Serum albumin to globulin ratio is related to cognitive decline via reflection of homeostasis: a nested case-control study

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Abstract

Background: Recent research suggests that several pathogenetic factors, including aging, genetics, inflammation, dyslipidemia, diabetes, and infectious diseases, influence cognitive decline (CD) risk. However, no definitive candidate causes have been identified. The present study evaluated whether certain serum parameters predict CD.

Methods: A total of 151 participants were assessed for CD using the Mini-Mental State Examination (MMSE), and 34 participants were identified as showing CD.

Results: Among CD predictive risk factors, *Helicobacter pylori* seropositivity was significantly predictive of CD risk, more so than classical risk factors, including white matter lesions and arterial stiffness [adjusted odds ratio (OR) = 4.786, 95% confidence interval (CI) = 1.710–13.39]. A multivariate analysis indicated that the albumin to globulin (A/G) ratio was the only factor that significantly lowered CD risk (OR = 0.092, 95% CI = 0.010–0.887). A/G ratio also was positively correlated with MMSE scores and negatively correlated with disruption of homeostatic factors (i.e., non-high-density lipoprotein, hemoglobin A1c, and high-sensitive C-reactive protein).

Conclusions: The current study results suggest that the A/G ratio is related to cognitive decline and may reflect homeostatic alterations.

Keywords: Albumin to globulin ratio, Cognitive decline, *Helicobacter pylori*, Homeostatic alteration, Mini-mental state examination

Background

Improvements in health care support have greatly extended average life expectancy, resulting in a substantial increase in the number of elderly individuals worldwide. Some forms of memory impairment are observed among elderly adults and can be predictive of age-related cognitive decline associated with Alzheimer’s disease (AD) [1] and other dementias. Rate of memory impairment varies based on several factors, including age, sex, types of cognitive tasks assessed, education, and emotional state [2].

Previous reports have noted several causes for cognitive decline (CD). For instance, infection can cause both direct and indirect decrements. The association between *Helicobacter pylori* (*H. pylori*) infection and AD has recently been addressed [3], and other infections [i.e., *Chlamydia pneumoniae* (*C. pneumoniae*), cytomegalovirus, and herpes simplex virus type1] may influence AD manifestation [4]. Furthermore, inflammation-mediated damage in the apolipoprotein E (ApoE) allele 4 suggests a plausible marker for cognitive impairment, possibly due to increased viral replication, which could eventually lead to AD [5]. One way to affect this relationship is by controlling risk factors (e.g., diabetes, cholesterol, hypertension, stroke, or smoking) that could help alleviate physiological dementia risk factors [6]. A common factor is chronic and systemic...
inflammation, which leads to increased levels of several proinflammatory cytokines that subsequently promote CD progression [7]. Chronic and systemic inflammation also induces atherosclerosis [8] and atherosclerosis-promoted cognitive impairment [9].

There is growing interest in identifying individuals who have not yet demonstrated CD but could be at greater risk for developing dementia. This is because cognitive impairment responds much better to treatment during early compared to advanced illness stages. With substantial increases in dementia incidence, early detection of possible precursors, diagnostics, treatment, and control of modifiable risk factors are highly important [10]. Insight is needed regarding the specific risk factors that predict CD incidence. Elucidation of these factors will help identify individuals with CD who are at the highest risk for developing AD in the near future.

Thus, the aim of the present nested case control study was to evaluate whether certain serum parameters, commonly measured during routine health checkups including magnetic resonance imaging (MRI) and pulse wave velocity as a marker of arterial stiffness, could be viable predictors of CD incidence.

Methods

Study participants

The present study consisted of self-administered questionnaires and medical examinations, including blood tests, conducted at the Kyoto Industrial Health Association. From 2003 to 2004, 488 Japanese participants completed a baseline epidemiological survey [11]. Basic cognitive functioning was assessed for 273 participants from 2006 to 2008 and for 290 participants from 2012 to 2014. A group of 151 participants (101 men and 50 women), with normal cognition in 2006–2008, attended follow-up visits during both the 2006–2008 and 2012–2014 periods. We included all of these 151 patients in our study in order to avoid selection bias. The Ethics Board from the Kyoto Prefectural University of Medicine approved the study protocol (G-144). After we explained the purpose of the study, written informed consent was obtained from all participants.

