Pathogenesis of AD is similar to that of uremic encephalopathy of Homocysteic acid.
(Homocysteic acid is a pathogen of Alzheimer's disease in human)

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Abstract

Homocysteic acid (HA) has been established as a pathogen in Alzheimer's disease (AD) in 3xTg-AD model mice. However, it is not established whether HA is involved in the AD pathogenesis in humans. We investigated the relationship between urinary HA levels and Mini-Mental State Examination (MMSE) scores in patients with AD (n = 110, normal =22, AD=88) and found a positive, statistically significant relationship between the two variables (r = 0.39, p =0.00003, n = 110). This relationship was stronger in females. (r = 0.47, p = 0.00008, n = 65 in females; r = 0.39, p = 0.02, n =45 in males). Urinary HA level difference between normal and AD in female was statistical significant (normal:18.4 $\pm$ 10.6vsAD: 9.1 $\pm$ 7.2 mM,$p<0.01$), but this difference of male was not statistical significant. (normal:12.7 $\pm$ 6 vs AD 8.8 $\pm$ 7.8). Smoking, hypertension, and diabetes mellitus decreased urinary HA excretion. On the basis of these results, we showed that HA is usually excreted into urine in humans and did not affect brain function in normal individuals. However, when urinary HA excretion was decreased, HA was shown to damage brain function, particularly cognitive ability. To confirm our hypothesis, that is, when urinary HA is suppressed to be excreted into urine, the blood HA level will be increased, we examined the relationship between the urinary HA level and blood HA level in 19 patients. The result was shown that the negative statistical significant relationship between them was observed (r=-0.6, p=0.007, n=19). From this result, the uremic encephalopathy of HA toxicity was confirmed. However it is a question that how the blood HA affected the brain cognitive function.

The direct effect of green tea leaves ingestion on HA level and MMSE score were also observed. Ingested green tea leaves decreased HA level in blood and concomitantly increased MMSE score, suggesting that blood HA could affect the cognitive function. But How? Some papers showed the exogenous NMDA including HA disrupted blood brain barrier and entered into brain and affected the cognitive function to decrease MMSE score.

These results indicate that human AD pathogenesis is influenced by HA, and that HA is a human pathogen in AD, indicating that the pathogenesis of AD is similar to that of uremic encephalopathy.

Also urinary HA can be used as a biomarker for Alzheimer's disease.

Introduction

Amyloid treatment in mice has been shown to be successful in recovering memory impairment (1,2). However, many human clinical trials on amyloid treatment for Alzheimer's disease (AD) have failed recently(3). This suggests that another pathogenic process must be active in humans.

We have recently found that homocysteic acid (HA) is an AD pathogen in 3xTg-AD model mice (with *APP*, *Presenilin* and *Tau* genes) (4). In the present study, we investigated whether the pathogenic action of HA is observed in humans. The results showed that HA is a human AD pathogen as well.

Materials and Methods

The study protocol was approved by the Juntendo University Hospital Ethics Committee, and all patients agreed to the procedure by providing informed written consent.

The patient profiles are shown in Table 1. Urine samples were collected from patients in the hospital and the specific gravity of samples was measured. Urinary HA level was adjusted to that of 1.020 specific gravity.

Urinary HA level

High performance liquid chromatography (HPLC) with an ECD detector was performed using a previous method with modifications (5). HA (4 mg) was added to the urine samples as an internal standard. Urine was diluted 10 times with water, and 20 µl of diluted urine sample was added to the measurement solution, which was composed of 150 µl o-phthaldialdehyde reagent and 150 µl mercaptoethanol. Fifteen minutes after mixing the diluted urine sample with the measurement solution, the sample was injected into the HPLC system.

Blood HA level was measured according to the method of (5).

Green tea leaves ingestion

16 Alzheimer's patients (Male5, Female 11) ingested 1 g of green tea leaves at every meal for one month. Before ingestion, their MMSE score and HA level in blood were measured. After one month, these parameters were measured.

