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A Missense Variant p.Ala117Ser in the Transthyretin Gene of a Han Chinese Family with Familial Amyloid Polyneuropathy

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Abstract Familial amyloid polyneuropathy (FAP) is a dominantly inherited disorder. This study aims to explore the genetic features of a Han Chinese family with FAP, characterized by bloating, alternating diarrhea and constipation, and weakness in his feet. Amyloid presented histologically in the vessel walls of hepatic portal area and nerves of the surgically excised liver specimens from the proband by hematoxylin and eosin staining. Amyloid deposition was further confirmed with Congo red treatment. A c.349G>T transversion (p.Ala117Ser) in *TTR* gene exon 4 was identified in the proband with typical autonomic neuropathy and peripheral motor neuropathy, as well as in his asymptomatic son. The variant was not detected in 200 normal ethnically matched controls. These findings provide new insights into FAP cause and diagnosis and have implications for genetic counseling.

Keywords Familial amyloid polyneuropathy · The *TTR* gene · Missense variant · Liver transplantation

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Introduction

Familial amyloid polyneuropathy (FAP, OMIM 105210) is a rare, autosomal-dominant disease, characterized by amyloid accumulation in the peripheral nerves and other organs, including the heart, kidneys, and eyes [1]. FAP was first described in Portugal in 1952 and was originally thought to be endemic in only a few countries including Portugal, Japan, and Sweden. It was later reported in other locations [2, 3]. FAP can be caused by the following four genes: the transthyretin gene (*TTR*, OMIM 176300), the apolipoprotein A1 gene (*APOA1*, OMIM 107680), the gelsolin gene (*GSN*, OMIM 137350), and the beta-2-microglobulin gene (*B2M*, OMIM 109700) [4]. Of these, the *TTR* amyloidosis is the most common form [5]. The *TTR* p.Val50Met mutation was firstly described as the cause of FAP in 1984 [6]. Currently, more than 125 *TTR* mutations have been identified, of which 13 *TTR* mutations seem to be non-amyloidogenic. All are missense point mutations except for one microdeletion p.Val142del (http://www.amyloidosismutations.com/main_menu.html). The p.Val50Met mutation is the most common *TTR* mutation reported in 85% of the FAP patients from the Familial Amyloidotic Polyneuropathy World Transplantation Registry (FAPWTR) [5, 7].

Nerve length-dependent, sensory-motor, and autonomic polyneuropathy beginning in the feet is the neurological feature of *TTR*-FAP [1, 5]. The phenotypes vary dramatically between kindreds with different variants. It is difficult to establish a firm genotype-phenotype correlation in FAP. Clinical phenotype variability exists even among the same family and individuals with the same point mutation [3, 8, 9]. Though patients with *TTR* p.Ile127Val mutation were reported to have severe FAP with shorter median survival [10], individuals with compound heterozygous p.Val50Met and p.Thr139Met or p.Arg124His variants present with a mild form of FAP [8],

indicating that the phenotype modifiers may be involved. *TTR*-FAP prevalence varies in different populations. There is a relatively high prevalence, 0.09% (1/1108) in northern Portugal, which is lower in the rest of Europe and USA (approximately 1/100,000) [3, 11]. *TTR*-FAP may present as sporadic cases in other non-endemic regions [12]. Sex ratio varied in different regions. Male-to-female ratios were significantly higher (10.7:1) in late-onset FAP *TTR* p.Val50Met Japanese patients, but much lower (0.9:1) in FAP *TTR* p.Val50Met Portuguese patients [10, 13]. *TTR*-FAP onset age ranges widely from 16 to 80 years old [12–14]. Disorder duration ranges from 2 to 21 years [14]. Age-dependent and geography-based penetrance has been described in the literature [14–16]. The penetrance was also significantly higher with maternally inherited *TTR* mutations [15, 17, 18].

In this article, we describe a Han Chinese family with c.349G>T (p.Ala117Ser) variant in the *TTR* gene. Bioinformation analysis along with the absence of the variant in 200 ethnically matched normal controls suggests that it may be a pathogenic variant.

