Supplemental Materials

HOMOCYSTEINE, ANOTHER RISK FACTOR FOR ALZHEIMER'S DISEASE, IMPAIRS APOLIPOPROTEIN E3 FUNCTION

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Methods

1) Analysis of DNA fragmentation

DAN fragmentation was evaluated by FACS analysis. Astrocytes derived from mouse cortices were plated in DMEM containing 10% FBS for 72 h. The cultures were then washed three times with DMEM and cultured in a serum-free DMEM medium in the absence or presence of Hcy at various concentrations for 48 h. The cells were trypsinized and fixed in ice-cold 70% ethanol overnight at 4°C. The cells were then washed three times with PBS, stained with propidium iodide (PI, 30 mg/ml) for 30 min at room temperature and subjected to FACS analysis on a FACScan flow cytometer (BD PharMingen). Data were analyzed using the CellQuest software. For each sample, 10,000 events were collected. The percentages of DNA fragmentation reflecting apoptotic cells were determined by measuring the fraction of nuclei containing a hypodiploid DNA content (sub-G1 fraction).

2) Cell viability analysis

Cell viability was evaluated using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA). Briefly, astrocytes derived from mouse cortices were seeded into 96-well plates in DMEM containing 10% FBS for 72h. The cultures were then washed three times with DMEM and cultured in a serum-free DMEM medium in the absence or presence of Hcy at various concentrations for 48 h. Cell viability was determined according to the manufacturer's instruction.

3) Correlation between levels of CSF or serum Hcy and the ratio of apoE3 dimer

The levels of Hcy in the CSF and the serum of the patients with normal serum Hcy or hyperhomocystenemia were determined as described in the Experimental Procedures in

the text. The ratio of apoE3 dimer was determined by Western blot analysis under non-reducing conditions as described in the text.

Legends

Supplemental Fig. 1

Effect of Hcy on the viability (A) and apoptosis (B) of astrocytes derived from mouse cortices. (A) Astrocytes were exposed to indicated concentrations of Hcy for 48h in serum-free DMEM. Cell viability was determined using CellTiter-Glo Luminescent Cell Viability Assay and is shown as a percentage of living cells. (B) Flow cytometry analysis was used to assess fragmentation after PI staining. Cells within the sub-G1 population contain fragmented DNA. Such cells are considered undergoing apoptosis. Data are means ± S.E. of three independent experiments. *p<0.005, **p<0.001 *vs* control.

Supplemental Fig. 2

The correlation between the level of Hcy and the ratio of apoE3 dimer is shown. (A) The level of Hcy in the CSF and the ratio of apoE3 dimer in each sample are plotted. (B) The level of Hcy in the serum and the ratio of apoE3 dimer in each sample are plotted. There is a tendency for negative correlation between the dimer ratio and Hcy value in CSF and serum samples, although it does not reach spastically significant. The tendency for these negative-correlations becomes stronger when the separately distributed data, such as a, b, or c, are removed.

Supplemental Fig. 3

The levels of total cholesterol, HDL-cholesterol, and triglyceride (TG) in plasma obtained from patients with hyperhomocysteinemia and normal Hcy are shown in Supplemental Fig. 3A. The level of plasma total cholesterol is significantly higher in patients with hyperhomocysteinemia than in normal controls. In contrast, there is no difference in the levels of HDL-cholesterol and TG between these two groups. The correlations between the ratio of apoE3 dimer and the levels of total cholesterol, HDL-cholesterol, and TG are shown in Supplemental Figs. 3B, C, and D, respectively. There is a significant negative correlation between the ratio of apoE3 dimer in CSF and the level of plasma total cholesterol (Supplemental Fig. 3B). However, there is no correlation between the ratio of apoE3 dimer and the levels of HDL-cholesterol and TG (Supplemental Figs. 3C, D). Although this study

shows that Hcy inhibits apoE3 dimerization, which impairs HDL generation, there is no difference in the level of HDL-cholesterol between the samples from patients with hyperhomocysteinemia and normal Hcy. This is because apolipoprotein AI, but not apoE, plays a major role in HDL generation in systemic circulation. There is a negative correlation between the ratio of apoE3 dimer and the level of plasma total cholesterol; however, it is unlikely that there is a cause-and-effect relationship between these two parameters. Because cholesterol metabolism in the brain is separated from that of systemic circulation by the blood-brain barrier, the cholesterol level in the plasma does not correlate with that in the brain. Thus, it is necessary to determine the lipid profile, including apoE-mediated HDL generation, in CSF in a future study.

