Uchida, K. *et al*.

Supplementary Materials

Supplementary Methods

1. Tone cohort

1.1. Neuropsychological battery in the longitudinal study

All participants in the Tone cohort underwent group assessment, named the 5-Cog, which used a set of five tests that measured the following cognitive domains: attention, memory, visuospatial function, language, and reasoning. Attention was evaluated by a Japanese version of a set dependency activity [1]. The category-cued recall test [2] was used to assess memory ability. The clock-drawing test, which required participants to draw the hands of a clock to depict the time at “ten after eleven” [3], was used to assess visuospatial function. Language ability was examined using a category fluency test [4]. The similarities subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [5] was employed to assess abstract reasoning ability.

1.2. Demographics and medical issues

Information on age, sex, education, occupation, marital status, family members, daily activities, previous medical and psychiatric diseases, medication, alcohol, and smoking as dementia risk factors were included in the questionnaire.

1.3. Assessment of mood status

To assess depression, a 15-item short version of the Geriatric Depression Scale for

mood evaluation was required [6]. The participants who scored six were considered to have depressive symptoms.

1.4. Complaints of memory loss

Nineteen items from the Deterioration de Cognitive Observed were conducted to decide whether participants had memory difficulties. If the participants indicated problems on ≥1 item, memory difficulties were considered to be present.

1.5. Assessment of activities of daily living

Nishimura's Activities of Daily Living (N-ADL) test [7] was used. This test reviewed five daily activities: walking/transferring, going outside, dressing/ bathing, feeding, and toileting. No difficulties were reported for any of the five items of N-ADL; thus, the participants were considered functionally normal.

1.6. Assessment of cognition function

All the participants underwent an assessment named the 5-Cog, which used a set of tests to measure five cognitive domains: attention, memory, visuospatial function, language, and reasoning. The 5-Cog cognitive assessment was conducted by an examiner for a group of 50 participants (maximum) and with the use of a projector. All the participants were asked to record their answers on an answer sheet. Mean duration of the 5-Cog examination was 35 min. For participants who had difficulty understanding the tasks or

impaired hearing or vision, the 5-Cog examination was individualized in a face-to-face setting. During the interview, the participants who could not respond to our instructions or to some of the scales because of obvious cognitive impairment were also identified.

2. Serum sampling

Blood was sampled from the cubital veins and was placed into blood collection tubes (Venoject-II® Autostep; Terumo Corporation, Tokyo, Japan). After coagulation of the blood sample for 30 min at room temperature followed by centrifugation at 1,300 g for 15 min at 20°C, serum was transferred to 1.5 ml tubes (TreffLab, Degersheim, Switzerland) and was then stored at −80°C until further use.

3. Immunoassay and *APOE* genotyping

The serum samples were analyzed using multiplex microsphere-based Luminex xMAP (Luminex Corp, Austin, TX) using HNDG1-36K [complement C3 (C3), TTR, apoE, apoA1] and HNDG2-36K kits [complement C4 (C4), macrophage inflammatory protein (MIP)-4] (EMD Millipore, Billerica, MA). This multi-immunoassay allowed the simultaneous quantification of all proteins in each kit described above. In the determination of serum levels of proteins, quality control proteins of each analyte were

included in each assay. We also added control serum samples to monitor reproducibility in each experiment.

For apoA1, C3, and TTR, the analyte proteins bound to immunobeads by immunoprecipitation (IP)-Western blotting were characterized. Based on IP-Western blotting, the molecular weights of apoA1 and TTR were 27 kDa and 15 kDa, respectively, and that of C3 corresponded to the weight of a full-length C3, not the inactivated form. These measurements approximately matched the calculated molecular masses (data not shown). *APOE* genotyping was performed using standard procedures [8].

4. Statistical analysis

In the statistical analysis, we first analyzed for the normality of distribution by Bartlett test, and non-parametric test was applied for data that were non-normal in distribution. For data consisting of more than three groups, Kruskal-Wallis test was used. Bonferroni correction was applied when two groups were compared. In the longitudinal analysis, Wilcoxon signed-rank test was used to analyze for significant differences between 2005 and 2008 in each individual.

