ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

RESEARCH ARTICLE

Clinical and genetic profiles of hereditary transthyretin amyloidosis in Taiwan

Hua-Chuan Chao[1,2], Yi-Chu Liao[3,4], Yo-Tsen Liu[3,4,5,6], Yuh-Cherng Guo[7,8], Fu-Pang Chang[2,9], Yi-Chung Lee[3,4,5] & Kon-Ping Lin[3,4]

[1]Division of Neurology, Department of Medicine, Taipei Veterans General Hospital, Taoyuan branch, Taoyuan, Taiwan
[2]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan
[3]Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan
[4]Department of Neurology, National Yang-Ming University School of Medicine, Taipei, Taiwan
[5]Brain Research Center, National Yang-Ming University, Taipei, Taiwan
[6]Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan
[7]Neuroscience Laboratory, Department of Neurology, China Medical University Hospital, Taichung, Taiwan
[8]School of Medicine, Medical College, China Medical University, Taichung, Taiwan
[9]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

Correspondence
Kon-Ping Lin and Yi-Chung Lee, No. 201, Sec.2, Shihpai Road, Peitou District, Taipei, 112, Taiwan, R.O.C. Tel: +886-2-28757585; Fax: +886-2-28757584; E-mails: ycli@vghtpe.gov.tw and kplin@vghtpe.gov.tw

Funding Information
This work was supported by the grants from Ministry of Science and Technology, Taiwan, ROC (MOST105-2628-B-075-002-MY3), and Taipei Veterans General Hospital (V108C-011). This work was financially supported by the Brain Research Center, National Yang-Ming University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

Received: 28 December 2018; Revised: 14 March 2019; Accepted: 19 March 2019

Annals of Clinical and Translational Neurology **2019; 6(5): 913–922**

doi: 10.1002/acn3.778

Abstract

Objective: The clinical and genetic profiles of hereditary transthyretin amyloidosis (ATTR) in Chinese populations remain elusive. We aim to characterize the features of ATTR in a Taiwanese cohort of Han Chinese descent. **Methods**: Seventy-nine patients with molecularly confirmed ATTR from 57 Taiwanese families were identified by sequencing the transthyretin gene (*TTR*). The clinical and electrophysiological data were scrutinized. Cardiac involvement of ATTR was evaluated by echocardiography and cardiac scintigraphy. Four microsatellite and seven single-nucleotide polymorphism markers flanking *TTR* were genotyped to investigate the founder effect of the *TTR* Ala97Ser mutation. **Results**: Most of the patients had a peripheral neuropathy with variable autonomic symptoms. The average age at disease onset (AO) was 58.2 ± 7.2 years, and the male patients had an earlier AO than female patients (56.6 ± 5.7 years vs. 61.8 ± 8.9 years, $P = 0.013$). Electrophysiological studies revealed a generalized axonal sensorimotor polyneuropathy and isolated median neuropathy in 84.5% and 15.5% of the patients, respectively. Up to 80% of the patients with ATTR had symptomatic or subclinical cardiac involvement. Six *TTR* mutations were identified in the participants including one novel mutation Glu89Asp. Among them, Ala97Ser was the most common mutation, accounting for 91.2% of the ATTR pedigrees. Detailed haplotype analyses demonstrated a shared haplotype in the 47 patients with the Ala97Ser mutation, suggesting a founder effect. **Interpretation**: The present study delineates the distinct features of ATTR in Taiwan and provides useful information for the diagnosis and management of ATTR, especially in patients of Chinese descent.

Introduction

Hereditary transthyretin amyloidosis (ATTR) is an autosomal-dominantly inherited disorder caused by a mutation in the transthyretin gene (*TTR*), which alters the biochemical property of transthyretin to increase its propensity to be misfolded and lead to formation of pathologic amyloid aggregates. ATTR preferentially manifests with peripheral and autonomic neuropathy, also known as familial amyloidotic polyneuropathy (FAP); however, it may also involve other vital organs.[1,2] Cardiac transthyretin amyloidosis, another important phenotype of ATTR, is a devastating cardiomyopathy characterized by concentric ventricular thickening, low cardiac output, atrial dilatation, and disturbed conduction system.[3] Usually, ATTR patients may develop both cardiac and neuropathic phenotypes during their disease courses.[4]

More than 120 *TTR* mutations have been reported worldwide, and there are considerable genotype-phenotype variations among different ethnic groups.[1] *TTR* Val30Met is the most common variant in the Portugal, Swedish, and Japanese populations,[1] while Val122Ile mutation is endemic in African-Americans.[5,6] Patients harboring *TTR* Val30Met mutation usually present with peripheral neuropathy, but those carrying the Val122Ile mutation commonly manifest with late-onset cardiomyopathy.[3] *TTR* Thr60Ala, the most common *TTR* mutation in the United Kingdom population, is associated with early cardiac involvement, autonomic dysfunction and peripheral neuropathy of variable severity.[7,8] To be noticed, certain *TTR* mutations, such as Leu58His, Ile84Ser, and Tyr114His, are associated with neuropathy starting in the upper limbs as carpal tunnel syndrome (CTS).[1] These findings suggest a broad genetic and phenotypic spectrum in ATTR and stress on the importance of clarifying the ethnic-specific spectrum of *TTR* mutations and the corresponding phenotype–genotype correlations.

There may be a distinct spectrum of *TTR* mutations in Taiwan. Several small-scaled studies only identified the Ala97Ser mutations in Taiwanese patients with FAP,[9–12] and this mutation has never been reported in Caucasian populations. Furthermore, 98% of Taiwanese populations are of Han Chinese descent and there is no large cohort study about ATTR in Chinese populations. Hence, it is intriguing and important to have a comprehensive knowledge of genotypic and phenotypic spectrum of *TTR* mutations in Taiwan, which may help diagnosis and management of ATTR, especially in patients of Chinese descent.

In this study, we characterized the clinical and genetic features of 79 Taiwanese patients with ATTR, including the cardiac manifestations. We also investigated the founder effect of the *TTR* Ala97Ser mutation.