Supplementl Figure 1
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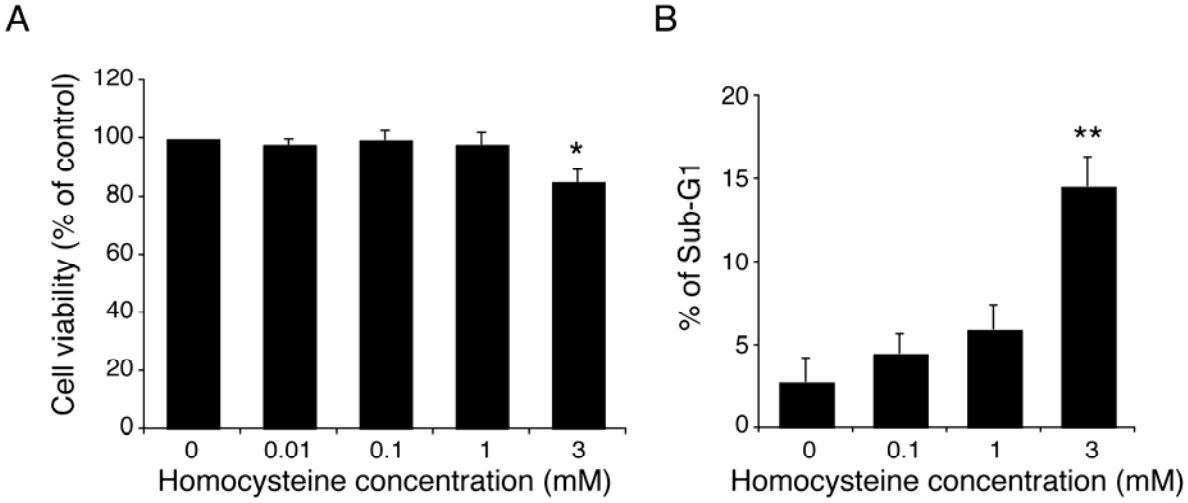

Supplemental Figure 2

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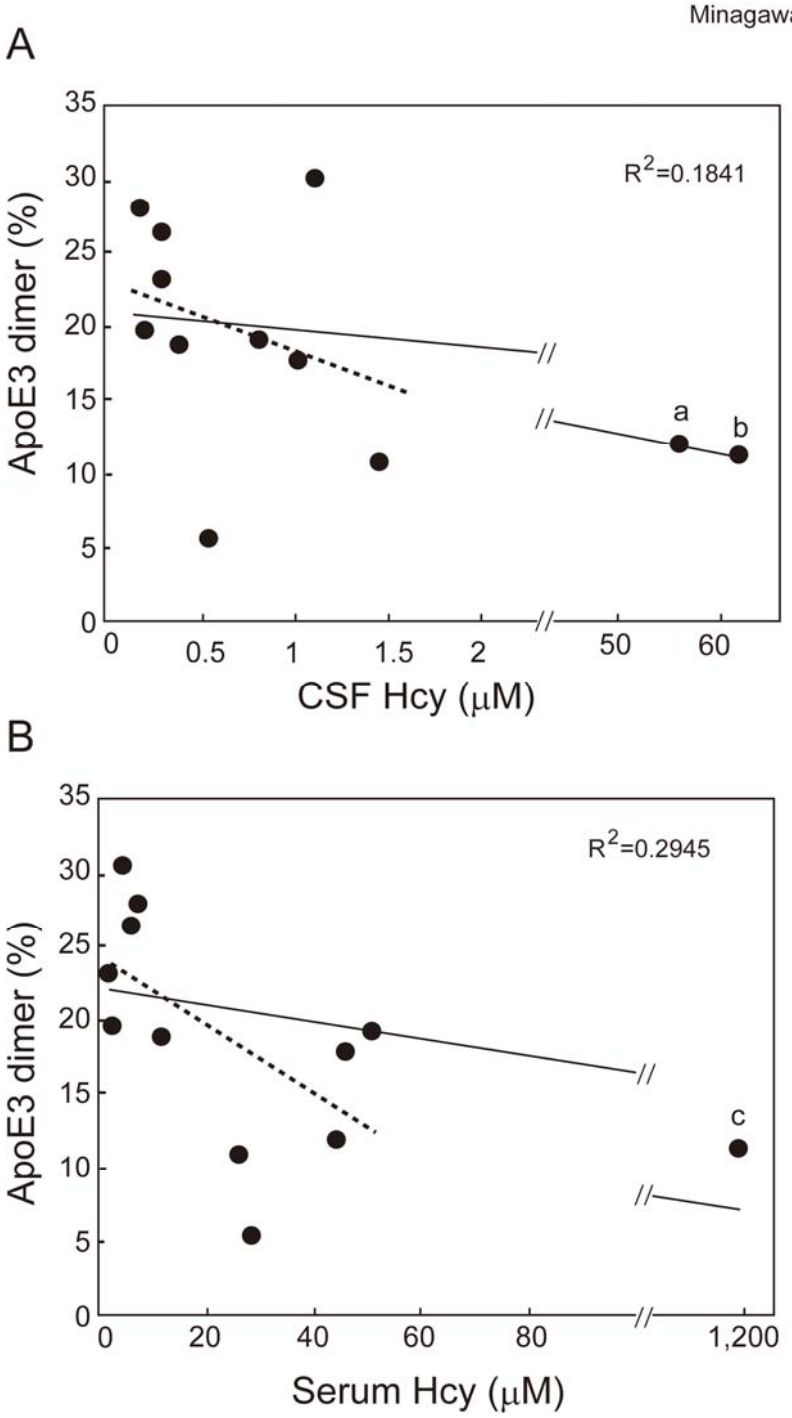