Methods

A dominant Han Chinese family (Taiwanese originally from Fujian) with FAP was enrolled in the Third Xiangya Hospital, Central South University, China (Fig. 1). The proband (Fig. 1, III-1) received a liver transplantation. Blood samples were collected from two members of the family and 200 unrelated, ethnically matched mainland Chinese, normal controls (age 40–70 years old). Informed consent was obtained from the individuals. The study received approval from the Ethics Committee of the Third Xiangya Hospital, Central South University, Changsha, Hunan, China.

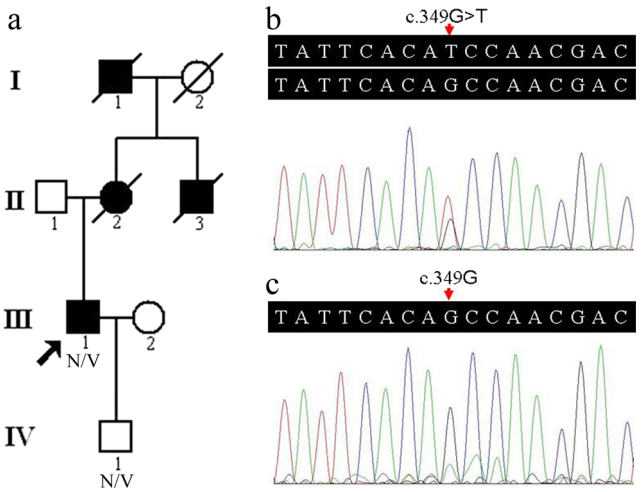

Fig. 1 **a** Pedigree with FAP. *Squares* represent males; *circles* represent females; *white symbols* symbolize unaffected individuals; *black symbols* indicate individuals with FAP; *slashed symbols* represent deceased individuals; *arrow* presents proband. *N* normal allele, *V* c.349G>T variant. **b** DNA sequencing of the c.349G>T variant in the *TTR* gene. **c** The sequencing electropherograms of wild-type *TTR* gene

Clinical Data

The proband presented with complaints of bloating, alternating diarrhea and constipation, and muscular weakness in his feet, over a year (Table 1). Neurological examination revealed muscle weakness in the lower extremities. Kidney function and ophthalmological examinations were normal. Cardiac ultrasound showed suspicious amyloid deposition. The proband's son (IV-1) did not complain of any sensory-motor problem or similar gastrointestinal symptoms. The proband's maternal grandfather (I-1), mother (II-2), and uncle (II-3) were unable to walk in their later years.

Light Microscope and Electron Microscopy Analysis

The hepatic specimens from the proband were fixed in 10% formalin and embedded in paraffin. The tissues were sectioned into 3 μm and stained with hematoxylin and eosin (HE) and Congo red. Electron microscopy samples were fixed in a 2.5% glutaraldehyde buffer for 2 h, then with osmium acid, dehydrated in acetone, and embedded with epoxy resin. The sections were observed under an electron microscope and photographed.

Gene Analysis

Genomic DNA was isolated from lymphocytes using the standard method [19]. Polymerase chain reaction (PCR) amplified the *TTR* gene (NCBI Reference Sequence: NG_009490.1, NM_000371.3) using a 9700 Thermal Cycler System (Applied Biosystems Inc., Foster City, USA), and PCR conditions were 95 °C for 3 min, followed by 35 cycles of 95 °C for 40 s, 58 °C for 35 s, 72 °C for 45 s, and a final extension step at 72 °C for 5 min. The primers used for PCR amplification cover all *TTR* gene coding regions and exon/intron boundaries, which were synthesized by Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China (Table 2). PCR products of 8.5 μl were digested by 0.8 U shrimp alkaline phosphatase and 8 U exonuclease I (Fermentas Inc., Burlington, Canada) in a 10-μl reaction volume. They were then sequenced directionally using an 8-capillary 3500 genetic analyzer (Applied Biosystems Inc., Foster City, USA). Three online tools, MutationTaster prediction (http://www.mutationtaster.org/), Sorting Intolerant from Tolerant (SIFT) prediction (http://sift.jcvi.org/), and HumVar-trained PolyPhen-2 (Polymorphism Phenotyping v2, http://genetics.

