Polymorphisms of Renin-Angiotensin System Genes With High-Altitude Pulmonary Edema in Japanese Subjects*

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**Study objectives:** The renin-angiotensin system (RAS), including angiotensin-converting enzyme (ACE) and angiotensin II type I receptor (AT1R), plays an important role in the pathogenesis of pulmonary hypertension, which is suggested to be critical in the development of high-altitude pulmonary edema (HAPE). Investigating the associations of the polymorphisms in the genes of RAS with HAPE is to elucidate the genetic background underlying this disease.

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

**Setting:** Shinshu University Hospital, Matsumoto, Japan.

**Participants:** Forty-nine HAPE-susceptible (HAPE-s) subjects with a history of HAPE, and 55 healthy climbers with HAPE resistance (HAPE-r).

**Interventions:** Twenty-one of 49 HAPE-s subjects underwent right cardiac catheterization.

**Measurements and results:** The insertion/deletion polymorphism in the ACE gene (ACE-I/D) was investigated by polymerase chain reaction (PCR). There was no significant difference of the distribution of the ACE-I/D polymorphism between the HAPE-s and HAPE-r groups. The A1166C and G1517T single-nucleotide polymorphisms (SNPs) in AT1R gene were investigated by the PCR following digested by corresponding restricted endonuclease enzymes. The distribution of the G1517T SNP was significantly different between the two groups ($p = 0.012$). The pulmonary hemodynamics of the 21 HAPE-s subjects were retrospectively examined. The pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR), and PVR index (PVRI) were all significantly increased on hospital admission. Moreover, the PVR and PVRI were significantly higher in the HAPE-s subjects with D positivity than in the HAPE-s subjects with I positivity (PVR, $p = 0.015$; PVRI, $p = 0.028$), while the PAP did not show any significant difference between the two subgroups.

**Conclusions:** The ACE-I/D polymorphism is not associated with HAPE susceptibility in Japanese subjects. The AT1R gene polymorphisms may likely associate with HAPE susceptibility. The D allele of the ACE-I/D polymorphism probably contributes to the hyperresponsive PVR and PVRI to acute hypoxia.

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**Key words:** high-altitude; Japanese; polymorphisms; pulmonary hemodynamics; renin-angiotensin system

**Abbreviations:** ACE = angiotensin-converting enzyme; ACE-I/D = insertion/deletion polymorphism in the ACE gene; Ang II = angiotensin II; AT1R = angiotensin II type 1 receptor; CI = confidence interval; CO = cardiac output; HAPE = high-altitude pulmonary edema; HAPE-r = HAPE resistant; HAPE-s = HAPE susceptible; HWE = Hardy-Weinberg equilibrium; I/D = insertion/deletion; PAP = pulmonary artery pressure; PAWP = pulmonary artery wedge pressure; PCR = polymerase chain reaction; PVR = pulmonary vascular resistance; PVRI = pulmonary vascular resistance index; RAS = renin-angiotensin system; SNP = single-nucleotide polymorphism

High-altitude pulmonary edema (HAPE) is an accelerated, noncardiogenic, permeability pulmonary edema developing in unacclimatized individuals rapidly exposed to altitudes > 2,500 m. It has been regarded as a multifactorial condition whose onset and progress are influenced by both genetic and environmental factors. The pulmonary vascular resistance (PVR) in HAPE-susceptible (HAPE-s) subjects showed a significant hypersensitive response

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to hypoxia than that in HAPE-resistant (HAPE-r) subjects. It is suggested that the constitutional susceptibility to HAPE may be based on some unidentified insufficient genetic background related to the exaggerated hypoxic pulmonary hypertension. The renin-angiotensin system (RAS), including angiotensin-converting enzyme (ACE) and angiotensin II (Ang II) type 1 receptor (AT1R), plays important roles in the pathogenesis of pulmonary hypertension through the mechanism involving with the regulation of pulmonary vascular tone. ACE generates the vasoconstrictor Ang II that modulates the pulmonary vasoconstrictive response to hypoxia via the interaction with AT1R. The concentration of Ang II was significantly elevated in patients with HAPE, and in individuals during prolonged altitude exposure. In addition, Ang II induces pulmonary edema in a rabbit model. It is suggested that the RAS plays an important part in the development of the hypoxic pulmonary hypertension in HAPE. Therefore, the genes for encoding the ACE and AT1R molecules are reasonably suspected to be the potential unidentified insufficient ones associated with the constitutional susceptibility to HAPE.

