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Case report

Familial exploration: A novel association of Thr 60Pro mutations in the Transthyretin gene with familial amyloidosis polyneuropathy

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Abstract: In this study, we present a rare case of familial amyloidosis polyneuropathy (FAP) caused by a novel mutation in the transthyretin protein (TTR) gene. A 40-year-old female patient presented with a complaint of numbness and weakness in both lower limbs that had persisted for a period of 18 months, accompanied by intermittent diarrhea. An analysis of her family history revealed that her father had succumbed to an instance of unexplained heart failure, while one of her siblings exhibited neurological symptoms of a comparable nature. A physical examination was conducted, which revealed sensory loss of distal symmetry, diminished tendon reflexes, and significant autonomic dysfunction. The skin biopsy revealed the presence of amyloid material deposits. Whole-exome sequencing revealed a novel mutation (c.179A>C, p.Thr60Pro), which replaces threonine with a proline at position 60 in the TTR gene. Subsequent screening revealed that other relatives carrying the same mutation also exhibited similar symptoms, thereby supporting the association between this mutation and FAP. This case underscores the significance of identifying novel TTR gene mutations, offers a novel perspective on the understanding of FAP, and posits that an early diagnosis and intervention are pivotal to enhance the prognosis.

Keywords: familial amyloidosis polyneuropathy (FAP); transthyretin protein (TTR); Thr 60Pro mutation

1. Introduction

Familial amyloidosis polyneuropathy (FAP) is a rare genetic disorder primarily caused by mutations in the transthyretin protein (TTR) gene. These mutations lead to abnormal protein deposition in the peripheral nervous system and other organs, thus resulting in progressive neurological impairment and multisystem dysfunction. Globally, over 100 distinct TTR gene mutations have been linked to FAP, with

Val30Met and Thr60Ala being among the most common [1]. Despite advancements in understanding TTR gene mutations, many aspects of FAP remain unexplored. An estimated 50,000–100,000 individuals worldwide are affected by FAP, and identifying new mutations is crucial as it deepens our understanding of the disease's genetic underpinnings and paves the way for personalized treatment strategies.

This report presents a rare case of FAP caused by a TTR gene mutation which has not been reported in the past. The aim is to emphasize the significance of an accurate diagnosis, the appropriate antimicrobial therapies, and to provide an overview of reported cases to guide evidence-based clinical decision-making.

2. Case presentation

The patient is a 40-year-old Han Chinese female from a city in southern China. The patient presented with progressive symptoms of numbness and weakness in both lower limbs over the past 18 months, with intermittent diarrhea and weight loss. The initial symptoms included mild toe numbness, then gradually spread upward into the calves and thighs, which seriously affected her daily life and work ability. The patient had no history of smoking or alcohol consumption and denied any comorbid chronic diseases. A review of the patient's medical history revealed that her father died at the age of 40 due to an unexplained heart failure, with no documented diagnosis. One of her siblings (aged 48 years) presented with neurological symptoms of numbness and weakness in both lower limbs; however, a detailed medical evaluation had not been performed. The patient's grandfather and maternal grandfather both died of heart disease at the approximate age of 60. The occurrence of early-onset cardiovascular events and neurological symptoms in these family members suggests a genetic basis for the disease.

The patient's blood pressure was 110/70 mmHg in the prone position and 70/40 mmHg in the standing position. The laboratory test results showed that the patient's blood routine, liver and kidney function, blood sugar, blood lipid, and other indicators were within the normal range. The electrocardiogram showed multiple premature atrial beats, and the echocardiogram results showed that the mitral flow Vmax(E) = 51 cm/s, Vmax(A) = 86 cm/s, E/A = 0.59, which suggested impaired diastolic function of the left ventricle.

A nerve conduction study (NCS) showed the following: the bilateral tibial nerve compound muscle action potentials (CMAP) nearly disappeared, a CMAP amplitude of the left tibial nerve was 0.5 mV (normal 8–20 mV), and the right did not lead; a motor nerve conduction velocity of the left was 28 m/s (normal 40–60 m/s) and the right was 26 m/s; and the distal motor latency of the left was 9.5 ms and the right was 10.2 ms (normal 3–5 ms). Neither peroneal nerve sensory nerve action potential (SNAP) was elicited bilaterally; the bilateral posterior tibial nerve SNAP amplitude was 0.3 μV on the left side and 0.2 μV on the right side (normal 10–35 μV), and the conduction velocity was 30 m/s on the left side and 29 m/s on the right side. Using electromyography (EMG), at rest, a large number of fibrillation potentials (++++) and positive sharp waves were seen in the quadriceps, tibialis anterior, and gastrocnemius muscles of both lower limbs. During light contraction, the tibialis anterior motor unit action potential (MUAP) had a time limit of 25 ms (normal 5–15 ms), a wave amplitude of 9 mV (normal 1–5 mV), and a polyphasic wave ratio of 50% (normal < 10%). During heavy contractions, only simple phases were elicited, and the motor unit recruitment was significantly reduced. For a definitive diagnosis, a skin biopsy was performed. The pathological examination

