DOI: 10.31276/VJSTE.66(2).76-81

A novel mutation in the transthyretin gene in amyloidosis: A cluster case report in Vietnam

Ngoc Lan Thi Nguyen[1, 2*], Thu Anh Nguyen[3], Quy Van Vu[1], Huyen Thi Vu[2], Phuong Lan Thi Dam[4], Duc Tuan Nguyen[1, 2]

[1]Biochemistry Department, Hanoi Medical University, 1 Ton That Tung Street, Trung Tu Ward, Dong Da District, Hanoi, Vietnam
[2]Clinical Laboratory, Hanoi Medical University Hospital, 1 Ton That Tung Street, Trung Tu Ward, Dong Da District, Hanoi, Vietnam
[3]Vinmec International Hospital, 458 Minh Khai Street, Vinh Tuy Ward, Hai Ba Trung District, Hanoi, Vietnam
[4]Biochemistry Department, 103 Military Hospital, 261 Phung Hung Street, Phuc La Ward, Ha Dong District, Hanoi, Vietnam

Received 22 September 2023; revised 3 December 2023; accepted 15 February 2024

Abstract:

Transthyretin amyloidosis (ATTR) is a slowly progressive condition characterised by the abnormal accumulation of a protein called amyloid in the body's organs and tissues. There are three main subtypes of amyloidosis (AL), including primary or AL, secondary or AA AL, and hereditary or familial AL. Hereditary AL, which is less common, is caused by an autosomal-dominant mutation, most frequently in the transthyretin (TTR) gene, and has a more favourable prognosis. We report the case of a 51-year-old Vietnamese male who presented with severe dizziness, fainting episodes, low BMI, slow heart rate, and a blood pressure of 60/40 mmHg. The patient had a history of digestive disorders, weakness, and pain in the legs even after disc spine surgery. All symptoms onset occurred at 43 years old. A pathogenic mutation in the TTR gene c.209G>T was confirmed in the patient and 7 out of 15 individuals in his extended family. This is the first case reported in Vietnam diagnosing a new variant of TTR causing typical AL. Based on this study, we conclude that the TTR variant c.209G>T is a pathogenic gene. This study also emphasises the need for increased knowledge regarding AL associated with this pathogenic variant. However, further extensive research is required to provide a comprehensive understanding of the pathogenesis of TTR in the future.

Keywords: **amyloid, digestive disorders, hereditary, slow heart rate.**

Classification number: **3.2**

1. Introduction

AL is a heterogeneous disease, acquired or hereditary in nature. Its common cause lies in the accumulation of toxic insoluble beta-sheet fibrillar protein aggregates within various organs, resulting in impaired organ function. The process of amyloid formation originates from a wide array of misfolded proteins converging towards a similar fibril structure. This accumulation of amyloid induces cellular stress and death, altering tissue architecture, and leading to organ dysfunction and eventual mortality [1]. Organs frequently affected by AL encompass the heart, kidneys, liver, spleen, nervous system, and digestive tract, presenting with diverse clinical symptoms such as cardiomyopathy, hepatomegaly, proteinuria, macroglossia, autonomic dysfunction, ecchymoses, neuropathy, renal failure, hypertension, and corneal and vitreous abnormalities. The disease can manifest as localized or systemic, with certain types of AL showing comorbidity with other diseases [2].

AL is classified into numerous subtypes based on the precursor protein involved, including AL with monoclonal immunoglobulin light chain, ATTRm with mutant TTR, and ATTRwt with wild-type TTR. Among hereditary AL, TTR is the most prevalent subtype, with over 100 pathogenic TTR mutations reported to date. Mutations in the TTR gene are accountable for hereditary AL, with the TTR gene located on 18q.12.1. TTR is a 55kDa tetrameric transport protein comprising four identical subunits of 127 amino acids. It is secreted from the liver into the blood and cerebrospinal fluid to facilitate the transportation of thyroid hormone thyroxin and retinol to the liver. Various mutations in the TTR gene have been identified in patients with familial amyloid polyneuropathy (FAP) or familial cardiac AL, which typically manifests with adult onset, follows an autosomal dominant inheritance pattern, and is primarily characterised by progressive and irreversible peripheral nerve damage.

