Concurrent cardiac transthyretin and brain β amyloid accumulation among the older adults: The Hisayama study

Hideomi Hamasaki1 | Masahiro Shijo1,2 | Ayaka Nakamura1 | Hiroyuki Honda1 | Yuichi Yamada3 | Yoshinao Oda3 | Tomoyuki Ohara4,5 | Toshiharu Ninomiya5,6 | Toru Iwaki1

1Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
2Department of Internal Medicine, Fukuoka Dental College Medical and Dental Hospital, Fukuoka, Japan
3Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
4Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
5Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
6Department of Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence
Toru Iwaki, Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, Email: iwaki@np.med.kyushu-u.ac.jp

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Abstract
Previous studies have revealed risk for cognitive impairment in cardiovascular diseases. We investigated the relationship between degenerative changes of the brain and heart, with reference to Alzheimer's disease (AD) pathologies, cardiac transthyretin amyloid (ATTR) deposition, and cardiac fibrosis. A total of 240 consecutive autopsy cases of a Japanese population-based study were examined. β amyloid (Aβ) of senile plaques, phosphorylated tau protein of neurofibrillary tangles, and ATTR in the hearts were immunohistochemically detected and graded according to the NIH-AA guideline for AD pathology and as Tanskanen reported, respectively. Cerebral amyloid angiopathy (CAA) was graded according to the Vonsattel scale. Cardiac fibrosis was detected by picrosirius red staining, followed by image analysis. Cardiac ATTR deposition occurred after age 75 years and increased in an age-dependent manner. ATTR deposition was more common, and of higher grades, in the dementia cases. We subdivided the cases into two age groups: ≤90 years old (n = 173) and >90 years old (n = 67), which was the mean and median age at death of the AD cases. When adjusted for age and sex, TTR deposition grades correlated with Aβ phase score (A2–3), the Consortium to Establish a Registry for AD score (sparse to frequent), and high Braak stage (V–VI) only in those aged ≤90 years at death. No significant correlation was observed between the cardiac ATTR deposition and CAA stages, or between cardiac fibrosis and AD pathologies. Collectively, AD brain pathology correlated with cardiac TTR deposition among the older adults ≤90 years.

KEY WORDS
Alzheimer's disease, amyloid β, ATTRwt, brain-heart interaction, transthyretin

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1 | INTRODUCTION

Alzheimer's disease (AD) is a major cause of dementia among older adults and is listed among the biggest public health issues worldwide. In Japan, the prevalence of AD has increased with longevity. Our pathological cohort study of autopsied Hisayama residents recently revealed a trend of dementia-related pathology [1–3]. Lifestyle-related diseases, such as abnormal lipid metabolism [4], insulin resistance [5], diabetes mellitus [6], and obesity [7] are risk factors for AD. Some of these overlap as risks for cardiovascular disease [8, 9]. Aging is a risk for both amyloidogenic β-amyloid (Aβ) deposition in the brain and ATTR deposits in the heart [10].

Cardiovascular diseases, such as atrial fibrillation (AF) and heart failure (HF), are risk factors for cerebral and cardiovascular systems, known as brain–heart axis, has been studied worldwide [11–13]. Although AF and HF are risk factors for all-cause dementia [14, 15], the causal relationship between AF and AD or HF and AD is controversial [16–18]. Recent studies have demonstrated that markers of cardiac stretch/stress are correlated with the prevalence of dementia. Higher serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) is an independent risk marker for dementia [19] and AD [20]. In addition, another study revealed the correlation between the elevation of serum NT-proBNP and myocardial fibrosis, one of the common histological features in HF, from the subclinical stage [21]. Interstitial myocardial fibrosis has been reported to be important early in failed heart [22].

Transthyretin (TTR) is a major carrier of thyroxine (T4) in cerebrospinal fluid and blood. More than 120 mutations in the TTR gene contribute to its deposition in tissues in the form of amyloids. Wild-type TTR (ATTRwt) is the amyloid precursor in the cardiac deposits found in older individuals. In the central nervous system, TTR is mainly produced in choroid plexus epithelial cells and in neurons under stress [23]. TTR can directly interact with the Aβ of AD in vitro and over expression of human TTR in human AD transgenic mouse models can suppress the AD phenotype [24, 25]. However, the relationship between cardiac TTR amyloid (ATTR) and brain Aβ deposition in human has not yet been clarified.

