Clinicopathological features of senile systemic amyloidosis: an ante- and post-mortem study

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Senile systemic amyloidosis is a common age-related amyloidosis that involves accumulation of wild-type transthyretin, with cardiac dysfunction being a predominant result. The importance of obtaining an accurate diagnosis of senile systemic amyloidosis has been increasingly recognized, so that novel treatments are being developed. However, the clinicopathological features of senile systemic amyloidosis remain to be completely understood. Here, we evaluated cardiac specimens from 181 consecutive post-mortem cases older than 40 years, including 6 cases of senile systemic amyloidosis, and 5 cases of familial amyloidotic polyneuropathy, which is a hereditary systemic amyloidosis caused by mutant forms of transthyretin. Furthermore, we studied ante-mortem clinicopathological findings of 11 senile systemic amyloidosis cases, in which 9 cases underwent gastrointestinal tract biopsy and/or subcutaneous tissue biopsy, at Kumamoto University Hospital. Of the autopsied cases of elderly Japanese (older than 80 years), 12% had senile systemic amyloidosis, with the percentage increasing with age. The occurrence of senile systemic amyloidosis in elderly Japanese patients was lower than that in previous reports, which suggests that a genetic background and/or environmental factor(s) may have important roles in the occurrence of senile systemic amyloidosis. Transthyretin amyloid deposits in familial amyloidotic polyneuropathy cases developed mainly in the pericardium and the surrounding muscle fascicles, whereas in cases with senile systemic amyloidosis the transthyretin amyloid deposits had a patchy plaque-like shape and developed mainly inside the ventricular wall. Biopsies from senile systemic amyloidosis patients evidenced amyloid deposits in 44% (4/9) of gastrointestinal tract and subcutaneous tissue samples combined. As myocardial biopsy may be dangerous for elderly people, the use of a combination of gastrointestinal tract and subcutaneous tissue biopsies may make diagnosis of senile systemic amyloidosis easier.

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Amyloidosis is characterized by the presence of extracellular deposits of insoluble amyloid fibrils.^{1,2} It is also classified as one of the protein conformational diseases, also called proteinopathies, in which specific proteins accumulate and affect organ functions.³ Thus far, 27 different protein precursors

of amyloid fibrils have been identified as the cause of different kinds of amyloidosis, such as Alzheimer's disease, prion disease, and type 2 diabetes.⁴ In those amyloidoses, several clinical features, such as aging, genetic mutation, inflammation, and tumors, can stimulate disease-causing polymerization of functional soluble proteins in the body.^{1,2}

Senile systemic amyloidosis, in which wild-type transthyretin forms amyloid deposits in various tissues, is an age-related nonhereditary systemic amyloidosis and affects mainly cardiac functions in elderly people.^{5,6} A few post-mortem studies carried out in the United State and European countries

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reported that the prevalence of senile systemic amyloidosis was 22–25% in patients older than 80 years.^{7–9} However, the prevalence of senile systemic amyloidosis in other populations, such as those in other Asian countries, remains to be elucidated.

Mutant forms of transthyretin cause familial amyloidotic polyneuropathy, which is a hereditary amyloidosis.¹⁰ To date, >120 transthyretin mutations have been identified, most of which lead to development of familial amyloidotic polyneuropathy; transthyretin Val30Met is the most common disease-causing transthyretin mutation in the world.^{11,12} Although clinical disorders of typical familial amyloidotic polyneuropathy patients, including peripheral neuropathy, autonomic dysfunction, renal dysfunction, gastrointestinal disorders, and ocular manifestations, are different from those of patients with senile systemic amyloidosis, several transthyretin variants such as transthyretin Val122Ile cause a cardiac amyloidosis that mimics senile systemic amyloidosis.¹³