Among the various types of AL that can culminate in life-threatening organ failure, amyloid cardiomyopathy stands out, characterised by the infiltration of amyloid into the heart muscle and other organs, leading to impaired cardiac and organ function [3, 4]. In the context of ATTR, amyloid fibril

*Corresponding author: Email: ngoclannguyen@hmu.edu.vn

deposition in the heart, stemming from the conversion of TTR homotetramers, precipitates heart failure, predominantly presenting as cardiomyopathy in the elderly. Extracellular deposition of amyloid fibres initiates intracellular stress responses, culminating in heightened inflammation, apoptosis, and organ dysfunction. Recent studies propose that cardiac amyloid derived from TTR may significantly contribute to the pathogenesis of heart failure with preserved ejection fraction. The incidence of this rare disease is estimated to be approximately 5-8 cases per 1 million people in the United States and the United Kingdom [5].

In this study, we diagnose and examine a family presenting with complex and diverse symptoms of TTR, particularly involving the cardiovascular system, commonly known as amyloid cardiomyopathy.

2. Case presentation

2.1. Chief complaints

A 51-year-old Vietnamese male presented to Hanoi Medical University with severe dizziness and fainting episodes persisting for one week.

2.2. History of past illness

In 2015, at 43 years old, the patient began experiencing digestive disorders, particularly loose stools postprandially, which progressively worsened over time, leading to significant debilitation and a weight loss of 20 kg. In 2018, at 46 years old, the patient underwent disc surgery in the L4-L5 region of the spine, after which his leg weakness and pain were exacerbated, especially during exertion. Approximately two years ago, in 2021, at 49 years old, the patient started experiencing increasing fatigue, shortness of breath, weakness, dizziness, and episodes of fainting upon sitting up. Additionally, the patient observed spontaneous bruising of the left eyelid. Despite seeking medical care at multiple hospitals in Vietnam and Singapore and undergoing comprehensive gastrointestinal endoscopy, cranial magnetic resonance imaging, and various tests, no definitive diagnosis was made, and treatments failed to alleviate symptoms.

2.3. Physical examination upon admission

Upon admission, the patient was 1.58 m tall, weighed 37 kg, resulting in a BMI of 14.8 kg/m². His heart rate was only 12-15 bpm, and his blood pressure measured 60/40 mmHg.

2.4. Imaging examinations

Electrocardiogram on admission revealed a 3rd-degree AV block with a very low ventricular rate of approximately 12-15 bpm (Fig. 1).

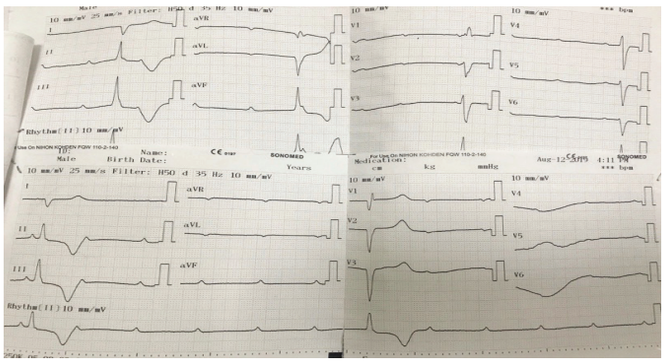

Fig. 1. Electrocardiogram in the emergency department. Third-degree heart block with an extremely slow heart rate of 12 bmp.

Echocardiography depicted a significantly thickened and brightly echogenic myocardium, with preserved left ventricular systolic function (EF 58%). However, severe diastolic dysfunction was noted, along with speckle pattern changes in total longitudinal strain (GLS) at the apex (Fig. 2).