Results

Urinary HA levels were significantly higher in normal than that in AD patients of female, but male urinary HA levels were not statistical significant between normal and patients. (Table 2).

Urinary HA levels were significantly and positively correlated with Mini-Mental State Examination (MMSE) scores (Fig. 1). This correlation was stronger in females than in males (Figs. 2 and 3). From Table 2, normal female excreted HA more higher than that of male.

Some smokers as well as patients with diabetes mellitus (DM) and hypertension (HT) were included in the study. Figures 4 and 5 clearly show that smoking, DM, and HT decreased HA excretion. Fig. 6 shows urinary HA of the mild cognitive impairment (MCI), indicating these urinary HA of MCI were around 20 mM. Fig. 7 shows the disease process of AD. I year was one year after detecting AD. 2 years indicate 2 years after detecting AD. From Fig. 7, it clearly shows that MCI preceeded AD for 1 year and 2 years, indicating that disease process was clearly showed by their urinary HA level. Table 3 shows the direct effect of green tea leaves ingestion on MMSE score and HA level in blood. Clearly shown in Table 3 , green tea leaves decreased HA level and concomitantly increased MMSE score, indicating that HA is a pathogen in human.

Now we are interested in the relationship between the decrease in urinary HA level and the increase in blood HA level. Fig. 8 shows the relationship between urinary HA level and blood HA level in 19 patients. From Fig. 8, the relationship between them was negative statistical significant was observed ($r=-0.6$, $p=0.007$, $n=19$).

From this result, patients showed urinary HA excretion was suppressed and their blood HA level increased, which indicated that patients showed an uremic encephalopathy of HA toxicity.

Discussion

Our results clearly indicate that urinary HA levels were positively correlated with MMSE scores. That is, when HA was excreted sufficiently into urine, cognitive function was normal. However, when HA excretion was decreased, cognitive function was impaired, suggesting that HA retained in the body caused cognitive impairment. This phenomenon was observed in Table 2. That is, Normal cognitive female persons excretes HA statistically

significantly higher than female AD patients. And Fig. 8 shows that the relationship between urinary and blood HA level is the negative statistical significant relation ($r=-0.6$, $p=0.007$, $n=19$), which indicates when the urinary HA excretion was suppressed in patients, the increase in blood HA level was observed. Then our hypothesis, a uremic encephalopathy of HA toxicity was confirmed. However it is still question how the blood HA affected MMSE score. Now some papers showed that exogenous NMDA including HA disrupted Blood Brain Barrier permeability (6,7). Then the increased level of HA in blood can enter into brain by BBB disruption and can suppress the cognitive function, which consequently induces the decrease in MMSE score.

Some studies have recently reported that the kidneys of patients with AD are impaired (8,9), suggesting that the decreased HA excretion due to renal failure could be a pathogenic factor in AD. Also green tea leaves ingestion simply but clearly indicated that HA is a pathogen of Alzheimer's disease in human. However we don't know why green tea leaves decreased HA level. But this effect is a real possible phenomenon, because one of authors (HT) already observed the green tea prevented Alzheimer's cognitive decline(10). Some powerful component of green tea leaves such as a pyrroloquinoline quinine (PQQ) can decrease HA level by inhibiting the HA production, or by stimulating HA metabolism.

Our recent study (4) showed that HA is the AD pathogen in the 3xTg-AD mouse model, but it is unclear whether HA is pathogenic in humans. Our observations reported in this study suggest that HA is related to AD pathogenesis in humans as well.