Table 1 Clinical and pathological features of the *TTR* variant patient and carrier in this study

Subjects	III-1	IV-1
Sex	Male	Male
Age (years)	69	43
Age of onset (years)	68	/
Symptom of onset	Bloating, alternating diarrhea, and constipation	/
Associated clinical features	Weakness in the lower extremities	/
Pathological features	Amyloid deposition presented in the vessel walls and nerves of the surgically excised liver specimen	/

bwh.harvard.edu/pph2/), were performed to estimate whether a variant affected protein structure or function [20, 21]. The structural and functional importance of the amino acid at the variant position was further assessed by National Center for Biotechnology Information-Basic Local Alignment Search Tool (NCBI-BLAST) in different species.

Results

Histopathologic evaluation of the excised liver specimens from the proband revealed that amyloid deposits were present in the perineurium and arteries of hepatic portal area by HE staining, further confirmed by Congo red treatment. There was no amyloid deposition in the hepatocytes. Electron microscopy revealed amyloid fibrils, which were crossed, or parallel-arranged, in bundles. Surrounding tissues were clear (Fig. 2).

A known heterozygous missense variant, c.349G>T (p.Ala117Ser), in the *TTR* gene, was identified in the proband (Fig. 1). Extended analysis of the family identified the identical c.349G>T variant in his asymptomatic son. This variant was absent in the 200 normal control subjects. This c.349G>T (p.Ala117Ser) variant was predicted to be disease causing, damaging, and probably damaging by MutationTaster, SIFT, and PolyPhen-2, respectively. The alanine at the mutated position (p.Ala117) is highly conserved in different species, suggesting its structural and functional importance (Fig. 3). Cartoon representation of the protein structure is shown in Fig. 4 created by PyMOL 1.7 based on the CPHmodels-3.3 [22]. Though recorded in the single nucleotide polymorphism database (rs267607161), there is no frequency data of this variant. The variant was absent in over 60,000 individuals in the Exome Aggregation Consortium (http://exac.broadinstitute.org/). According to the American College of Medical Genetics and Genomics guidelines [23], the c.349G>T (p.Ala117Ser) variant was classified as a "likely pathogenic" variant.

Discussion

This study detected the presence of amyloid deposits in the perineurium and arteries of the proband's hepatic portal area using HE staining. Amyloid deposition exhibited affinity for Congo red. A heterozygous missense variant c.349G>T (p.Ala117Ser), previously reported as Ala97Ser by other studies [24–30], was identified in the proband's and his asymptomatic son's *TTR* gene. Amyloid deposition in tissue and a proven amyloidogenic variant in the *TTR* gene confirmed this patient's diagnosis of *TTR*-FAP. Patients with p.Ala117Ser *TTR*-FAP usually had a late age at onset, different from those with *TTR* p.Val50Met mutation [25, 26]. Almost all *TTR* p.Ala117Ser patients have motor and sensory symptoms. Autonomic symptoms, such as gastrointestinal symptoms and orthostatic hypotension, are common (Table 3). The patient in our study showed gastrointestinal symptoms, autonomic nerve function damage, and lower limb weakness, which are typical manifestations in the *TTR*-FAP cases. He presented no significant sensory symptom. Disease progression is usually described as having three stages according to the patients' signs and symptoms. Stage I patients are ambulatory. Stage II patients are

Table 2 Primers for the *TTR* gene

Exon	Forward (5′-3′)	Reverse (5′-3′)	Product size (bp)
1	AGTGAGTATAAAAGCCCCAGG	TGCTCAGAGTTCAAGTCCCA	330
2	TCTTGTTTCGCTCCAGATTTCT	AGCAGATGATGTGAGCCTCT	313
3	TGCCATGCCATTTGTTTCCT	CCAAAACCAAAACAACCCTCG	231
4	TTTCGGGCTCTGGTGGAAAT	TTGTCTCTGCCTGGACTTCT	276