Cognitive testing

The Mini-Mental State Examination (MMSE) is a brief, but universal, 30-point measure of cognitive status [12]. The MMSE has become one of the most widely used cognitive screening instruments for CD, which covers various cognitive domains. Specifically, the MMSE is used to estimate the severity of cognitive impairment and assess longitudinal changes in cognitive status. Trained neurologists or a neuropsychologist determined the MMSE scores as described previously [13]. A score ≤ 27 is considered reflective of cognitive impairment [14]. We were able to identify 34 participants as suitable for the CD group as they produced MMSE scores between 28–30 points in 2006–2008 and scores from 24–27 in 2012–2014. Similarly, 117 participants were defined as the control group, with scores from 28–30 in 2006–2008 that did not decrease when assessed in 2012–2014. The time between the two cognitive evaluations was not significantly different between the control (mean = 5.74 years) and CD (mean = 5.76 years) groups.

The verbal fluency test is a well-established method for evaluation of cognitive function [15]. All participants also completed a verbal fluency test. In this task, as in previous reports, the participants were asked to provide as many words beginning with Ta and Ka as they could recall [13].

Medical information and blood biochemistry

The present study evaluated medical information obtained via self-administered questionnaires (education level, anamnesis at baseline and in 2012–2014, medication, frequency of depressive symptoms, smoking, and drinking habits). Instrumental activities of daily living (IADL) and metabolic equivalents (METs) were assessed as previously reported [16, 17]. The scoring guidelines recommend adding an additional point for people with less than 13 years of education [18]. Furthermore, blood chemistry data [triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), non-HDL-C, total protein, albumin, A/G ratio, creatinine, uric acid, hemoglobin A1c (HbA1c), high sensitive C-reactive protein (hsCRP), and antibodies against C. pneumoniae, and H. pylori antibodies] were assessed. An IgG index above 1.1, and an IgA index above 1.1, was defined as criteria for C. pneumoniae positivity [19], and a cutoff point greater than 2.3 for the ELISA VALUE indicated H. pylori positivity [20]. The following anthropometry data were also obtained during the health check-ups: weight, height, and systolic and diastolic blood pressure. Anamnesis and medication history were assessed using a questionnaire. Hypertension was resting systolic blood pressure ≥ 140 mmHg or being treated for hypertension. Diabetes mellitus was defined as HbA1c ≥ 6.5% and dyslipidemia as triglycerides ≥ 150 or HDL ≤ 40. Additionally, the pulse wave velocity [21], which is a potential marker of arterial stiffness, was measured in 2006–2008 and 2012–2014.

Apolipoprotein E genotyping

Genomic DNA was extracted from the buffy coat fraction of each participant’s blood sample. Genotyping was performed using polymerase chain reaction (PCR) with the following primers; forward: ACGAGACCATGAAG GAGTTGAA and reverse: ACCTGCTCCTTCACCTCG TCCAG. Amplification of the genomic DNA resulted in a PCR product = 514 bp, which was subjected to a direct
sequence or PCR-restriction fragment length polymorphism analysis [22]. The ApoE isoforms differed in cysteine and arginine content at positions 112 and 158: ApoE-e2: Cys (TGC), Cys (TGC), ApoE-e3: Cys (TGC), Arg (CGC), ApoE-e4: Arg (CGC), Arg (CGC). ApoE status was classified as ε4 carriers for participants with the ApoE4 isoform (phenotypes ε2/4, ε3/4, ε4/4) and as non-ε4 carriers for participants without the ApoE4 isoform (phenotypes ε2/2, ε2/3, ε3/3).

Scoring white matter and periventricular hyperintensities
Brain MRI was performed using a 1.5-T scanner. MRI was performed to assess different types of hyperintense signal abnormalities surrounding the ventricles, and deep white matter abnormalities were evaluated as deep white matter lesions (DWL) and periventricular hyperintensities (PVH), as previously reported [13]. MRI cerebrovascular staging was carried out using the Fazekas classification [23].