Materials and Methods

Subjects

A consecutive series of 79 individuals with ATTR from 57 pedigrees of Han Chinese descent were recruited from the Neurological Service in Taipei Veterans General Hospital, which is a 2947-bed national medical center in Taiwan, serving both veterans and regular citizens and accepting difficult neuromuscular disease cases referrals. The diagnosis of ATTR was based on the consensus from the European Network for TTR-FAP (ATTReuNET).[13] All the 79 participants had peripheral neuropathy with variable motor, sensory, and autonomic symptoms as well as a pathogenic *TTR* mutation. Another two asymptomatic gene carriers with a *TTR* Ala97Ser mutation were enrolled for the cardiac scintigraphic imaging study. The study was approved by the institutional review board of the Taipei Veterans General Hospital, and written informed consent was obtained from all participants.

Clinical evaluation, disability assessment and electrophysiology

The presence of relevant symptoms and neurological findings were recognized by detailed clinical evaluation, including history taking, medical record review, and physical examination. DN4 questionnaire, consisting of ten items with a total score of 10, was used to assess neuropathic pain with a cut-off value ≥ 4 points.[14] In addition, the clinical functional status and the severity of disability were evaluated according to the clinical stages of Transthyretin Familial Amyloidotic Polyneuropathy (TTR-FAP) with a scale of 0 to III (stage 0, no symptoms; stage I, unimpaired ambulation, mostly with mild sensory, motor, and autonomic neuropathy in the lower limbs; stage II, assistance for ambulation required, mostly with a moderate motor, sensory, and autonomic impairment of the four limbs; stage III, wheelchair-bound or bedridden status with severe sensory, motor, and autonomic involvement of all limbs).[15]

Electrophysiological studies were performed using a Medlec electromyograph. Our patients underwent motor nerve conduction studies in the upper and lower extremities, including median, ulnar, tibial, and peroneal nerves. Ring electrode recordings were carried out to assess sensory conduction of the median, ulnar, and sural nerves. The parameters, including distal latency, nerve conduction velocity, amplitude of compound muscle action potential, amplitude of sensory action potential, and minimal F response, were recorded.

Cardiac evaluation

Echocardiography was performed with two-dimensional and M mode settings. Left ventricular (LV) wall thickening, systolic function, diastolic function, and left atrial (LA) diameter were measured. To evaluate cardiac amyloidosis, the cardiac single photon emission computed tomography with computed tomography (SPECT/CT) and ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) scintigraphy were performed after intravenous injection of 15 mCi ^{99m}Tc-PYP for 1 h and 3 h, respectively. Myocardial PYP uptake was rated by semi-quantitative visual grading with the isotope intensity of rib as reference:[16,17] Grade 0: no uptake and normal bone uptake; Grade 1: uptake less than rib uptake; Grade 2: uptake equal to rib uptake; Grade 3: uptake greater than rib uptake with mild or absent rib uptake.

Pathological evaluation

Pathological diagnosis of amyloidosis was achieved in nine patients, including five with sural nerve biopsies, three with rectal biopsies, and one with bronchoalveolar lavage and bronchoscopic biopsy. Sections from paraffin-embedded biopsied tissues were stained with hematoxylin-eosin (H-E) and Congo red. Optical microscopy with polarized light was used to detect amyloid deposition. The presence of a pathogenic mutation in TTR, a family history, and either a typical clinical manifestation or pathological confirmation in another family member was sufficient for diagnosis of ATTR.

Genetic analysis

Genomic DNA was isolated from white blood cells. Mutation analyses were performed by polymerase chain reactions (PCR) amplification using intronic primers and Sanger sequencing. The intronic primers used to amplify all the exons of *TTR* were described previously.[9] All amplicons were sequenced for both sense and antisense strands using the Big Dye 3.1 dideoxy terminator method (Applied Biosystems, Foster City, CA) on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems). Mutations were identified by aligning the amplicon sequences with the published human *TTR* coding sequence (RefSeq NM_000371.3).

Haplotype analysis

To investigate whether there is a founder effect for the *TTR* Ala97Ser mutation, the haplotype analysis was performed in fifteen families carrying the mutation. Four polymorphic microsatellite and seven single nucleotide polymorphism (SNP) markers flanking *TTR* and covering a region of 1.5 Mb were genotyped. These markers are D18S49, rs12709651, rs7232282, rs7231553, D18S1242, rs1080093, rs1080094, rs8098191, D18S2021, rs614342, and D18S457. The first seven makers are centromeric and the following four markers are telomeric to *TTR*. All the information regarding these markers were obtained from the Genome Data Viewer (http://www.ncbi.nlm.nih.gov/genome/gdv/browser/).

Statistical analysis

The demographic data of the participants were included for descriptive statistics. For inferential statistics, continuous variables were compared between two defined groups using Student's t-test, while categorical variables were compared using Chi-square test or Fisher's exact test where appropriate. A *P*-value less than 0.05 in a two-tailed test was considered to be statistically significant.

Results

Clinical manifestations and electrophysiological findings

The demographic data and clinical features of the 79 ATTR patients are shown in Table 1. The mean age at onset (AO) was 58.2 ± 7.2 years (range: 40–75 years). The mean disease duration from symptom onset to first neurological evaluation was 4.1 ± 3.6 years (range: 0–13 years). The male participants had a significantly earlier AO than the female patients did (56.6 ± 5.7 vs. 61.8 ± 8.9, $P = 0.013$). The first symptoms usually were sensory abnormalities over bilateral upper limbs as carpal tunnel syndrome (CTS) (43%), asymmetric sensory symptoms presenting as radiculopathy pattern (13.9%), and symmetric sensory-motor complaints (13.9%). At evaluation, 81% of the patients had sensory-motor symptoms, and up to 70% had autonomic symptoms. Electrophysiological studies revealed generalized axonal sensorimotor polyneuropathy and isolated median neuropathy in 84.5% and 15.5% of the patients, respectively.[18,19] Approximately half of the Taiwanese ATTR patients had severe functional disability at the time of diagnosis, including 36.7% having impaired ambulation (FAP stage II) and another 13.9% being wheel-chair bound (FAP stage III). Furthermore, 22.8% of them had bulbar dysfunction and 44.3% had more than 5% of body weight loss within 6 months before diagnosis.[20,21]

Conventional cardiac evaluation and cardiac scintigraphic imaging

Only 5.1% of the ATTR patients reported cardiac symptoms as their first manifestations. However, up to 30% of all patients had heart failure or cardiac arrhythmia at the time of diagnosis (Table 1). Thirty-eight participants underwent echocardiography, and cardiac involvement was found in 73.7% of them with the findings of concentric left ventricular hypertrophy (CLVH), asymmetric septal hypertrophy, granular sparkling appearance of myocardium or systolic dysfunction.