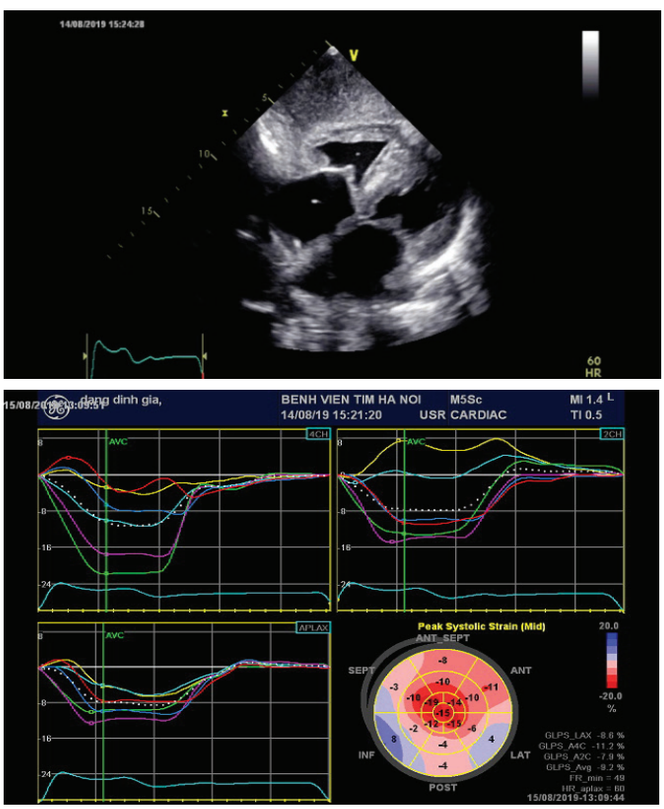

Fig. 2. Echocardiography.

Cardiac magnetic resonance displayed diffuse late gadolinium enhancement in two ventricles and two atriums, significant wall thickness exceeding 18 mm, minimal pericardial effusion, and preserved left ventricular function (EF≥60%) (Fig. 3).

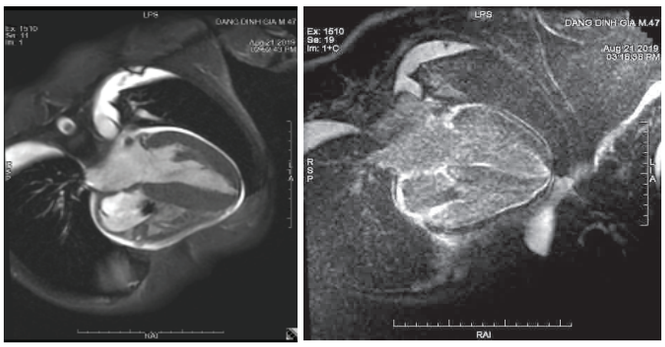

Fig. 3. Diffuse late gadolinium enhancement on cardiac magnetic resonance.

Coronary angiography revealed no signs of damage. The 99m Technitium-Pyrophosphate imaging strongly indicated the presence of TTR AL, with a semi-quantitative imaging score of 3 and a cardiac/contralateral ratio (H/CL ratio) of 2 (Fig. 4).

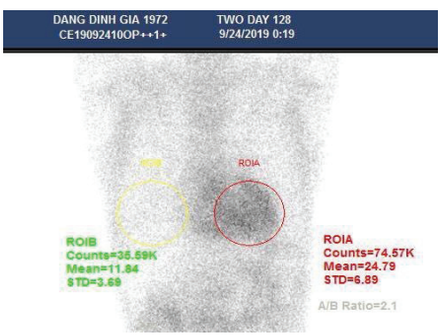

Fig. 4. The 99m Tc-PYP uptake by the heart using the contralateral heart-to-lung ratio (H/CL) =2.1.

2.5. Laboratory examinations

Blood tests revealed renal dysfunction (eGFR 34 ml/min), myocardial trauma (high-sensitive Troponin T: 252 pg/ml), and heart failure (NT-proBNP: 13259 pg/ml).

Serum immunofixation tests yielded normal results. Abdominal fat and tongue biopsies did not exhibit Congo red staining, indicating a negative result for AL.

2.6. Sequencing of TTR gene

Peripheral blood was collected from the patient and sent to the Green Cross laboratory in Korea for sequencing of the TTR gene. The Diagnostic Exome Sequencing method was utilised, scanning 5870 genes, revealing a heterozygous mutation for c.209G>T (p.Ser70Ile) on the TTR gene. Sanger sequencing was subsequently performed on samples harbouring the mutation. The primer sequences are shown in Table 1. The results of the samples are shown in Fig. 5.

