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Cardiac Amyloidosis Associated With a Novel Transthyretin Aspartic Acid-18 Glutamic Acid *De Novo* Mutation

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A 40-year-old man presented with initial symptoms of syncope caused by restrictive cardiomyopathy and autonomic nervous system impairment, but it was confirmed that he had a novel transthyretin (TTR) variant, aspartic acid-18 glutamic acid (Glu), and a *de novo* gene mutation. A polymerase chain reaction-induced mutation restriction analysis with a mismatched sense primer demonstrated that he was heterozygous for TTR Glu 18. Liver transplantation was not performed because of profound weakness and severe postural hypotension. Right-sided heart failure predominated in association with low output syndrome and a gradual decrease in total QRS voltage on electrocardiogram over 5 years of follow-up. Autonomic neuropathy developed and he eventually died of both-sided heart failure at the age of 45 years. Immunohistochemical and DNA studies are important to diagnose and treat TTR-related cardiac amyloidosis. (*Circ J* 2003; **67:** 965–968)

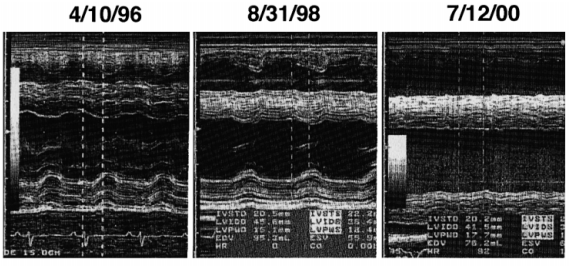
Key Words: Amyloidosis; Restrictive cardiomyopathy; Syncope; Transthyretin

Cardiac amyloidosis results from the deposition of insoluble amyloid protein fibrils that are usually derived from immunoglobulin light chains (AL amyloidosis), amyloid A protein (AA amyloidosis), wild-type transthyretin (TTR) (senile cardiac amyloidosis), or variant TTR with one amino acid substitution (familial cardiac amyloidosis)[1]. TTR is a plasma protein synthesized in the liver[2], transports thyroxin and retinol, and amyloidosis associated with variant TTR is usually caused by autosomal dominant inheritance[3], but it can arise sporadically[2]. Over 70 point mutations of the TTR gene have been described, the most frequent of which is the substitution of methionine (Met) for valine at position 30 that causes familial amyloid polyneuropathy (FAP)[4]. A sensory disturbance in the lower extremities is the usual initial symptom of FAP[5]. Recently, a patient with a TTR variant of aspartic acid (Asp)-18 glycine (Gly) with only central nervous impairment caused by meningocerebrovascular amyloid deposition was reported[6] and we present a patient with a different amino acid substitution at the same position (Asp-18), which is a new TTR variant Asp-18 glutamic acid (Glu). This patient primarily developed amyloid cardiomyopathy and autonomic nervous system impairment.

Case Report

A 40-year-old Japanese man presented with exertional dyspnea in December 1995 and developed orthostatic and exertional dizziness 1 month later. Because of an episode of unconsciousness while walking in March 1996, he was referred to hospital for further evaluation of the syncope.

On admission, chest radiography showed neither cardiomegaly (cardiothoracic ratio (CTR): 51%) nor pulmonary congestion. Electrocardiography (ECG) revealed a normal sinus rhythm, undetermined axis, a low voltage, incomplete right bundle branch block, Q waves in leads V_{1-3} with ST elevation in leads V_{1-4}, negative T waves in leads III and aV_F and biphasic T waves in leads II, V_5 and V_6. Echocardiography showed diffuse bilateral ventricular hypertrophy (thickness of interventricular septum (IVS), left ventricular (LV) posterior wall and right ventricular (RV) anterior wall: 20 mm, 16 mm and 5 mm, respectively) with an LV ejection fraction (EF) of 56% (Fig 1), granular sparkling of the IVS, and restricted mitral valve inflow (deceleration time (DT): 144 ms). ^{99m}Tc-pyrophosphate scintigraphy



	4/10/96	8/31/98	7/12/00
EF (%)	56	49	18
RVDd (mm)	12	20	22
IVSWT (mm)	20	21	20
LVPWT (mm)	16	15	18
LVDd (mm)	46	46	42

Fig 1. M-mode echocardiography over 5 years of follow-up shows a gradual decrease in the wall motion of both ventricles, and an increase in the chamber size of the right, but not the left ventricle. EF, ejection fraction; RVDd, right ventricular diastolic dimension; IVSWT, interventricular septum wall thickness; LVPWT, left ventricular posterior wall thickness; LVDd, left ventricular diastolic dimension.