Statistical analyses
Continuous variables are expressed as means ± standard deviations (SDs) or median [range], and categorical data are expressed as sums and percentages. Inter-group comparisons were performed using unpaired t-tests for continuous variables or Mann–Whitney U-tests, and the chi-square or Fisher’s exact tests for categorical variables (sex, ApoE4, education, depressive symptoms, baseline and 2012–2014 period anamnesis, C. pneumonia and H. pylori seropositivity, drinking and smoking prevalence, DWL, and PVH). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression analyses in which CD was the dependent variable and age, sex, ApoE4 status, education, smoking and drinking habits, and baseline anamnesis were the independent variables. Significant predictors from the logistic regression analysis were considered independent variables in the multiple logistic regression analysis using a stepwise forward selection method. A Spearman’s rank correlation coefficient was calculated to confirm whether serum A/G ratio was related to MMSE scores, pulse wave velocity, and hsCRP, as well as significant variables from the logistic regression analysis. All statistical tests were two-tailed, and differences with a p-value < 0.05 were considered statistically significant. SPSS software (version 18.0) was used for all statistical analyses.

Results
Participant characteristics
Table 1 shows participant characteristics, including anthropometric measures, blood chemistry data, questionnaire responses, and the number for each item between the control and CD groups. The mean age (± standard deviation: SD) for the control group was 59.4 (±5.9) years, compared to 61.2 (±4.6) years for the CD group. There were no significant differences between the anthropometric measures of the two groups. Although the CD group did not show significantly decreased scores on verbal fluency tasks in 2006–2008, their verbal fluency scores significantly decreased in 2012–2014. Furthermore, no significant differences in depressive symptoms, IADL, or METs were observed between the control and CD groups. The distribution of ApoE4 genotypes was in the Hardy–Weinberg equilibrium (control group: p = 0.621; CD group: p = 0.565). The ApoE4 allele distribution was not significantly different between the control and CD groups.

Associations between CD and control participant characteristics
Non-HDL-C, total protein, HbA1c, H. pylori seropositivity, and pulse wave velocity during both 2006–2008 and 2012–2014 were significantly higher in the CD group compared to the control group (Table 1). In contrast, the A/G ratio was significantly lower in the CD group (Table 1).

To determine variables significantly associated with CD, a logistic regression analysis adjusted for age, sex, ApoE4 status, education, smoking and alcohol drinking habits, and anamnesis was performed. The variables selected by this analysis were MRI evaluation, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), triglycerides, total cholesterol, HDL, non-HDL, total protein, albumin, A/G ratio, creatinine, uric acid, HbA1c, hsCRP, C. pneumoniae and H. pylori seropositivity, pulse wave velocity, education, and ApoE4 status (Tables 2 and 3). From a diagnostic imaging viewpoint (Table 2), the odds of DWL grade 1 and 2, which were evaluated by a Fazekas classification during the 2nd follow-up, showed significant higher values for CD group. As shown in Table 3, non-HDL-C, A/G ratio, HbA1c, and H. pylori seropositivity were predictive of CD.

Next, a multivariate analysis was performed with all of the significant variables considered simultaneously: non-HDL-C, A/G ratio, HbA1c, and H. pylori seropositivity (Table 4). Based on a stepwise forward selection method, A/G ratio was significantly predictive with a low OR (OR = 0.092, 95% CI = 0.010–0.887), and H. pylori seropositivity was significantly predictive with a high OR (OR = 4.468, 95% CI = 1.535–13.00). Therefore, A/G ratios were significantly positive correlation of MMSE scores (during both 2006–2008 and 2012–2014), and negative correlation with non-HDL-C, HbA1c, and hsCRP (Table 5).

