研究成果の刊行に関する一覧表

雑誌

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［V］研究成果の刊行物・別刷

Recipient Aging Accelerates Acquired Transthyretin Amyloidosis After Domino Liver Transplantation

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Running title: Recipient aging accelerates acquired TTR amyloidosis

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Abbreviations: ATTR, amyloidogenic transthyretin; BF, bright-field; DLT, domino liver transplantation; FAP, familial amyloid polyneuropathy; LT, liver transplantation; PL, polarized light; TTR, transthyretin

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Abstract

Introduction: Domino liver transplantation (DLT) with liver grafts from patients with hereditary transthyretin (TTR) amyloidosis has been performed throughout the world because of a severe liver graft shortage. Reports of acquired systemic TTR amyloidosis in domino liver recipients have been increasing; however, the precise pathogenesis and clinical course of acquired TTR amyloidosis remains unclear.

Results: We analyzed the relationship between the occurrence of acquired amyloidosis and clinical features in consecutive 22 domino liver donors with hereditary TTR amyloidosis [10 males and 12 females; mean age at DLT: 37.2 years; TTR mutations: V30M (n = 19), Y114C (n = 1), L55P (n = 1), and S50I (n = 1)] and 22 liver recipients (16 males and 6 females; mean age at DLT, 46.2 years). The mean times from DLT to amyloid first appearance and transplant recipient symptom onset were 8.2 years and 9.9 years, respectively. Kaplan-Meier analysis and quantification of the amyloid deposition revealed aging of recipients correlated with early *de novo* amyloid deposition. The sex of donors and recipients and the age, disease duration, and disease severity of donors had no significant effect on the latency of *de novo* amyloid deposition.

Conclusion: Our results demonstrate that recipient aging is associated with the early-onset *de novo* amyloidosis. Because acquired amyloidosis will likely increase, careful follow-up for early

amyloidosis detection and new treatments, including TTR stabilizers and gene-silencing therapies, are required.

Introduction

Hereditary transthyretin (TTR) amyloidosis is one of the most common causes of hereditary polyneuropathy and is distributed worldwide, with high-prevalence locations in Portugal, Sweden, and Japan.[1] TTR normally exists in blood as a soluble tetramer, by which it transports thyroxine and retinol-binding protein. *In vitro* studies of TTR amyloidogenesis led to the hypothesis that soluble TTR self-assembles into amyloid fibrils and that point mutations influence the rate of TTR amyloid fibril formation.[2] As of today, more than 130 point mutations in the TTR gene have been reported, and many of these mutations are amyloidogenic, with amyloidogenic transthyretin (ATTR) V30M (p. V50M) being the most common mutation that leads to hereditary TTR amyloidosis.[1] ATTR V30M (p. V50M) affects various systemic organs and tissues, including the peripheral nerves, autonomic nerve system, heart, gastrointestinal system, kidneys, and eyes, and the mean time from disease onset to death, without treatment, is approximately 10 years.[3]

Because the liver produces more than 95% of serum TTR, liver transplantation (LT) has served as the standard treatment of hereditary TTR amyloidosis since 1990.[4] Data in the FAP World Transplant Registry show that approximately 120 LTs are performed worldwide each year. LT usually halts the progression of symptoms, prolongs the survival duration, and increases the quality of life of patients with hereditary TTR amyloidosis,[5,6] but recent studies have revealed that LT

occasionally failed to prevent progression of amyloid cardiomyopathy, especially in elderly men and non-V30M patients.[7,8] In view of the severe shortage of liver grafts worldwide and the fact that livers from patients with hereditary TTR amyloidosis are functionally and morphologically normal except for the production of mutant TTR, explanted livers from patients with hereditary TTR amyloidosis are used as domino liver grafts for patients with fetal hepatic disorder.[9] From 1995 to the end of 2013, 1112 domino liver transplantations (DLTs) were performed worldwide (FAP World Transplant Register; retrieved from http://www.fapwtr.org/index.htm).

Because patients with hereditary TTR amyloidosis usually develop amyloid deposition after age 20, liver grafts explanted from hereditary TTR amyloidosis patients were expected to not cause amyloidosis for a long period. However, in 2004 Sousa et al reported the surprising finding that asymptomatic *de novo* amyloid deposition occurred in the skin 3 years after domino liver transplantations (DLT).[10] In 2005, Stangou et al reported the first case of symptomatic acquired TTR amyloidosis, confirmed by nerve biopsy, 8 years after DLT.[11] Since then, several patients with symptomatic and asymptomatic amyloid deposition after DLT were reported, with the time interval between DLT and the development of amyloidosis ranging from 3 to 9 years.[12-22] This time interval was believed to be unexpectedly short, because of the usual time to develop amyloid deposition in hereditary TTR amyloidosis patients. The number of patients with acquired amyloidosis after DLT continues to increase, but the exact rate of increase and the risk factors for early-onset amyloidosis

remain unclear. In our study here, we analyzed the occurrence and risk factors for development of amyloid deposits and symptoms in domino liver recipients.