revealed amyloid deposits in the dermis, positive Congo red staining, and apple green birefringence under a polarized microscope.

To identify genetic factors, whole-exome sequencing (WES) was performed on the patient and her family members. The gene sequencing results showed that the patient had developed a threonine to proline mutation (c.179A>C, p.Thr60Pro) at site 60 in the TTR gene. Subsequently, the screening of her family members found that one of the patient's siblings carried the same mutation and showed similar neurological symptoms, thus supporting the association between this mutation and FAP (Figure 1).

Due to financial reasons, the treatment was halted after the patient was diagnosed. The patient became hemiplegic, was confined to the bed at home, and was taken care of by her family members.

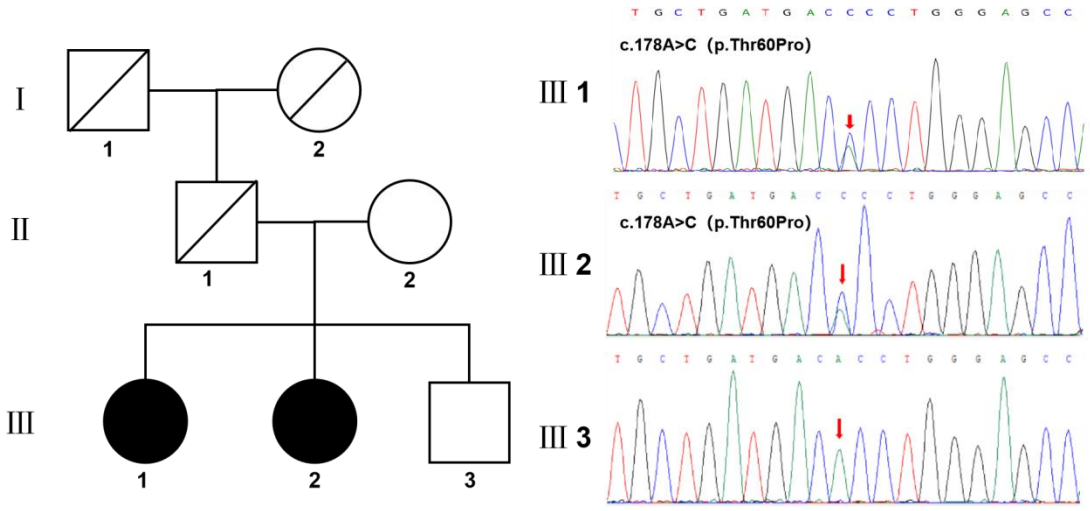

Figure 1. Patient's family tree (left), patient III 1 and sibling III 2, both with TTR gene mutations (c.179A>C, p.Thr60Pro); patient's NGS results (right).

3. Discussion

FAP was first reported by Andrade in 1952 in northern Portugal [2], followed by successive discoveries in Japan in 1968 [3] and in Sweden in 1976 [4]. As a typical protein misfolding disease, the pathogenesis of hereditary transthyretin-mediated amyloidosis (ATTRv) is centered on the mutation-induced dissociation of the TTR subunit, which triggers the misfolding and aggregation of TTR amyloid fibrils in the extracellular space, thus resulting in multi-organ dysfunction [5].

3.1. Pathophysiology and genetics

TTR is a polypeptide chain consisting of 127 amino acid residues, which is assembled to form a homotetrameric protein with a molecular weight of 56 kDa, and shows a prominent beta-folded secondary structure. Its main site of synthesis is the liver, with small amounts produced by the choroid plexus and retinal cells, and its normal physiological function is to transport thyroxine and retinol [6]. Pathogenic mutations destabilize the structure of TTR tetramers, which prompts their dissociation into monomers. These free monomers self-aggregate in the extracellular space to form non-fibrillar soluble oligomers and fibrils, which eventually assemble into insoluble amyloid fibrils [7].

