A Genome Wide DNA Microsatellite Association Study and Association of TIMP3 gene Polymorphism in Japanese Patients with High Altitude Pulmonary Edema

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Conflict of interest disclosure: I have no real or perceived conflicts of interest that relate to the presentation.

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**Background**

- High altitude pulmonary edema (HAPE) is a non-cardiogenic pulmonary edema that develops in susceptible people who ascend to high altitude.
- The pathogenesis remains to be conclusively elucidated and genetic polymorphisms were highly proposed to be associated with HAPE. There have been many reports about gene polymorphisms in association with pathogenesis of HAPE, but few of them suggested clear-cut HAPE genes.
- The aim of this study is to attempt to identify one or more candidate genes that might be associated with susceptibility to resistance to HAPE by genomewide study and single nucleotide polymorphisms (SNPs) genotyping within candidate genes.

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**Methods - Microsatellite study**

- **HAPEx-susceptible subjects (HAPEx-s):** 53 Japanese
  - All of them had previously developed HAPE in their mountaineering histories of climbing Japan North Alps higher than 2500m.
  - All of them usually climb mountains vigorously over 3,000m who never developed acute mountain sickness including HAPE during their mountaineering histories.
  
  **Preparation of genomic DNA**
  - Genomic DNA from all subjects were extracted from venous blood by phenol extraction of sodium dodecyl sulfate (SDS) - lysed and protein K-treated cells

| Chromosome / locus | allele | HAPEx-s (%) | HAPE-r (%) | OR [95% CI] | p [Pc]
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>D1S2697</td>
<td>284</td>
<td>11 (14.7)</td>
<td>2.17</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>D1S2570</td>
<td>156</td>
<td>18 (17.5)</td>
<td>0.98</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td>D5S424</td>
<td>212</td>
<td>5 (4.8)</td>
<td>0.15</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>D5S425</td>
<td>179</td>
<td>26 (24.5)</td>
<td>0.73</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>D1S2697</td>
<td>202</td>
<td>10 (9.8)</td>
<td>0.11</td>
<td>0.962</td>
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<tr>
<td></td>
<td>D16S3103</td>
<td>136</td>
<td>24 (22.6)</td>
<td>0.22</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>D1S2697</td>
<td>322</td>
<td>12 (12.0)</td>
<td>0.11</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td>D14S283</td>
<td>216</td>
<td>6 (6.0)</td>
<td>0.83</td>
<td>0.627</td>
</tr>
</tbody>
</table>

**Candidate Genes**

- The National Center for Biotechnology Information (NCBI) Map Viewer, National Library of Medicine, National Institute of Health were managed to select candidate genes. The PCR-amplified products were sequenced automatically by ABI 3130 DNA Analyzer of Sequencing Core Facility.

**SNP database of Applied Biosystems**

- The markers were amplified by polymerase chain reaction (PCR) according to the manufacturer’s protocols.
- The PCR-amplified products were sequenced automatically by ABI 3130 DNA Analyzer.
- The significance of the difference in the distributions of alleles between HAPE-s and HAPE-r was determined by a chi square test.
- p-values were corrected by multiplying the number of alleles observed at each locus (Pc).

**Microsatellite typing**

- A case-control association genome study was performed using 400 polymorphic microsatellite markers (ABI PRISM® Linkage Mapping Set v2.5-MD10 kit)
- The frequency of the allele C in rs130293 showed statistically significant association with HAPE.

**Results - Microsatellite study**

- The frequency of haplotype CAC was significantly lower in HAPE-s than the HAPE-r for the strength of association of the haplotype C with the HAPE-s group.

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**Discussion**

- This is the first case-control genome-wide association study aimed at identifying candidate genes for HAPE pathogenesis.
- TIMP-3 is one of four tissue inhibitor of matrix metalloproteinases (TIMPs). TIMP-3 was already reported to function as pro-MMP-2 and pro-MMP-9. Both are activated to MMP-2 and to MMP-9. Pirrone et al suggested that hypoxia stimulation induced significant higher activation of MMP-2, and histological analysis revealed alveolar emphysema, interstitial edema and a moderate inflammation.
- TIMP-3 has also demonstrated as an inhibitor of a disintegrin and metalloproteinase 17 (ADAM17). ADAM17 was reported to modulate the alveolar epithelial barrier through neutrophil-1 and human epidermal growth factor receptor-2 in the pathophysiology of acute lung injury.
- In this study, the rs130293 SNP is located in intron-1 of the TIMP3 gene, this SNP would have any direct influence on the conformation of the TIMP3 protein. However, it remains possible that this SNP may have an effect on mRNA stability and transcription and/or translation efficiency. This might cause some influences to the TIMP3 molecule and interfere its regulation of MMP2, MMP-9 and ADAM17, thus might induce inflammation, high permeability in pulmonary artery, modulates the alveolar epithelial barrier, consequently correspond to development of HAPE.