Sixteen patients with ATTR and two asymptomatic gene carriers with a *TTR* Ala97Ser mutation received ^{99m}Tc-PYP scintigraphy. We categorized these participants into three groups according to the presence of neuropathy and cardiac symptoms. Group 1 included three patients with neuropathy and class 2 or more severe heart failure defined by New York Heart Association Functional Classification (NYHA Fc) (ATTR with HF). Group 2 contained

Table 1. Demographic data and clinical characterization of ATTR patients in Taiwan

	Mean (SD) or patient number (%)	ATTR patients (n = 79)
Demographics	Male	55 (69.6%)
	Family History	63 (79.7%)
	Age at onset (y)	58.2 (7.2)
	Age at evaluation (y)	62.3 (7.2)
First symptoms[1]	Sensory symptoms of ULs	34 (43%)
	Sensory, asymmetry	11 (13.9%)
	Sensory-motor	11 (13.9%)
	Sensory, symmetry	9 (11.4%)
	Motor	5 (6.3%)
	Heart	4 (5.1%)
	Autonomic symptoms	4 (5.1%)
	Generalized	1 (1.3%)
Presentation at evaluation	Sensory-motor	64 (81.0%)
	Autonomic dysfunction	55 (69.6%)
	Neuropathic pain (DN4)	48 (60.8%)
	Body weight loss	35 (44.3%)
	Heart	24 (30.4%)
	Heart failure	15 (19.0%)
	Cardiac arrhythmia	11 (13.9%)
	Bulbar symptoms	18 (22.8%)
	Sensory	13 (16.5%)
	Motor	2 (2.5%)
Clinical severity (FAP stage)	Stage I	39 (49.4%)
	Stage II	29 (36.7%)
	Stage III	11 (13.9%)
Electrophysiology		(n = 71)
	Isolated median neuropathy	11 (15.5%)
	Axonal sensorimotor polyneuropathy	60 (84.5%)
Echocardiography[2]		(n = 38)
	EF (%)	54.0%
	LV thickness IVS/PW (mm)	14.1/13.0
	Abnormal Echocardiography (n,%)	28 (73.7%)

[1]Sensory symptoms of ULs: symmetrically distal sensory symptoms of upper limbs as carpal tunnel syndrome; Sensory, asymmetry: asymmetric sensory symptoms of upper or lower limbs, referred as a radiculopathy pattern; Sensory-motor: symmetrically distal sensory-motor symptoms of four limbs; Motor: exclusive weakness or clumsiness involving upper or lower limbs; Autonomic symptoms: one of related symptoms including orthostatic intolerance, gastrointestinal symptoms (diarrhea, constipation, or alternative symptoms), urinary bladder dysfunction (dysuria and catheterization), impotence, hypohidrosis or anhidrosis; Generalized: symmetric sensory-motor complaints and autonomic symptoms.

[2]EF, left ventricular ejection fraction; IVS/PW, interventricular septal thickness/posterior wall thickness.

13 ATTR patients with neuropathy but no clinically evident heart failure. Group 3 consisted of two asymptomatic carriers. Their clinical information, neuropathy pattern and scintigraphic grading were demonstrated (Table 2). The clinical severity between the ATTR patients with or without heart failure was similar in their FAP staging. Significant amyloid deposition was identified in all the patients with heart failure and in 76.9% of patients without clinically evident heart failure. The two asymptomatic gene carriers both had an intermediate degree of cardiac amyloid deposition on ^{99m}Tc-PYP scan. Furthermore, there was no significant association between the pattern of peripheral nerve involvement and severity of cardiac amyloid deposition.

Mutational analysis of Taiwanese ATTR patients

Mutation analysis of the 57 Taiwanese ATTR pedigrees revealed six missense *TTR* mutations, including Ala45Thr (c.193G>A), Thr60Ala (c.238A>G), Ile73Val (c.277A>G), Ser77Try (c.290C>A), Glu89Asp (c.327G>T), and Ala97Ser (c.349G>T) (Fig. 1A). All the six mutations were present with a heterozygous form. The Glu89Asp mutation was novel, and the other five mutations had been reported as pathogenic mutations before.[8,22–25] The Ala97Ser mutation was the most common mutation in this ATTR cohort, accounting for 52 of the 57 ATTR pedigrees (91.2%). The pedigrees harboring the other five *TTR* mutations were demonstrated in Fig. 1B. The clinical characteristics of patients with different TTR mutations were shown in Table 3.