The importance of accurate diagnosis of senile systemic amyloidosis has been increasingly recognized, and a novel specific transthyretin stabilizer, tafamidis, has been developed and is undergoing phase II and III clinical trials.¹⁴ This chemical compound reportedly prevented dissociation of native transthyretin tetramers into monomers, which is a key factor in promoting amyloid fibrillogenesis.¹⁵ Effective medical treatment of patients with transthyretin amyloidosis requires an accurate diagnosis with the use of various examinations, including histopathology, echocardiography, scintigraphy, genetic testing, mass spectrometry, and serum protein analyses.⁶ Histopathological examinations have a critical role in obtaining direct evidence of amyloid deposits and determining the type of amyloid-causing protein. As the heart is the most commonly affected organ, myocardial biopsy is thought to be the most sensitive method for detecting amyloid deposits in patients with senile systemic amyloidosis, but this type of biopsy is too invasive and carries a risk of complications, especially for elderly patients. In patients with other types of systemic amyloidosis, less invasive biopsy, such as of the gastrointestinal tract, labial salivary gland, and subcutaneous tissue, has been preferred, because amyloid deposits in those tissues occur early in the course of disease.¹ However, the histopathological features of amyloid deposits in systemic tissues in senile systemic amyloidosis remain to be clarified.

In this study, we investigated the clinicopathological features of senile systemic amyloidosis, including the occurrence of senile systemic amyloidosis and the distribution of amyloid deposits. We found major histopathological differences between senile systemic amyloidosis and familial amyloidotic polyneuropathy. We also describe how to perform histopathological examinations to

achieve an accurate diagnosis of senile systemic amyloidosis.

Patients and methods

Patients

To determine the occurrence of cardiac amyloidosis, we evaluated cardiac specimens from 181 consecutive post-mortem cases. Specimens were available for patients who had died at the age of 40 years or older (11 patients in their forties, 35 in their fifties, 44 in their sixties, 65 in their seventies, 20 in their eighties, 5 in their nineties, and 1 centenarian) and who were autopsied at Kumamoto University Hospital between January 2001 and March 2008, which included 5 cases of familial amyloidotic polyneuropathy who had died at the age of 40 years or older. Patient selection process for post-mortem studies was shown schematically in Figure 1. Patients' profiles of senile systemic amyloidosis and familial amyloidotic polyneuropathy were described in Table 1. To determine the distribution

Figure 1 Schematic presentation of patient selection for post-mortem studies. *Four cases of double amyloidosis include amyloid A + atrial natriuretic factor in atrium (two cases), β_2 -microglobulin + unclassified valvular amyloidosis (one case), and atrial natriuretic factor in atrium + unclassified ventricular amyloidosis (one case). **Detailed histopathological findings of familial amyloidotic polyneuropathy cases were also showed in Supplementary Table S2.

Table 1 Clinical features for 6 autopsied and 11 ante-mortem biopsied cases of senile systemic amyloidosis and for 6 autopsied cases of familial amyloidotic polyneuropathy

