



RESEARCH ARTICLE

Cutaneous amyloid is a biomarker in early ATTRv neuropathy and progresses across disease stages

Roy Freeman¹ , Alejandra Gonzalez-Duarte², Fabio Barroso³, Marta Campagnolo¹, Sharika Rajan¹, Jennifer Garcia¹, Jee Young Kim¹, Ningshan Wang¹, Lucas Orellana³ & Christopher Gibbons¹ 

¹Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

²Instituto Nacional de Ciencias Medicas y Nutrición Salvador Zubirán, Mexico

³Raúl Carrea Institute for Neurological Research, FLENI, Ciudad Autónoma de Buenos Aires, Argentina

Correspondence

Roy Freeman, Autonomic and Peripheral Nerve Laboratory, Department Neurology, Beth Israel Deaconess Medical Center, 1 Deaconess Road, Boston, MA 02215, USA. Tel: (617) 632-8454; Fax: (617) 632-8454; E-mail: rfreeman@bidmc.harvard.edu

Funding Information

This work is supported in part by a grant from Pfizer (RF).

Received: 29 April 2022; Revised: 22 June 2022; Accepted: 14 July 2022

Annals of Clinical and Translational Neurology 2022; 9(9): 1370–1383

doi: 10.1002/acn3.51636

Abstract

Objective: To determine the sensitivity and specificity of cutaneous amyloid deposition in relation to patient-reported measures in the earliest disease stage of hereditary ATTR amyloidosis (ATTRv). **Methods:** In a cross-sectional study, we analyzed 88 individuals with TTR mutations, 47 of whom were in the earliest disease stage and without clinically evident neuropathy, 12 healthy controls, and 13 disease controls with diabetes. All participants' neuropathy symptoms and signs were assessed using validated patient and clinician-reported measures and 3-mm skin punch biopsies were immunostained using protein gene product 9.5 and Congo Red. **Results:** Amyloid can be detected in the earliest disease stages in up to 86% of patients with ATTRv amyloidosis. Amyloid was not detected in healthy individuals or individuals with diabetic peripheral neuropathy supporting a sensitivity of 86% and a specificity of 100%. The cutaneous deposition of amyloid correlates with neuropathy sensory symptoms, measured with the Neuropathy Total Symptom Score-6 ($R = 0.46$, $p < 0.01$); pain measured with the Brief Pain Symptom Inventory ($R = 0.44$, $p < 0.05$); autonomic symptoms, measured with the Boston Autonomic Symptom Questionnaire ($R = 0.38$, $p < 0.05$); and quality of life measured with the Norfolk Diabetic Neuropathy Quality of Life Questionnaire ($R = 0.44$, $p < 0.05$). Individuals with amyloid deposition were more likely to have sensory symptoms, pain, autonomic impairment, and reduced quality of life than ATTRv patients without amyloid deposition. **Interpretation:** These findings have implications for understanding the earliest manifestations of the clinical phenotype of ATTRv-associated neuropathy, for the pathophysiological construct of disease staging, and for timing the introduction of disease-modifying therapy.

Introduction

Hereditary ATTR amyloidosis (ATTRv) is a progressive, debilitating, multisystem fatal disease caused by the extracellular deposition of misfolded, mutant transthyretin in tissues throughout the body.^{1,2} The clinical manifestations of ATTRv are heterogeneous and vary by mutation, geographical region, and other genetic and environmental factors. This heterogeneity, coupled with low penetrance of the trait, anticipation, and the late onset of the clinical manifestations of the disease in some patients, has led to poor recognition of the disease, particularly in

nonendemic areas, and highlights the need for sensitive, specific, and readily available diagnostic tests.