Several lines of evidence support the pathogenicity of the *TTR* Glu89Asp mutation. First, the mutation is not present in the 125,748 exome, and 15,708 whole-genome sequences from unrelated individuals in the Genome Aggregation Database (gnomAD) as well as the genome data of 1517 Taiwanese controls from Taiwan biobank database (http://taiwanview.twbiobank.org.tw/index). Second, two different mutations at the same Glu89 amino acid residue, including Glu89Gln and Glu89Lys, had been identified as pathogenic mutations for ATTR.[26,27] Third, two of the three popular bioinformatics tools for predicting the pathogenicity of nucleotide variants support the pathogenic role of *TTR* Glu89Asp. MutationTaster (http://www.mutationtaster.org) and PolyPhen-2 (http://genetics.bwh.harvard. edu/pph2) predicted *TTR* Glu89Asp as a disease-causing variant, whereas SIFT (https://sift.bii.a-star.edu.sg) predicted it as a tolerated one. Fourth, the patient with the Glu89Asp mutation has a typical phenotype and pathological finding of ATTR, which is highly specific for *TTR* mutations. Therefore, *TTR* Glu89Asp is classified as a likely pathogenic variant according to the American College of Medical Genetics and Genomics (ACMG) guideline.[28]

Table 2. Clinical information of the individuals receiving scintigraphic analysis of cardiac involvement.

n	ATTR with HF *n* = 3	ATTR without HF *n* = 13	Asymptomatic carriers *n* = 2
Male (%)	2 (66.7%)	9 (69.2%)	0
FAP stage			
Stage 1	2 (66.7%)	9 (69.2%)	0
Stage 2 & 3	1 (33.3%)	4 (30.8%)	0
PYP scan			
Intermediate (Grade 1)	0 (0%)	3 (23.1%)	2 (100%)
Significant (Grade 2 & 3)	3 (100%)	10 (76.9%)	0
Peripheral neuropathy			
Isolated median neuropathy	1 (33.3%)	5 (38.5%)	0
Sensorimotor polyneuropathy	2 (66.7%)	8 (61.5%)	0

HF, Heart failure defined by New York Heart Association Functional Classification (NYHA Fc) class 2 or more; ATTR, transthyretin amyloidosis; FAP, familial amyloidotic polyneuropathy; PYP scan, ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) scintigraphy.

Clinical information of the patient carrying the *TTR* Glu89Asp mutation

The patient with the *TTR* Glu89Asp mutation presented with distal paresthesia since age 59 years and intermittent chest tightness, diarrhea, and erectile dysfunction since age 64. Progressively ascending numbness, tingling pain and weakness of four limbs accompanying with loss of body weight up to 12 kg developed in the following 2 years. He was wheelchair-bound by age 68. The neurological examination at age 66 revealed severe hypophonia, generalized areflexia, weakness and muscle wasting and impaired sensation of all modalities in the distal four limbs. The nerve conduction study showed severe axonal polyneuropathy (Table S1). The sympathetic skin responses (SSR) were absent over lower limbs and diminished with prolonged latencies over upper limbs. The heart rate variability test revealed decreased variability on

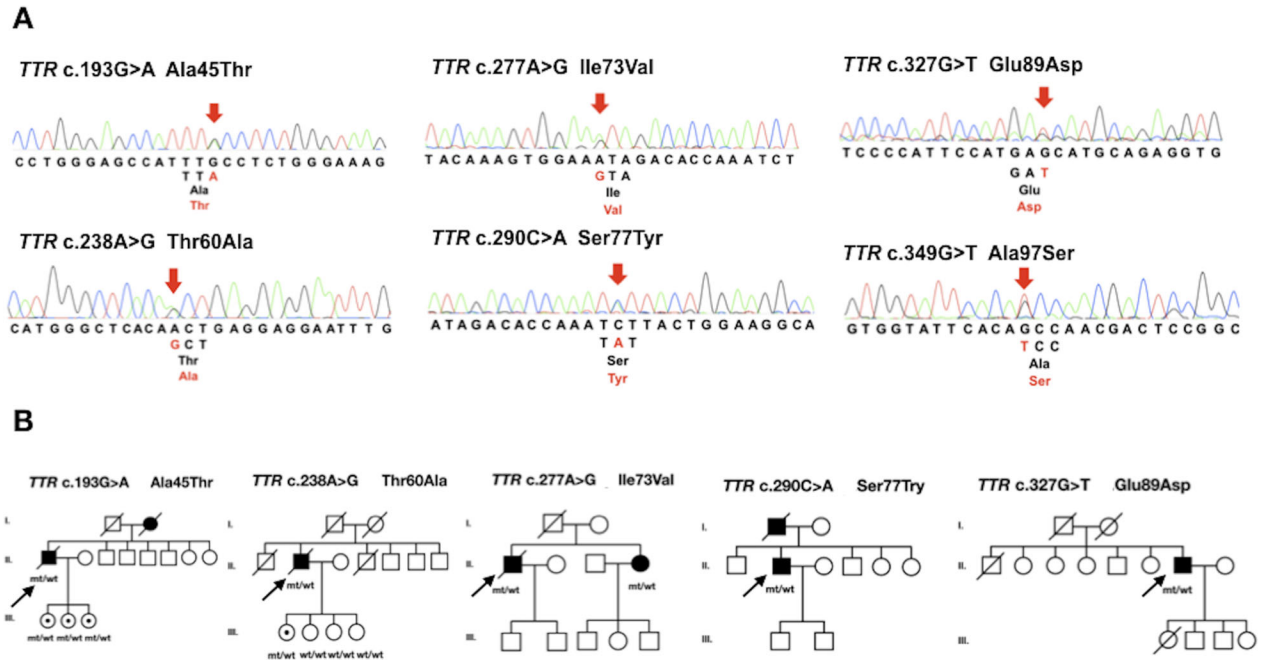

Figure 1. Pedigrees and electropherograms of the *TTR* mutations in this study. (A) The six *TTR* mutations identified in this study, with the sense strand electropherogram shown on the top and a limited reading frame depicting the corresponding amino acid substitutions shown below. (B) The pedigrees of the patients carrying the *TTR* mutations, including the Ala45Thr, Thr60Ala, Ile73Val, Ser77Tyr, and Glu89Asp mutations. The arrows indicate the probands. The squares and circles denote males and females, respectively. The filled and open symbols represent affected and unaffected members. Dotted symbols represent asymptomatic carriers. Slashed indicate deceased individuals.