Discussion
Although no single cause for cognitive impairment has been identified, recent research suggests that several pathogenetic factors such as aging, genetics, inflammation,
| Baseline                      | All | Male | Female |
|------------------------------|-----|------|--------|
| Age (years)                  | 117 | 34   | 82     |
| Sex (Female)                 | 35  | 15   | 28     |
| BMI (kg/m²)                  | 117 | 34   | 82     |
| SBP (mmHg)                   | 117 | 34   | 82     |
| DBP (mmHg)                   | 117 | 34   | 82     |
| Triglyceride (mg/dl)         | 115 | 34   | 81     |
| Total cholesterol (mg/dl)    | 115 | 34   | 81     |
| HDL-C (mg/dl)                | 115 | 34   | 81     |
| non-HDL-C (mg/dl)            | 115 | 34   | 81     |
| Total Protein (g/dl)         | 115 | 34   | 81     |
| Albumin (g/dl)               | 115 | 34   | 81     |
| A/G ratio                    | 99  | 30   | 71     |
| Creatinine (mg/dl)           | 115 | 34   | 81     |
| Uric acid (mg/dl)            | 115 | 34   | 81     |
| HbA1c                        | 117 | 34   | 82     |
| hsCRP (mg/dl)                | 71  | 26   | 45     |
| C. pneumoniae seropositivity | 39  | 14   | 25     |
| H. pylori seropositivity     | 58  | 27   | 31     |
| ApoE4 carrier                | 25  | 6    | 15     |
| ApoE4 not determined         | 2   | 1    | 1      |

### Anamnesis

| Hypertension                 | 35  | 14   | 21     |
| Hyperlipidemia               | 18  | 6    | 11     |
| Diabetes                     | 21  | 8    | 13     |
| History of stroke            | 1   | 0    | 0      |
| Education          | < 13 year | 53 | 23 | 45.3 (%) | 67.6 (%) | 0.049 | 32 | 12 | 39.0 (%) | 63.2 (%) | 0.122 | 21 | 11 | 60.0 (%) | 73.3 (%) | 0.526 |
|--------------------|-----------|----|----|----------|----------|--------|----|----|----------|----------|--------|----|----|----------|----------|------|
|                    | ≥ 13 year | 60 | 11 | 51.3 (%) | 32.4 (%) |        | 47 | 7  | 57.3 (%) | 36.8 (%) | 13     | 4  | 37.1 (%) | 26.7 (%) |        |
|                    | Not determined | 4 | 0  | 3.42 (%) | 0 (%)    |        | 3  | 0  | 3.66 (%) | 0 (%)    | 1      | 0  | 2.86 (%) | 0 (%)    |      |

| Alcohol drinking   | Current   | 78 | 15 | 66.7 (%) | 44.1 (%) | 0.0067 | 67 | 13 | 81.7 (%) | 68.4 (%) | 0.301 | 11 | 2  | 31.4 (%) | 13.3 (%) | 0.297 |
|                    | Former    | 3  | 2  | 25.6 (%) | 5.88 (%)  |        | 3  | 2  | 3.66 (%) | 10.5 (%)  | 0      | 0  | 0  | 0.0 (%)  | 0.0 (%)   |      |
|                    | Never     | 35 | 16 | 29.9 (%) | 47.0 (%)  |        | 11 | 4  | 13.4 (%) | 21.1 (%)  | 24     | 12 | 68.6 (%) | 80.0 (%) |        |
|                    | Not determined | 1 | 1  | 0.86 (%) | 2.94 (%)  |        | 1  | 0  | 1.22 (%) | 0.0 (%)   | 0      | 1  | 0  | 0.0 (%)  | 667 (%)   |      |

| Smoking (%)        | Current   | 21 | 7  | 179 (%)  | 20.6 (%) | 0.758  | 20 | 7  | 24.4 (%) | 36.8 (%) | 0.561 | 1  | 0  | 2.86 (%) | 0 (%)    | 0.221 |
|                    | Former    | 40 | 9  | 342 (%)  | 26.5 (%) |        | 40 | 8  | 48.8 (%) | 42.1 (%) | 0      | 1  | 0  | 667 (%)   | 0 (%)    |      |
|                    | Never     | 54 | 16 | 462 (%)  | 47.1 (%) |        | 21 | 4  | 25.6 (%) | 21.1 (%) | 33     | 12 | 94.3 (%) | 80.0 (%) |        |
|                    | Not determined | 2 | 2  | 1.71 (%) | 5.88 (%)  |        | 1  | 1  | 1.22 (%) | 0.0 (%)   | 1      | 2  | 2.86 (%) | 13.3 (%) |      |