The ACE protein is encoded by a 21-kilobase, 26-exon gene located on chromosome 17 at q23. A polymorphism in which the deletion rather than the insertion of a 287–base-pair sequence in intron 16 of the human ACE gene (insertion/deletion polymorphism in the ACE gene [ACE-I/D]) was confirmed to be associated with the increased serum ACE activity. The AT1R gene maps to the long arm of chromosome 3 containing five exons. A number of polymorphisms of the AT1R gene have been identified. To elucidate the association regarding the polymorphisms in RAS genes with HAPE, we examined the insertion/deletion (I/D) polymorphism in the ACE gene and the A1166C and G1517T (the upper number indicates the position of the substituted nucleotide that was given from the start of coding exon; A = adenine; T = thymine; C = cytosine; G = guanine) single-nucleotide polymorphisms (SNPs) in the AT1R gene in a selected Japanese HAPE-s and HAPE-r cohorts.

Materials and Methods

Study Populations

Two comparable groups in terms of age, gender, ethnicity, and environmental hypoxic exposure were selected in this cross-sectional, case-control, genetic association study. The case group consisted of 49 HAPE-s subjects (42 male and 7 female subjects; average age, 33.4 years), while the control group was composed of 55 HAPE-r mountaineers (46 male and 9 female subjects; average age, 38.6 years). This study and its investigational protocol were approved by the institutional ethics review board of Shinshu University for human study, and written informed consent was obtained from each study patient and control subject after a full explanation of the study. The procedures used in this human study were in accordance with the recommendations found in the Helsinki Declaration of 1975. All subjects were unrelated natives of Japan, born and residing at low altitudes less than our institute (610 m above sea level) where the blood samples were obtained.

The HAPE-s subjects in the present study were defined as the individuals who experienced HAPE while climbing the Japan Alps, ranging from 2,758 to 3,190 m, and were admitted to our hospital between July 1979 and September 2002. Almost all of them were rescued by helicopter because they could not walk down by themselves. Due to the efficient rescue system and the geographic advantage around the Japan Alps region, it took approximately 1 h to transport the patients from the mountains to the Shinshu University Hospital by helicopter. On hospital admission, the average P_O2 in arterial blood was 46.15 ± 14.55 torr (± SD), and average oxygen saturation in peripheral blood was 79.97 ± 9.94% breathing room air, indicating a severe hypoxemia in the early stage of HAPE. The diagnosis of HAPE was based on the following criteria: onset at high altitude of the typical symptoms, including cough and dyspnea at rest; absence of signs of infection; presence of pulmonary rales and cyanosis; disappearance of symptoms and signs within 3 days of the start of treatment with bed rest and supplemental oxygen; and chest radiographic infiltrates consistent with pulmonary edema. All subjects with HAPE met all criteria at the onset of the disorder and recovered promptly and well with hospitalization. Examinations and cardiovascular tests were conducted in the hospital after recovery for excluding any preexisting cardiopulmonary diseases. All subjects were in healthy condition at the time of study.

The control group was critical and required strict criteria to be selected, so we recruited elite mountaineers from the Mountain-eering Association of Nagano Prefecture and the Alpine Club of Shinshu University, Japan. The members of the “Association” and “Club” are all elite mountaineers and climb mountains >3,000 m in Japan and overseas year round (maximum, 200 climbs per year). Some of them also successfully challenged mountains >5,000 m several times. No subjects reported history of HAPE or other cardiopulmonary disorders in a questionnaire sheet that contained the components of Lake Louise Score during the recruitment. We defined them as the HAPE-r group due to their resistance to HAPE while exposed to high-altitude environments.

Identification of the I/D Polymorphism in the ACE Gene

The venous blood samples were obtained from all study patients and control subjects and stored below −80°C in our institute until the sample sizes reached relative sufficiency that could be estimated statistically to detect an association if the relative risk factor is ≥5 with 80% power at the 5% significance level. Genomic DNA was extracted from venous blood by phenol extraction sodium dodecyl sulfate-lysed and protease K-treated cells as the standard procedure. The I/D polymorphism was examined by the polymerase chain reaction (PCR) as the method described by Rigat et al. Since the D allele in heterozygous samples is preferentially amplified, each sample found to have the DD genotype was subjected to a second independent PCR amplification using the insertion-specific primers as described by Lindpaintner et al. This procedure correctly identified the 4 to 5% of samples with ID genotype that are misclassified as DD in the first PCR amplification. In total, 12 uncertain samples were analyzed by the second PCR reaction in the present examination.
Identification of the A1166C and G1517T SNPs in the AT1R Gene

