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【 CASE REPORT 】

Late-onset Hereditary ATTR Amyloidosis with a Novel p.P63S (P43S) *Transthyretin* Variant

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Abstract:
The patient was an 82-year-old Japanese man with no family history suggestive of amyloidosis. He developed bilateral leg edema and shortness of breath and was referred to our hospital. An electrocardiogram showed atrial fibrillation with right bundle branch block. Echocardiography showed concentric LV hypertrophy. An endomyocardial biopsy showed severe ATTR amyloid deposits. A genetic analysis of the *transthyretin* (*TTR*) gene revealed a heterozygous c.187C>T missense variant resulting in p.P63S (P43S). *In silico* analyses predicted that this variant only modestly altered the structure and function of the TTR protein. The p.P63S variant might be associated with an elderly-onset cardiac-dominant ATTRv phenotype.

Key words: hereditary ATTR amyloidosis, transthyretin, cardiomyopathy

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Introduction

Transthyretin amyloidosis (ATTR amyloidosis) is a life-threatening, gain-of-toxic-function disease characterised by extracellular deposition of amyloid fibrils composed of transthyretin (TTR) (1, 2). Wild-type TTR deposition leading to the sporadic amyloid disease, wild-type ATTR (ATTRwt) amyloidosis, occurs in the elderly (3, 4). In contrast, deposition of variant TTR results in autosomal dominant hereditary amyloidosis, known as hereditary ATTR (ATTRv) amyloidosis (5, 6). ATTRv amyloidosis typically presents earlier and is often more severe than ATTRwt amyloidosis. Indeed, ATTRv amyloidosis used to be considered an incurable, fatal disease until liver transplantation was developed in the 1990s (7).

The clinical effects of the TTR tetramer stabilizers [i.e., diflunisal (8) and tafamidis (9, 10)] and nucleic acid drugs target for *TTR* mRNA [i.e., patisiran (11) and inotersen (12)] have recently been described. Therefore, the early diagnosis and timely intervention of ATTRv amyloidosis are becoming critical to improving the prognosis.

We herein report the clinical, pathological, biochemical, and molecular biological findings of an 82-year-old Japanese ATTRv amyloidosis patient with a novel missense variant in the *TTR* gene.

Case Report

The patient was an 82-year-old Japanese man with no family history suggestive of amyloidosis, including neuropathy and cardiomyopathy. He had previously undergone chemotherapy for prostate cancer. He was referred to the Department of Cardiology at National Hospital Organization Kyoto Medical Center for the further investigation of bilateral leg edema and shortness of breath (New York Heart As-

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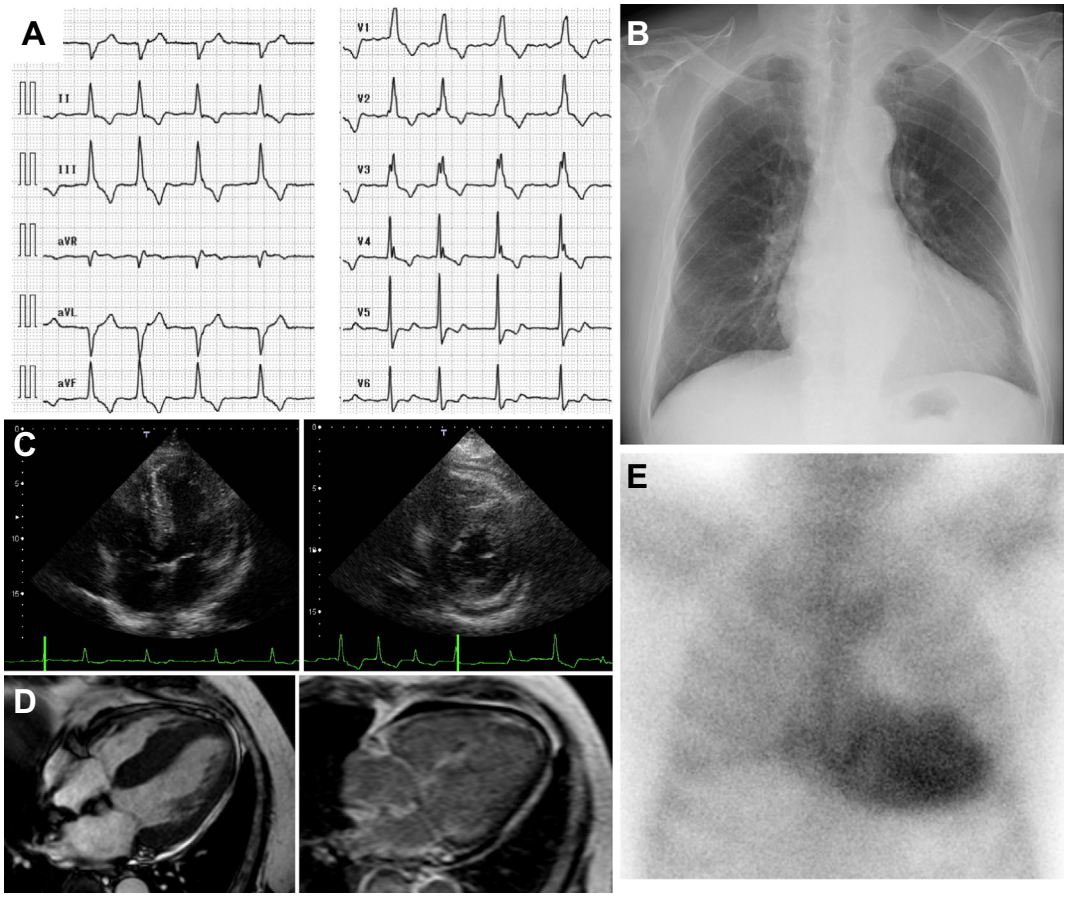