In this study, we evaluated cardiac fibrosis using picrosirius red (PSR) staining, followed by image analysis. The deposition of cardiac amyloid was screened using microscope with polarizer filters on direct first scarlet staining and Congo red staining. The degree of amyloid deposition was graded against TTR by immunohistochemistry. The relative burden of cardiac amyloid was compared statistically with the relative severity of AD pathology as determined by conventional pathologic criteria. Considering that dementia is an urgent issue in aging cohort, it is extremely important to accurately identify the risk factors for dementia. Therefore, this study aimed to clarify the association of cardiac lesions with AD pathologies.

2 | MATERIALS AND METHODS

2.1 | Subjects

This study included pathological specimens from a total of 240 autopsies performed between 2009 and 2014 on residents of Hisayama town in Fukuoka Prefecture, Japan (mean age at death: 83.0 ± 10.6 years; age range: 42–105 years). The participation rate was 80% of all residents aged 40 years or more, and the autopsy examination rate was approximately 75%. Characteristics of the examined cases are shown in Table 1. The clinical diagnosis of cognitive disorders was based on the Diagnostic and Statistical Manual of Mental Disorders, Third Edition—Revised (DSM-III-R) [26]. In addition, we also performed the revised version of Hasegawa's dementia scale (HDS-R) [27] to assess the cognitive function. The presence of dementia was defined clinically using the DSM-III-R and HDS-R. Details of the clinical survey have been previously described [28]. This study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University, and was conducted in accordance with the ethical standards described in the fifth revision of the Declaration of Helsinki, 2000.

2.2 | Histopathological study of the brain

Brains were weighed and fixed in 10% buffered formalin for at least 2 weeks. The brain specimens included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobe, hippocampus with entorhinal cortex, and transentorhinal cortex (at the level of the lateral geniculate body), calcarine cortex, and basal ganglia, including the nucleus basalis of Meynert, thalamus, midbrain, pons, medulla oblongata, and cerebellum. Sections were embedded in paraffin and routinely stained with hematoxylin and eosin stain and Klüver–Barrera stain. The specimens were immunostained with antibodies against phosphorylated MAPT (p-MAPT; clone AT8, mouse monoclonal, 1:500; Innogenetics, Ghent, Belgium) and Aβ (clone 6F/3D, mouse monoclonal, 1:200; Dako, Glostrup, Denmark). Antigen retrieval for Aβ was performed by incubation in 90% formic acid for 1 h and that of other antibodies was performed by autoclaving in 0.01 M citrate buffer (pH 6). Sections were incubated with primary antibodies overnight at 4°C, and immunoreaction products were detected by employing the polymer immunocomplex method using the Envision system (Dako). Nuclei were counterstained with hematoxylin, and immunoreactivity was visualized using 3, 3′-diaminobenzidine (DAB,
Dojindo, Kumamoto, Japan). Senile plaques were detected using both a modified Bielschowsky method and immunohistochemistry for Aβ. The assessment of AD pathology was according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) guidelines [29], Braak and Braak stage [30, 31], and Thal scores [32]. These scores were combined to estimate the likelihood of AD pathological changes. The pathological diagnosis of AD was made according to the National Institute on Aging and the Alzheimer’s Association (NIA-AA) guidelines [32]. A diagnosis of AD was determined clinicopathologically according to the presence of AD pathologic changes and clinical evidence of dementia. A pathological examination for amyloid angiopathy was carried out in 228 out of 240 autopsy cases. The severity of cerebral amyloid angiopathy (CAA) was classified into three stages according to the Vonsattel scale [33], as follows:

1. Mild: Patchy Aβ deposition in vessel walls.
2. Moderate: Media is replaced by Aβ, without evidence of blood leakage.
3. Severe: There are double-barreled vessels, endothelial involvement, or perivascular leakage evidenced by the presence of erythrocytes or hemosiderin, or both.