References

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Supplementary Table 1. Demographic characteristics of participants in the longitudinal study of the Tone cohort

Characteristics	NDC during follow up (n = 20)	NDC-MCI (n = 9)	MCI-sMCI/AD (n = 6)	*P* value*
Age in 2005	76.4 ± 5.8†	73.7 ± 6.4	74.7 ± 5.7	0.5231
Age in 2008	80.3 ± 5.8	77.6 ± 6.4	78.3 ± 5.7	0.43784
Male / Female	7/13	2/7	2/4	
Years of education	10.3 ± 2.4	9.8 ± 1.9	11.5 ± 2.9	0.4752
BMI‡	22.6 ± 2.4	23.8 ± 5.0	24.3 ± 5.0	0.35639
GDS Score‡	2.1 ± 1.7	3.6 ± 3.5	3.6 ± 2.6	0.31795
Cigarette smoking	4	1	2	
Alchohol drinking	7	1	2	
APOE ε4 carrier	3	0	2	
History of disease				
Cardiovascular disease	1	0	0	
Diabetes mellitus	1	0	1	
Hyperlipidemia	0	0	0	
Hypertension	2	2	1	

*Kruskal-Wallis test. Significant differences among the groups are indicated.

†mean ± SD

‡Values in 2008

Supplementary Table 2. Serum levels of ApoE, ApoA1, C3, C4, TTR, MIP4 in the longitudinal analysis of the Tone cohort

Analyte	2001	2005	2008	2005	2008	*P* value*
ApoE	NDC	NDC	NDC	108.5 ± 35.6†	83.3 ± 19.1	4.19E-04
	NDC	NDC	MCI	111.2 ± 30.1	80.0 ± 33.9	0.01285
	NDC	MCI	sMCI / AD	124.0 ± 25.5	68.1 ± 15.9	0.03603
ApoA1	NDC	NDC	NDC	1687.4 ± 593.0	1315.3 ± 444.6	0.00109
	NDC	NDC	MCI	2025.6 ± 222.3	1199.4 ± 401.5	0.00915
	NDC	MCI	sMCI / AD	1958.2 ± 288.0	1236.4 ± 160.2	0.03603
C3	NDC	NDC	NDC	100.4 ± 52.2	71.6 ± 32.6	0.04579
	NDC	NDC	MCI	100.3 ± 42.4	78.7 ± 46.7	0.40694
	NDC	MCI	sMCI / AD	71.8 ± 76.4	59.1 ± 27.7	1.00
C4	NDC	NDC	NDC	30.3 ± 63.7	63.7 ± 26.8	3.15E-04
	NDC	NDC	MCI	37.9 ± 30.0	71.6 ± 24.0	0.03297
	NDC	MCI	sMCI / AD	22.4 ± 22.8	62.6 ± 33.0	0.05906
TTR	NDC	NDC	NDC	299.3 ± 58.7	288.7 ± 69.2	0.66769
	NDC	NDC	MCI	352.9 ± 61.0	312.6 ± 73.7	0.15513
	NDC	MCI	sMCI / AD	280.0 ± 60.6	218.6 ± 32.4	0.09349
MIP-4	NDC	NDC	NDC	0.12 ± 0.07	0.11 ± 0.07	0.40092
	NDC	NDC	MCI	0.11 ± 0.07	0.12 ± 0.07	0.72228
	NDC	MCI	sMCI / AD	0.12 ± 0.06	0.11 ± 0.05	0.40168

*Wilcoxon test

†mean ± SD

Supplementary Table 3. Characteristics of participants and serum levels of apoE, apoA1, C3, C4, TTR and MIP-4 in the cross-sectional analysis of Tone cohort

Analyte	NDC (n = 49)	MCI (n = 15)	sMCI / AD (n = 6)	*P* value*
Age	77.5 ± 6.3[†]	76.4 ± 6.1	78.3 ± 5.7	0.73634
Male/Female	16/33	4/11	2/4	
ApoE[§]	98.7 ± 31.1	97.6 ± 37.2	68.1 ± 15.9	0.0278
ApoA1[§]	1597.7 ± 544.6	1502.9 ± 519.4	1236.4 ± 160.2	0.23319
C3[§]	88.6 ± 44.8	75.9 ± 57.8	59.1 ± 27.7	0.13999
C4[§]	45.4 ± 30.4	53.5 ± 32.0	66.8 ± 35.0	0.33774
TTR[§]	304.8 ± 66.6	299.5 ± 68.5	218.6 ± 32.4[¶]	0.01264
MIP-4[§]	0.11 ± 0.07	0.12 ± 0.07	0.10 ± 0.05	0.85988

*Kruskal-Wallis test. Significant differences among the three groups are indicated.