Table 1. Summary of clinical and genetic findings in patients with TTR variant c.2009G>T.

Clinical symptoms	1st Cousin (III:4)	1st older sister (III:5)	2nd Cousin (III:6)	3rd Cousin (III:7)	2nd older sister (III:10)	Granddaughter (IV:8)	Grandson (IV:9)	Patient (III:15)
Sex	M	M	F	M	F	F	M	M
Year old	1970	1976	1969	1972	1981	1999	2004	1972
Fainting	+	-	+		-	-	-	+
Low blood pressure	+	-	+		-	-	-	+
Heart failure	+	-	+		-	-	-	+
Chronic kidney disease	-	-	+		-	-	-	+
Heart arrhythmia	+	-	+		-	-	-	+
Cardiovascular symptoms: tricuspid regurgitation, myocardial hypertrophy...	+	+/-	+	+	-	-	-	+
Age of onset of digestive disorders	47	+/-	48	50	-	-	-	42
Digestive symptoms: colon, stomach, liver...	+++	+/-	+++	+	+/-	-	-	+++

(-): none; (+): yes; (+++): yes in severe degree.

2.7. Medical management

A permanent chamber pacemaker compatible with MRI was implanted, and low-dose vasopressors were administered to stabilise blood pressure. Digestive enzyme supplements and multivitamins were prescribed to alleviate gastrointestinal

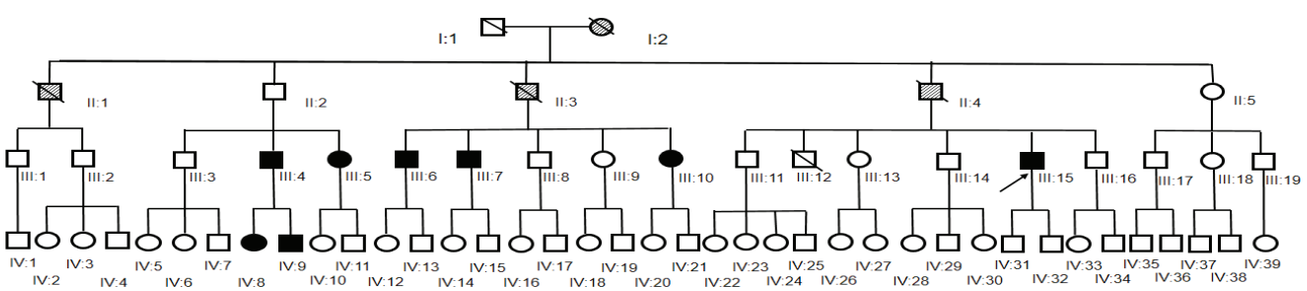

Fig. 5. Pedigree of an extended family with four generations. The proband was indicated by black arrow. Male individuals were represented by squares, while female individuals were represented by circles. Individuals with mutation were denoted by black color; individuals without mutation were denoted by white color; individuals who had died were denoted with the cross; individuals who had the similar clinical symptoms were denoted by grey color.

disturbances. Although fainting episodes improved post-pacemaker implantation, the patient continued to experience low blood pressure (around 80/60 mm Hg) and dizziness upon position changes, with persistent loose stools following meals. Given the laboratory and imaging findings, the patient was diagnosed with ATTR-type AL.

Since treatment for this disease had not been reported in Vietnam, the patient's records were forwarded to colleagues in the United States for further evaluation. Upon reviewing the tests and imaging conducted in Vietnam, the American colleagues confirmed the diagnosis of ATTR AL without the need for additional testing. The patient was fortunate to be enrolled in a clinical trial providing specific treatment free of charge. The treatment involved a short double-stranded interfering RNA targeting the liver, which led to a notable improvement in symptoms shortly after initiation.

3. Final diagnosis

ATTR Amyloidosis.