Supplemental Figure 3

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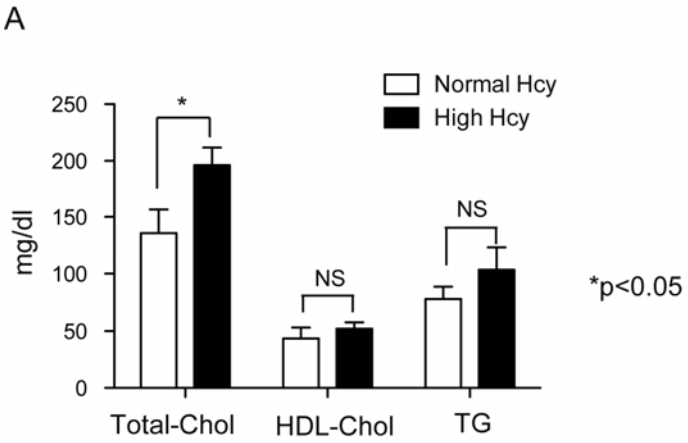

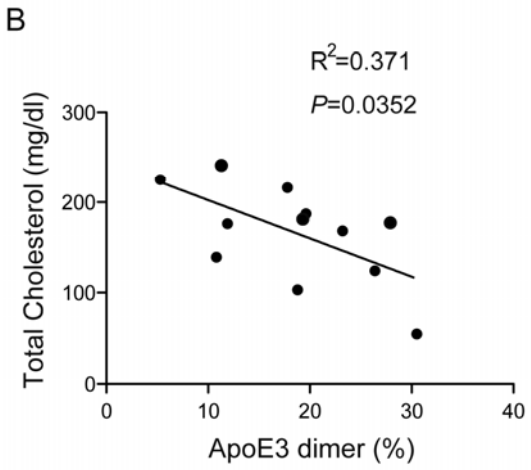

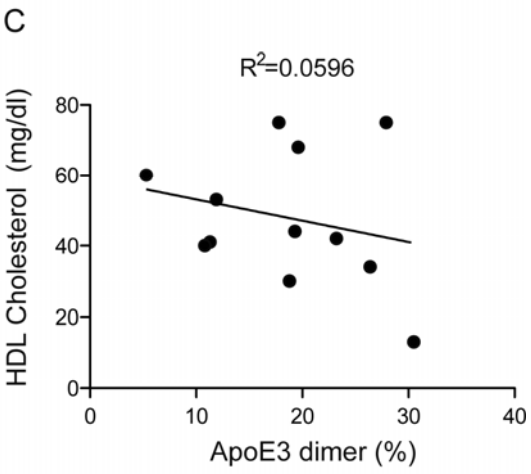

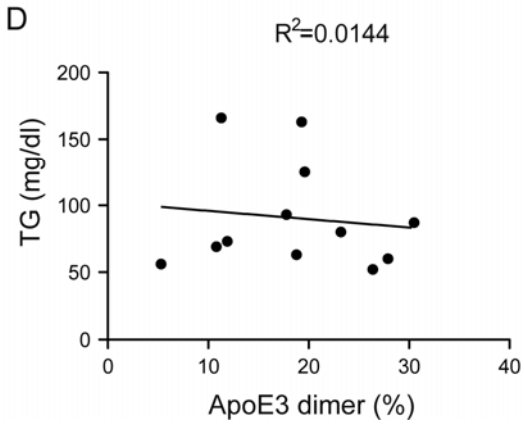

Supplemental Table 1. Plasma lipid profile of the patients

	NO	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal Hcy	1	55	13	87
	2	103	30	63
	3	168	42	80
	4	124	34	52
	5	177	75	60
	6	187	68	125
High Hcy	7	176	53	73
	8	181	44	163
	9	241	41	166
	10	216	75	93
	11	139	40	69
	12	225	60	56