In the presence of a family history of ATTRv or a suggestive clinical phenotype, a pathogenic mutation can be detected by TTR gene sequencing. Pathological confirmation of the disease is obtained by histological confirmation of amyloid deposition. Skin biopsy assessment of intraepidermal nerve fiber density has become the de facto gold standard for the diagnosis of small fiber neuropathy, yet few reports of skin biopsy assessments of cutaneous amyloid exist. Initial studies failed to support the use of skin biopsy to document amyloid deposition,^{3,4}

however, more recent studies have observed amyloid deposition in ATTRv patients^{5–7} although rarely in the absence of the clinically evident disease.⁵

We studied skin biopsy detection of cutaneous amyloid deposition in a large series of ATTRv patients from different geographic regions with different TTR mutations. The patient perspective is of increasing importance in health-care delivery and clinical trial design. Thus, with patient-centricity in mind, we focus our results on patient input using validated patient-reported outcome (PRO) and quality of life (QOL) questionnaires. We show that amyloid deposition occurs early in the clinical course, even before the appearance of clinically evident disease. In doing so, we determine the optimum number of sections and biopsy sites to maximize diagnostic sensitivity. We also report the relationship between amyloid detection on skin biopsy and patient- and clinician-reported measures with a focus on the earliest manifestations of ATTRv neuropathy. Finally, we determine the relationship between the clinical neuropathy features and their structural correlates in the skin and introduce an index of amyloid deposition that could function as a therapeutic biomarker and measure of target engagement. These findings provide the basis for a diagnostic and therapeutic approach to individuals with ATTRv with neuropathy across disease stages and mutations.

Materials and Methods

One hundred and thirteen participants (88 individuals with TTR pathogenic variants, 12 healthy controls, and 13 disease controls with diabetes) from the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; the Raúl Carrea Institute for Neurological Research, FLENI, Ciudad Autónoma de Buenos Aires, Argentina; and the Beth Israel Deaconess Medical Center, Boston, USA. Participants were screened for other potential causes of neuropathy, including diabetes, neurotoxins, thyroid dysfunction, and vitamin B12 deficiency. Subjects were excluded if laboratory abnormalities confirmed another potential cause of neuropathy. Control subjects were healthy individuals of similar age with no known medical illness. Disease controls had diabetic neuropathy with a range of neuropathy severity. The study protocol was approved by local ethical and research committees and all participants gave their written informed consent.

Patient-reported scales

All participants completed symptom scores including the Neuropathy Total Symptom Score (NTSS-6),⁸ the Brief Pain Symptom Inventory Short Form (BPI-SF),⁹ the

Boston Autonomic Symptom Questionnaire,¹⁰ and the Norfolk Diabetic Neuropathy Quality of Life Questionnaire (Norfolk-DN QoL).¹¹

The NTSS-6 evaluates the frequency and intensity of individual neuropathy painful and nonpainful sensory symptoms, for example, numbness and/or insensitivity, prickling, and/or tingling sensation.⁸

The BPI-SF assesses the severity of pain and the impact of pain on daily functioning.⁹

QoL was measured with the Norfolk-DN QoL, a 47-item questionnaire that groups items according to small fiber, large fiber, and autonomic nerve function, symptoms, and activities of daily living (ADL).¹¹

Clinician-reported scales

All participants had complete history and physical examinations to confirm study eligibility. Neuropathy-specific examinations were quantified by the Neuropathy Impairment Score in the lower limbs (NIS-LL)¹² and the Utah Early Neuropathy Score (UENS).¹³ The NIS-LL is a neuropathy score weighted toward motor testing with a range of 0 (no neuropathy) to 88 (severe sensory and motor neuropathy in the lower legs). The Utah Early Neuropathy Score (UENS) is a small fiber sensory examination with a score of 0 (no neuropathy) to 24 (severe bilateral sensory neuropathies). Functional status was assessed by Coutinho staging.¹⁴

Laboratory studies

Laboratory studies included blood glucose levels, thyroid function tests, vitamin B12 levels, and serum protein electrophoresis with immunofixation to exclude other causes of peripheral neuropathy.¹⁵

Genetic analysis of TTR mutations

Genomic DNA was extracted from the peripheral venous blood using direct sequencing or saliva testing, using commercial genetic laboratories (Ambry Genetics).