4. Other cases in the patient's family

The pedigree of the extended family spanning four generations is depicted in Fig. 6. Following receipt of the sequencing results, samples were collected from an additional 15 members of the patient's extended family, including five cousins (III:4, III:5, III:6, III:7, III:10) and some of their offspring (IV:5, IV:6, IV:7, IV:8, IV:9, IV:10, IV:11, IV:12, IV:13, IV:14, IV:15), for sequencing tests. Clinical data were obtained for all members of the extended family. The patient's paternal grandmother (I:2), uncles (II:1, II:3), and father (II:4) all exhibited similar symptoms, unfortunately resulting in their demise.

The sequencing test results revealed that five other individuals, who were cousins of the patient (III:4, III:5, III:6, III:7, III:10), exhibited identical results. Among them, one family from the patient's uncle (II:2) had two out of three children (III:3, III:4) carrying the disease gene. Furthermore, one child with the disease gene from this family (III:4) had a son and a daughter (IV:8, IV:9), both of whom carried the disease gene and manifested symptoms (Table 1).

5. Discussion

ATTR arises from abnormal fibres derived from TTR, a protein primarily synthesised by the liver, which aggregate and deposit in tissues and organs. The clinical manifestations of this disease are diverse, contingent upon the organs affected. In the patient's history, the onset of gastrointestinal and radicular symptoms preceding cardiovascular symptoms suggests a singular association with AL. However, definitive testing confirming the disease was not performed at that time. All clinical features observed in this patient are consistent with sensory-autonomic symptoms.

Upon the patient's admission to the hospital, cardiovascular symptoms were most pronounced. In his extended family, digestive and cardiovascular symptoms were more prevalent and readily identifiable than other manifestations. Cardiomyopathy, particularly ATTR-associated cardiomyopathy (ATTR-CM), is a common presentation of ATTR AL and is linked to a notably diminished life expectancy of 2 to 6 years post-diagnosis. Patients with ATTR-CM endure debilitating physical symptoms akin to heart failure, such as exercise intolerance and fatigue, culminating in reduced activity capacity, compromised quality of life, and eventual mortality. ATTR-CM can manifest as either the wild-type TTR subset (ATTRwt) or be inherited from multiple genetic variants of TTR (mutant transgenic AL ATTRm; also recognised as hereditary ATTR) [6].

Conforming to the consensus recommendations for the suspicion and diagnosis of cardiac AL published in Circulation Heart Failure in September 2019, screening for monoclonal protein AL is imperative in symptomatic patients exhibiting suggestive electrocardiography, echocardiography, cardiac MRI, and AL biomarkers. This involves conducting three tests: serum kappa/lambda free light chain ratio (abnormal if the ratio falls below 0.26 or exceeds 1.65), serum immunofixation (abnormal if monoclonal protein is detected), and urine immunofixation (abnormal if monoclonal protein is detected). These tests typically yield normal results for patients with ATTR AL [2].

Our patient underwent treatment for various digestive and neurological ailments across multiple medical facilities, yet no definitive diagnosis was reached. It was only upon the onset of cardiovascular symptoms, with echocardiogram findings indicative of thickened and brightened heart walls, that suspicion of this disease arose. However, our understanding of the disease at that juncture was limited. Subsequent testing confirmed the diagnosis, based on myocardial perfusion with 99m pyrophosphate, indicating TTR AL. An abnormal genetic test further confirmed the hereditary pattern of the patient's ATTR.

The genetic mutation detected in our patient is seldom documented in the literature. The most prevalent variant worldwide is Val122Ile (or pV142I), occurring in 3-4% of black Americans with an indeterminate gene penetrance [7]. The cardinal clinical presentation of this TTR Val122Ile variant is cardiomyopathy, with an estimated 10% of Black Americans over 60 years old with heart failure harbouring this variant. The Val30Met variant is the most prevalent in manifesting neuropathy [8]. Our patient's genetic mutation is heterozygous c.209G>T (p.Ser70Ile), previously reported in select Asian cases. For example, ATTR Ser50Ile was reported in two Japanese patients by Nishi and Saeki. Briefly, TTR gene PCR products were denatured in the presence of formamide and were electrophoresed in undenatured polyacrylamide gels to detect electrophoretic changes due to sequence variation. An abnormal DNA fragment was visualised by silver staining in exon 3 PCR products from the patient. Subsequent

sequencing analysis revealed a T-to-A transition and resulted in a substitution of Ser by Ile at codon 50 of the TTR gene. Saeki found the exon 3 variant at the 50th codon, and the AGT encoding for Ser was changed to the ATT encoding for Ile. The mechanism through which variant TTR molecules are deposited remains incompletely understood, although mutations at phylogenetically conserved sites of the TTR molecule may play a pivotal role in amyloid formation [9, 10].