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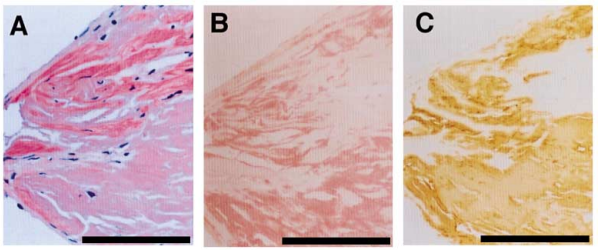

Fig 2. Histopathology of a myocardial specimen. (A) Left ventricular endomyocardium (H&E). (B) Massive amyloid deposits adjacent to (A) (Congo-red staining). (C) Immunohistochemical staining adjacent to (A) with anti-transthyretin antibody. Bar = 100 μm.

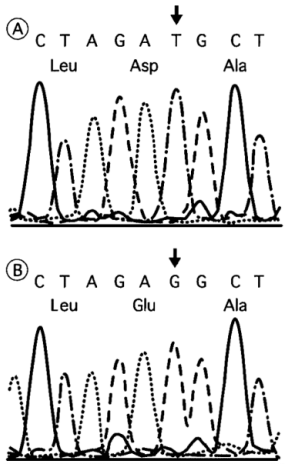

Fig 3. DNA sequencing of transthyretin (TTR) codon 18. Samples: (A) wild and (B) mutant types. Raw data are for sense allele of normal or mutant TTR genes. Single base change (T→G) is present at codon 18 base 3 in mutated TTR gene. – – –: G, –·–: T, ·····: A, —: C

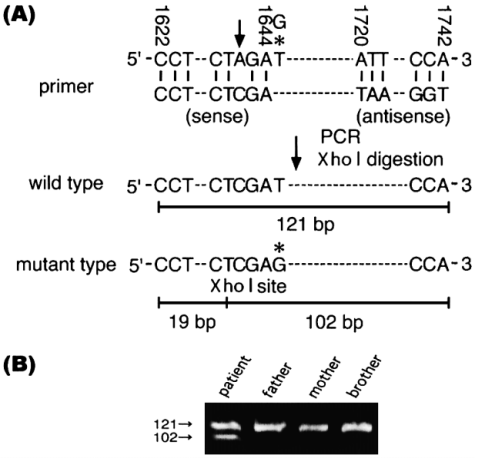

Fig 4. (A) Polymerase chain reaction (PCR)-induced mutation restriction analysis of transthyretin (TTR) glutamic acid (Glu)18. Sense primer has one base substitution: A→C replacement at position 1642 (↓). Mutant TTR gene with T→G transition at position 1645 (*) creates a new restriction site for *Xho* I, yielding 2 fragments of 102 and 19 base pairs (bp) on *Xho* I digestion. (B) TTR exon 2 digested with *Xho* I. TTR Glu18 from patient gave 2 abnormal bands of 102 and 19 bp in addition to the normal 121 bp band, whereas that from his parents and brother yielded only normal bands. Bp sizes of the PCR products are shown at left.

showed diffuse uptake by the LV and RV. His blood pressure (BP) decreased from 124/84 mmHg in the supine position to 90/60 mmHg at the upright position accompanied by dizziness. The treadmill exercise test showed neither an ST-T change nor arrhythmia, but a decrease in systolic BP of 33 mmHg and an increase in heart rate of 27 beats/min. Cardiac catheterization was performed in April 1996 and all pressure values except for a LV end-diastolic pressure of 14 mmHg were all within normal limits. Left ventriculography showed neither mitral regurgitation nor wall motion abnormality and the calculated LVEF was 60%. Coronary angiography showed no organic lesion. An endomyocardial biopsy of the LV (Fig 2A) revealed massive amyloid deposits that were positive with Congo red (Fig 2B), resistant to $KMnO_4$ and positive for anti-TTR antibody (Fig 2C), indicating a diagnosis of TTR-related cardiac amyloidosis. An endomucosal biopsy of the stomach and colon also showed amyloid deposits. His parents were healthy and the family did not have a history of amyloidosis.

