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-
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/m². Cardiac magnetic resonance imaging revealed elevated myocardial native T1 values with wide-spreading late gadolinium enhancement at the left ventricle and atrium (Fig. 1D). ⁹⁹mTc-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.

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).
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MS/MS analysis of the biopsied tissue revealed that the no variant of this residue has been reported to date. An LC-

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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 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 have been identified. The three main phenotypes of ATTRv amyloidosis are familial amyloid polyneuropathy, familial amyloid cardiomyopathy, and familial leptomeningeal amyloidosis.

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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