

**Longitudinal Genome-wide Association Study of Cognitive Impairment  
After Subarachnoid Hemorrhage: A Long-term Prospective Cohort Study**

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on behalf of the First Korean Stroke Genetics Association Research (the FirstKSGAR) Study

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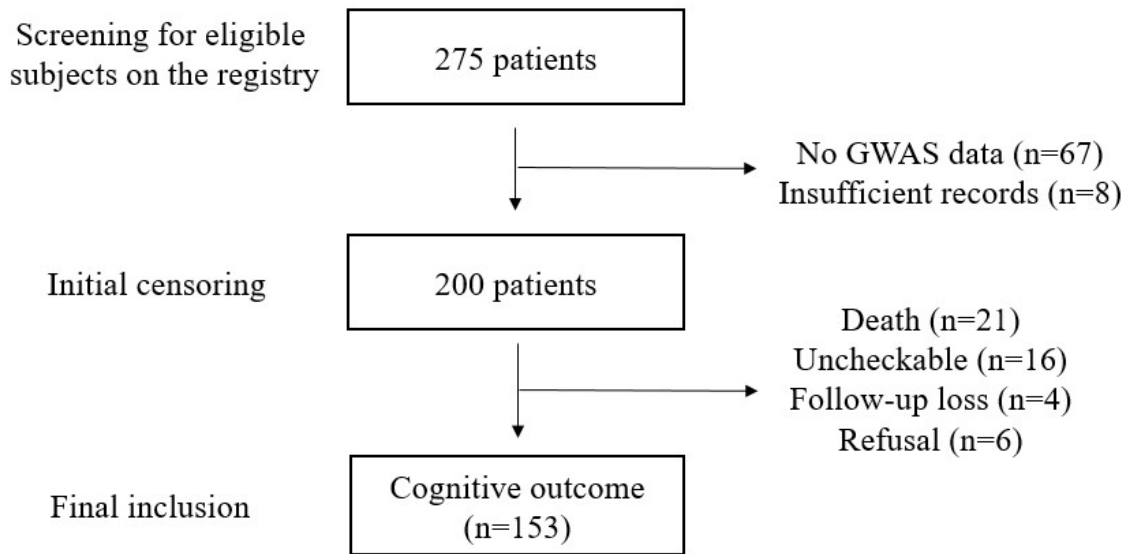
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## SUPPLEMENTAL DATA

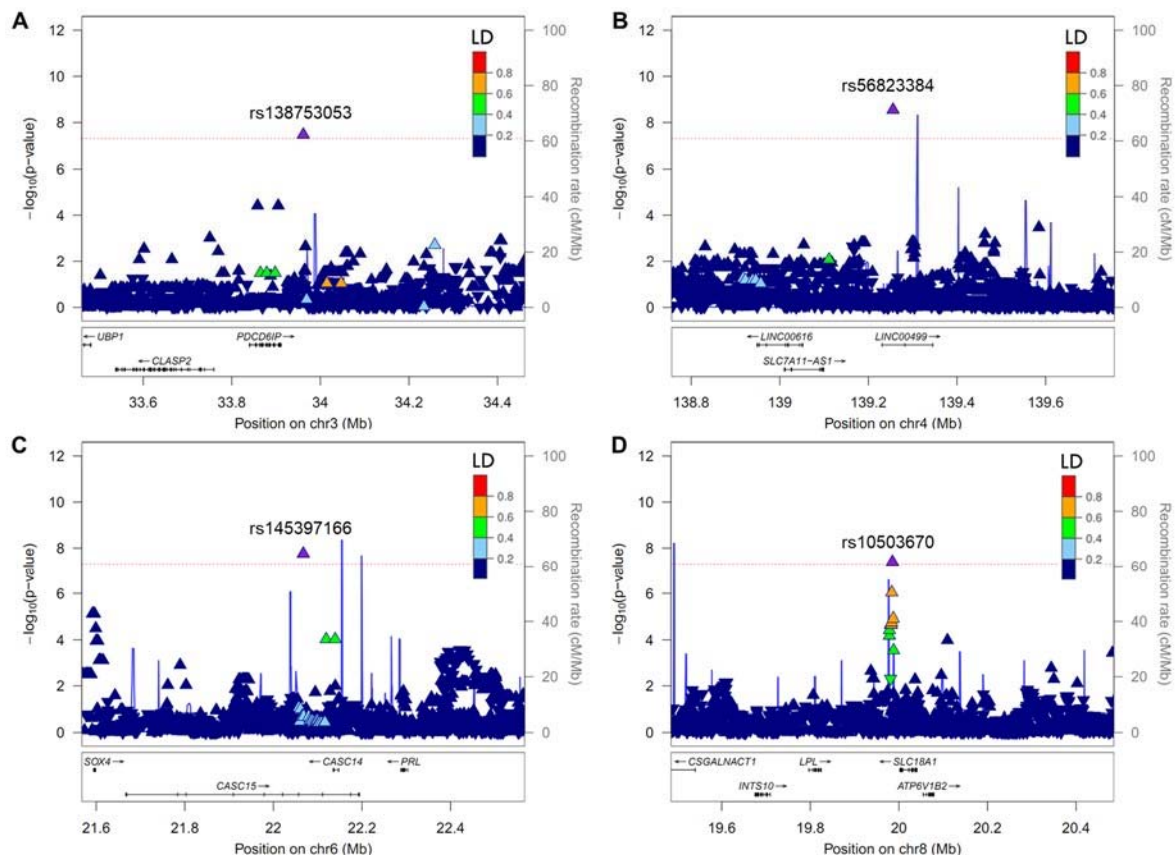
**Supplementary Figure 1.** Flow chart of included and excluded patients.