Figure 1. **(A) An electrocardiogram, (B) chest X-ray, and (C) echocardiography findings at the end of diastole, (D) cine (left panel) and late gadolinium enhanced (right panel) magnetic resonance imaging, and (E) ^{99m}Tc-pyrophosphate scintigraphy of the patient.**

sociation functional class II).

General and neurological examinations upon admission revealed no abnormal findings apart from edema in both legs. Other symptoms suggestive of amyloidosis, such as carpal tunnel syndrome, neuropathy, diarrhea, or protein urea, were unremarkable. A 12-lead electrocardiogram showed atrial fibrillation with right bundle branch block (Fig. 1A). Chest X-ray uncovered cardiomegaly and a cardio-thoracic ratio of 58% (Fig. 1B). Serum N-terminal pro B-type natriuretic peptide and high-sensitive troponin I levels were elevated at 2,103 pg/mL and 81.9 pg/mL, respectively.

Transthoracic echocardiography showed a preserved left ventricular (LV) function (LV end-diastolic dimension/LV end-systolic dimension: 47/32 mm; LV ejection fraction calculated using Teichholz's formula: 60%) and concentric LV hypertrophy with an interventricular septum thickness of 16 mm and posterior wall thickness of 15 mm. Enlargement of both atria and slight pericardial effusion were also noted (Fig. 1C). Coronary angiography showed a normal coronary artery, and right heart catheterization revealed a slightly high pulmonary capillary wedge pressure of 18 mmHg and a normal cardiac index of 2.5 L/min/m^2. Cardiac magnetic resonance imaging revealed elevated myocardial native T1 values with wide-spreading late gadolinium enhancement at the left ventricle and atrium (Fig. 1D). ^{99m}Tc-pyrophosphate scintigraphy displayed a markedly increased isotope uptake in the heart (Fig. 1E). He had a normal serum immunoglobulin free light chain ratio without monoclonal proteins in serum and urine analyses. An endomyocardial biopsy of the interventricular septum showed severe amyloid deposits that were specifically immunolabelled with anti-TTR antibody (Fig. 2).

We suspected the patient of having ATTRwt amyloidosis, as he showed an elderly-onset sporadic cardiac phenotype. However, a genetic analysis of the *transthyretin (TTR)* gene revealed a heterozygous c.187C>T missense variant resulting in p.P63S (P43S). Based on these findings, we diagnosed him with ATTRv amyloidosis.

The c.187C>T (p.P63S) variant has not been described in disease-causing variant databases, such as the Human Gene Mutation Database Professional (http://www.hgmd.org/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), or in healthy individual databases, including the 1,000 Genomes Project (http://www.internationalgenome.org) and Exome Aggregation Consortium (https://gnomad.broadinstitute.org) (Table 1). The P63 amino acid is conserved across mammalian species (Table 2). The possible impact of the novel variant on the structure and function of TTR was assessed using bioinformatics tools, such as SIFT (https://sift.bii.a-star.edu.

