homozygotes observed in patients indicates that the GCG allele may lead to a probable alteration in a vital function.

The other two redundant SNPs of *SP-A1* showing significant association with HAPE (SP-A1 T3192C and SP-A1 T3234C) are present in the carbohydrate recognition domain of SP-A, which participates in lipid binding and pH-dependent lipid aggregation needed for a homogeneous surfactant layering at the air-liquid interface.[20,21] However, the levels of MDA, GSH, and LDH were not significantly different in patients carrying different alleles, suggesting that SP-A1 T3192C and SP-A1 T3234C could be simply surrogate markers. The coexistence of TAC (SP-A1 T3192C) with GAC (SP-A1 T3234C) genotypes and, also, their heterozygotes in all of the subjects indicate that they may be in linkage disequilibrium with each other.

SP-A2 A3265C, located in exon 6 of *SP-A2* coding for the carbohydrate recognition domain, leads to a change in amino acid from glutamine to lysine. Both the CAG allele (coding for glutamine) and the CAG genotype were associated with patients, and the CAG patients showed increased oxidative damage in comparison with the NAG patients suggesting that the CAG allele may be genetically predisposing the LAN subjects to HAPE.

The heterozygotes at SP-A1 C1101T and at SP-A2 A3265C showed reduced oxidative damage, which suggests a heterozygote advantage. All of the patients who were heterozygous at locus SP-A1 C1101T were also heterozygous at locus SP-A2 A3265C, and these double-heterozygous subjects showed additionally decreased oxidative damage, which indicates that heterozygosity at both of these loci may have a synergistic effect. The observation is supported by the fact that SP-A is composed of gene products of both *SP-A1* and *SP-A2*.

The SNPs SP-A2 G1649C and SP-A2 A1660G, associated with patients of allergic bronchopulmonary aspergillosis and pulmonary tuberculosis in our earlier studies, did not show association with

HAPE patients.[10,11] SP-A1 C1193G (Leu to Val) was identified as one of the polymorphisms associated with tuberculosis in the Mexican population;[22] however, in the present study we did not find any significant association of this SNP with HAPE.

The lung functions of highlanders have been observed to be superior to the lowlanders, and genetic differences among the HAN and LAN populations might have a role in the distinct mechanisms of high-altitude acclimatization of these ethnically segregated populations.[23] The SNP profile of *SP-A1* and *SP-A2* of the LAN and HAN populations varied significantly, which suggests that two populations are genetically very different from each other with respect to these two genes. However, the frequency of susceptibility alleles of *SP-A1* and *SP-A2* in HAN control subjects was lower than in the LAN HAPE subjects, as expected (allele frequency of C allele of SP-A1 C1101T in LAN HAPE subjects, 36.3% and in HAN control subjects, 20%; allele frequency of C allele of SP-A2 C2474T in LAN HAPE subjects, 100% and in HAN control subjects, 83.3%; allele frequency of A allele of SP-A2 A2491C in LAN HAPE subjects, 100% and in HAN control subjects, 83.3%; allele frequency of C allele of SP-A2 A3265C in LAN HAPE subjects, 78.6% and in HAN control subjects, 63.6%). Such genetic differences in the HAN and LAN populations with respect to other genetic loci, such as the renin-angiotensin system and the β-2-andrenergic receptor alleles, have been reported earlier.[24,25] The mean values of two biochemical parameters for oxidative damage, namely, MDA and GSH, showed that in the HAN control subjects there was lower oxidative damage in comparison with the LAN control subjects as observed by the decreased MDA levels, and perhaps it was on account of the increased GSH levels. To date, there are no studies comparing the LAN and HAN populations with respect to their response to high-altitude-induced oxidative stress.

The exposure of the lung surfactant to high-altitude-induced oxidative stress may result in the peroxidation of unsaturated phospholipids, surfactant inactivation, airspace collapse, and impaired gas exchange. SP-A protects surfactant phospholipids from oxidative damage.[6] Oxidative stress, such as ozone exposure of SP-A gene variants, resulted in the production of significantly different amounts of proinflammatory cytokines interleukin-8 and tumor necrosis factor-α.[12] The results of the present study propose that genetic variants of *SP-A1* (C1101T, T3192C, and T3234C) and *SP-A2* (A3265C) are associated with HAPE in LAN sojourners in the Indian population. Furthermore, two of the alleles GCG (SP-A1 C1101T) and CAG (SP-A2 A3265C) may have adverse effects on the protective role of SP-A against oxidative damage, because higher levels of MDA and LDH and lower levels of GSH were observed in HAPE patients carrying these polymorphisms.