The A1166C and G1517T SNPs of the AT1R gene were identified by PCR amplification following subsequent restriction digestion using NlaIII and Hinf I endonuclease enzymes correspondingly according to the method described by Bonnardeaux et al. 11

Pulmonary Hemodynamics

Right cardiac catheterization during room air breathing was undergone within 6 h after admission to Shinshu University Hospital, and repeated on the fourth to seventh day of recovery as well. A thermodilution Swan-Ganz catheter (Becton, Dickinson and Company; Franklin Lakes, NJ) was introduced percutaneously into the pulmonary artery via the right internal jugular vein. The pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PAWP), and cardiac output (CO) were measured.10 PVR was calculated by subtracting PAWP from PAP and dividing by the CO. The PVR and CO were further corrected per square meter of body surface area and expressed as PVR index (PVRI) and CO index, respectively.

Statistical Analysis

Regarding the phenotype of HAPE-s, the frequencies of the alleles, genotypes, and the allelic positivities were counted and compared by the χ² test between the HAPE-s and HAPE-r groups. The positivity was defined as the frequency of individuals having one or two of the particular alleles. The exact test of Hardy-Weinberg equilibrium (HWE) was performed by the Markov chain method, which was reported to have an advantage of obtaining a complete enumeration for testing HWE in cases where the number of alleles and the sample size are small.20 Therefore, the possibility of type I error due to our relative small sample sizes was limited to a minimum. The odds ratio was calculated as cross-product ratio of a particular allele in the sample sizes was limited to a minimum. The odds ratio was calculated by expected frequency (0.5) and sample size (20) where the number of alleles and the sample size are small.20 The p value was calculated by Markov chain method, which was reported to have an advantage of obtaining a complete enumeration for testing HWE in cases where the number of alleles and the sample size are small.20 Therefore, the possibility of type I error due to our relative small sample sizes was limited to a minimum. The odds ratio was calculated as cross-product ratio of a particular allele in the sample.

Results

Identification of the I/D Polymorphism in the ACE Gene

The frequencies of genotype, allele and the allelic positivity of the ACE-I/D polymorphism in each group are summarized in Table 1. The observed genotypic frequencies were in agreement with the frequencies predicted by HWE in each group. The prevalence of the D allele was 35.7% in HAPE-s subjects and 30.0% in HAPE-r subjects. There were no significant differences of the genotypic and the allelic frequencies in the ACE-I/D polymorphism between the HAPE-s and HAPE-r groups. Furthermore, the effects assuming the D allele of the ACE-I/D polymorphism in the additive, recessive, and dominant modes on the phenotype of HAPE-s showed no significant association of the ACE-I/D polymorphism with the HAPE-s phenotype (Table 2; all p values > 0.05).

Identification of the A1166C and G1517T SNPs in the AT1R Gene

The identification of the A1166C and G1517T polymorphisms were obtained only in 43 HAPE-s and 49 HAPE-r samples. The frequency of the C allele in subjects and 30.0% in HAPE-r subjects. There were no significant differences of the genotypic and the allelic frequencies in the ACE-I/D polymorphism between the HAPE-s and HAPE-r groups. Furthermore, the effects assuming the D allele of the ACE-I/D polymorphism in the additive, recessive, and dominant modes on the phenotype of HAPE-s showed no significant association of the ACE-I/D polymorphism with the HAPE-s phenotype (Table 2; all p values > 0.05).
A\textsuperscript{1166}C polymorphism in HAPE-s (22.6\%) was statistically similar to that in HAPE-r group (24.0\%) [Table 3; \( p = 0.832 \)]. However, the frequency of the T allele in G\textsuperscript{1517}T polymorphism in the HAPE-s group (40.7\%) was significantly higher than that in the HAPE-r group (23.5\%) [Table 3; \( p = 0.012 \)], with an odds ratio of 2.24 (95% CI, 1.19 to 4.21).

**Pulmonary Hemodynamics**

Pulmonary hemodynamics were examined in 21 of the 49 HAPE patients on hospital admission and at recovery, respectively. The pulmonary hemodynamic data showed that the mean PAP, PVR, and PVRI of HAPE-s subjects on admission (such as mean PAP, PAWP, and CO index) did not show any significant differences between the two subgroups. The PVR and PVRI in the early stage of HAPE were significantly increased in the D-positivity subgroup than in the I-positivity subgroup, while the mean PAP did not show any significant difference between the two subgroups, suggesting a possible association of the D allele with the intermediate phenotype of the enhanced pulmonary vasoreactivity to hypoxia.