Case no.	Transthyretin gene	Molecular mass of serum transthyretin (Da)	Age (year)	Sex	Heart weight (g)	RV thickness (mm)	LV thickness (mm)	Main causes of death	Histopathological features of transthyretin amyloid deposits in the LV wall
<i>Senile systemic amyloidosis (autopsied cases)</i>									
A-case 1	Wild type	NE	73	M	830	5	NE	Sepsis	Patchy plaque-like shape (small)
A-case 2	Wild type	NE	73	M	550	2	15	Interstitial pneumonia, pneumothorax, pulmonary hemorrhage	Patchy plaque-like shape (small to large)
A-case 3 ^a	Wild type	13 761	75	M	750	7	20	Cardiac failure, sepsis	Combined patchy deposits (diffuse pericellular deposition)
A-case 4	Wild type	NE	83	F	210	2	10	Carcinomatous peritonitis	Patchy plaque-like shape (small to large)
A-case 5	Wild type	NE	93	M	400	4	15	Cardiac failure	Patchy plaque-like shape (small)
A-case 6	Wild type	NE	102	F	NE	NE	NE	Cardiac failure	Patchy plaque-like shape (small to large)
Average			83.2		548.0	4.0	15.0		
S.d.			12.0		253.2	2.1	4.1		
<i>Senile systemic amyloidosis (biopsied cases)</i>									
B-case 1 ^b	Wild type	13 761	56	M	NA	NE	19.4 ^c	Alive	Patchy plaque-like shape (moderate)
B-case 2 ^b	Wild type	13 761	69	M	NA	NE	15.8 ^c	Alive	Patchy plaque-like shape (small)
B-case 3 ^b	Wild type	13 761	70	M	NA	NE	24.9 ^c	Alive	NE
B-case 4 ^b	Wild type	13 761	71	M	NA	NE	11.9 ^c	Alive	Patchy plaque-like shape (moderate)
B-case 5 ^{a,b}	Wild type	13 761	72	M	NA	NE	22.7 ^c	Cardiac failure, sepsis	Combined patchy deposits (diffuse pericellular deposition)
B-case 6 ^b	Wild type	13 761	73	M	NA	5.0 ^c	13.6 ^c	Alive	Patchy plaque-like shape (moderate)
B-case 7 ^b	Wild type	13 761	74	M	NA	NE	10 ^c	Alive	Patchy plaque-like shape (small)
B-case 8 ^b	Wild type	13 761	75	M	NA	NE	11.4 ^c	Alive	Patchy plaque-like shape (moderate to large)
B-case 9 ^b	Wild type	13 761	76	M	NA	NE	10.4 ^c	Alive	Patchy plaque-like shape (moderate)
B-case 10 ^b	Wild type	13 761	83	M	NA	NE	14.4 ^c	Alive	NE
B-case 11 ^b	Wild type	13 761	85	M	NA	NE	15.1 ^c	Alive	NE
Average			73.1		5.0	15.4			
S.d.			7.6		NA	5.0			
<i>Familial amyloidotic polyneuropathy (autopsied cases)</i>									
F-case 1 ^d	V30M	13 761/13 793	42	F	320	7	17	Sudden death	Deposits in pericardial border and surrounding muscle fascicles
F-case 2 ^d	Y114C	13 701/13 761	53	F	580	15	25	Sudden death	Deposits in border area surrounding muscle fascicles, and severe deposits in vascular walls
F-case 3 ^d	S50I	13 761/13 781	54	M	570	NE	NE	Cardiac failure	Severe diffuse pericellular deposition
F-case 4 ^d	V30M	13 761/13 793	67	M	438	NE	20	Cardiac failure	Deposits in pericardial border and surrounding muscle fascicles

Table 1 Continued

Case no.	Transthyretin gene	Molecular mass of serum transthyretin (Da)	Age (year)	Sex	Heart weight (g)	RV thickness (mm)	LV thickness (mm)	Main causes of death	Histopathological features of transthyretin amyloid deposits in the LV wall
F-case 5 ^d	V30M/R104H	13 742/13 793	73	M	580	11	20	Cardiac failure, twisting of the sigmoid colon	Deposits in area surrounding pericardial border, and severe diffuse deposits
Average			57.8		496.7	11.0	20.4		
S.d.			12.3		116.1	2.8	3.3		

Abbreviations: LV, left ventricle; NE, not examined; NA, not applicable; RV, right ventricle.

^aA-case 3 and B-case 5 were the same patient.^bThose senile systemic amyloidosis cases were diagnosed at Kumamoto University Hospital, Department of Cardiology during January 2005 and December 2010.^cDetermined by echocardiography.^dDetailed histopathological findings of familial amyloidotic polyneuropathy cases were also showed in Supplementary Table S2.

of amyloid deposits in the body, available other tissue specimens of those senile systemic amyloidosis and familial amyloidotic polyneuropathy cases were also examined (Table 2, and Supplementary Table S2).

Furthermore, to determine ante-mortem clinicopathological findings of senile systemic amyloidosis, we also studied 11 senile systemic amyloidosis patients who were diagnosed at Kumamoto University Hospital, Department of Cardiology from January 2005 to December 2010.

Congo Red Staining

Tissue samples were embedded in paraffin, serially sectioned at a thickness of 4 μ m, and placed on microscope slides. Sections were stained with hematoxylin–eosin and alkaline Congo red and were also examined under polarized light for the presence of green birefringence. The degree of amyloid deposition was determined by computer measurement of Congo red-positive areas via the public domain ImageJ program developed by the US National Institutes of Health and available at <http://rsb.info.nih.gov/ij/index.html>, as described previously.¹⁶ For each cardiac specimen, three different visual fields were studied at $\times 100$ magnification, and pictures were saved via a digital camera (Olympus DP70; Olympus, Tokyo, Japan).