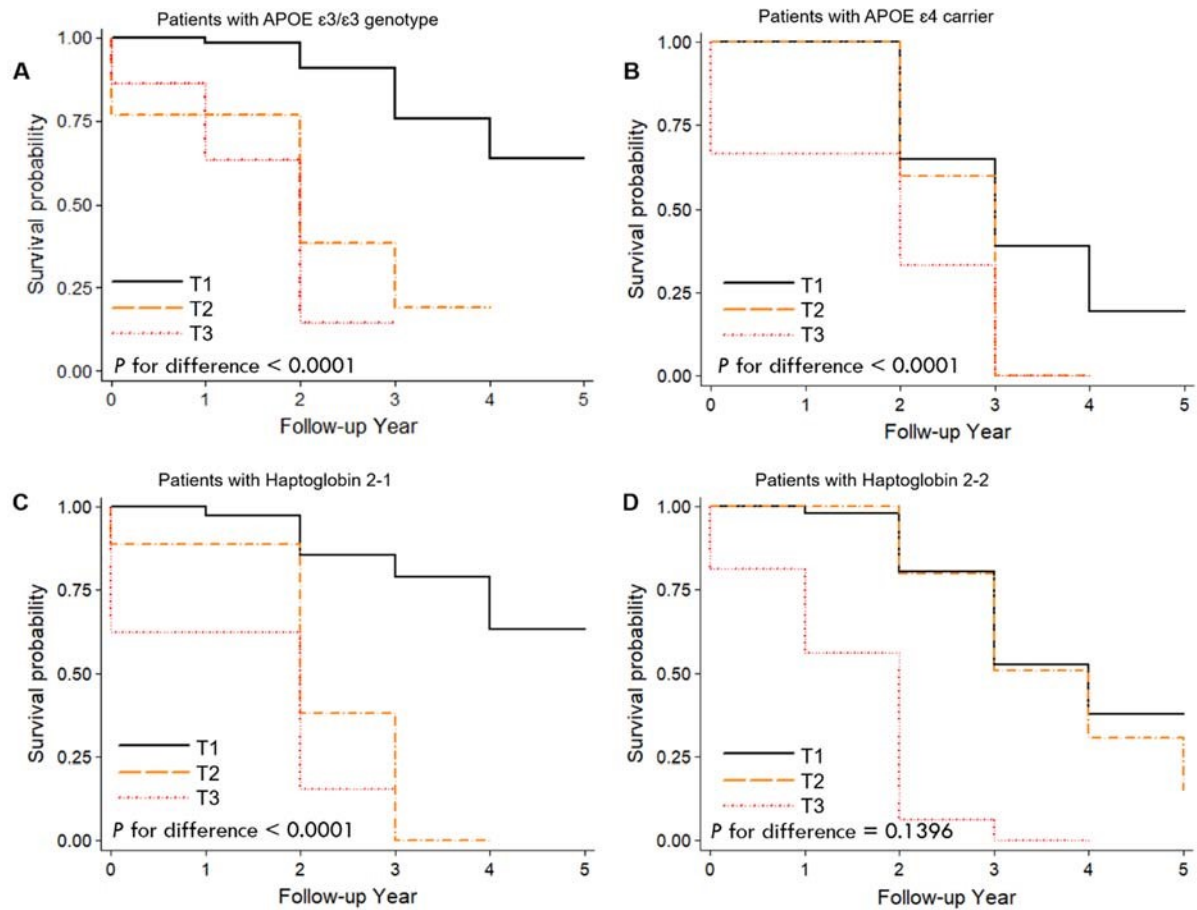
**Supplementary Figure 2.** Western blotting analysis of haptoglobin (Hp) phenotypes. Hp alleles were confirmed using polyacrylamide gel electrophoresis, followed by immunoblotting to identify  $\alpha 1$  and  $\alpha 2$  chains referring to our previous protocols<sup>13,19</sup>: Three types of haptoglobin were classified, and then western blotting was performed again targeting only Hp2-1. For analysis, a 1:75 dilution of serum was made by adding a 1  $\mu$ l of serum to 74  $\mu$ l of phosphatebuffered saline. Samples were prepared by mixing a serum diluent with an equal volume of 2xSDS sample buffer (Bio-Rad, CA, USA) and boiled at 95 °C for 8 min. After boiling, 10  $\mu$ l of sample was loaded on 15 % polyacrylamide gel and electrophoresed for 150 min at 100 V (BioRad, CA, USA). After transfer, the membranes were blocked with 5 % BSA in TBST (10 mM Tris-HCl pH8.0, 150 mM NaCl) including 0.01 % Tween-20 for 1 hr. The membranes were incubated overnight with polyclonal rabbit anti-human haptoglobin antibody (Dako, Denmark) diluted 1:10,000 in blocking buffer at 4 °C. After three washes with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Abcam, UK) in a 1:10,000 for 1 hr at room temperature. Following a final washing steps, HRP substrate (Thermo, USA) was added to a membrane and chemiluminescence was detected using X-ray film (Kodak, USA). To determine the polymeric composition of Hp2-1 based on molecular size, additional immunoprecipitation was carried out with anti-Hp antibody. To immunoprecipitate Hp, 0.3 mg/ml of the serum from patient with Hp2-1 was incubated overnight with anti-Hp antibody at 4 °C. The immune complexes were precipitated with protein A/G Sepharose (Santa Cruz, USA) and analyzed by western blotting. Anti-albumin antibody (Abcam, UK) was used as a loading control. The band intensities of  $\alpha 1$ - and  $\alpha 2$ - chain were analyzed using ImageJ software (Version 1.49v, National Institutes of Health, USA).