Table 3. Phenotype-genotype characterization of individual patients with different *TTR* mutations

	Mean (SD); %	Ala45Thr (n = 1)	Thr60Ala (n = 1)	Ile73Val (n = 2)	Ser77Try (n = 1)	Glu89Asp (n = 1)	Ala97Ser (n = 73)
Demographics	Age at onset	59	59	52 (1.4)	54	59	58.3 (7.4)
	Symptom duration (years)	9	2	1.5 (2.1)	10	6	4(3.6)
First symptoms		HF[1]	HF	Sensory UL; Sensory-motor	Sensory UL	Sensory	Sensory UL (43.8%)
Presentation at evaluation	Neuropathy	S-M poly	S-M poly	S-M poly; CTS	S-M poly	S-M poly	S-M poly (80.8%)
	Autonomic symptoms	OI, GI, UB	OI, UB, Vasomotor	GI, UB, Sweat	GI, UB, Sex	OI, UB, Sex, Sweat	68.5%
	Clinical severity	FAP stage 2	FAP stage 2	FAP stage 2 & 1	FAP stage 2	FAP stage 2	FAP (2 + 3) 48%
	Neuropathic Pain	100%	100%	50%	100%	100%	58.9%
	Heart	HF, Arrhythmia	HF, Arrhythmia	HF/NR	NR	HF	HF (16.4%) Arrhythmia (15.1%)
Echocardiogram	Ejection fraction	49%	33%	43%; 63%	–	50%	55.2%
	IVS/PW (mm)	19/13	14/14	20/17; 10/10	–	12/11	14/12.9
Nerve conduction study		Axonal S-M poly.	Axonal poly.	Axonal poly.; Median neuropathy	Axonal poly.	Axonal poly.	Axonal poly.(84.6%)

[1]HF: heart failure; Sensory UL: sensory symptoms of upper limbs; S-M poly: sensorimotor polyneuropathy; OI: orthostatic intolerance; GI: gastrointestinal symptoms (diarrhea, constipation or alternative symptoms); UB: urinary bladder dysfunction (dysuria and catheterization); Sex: impotence; Sweat: hypohidrosis or anhidrosis; Vasomotor: skin color change; FAP stage: stages of Transthyretin Familial Amyloidotic Polyneuropathy; NR: not reported; nl.:normal; EF: ejection fraction; CLVH: concentric left ventricular hypertrophy
Axonal poly.: axonal sensory-motor polyneuropathy; CTS: carpal tunnel syndrome

R-R intervals. Severe postural hypotension (54/27 mmHg at 3rd minutes on standing) was demonstrated by head-up tilt test. Rectal biopsy of the patient revealed characteristic apple-green birefringence of amyloid deposits in the Congo red stain (Fig. 2A and B). The serum and urine protein electrophoresis with immunofixation did not find monoclonal band and the serum free light chain ratio analysis revealed normal values. The echocardiography showed preserved ejection fraction with mild CLVH at age 66 and mild systolic dysfunction at age 69. ^{99m}Tc-PYP scintigraphy and SPECT/CT imaging showed prominent amyloid deposition with visual score of grade 3, mainly locating in the left ventricle, at age 69 (Fig 2C and D).

Founder Effect of *TTR* Ala97Ser in Taiwan

Haplotype analysis was performed in 91 individuals from 15 pedigrees harboring the p.Ala97Ser mutation, including 47 patients, ten asymptomatic carriers and 34 unaffected subjects. The families were denoted as A to P (Fig. 3). These families shared a common haplotype covering a region of 0.48 Mb containing loci rs12709651, rs7232282, rs7231553, D18S1242, rs1080093, rs1080094, rs8098191, D18S2021, and rs614342 linked to *TTR* Ala97-Ser (C-C-A-1-C-A-mutation-G-1-C), suggesting the presence of a founder effect for the Ala97Ser mutation.

Discussion

The present study comprehensively investigates the clinical features and mutational spectrum of a cohort of 79 patients with ATTR from 57 pedigrees of Han Chinese descent in Taiwan. The studied cohort is the largest ATTR cohort in Han Chinese populations. There are several intriguing findings in this study. Firstly, *TTR* Ala97-Ser mutation is the most common cause of ATTR in Taiwan, accounting for 91.2% of the pedigrees. The patients with the Ala97Ser mutation usually present with a late-onset peripheral neuropathy and autonomic dysfunction at their late fifties, and approximately half of them have CTS as the initial presentation. Upon the time of diagnosis, majority of them had significant cardiac amyloid deposition and abnormal cardiac echocardiographic findings despite less than one-third of patients report heart symptoms. Secondly, *TTR* Glu89Asp is a novel pathogenic mutation for ATTR. Furthermore, there is a founder effect for the Ala97Ser mutation in Taiwan. Moreover, there was no correlation between the pattern or severity of neuropathy and the severity of cardiac amyloidosis in the ATTR cohort. Cardiac evaluation is mandatory in every patient with ATTR, even in those with very early-stage disease and trivial symptom.