### 2.3 Histopathological study of the heart

Hearts were weighed and fixed in 10% buffered formalin for at least 1 week. The cardiac specimens...
were collected from the wall of the left ventricle and were routinely stained with hematoxylin and eosin stain, Congo red stain, PSR stain, and direct fast scarlet stain. Immunohistochemistry for TTR was performed using anti-TTR antibody (EP2929Y, rabbit monoclonal, 1:3000, Abcam, Cambridge, UK) and was detected by employing an enhanced indirect immunoperoxidase method (Histofine Simple Stain MAX PO MULTI, Nichirei Biosciences, Tokyo, Japan). Cases with cardiac amyloid deposition were screened microscopically with Congo red and direct first scarlet staining using polarizer filters. Then, the degree of cardiac ATTR deposition was assessed immunohistochemically. The cardiac ATTR was graded as reported by Tanskanen [34]: 0 = no amyloid; 1 = small amounts of amyloid in the vascular walls or between the heart muscle cells; 2 = clearly detectable areas of amyloid in several visual fields, including vascular deposits; and 3 = large amounts of amyloid. Representative images of each ATTR grade are shown in Figure S1.

2.4 | Quantitative analysis of cardiac pathologies

For quantification of cardiac fibrosis, we performed image analysis of PSR-stained images using the ImageJ software (http://imagej.nih.gov/ij/; National Institutes of Health, Bethesda, MD, USA). To detect fine collagen fibers between cardiomyocytes, we examined three locations in each case and captured the images using a 20× objective lens with a total field size of 704 × 528 μm², which comprised 1600 × 1200 pixels (0.44 μm × 0.44 μm/pixel). The picric acid staining was separated from the Sirius-Red coloring using the Color Deconvolution plugin [35]. We then automatically set the threshold using the modified isodata method, extracted positive objects, and calculated the ratio of cardiac fibrosis to the total area of the region of interest using the ImageJ software.

2.5 | Statistical analysis

Statistical analysis was performed using JMP Pro 15 (SAS Institute, Cary, NC, USA). P values were calculated using Mann–Whitney U tests and likelihood ratio test. Logistic regression analysis was used to assess the odds ratio of cardiac ATTR deposition. ATTR grades were set as dependent variables in logistic regression analysis, which included age and sex; and other values were set as independent variables, such as presence of dementia, Aβ phase score, Braak stage, CERAD score, CAA stage, and history of AF or HF. Significance was defined as p < 0.05. Characteristics of overall and adjusted group are listed in Table 1.

3 | RESULTS

3.1 | ATTR deposition and AD-related pathologies in overall group

A total of 54 out of 240 autopsy cases (22.5%) were positive for cardiac ATTR. Cardiac ATTR deposition was not seen in anyone less than 75 years of age. Both the prevalence and grade of the deposition increased with aging (Figure 1A). ATTR deposition was more common in dementia cases (Figure 1B). There was no significant difference in ATTR deposition between male and female patients (Figure 1C). Regarding AD pathologies, cases with severe cardiac ATTR deposition was more frequently observed to be those with higher Aβ phase scores, Braak NFT stages, and CERAD scores (Figure 1D–F). A total of 82 out of 228 autopsy cases (36.0%) were positive for CAA. When subdivided by the presence of dementia, the prevalence of CAA was 22.3% (n = 25) in the subjects without dementia (n = 112) and 49.1% (n = 57) in the patients with dementia (n = 116). The CAA grades were positively correlated with Aβ phase scores, Braak NFT stages, and CERAD scores (Figure S2A–C). However, the CAA stages were not correlated with cardiac ATTR deposition (Figure 1G).

TTR grade 1 included cases with extremely small amounts of ATTR deposition. Because small amounts of cardiac ATTR rarely affect the cardiac function, we further investigated the cases with ATTR grades 2–3. For the statistical analysis, we subdivided Aβ phase score into A0–A1 (minimal cortical involvement of Aβ deposition) and A2–A3 (widespread involvement of Aβ deposition); CERAD stages into CERAD none (no neuritic plaques) and CERAD sparse to frequent (any neuritic plaques); and Braak NFT stage into stages 0–4 (none or limbic involvement of NFT) and stages 5–6 (cortical involvement of NFT). Logistic regression analysis demonstrated a positive association between ATTR deposition and age at death, the presence of dementia, and AD-related pathologies (Table 2). The cardiac ATTR deposition was not correlated with CAA stages (Table 2, p = 0.309). ATTR deposition became more severe according to the presence of HF (Table 3, p = 0.079). However, the correlation between ATTR deposition and AF was not significant (Table 3, p = 0.180).

