

Supplementary Data

Human Serum Transthyretin Levels Correlate Inversely with Alzheimer's Disease

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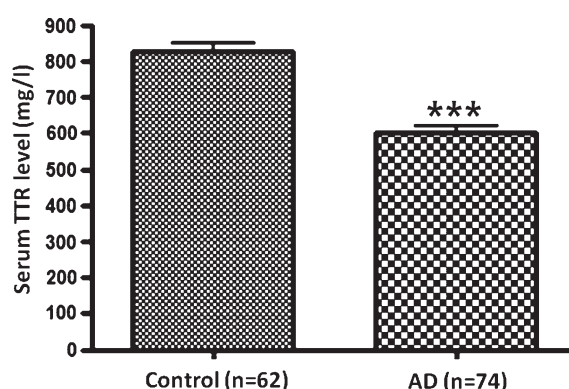
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SUPPLEMENTARY METHODS

Western blot

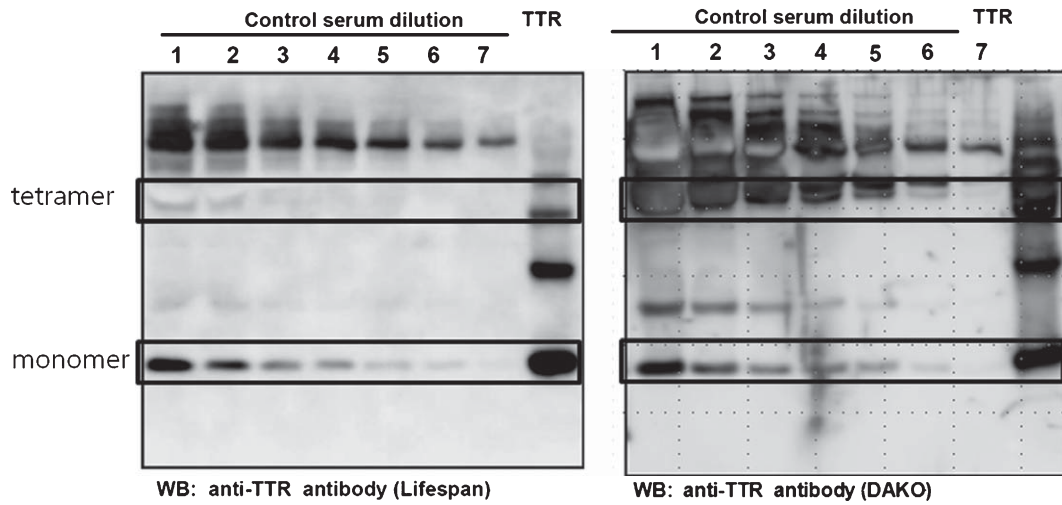
Serum was diluted in 1× Phosphate buffered saline (PBS), and serial diluted sera were loaded on 4–12% NuPAGE MES gel (Invitrogen) to separate proteins. Standard TTR protein (Sigma, St. Louis, MO) was used as a positive control. After transfer to polyvinylidene difluoride (PDVF) membranes, nonspecific binding was blocked by incubating the membranes in Tris-



Supplementary Figure 1. Selected subpopulation of nondemented control and AD patients with similar average age (average age of control = 69.21 ± 7.86 , that of AD = 69.49 ± 6.84) showed significantly decreased serum TTR level in AD. Serum TTR level: control (mg/l) = 827.3 ± 22.66 , AD = 600.9 ± 18.01 , *** $p < 0.001$.

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Supplementary Figure 2. Isomeric specificity of anti-TTR antibodies. Two anti-TTR antibodies were tested for binding specificity to TTR isoforms. Dilution of control serum: 1: 1/50, 2: 1/100, 3: 1/200, 4: 1/400, 5: 1/800, 6: 1/1600, 7: 1/3200.

buffered saline with Tween-20 (TBST, 137 mM NaCl, 20 mM Tris, pH 7.8, and 0.05% Tween 20) and 5% nonfat skim milk. The membrane was reacted with anti-human TTR (DAKO, Glostrup, Denmark or Lifespan, Seattle, WA), diluted 1:2000, for 12 h at 4°C and horseradish peroxidase-conjugated anti-rabbit IgG (Amersham Pharmacia Biotech, Buckinghamshire,

UK, 1:5000) for 1 h at RT. Immunoreactive proteins were detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Buckinghamshire, UK). Densitometric measurement data was performed using a digital image analyzer (LAS-3000, Fuji, Japan).