Electron Microscopy

Post-mortem cardiac samples were fixed first in 4% paraformaldehyde and 1% glutaraldehyde solution and then in 1% OsO₄, after which they were embedded in Epon. Ultrathin sections were stained with 4% uranyl acetate and lead citrate and were examined with a transmission electron microscope (H-7500; Hitachi, Tokyo, Japan) at an accelerating voltage of 100 kV, as described previously.¹⁷

Immunohistochemistry

To clarify the types of amyloid proteins, cardiac specimens with amyloid deposits were stained with antibodies that react with transthyretin, atrial natriuretic factor, immunoglobulin light chain (lambda and kappa), amyloid A, and β_2 -microglobulin (Supplementary Table S1), as described previously.¹⁶ To show morphological features of the ventricle as a whole, we also created a joint image with the image stitching function of the BZ-8100 microscope (Keyence, Osaka, Japan), which can join high-resolution images into a single wide-field image.

Genetic and Mass Spectrometric Analyses for Detecting Transthyretin Mutations

PCR amplification by direct DNA sequencing was accomplished with the use of ABI BigDye

Table 2 Presence of amyloid deposits in various tissues from six autopsied senile systemic amyloidosis cases

	<i>A-case 1</i> (73 years)	<i>A-case 2</i> (73 years)	<i>A-case 3</i> (75 years)	<i>A-case 4</i> (83 years)	<i>A-case 5</i> (93 years)	<i>A-case 6</i> (102 years)	% Positive samples including vascular deposits (no./total no.)	% Deposits in extracellular interstitium (no./total no.)
<i>Heart</i>								
Left ventricle	+	+++	+++	++	+	+++	100 (6/6)	100 (6/6)
Right ventricle	—	+++	+++	+	NE	NE	75 (3/4)	75 (3/4)
Interventricular septum	+	NE	+++	NE	+	NE	100 (3/3)	100 (2/2)
Left atrium	—	NE	+++	NE	NE	NE	50 (1/2)	50 (1/2)
Right atrium	—	NE	+++	NE	NE	NE	50 (1/2)	50 (1/2)
<i>Artery</i>								
Aorta	—	+	+	v+	+	NE	80 (4/5)	60 (3/5)
Coronary artery	—	NE	—	—	NE	—	0 (0/4)	0 (0/4)
Common carotid artery	—	NE	+	NE	NE	NE	50 (1/2)	50 (1/2)
Renal artery	—	NE	++	—	+	NE	50 (2/4)	50 (2/4)
Lung	+	+++	+++	+	++	++	100 (6/6)	100 (6/6)
Tongue	NE	v+	++	NE	v+	NE	100 (3/3)	33 (1/3)
Esophagus	—	v+	+	—	v+	NE	60 (3/5)	20 (1/5)
Stomach	—	v+	+	v+	+	NE	80 (4/5)	40 (2/5)
Duodenum	—	NE	+	NE	v+	v+	75 (3/4)	25 (1/4)
Jejunum	—	v+	v+	+	v+	NE	80 (4/5)	20 (1/5)
Ileum	—	v+	v+	v+	v+	NE	80 (4/5)	0 (0/5)
Colon	—	v+	v+	v+	v+	NE	80 (4/5)	0 (0/5)
Rectum	—	v+	v+	v+	NE	NE	75 (3/4)	0 (0/4)
Liver	—	—	v+	—	v+	NE	40 (2/5)	0 (0/5)
Gallbladder	—	v+	v+	+	NE	NE	75 (3/4)	25 (1/4)
Kidney	—	—	+	—	v+	NE	40 (2/5)	20 (1/5)
Glomerulus	—	—	—	—	—	NE	0 (0/5)	0 (0/5)
Adrenal gland	—	NE	v+	v+	v+	NE	75 (3/4)	0 (0/4)
Ureter	NE	NE	+	v+	NE	NE	100 (2/2)	50 (1/2)
Bladder	—	+	+	+	+	NE	80 (4/5)	80 (4/5)
Prostate	—	+	v+	NA	NE	NA	67 (2/3)	33 (1/3)
Testis	—	—	—	NA	NE	NA	0 (0/3)	0 (0/3)
Uterus	NA	NA	NA	v+	NA	NE	100 (1/1)	0 (0/1)
Thyroid gland	—	v+	v+	NE	v+	NE	75 (3/4)	0 (0/4)
Pancreas	—	v+	v+	—	v+	NE	60 (3/5)	0 (0/5)
Spleen	—	v+	—	NE	NE	NE	33 (1/3)	0 (0/3)
Spinal ligament	NE	NE	+++	NE	NE	NE	100 (1/1)	100 (1/1)
Bone marrow	—	—	—	NE	—	NE	0 (0/4)	0 (0/4)

Abbreviations: —, not detected; v+, amyloid deposits limited to vascular walls; + (mild), ++ (moderate), and +++ (severe), amyloid deposits in both vascular walls and extracellular interstitium; NE, not examined; NA, not applicable. Patients' profiles were described in Table 1.