Recent clinical trials on amyloid treatment for AD have all failed. Because all AD model mice show a successful recovery from cognitive impairment following amyloid treatment, a different pathogenic process for AD must be present in humans. We suggest that this process is related to HA considering that HA does not induce its toxicity in mice (11). However amyloid treatment results in the recovery of cognitive impairment because the brain HA level in mice is very low, and it was not toxic (11). But in humans, the brain HA level is very high. For example, the urinary HA level in mice is in micromolar concentrations (4), whereas that in humans is tens of millimolar concentrations, indicating very high levels of HA in the body. These results suggest that we should not neglect HA toxicity even after amyloid treatment. Our observations also provide other interesting insights that DM and HT

decreased the urinary HA level, and these diseases are well-known high risk factors for AD (12,13). Furthermore, smoking decreased urinary HA excretion, and heavy smoking can result in AD (14). Our results suggest that DM, HT, and smoking can induce AD by decreasing urinary HA excretion. It is presently unclear how these pathologies suppress HA excretion. However, it is known that organic anion transporters in the kidney play an important role in the excretion of toxic substances (15). Perhaps DM, HT, and smoking affect this transporter system.

More over, there are many evidences which show the homocysteic acid (HA) involvement in human Alzheimer's pathogenic processes.

First Vlassenco et al has reported the possible link between regional aerobic glycolysis and amyloid deposition in normal brain (16).

This phenomenon may be induced by HA. First, HA is known as a neurotransmitter in normal brain (17). Second, HA induced a seizure of immature pup of rat. This brain metabolism was changed to be strong glycolysis. (18). Third, HA is published to be a pathogen of Alzheimer's disease (4) and HA induced amyloid beta42. (5)

From these evidences, Vlassenco's report, the possible link between a regional glycolysis and amyloid deposition in normal brain, can be induced by HA, whose brain will be later Alzheimer's one, because HA is a pathogen of Alzheimer's disease.

Second, it has reported that homocysteine-lowering by B vitamins slow the rates accelerated brain atrophy in mild cognitive impairment (19). This report shows that homocysteine induced brain atrophy, which induced the neurodegeneration. But we observed that a physiological level of homocysteine did not induce the neurodegeneration (20), then what did induce the brain atrophy?

HA is the possible compound which can induce the brain atrophy. Because HA is produced by homocysteine.

Third, some special food, that is, high fat diet and low carbohydrate diet can recover the cognitive impairment of Alzheimer's patient (21). This special food induced the ketone body in human, especially beta-hydroxybutyrate (BHB) was induced (21). This BHB is very similar chemical structure as that of HA and BHB, then can compete HA toxicity, which induces the cognitive impairment (4).

In conclusion, our findings indicate that when urinary HA excretion is

decreased, HA rises to a toxic level in the body, particularly in the brain. This retention of HA induces the AD pathogenic process and cognitive impairment, suggesting that the pathogenesis of AD is similar to that of uremic encephalopathy.

Moreover, our findings suggest that urinary HA can be used as a biomarker of Alzheimer's disease. That is, first normal person's urinary HA level should be observed and if their urinary HA level will be decreased by around 20 mM , their Alzheimer's process will be started (Fig. 6). So we should know our normal HA level, because our observation indicates that normal HA level shows strong individual variation.

Also our observations indicated that mild cognitive impairment (MCI) patients showed their HA levels were around 20 mM, (Fig. 6), indicating that urinary HA level at 20 mM is very useful for detecting first Alzheimer's disease process, because disease process of Alzheimer's disease was started around 20 mM of urinary HA (Fig. 7).

References

(1) Brendza RP, Bacskai BJ, Cirrito JR, Simmons KA, Skoch JM, Klunk WE, Mathis CA, Bales KR, Paul SM, Hyman BT and Holtzman DM (2005). Anti-abeta antibody treatment promotes the rapid recovery of amyloid-associated neuritic dystrophy in pdapp transgenic mice. J Clin Invest; 115: 428-433.

(2) J. Sanchez-Ramos, S. Song, V. Sava, B. Catlow, , X. Lin, T. Mori, C. Cao and G.W. Arendash, Granulocyte colony stimulating factor decreases brain amyloid burden and reverses cognitive impairment in Alzheimer's mice Neuroscience Volume 163, Issue 1, 29 September 2009, Pages 55-72

(3) Gina Kolata; Doubt in Tactic in Alzheimer's Battle, http://www.nytimes.com/2010/o8/19/health/19alzheimers.html

(4) Hasegawa T, Mikoda N, Kitazawa M, LaFerla FM (2010) Treatment of Alzheimer's disease with anti-homocysteic acid antibody in 3xTg-AD male mice. PLoS ONE 5(1): e8593. doi:10.1371/journal.pone.0008593.