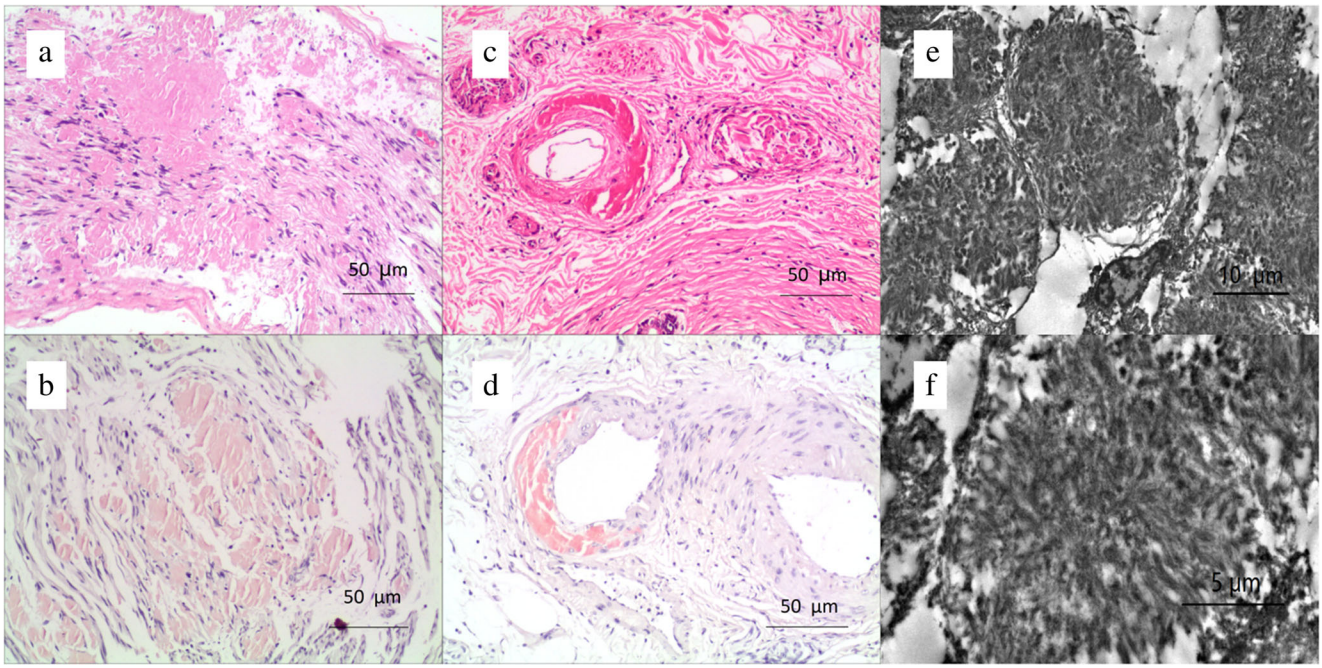

Fig. 2 Pathological imaging of the proband's liver. **a** HE-stained section demonstrates that amyloid deposition is accumulated in nerve fascicle. **b** The amyloid deposition exhibits affinity for Congo red within a nerve fascicle. **c** HE-stained section demonstrates that amyloid materials are accumulated in the vessel. **d** The amyloid deposition was positive for Congo red staining within a vessel. **e** Amyloid fibrils under electron microscope. **f** Amyloid fibrils were crossed or parallel arranged in bundles under electron microscope

ambulatory but require assistance. Stage III patients are either bedridden or wheelchair-bound [3]. The proband in this study is in the early stages of the disease and suffers mild motor impairment of the lower extremities, moderate autonomic manifestations, and full ambulation.