[†]mean ± SD

[§]μg/ml

[¶]Bonferroni test. A significant difference between NDC and sMCI/AD was observed inTTR (*P* = 0.02025).

Supplementary Table 4. Characteristics of paticipants from the Tone and Tsukuba cohorts with MMSE score and serum levels of apoA1,C3, TTR, apoE, C4, and MIP-4

Characteristics	MMSE score				*P* value*
	27-30 (n=71)	24-26 (n=34)	20-23 (n=17)	<20 (n=20)	
Age	69.7 ± 11.0[†]	72.8 ± 8.2	76.0 ± 6.7	72.1 ± 7.8	0.14277
Male/Female	39 / 32	17 / 17	13 / 4	16 / 4	
ApoA1[‡]	2148.5 ± 857.1	1940.4 ± 645.0	1710.5 ± 528.8	1645.1 ± 328.5[§]	0.00359
C3[‡]	23.8 ± 21.6	15.2 ± 10.1	20.4 ± 16.9	22.3 ± 15.4	0.32561
TTR[‡]	478.6 ± 182.4	455.6 ± 178.9	380.5 ± 108.5	364.3 ± 138.0[§]	0.01894
ApoE[‡]	102.4 ± 31.2	114.5 ± 47.4	120.1 ± 38.0	114.8 ± 35.7	0.46029
C4[‡]	67.2 ± 34.3	80.6 ± 29.5	85.3 ± 40.5	69.8 ± 27.3	0.35412
MIP-4[‡]	0.41 ± 0.19	0.53 ± 0.14	0.41 ± 0.14	0.39 ± 0.18	0.04523

*Kruskal-Wallis test. Significant differences among the three groups are indicated.

[†]mean ± SD

[‡]μg/ml

[§]Bonferroni test. Significant differences in MMSE score 27-30 vs. <20 were obserbed in ApoA1 (P = 0.0396) and TTR (P = 0.05074).

Supplementary Table 5. Characteristics of participants and serum apoA1, C3, and TTR levels in the prospective stuty for MCI and AD risk analysis in the Uji cohort

Total number of participants	258
Age	68.9 ± 10.9*
Male / Female	98 / 160
APOE e4 carrier, %	23.6
MMSE score	27.3 ± 4.0
ApoA1†	1588.5 ± 412.2
C3†	6.4 ± 2.7
TTR†	388.4 ± 149.1