(5) Quinn CT, Griener JC, Bottiglieri T, Hyland K, Farrow A, Kamen BA. Elevation of homocysteine and excitatory amino acid neurotransmitters in the CSF of children who receive methotrexate for the treatment of cancer. J Clin Oncol. 1997 Aug;15(8):2800-6.

(6) Chi OZ, Hunter C, Liu X, Weiss HR: Effect of exogenous excitatory amino acid neurotransmitters on blood-brain barrier disruption in focal cerebral ischemia. Neurochem Res. 2009 ;34(7):1249-54.

(7) Liu X, Hunter C, Weiss HR, Chi OZ. Effect of blocade of ionotropic glutamate receptors on blood-brain barrier disruption in focal cerebral ischemia. Neurol Sci. 2010 ;31(6):699-703.

(8) Tsai C-F, Wang S-J, Fuh J-L. Moderate chronic kidney disease is associated with reduced cognitive performance in midlife women. Kidney International 2010 78, 605-610 (September (2) 2010) | doi:10.1038/ki.2010.185.

(9) Madero M, Gul A, Sarnak MJ. Cognitive function in chronic kidney disease. Semin Dial. 2008;21(1):29-37.

(10) Hasegawa, Tohru., Okello, Edward. and Yamada, Tatsuo. Protective effect of Japanese green tea against cognitive impairment in the elderly, a two-years follow-up observation. *Alzheimer's and Dementia*, 1 (1 Supplement 1), p.S100, Jul 2005

(11) Hasegawa T, Ukai W, Jo DG, Xu X, Mattson MP, Nakagawa M, et al. (2005) Homocysteic acid induces intraneuronal accumulation of neurotoxic Ab42: implication for the pathogenesis of Alzheimer's disease. J Neurosci Res.

(12) Akomolafe A, Beiser A, Meigs JB, Au R, Green RC, Farrer LA, Wolf PA,

Seshadri S. Diabetes mellitus and risk of developing Alzheimer disease: results from the Framingham Study. Arch Neurol. 2006 Nov;63(11):1551-5.

(13) Goldstein FC, Ashley AV, Freedman LJ, Penix L, Lah JJ, Hanfelt J, Levey AI. Hypertension and cognitive performance in African Americans with Alzheimer disease. Neurology 2005 vol. 64 no. 5 899-901.

(14) Cataldo JK, Prochaska JJ, Glantz SA. Cigarette smoking is a risk factor for Alzheimer's disease: an analysis controlling for tobacco industry affiliation. J Alzheimers Dis. 2010;19(2):465-80.

(15) Robertson EE, Rankin GO. Human renal organic anion transporters: Characteristics and contributions to drug and drug metabolite excretion Pharmacology & Therapeutics Volume 109, Issue 3, March 2006, Pages 399-412.

(16) Andrei G. Vlassenko, S. Neil Vaishnavi, Lars Couture, Dana Sacco, Benjamin J. Shannon, Robert H. Mach, John C. Morris, Marcus E. Raichle, and Mark A. Mintun Spatial correlation between brain aerobic glycolysis and amyloid-ß (Aß) deposition. Proc Natl Acad Sci U S A. 2010 Sep 13. [Epub ahead of print]