The human *TTR* gene, located on 18q12.1, includes 4 exons spanning over 7 kb and encodes 147 amino acids. The TTR protein is a 56 kDa homotetrameric protein formed by the 127-residue polypeptides. It is a soluble protein circulating in peripheral blood and cerebrospinal fluid [5]. Half of the residues in each monomer are composed of two β-sheets, each of which is composed of four strands. The remaining residues loop attaches to the β-strands [31]. TTR, as a plasma-transport protein for thyroxin (T4) and vitamin A, is primarily synthesized in the liver [5, 7]. The remainder is in choroid plexus

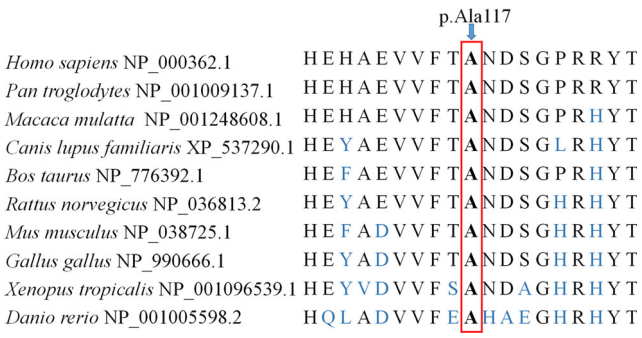

Fig. 3 Conservation analysis of TTR p.Ala117 amino acid residue

cells and retinal cells [5]. Energetic studies of a large number of recombinant *TTR* variants suggested that amyloidogenic mutations destabilize the native quaternary and tertiary structures of TTR, thereby inducing conformational changes [32, 33]. When the *TTR* gene mutates, TTR tetramer dissociates into monomers as the initial step which allows subsequent partial misfolding and misassembly. This leads to the formation of TTR amyloid fibrils and several aggregate morphologies [32, 34]. Dissociation of TTR tetramer into monomers depends on pH. Under acidic conditions, tetrameric TTR mutant dissociates into monomers to a much greater extent than that of wild-type TTR [33, 35]. The mutated alanine p.Ala117 located on the carboxy terminus, the F-strand of the TTR molecule, is part of the hydrophobic core [26, 36]. A misTTR antibody and a peptide inhibitor that selectively target TTR residues in the F-strand can inhibit fibrillogenesis or protein aggregation [37, 38], which supports the importance of F-strand for TTR protein aggregation. The substitution of alanine p.Ala117 with the less hydrophobic serine might destabilize the structure and cause the dissociation of the TTR tetramer.

Transgenic Drosophila melanogasters, with *TTR* Leu55Pro or engineered *TTR* Val14Asn/Val16Glu, showed peripheral toxicity, accompanied by premature death and locomoter behavioral alterations [39]. In transgenic mice carrying human *TTR* mutants, amyloid deposition was detected in the gastrointestinal tract and other organs and tissues, which became more remarkable with aging [40, 41].