In a study detailing the distinctive phenotypic and genetic features of hereditary ATTR within a multiracial South - East Asian cohort in Singapore, the variant ATTR-S50I (p.Ser70Ile) was identified in patients exhibiting somatic neuropathy diseases, including carpal tunnel syndrome, autonomic dysfunction, and cardiac dysfunction. These cases were characterised by early onset and a positive parental history [11].

Data on the occurrence of hypertrophic cardiomyopathy (HCM) in individuals with hereditary ATTR AL have been collected by the Hypertrophic Cardiomyopathy Genetics Oversight Committee. This data indicates that some individuals with hereditary ATTR AL exhibit characteristics of HCM and/or restrictive cardiomyopathy (RCM). The prevalence of HCM and/or RCM in hereditary ATTR AL is high, but the exact percentage has not been accurately determined due to the high prevalence of HCM and RCM (approximately 25%) in cardiac AL associated with aging. This condition is caused by the accumulation of misfolded TTR in the heart over time. Certain variants, such as TTR-V30M, TTR-T60A, and TTR-V122L, are commonly associated with cardiac AL.

The first autopsy case report by N. Sakashita, et al. (2000) [10] described the clinical pathological findings for two cases of FAP with the single amino acid mutation ATTR Ser50Ile. The report demonstrated systemic amyloid deposition in various organs and tissues of the autopsy cases. Initially, signs and symptoms in FAP (ATTR Ser50Ile) did not differ from those of typical FAP (ATTR Val30Met). However, cardiac symptoms, particularly congestive heart failure, became prominent in the early clinical stage. Consistent with the present cases, previous reports have highlighted severe heart failure and fatal arrhythmias as the most serious issues in this type of mutation. The transplantation of pacemakers may improve prognosis. This present examination revealed significant amyloid deposition in the cardiovascular system, akin to that described in previous autopsy reports of the TTR-associated FAPs of Ser50Arg and Tyr114Cys [12-14]. The total amount of amyloid in the current autopsy case was immeasurable compared to the 20 FAP cases (ATTR Val30Met) previously reported [13-15].

The ATTR Ser50Ile mutation has been documented in several studies conducted in Japan [12-16]. DNA sequence analysis by K. Sadamatsu, et al. (1997) [12] revealed the presence of the 50TTR Ile mutation in one patient. This individual presented clinical features of hereditary sensory and autonomic neuropathy, alongside autonomic dysfunction and amyloid cardiomyopathy. The 58-year-old Japanese woman experienced weakness, numbness, and chest pain during exertion, alongside sensory disturbances in her lower limbs over four years. A notable amyloid deposition was detected in her colon. The onset age for these four patients ranged from the fourth to fifth decade of life. Hence, FAP associated with the TTR Ile 50 mutation is likely to manifest in middle age, predominantly affecting peripheral nerves and the heart [12].

Due to financial constraints, the patient's family could only conduct testing on 16 individuals, revealing that 50% of them carried the disease-causing gene. Among those carrying the gene, two-thirds exhibited cardiovascular and gastrointestinal symptoms. Moreover, a case within the patient's extended family was identified where the father carried the disease-causing gene, and both the son and daughter were also affected. This, alongside relevant clinical evidence, supports the attribution of disease-causing potential to the TTR c.209G>T (p.Ser70Ile) variant, the first variant identified in Vietnam for diagnosing hereditary ATTR disease. These findings contribute to genetic counselling and prevention strategies for individuals carrying the disease-causing gene, aiming to mitigate the risk of future disease occurrence.