After receiving written, informed consent to participate in the study, blood samples were obtained from the patient and his family. Total DNA was extracted from peripheral leukocytes, and the entire TTR genome was sequenced using an automated fluorescent sequencer as described[7] Direct DNA sequencing showed that the patient had a T→G transition at position 1645 in the TTR exon 2, which replaced Asp (GAT)-18 with Glu (GAG) (Fig 3). We used polymerase chain reaction (PCR)-induced mutation restriction analysis[8] for the TTR Asp-18 Glu missense mutation to facilitate its detection. A primer with one mismatched base pair (bp) at position 1642 adjacent to the mutated thymine created an artificial restriction site for *Xho* I in the mutant Glu-18 allele (Fig 4A). The PCR product gave 2 fragments of 102 and 19 bp in addition to the normal band at 121 bp after *Xho* I digestion, indicating that the patient was heterozygous for the TTR Glu-18 allele (Fig 4B). Controls, as well as the parents and brother of the patient, had only the normal fragment of 121 bp (Fig 4B). Paternity was confirmed by blood typing and short tandem repeat analysis. DNA sequencing of the TTR gene of both parents of the patient and his brother also identified normal TTR genes. These findings indicated a diagnosis of cardiac amyloidosis caused by a novel *de novo* mutation of TTR Asp-18 Glu.

Although liver transplantation is considered the most effective treatment for FAP[9] this patient was too weak to undergo the procedure. He was therefore, conservatively treated with droxidopa, digoxin, pimobendan, docarpamine, furosemide, and spironolactone to control the orthostatic hypotension and heart failure. However, he developed digoxin sensitivity. Very small doses of β-blocker (metoprolol, 1.25 mg) and angiotensin converting enzyme inhibitor (ACEI) (quinapril, 2.5 mg) were also administered to try and control heart failure, but failed because of further weakness and hypotension. A bifascicular block (complete right-bundle branch block and left posterior hemiblock) and PQ prolongation on ECG suddenly developed in August 1998 and a permanent pacemaker was implanted in October 1998 because an electrophysiological study revealed an His-ventricular (H-V) block (H-V interval: 78 ms) associated with sinus node dysfunction (corrected sinus node recovery time: 3510 ms). Total QRS voltage on ECG decreased over the years (10.5 mV to 7.4 mV), and echocardiography over the 5 years of follow-up showed a gradual decrease in the wall motion of both ventricles, and an increase in the size of the RV chamber but not that of the

LV (Fig 1). Because the systolic BP was less than 90 mmHg despite several inotropic agents, a low dose of intravenous dopamine was intermittently administered to treat the pleural effusion and massive ascites and peripheral edema. The inferior vena cava was enlarged to a diameter of 24 mm without respiratory movement. A chest X-ray revealed cardiomegaly (CTR = 60–68%) without pulmonary congestion. He also complained of progressive malaise and a decrease in urine volume. Gastrointestinal symptoms such as abdominal fullness, nausea, vomiting, dysphagia, and constipation gradually developed and parenteral nutrition and enemas were eventually required to control intestinal amyloidosis, including paralytic ileus in June 2000. He was readmitted to hospital in December 2000 because of systemic collapse and acute renal failure. He was placed on an artificial ventilator, but the heart did not contract in response to cardiac pacing, and he died 5 days later. His family refused an autopsy.