(17) Lehmann J, Tsai C, Wood PL. Homocysteic acid as a putative excitatory

amino acid neurotransmitter: I. Postsynaptic characteristics at N-methyl-D-aspartate-type receptors on striatal cholinergic interneurons. J Neurochem. 1988 Dec;51(6):1765-70.
(18) Jaroslava Folbergrová, Renata Haugvicová and Pavel Mare Attenuation of seizures induced by homocysteic acid in immature rats by metabotropic glutamate group II and group III receptor agonists,Brain Res. 908 (2001) 120-129
(19) Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, et al. (2010) Homocysteine-Lowering by B Vitamins Slows the Rate of Accelerated Brain Atrophy in Mild Cognitive Impairment: A Randomized Controlled Trial. PLoS ONE 5(9): e12244. doi:10.1371/journal.pone.0012244
(20) Ziemińska E, Stafiej A, Łazarewicz JW. Role of group I metabotropic glutamate receptors and NMDA receptors in homocysteine-evoked acute neurodegeneration of cultured cerebellar granule neurons . Neurochem Int. 2003 43(4-5):481-92
(21) Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, Hyde K, Chapman D, Craft S. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. Neurobiol Aging. 2004 Mar;25(3):311-4

Figure legends

Fig. 1 Relationship between urinary homocysteic acid (HA) level and Mini-Mental State Examination (MMSE) scores. The patients were all admitted to the Juntendo University Hospital (n = 110; females, n = 65, males, n = 45; age, 75.8 ± 8 years).

Fig. 2 Relationship between urinary HA levels and MMSE) scores in females (n = 65; age, 77 ± 7.8 years).

Fig. 3 Relationship between urinary HA levels and MMSE scores in males (n = 45; age, 74 ± 8.6 years).

Fig. 4 Effect of diabetes mellitus (DM) and hypertension (HT) on urinary HA levels and MMSE scores. All patients were female. Green: DM, Red: HT

Fig. 5 Effect of smoking on urinary HA levels and MMSE scores. All patients were male. Red: Smokers

Fig. 6 Effect of MCI on urinary HA level and MMSE score. Three MCI patients showed their sample by red.

Fig. 7 Disease process from MCI to AD. 1 y indicate one year after AD. 2 y indicate two years after AD. Brown square indicates average of MCI, 1 year, 2 years points. This figures shows the disease process with arrow marks.

Fig. 8 The relationship between urinary HA level and blood HA level.
19 AD patients submitted their urine and blood. Their urinary HA and blood HA were measured.

Table 1 Patient profile			
	Number	Age	Body weight
Total	110	75.8 ± 8 years	53.7 ± 11.4 Kg
Male	45	74 ± 8.6 years	61.5 ± 7.8 Kg
Female	65	77 ± 7.8 years	49 ± 10.7 Kg

Table 2

	Normal MMSE score (30-29)	Urinary HA level (mM)	p value
Male	n=9	12.7 ± 6	
Female	n=13	18.4 ± 10.6	
	Alzheimer's patients		
Male	n=36	8.8 ± 7.8	
Female	n=52	9.1 ± 7.2	p<0.01 vs normal

HA, homocysteic acid; MMSE, Mini-Mental State Examination

Table 3 Effect of green tea leaves on MMSE score and HA level in blood

	MMSE score	HA level in blood (μM)
Before	14 ± 5	11.6 ± 6
	P<0.05	p<0.001
After	18 ± 4	1.2 ± 1

Patients agreed with this experiment.
Male (72.2 ± 3.6years, n=5) Female (75.4 ± 4.7 years, n=11)
Green tea leaves (1g) were ingested at every meal for one month. Before the ingestion of green tea leaves, their MMSE score and HA level in blood were measured. After one month, their MMSE score and HA level in blood were measured.