Terminator reagents (Applied Biosystems, Foster City, CA, USA) on the 3130 Genetic Analyzer (Applied Biosystems). We processed the transthyretin gene by using DNA prepared from peripheral blood leukocytes or paraffin-embedded formalin-fixed specimens with the following specific primers: exon 1 forward: 5'-TCAGATTGGCAGGGATAAGC-3', reverse: 5'-GCAAAGCTGGAAGGAGTCAC-3'; exon 2 forward: 5'-CGTTCCTGATAATGGGATCA-3', reverse: 5'-CAAAAAGAGGGCATCCTTCA-3'; exon 3 forward: 5'-TCTGACTTAGTTGAGGGGAAATG-3', reverse: 5'-AGGGAACCTTTGGTCATTCA-3'; exon 4 forward: 5'-TGCCAGCATATTTGAGCTTTT-3', reverse: 5'-ATGAAAAGTGCCTTTCACAGGA-3'.

To measure the molecular mass of serum transthyretin, we performed mass spectrometric analyses with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS; PCS 4000 SELDI-TOF MS instrument; Bio-Rad, Hercules, CA, USA), as previously described.¹⁸

Ethics

The Human Ethics Review Committee of Kumamoto University approved the study protocol, and signed consent forms were obtained from families of subjects. All patients' family members gave informed consent for performance of an autopsy.

Statistical Analysis

Data were evaluated with Student's *t*-test and ANOVA. All analyses were performed with JMP version 5.1 (SAS Institute Japan, Tokyo, Japan). *P*-values of <0.05 were regarded as statistically significant.

Results

All cardiac tissues examined from 48 of 181 (27%) autopsied cases showed amyloid deposits. We determined the occurrence of cardiac amyloidosis in each generation older than 40-year old (Supplementary Figure S1). As shown in Figure 1, we determined types of cardiac amyloidosis by means of the immunohistochemical stainings. To distinguish senile systemic amyloidosis from familial amyloidotic polyneuropathy, we analyzed the transthyretin gene sequence and/or the shift in molecular mass of transthyretin in all cases with transthyretin amyloid deposits (Figure 2, Table 1). The occurrence of senile systemic amyloidosis in elderly Japanese cases (older than 80 years) was 11.5% (3/26) and increased with age (Figure 1). Of the 181 autopsied cases, none younger than 70 years showed evidence of senile systemic amyloidosis.

In this study, we also evaluated clinicopathological findings for autopsied cases and ante-mortem biopsied cases of senile systemic amyloidosis and

Figure 2 Detection of variant transthyretins by means of genetic and protein analyses. Transthyretin DNA sequences were examined for senile systemic amyloidosis (a) and familial amyloidotic polyneuropathy (b) by using ABI BigDye Terminator reagents. A heterozygous transversion of G to A was predicted to change valine to methionine at residue 30 of the mature protein (Val30Met) (underlined). Mass spectrometric analysis of transthyretin from serum samples of senile systemic amyloidosis (c) and familial amyloidotic polyneuropathy (d) cases was performed by means of the PCS 4000 SELDI-TOF MS instrument. (a) Wild-type transthyretin (13 761 Da); (b) mutant transthyretin Val30Met (13 793 Da).

autopsied cases of familial amyloidotic polyneuropathy (Table 1). A significant difference in age was observed for the senile systemic amyloidosis and familial amyloidotic polyneuropathy autopsied cases (83.2 ± 12.0 years *versus* 57.8 ± 12.3 years, respectively, $P < 0.01$), but no significant difference in LV thickness or heart weight was found (Table 1). Three of autopsied senile systemic amyloidosis cases (3/6) died of cardiac failure, which is the main clinical feature of senile systemic amyloidosis. In addition, one of those senile systemic amyloidosis cases died of interstitial pneumonia with pulmonary hemorrhage.