Patients and Methods

Domino liver donors (hereditary TTR amyloidosis patients)

We evaluated data for 22 consecutive hereditary TTR amyloidosis patients (10 men and 12 women, 27–58 years old) who underwent living donor LTs and whose explanted livers were used as domino liver grafts for patients with potentially fatal hepatic disorders between 1998 and 2014 (Table 1). The diagnosis was made on the basis of clinical manifestations, histopathological detection of amyloid deposits in biopsied tissues, and the presence of a *TTR* gene mutation. Nineteen patients had the V30M (p. V50M) TTR mutation, the most common type of TTR mutation in the world, and three had other mutations (one each of Y114C, L55P, and S50I). The mean disease duration before LT was 2.4 years.

Domino liver recipients

Twenty-two patients (16 men and 6 women, 18–62 years old) who had end-stage liver cirrhosis, hepatocellular carcinoma, or congenital biliary atresia underwent DLT with liver grafts obtained from hereditary TTR amyloidosis patients. DLTs were performed at Kumamoto University

Hospital (n = 18) and Kyoto University Hospital (n = 4), and all patients were followed at Kumamoto University hospital. To detect early symptoms of acquired amyloidosis, such as autonomic dysfunction, sensorimotor polyneuropathy, gastrointestinal dysfunction, arrhythmia, and vitreous opacity, we periodically monitored DLT recipients. We regularly used nerve conduction studies, echocardiography, electrocardiography, and biopsy of abdominal fat, skin, and gastric mucosa (supplemental Table 1).

Histopathological evaluation

We evaluated explanted livers from hereditary TTR amyloidosis patients and biopsied liver samples from domino liver recipients by means of Congo red staining and immunohistochemistry with an anti-TTR antibody (Dako, Glostrup, Denmark). Degrees of perivascular amyloid deposition in the liver were classified into three categories: (a) whole circumferential deposition, (b) noncircumferential deposition, and (c) no amyloid deposition. To monitor *de novo* amyloid deposition in DLT recipients, biopsy samples were obtained annually from abdominal fat, skin, and gastric mucosa and were evaluated with Congo red staining. Additional immunohistochemistry for transthyretin, immunoglobulin light chain, and serum amyloid A protein was performed for cases with amyloid deposition. Calculation of the percent area occupied by Congo red-positive area fractions were performed using the public domain

NIH ImageJ.

Statistical analysis

Kaplan-Meier analysis was used to estimate the cumulative percentage of TTR amyloid deposition and new-onset amyloid symptoms of domino liver recipients, and the difference between groups was evaluated by using the log-rank test. The difference between groups was also analyzed by means of the Student t-test. Differences with a p value of less than 0.05 (two-tailed) were considered statistically significant.

Results

Occurrence of amyloid deposition and symptoms of amyloidosis of DLT recipients

Kaplan-Meier analysis revealed the estimated occurrence of amyloid deposition and symptoms of amyloidosis after DLT in all domino liver recipients (Fig. 1). The mean time interval between the first detection of amyloid (at 3.2-9.0 years, mean: 8.2 years) and the onset of symptoms (at 5.7-11.8 years, mean: 9.9 years) was 1.7 years. In the recipients of V30M liver, the mean times from DLT to the first detection of amyloid and symptom onset were 8.2 years and 10.8 years, respectively. Because of small numbers of non-V30M mutations, differences between patients with V30M and non-V30M mutations could not be determined.

Five domino recipients developed decreased sensation in the distal extremities, two of whom had mild gastrointestinal symptoms. Other symptoms that are characteristic of hereditary TTR amyloidosis, such as muscle weakness, orthostatic hypotension, cardiac conduction disturbance, heart failure, and vitreous opacity were not observed during the observation period (Table 2).

Impact of clinical characteristics of DLT donors (patients with hereditary TTR amyloidosis) on* de novo *amyloid deposition in DLT recipients

Because of the phenotypic differences, including age at disease onset, clinical symptoms, and disease progression rate, among patients with TTR mutations, we excluded the small number of patients with non-V30M mutations and evaluated 19 patients with ATTR V30M (p. V50M). Kaplan-Meier analysis revealed no significant sex-related differences for the time of onset of amyloid deposition in domino liver recipients (Fig. 2A). No significant differences in age, duration of disease, or degree of disease severity (FAP clinical score[23]) at the time of the DLT were seen between domino liver recipients with and without *de novo* amyloid deposition (Table 3).