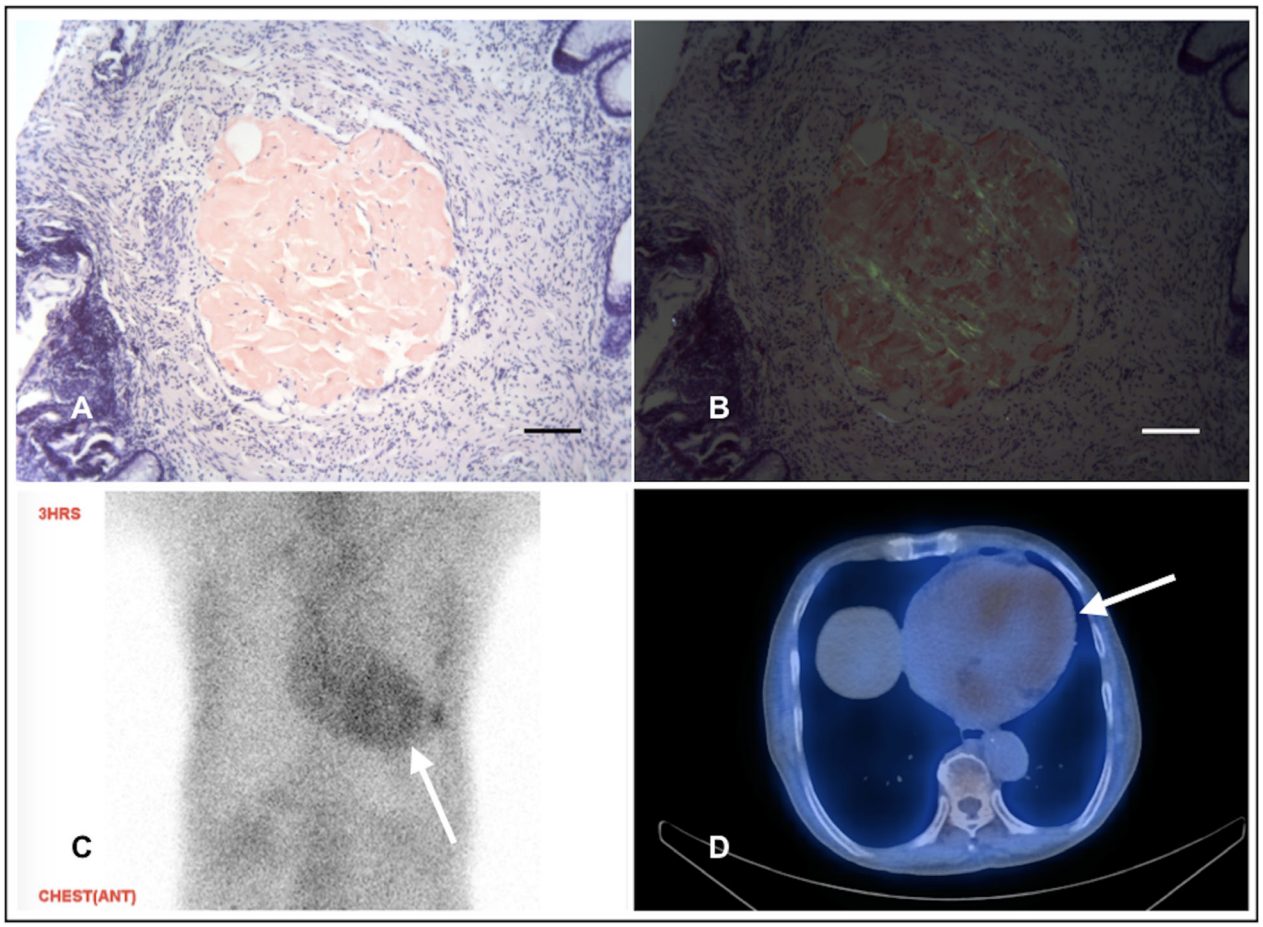

Figure 2. Rectal biopsy and the 99mTc-PYP scintigraphy and SPECT/CT imaging of the patient with the novel *TTR* Glu89Asp mutation. (A) Pathological sections with the Congo red stain showed an amorphous, small focus of amyloid deposition in submucosa of rectum. (B) Under polarized light examination, the amyloid deposition showed apple-green birefringence. (C) ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) scintigraphy reveals increased PYP uptake in the heart with visual score of grade 3. (D) The SPECT/CT image shows the amyloid deposition mainly located in the left ventricle. Bars = 100 μm.

Taiwanese ATTR population is particular because of the *TTR* Ala97Ser mutation, which contributes to the majority of ATTR in Taiwan but is absent in other populations. Several other population-specific *TTR* mutations have also been identified.[7,8,22,25,29] For example, the Thr60Ala mutation, which is prevalent in British patients with ATTR, is associated with early cardiac and autonomic symptoms and variable severity of peripheral neuropathy in advanced disease stage.[7,8]

In addition to the Ala97Ser mutation, five other *TTR* mutations were identified in the Taiwanese ATTR cohort, including the novel Glu89Asp mutation, manifesting as late-onset neuropathy with obvious autonomic and cardiac involvement. Our work also demonstrated a common haplotype consisting of nine neighboring loci of the microsatellite or SNP markers linked to the *TTR* Ala97Ser mutation in the 15 ATTR families. These findings suggest that the Taiwanese patients harboring the Ala97Ser mutation may be descendants from a common ancestor. A founder effect for the *TTR* Val30Met mutation has also been shown in the Japanese, Swedish and Portuguese populations.[30,31] The late disease onset of the Ala97Ser mutation-associated ATTR makes it easy to inherit the mutation from patients to their offspring.

Our study underlines the importance of cardiac evaluation in every patient with ATTR, even in those without heart symptoms. In the Taiwanese ATTR cohort, less than one-third of the patients had cardiac symptoms, but near three-fourths of the ATTR patients receiving echocardiography had abnormal findings. Furthermore, ^{99m}Tc-PYP scintigraphy revealed significant cardiac amyloid deposition in 76.9% of ATTR patients without an overt heart failure symptom. Even for the two asymptomatic *TTR* mutation carriers, an intermediate degree of cardiac