In 2006-2008

| MMSE              | 34 | 295 ± 0.71 | 290 ± 0.75 | <0.001 | 82 | 19 | 29.6 ± 0.64 | 29.3 ± 0.67 | 0.092 | 35 | 15 | 29.4 ± 0.85 | 286 ± 0.72 | 0.004 |
| Ta                | 34 | 10 [4-18]  | 9 [4-18]   | 0.274  | 82 | 19 | 10 [4-18]  | 9 [4-18]   | 0.501 | 35 | 15 | 10 [4-18]  | 8 [4-18]   | 0.469 |
| Ka                | 34 | 12 [2-21]  | 11 [5-17]  | 0.052  | 82 | 19 | 12 [4-21]  | 11 [5-17]  | 0.100 | 35 | 15 | 12 [2-21]  | 10 [5-17]  | 0.425 |

Pulse wave velocity (m/sec)

| 116 | 34 | 152 ± 2.68 | 166 ± 2.73 | 0.013 | 81 | 19 | 15.4 ± 2.56 | 17.8 ± 2.94 | <0.001 | 35 | 15 | 14.9 ± 2.95 | 149 ± 1.25 | 0.959 |

In 2012-2014

| MMSE              | 34 | 295 ± 0.67 | 260 ± 0.88 | <0.001 | 82 | 19 | 29.6 ± 0.54 | 25.8 ± 0.87 | <0.001 | 35 | 15 | 29.3 ± 0.87 | 26.1 ± 0.91 | <0.001 |

Verbal fluency tasks

| Ta                | 34 | 9 [2-17]   | 8 [3-24]   | 0.026  | 82 | 19 | 9 [2-15]   | 8 [4-13]   | 0.055 | 35 | 15 | 9 [2-17]   | 8 [3-24]   | 0.237 |
| Ka                | 34 | 10 [3-20]  | 9 [5-18]   | 0.038  | 82 | 19 | 10 [3-20]  | 9 [5-18]   | 0.440 | 35 | 15 | 10 [3-20]  | 8 [5-14]   | 0.017 |

Pulse wave velocity (m/sec)

| 116 | 34 | 170 ± 2.99 | 185 ± 3.28 | 0.011 | 81 | 19 | 17.2 ± 2.65 | 19.4 ± 3.76 | 0.026 | 35 | 15 | 16.4 ± 3.64 | 17.4 ± 2.20 | 0.329 |

IADL              | 34 | 13 [9-13]  | 13 [7-13]  | 0.463  | 81 | 19 | 13 [9-13]  | 13 [9-13]  | 0.595 | 35 | 15 | 13 [10-13] | 13 [7-13]  | 0.580 |

METS              | 117 | 145 [2.0-48.4] | 16.9 [1.5-50.4] | 0.161 | 81 | 19 | 15.2 [2.5-48.4] | 17.1 [1.5-50.4] | 0.195 | 35 | 15 | 14.5 [2.0-42.5] | 142 [1.5-400] | 0.335 |

Anamnesis

| Hypertension     | 63 | 20 | 53.9 (%) | 58.8 (%) | 0.0697 | 45 | 15 | 54.9 (%) | 78.9 (%) | 0.071 | 18 | 5  | 51.4 (%) | 33.3 (%) | 0.355 |
| Hyperlipidemia   | 61 | 25 | 52.1 (%) | 73.5 (%) | 0.031  | 41 | 14 | 50.0 (%) | 73.7 (%) | 0.076 | 20 | 11 | 57.1 (%) | 73.3 (%) | 0.351 |
### Table 1 Participant characteristics at baseline and follow-up according to CD condition (Continued)

| Category         | Count | Rate (%) | Count | Rate (%) | Test Statistic | p-value |
|------------------|-------|----------|-------|----------|----------------|---------|
| Diabetes         | 26    | 10       | 22.2  | 29.4     | 0.372          | 0.415   |
| History of stroke| 6     | 1        | 5.13  | 2.94     | 0.822          | 0.640   |
| Feel depression  |       |          |       |          |                |         |
| Nothing          | 108   | 31       | 92.3  | 91.2     | 0.804          | 0.888   |
| Sometimes        | 8     | 3        | 6.84  | 8.82     | 0.448          |         |
| Always           | 1     | 0        | 0.86  | 0 (%)    | 0.162          |         |