Despite study limitations, such as a small sample size, it still underscored the disease-causing impact of the variant from a genetic standpoint. Furthermore, the inability to analyse the patient's parents and siblings to explore potential associated variants, lack of biopsy to confirm the diagnosis, and challenges in investigating the patient's neurological symptoms complicated the assessment of the relationship between neuro symptoms and the disease. Moreover, diagnosing hereditary ATTR disease within a clinical setting poses challenges. Although efforts were made to analyse relevant reports on the TTR c.209G>T (p.Ser70Ile) variant, currently, no documented reports on this variant exist in the literature worldwide or in Vietnam. Thus, establishing the role of this variant in the disease's pathogenesis remains challenging. With the advancements in molecular genetic testing, new technologies in genetic sequencing could facilitate the identification of multigene panels, including the TTR gene and other genes with numerous variants [17-20]. If this multigene panel could be implemented in diagnostics, genotype-phenotype correlations and disease causation could be elucidated more clearly.

Currently, there have been no reports published regarding the penetrance of the TTR gene. However, based on the findings from a cluster of cases and some related reports, we can cautiously suggest that hereditary ATTR disease may exhibit incomplete penetrance, depending on the age of the affected individuals and the varying levels of expression, which often occur later in life. Therefore, identifying individuals carrying the disease-causing gene is of significant importance in preventing disease progression and plays a crucial role in counselling, screening, prenatal diagnosis, and premarital assessment.

6. Conclusions

Hereditary TTR AL is a rare disease with diverse clinical manifestations, but its nonspecific nature makes diagnosis a significant challenge for clinicians. We report a cluster of hereditary TTR AL cases in Vietnam with a different gene mutation than those previously reported worldwide. This study emphasises the importance of genetic testing in disease diagnosis, as hereditary TTR AL presents with late-onset and multi-organ involvement. Diagnosis often requires a multidisciplinary approach, and treatment remains a complex issue due to the high cost of targeted therapies. Further in-depth research should be conducted to improve diagnostic capabilities and gain a better understanding of the underlying disease mechanisms, aiming to reduce treatment costs for patients when the disease is in advanced stages.

CRediT author statement

Ngoc Lan Thi Nguyen: Reviewing, Methodology, Data analysis, Data collection, Writing, Editing; Thu Anh Nguyen: Data collection; Quy Van Vu: Data analysis; Huyen Thi Vu: Writing, Editing; Phuong Lan Thi Dam: Writing, Editing; Duc Tuan Nguyen: Data analysis.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] G. Merlini, V. Bellotti (2003), "Molecular mechanisms of amyloidosis", *The New England Journal of Medicine*, **349(6)**, pp.583-596, DOI: 10.1056/NEJMra023144.

[2] M.S. Maurer, S. Bokhari, T. Damy, et al. (2019), "Expert consensus recommendations for the suspicion and diagnosis of transthyretin cardiac amyloidosis", *Circulation Heart Failure*, **12(9)**, DOI: 10.1161/CIRCHEARTFAILURE.119.006075.

[3] Y. Ando, T. Coelho, J.L. Berk, et al. (2013), "Guideline of transthyretin-related hereditary AL for clinicians", *Orphanet J. of Rare Dis.*, **8**, DOI: 10.1186/1750-1172-8-31.

[4] Y. Sekijima, M. Ueda, H. Koike, et al. (2018), "Diagnosis and management of transthyretin familial amyloid polyneuropathy in Japan: Red-flag symptom clusters and treatment algorithm", *Orphanet J. Rare Dis.*, **13(1)**, DOI: 10.1186/s13023-017-0726-x.

[5] T.P. Quock, T. Yan, E. Chang, et al. (2018), "Epidemiology of AL amyloidosis: A real-world study using US claims data", *Blood Advances*, **2(10)**, pp.1046-1053, DOI: 10.1182/bloodadvances.2018016402.

[6] W. Hassan, H.A. Sergani, W. Mourad, et al. (2005), "Amyloid heart disease: New frontiers and insights in pathophysiology, diagnosis, and management", *Texas Heart Inst. J.*, **32(2)**, pp.178-184.

[7] F.L. Ruberg, M. Grogan, M. Hanna, et al. (2019), "Transthyretin amyloid cardiomyopathy: JACC state-of-the-art review", *Journal of The American College of Cardiology*, **73(22)**, pp.2872-2891, DOI: 10.1016/j.jacc.2019.04.003.