3.2 | Cardiac fibrosis in overall group

Area of cardiac fibrosis slightly increased with aging (Figure 2A); however, significance was not observed between the area of cardiac fibrosis and variables, including aging, sex, dementia, Aβ phase score, Braak NFT stage, CERAD score, and CAA stage (Figure 2B–G). The area of cardiac fibrosis was significantly higher in the cases with HF compared with those without HF (ratio of cardiac fibrosis = 5.71 ± 0.65% vs. 4.12 ± 0.22%,
respectively, $p = 0.006$). However, the area of cardiac fibrosis was not significantly higher in the cases with AF compared with those without AF (ratio of cardiac fibrosis $= 5.22 \pm 0.67\%$ vs. $4.26 \pm 0.23\%$, respectively, $p = 0.179$).

### 3.3 Relationship between ATTR deposition and AD pathology according to age

When adjusted for age and sex, the ATTR grades were significantly higher in Braak NFT stages 5–6 only. The ATTR grades followed an upward trend in the Aβ deposition groups (Table 4). To clarify the association between cardiac ATTR deposition and AD pathologies among the older adults, we subdivided the cases into two age groups: ≤90 years old (n = 173) and >90 years old (n = 67), which was the mean and median age at death of the AD cases. In the ≤90 years age group, cardiac ATTR deposition was significantly correlated with Aβ phase score (Table 4, $p = 0.020$), Aβ deposition (Table 4, $p = 0.017$), and Braak NFT stage (Table 4, $p < 0.001$). Conversely, there was no association between cardiac ATTR deposition and AD-related pathological changes among those in the >90 years age group. Likewise, ATTR deposition both in the ≤90 and >90 years old age groups were not significantly correlated with the CAA stage ($p = 0.221$ and 0.097, respectively), although the risk of ATTR deposition was slightly increased in cases of CAA in the ≤90 years old age group.


**DISCUSSION**

Given that the brain-heart axis is important for the development of AD [13, 36, 37], we first studied the ATTR burden and fibrosis of the heart in the general Japanese population, and then compared the cardiac pathologies with the AD-related brain pathologies. The prevalence of cardiac ATTR in this study was 4.3% in septuagenarians and 20.2% in octogenarians. Some Japanese reports stated that the prevalence of cardiac ATTR in Japanese octogenarians was approximately 16%, which is consistent with our result. A previous review, which summarized data from western countries, showed that ATTR was present in the heart of 25%–30% of septuagenarians and octogenarians [38], suggesting that the prevalence may reflect either genetic or environmental differences or both.

We observed positive correlations between the cardiac ATTR deposition and AD-related brain pathologies. Aging is the common risk factor for AD and ATTR in older adults. It is noteworthy that the concurrence of AD and cardiac ATTR pathologies in those aged ≤90 years at death was significant, when adjusting for

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**TABLE 2** Odds ratios of cardiac ATTR deposition by univariate analysis

| ATTR grade 0–1 vs. 2–3 | OR     | 95% CI  | \( p \) value |
|------------------------|--------|---------|--------------|
| Age at death           | 1.17   | 1.11–1.25| <0.001       |
| Dementia               | 3.50   | 1.71–7.63| <0.001       |
| Aβ phase score         | <0.001 |         |              |
| A0, A1                 | 1.00   | (Reference) |          |
| A2, A3                 | 3.82   | 1.86–8.18|              |
| Braak stage 0–4        | 1.00   | (Reference) |          |
| 5 and 6               | 6.65   | 3.03–16.2|              |
| CERAD score           | 0.002  |         |              |
| None                  | 1.00   | (Reference) |          |
| Sparse                | 8.78   | 1.52–166|              |
| Moderate              | 10.2   | 1.53–202|              |
| Frequent              | 14.4   | 2.91–262|              |
| CERAD none vs. others | 0.015  |         |              |
| None                  | 1.00   | (Reference) |          |
| Sparse to frequent    | 12.2   | 2.53–219|              |

| CAA stage | OR     | 95% CI  | \( p \) value |
|-----------|--------|---------|--------------|
| 0         | 1.00   | (Reference) |          |
| 1         | 2.01   | 0.71–5.19|              |
| 2         | 1.26   | 0.43–3.26|              |
| 3         | 2.25   | 0.79–5.89|              |

Abbreviations: ATTR, transthyretin amyloid; CAA, cerebral amyloid angiopathy; CERAD, The Consortium to Establish a Registry for Alzheimer’s Disease; CI, confidence interval; OR, odds ratio.