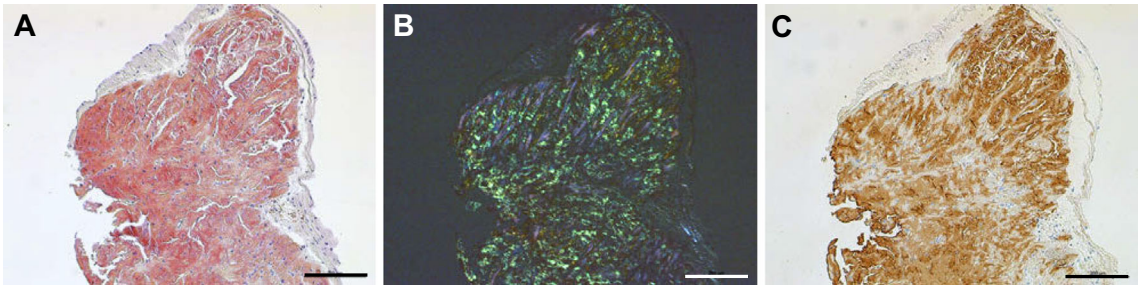

Figure 2. **Pathological findings of biopsied cardiac tissue. (A, B) Congo red staining. (C) Immunohistochemical staining with anti-TTR antibody. Scale bars: 200 μm.**

Table 1. ***In silico*** **Analyses of the c.187C>T (p.P63S) Variant.**

Base change	Amino acid change	dbSNP#	1000G* Freq	ExAC* Freq	SIFT**	Polyphen2**	Mutation Tester**	CADD**
c.187C>T	p.P63S	rs1803083	0	0	T	B	D	10.35

*The c.187C>T (p.P63S) variant has not been described in healthy individual databases, including the 1000 Genomes Project and Exome Aggregation.

**The c.187C>T (p.P63S) variant was predicted to be only modestly altered the structure and function of the TTR protein based on the analysis by bioinformatics tools including SIFT, Polyphen2, Mutation Tester, and CADD.

dbSNP#: Single Nucleotide Polymorphism Database reference number, 1000G: 1000 Genomes Project, Freq: allele frequency, ExAC: Exome Aggregation Consortium, T: tolerated, B: benign, D: disease causing

Table 2. **Homologs of the *TTR* Gene at the P63 Residue.**

Species	P63																				
Human	V	F	R	K	A	A	D	D	T	W	E	P	F	A	S	G	K	T	S	E	S	G	E
Chimpanzee										W	E	P	F	A	S	G	K	T	S	E	S	G	E
Rhesus macaque										W	A	P	F	A	S	G	K	T	S	E	S	G	E
Cat										W	E	P	F	A	S	G	K	T	S	E	F	G	E
Mouse										W	E	P	F	A	S	G	K	T	A	E	S	G	E
Chicken							D	G	T	W	Q	D	F	A	T	G	K	T	T	E	F	G	E
Pufferfish						A	D	G	G	W	T	Q	V	A	N	G	M	T	D	A	S	G	E
Drosophila	V	S	R	L	D	E	I	Q	E	W	R	S	L	R	A	A	Q	T	D	A	D	G	R
Western clawed frog	V	F	R	N	T	-	E	G	N	W	E	L	I										

The P63 amino acid is conserved across mammalian species, such as human, chimpanzee, rhesus macaque, cat, and mouse.

sg/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Tester (http://www.mutationtaster.org/), and CADD (https://cadd.gs.washington.edu/), which predicted the protein to be only modestly altered (Table 1). We further investigated the biopsied heart tissue using laser microdissection and liquid-chromatography tandem mass spectrometry (LC-MS/MS) to analyze the composition ratio of wild-type TTR (P63) to variant TTR (S63) in the amyloid fibrils, as previously reported (13, 14). The tryptic peptide mass peak ratio of wild-type TTR (P63) to variant TTR (S63) in the cardiac amyloid deposits was 37:63 (Fig. 3).

Discussion

In the present case, amyloid cardiomyopathy (15) was the primary and initial presentation at 82 years old without a family history suggestive of amyloidosis. We initially suspected him of having wild-type ATTR amyloidosis. However, a molecular genetic analysis of the *TTR* gene revealed heterozygosity for the novel variant, c.187C>T (p.P63S). *TTR* gene sequencing is necessary in all cases of ATTR-type cardiac amyloidosis because ATTRv amyloidosis cannot be distinguished from ATTRwt amyloidosis on clinical grounds alone, and a family history indicating autosomal dominant inheritance is often absent because of incomplete and late disease penetrance (16). Differentiating between ATTRv and ATTRwt is important for two reasons. First, treatment options for ATTRv amyloidosis are different from those for ATTRwt amyloidosis (i.e., liver transplantation and nucleic acid drugs are approved only for ATTRv amyloidosis). Second, we can provide genetic counseling and predictive genetic testing for other family members at risk of developing ATTRv amyloidosis (17-19). As several disease-modifying therapies are available for ATTRv amyloidosis, family screening is very important for the early diagnosis and timely intervention (17-19).