Regarding the association of the ACE-I/D polymorphism with high altitudes, Montgomery et al\textsuperscript{21} first reported that the I allele was associated with elite British male mountaineers who could reach beyond 7,000 m without the use of supplemental oxygen. Subsequently, Woods et al\textsuperscript{22} showed that the I allele was associated with the maintenance of the oxygen saturation at high altitudes. Such associations were interpreted by the mechanism through the low ACE activity that was genetically determined by the I allele of the ACE-I/D polymorphism. The low Ang II cascaded by the low ACE activity in the I-positivity elite mountaineers may improve the local muscle efficiency and the maximal oxygen uptake rather than affect the central cardiopulmonary system,\textsuperscript{23} because the maximum altitudes reached by elite Himalayan mountaineers are correlated with the aerobic fitness assessed by maximal oxygen up-
ACE-I/D polymorphism was 16.3% in Kyrgyz high-
in serum ACE activity that could be attributed to the
serum ACE activity partly. The percent variance
the ACE-I/D polymorphism was not associated with
cause the PAP in the study by Dehnert et al.24 was
individuals vs those nonsusceptible at 4,559 m inde-
a marked (fivefold) increase in the serum ACE
protein led the membrane-bound ACE to be
efficiently clipped from the cell surface, resulting in
a marked (fivefold) increase in the serum ACE
activity in eight families by an autosomal dominant
inheritance. Zhu et al.28 reported an A/G mutation at
base 2350 in intron 17 of the ACE gene was strongly
correlated with circulating ACE concentration.
Indeed, the I/D polymorphism is located at the intron
of the ACE gene and does not appear to be func-
tional, so the ACE-I/D polymorphism may have no
association with the phenotype of HAPE-s.

The pulmonary hemodynamic data confirm the
fact that HAPE is a noncardiogenic form of pulmo-
nary edema with an increased PAP, PVR, and a
normal PAWP. The individual susceptibility may be
associated with the enhanced pulmonary vascular
reactivity to hypoxia and exercise.3 Notably, further
analysis of the pulmonary hemodynamic data
showed that the PVR and PVRI of the HAPE-s
subjects on hospital admission were significantly
higher in D-positivity individuals than in I-positivity
individuals, while the mean PAP was not significant
different between the D-positivity and I-positivity
individuals, implying that the D allele was associated
with increased PVR and PVRI in a dominant mode
but not PAP. Consistently, the study of Dehnert et
al.24 also found a significant higher PAP in HAPE-s
individuals vs those nonsusceptible at 4,559 m inde-
pendent of the ACE-I/D polymorphism. But be-
cause the PAP in the study by Dehnert et al.24 was
mainly obtained retrospectively from their previous
noninvasive Doppler echocardiographic examina-
tions, they could not provide other pulmonary he-
modynamic data including the PVR and PVRI.
Although increased PAP plays an important role in
the development of HAPE, it is a pathophysiologic
consequence resulting from the interactions among
vasoconstrictors, blood redistribution, and micro-
thrombin, etc., within the pulmonary circulation
system rather than the initial reason of HAPE. PVR
and PVRI are intermediate phenotypes correlated to
pulmonary vascular tone, and are highly controlled
by pulmonary vasoconstrictors including the Ang II.
The increased endogenous Ang II vasoconstrictor
in HAPE-s with D positivity may potentially up-
regulate the basic pulmonary vascular tone, resulting
in hyperresponsive PAP evoked by hypoxic chal-
To date, there is no report about the G1517T variant
in Japanese. The primary data presented here suggest
that the A1166C variant does not significantly contrib-
ute the development of HAPE. Nevertheless, the
G1517T variant is in the 3′-untranslated region of
the gene, and therefore the amino-acid sequence of
the receptor is not altered. It is possible that the
variant is in linkage disequilibrium with some un-
identified functional variants of the AT1R gene, or
earlier genes those are responsible for the associa-
tion with HAPE.

In conclusion, our study does not suggest any
associations of the ACE-I/D polymorphism with the
phenotype of HAPE in Japanese. The polymorphism
in the AT1R gene may associate with the HAPE-s via
linkage disequilibrium with some unidentified func-

tional variants in the same gene or nearby genes those are associated with HAPE. Of note, the D positivity is associated with the intermediate phenotype (PVR and PVRI) of the hyperresponsive PAP to acute hypoxia in HAPE-s, possibly as a potential genetic marker to predict the exaggerated hypoxic pulmonary hypertension in HAPE.

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