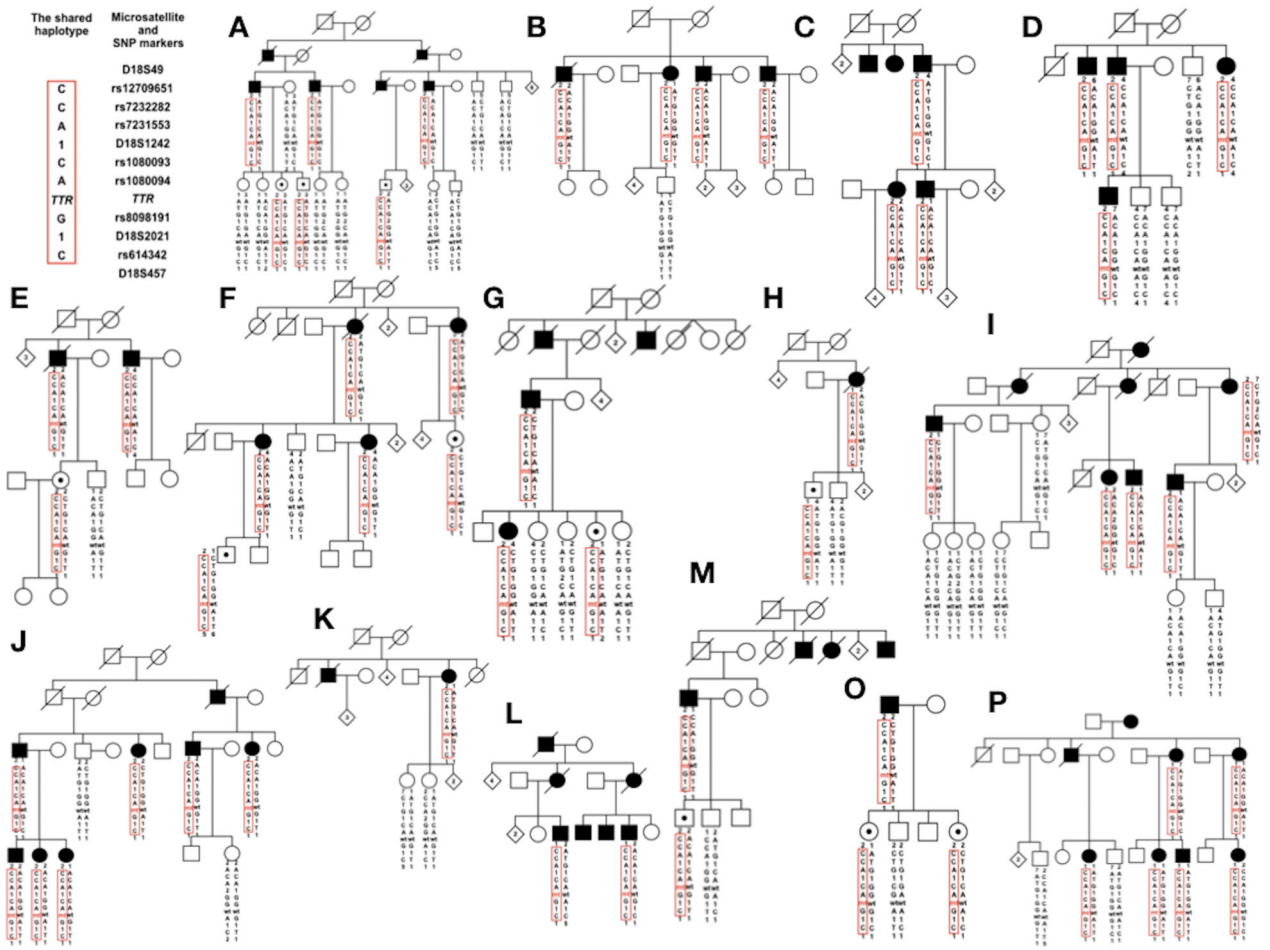

Figure 3. Haplotype analysis. Haplotype analysis of the microsatellite and single nucleotide polymorphism (SNP) markers flanking the *TTR* gene in 15 ATTR pedigrees (A–P) carrying the *TTR* Ala97Ser mutation. The squares and circles denote males and females. The filled and open symbols represent affected and unaffected members. Dotted symbols indicate asymptomatic gene carriers and the numbers in diamonds are the numbers of unaffected individuals. A slash represents deceased members. The gender and birth order have been partially hidden because of confidentiality.

amyloidosis was detected by ^{99m}Tc-PYP imaging in both of them. To be noticed, there was no correlation between the pattern or severity of neuropathy and the severity of cardiac amyloidosis in the Taiwanese ATTR cohort. Similar findings were also found in an ATTR cohort in US. Within this cohort, 10 of the 12 (84%) asymptomatic *TTR* mutation carriers had cardiac amyloidosis revealed by ^{99m}Tc-PYP scintigraphy.[32] Since newly emerging RNA interference and antisense oligonucleotide therapies have changed the therapeutic paradigm for ATTR,[33,34] routinely genetic testing for *TTR* mutations in individuals at risk and aggressively monitoring cardiac amyloidosis in asymptomatic *TTR* mutation carriers may allow early intervention.[35]

In conclusion, we demonstrated the distinct clinical and genetic profiles of ATTR in Taiwan, where *TTR* Ala97Ser accounts for approximately 90% of ATTR and is associated with a late-onset neuropathy with autonomic and cardiac involvement. There is a founder effect for the Ala97Ser mutation in Taiwan. One novel mutation for ATTR, Glu89Asp, was identified. Cardiac evaluation is important in ATTR patients, even in those without heart symptoms. These findings provide useful information for the diagnosis and management of ATTR, especially in patients of Chinese descent.

Acknowledgments

We thank for the patients who participated in this study. This work was supported by the grants from Ministry of Science and Technology, Taiwan, ROC (MOST105-2628-B-075-002-MY3), and Taipei Veterans General Hospital (V108C-011). This work was financially supported by the Brain Research Center, National Yang-Ming

University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

Conflict of Interest

None declared.