Skin biopsies

All patients underwent 3-mm punch skin biopsies at the lateral distal leg and distal thigh using standard techniques.¹⁶ All specimens were analyzed at Beth Israel Deaconess Medical Center. Specimens were fixed and stained with protein gene product 9.5 (1:1000, rabbit anti PGP 9.5, Chemicon International Inc.). Skin biopsies were fixed and stained in Zamboni fixative, using protein gene product 9.5 (PGP9.5) as previously reported^{17,18} and Congo Red (using a modified Highman's Congo Red

protocol)^{5,19} using 50- μ m-thick tissue sections. Briefly, sections were stained in Congo Red solution for 10 min, rinsed, differentiated in alkaline alcohol, counterstained with Gill's hematoxylin, dehydrated, dipped in xylene, and mounted. A total of four tissue sections per biopsy were used to quantify the intraepidermal nerve fiber density (IENFD) using immunofluorescent microscopy.¹⁷ A total of six tissue sections per biopsy were stained with Congo Red. Biopsies were viewed at high power (40x) through all tissue sections under polarized light to detect amyloid. Biopsy sections were viewed under polarized light and were imaged in entirety using a 6.2 megapixel Pixelink camera at 20x resolution to encompass the entire biopsy. The number of amyloid deposits was measured in each image, with a maximum of two amyloid deposits per field of view (to prevent overcounting of large amorphous sheets of amyloid—thus each field of view had 0–2 amyloid deposits). The total number of deposits was then divided by the total length of tissue studied in millimeters multiplied by 100 (for ease of data reporting), with the results expressed as the Amyloid Deposition Index (ADI—the number of amyloid deposits per linear millimeter of tissue measured). The number of tissue sections reviewed in order to detect amyloid was recorded for each subject. The pathology reviewer (C.H.G.) was blinded to the clinical data.

Intraepidermal nerve fiber density (IENFD)

IENFD counting was performed by a physician blinded to diagnosis, and the results were expressed as a linear density (number of fibers per millimeter) as previously described.¹⁷

Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Statistical analysis

Statistical data analysis was performed using SPSS v17.0 (IBM, Chicago, IL). Results are reported in aggregate, but also by mutation type, Coutinho stage, NIS-LL stage, and amyloid deposition. The amyloid deposition index groups were defined as <15, 15–150, and >150 based on the normal distribution of amyloid index data into three groups. Data are reported as mean, median, range, or standard deviation as noted. Between-group comparisons were made by one-way analysis of variance (ANOVA) with Tamhane T2 post hoc test. Correlations between tests of nerve fiber structure, nerve fiber function, amyloid deposition, and symptom scores were measured using Pearson

correlation coefficient (R) if data were normally distributed, with corrections for multiple comparisons.

Results

Demographic information

One hundred and thirteen participants enrolled in the study (88 ATTRv, 12 controls, and 13 individuals with type 2 diabetes). The distribution of TTR mutations is listed in Table 1 and includes 43 participants with Val30-Met mutations and 30 with Ser50Arg mutations. Other mutations, reported as a group, include 2 F64L mutations, 6 G47A mutations, 5 S52P mutations, 1 Y136H, mutation, and 1 V142I mutation. Most participants were in the early disease stage (Coutinho stage 0: $n = 47$, Coutinho stage 1: $n = 32$, Coutinho stage 2: $n = 8$, and Coutinho stage 3: $n = 1$). The controls were healthy and individuals with diabetes who were otherwise healthy with normal renal function, well-controlled blood pressure, and lipids. Complete demographic information on all participants is provided in Table 1.

Amyloid detection by skin biopsy

Amyloid was detected in skin biopsies of 76 of 88 (86%) individuals with ATTRv, and none of the healthy control participants or individuals with diabetes when using two skin biopsies from different sites with six tissue sections per biopsy (86% sensitivity, 100% specificity). Skin biopsies positive for amyloid deposition are shown in Figure 1A–D. In Figure 1E and 1F the number of skin biopsies obtained and the number of tissue sections required for the detection of amyloid is illustrated. At the distal leg biopsy site, there is a 37% chance that amyloid is detected within a single tissue section, which increases to 74% with six tissue sections (Fig. 1E). By adding a second biopsy with an additional six tissue sections, the positive detection rate of amyloid increases to 86%. Additional results are provided in Table 1.

In patients with no clinical signs of neuropathy (i.e., NIS-LL score of 0), a single tissue section at the distal leg had a 28% amyloid detection rate (Fig. 1F). With six tissue sections from a single biopsy the amyloid detection rate was 52%, with the addition of a second skin biopsy, also with six tissue sections measured, the detection rate increased to 77% (Fig. 1F).

Similar results were obtained for Coutinho grade 0 (i.e., patients graded by their physicians as asymptomatic), a single tissue section at the distal leg had a 48% chance of detecting amyloid (Fig. 1E). Using six tissue sections from a single biopsy, amyloid was detected

Table 1. Demographic information and participant characteristics.