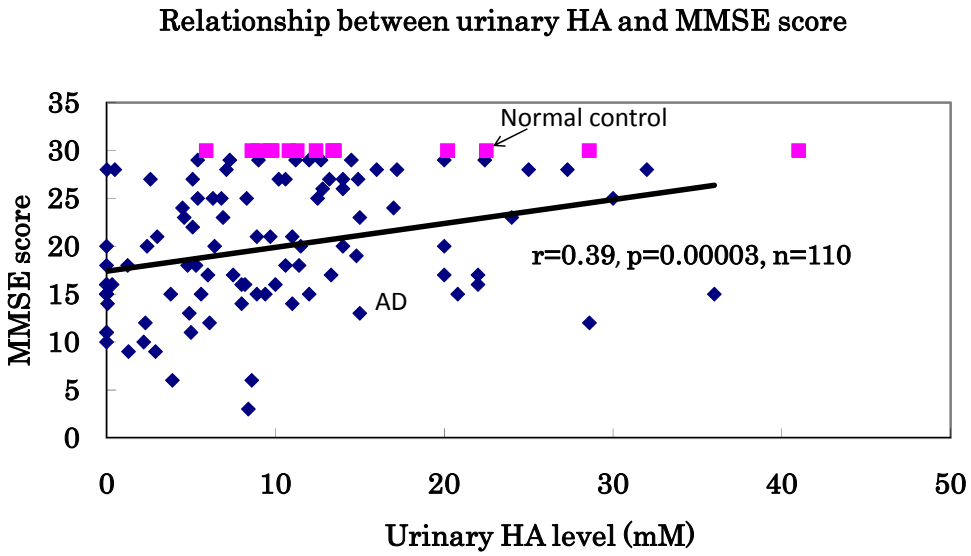
Relationship between urinary HA and MMSE score
MMSE score
Urinary HA level (mM)
Normal control
AD
r=0.39, p=0.00003, n=110
0
5
10
15
20
25
30
35
0
10
20
30
40
50

Fig. 1

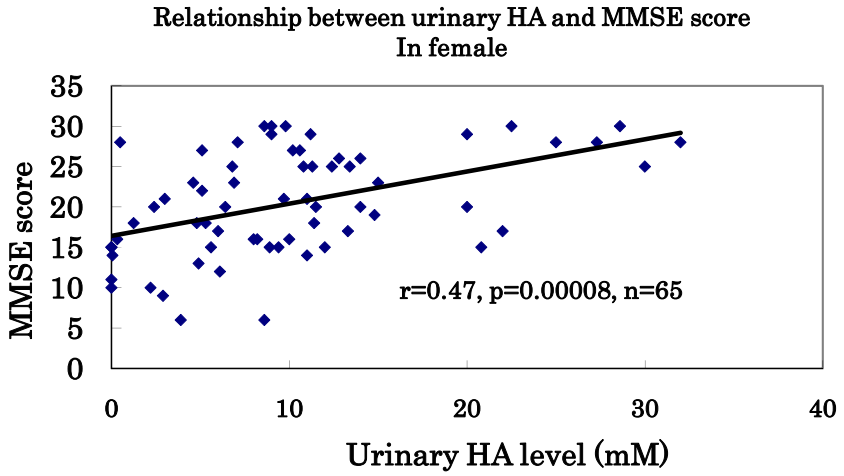
Relationship between urinary HA and MMSE score
In female
MMSE score
35
30
25
20
15
10
5
0
0
10
20
30
40
Urinary HA level (mM)
r=0.47, p=0.00008, n=65

Fig. 2

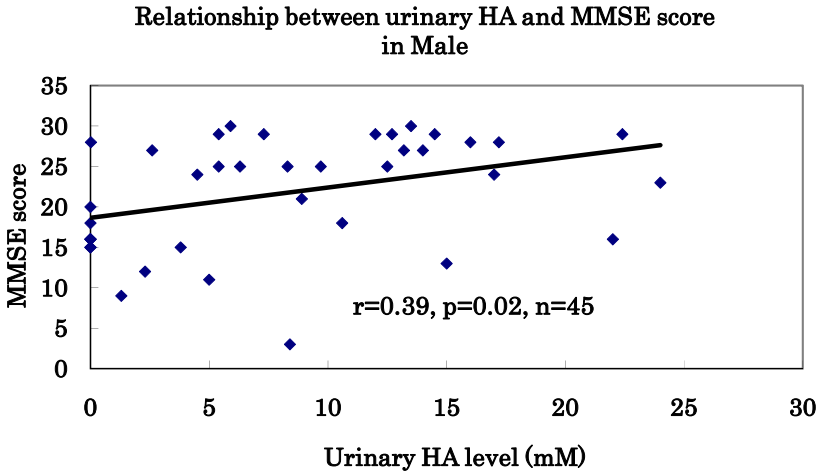
Relationship between urinary HA and MMSE score in Male
MMSE score
Urinary HA level (mM)
r=0.39, p=0.02, n=45
0
5
10
15
20
25
30
35