We used histopathology to analyze the degrees of amyloid deposition in explanted livers of 12 of 19 domino liver donors. Because of the small sample number and the variation in

observation period, we could not perform a statistical analysis, but the presence and degrees of amyloid deposition in explanted livers showed no tendency to promote the development of *de novo* amyloid deposition in domino liver recipients (Fig. 2B).

Because 17 of 19 ATTR V30M (p. V50M) liver donors were patients from high-prevalence areas and had a family history of hereditary TTR amyloidosis, we could not analyze the effect of a difference in the place of origin high-prevalence versus low-prevalence areas—of these liver donors.

Impact of clinical characteristics of DLT recipients on* de novo *amyloid deposition

Kaplan-Meier analysis revealed no significant correlation between sex and *de novo* amyloid deposition in domino liver recipients (Fig. 3A). The mean time to first detection of amyloid deposition, however, occurred earlier in older (more than 50 years old at the time of DLT) domino liver recipients compared with younger (less than 50 years at the time of DLT) domino liver recipients (5.8 years vs. 8.6 years, respectively). Among domino liver recipients with amyloid deposition, we found a negative correlation between the age at the time of DLT and the time to first detection of amyloid deposition (Fig. 3C). And, more severe amyloid deposition was observed in aged recipients (Fig. 3D, E).

We found no significant correlation between types of immunosuppressants (tacrolimus,

mycophenolate mofetil, or prednisolone) and *de novo* amyloid deposition in domino liver recipients.

Discussion

In this study, we determined the occurrence of amyloid deposition and symptoms of amyloidosis in DLT recipients, and we found that aging of liver recipients promoted *de novo* amyloid formation.

Our data on the time to the first detection of amyloid and time to the onset of symptoms in domino recipients were similar to those previously reported.[10-22] This short latent period, compared with that found in patients with hereditary TTR amyloidosis, had been thought to be related to the older age of the recipients or the seeding effect of already formed amyloid fibrils that were present in donor livers. Our results indicate that older recipients tended to develop amyloid deposition earlier than younger recipients. Accumulating evidence has revealed that aging is a general risk factor for various types of amyloidosis including Alzheimer's disease,[24] apolipoprotein AI amyloidosis,[25] islet amyloid polypeptide amyloidosis,[26] AA amyloidosis,[27] and TTR amyloidosis.

TTR-related amyloidosis is divided into two types by the presence or absence of TTR mutation. The age at onset of hereditary ATTR V30M (p. V50M) amyloidosis differs between

high-prevalence and low-prevalence areas in Japan: it is 20-40 years in high-prevalence areas and older than 50 years in low-prevalence areas. Aging has been thought to be related to both types of cases; however, biochemical and histopathological differences between early- and late-onset cases were reported.[28,29] Furthermore biochemical and pathological investigations of amyloid fibrils in DLT recipients may provide insight into the pathogenesis of acquired TTR amyloidosis.

Wild-type TTR amyloidosis is a common age-related amyloidosis, in which amyloid fibrils derived from wild-type TTR deposit mainly in the heart, ligaments, tendons, and cartilage.[30,31] In general, age-related amyloid deposition may result from either accelerated production or slowed clearance of amyloidogenic proteins. In the setting of TTR-related amyloidosis, because production and serum levels of TTR decrease with age,[32] a reduced TTR clearance is presumably caused by aging.

A seeding effect of already formed amyloid fibrils, which were present in donor livers, is another possible cause of early development of *de novo* amyloidosis. In our histopathological study, the presence or degrees of amyloid deposition in explanted livers of domino liver donors showed no correlation with the development of *de novo* amyloid deposits in domino liver recipients. Also, a long duration of the disease and the severity of the disease in domino liver donors (patients with hereditary TTR amyloidosis) at the time of the DLT did not accelerate

acquired TTR amyloidosis. We believe that these results are consistent with previous *in vitro* studies in which TTR fibrils showed no or few seeding effects.[33] Our data also showed that the mean time interval from the first detection of amyloid to the onset of symptoms in liver recipients was 1.7 years. This interval is extremely short compared with that in Alzheimer's disease, in which Aβ deposition begins more than 20 years before the clinical onset of dementia.[34]

Despite the potential risk of causing acquired TTR amyloidosis in domino liver recipients much sooner than expected, DLTs with liver grafts from hereditary TTR amyloidosis patients are still critically needed because of the severe liver graft shortage. Therefore, informing patients about the risk of *de novo* amyloidosis is quite important, as is continuing long-term evaluation of clinical symptoms and histopathological findings. Investigation of the precise clinical course of acquired TTR amyloidosis is also necessary. The effect of new treatments of TTR amyloidosis, such as tafamidis meglumine, which is a TTR stabilizer, and gene-silencing therapies with small interfering RNA and antisense oligodeoxynucleotides, also warrants study in the near future.

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