Discussion

A 40-year-old man died of cardiac amyloidosis caused by a new TTR variant consisting of the substitution of Glu for Asp at position 18 (Asp-18 Glu). Cardiac amyloidosis is characterized as a 'stiff heart' syndrome with diastolic dysfunction and exercise intolerance is common because of the inability to increase cardiac output as a result of the restrictive cardiomyopathy.[10] The present patient also developed severe postural hypotension because of autonomic nerve involvement by the amyloid protein. The combination of postural hypotension and restrictive cardiomyopathy is thought to cause a profound decrease in cardiac output, which can be demonstrated by the treadmill exercise test, and may explain the cause of syncope in this patient. A permanent pacemaker was implanted 2.5 years after the initial symptom of syncope. Involvement of the cardiac amyloidosis in the conduction system did not seem to be a cause of syncope because the bifascicular block associated with PQ prolongation developed 2.5 years after admission. A low voltage ECG, despite the increased thickness of the left ventricular wall, is characteristic of myocardial infiltration by amyloid fibrils[11–13] and was evident in our patient. The magnitude of the decrease in total QRS voltage (10.5 mV to 7.4 mV) was greater the increase in wall thickness (interventricular septum + left ventricular posterior wall: 36 mm to 38 mm) over the 5 years of follow-up.

The prognostic significance of DT as a parameter of diastolic dysfunction in patients with cardiac amyloidosis has been emphasized by Klein et al[14] and Doppler echocardiography of the present patient showed a decrease in DT, indicative of restrictive physiology. Echocardiographic tracking over the 5 years of follow-up showed thickening of the RV wall, decreased RV wall motion and an increase in the size of RV chamber, which are all consistent with predominantly right-sided heart failure in patients with cardiac amyloidosis.[15]

Patients with cardiac amyloidosis can become sensitized to digoxin,[10] which binds to amyloid fibrils,[16] and despite very cautious administration, this occurred in the present patient. ACEI and β-blockers failed to control the heart failure because of further weakness and hypotension. Activation of the cardiac renin–angiotensin system contributes to LV diastolic dysfunction in hypertensive or hypertrophic cardiomyopathy, suggesting that ACEI and/or angiotensin receptor blocker should be beneficial for diastolic heart failure.[17–19] Careful use of low doses of diuretics and vasodilators for patients with cardiac amyloidosis may afford a degree of symptomatic benefit at the risk of hypotension,[15] but Gillmore et al cautioned that overzealous use of ACEI for patients with cardiac amyloidosis must be avoided.[20] The median survival of patients with primary systemic amyloidosis who develop heart failure is only 5 months,[21] but the prognosis of cardiac amyloidosis associated with the TTR variant is better.[22] Indeed, this patient survived 5 years after the diagnosis was confirmed. Nonetheless, successful management using digoxin, ACEI and β-blockers seems unlikely for the predominantly right-sided type of heart failure caused by the cardiac amyloidosis of the present patient. Although liver transplantation was considered,[9] it was not applicable for this patient because of severe heart failure, weakness and postural hypotension.

A missense mutation of the TTR gene (Asp 18 → Glu) was identified by DNA sequencing analysis and because this mutation did not create a new restriction site or abolish an established site, a modified primer with one base substitution was designed to create a new artificial restriction site for *Xho* I. When a patient is diagnosed as having TTR-related amyloidosis, the genes of all relatives should be analyzed because early diagnosis of TTR-related amyloidosis is necessary for successful liver transplantation. An amino acid substitution at the same position of Asp-18 has been reported,[6] but the clinical phenotype of the present patient was quite different from that patient, who had a TTR variant of Asp-18 Gly and whose clinical manifestations were restricted to the central nervous system.[6] Different amino acid substitutions at the same position of TTR molecule often cause diverse clinical features of amyloidosis,[23] and moreover, even the same TTR mutations can manifest as dissimilar clinical features in affected individuals.[24,25] Unidentified genetic factors might also be involved in the development of the clinical manifestations of TTR-related amyloidosis.

In summary, this is the first report of the TTR variant Asp-18 Glu being associated with a cardiac manifestation and postural hypotension, onset at age 40 years of age, and a *de novo* gene mutation. Immunohistochemical and DNA studies are important for diagnosis because TTR-related cardiac amyloidosis is usually autosomal dominant inheritance.[3] In particular, the DNA of all relatives should be studied, because early diagnosis and liver transplantation remains the radical treatment of choice for patients with TTR-related amyloidosis. We emphasize the need for medical attention to this disease because increasing awareness will help in terms of earlier diagnosis and treatment.

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