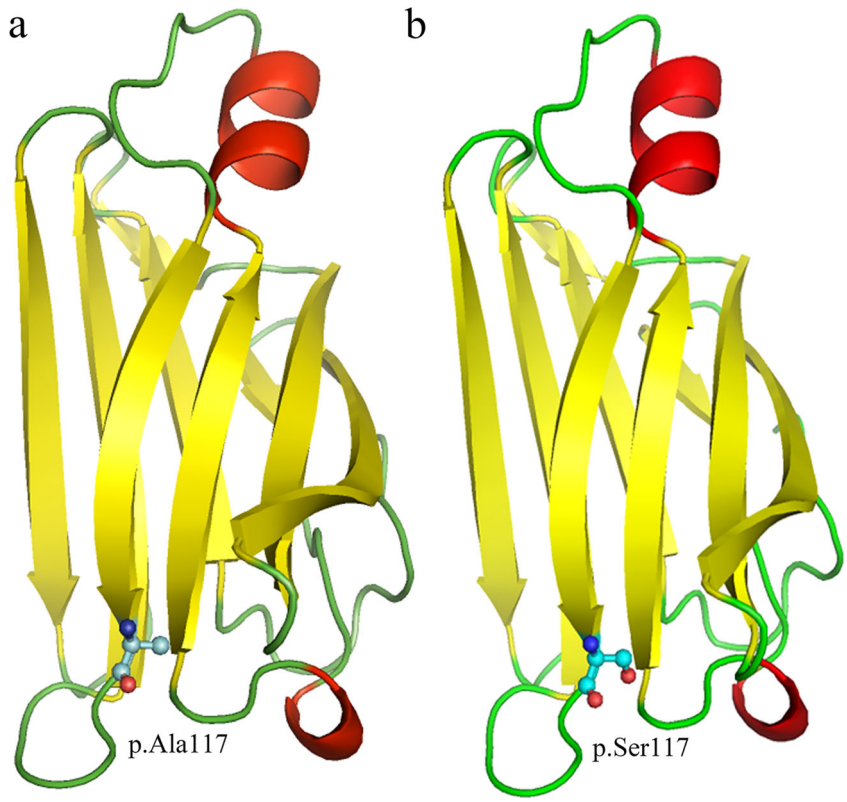

Fig. 4 Cartoon representation of the model structure of TTR by PyMOL 1.7 based on the CPHmodels-3.3: the wild-type alanine (**a**) and mutated serine (**b**) located at position 117 are shown as ball-and-stick models

The current reference treatment for *TTR*-FAP is liver transplantation [12, 32]. Liver transplantation is recommended to early onset *TTR*-FAP patients with p.Val50Met mutation before 50 years old except for women, aiming to remove the main source of systemic mutant TTR [12]. To our knowledge, this is the first liver transplantation case reported with a *TTR* p.Ala117Ser variant, and the patient had a liver transplantation at an early stage of *TTR*-FAP. By 6 months after surgery, patient's gastrointestinal symptoms eased. There had been no further progression of the neuropathy though long-term effects need further follow-up observation.

Recently, some new therapeutic strategies intended to stabilize TTR have become available. Tafamidis, a specific TTR stabilizer, is the first *TTR*-FAP drug approved for use in Europe and some other countries (Japan, Mexico, and Argentina) [32, 42]. In the latest study of early treatment with tafamidis over a 5.5-year period, it resulted in delay in neurologic progression and long-term preservation of nutritional

Table 3 Clinical presentations of the patients with c.349G>T variant of *TTR* gene

References	Lai et al. [25]	Yang et al. [26]	Liu et al. [27]	Chao et al. [28]	Tachibana et al. [29]	Klein et al. [30]	Our study
Origin	Taiwan	Taiwan	Taiwan	Taiwan	Taiwan	USA	Mainland China
Sex ratio (male:female)	14:4	16:3	3:2	25:3	1:0	1:0	1:0
Age-onset (years)	65.2 ± 5.4	59.5 ± 5.7	51.2	59.9 ± 6.0	68	64	68
Motor symptom	N/A	19/19	5/5	28/28	+	N/A	+
Sensory symptoms	N/A	19/19	5/5	28/28	+	N/A	−
Paresthesia	N/A	19/19	5/5	28/28	+	N/A	−
Pain	N/A	11/19	N/A	15/28	−	N/A	−
Autonomic symptoms	N/A	19/19	5/5	22/28	+	N/A	+
Gastrointestinal symptoms	N/A	18/19	5/5	N/A	+	N/A	+
Orthostatic hypotension	N/A	14/19	2/5	N/A	−	N/A	−
Cardiac involvement	N/A	N/A	3/5	N/A	+	N/A	+

N/A not applicable, "+" with this symptom, "−" without this symptom

status [43]. Some other new therapeutic strategies for *TTR* amyloidosis including antibody [44, 45], *TTR* siRNA treatment [46], and tauroursodeoxycholic acid and curcumin [47] are currently being explored, which may shed a new light on the therapy of *TTR*-FAP.

Conclusions

The missense variant c.349G>T (p.Ala117Ser) of the *TTR* gene may be responsible for the Han Chinese family with FAP. Sanger sequencing of *TTR* gene provides a cost-effective approach to identify variant responsible for patients of FAP. These findings provide new insights into FAP cause and diagnosis and have implications for genetic counseling.

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Compliance with Ethical Standards Informed consent was obtained from the individuals. The study received approval from the Ethics Committee of the Third Xiangya Hospital, Central South University, Changsha, Hunan, China.

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Conflict of Interest The authors declare that they have no conflict of interest.

Financial Disclosures None to declare.

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