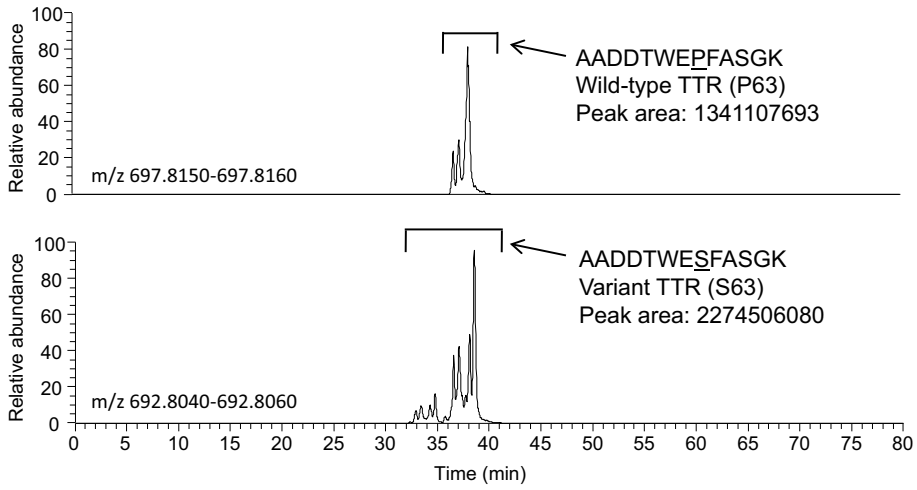

Figure 3. The composition ratio of wild-type TTR (P63) to variant TTR (S63) in cardiac amyloid fibrils analyzed by ion chromatograms of liquid chromatography-tandem mass spectrometry. Upper and lower chromatograms indicate peaks of tryptic peptides containing wild-type (P63) and variant (S63) TTR, respectively. The composition ratio of wild-type to variant TTR was calculated by the ion amounts of wild-type (P63) TTR and variant (S63) peptides evaluated by their corresponding peak areas (wild-type: 1341107693; variant: 2274506080). The cardiac amyloid was predominantly composed of the variant TTR (ratio of wild-type TTR to variant TTR was 37: 63).

In silico analyses predicted that the p.P63S variant only modestly altered the structure and function of the TTR protein. P63 is a relatively conserved amino acid residue, and no variant of this residue has been reported to date. An LC-MS/MS analysis of the biopsied tissue revealed that the amyloid was predominantly composed of the variant TTR. Taken together, these findings suggested that p.P63S was a modestly pathogenic variant.

To date, more than 130 *TTR* gene variants have been reported, and considerable genotype-phenotype correlations have been identified. The three main phenotypes of ATTRv amyloidosis are familial amyloid polyneuropathy, familial amyloid cardiomyopathy, and familial leptomeningeal amyloidosis (1, 17). Among the variants responsible for cardiomyopathy, p.V142I (V122I) is notable for its prevalence in African-Americans. Approximately 3.0-3.9% of African-Americans are heterozygous for p.V142I and develop elderly-onset cardiac amyloidosis (20, 21). It was shown that p.V142I TTR was slightly destabilized as compared with wild-type TTR (22, 23), which may explain the elderly-onset phenotype. The clinical characteristics of our patient as well as the results of pathological, biochemical, and *in silico* analyses suggested that p.P63S is a modestly pathogenic variant and might be associated with an age-dependent cardiac-dominant ATTRv phenotype with a low penetrance, similar to p.V142I (24, 25). However, the possibility that this patient had a *de novo* variant could not be excluded, since the parents were unavailable for testing.

In summary, we reported an 82-year-old Japanese ATTRv amyloidosis patient with a novel p.P63S variant in the *TTR* gene, who showed an elderly-onset cardiomyopathy phenotype. The further accumulation of patients with this variant is warranted in order to elucidate the detailed clinical characteristics of p.P63S ATTRv amyloidosis.

The authors state that they have no Conflict of Interest (COI).