**Supplementary Figure 3.** Regional association plots of four loci  $\pm 500$  kb including rs138753053 (*PDCD6IP-LOC101928135*), rs56823384 (*LINC00499*), rs145397166 (*CASC15*), and rs10503670 (*LPL-SLC18A1*) that showed a genome-wide association with cognitive impairment following subarachnoid hemorrhage. Triangles and reverse triangles indicate positive and negative effect sizes, respectively, and each color shows pairwise linkage disequilibrium. The purple up-triangle indicates the top SNP in each region. Other colors indicate pairwise linkage disequilibrium (LD,  $r^2$ ): navy, 0 – 0.2; green, 0.2 – 0.4; sky-blue, 0.4 – 0.6; orange, 0.6 – 0.8; red, 0.8 – 1.



**Supplementary Figure 4.** Kaplan-Meier survival curves without cognitive impairment based on the weighted polygenic risk score model in subarachnoid hemorrhage patients with APOE  $\epsilon 3/\epsilon 3$  (A), APOE  $\epsilon 4$  carriers (B), haptoglobin 2-1 (C), and haptoglobin 2-2 (D). Lines indicate low-risk (black solid line), middle-risk (orange dash-dot line), and high-risk (red dot line) groups. The p value for differences between the risk groups is given.



**Supplementary Table 1.** Baseline characteristics of patients enrolled in this study.

| Variables     | Cognitive impairment<br>(n = 65) | Non-cognitive impairment<br>(n = 88) | HR (95% CI) <sup>a</sup> | P     |
|---------------|----------------------------------|--------------------------------------|--------------------------|-------|
| APOE rs429358 |                                  |                                      | 1.22 (0.73-2.02)         | 0.454 |
| TT            | 47 (72.3%)                       | 73 (83.0%)                           |                          |       |
| CT            | 18 (27.7%)                       | 14 (15.9%)                           |                          |       |
| CC            | 0 (0%)                           | 1 (1.1%)                             |                          |       |
| APOE, rs7412  |                                  |                                      | 1.11 (0.59-2.11)         | 0.740 |
| CC            | 55 (84.6%)                       | 76 (86.4%)                           |                          |       |
| CT            | 10 (15.4%)                       | 11 (12.5%)                           |                          |       |
| TT            | 0 (0%)                           | 1 (1.1%)                             |                          |       |
| Haptoglobin   |                                  |                                      |                          |       |
| Hp 1-1        | 1 (1.5%)                         | 13 (14.8%)                           | Reference                |       |
| Hp 2-1        | 18 (27.7%)                       | 39 (44.3%)                           | 6.58 (0.88-49.36)        | 0.067 |
| Hp 2-2        | 46 (70.8%)                       | 36 (40.9%)                           | 9.82 (1.35-71.30)        | 0.024 |

Data are described with the number of subjects (percentage). <sup>a</sup>

HR indicates Hazard ratio; CI, confidence interval.

**Supplementary Table 2.** Subgroup analysis of the five genome-wide significant single nucleotide polymorphisms (SNPs) associated with cognitive impairment following subarachnoid hemorrhage according to apolipoprotein E (APOE) and haptoglobin (Hp) subtypes.

| SNP         | M/m <sup>a</sup> | APOE ε3/ε3 |             |                      | APOE ε4 carrier |             |          | Hp2-1 |             |                      | Hp2-2  |               |                      |
|-------------|------------------|------------|-------------|----------------------|-----------------|-------------|----------|-------|-------------|----------------------|--------|---------------|----------------------|
|             |                  | HR         | 95% CI      | <i>P</i>             | HR              | 95% CI      | <i>P</i> | HR    | 95% CI      | <i>P</i>             | HR     | 95% CI        | <i>P</i>             |
| rs138753053 | G/A              | 24.61      | 6.82-88.84  | 1.0×10 <sup>-6</sup> | NA              | NA          | NA       | 24.77 | 3.25-188.79 | 0.002                | 141.64 | 11.42-1756.64 | 1.2×10 <sup>-4</sup> |
| rs56823384  | T/C              | 13.72      | 5.23-36.00  | 1.0×10 <sup>-7</sup> | NA              | NA          | NA       | 24.74 | 5.50-111.38 | 2.9×10 <sup>-5</sup> | 8.71   | 2.71-28.03    | 2.8×10 <sup>-4</sup> |
| rs145397166 | C/G              | 12.01      | 4.35- 33.15 | 1.6×10 <sup>-6</sup> | 11.42           | 0.97-134.36 | 0.053    | 12.51 | 1.82-85.82  | 0.01                 | 10.02  | 3.60-27.85    | 1.0×10 <sup>-5</sup> |
| rs10503670  | G/A              | 4.24       | 2.48-7.24   | 1.2×10 <sup>-7</sup> | 1.76            | 0.97-3.20   | 0.063    | 2.60  | 1.27-5.30   | 0.009                | 3.62   | 2.19-6.00     | 5.5×10 <sup>-7</sup> |
| rs76507772  | A/C              | 5.38       | 2.54-11.42  | 1.2×10 <sup>-5</sup> | 8.48            | 0.63-114.03 | 0.107    | 7.41  | 1.70-32.21  | 0.008                | 5.70   | 2.67-12.17    | 6.7×10 <sup>-6</sup> |

<sup>a</sup> M/m indicates a major and minor allele, respectively. <sup>b</sup>HR indicates Hazard ratio; CI, confidence interval