Fig. 3

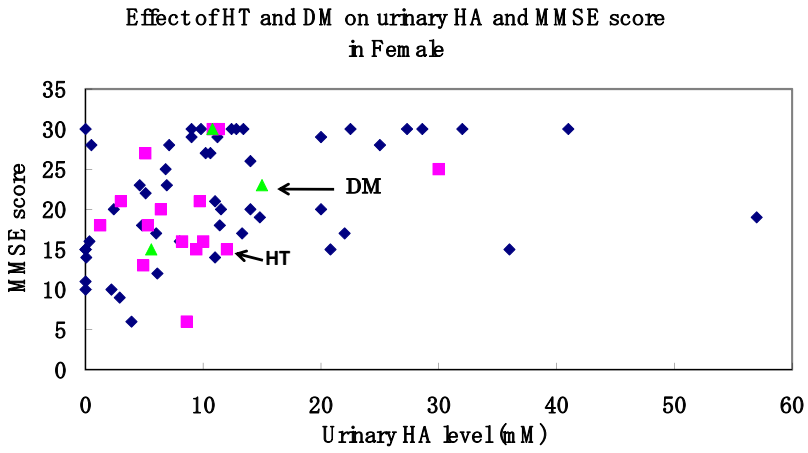
Effect of HT and DM on urinary HA and MMSE score in Female
MMSE score
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Urinary HA level (mM)
DM
HT

Fig. 4

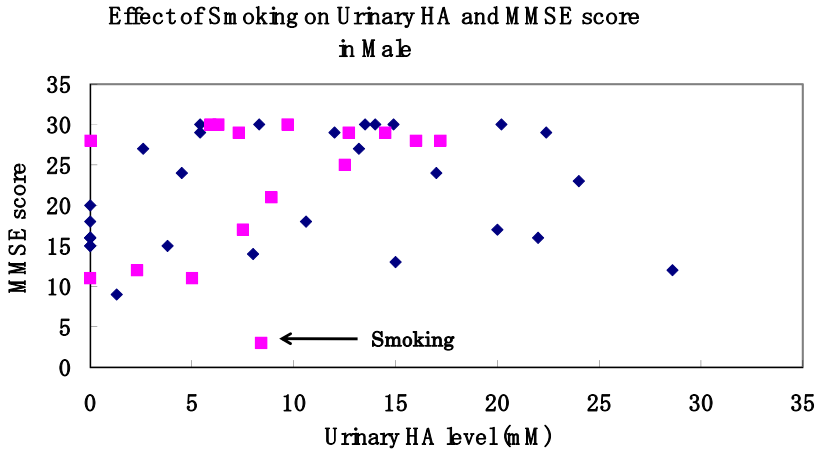
Effect of Smoking on Urinary HA and MMSE score in Male
MMSE score
35
30
25
20
15
10
5
0
Smoking
0
5
10
15
20
25
30
35
Urinary HA level (mM)