Category differences are analyzed by t-test, IADL, METs and Verbal fluency tasks are analyzed by U-test. 
Chi-square test for ApoE, smoking and alcohol drinking habit, or Fisher's exact test for sex, H. pylori seropositivity, C. pneumoniae seropositivity, hypertension, hyperlipidemia, diabetes, history of stroke, education CD cognitive decline, MMSE Mini-Mental State Examination, A/G albumin to globulin, H. pylori Helicobacter pylori, C. pneumoniae Chlamydia pneumoniae, ApoE apolipoprotein E, SD standard deviation, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, IADL instrumental activities of daily living, METs metabolic equivalents, HDL-C high-density lipoprotein cholesterol, HbA1c hemoglobin A1c, hsCRP high sensitive C-reactive protein.
dyslipidemia, diabetes, and infectious diseases are plausible candidates. The present results revealed that *H. pylori* seropositivity tended to be related to more severe CD incidence. Furthermore, the present study explored, for the first time, an association between A/G ratios and CD incidence.

Growing evidence has underscored a mechanistic link between cholesterol metabolism in the brain and the formation of amyloid plaques. Excess brain cholesterol has been associated with increased formation and deposition of \( \beta \)-amyloid from amyloid precursor proteins. Indeed, non-HDL-C was associated with CD incidence in the present study. Cholesterol-lowering statins have become a focus for AD research [24]. Moreover, genetic polymorphisms associated with pivotal points in cholesterol metabolism within brain tissues may contribute to AD risk and pathogenesis. A recent meta-analysis indicated the positive predictive value of the ApoE4 allele for progression from cognitive impairment to AD-type dementia [25]. Although there is convincing evidence to suggest that ApoE4 is the main predictor for progression from CD to AD, ApoE4 may not be a risk factor for CD incidence. For instance, the present findings revealed that ApoE4 status was not associated with CD incidence.

Cognitive impairment can present with mild deficits affecting one or multiple cognitive domains. Size and location of white matter lesions and ischemic and hemorrhagic strokes are associated with varying clinical presentation in these patients [26]. Concerning the link between CD incidence and cerebrovascular lesion occurrence, we found that the CD group showed not only decreased MMSE scores but also progression of DWL Fazekas grade. In general, white matter lesions are a key vascular, cognitive impairment marker. Although DWL and PVH were not predictive of CD incidence in the present study, CD group indicated DWL grade progression.

Recent studies have shown that *H. pylori* infection leads to cognitive impairment [3]. *H. pylori* infection likely influences cognitive impairment by increasing neurodegenerative lesions, especially neurofibrillary tangles.
and neuronal loss via ischemic lesions. *H. pylori* infection evolving over many years could also cause chronic gastric and plasmatic inflammation, thus inducing a chronic inflammation model plausibly responsible for cerebrovascular lesions and the exacerbation of neurodegeneration [3]. Moreover, when accomplished, *H. pylori* eradication is beneficial for improving cognitive and functional states among patients, perhaps altering the progressive nature of AD [27]. Additionally, chronic inflammation might be an underlying factor for an association between metabolic syndrome and CD [28]. The present study suggests a relationship between inflammation, disruption of homeostatic factors [e.g., cholesterol metabolism (dyslipidemia), HbA1c