Figure 3 clearly shows differences in the morphology of cardiac transthyretin amyloid deposits in senile systemic amyloidosis and familial amyloidotic polyneuropathy. Transthyretin amyloid deposits developed predominantly in the pericardium and surrounding muscle fascicles in familial amyloidotic polyneuropathy, whereas they appeared as patchy plaque-like shapes and developed predominantly inside the ventricular wall in senile systemic amyloidosis (Figures 3a–i, Table 1). Electron microscopic examination revealed differences in morphology of amyloid fibrils in greater detail

Figure 3 Morphological features of cardiac transthyretin amyloid deposits in senile systemic amyloidosis and familial amyloidotic polyneuropathy, which demonstrate a major difference between these disorders. (a–i) Immunohistochemical staining with an anti-transthyretin antibody (Dako). (a–f) Patchy plaque-like deposits in the ventricles of senile systemic amyloidosis cases (a–c: A-case 4, d–f: A-case 2). (g–i) Deposits in the pericardial border and surrounding muscle fascicles in the ventricle of an familial amyloidotic polyneuropathy patient (F-case 4). (b, e, h) Epicardial side of the left ventricular wall. (c, f, i) Endocardial side of the left ventricular wall. (a, d, g) Joint images created with the image stitching function of the BZ-8100 microscope (Keyence); scale bars: 3 mm. (b, c, e, f, h, i) Scale bars: 200 μ m. (j–m) Electron micrographs of amyloid fibrils in cardiac specimens. (j, l) Short, rigid, haphazardly arranged fibrils in senile systemic amyloidosis (A-case 3). (k, m) Long, straight, parallel fibrils in familial amyloidotic polyneuropathy transthyretin Val30Met (F-case 4). (j, k) Scale bars: 500 nm. (l, m) Scale bars: 200 nm.

(Figures 3j–m): long, straight fibrils were arranged in parallel in familial amyloidotic polyneuropathy, whereas short, rigid fibrils were haphazardly arranged in senile systemic amyloidosis. In all senile systemic amyloidosis cases, the endocardial side of the left ventricular wall had larger areas of amyloid deposits than did the epicardial side of the left ventricular wall (Figure 4).

We also investigated the distribution of transthyretin amyloid deposits in various tissues in autopsied senile systemic amyloidosis cases (Table 2) and

familial amyloidotic polyneuropathy (Supplementary Table S2). Amyloid deposits occurred in the left ventricle and lungs of all these senile systemic amyloidosis cases (6/6). Amyloid deposits were also observed in the 5/6 cases of the bladder (Table 2). In thyroid gland, pancreas, liver, gallbladder, adrenal gland, and gastrointestinal tract, they were detected predominantly in the walls of small arteries (Table 2). In the gastrointestinal tract of familial amyloidotic polyneuropathy cases, amyloid deposits were found both in basal laminae and in walls of

small arteries (Figure 5, Supplementary Table S2). However, in senile systemic amyloidosis cases, amyloid deposits were detected predominantly in walls of small arteries (Figure 5, Table 2). Furthermore, in the glomerulus of kidney, all of familial amyloidotic polyneuropathy cases (5/5) showed amyloid deposits (Supplementary Table S2), but none of senile systemic amyloidosis cases developed amyloid deposits (Table 2). One senile systemic amyloidosis case (A-case 3) also evidenced

Figure 4 Areas with amyloid deposits in the endocardial and epicardial sides of the left ventricular wall in autopsied senile systemic amyloidosis cases. Areas with amyloid deposition were measured as described in the text.

severe amyloid deposits in spinal ligaments (Supplementary Figure S2).

Transthyretin amyloid deposits were detected in 100% (8/8) of myocardial samples obtained from senile systemic amyloidosis patients who had ante-mortem biopsies (Table 3). However, much less amyloid was detected in the gastrointestinal tract and subcutaneous tissue than in the myocardium. Analysis of both gastrointestinal tract and subcutaneous tissues combined indicated amyloid deposits in 44.4% (4/9) of senile systemic amyloidosis patients (Table 3).