Fig. 5

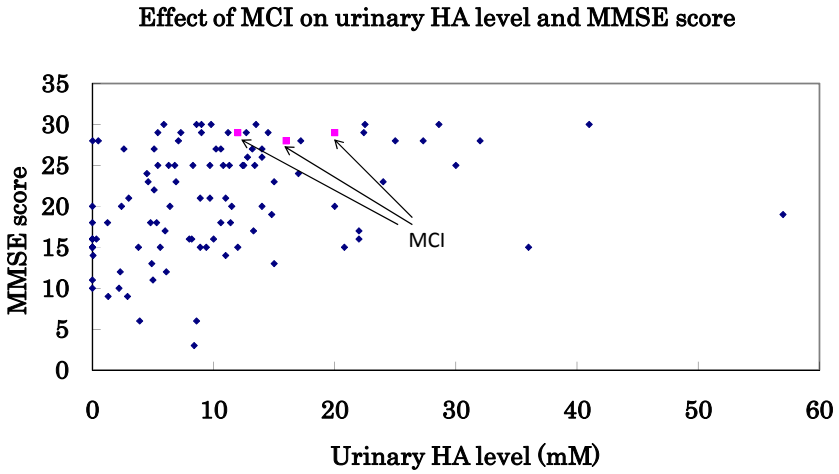
Effect of MCI on urinary HA level and MMSE score
MMSE score
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Urinary HA level (mM)
MCI

Fig. 6

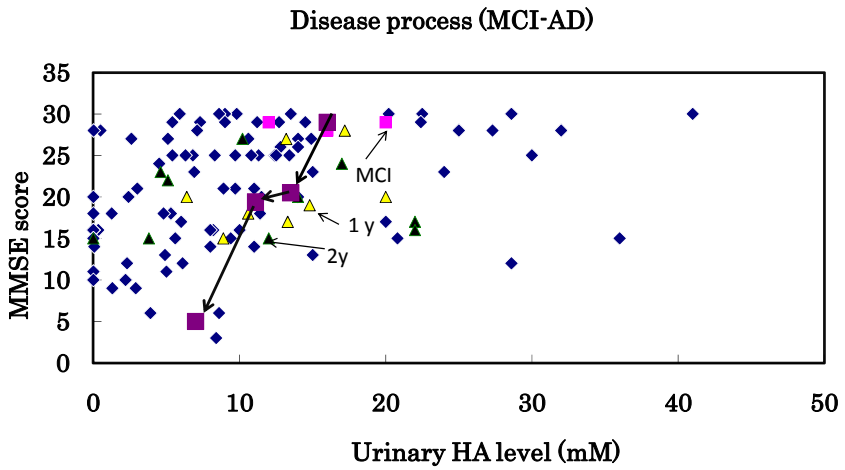
Disease process (MCI-AD)
MMSE score
35
30
25
20
15
10
5
0
0
10
20
30
40
50
Urinary HA level (mM)
MCI
1 y
2y

Fig. 7

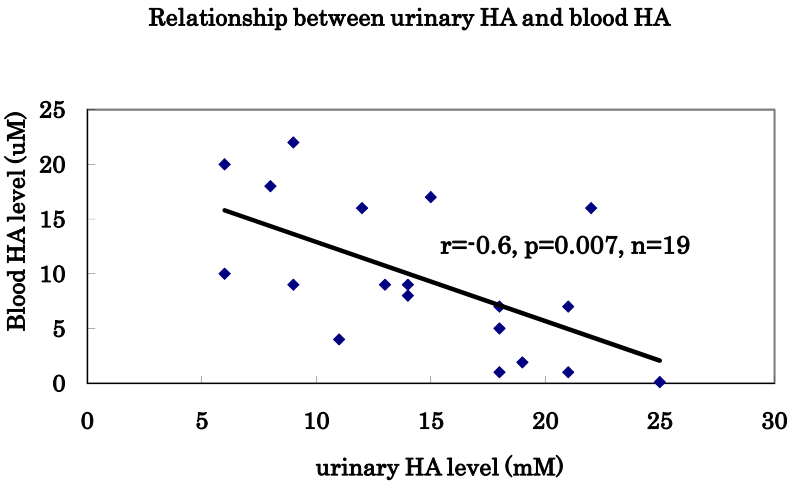
Relationship between urinary HA and blood HA
Blood HA level (uM)
25
20
15
10
5
0
0
5
10
15
20
25
30
urinary HA level (mM)
r=-0.6, p=0.007, n=19

Fig. 8