References

1 Askew EW. Work at high altitude and oxidative stress: antioxidant nutrients. Toxicology 2002; 180:107–119
2 Menon ND. High altitude pulmonary edema. N Engl J Med 1965; 273:66–73
3 Singh I, Kapila CC, Khanna PK, et al. High altitude pulmonary edema. Lancet 1965; 1:229–234
4 Sinha SB, Viswanathan R. The role of alveolar surfactant in the development of high altitude pulmonary oedema. Indian J Chest Dis Allied Sci 1985; 27:4–10
5 Droma Y, Hanaoka M, Hotta J, et al. Pathological features of the lung in fatal high altitude pulmonary edema occurring at moderate altitude in Japan. High Alt Med Biol 2001; 2:515–523
6 McCormack FX. Structure, processing and properties of surfactant protein A. Biochim Biophys Acta 1998; 1408:109–131
7 Bridges JP, Davis HW, Daodarasamy M, et al. Pulmonary surfactant proteins A and D are potent endogenous inhibitors of lipid peroxidation and oxidative cellular injury. J Biol Chem 2000; 275:38848–38855
8 Hallman M, Haataja R, Marttila R. Surfactant proteins and genetic predisposition to respiratory distress syndrome. Semin Perinatol 2002; 26:450–460
9 Guo X, Lin HM, Lin Z, et al. Surfactant protein gene A, B, and D marker alleles in chronic obstructive pulmonary disease of a Mexican population. Eur Respir J 2001; 18:482–490
10 Saxena S, Madan T, Muralidhar K, et al. Association of polymorphisms in the collagen region of *SP-A2* with elevated levels of total IgE antibodies and eosinophilia in allergic bronchopulmonary aspergillosis patients. J Allergy Clin Immunol 2003; 111:1001–1007
11 Madan T, Saxena S, Murthy KJ, et al. Association of polymorphisms in the collagen region of human *SP-A1 and SP-A2* genes with pulmonary tuberculosis in Indian population. Clin Chem Lab Med 2002; 40:1002–1008
12 Wang G, Umstead TM, Phelps DS, et al. The effect of ozone exposure on the ability of human surfactant protein a variants to stimulate cytokine production. Environ Health Perspect 2002; 110:79–84
13 Hanaoka M, Kubo K, Yamazaki Y, et al. Association of high-altitude pulmonary edema with the major histocompatibility complex. Circulation 1998; 97:1124–1128
14 Droma Y, Hanaoka M, Ota M, et al. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. Circulation 2002; 106:826–830
15 Fu Z, Jiang P, Ren Y, et al. Changes of antioxidative capacity and endothelial function before and after treatment among patients with high altitude pulmonary edema. Zhonghua Jie He He Hu Xi Za Zhi 2002; 25:33–35
16 Dobbeling U, Boni R, Haffner A, et al. Method for simultaneous RNA and DNA isolation from biopsy material, culture cells, plants and bacteria. Biotechniques 1997; 22:88–90
17 Lin Z, Pearson C, Chinchilli V, et al. Polymorphisms of

human *SP-A*, *SP-B*, and *SP-D* genes: association of SP-B Thr131Ile with ARDS. Clin Genet 2000; 58:181–191
18 Dousset JC, Trouilh M, Foglietti MJ. Plasma malonaldehyde levels during myocardial infarction. Clin Chem Acta 1983; 129:319–322
19 Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882–888
20 McCormack FX, Festa AL, Andrews RP, et al. The carbohydrate recognition domain of surfactant protein A mediates binding to the major surface glycoprotein of *Pneumocystis carinii*. Biochemistry 1997; 36:8092–8099
21 McCormack FX, Stewart J, Voelker DR, et al. Alanine mutagenesis of surfactant protein A reveals that lipid binding and pH-dependent liposome aggregation are mediated by the carbohydrate recognition domain. Biochemistry 1997; 36: 13963–13971
22 Floros J, Lin HM, Garcia A, et al. Surfactant protein genetic marker alleles identify a subgroup of tuberculosis in a Mexican population. J Infect Dis 2000; 182:1473–1478
23 Malik SL, Pandey AK. Respiratory adaptation to high altitude in adolescent Bod girls of the Western Himalayas. Ann Hum Biol 1993; 20:575–581
24 Pasha MA, Khan AP, Kumar R, et al. Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in Indian populations of different ethnic origins. J Biosci 2002; 27:67–70
25 Rupert JL, Monsalve MV, Devine DV, et al. Beta2-adrenergic receptor allele frequencies in the Quechua, a high altitude native population. Ann Hum Genet 2000; 64:135–143

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

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