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**TABLE 3** Association of ATTR grade with heart failure or atrial fibrillation

| ATTR grade | Gender | Heart failure | OR (n = 196) | 95% CI | \( p \) value |
|------------|--------|---------------|--------------|-------|--------------|
| 0          | Male/Female | − (n = 196) | + (n = 44) | 1.00 (Reference) | 0.079 |
| 1          | Male/Female | − (n = 162) | + (n = 22)  | 2.35 | 0.98–8.17 |
| 2          | Male/Female | − (n = 147) | + (n = 11)  | 3.87 | 0.65–11.5 |
| 3          | Male/Female | − (n = 147) | + (n = 11)  | 2.15 | 0.03–3.22 |

| ATTR grade | Gender | Atrial fibrillation | OR (n = 207) | 95% CI | \( p \) value |
|------------|--------|---------------------|--------------|-------|--------------|
| 0          | Male/Female | − (n = 207) | + (n = 33) | 1.00 (Reference) | 0.180 |
| 1          | Male/Female | − (n = 165) | + (n = 20)  | 1.00 (Reference) | 0.079 |
| 2          | Male/Female | − (n = 165) | + (n = 20)  | 1.00 (Reference) | 0.079 |
| 3          | Male/Female | − (n = 165) | + (n = 20)  | 1.00 (Reference) | 0.079 |

Abbreviations: ATTR, transthyretin amyloid; CI, confidence interval; OR, odds ratio; SE, standard error.
age and sex. This suggests risk factors other than aging which are common in the cardiac ATTR deposition and AD pathologies. We consider lifestyle-related disorders to be one of the candidates because our previous study found a positive correlation between CERAD scores and lifestyle-related disorders [4]. Thus, a combinatory approach using pathology and epidemiology may be suitable to identify lifestyle-related risk factors that increase the amyloid burden in different organs.

As a molecular interaction, TTR is a major carrier of T4 and directly binds with Aβ to prevent fibril formation [24, 25, 39]. Furthermore, serum TTR levels have been reported to correlate inversely with Aβ in patients with AD [40]. Thus, lower TTR levels in combination with a reduction of apolipoprotein A1 and complement C3 could be a peripheral biomarker for AD diagnosis [41], although TTR alone is not a predictor of AD progression [42]. While a relationship between aging and amyloid deposition in various organs including the brain and heart was suggested many years [10], until now, the relationship between ATTR deposition in the heart or other organs and AD pathological changes has not been clarified. In this study, we found that ATTR deposition was correlated with AD pathology in those aged ≤90 years.

Pathologically defined CAA is common in older adults, especially in patients with AD [43]. The prevalence of CAA in our study was roughly in line with the findings of previous population-based autopsy studies in older adults [44, 45]. We found that the odds ratio of cardiac ATTR deposition was slightly higher in adults with CAA who were younger than 90 years of age at death, but this relationship was not significant. This suggests that cardiac ATTR deposition could be modestly associated with CAA at a younger age. Although ATTR is known to accumulate in the vascular walls of various organs, ATTR deposition in intracranial blood vessels are quite rare. Considering this evidence, it seems unlikely that cardiac ATTR deposition directly affect the cerebral blood vessels.
Rather, it is more likely that there are shared risk factors for cardiac ATTR deposition and senile plaque formation. Regarding cardiac fibrosis, the fibrotic response can be subdivided into replacement fibrosis and reactive fibrosis [46, 47]. Replacement fibrosis, or scar formation, is a process that maintains the structural integrity of the ventricular wall after ischemic injury and prevents rupture. Reactive fibrosis is defined as the expansion of extracellular matrix caused by chronic stress or inflammation without a significant loss of cardiomyocytes. An exaggerated fibrotic response outside the injured area contributes to left ventricular systolic and diastolic dysfunction, which eventually leads to HF [48]. We found a positive correlation between the presence of HF and cardiac fibrosis, which is consistent with previous findings that cardiac fibrosis is a risk factor for HF [48]. However, cardiac fibrosis was not correlated with the presence of AD pathologies in this study. This suggests that fine cardiac fibrosis may not be closely related to the presence of AD pathologies, as our assessment was mainly focused on fine collagen fibers between cardiomyocytes.

AF has been reported to be a relatively common complication in ATTRwt amyloidosis (27%–67%) [49, 50]. In our study, the incidence of AF in patients with cardiac TTR accumulation was 23.6%, which is slightly lower but consistent with that in previous studies. Although several epidemiological studies on AD and AF have been conducted, the association of these variables remains controversial. An association between AD and AF in younger population, which was not apparent in groups including individuals aged >70 years, has been reported [16]. Considering that this was a general population-based cohort study, the relatively old age at death may have contributed to the lack of correlation between cardiac TTR deposition and AF and between AD and AF.