References

1. Sekijima Y. Transthyretin (ATTR) amyloidosis: clinical spectrum, molecular pathogenesis and disease-modifying treatments. J Neurol Neurosurg Psychiatry 2015;86:1036–1043.
2. Sipe JD, Benson MD, Buxbaum JN, et al. Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. Amyloid 2014;21:221–224.
3. Patel KS, Hawkins PN. Cardiac amyloidosis: where are we today? J Intern Med 2015;278:126–144.
4. Rapezzi C, Quarta CC, Obici L, et al. Disease profile and differential diagnosis of hereditary transthyretin-related amyloidosis with exclusively cardiac phenotype: an Italian perspective. Eur Heart J 2013;34:520–528.
5. Jacobson DR, Pastore R, Pool S, et al. Revised transthyretin Ile 122 allele frequency in African-Americans. Hum Genet 1996;98:236–238.
6. Yamashita T, Hamidi Asl K, Yazaki M, Benson MD. A prospective evaluation of the transthyretin Ile122 allele frequency in an African-American population. Amyloid 2005;12:127–130.
7. Sattianayagam PT, Hahn AF, Whelan CJ, et al. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. Eur Heart J 2012;33:1120–1127.
8. Carr AS, Pelayo-Negro AL, Evans MR, et al. A study of the neuropathy associated with transthyretin amyloidosis (ATTR) in the UK. J Neurol Neurosurg Psychiatry 2016;87:620–627.
9. Liu YT, Lee YC, Yang CC, et al. Transthyretin Ala97Ser in Chinese-Taiwanese patients with familial amyloid polyneuropathy: genetic studies and phenotype expression. J Neurol Sci 2008;267:91–99.
10. Yang NC-C, Lee M-J, Chao C-C, et al. 2010_Clinical presentations and skin denervation in amyloid neuropathy due to transthyretin Ala97Ser. Neurology. 2010;75:532–538.
11. Chao CC, Huang CM, Chiang HH, et al. Sudomotor innervation in transthyretin amyloid neuropathy: Pathology and functional correlates. Ann Neurol 2015;78:272–283.
12. Hsu HC, Liao MF, Hsu JL, et al. Phenotypic expressions of hereditary Transthyretin Ala97Ser related Amyloidosis (ATTR) in Taiwanese. BMC Neurol 2017;17:178.
13. Adams D, Suhr OB, Hund E, et al. First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy. Curr Opin Neurol 2016;29(Suppl 1):S14–S26.
14. Bouhassira D, Attal N, Alchaar H, et al. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). Pain 2005;114(1–2):29–36.
15. Coutinho P, De Silva AM, Lima JL, Barbosa AR. Forty years of experience with type I amyloid neuropathy: review of 483 cases. In: G. G. Glenner, P. P. Costa, and A. F. de Freitas, eds. Amyloid and amyloidosis. pp. 88–98. Amsterdam: Excerpta Medica, 1980.
16. Points AP. 99mTechnetium-Pyrophosphate Imaging for Transthyretin Cardiac Amyloidosis. Released Feb., 2016. From American Society of Nuclear Cardiology. https://www.asnc.org/practicepoints
17. Perugini E, Guidalotti PL, Salvi F, et al. Noninvasive etiologic diagnosis of cardiac amyloidosis using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. J Am Coll Cardiol 2005;46:1076–1084.
18. commitee Aqa. Literature review of the usefulness of nerve conduction studies and electromyography for the evaluation of patients with carpal tunnel syndrome. Muscle Nerve. 1993;16:1392–1414.
19. Jablecki CK, Andary MT, Floeter MK, et al. Practice parameter: Electrodiagnostic studies in carpal tunnel syndrome. Report of the American Association of Electrodiagnostic Medicine, American Academy of Neurology, and the American Academy of Physical Medicine and Rehabilitation. Neurology 2002;58:1589–1592.
20. Gaddey HL, Holder K. Unintentional weight loss in older adults. Am Fam Physician 2014;89:718–722.
21. Lankisch P, Gerzmann M, Gerzmann JF, Lehnick D. Unintentional weight loss: diagnosis and prognosis. The first prospective follow-up study from a secondary referral centre. J Intern Med 2001;249:41–46.
22. Parman Y, Adams D, Obici L, et al. Sixty years of transthyretin familial amyloid polyneuropathy (TTR-FAP) in Europe: where are we now? A European network approach to defining the epidemiology and management patterns for TTR-FAP. Curr Opin Neurol 2016;29(Suppl 1):S3–S13.
23. Booth DR, Gillmore JD, Persey MR, et al. Transthyretin Ile73Val is associated with familial amyloidotic polyneuropathy in a Bangladeshi family. Mutations in brief no. 158. Online. Hum Mutat 1998;12:135.
24. Saraiva MJ, Almeida Mdo R, Sherman W, et al. A new transthyretin mutation associated with amyloid cardiomyopathy. Am J Hum Genet 1992;50:1027–1030.
25. Zhen DB, Swiecicki PL, Zeldenrust SR, et al. Frequencies and geographic distributions of genetic mutations in transthyretin- and non-transthyretin-related familial amyloidosis. Clin Genet 2015;88:396–400.

26. Almeida MR, Ferlini A, Forabosco A, et al. Two transthyretin variants (TTR Ala-49 and TTR Gln-89) in two Sicilian kindreds with hereditary amyloidosis. Hum Mutat 1992;1:211–215.
27. Nakamura M, Hamidi Asl K, Benson MD. A novel variant of transthyretin (Glu89Lys) associated with familial amyloidotic polyneuropathy. Amyloid 2000;7:46–50.
28. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–424.
29. Koike H, Misu K, Ikeda S, et al. Type I (transthyretin Met30) familial amyloid polyneuropathy in Japan: early- vs late-onset form. Arch Neurol 2002;59:1771–1776.
30. Ohmori H. Common origin of the Val30Met mutation responsible for the amyloidogenic transthyretin type of familial amyloidotic polyneuropathy. J Med Genet 2004;41:e51–e.
31. Zaros C, Genin E, Hellman U, et al. On the Origin of the Transthyretin Val30Met Familial Amyloid Polyneuropathy. Ann Hum Genet 2008;72:478–484.
32. Haq M, Pawar S, Berk JL, et al. Can (99m)Tc-Pyrophosphate Aid in Early Detection of Cardiac Involvement in Asymptomatic Variant TTR Amyloidosis? JACC Cardiovasc Imaging 2017;10:713–714.
33. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med 2018;379:11–21.
34. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. N Engl J Med 2018;379:22–31.
35. Obici L, Kuks JB, Buades J, et al. Recommendations for presymptomatic genetic testing and management of individuals at risk for hereditary transthyretin amyloidosis. Curr Opin Neurol 2016;29(Suppl 1):S27–S35.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Serial clinical information of the patient with the *TTR* Glu89Asp mutation was demonstrated in the supplementary table, including nerve conduction study, echocardiography, and cardiac scintigraphy at the age of 66 and 69.