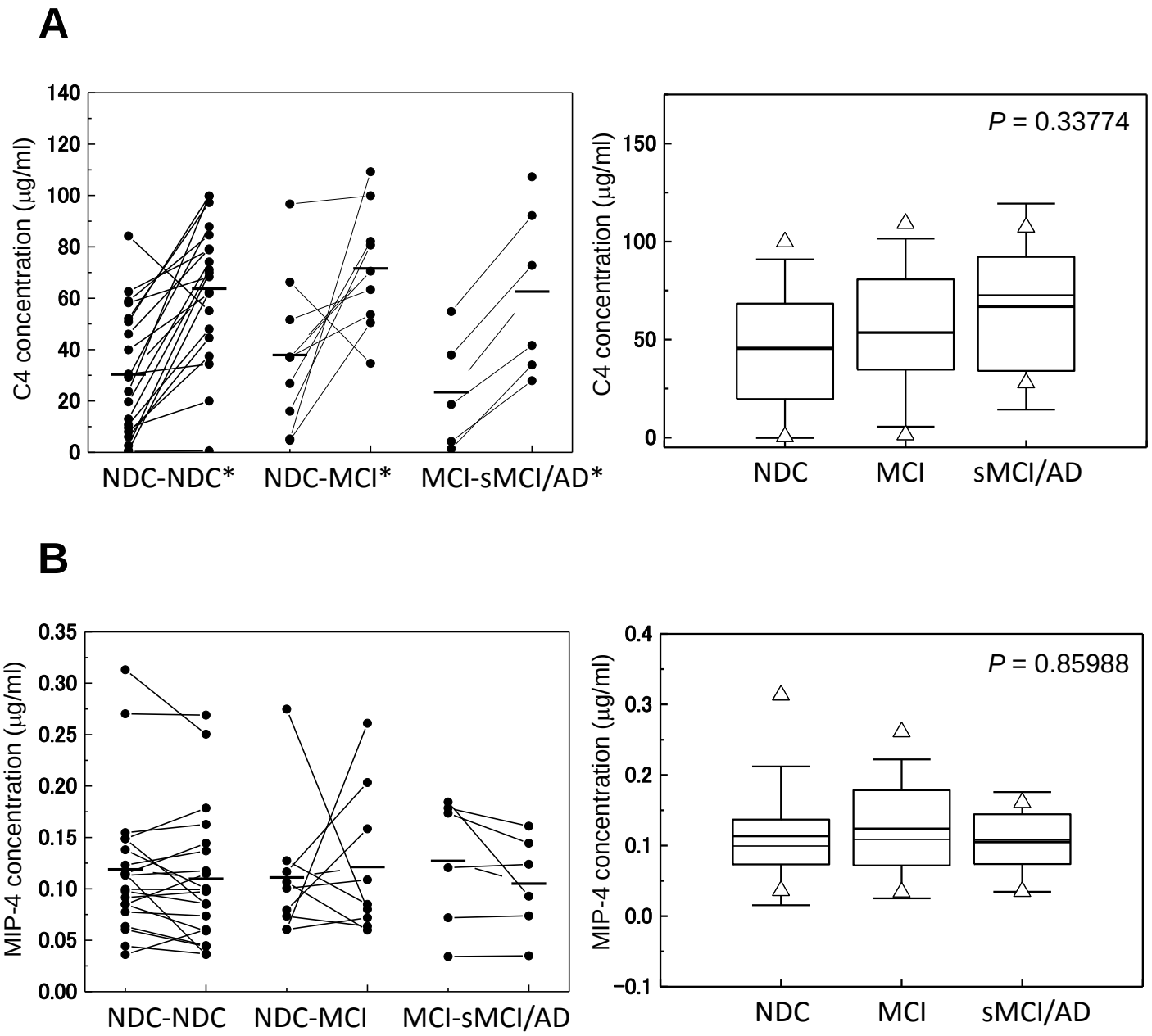
*mean ± SD

†μg/ml

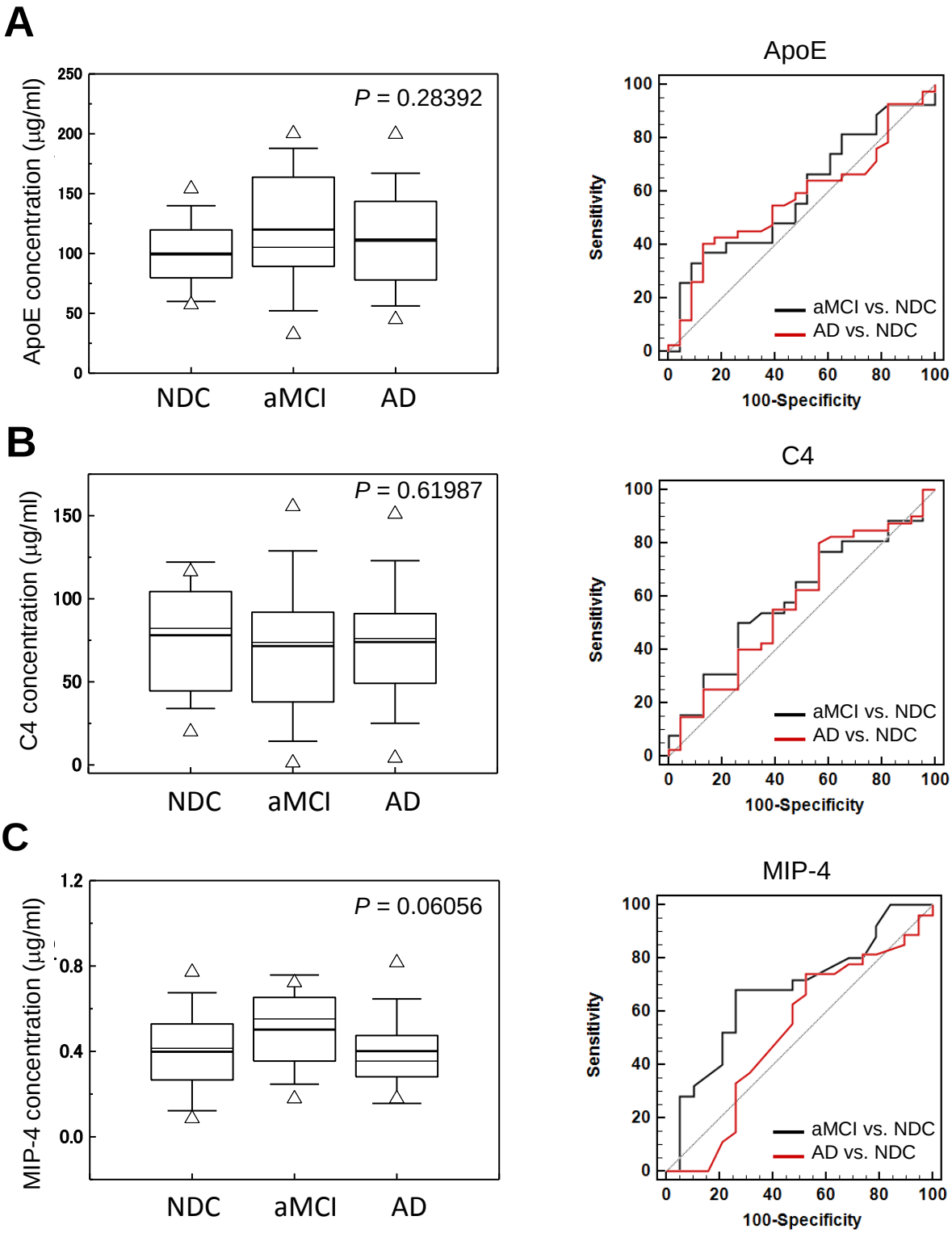
Supplementary Table 6. Summary of peripheral sequester protein levels in MCI and AD in the longitudinal, cross-sectional, and prospective studies