One of those patients (B-case 11) showed a nodular transthyretin amyloid deposit mimicking a lung cancer mass (Figure 6a). A lung biopsy revealed that the mass lesion consisted of severe transthyretin amyloid deposits without malignant cells (Figures 6b–d).

Discussion

In this report, we investigated the clinicopathological features of senile systemic amyloidosis in elderly Japanese patients by means of post-mortem and ante-mortem studies. We also demonstrated major histopathological differences between senile systemic amyloidosis and familial amyloidotic polyneuropathy.

In studies of post-mortem specimens, we determined that the occurrence of senile systemic amyloidosis in elderly Japanese cases (older than

Figure 5 Transthyretin amyloid deposits in the duodenum: major differences between senile systemic amyloidosis and familial amyloidotic polyneuropathy. (a–c) Senile systemic amyloidosis (A-case 3). (d–f) Familial amyloidotic polyneuropathy (F-case 4). (a, d) Congo red staining, (b, e) Congo red staining viewed under polarized light, and (c, f) immunohistochemical staining using an anti-transthyretin antibody. Arrows point to vascular amyloid deposits; arrowheads, to amyloid deposits in the basal laminae. Scale bars: 200 μ m.

Table 3 Occurrence of amyloid deposits in biopsied tissues from 11 men with senile systemic amyloidosis

Tissue	B-case 1 (56 years)	B-case 2 (69 years)	B-case 3 (70 years)	B-case 4 (71 years)	B-case 5 (72 years)	B-case 6 (73 years)	B-case 7 (74 years)	B-case 8 (75 years)	B-case 9 (76 years)	B-case 10 (83 years)	B-case 11 (85 years)	% Positive samples (no./total no.)
Heart	+	+	NE	+	+	+	+	+	+	NE	NE	100 (8/8)
% Area with amyloid deposits	25	5	NE	16	61	33	11	46	16	NE	NE	
Stomach	NE	NE	NE	—	v+	NE	—	NE	—	NE	—	20 (1/5)
Duodenum	—	NE	v+	—	v+	NE	—	NE	—	v+	—	38 (3/8)
Rectum	—	NE	NE	—	NE	—	NE	NE	NE	NE	NE	0 (0/3)
Subcutaneous tissue	—	NE	f+, v+	NE	NE	NE	—	NE	—	—	f+	33 (2/6)

Abbreviations: —, not detected; v+, amyloid deposits in vascular walls; f+, amyloid deposits in fat tissue; +, amyloid deposits in extracellular interstitium; NE, not examined. All cases were diagnosed at Kumamoto University Hospital, Department of Cardiology from January 2005 to December 2010. Patients' profiles were described in Table 1.

80 years) was 11.5%, which was lower than in cases investigated by the same means in the United States and European countries (22–25%).^{7–9} Although we did not identify the reasons for this difference, we suspect that genetic and/or environmental factors may affect the occurrence of the disease. However, as Table 1 shows almost all senile systemic amyloidosis patients (5/6), except for A-case 3, had never been diagnosed as having senile systemic amyloidosis, although two of those patients (2/5) died of cardiac failure, which is the main clinical feature of senile systemic amyloidosis. This finding indicates that more attention should be paid to senile systemic amyloidosis when evaluating and diagnosing cardiac failure in the elderly.

For these same post-mortem senile systemic amyloidosis cases, we provided detailed histopathological findings of amyloid deposits in various organs. Several post-mortem studies of senile systemic amyloidosis have been reported,^{5,7,19,20} but our careful study elucidated specific histopathological features of senile systemic amyloidosis in various systemic tissues. We also demonstrated major histopathological differences, including the distribution of amyloid deposits in various organs and the morphological features of amyloid fibrils in cardiac specimens, between senile systemic amyloidosis and mutant transthyretin-derived familial amyloidotic polyneuropathy. These results suggest that the amyloid formation mechanisms in familial amyloidotic polyneuropathy and senile systemic amyloidosis may differ. Bergström *et al*²¹ reported finding morphologically different amyloid fibrils in cardiac specimens from Swedish patients with senile systemic amyloidosis and those with familial amyloidotic polyneuropathy: short, rigid, haphazardly arranged fibrils predominantly in senile systemic amyloidosis, and long, straight, parallel fibrils in familial amyloidotic polyneuropathy. They proposed that fragmentation of C-terminal portions of transthyretin in senile systemic amyloidosis may generate the different amyloid fibrils. In fact, our electron microscopic findings were consistent with data from their studies of Swedish patients.