The TTR gene (18q11.2-12) is approximately 7 kb in length and contains four exons, which

encode monomers that form soluble TTR tetramers by non-covalent binding. Current studies have identified 119 point mutations in the TTR gene, 113 of which are amyloidogenic [8]. The mutation spectrum shows significant differences by the geographic region, as the most common mutation type, TTR Val30Met results from a base substitution from guanine to cytosine in the exonic region of the gene, and is almost the only mutation detected in Portuguese, Brazilian, and Swedish populations, whereas up to 30 different TTR variants have been reported in Japan and France [8–10]. This study reports a novel mutation type, c.178A>C (p.Thr60Pro), in the TTR gene. This mutation has not been reported in the previous literature, and its discovery provides an important addition to the study of the genetic diversity of ATTRv amyloidosis, as well as a new perspective to expand the study of the disease diagnosis and pathological mechanisms.

3.2. Clinical manifestations

Autonomic neuropathy is common in patients with early-onset FAP and often involves the cardiac circulatory, gastrointestinal, and genitourinary systems. With the exception of intracardiac conduction failure, functional deficits in these systems most often appear later than sensory-motor symptoms. Cardiac autonomic dysfunction can lead to heterogeneous upright hypotension, and gastrointestinal symptoms manifest as alternating diarrhea and constipation, or dehydration and weight loss triggered by gastroparesis.

Extra-neurological manifestations mainly involve the heart, eyes, and kidneys. Literature data show that cardiac involvement is present in about 80% of TTR-related FAP cases, which can induce restrictive cardiomyopathy, arrhythmias, and severe conduction disorders such as an atrioventricular block with syncope, fainting, and even sudden death [11]. Ocular abnormalities are seen in about 10% of patients with TTR-associated FAP, which mainly include vitreous clouding and chronic open-angle glaucoma [12]. In a Portuguese study, renal involvement was seen in about one-third of patients, which manifested as nephritic syndrome and progressive renal failure [13]. Of interest, the patient in this case showed only symptoms of autonomic neuropathy, mainly as numbness and weakness of both lower limbs and intermittent diarrhea, and has not yet developed the typical extra-neurological manifestations of the multiple systems commonly described above, thus providing a unique case study of the diversity of clinical manifestations of FAP.

3.3. Diagnosis

A diagnosis of ATTRv amyloidosis can be confirmed by a biopsy detection of amyloid deposits combined with TTR gene sequencing to identify amyloidogenic variants [14,15]. These assays help to differentiate ATTRv amyloidosis from a variety of peripheral neuropathies [16]. Its formal diagnosis requires the detection of characteristic amyloid deposits in the biopsy samples [17], which usually appear greenish-yellow and birefringent when viewed using Congo red staining combined with polarised microscopy. Although immunohistochemistry can determine the biochemical nature of amyloid deposits, there is a risk of either false negatives or false positives. Among TTR gene sequencing methods, Sanger sequencing remains the gold standard due to its effectiveness in detecting and identifying rare or unknown variants [18]. ATTRv amyloidosis should be suspected in patients with a progressive symmetric sensorimotor neuropathy who fulfil at least one of the following criteria: A positive family history of ATTRv amyloidosis, early autonomic dysfunction, gastrointestinal distress,

unexplained weight loss, renal abnormalities, and vitreous clouding [19]. In this case, screening of the patient's family members showed that other relatives who carried the same mutation exhibited similar symptoms; this suggests the important role of family screening in the early diagnosis and prevention of FAP [20]. In this case study, histopathology of the peroneal nerve and skin of the patient revealed local fibrous tissue amyloidosis (positive Congo red staining), which was diagnosed by gene sequencing as a novel TTR gene mutation--Thr 60Pro. The patient's sister had the same mutation type, but fortunately, the patient's sister only had mild neurological symptoms.

3.4. Treatment

In ATTRv, mutations in the TTR gene can lead to misfolding of the TTR protein, which depolymerises from a stable tetrameric structure into either monomers or oligomers. These abnormal proteins further aggregate to form insoluble amyloid fibrils with a β- folded structure and are deposited in various tissues and organs such as the heart, nerves, and kidneys, thus destroying the normal tissue structure and impairing organ function. Based on an in-depth understanding of the different pathological stages of the disease, a series of therapeutic options have been developed to intervene in the pathogenic stages, alleviate the symptoms, and slow down the progression of the disease.