Group	Tone cohort: longitudinal study*			Tone cohort: cross-sectional study	Tsukuba cohort: cross-sectional study		Uji cohort: prospective study	Function
	NDC-NDC	NDC-MCI	MCI-sMCI / AD	NDC vs. MCI vs. sMCI/AD	NDC vs. aMCI	NDC vs. AD	NDC vs. MCI	
Apolipoprotein	ApoA1 ↓ 0.00109	ApoA1 ↓ 8.41E-04	ApoA1 ↓ 0.00179	ApoA1 ↓ 0.23319	ApoA1 ↓ 0.00265	ApoA1 ↓ 1.98E-05	ApoA1 ↓ 0.03907	ApoA1 is involved in Aβ clearance via cholesterol/lipoprotein transporter ATP-binding cassette transporter A1 (ABCA1).
	ApoE ↓ 0.00126	ApoE → 0.00162	ApoE ↓ 0.00161	ApoE ↓ 0.0278	ApoE → 0.19892	ApoE → 0.72119	—	At the blood–brain barrier, soluble Aβ is predominantly transported from the interstitial fluid into the blood stream via LRP1 and p-glycoprotein.
Complement	C3 ↓ 0.04671	C3 ↓ 0.38647	C3 → 0.75317	C3 ↓ 0.13999	C3 ↓ 3.74E-04	C3 ↓ 0.23189	C3 ↓ 0.03305	Complement activation clears immune complexes including Aβ. The complement system may also lead to host cell damage and to neurons being particularly susceptible to complement–mediated damage
	C4 ↑ 1.98E-05	C4 ↑ 0.03374	C4 ↑ 5.02E-04	C4 ↑ 0.33774	C4 → 1.00	C4 → 1.00	—	
Aβ-binding protein	TTR → 0.53406	TTR → 0.18291	TTR ↓ 0.06541	TTR ↓ 0.01264	TTR ↓ 0.0634	TTR ↓ 1.08E-05	TTR ↓ 0.25651	TTR binds to all forms of soluble Aβ, monomer, oligomer, and fibrils. TTR inhibits Aβ deposition and reduces its toxicity.