| Table 3 | Logistic regression analysis according to CD condition |
|---------|-------------------------------------------------------|
| Number  | Control | CD | OR | 95% CI | Control | CD | OR | 95% CI | Control | CD | OR | 95% CI |
| BMI     | 117     | 34 | 1.073 | 0.998-1.154 | 1.060 | 0.910-1.235 | 1.024 | 0.868-1.207 |
| SBP     | 117     | 34 | 0.999 | 0.938-1.151 | 0.994 | 0.971-1.017 | 0.971 | 0.941-1.002 |
| DBP     | 117     | 34 | 1.010 | 0.970-1.052 | 0.998 | 0.954-1.044 | 0.997 | 0.941-1.036 |
| Triglyceride | 115 | 34 | 1.003 | 0.969-1.011 | 1.002 | 0.994-1.010 | 1.002 | 0.989-1.015 |
| Total cholesterol | 115 | 34 | 1.001 | 0.998-1.102 | 1.009 | 0.995-1.102 | 1.009 | 0.995-1.102 |
| HDL-C   | 115     | 34 | 0.972 | 0.946-0.999 | 0.977 | 0.950-1.004 | 0.973 | 0.942-1.005 |
| non-HDL-C | 115 | 34 | 1.014 | 0.984-1.051 | 1.002 | 0.978-1.027 | 1.002 | 0.989-1.018 |
| Total Protein | 115 | 34 | 2.971 | 1.023-8.622 | 3.575 | 1.088-11.74 | 3.219 | 0.938-11.04 |
| Albumin | 115     | 34 | 2.035 | 0.921-1.371 | 1.980 | 0.923-1.482 | 1.852 | 0.921-1.627 |
| A/G ratio | 99   | 30 | 0.063 | 0.006-0.619 | 0.032 | 0.003-0.379 | 0.037 | 0.003-0.470 |
| Creatinine | 115  | 34 | 2.688 | 0.207-34.86 | 2.852 | 0.185-43.87 | 2.235 | 0.135-36.88 |
| Uric acid | 115   | 34 | 1.007 | 0.695-1.549 | 1.037 | 0.707-1.520 | 1.068 | 0.730-1.563 |
| HbA1c   | 117     | 34 | 2.433 | 1.156-5.118 | 2.405 | 1.131-5.112 | 2.586 | 1.036-6.455 |
| hsCRP   | 71      | 26 | 12.95  | 0.104-1617 | 66.97  | 0.303-14824 | 42.42  | 0.127-14225 |
| C. pneumoniae seropositivity | 117 | 34 | 3.507 | 1.398-8.801 | 4.867 | 1.754-13.50 | 4.786 | 1.710-13.39 |
| H. pylori seropositive | 117  | 34 | 4.255 | 1.422-12.73 | 4.468 | 1.422-13.32 | 1.125 | 0.963-1.313 |
| Pulse wave velocity in 2006-2008 | 116 | 34 | 1.184 | 1.021-1.371 | 1.209 | 1.028-1.422 | 1.179 | 0.989-1.404 |
| Pulse wave velocity in 2012-2014 | 116 | 34 | 1.158 | 1.015-1.322 | 1.145 | 0.989-1.327 | 1.125 | 0.963-1.313 |
| Education | 113  | 34 | 2.129 | 0.927-4.890 | 2.035 | 0.921-4.890 | 2.359 | 1.092-5.132 |
| ApoE4 carrier | 115  | 33 | 0.781 | 0.310-1.970 | 0.781 | 0.310-1.970 | 0.781 | 0.310-1.970 |

Model I: Adjusted for age and sex  
Model II: Adjusted for age, sex, apolipoprotein E4, education, smoking and alcohol drinking habits  
Model III: Adjusted for age, sex, apolipoprotein E4, education, smoking and alcohol drinking habits, hypertension, hyperlipidemia and diabetes  
CD cognitive decline, OR odds ratio, CI confidence interval, A/G albumin to globulin, H. pylori *Helicobacter pylori*, C. pneumoniae *Chlamydia pneumoniae*, ApoE apolipoprotein E, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HDL-C high-density lipoprotein cholesterol, HbA1c hemoglobin A1c, hsCRP high sensitive C-reactive protein

| Table 4 | Multiple logistic regression analysis with stepwise forward selection based on CD condition |
|---------|----------------------------------------------------------|
| Multivariate | OR | 95% CI | Stepwise forward selection | OR | 95% CI |
| non-HDL-C | 1.011 | 0.999-1.024 | 1.011 | 0.999-1.024 |
| A/G ratio | 0.265 | 0.022-3.215 | 0.092 | 0.010-0.887 |
| HbA1c | 1.743 | 0.782-3.883 | 1.743 | 0.782-3.883 |
| H. pylori seropositive | 2.455 | 1.422-12.73 | 1.535-13.00 |
| Sex | 1.493 | 0.522-4.270 | 1.493 | 0.522-4.270 |
| Age | 1.079 | 0.983-1.183 | 1.079 | 0.983-1.183 |

Non-HDL-C, A/G ratio, HbA1c, H. pylori seropositive, Sex, Age: All variables were entered into models, and variables that were not statistically significant (p > 0.05) were eliminated stepwise.  
MMSE score in 2006-2008: 0.010.  
MMSE score in 2012-2014: 0.010.  
hsCRP: 0.369.  
Pulse wave velocity in 2006-2008: 0.579.  
Pulse wave velocity in 2012-2014: 0.579.