3.4.1. Liver transplantation

Liver transplantation is the classic treatment option for ATTRv amyloidosis, and patients with surgical indications can opt for liver transplantation to reduce the synthesis of variant transthyretin proteins, thus effectively inhibiting amyloid formation and prolonging the patient survival. In a 20-year retrospective analysis, the study found a 20-year survival rate of 55.3% after liver transplantation. A multivariate analysis showed that a higher BMI, an age <50 years at presentation, a shorter duration of disease prior to liver transplantation, and the TTR Val30Met mutation were independent predictors of survival [21].

3.4.2. TTR stabilizers

TTR stabilizers prevent dissociation, misfolding, and amyloid fiber formation by stabilizing the tetrameric structure of TTR. Two types of TTR stabilizers are currently available: Tafamidis and Diflunisal. Tafamidis is an oral, highly specific stabilizer of thyroxine transport. A clinical study showed that early intervention with tafamidis resulted in a long-term delay in the neurological progression of ATTRv amyloidosis [22]. Diflunisal is a non-steroidal anti-inflammatory drug that stabilizes thyroxine tetramers in vitro and prevents the formation of amyloid fibrils. A randomized controlled clinical trial found that 2 years of Diflunisal use reduced the rate of progression of neurological impairment and maintained the quality of life in patients with familial amyloid polyneuropathy compared to placebo [23].

3.4.3. Gene silencing therapy

RNA interference (RNAi) mediates the cleavage of specific mRNAs, which leads to robust and durable reductions in the gene target expression. In a phase II study that included 29 patients with

ATTRv amyloidosis, the administration of the RNAi drug patisiran at 0.3 mg/kg body weight every 3 weeks reduced the mean serum levels of TTR by approximately 80% [24]. A multicenter observational study in Italy found that the majority of patients demonstrated stable progression of neuropathy following patisiran treatment, regardless of the baseline disease severity or genotype, and the treatment was well tolerated, with 90% of patients reporting no adverse events [25].

Inotersen is a second-generation antisense oligonucleotide that targets and reduces levels of TTR RNA transcripts, and reduces the levels of TTR RNA transcripts. In a Phase I study in healthy volunteers, 4 weeks of inotersen treatment was well tolerated and reduced the plasma TTR levels by up to 96% [26].

3.4.4. Novel therapy

An alternative strategy to treat ATTRv amyloidosis is to promote the clearance of amyloid deposits with monoclonal antibodies directed against components of these deposits. Serum amyloid P component (SAP), a ubiquitous non-profibrillar plasma glycoprotein present in amyloid deposits, can be depleted of residual SAP with miridesap, a small molecule drug that depletes circulating SAP, in combination with dezamizumab, a fully humanized anti-SAP monoclonal antibody. A single-dose incremental phase I trial demonstrated the safety and efficacy of dezamizumab in 15 patients with amyloidosis [27].

3.5. Limitations

There were two limitations within in this study. First, the key pathological images of the patient's histological examinations, such as immunohistochemistry, which were completed at a later stage in an outside hospital, could not be included in this paper; this makes the presentation of the characteristic pathological changes of the disease incomplete, and weakens the visual evidence of the pathological diagnosis and the completeness of the study. Second, the patient was not able to receive systemic treatment due to financial constraints; although the follow-up showed that she maintained basic life functions under home care, the lack of data on the evolution of the disease course under interventions made it difficult to assess the impact of treatment on the disease due to this rare mutation. Nonetheless, this study reports a novel de novo mutation c.178A>C (p.Thr60Pro) in the TTR gene, which still provides important clues for ATTRv-related studies.

4. Conclusion

This case is the first to identify and report a de novo mutation c.178A>C (p.Thr60Pro) in the TTR gene, which was significantly associated with neurological damage in the patient, thus further supporting the central role of TTR gene mutations in the process of FAP triggered by ATTRv. This finding not only expands the spectrum of ATTRv-causing mutations, but also provides a new diagnostic perspective for patients with similar clinical manifestations that are difficult to diagnose by conventional tests.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Guoyan Chen conducted patient admission, medical history collection, physical examinations, and auxiliary examinations; drafted the initial manuscript. Jing Gao organized clinical data and analyzed case characteristics. Ziyao Li performed data analysis and interpretation of results; assisted in data organization. Jine He designed the research framework, reviewed the full manuscript, coordinated the submission process, and ensured the integrity of the study.

Ethics approval of research

This research has received ethical approval by the Ethics Commision of Yanan University Affiliated Hospital (IIT-R-20250066). A Written informed consent was obtained from the patient.

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