5 | LIMITATION

It is necessary to mention the limitations of this study. All participants were residents of Hisayama town, Japan. Hisayama town has demographic characteristics representative of the national average in Japan, such as age, occupation, and distribution of nutrient intake [51], which may not reflect the rest of the population or race.

In Japan, pathological confirmation of ATTRwt is performed according to the revised diagnostic criteria for systemic amyloidosis, which requires confirmation of amyloid deposition by Congo red and presence of TTR as well as the absence of ALκ and ALλ. In this study, we did not investigate the absence of ALκ and ALλ or the genetic background of amyloidosis; therefore, we could not eliminate the influence of other amyloidosis. Moreover, a recent study reported that TTR and AL amyloidosis could be present in a single patient [52]. Detailed typing of other amyloidosis will be needed to examine the effect of TTR accumulation in detail.

6 | CONCLUSION

We found cardiac ATTR deposition to be correlated with brain AD pathologies in those aged ≤90 years at

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**TABLE 4** Odds ratio of cardiac ATTR deposition in total cases and in individuals aged ≤90 years and >90 years of age at death by logistic regression analysis

| AD pathology                  | Total            | 90 years old and younger | Above 90 years old |
|-------------------------------|------------------|--------------------------|---------------------|
|                               | OR 95% CI p value| OR 95% CI p value        | OR 95% CI p value   |
| Aβ phase score                | 0.076            | 0.005                    | 0.872               |
| A0, A1                        | 1.00 (Reference) | 1.00 (Reference)         | 1.00 (Reference)    |
| A2, A3                        | 2.03 0.92–4.55   | 5.49 1.63–22.4           | 1.02 0.36–3.33      |
| Braak stage                   | 0.008            | <0.001                   | 0.714               |
| 0–4                           | 1.00 (Reference) | 1.00 (Reference)         | 1.00 (Reference)    |
| 5, 6                          | 3.28 1.33–7.78   | 9.62 2.32–65.9           | 1.26 0.37–4.68      |
| CERAD                         | 0.061            | 0.020                    | 0.989               |
| None                          | 1.00 (Reference) | 1.00 (Reference)         | 1.00 (Reference)    |
| Sparse to frequent            | 4.96 0.94–91.6   | 7.63 1.02–1130           | 1.02 0.11–22.3      |
| CAA stage                     | 0.600            | 0.221                    | 0.097               |
| None                          | 1.00 (Reference) | 1.00 (Reference)         | 1.00 (Reference)    |
| Mild to severe                | 0.81 0.35–1.79   | 1.97 0.66–5.87           | 0.39 0.12–1.18      |

Abbreviations: ATTR, transthyretin amyloid; CAA, cerebral amyloid angiopathy; CERAD, The Consortium to Establish a Registry for Alzheimer’s Disease; CI, confidence interval; OR, odds ratio.
death, suggesting that there is a common risk between these pathologies. Further elucidation of the common mechanisms, such as microenvironmental acidic events inducing protein misfolding and reduced clearance of insoluble amyloid materials, will be needed to reduce the future burden of dementia and cardiac failure.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS
Hideomi Hamasaki and Masahiro Shijo conceptualized the study, conducted the histopathological examinations, analyzed data, and wrote portions of the manuscript. Ayaka Nakamura, Hiroyuki Honda, Yuichi Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Tomoyuki Ohara and Toshiharu Yamada, and Yoshinao Oda conducted the histopathological examinations. Toru Iwaki conceptualized the study, analyzed data, and wrote portions of the manuscript. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Hideomi Hamasaki https://orcid.org/0000-0002-6578-8092
Masahiro Shijo https://orcid.org/0000-0001-6851-2860
Hiroyuki Honda https://orcid.org/0000-0002-4225-7248
Toru Iwaki https://orcid.org/0000-0001-7826-870X

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 Representative images of ATTR. (A) grade 1, (B) grade 2, and (C) grade 3

FIGURE S2 Histograms showing CAA grades versus AD pathologies. (A) Ap phase score of the NIA-AA criteria, (B) Braak stage, and (C) CETAD on the horizontal axes, respectively. The light gray, slash-filled, and black columns represent the cases with Mild, Moderate, and Severe CAA stages, respectively.