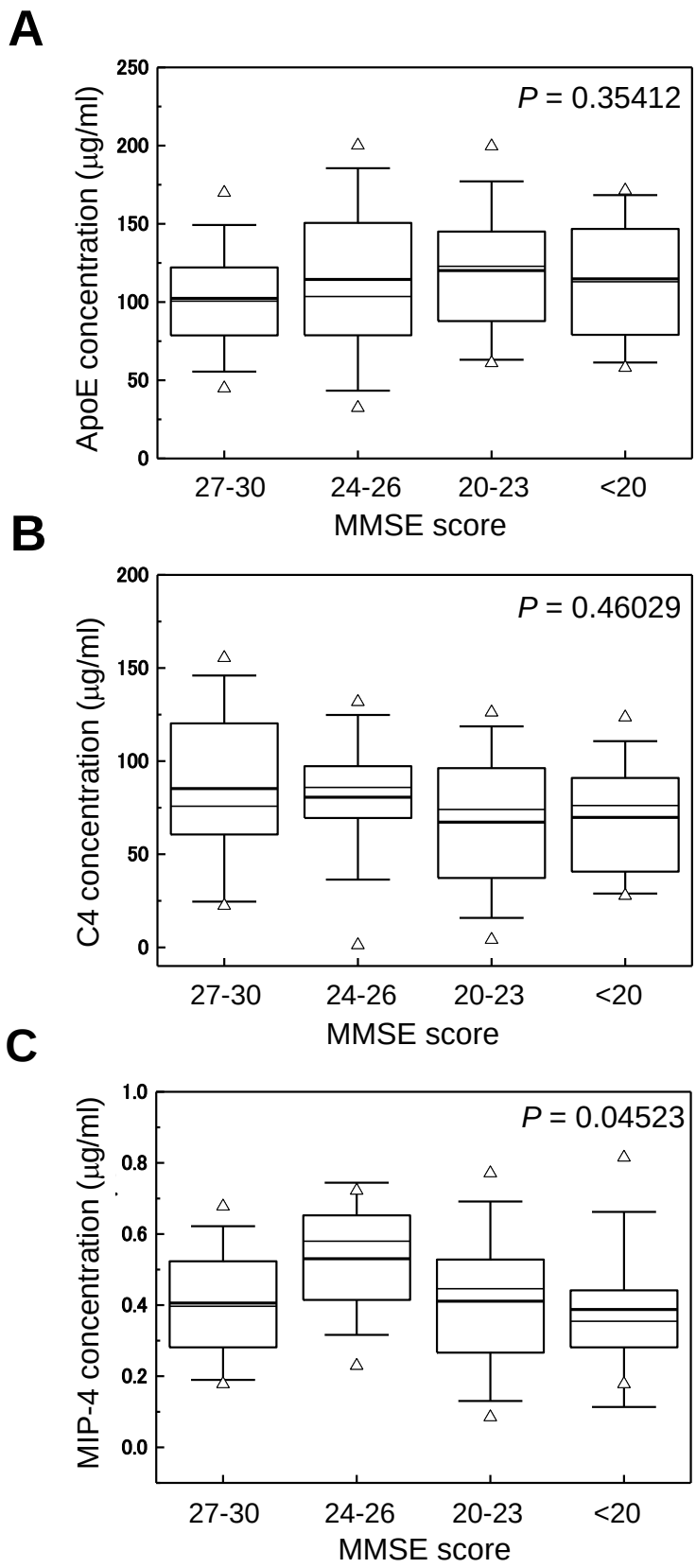
*MIP-4 levels did not significantly change with cognitive decline.



Supplementaryl Fig. 1. Serum levels of C4 (**A**) and MIP-4 (**B**) in NDC, MCI, sMCI/AD in the longitudinal (left panel) and cross-sectional (right panel) analyses of Tone cohort participants. Significant differences were observed in C4: NDC-NDC (P = 1.98E-05), NDC-MCI (P = 0.03374), and MCI-sMCI/AD (P = 5.02E-04).



Supplementary Fig. 2. Serum levels of apoE (**A**), C4 (**B**), and MIP-4 (**C**) in NDC (n = 49), aMCI (n = 51), and AD (n = 42) groups in a cross-sectional study (left panel) of the Tsukuba cohort and their corresponding *C*-statistics (right panel).



Supplementary Fig. 3. Relationship among apoE (**A**), C4 (**B**), and MIP-4 (**C**) levels and the MMSE scores in a cross-sectional study of the Tsukuba cohort.