[8] M. Bruno, A. Castano, A. Burton, et al. (2021), "Transthyretin amyloid cardiomyopathy in women: Frequency, characteristics, and diagnostic challenges", *Heart Failure Reviews*, **26(1)**, pp.35-45, DOI: 10.1007/s10741-020-10010-8.

[9] H. Nishi, A. Kimura, H. Harada, et al. (1992), "Novel variant transthyretin gene (Ser50 to Ile) in familial cardiac amyloidosis", *Biochemical and Biophysical Research Communications*, **187(1)**, pp.460-466, DOI: 10.1016/S0006-291x(05)81516-5.

[10] N. Sakashita, Y. Ando, K. Obayashi, et al. (2000), "Familial amyloidotic polyneuropathy (ATTR Ser50Ile): The first autopsy case report", *Virchows Archiv: An International Journal of Pathology*, **436(4)**, pp.345-350, DOI: 10.1007/s004280050457.

[11] Z. Chen, J.S. Koh, M. Saini, et al. (2021), "Hereditary transthyretin amyloidosis - Clinical and genetic characteristics of a multiracial south-east Asian cohort in Singapore", *Journal of Neuromuscular Diseases*, **8(4)**, pp.723-733, DOI: 10.3233/JND-210656.

[12] K. Sadamatsu, Y. Hayashi, M. Nakamura (1997), "Three siblings of familial amyloid cardiomyopathy with isoleucine-50 transthyretin mutation", *International Journal of Cardiology*, **61(2)**, pp.151-155, DOI: 10.1016/S0167-5273(97)00145-9.

[13] K. Takahashi, S. Yi, Y. Kimura, et al. (1991), "Familial amyloidotic polyneuropathy type 1 in Kumamoto, Japan: A clinicopathologic, histochemical, immunohistochemical, and ultrastructural study", *Human Pathology*, **22(6)**, pp.519-527, DOI: 10.1016/0046-8177(91)90227-g.

[14] S. Ueno, H. Fujimura, S. Yorifuji, et al. (1992), "Familial amyloid polyneuropathy associated with the transthyretin Cys114 gene in a Japanese kindred", *Journal of Neurology*, **115**, Pt. 5, pp.1275-1289, DOI: 10.1093/brain/115.5.1275.

[15] N. Takahashi, S. Ueno, T. Uemichi, et al. (1992), "Amyloid polyneuropathy with transthyretin Arg50 in a Japanese case from Osaka", *J. Neurol. Sci.*, **112(1-2)**, pp.58-64, DOI: 10.1016/0022-510X(92)90132-5.

[16] Y. Date, M. Nakazato, K. Kangawa, et al. (1997), "Detection of three transthyretin gene mutations in familial amyloidotic polyneuropathy by analysis of DNA extracted from formalin-fixed and paraffin-embedded tissues", *J. Neurol. Sci.*, **150(2)**, pp.143-148, DOI: 10.1016/s0022-510x(97)00077-4.

[17] P. Han, G. Wei, K. Cai, et al. (2020), "Identification and functional characterisation of mutations in LPL gene causing severe hypertriglyceridaemia and acute pancreatitis", *J. Cell. Mol. Med.*, **24(2)**, pp.1286-1299, DOI: 10.1111/jcmm.14768.

[18] R. Zhang, S. Chen, P. Han, et al. (2020), "Whole exome sequencing identified a homozygous novel variant in CEP290 gene causes Meckel syndrome", *J. Cell. Mol. Med.*, **24(2)**, pp.1906-1916, DOI: 10.1111/jcmm.14887.

[19] Y. Dai, S. Liang, X. Dong, et al. (2019), "Whole exome sequencing identified a novel DAG1 mutation in a patient with rare, mild and late age of onset muscular dystrophy-dystroglycanopathy", *J. Cell. Mol. Med.*, **23(2)**, pp.811-818, DOI: 10.1111/jcmm.13979.

[20] Y. Zheng, J. Xu, S. Liang, et al. (2018), "Whole exome sequencing identified a novel heterozygous mutation in *HMBS* gene in a Chinese patient with acute intermittent porphyria with rare type of mild anemia", *Front Genet*, **9(129)**, DOI: 10.3389/fgene.2018.00129.