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**Conclusion**

- Microsatellite association study revealed 5 markers associated with susceptible to HAPE and 4 markers associated with resistant to HAPE.
- This study also demonstrated that the rs130293 (C/T) SNP in the TIMP3 gene were likely associated with developing HAPE.
Pulmonary artery pressure and serum biomarkers in high-altitude pulmonary edema susceptible subjects during acute hypoxic exposure

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Introduction
High-altitude pulmonary edema (HAPE) is a life-threatening form of noncardiogenic edema characterized by exaggerated hypoxic pulmonary hypertension. Decreased vasodilator nitric oxide (NO) and increased vasoconstrictors peptide endothelin-1 (ET-1), angiotensin-converting enzyme (ACE) and an altered vascular endothelial growth factor (VEGF) were thought to play important roles in the exaggerated pulmonary hypertension in HAPE susceptible subjects (HAPE-s) at high altitude. It is not yet clear the overall status of the vasoconstrictors and vasodilators in HAPE-s under hypoxic exposure.

Materials and Methods
HAPE-s: 17 Japanese who had previously developed at least one episode of HAPE while climbing the Japanese Alps of height from 2,758 to 3,190m above sea level.
HAPE-r: 10 Japanese who usually climb mountains vigorously over 3,000 m. None of them reported history of HAPE or acute mountain sickness and other cardiopulmonary disorders according to their answers to the questionnaire worksheet of Lake Louise Score.

The hypoxic gas (mixed by O2, CO2 and N2; PETO2 = 60 mmHg) was supplied to the subjects for 30 minutes in the oxygen saturation around 75-80% corresponding to altitude about 4,000 m.

Systolic pulmonary arterial pressure (sPAP) was measured by doppler echocardiography and venous blood were collected before (normoxia) and after hypoxic breathing (hoxia). The serum levels of vasodilator nitro oxide and vasoconstrictors were measured:

- NO was measured by detecting the colorimetric Griess color reaction.
- ET-1 was measured by radioimmunoassay (RIA).

ACE (IU/L, 37-121) and VEGF (pg/mL, 216-341) were measured by colorimetric assays.

Results

### Table 1. Background of the subjects

<table>
<thead>
<tr>
<th></th>
<th>HAPE-s</th>
<th>HEPE-r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Ratio</td>
<td>13:4</td>
<td>9:1</td>
</tr>
<tr>
<td>Mean age, yr (Range)</td>
<td>50 (27-64)</td>
<td>51 (25-67)</td>
</tr>
</tbody>
</table>

### Table 2 Systolic PAP and levels of ET-1, ACE, NOx and VEGF

<table>
<thead>
<tr>
<th></th>
<th>Normoxic condition</th>
<th>Hypoxic condition</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>sPAP (mmHg)</td>
<td>24.2±8.91</td>
<td>33.5±11.5</td>
<td>0.001</td>
</tr>
<tr>
<td>ET-1 (pg/mL)</td>
<td>1.9±0.5 (#P=0.02)</td>
<td>2.5±0.8 (#P=0.04)</td>
<td>0.03</td>
</tr>
<tr>
<td>ACE (IU/L, 3Nox)</td>
<td>12.1±4.6</td>
<td>11.3±4.2 (n.s)</td>
<td></td>
</tr>
<tr>
<td>NOx (μmol/L)</td>
<td>60.8±35.3</td>
<td>41.7±26.2 (#P=0.006)</td>
<td>0.009</td>
</tr>
<tr>
<td>VEGF (pg/mL)</td>
<td>392.5±184.3 (#P=0.02)</td>
<td>327.1±255.8 (n.s)</td>
<td></td>
</tr>
<tr>
<td>sPAP (mg/dL)</td>
<td>25.8±8.49</td>
<td>32.3±14.4 (n.s)</td>
<td>0.02</td>
</tr>
<tr>
<td>ET-1 (pg/mL)</td>
<td>1.5±0.4</td>
<td>1.8±0.8 (n.s)</td>
<td></td>
</tr>
</tbody>
</table>

*Indicates normoxia vs. hypoxia, # indicates HAPE-s vs. HEPE-r

Discussion
The sPAP was significantly increased in both the HAPE-s and HEPE-r after hypoxic exposure compared to those of normoxia. However, it seems that the sPAP in HAPE-s was more sensitive in response to hypoxia than that in HEPE-r group.

The HAPE-s showed significantly decrease of NO (p = 0.009) and increase of ET-1 (p = 0.03) after hypoxic exposure compared to those of HEPE-r. The change as well as the percent change of NO from the normoxic to hypoxic conditions were in negative correlations with the hypoxia-induced elevated sPAP in the HAPE-s (r = 0.53 and 0.57, respectively). However, the change and the percent change of ET-1 did not show any correlations with the hypoxia-induced elevated sPAP in the HAPE-s. In contrast, we did not find any correlations of sPAP with serum levels of either NO or ET-1 in the control group of HEPE-r subjects. There were no significant changes of ACE activity and VEGF. This might be due to the distinction between their situations in pulmonary circulation and those in systemic circulation.

This study demonstrated that a coexistence of an increase of ET-1 and a decrease of NO concentrations induced by hypoxia was more vital than either of the single factor in the development of HAPE. The increased ET-1 might enhance the effect of vasoconstriction caused by the deficient NO, reversely, the reduced NO might amplify the vasoconstriction due to the increased ET-1. The conjugate effect of the decreased NO and increased ET-1 intensively activates pulmonary vasoconstriction, contributing to elevation in pulmonary artery pressure, eventually, leading to pulmonary edema in HAPE-s at high altitude.

Conclusion
The simultaneously reduced NO level with an augmented ET-1 level during acute hypoxic exposure may determine the pulmonary vascular status that eventually contributes to the increased sPAP in HAPE-s at high altitude by a predominant effect of NO.