We also demonstrated different organ distributions of amyloid deposits in post-mortem senile systemic amyloidosis cases compared with distributions previously reported for Japanese familial amyloidotic polyneuropathy patients.²² We found that amyloid deposits in senile systemic amyloidosis tended to develop predominantly in tissues with high mechanical stress, such as the cardiac ventricle (especially the endocardial side), alveolar wall of the lungs, bladder wall, and vascular walls, but they rarely developed in tissues without such mechanical stress, such as the glomerulus and thyroid gland, where severe amyloid deposits were often found in familial amyloidotic polyneuropathy (Supplementary Table S2).²² In addition, we also discovered severe amyloid deposits in the spinal ligaments of a post-mortem senile systemic

Figure 6 Nodular lesion in the lung of SSA. (a) Chest CT showed a nodular lesion (arrow) mimicking a tumor in the lung of a SSA patient B-case 11. (b–d) Microscopic biopsy findings. (b) Congo red staining, (c) Congo red staining viewed under polarized light, and (d) immunohistochemical staining using an anti-TTR antibody. Scale bars: 200 μ m.

amyloidosis case (Supplementary Figure S2). We recently demonstrated wild-type transthyretin-derived amyloid deposits in 35% (39/111 cases) of surgically resected ligaments from patients older than 40 years with orthopedic disorders.²³ Amyloid deposits in such ligaments of senile systemic amyloidosis patients may frequently develop without obvious symptoms. On the basis of those findings, we speculate that aging of the tissue matrix caused by long-term mechanical stress may have an important role in amyloid formation in senile systemic amyloidosis. Additional studies are needed to confirm this possibility.

In addition, we found a nodular transthyretin amyloid deposit that mimicked a cancer lesion in the lung of a senile systemic amyloidosis patient (Figure 6). This type of amyloid deposit in senile systemic amyloidosis lungs has not been described except for one account in a case report.²⁴ Furthermore, in post-mortem autopsied cases, one senile systemic amyloidosis case (A-case 2) died of interstitial pneumonia and pulmonary hemorrhage (Table 1) with severe transthyretin amyloid deposits in lungs (Table 2). Those findings suggest that attention should be paid to such amyloid deposits in the lung, which is another major amyloid-targeted site.

To obtain an accurate diagnosis of senile systemic amyloidosis, confirmation of the presence of transthyretin amyloid deposits is needed by means of tissue biopsies of suitable organs.^{1,2} Although we observed amyloid deposits in the heart and lung of all senile systemic amyloidosis cases in our post-mortem study, biopsies of these tissues are often difficult to perform because of the highly invasive biopsy methods needed. Although, in most of post-mortem senile systemic amyloidosis cases (5/6), amyloid deposits were observed predominantly in the vascular walls in the gastrointestinal tract (Table 2), which is one of the most common biopsy sites used for diagnosis of various types of systemic amyloidosis because of the less invasive method required,¹ we detected no amyloid deposits in the gastrointestinal tract of more than half of the ante-mortem senile systemic amyloidosis patients (Table 3). These results suggest that amyloid deposits in the gastrointestinal tract may develop in early stages of senile systemic amyloidosis. In addition, we found no amyloid in the basal laminae in the gastrointestinal tract, a site where amyloid deposits often developed in familial amyloidotic polyneuropathy (Figure 5, Supplementary Table S2). These histopathological findings lead to the conclusion that diagnosis of senile systemic amyloidosis requires obtaining a biopsy specimen from deeper sites containing submucosal vascular walls of the tract rather than using the common biopsy method. Moreover, biopsy of subcutaneous tissue may help detect transthyretin amyloid deposits, because one senile systemic amyloidosis patient (B-case 11) evidenced amyloid deposits in subcutaneous fat but not in the gastrointestinal tract (Table 3).

In conclusion, we provide here the first data showing the occurrence of senile systemic amyloidosis in an elderly Japanese population. The lower occurrence of amyloid deposition suggests differences in genetic and/or environmental factors. We also believe that our detailed clinicopathological findings for systemic tissues will provide novel insights into understanding senile systemic amyloidosis.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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