Molecular analysis of mutant high-temperature requirement serine protease A1 identified in patients with familial cerebral small vessel disease
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Background and Objectives
Loss-of-function mutations in high-temperature requirement A serine peptidase 1 (HTRA1) cause familial cerebral small vessel disease (ICSVD) in a recessive manner. However, we recently reported that dominant-negative (DN) mutations in HTRA1 also cause ICSVD in a dominant manner and these mutations impair trimer-associated HTRA1 activation. Although several HTRA1 mutations associated with CSVD have been reported, it remains unknown which mutations have DN effect.

The aim of this study was to clarify the issue and investigate whether DN mutations influence the clinical features of ICSVD.

Methods
15 HTRA1 mutations; 5 mutations were reported as homozygous or compound heterozygous state (R166C, A173T, G295R, A321T, L364P) and 10 mutations were reported as heterozygous state (S121R, A123S, R133G, R166L, A173P, S284G, S284R, P285Q, F286V, D450H). After purification of wild type HTRA1 (WT) and mutant HTRA1 proteins, the protease activity of each protein was measured by using fluorescein isothiocyanate–labeled casein as a substrate. We further evaluated the protease activity of purified protein from co-expression medium of WT and each mutant HTRA1. In addition, the oligomer structure of HTRA1 was analyzed by size-exclusion chromatography. Lastly, we retrospectively compared the clinical features of heterozygous carriers with and without DN mutation. It was defined that severe white matter lesions (WMLs) is corresponding to Fazekas grade 3 or III.

Results

Fig1. Protease activity of mutant HTRA1

Table. Comparison of previous reported heterozygous carriers of HTRA1 mutation with and without DN

<table>
<thead>
<tr>
<th></th>
<th>DN+ n=17 (%)</th>
<th>DN- n=9 (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe WMLs</td>
<td>14 (82.4)</td>
<td>4 (44.4)</td>
<td>0.078</td>
</tr>
<tr>
<td>Cognitive impairments</td>
<td>14 (82.4)</td>
<td>5 (55.6)</td>
<td>0.19</td>
</tr>
<tr>
<td>Gait disturbance</td>
<td>11 (64.7)</td>
<td>4 (44.4)</td>
<td>0.419</td>
</tr>
<tr>
<td>Symptomatic carriers</td>
<td>13 (76.5)</td>
<td>3 (33.3)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

* Symptomatic carriers were defined as the patients with severe white matter lesions (WMLs) in addition to cognitive impairments or gait disturbance

Conclusion

Five of 15 previously reported HTRA1 mutations have DN. The presence of DN positively influence the severity of CSVD.

These results suggested that it is not sufficient to decide the pathogenicity of mutated HTRA1 in a heterozygous states according to the results of protease activity or in silico analysis. Detailed molecular analysis is required to confirm the pathogenicity of mutated HTRA1 in a heterozygous states.

References
Hara K et al. NEJM. 2009.

We have no disclosures about this report.