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References

1. Sekijima Y. Transthyretin (ATTR) amyloidosis: clinical spectrum, molecular pathogenesis and disease-modifying treatments. J Neurol Neurosurg Psychiatry **86**: 1036-1043, 2015.
2. Benson MD, Buxbaum JN, Eisenberg DS, et al. Amyloid nomenclature 2018: recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. Amyloid **25**: 215-219, 2018.
3. Cornwell GG III, Murdoch WL, Kyle RA, Westermark P, Pitkanen P. Frequency and distribution of senile cardiovascular amyloid. A clinicopathologic correlation. Am J Med **75**: 618-623, 1983.
4. Westermark P, Sletten K, Johansson B, Cornwell GG III. Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci U S A **87**: 2843-2845, 1990.
5. Plante-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol **10**: 1086-1097, 2011.
6. Ando Y, Coelho T, Berk JL, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J Rare Dis **8**: 31, 2013.
7. Holmgren G, Ericzon BG, Groth CG, et al. Clinical improvement and amyloid regression after liver-transplantation in hereditary transthyretin amyloidosis. Lancet **341**: 1113-1116, 1993.
8. Berk JL, Suhr OB, Obici L, et al. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. JAMA **310**: 2658-2667, 2013.

9. Coelho T, Maia LF, Martins da Silva A, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology **79**: 785-792, 2012.

10. Maurer MS, Schwartz JH, Gundapaneni B, et al. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. N Engl J Med **379**: 1007-1016, 2018.

11. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med **379**: 11-21, 2018.

12. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for aatients with hereditary transthyretin amyloidosis. N Engl J Med **379**: 22-31, 2018.

13. Tsuchiya-Suzuki A, Yazaki M, Sekijima Y, Kametani F, Ikeda SI. Steady turnover of amyloid fibril proteins in gastric mucosa after liver transplantation in familial amyloid polyneuropathy. Amyloid **20**: 236-243, 2013.

14. Sekijima Y, Yazaki M, Oguchi K, et al. Cerebral amyloid angiopathy in posttransplant patients with hereditary ATTR amyloidosis. Neurology **87**: 773-781, 2016.

15. Ruberg FL, Grogan M, Hanna M, Kelly JW, Maurer MS. Transthyretin amyloid cardiomyopathy: JACC state-of-the-Art review. J Am Coll Cardiol **73**: 2872-2891, 2019.

16. Maurer MS, Elliott P, Comenzo R, Semigran M, Rapezzi C. Addressing common questions encountered in the diagnosis and management of cardiac amyloidosis. Circulation **135**: 1357-1377, 2017.

17. Sekijima Y. Hereditary Transthyretin Amyloidosis. In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. University of Washington, Seattle, 1993-2020 [Internet]. [updated 2018 Dec 20; cited 2020 Jun 10]. Available from: ht tp://www.ncbi.nlm.nih.gov/books/NBK1194/

18. Ueda M, Sekijima Y, Koike H, et al. Monitoring of asymptomatic family members at risk of hereditary transthyretin amyloidosis for early intervention with disease-modifying therapies. J Neurol Sci **414**: 116813, 2020.

19. Schmidt HH, Barroso F, González-Duarte A, et al. Management of asymptomatic gene carriers of transthyretin familial amyloid polyneuropathy. Muscle Nerve **54**: 353-360, 2016.

20. Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. N Engl J Med **336**: 466-473, 1997.

21. Yamashita T, Hamidi Asl K, Yazaki M, Benson MD. A prospective evaluation of the transthyretin Ile122 allele frequency in an African-American population. Amyloid **12**: 127-130, 2005.

22. Jiang X, Buxbaum JN, Kelly JW. The V122I cardiomyopathy variant of transthyretin increases the velocity of rate-limiting tetramer dissociation, resulting in accelerated amyloidosis. Proc Natl Acad Sci U S A **98**: 14943-14948, 2001.

23. Sekijima Y, Wiseman RL, Matteson J, et al. The biological and chemical basis for tissue-selective amyloid disease. Cell **121**: 73-85, 2005.

24. Quarta CC, Buxbaum JN, Shah AM, et al. The amyloidogenic V 122I transthyretin variant in elderly black Americans. N Engl J Med **372**: 21-29, 2015.

25. Buxbaum JN, Ruberg FL. Transthyretin V122I (pV142I)* cardiac amyloidosis: an age-dependent autosomal dominant cardiomyopathy too common to be overlooked as a cause of significant heart disease in elderly African Americans. Genet Med **19**: 733-742, 2017.