| Table 5 | Correlations between A/G ratio and variables used in the multivariate analysis |
|---------|---------------------------------|
| A/G ratio Coefficient p value | 0.187 | 0.034 |
| MMSE score in 2006-2008 | 0.026 | 0.003 |
| non-HDL-C | -0.230 | 0.009 |
| HbA1c | -0.193 | 0.029 |
| hsCRP | -0.369 | 0.001 |
| Pulse wave velocity in 2006-2008 | -0.047 | 0.598 |
| Pulse wave velocity in 2012-2014 | -0.001 | 0.989 |

A/G albumin to globulin, MMSE Mini-Mental State Examination, HDL-C high-density lipoprotein cholesterol, HbA1c hemoglobin A1c, hsCRP high sensitive C-reactive protein

MMSE cognitive decline, OR odds ratio, CI confidence interval, HDL-C high-density lipoprotein cholesterol, HbA1c hemoglobin A1c, A/G albumin to globulin, H. pylori *Helicobacter pylori*
targets. Secondly, we diagnosed widely conducted and can be used to identify candidate involvement gastric testing. The primary limitation of this case has a reduced likelihood of detecting a true effect, nested these variables. While a study with low statistical power the participants differed between the two groups, logistic male and female participants, and the education level of were a relatively small number of participants in the CD sum, the A/G ratio may be a very reliable index for CD incidence caused by disruption of homeostasis.

A few study limitations should be noted. First, there were a relatively small number of participants in the CD group. Therefore, an analysis of data from male and female participants separately would not be useful because of the low statistical power. Although, the proportion of male and female participants, and the education level of the participants differed between the two groups, logistic regression analysis was performed after adjusting for these variables. While a study with low statistical power has a reduced likelihood of detecting a true effect, nested case–control studies with small sample sizes are still widely conducted and can be used to identify candidate targets. Secondly, we diagnosed H. pylori infections via serum antibody detection, whereas the gold standard involves gastric testing. The primary limitation of this serologic test is its inability to discriminate between current and old infections. However, H. pylori induces humoral and cellular immune responses that can affect or perpetuate neural tissue damage [33]. This pathogen may influence the pathophysiology of AD by inducing vascular disorders that have been implicated in endothelial damage and neurodegeneration. Overall, the results of the present and previous studies suggest that both current and old H. pylori infections contribute to CD by inducing neural tissue damage. One other issue was that A/G ratios, as well as other biological markers, were only determined once, during the baseline survey. Conversely, cognitive data were available at both baseline and follow-up. Therefore, larger prospective trials are needed to better assess how A/G ratios are associated with CD incidence.

Conclusions
The current study observed that A/G ratios, which are part of routinely administered laboratory tests, could reflect changes in homeostatic factors. Additional investigations are expected to show that the modification of A/G ratios could lead toward novel and effective strategies for predictive CD screening.

Abbreviations
A/G: Albumin to globulin; AD: Alzheimer’s disease; ApoE: Apolipoprotein E; BMI: Body mass index; C. pneumoniae: Chlamydia pneumoniae; CD: Cognitive decline; CI: Confidence interval; DBP: Diastolic blood pressure; DWL: White matter lesions; H. pylori: Helicobacter pylori; Hba1c: Hemoglobin A1c; HDL-C: High-density lipoprotein cholesterol; hsCRP: High sensitive C-reactive protein; IADL: Instrumental activities of daily living; METs: Metabolic equivalents; MMSE: Mini-mental state examination; MRI: Magnetic resonance imaging; OR: Odds ratio; PVH: Periventricular hyperintensities; SBP: Systolic blood pressure; SD: Standard deviation

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Availability of data and materials
The dataset used in this article is not published, but anonymous data can be available at request to the authors.

Authors’ contributions
TKoyama analyzed the data, and wrote the manuscript. NK, MN, TM, YW designed the idea of the study. EO, DM, IW, FM, MK, TKasai, YO, TY, TT, IM, SM collected the samples. AT, KY, KT were in charge of the MR evaluations. All authors contributed to approval of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.
Ethics approval and consent to participate
The Ethics Board from the Kyoto Prefectural University of Medicine approved the study protocol (G-144). After we explained the purpose of the study, written informed consent was obtained from all participants.

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