TTR Mutation	V30M	V30M	V30M	V30M	V30M	V30M	V30M	V30M	V30M	V30M	V30M	S50R	S50R	S50R	S50R	S50R	S50R	Other	Other	Other	Healthy control	Disease control	
	All	Stage 0	Stage I	Stage II	Stage III	All	Stage 0	Stage I	Stage II	Stage III	All	Stage 0	Stage I	Total	Stage 0	Stage I	Stage 0	Stage I	Stage 0	Stage I	N/A	N/A	
Coutinho Stage	43	16	18	8	1	30	19	11	15	12	15	11	12	15	12	3	12	3	12	3	12	13	13
Number	40.8 ± 13.4 (19–73)	31.9 ± 9.1 (19–46)	44 ± 12 (25–73)	47.7 ± 11.8 (33–72)	70	38.6 ± 13 (21–61)	32.5 ± 9.3 (21–59)	50.4 ± 10.8 (25–61)	38.8 ± 11.2 (26–66)	49.6 ± 8.6 (33–66)	38.8 ± 11.2 (26–66)	50.4 ± 9.3 (21–59)	50.1 ± 13.3 (30–72)	63.4 ± 9.3 (52–79)	36.1 ± 8.6 (26–51)	49.6 ± 8.6 (33–66)	36.1 ± 8.6 (26–51)	49.6 ± 8.6 (33–66)	36.1 ± 8.6 (26–51)	49.6 ± 8.6 (33–66)	36.1 ± 8.6 (26–51)	50.1 ± 13.3 (30–72)	63.4 ± 9.3 (52–79)
Mean ± SD																							
Range																							
(years)																							
Sex	25 F	10 F	12 F	3 F	1 M	19 F	12 F	7 F	3 F	1 M	19 F	12 F	7 F	8 F	6 F	2 F	6 F	6 F	6 F	2 F	6 F	4 F	4 F
	18 M	6 M	6 M	5 M		11 M	7 M	4 M	5 M		11 M	7 M	4 M	7 M	6 M	1 M	6 M	6 M	6 M	1 M	6 M	7 M	7 M
Number of amyloid-positive biopsies (%)	37 (86%)	11 (68.8%)	17 (94.4%)	8 (100%)	1 (100%)	25 (83.3%)	16 (84.2%)	9 (81.8)	8 (100%)	1 (100%)	25 (83.3%)	16 (84.2%)	9 (81.8)	14 (93.3%)	11 (91.6%)	3 (100%)	11 (91.6%)	11 (91.6%)	11 (91.6%)	3 (100%)	0	0	0
NIS-LL mean ± SD (range)	18.9 ± 23.3 (0–73)	0	16.1 ± 13.3 (0–48)	56.9 ± 6.9 (51–73)	66.5	3.9 ± 5.3 (0–18)	2.3 ± 4.1 (0–6)	7.1 ± 5.8 (0–18)	3.4 ± 4.4 (0–14)	66.5	3.9 ± 5.3 (0–18)	2.3 ± 4.1 (0–6)	7.1 ± 5.8 (0–18)	3.4 ± 4.4 (0–14)	2.5 ± 3.7 (0–7)	7 ± 5.1 (2–14)	2.5 ± 3.7 (0–7)	2.5 ± 3.7 (0–7)	2.5 ± 3.7 (0–7)	7 ± 5.1 (2–14)	0	0	7.1 ± 5.1
UENS mean ± SD (range)	14.6 ± 15.8 (0–42)	0	16 ± 10.9 (0–40)	37.5 ± 4 (30–42)	40	5.4 ± 8.1 (0–32)	2.6 ± 4.9 (0–13)	10.2 ± 10.3 (0–32)	3.5 ± 6.0 (0–23)	40	5.4 ± 8.1 (0–32)	2.6 ± 4.9 (0–13)	10.2 ± 10.3 (0–32)	3.5 ± 6.0 (0–23)	1.5 ± 2.2 (0–7)	11.3 ± 10.4 (3–23)	1.5 ± 2.2 (0–7)	1.5 ± 2.2 (0–7)	1.5 ± 2.2 (0–7)	11.3 ± 10.4 (3–23)	0	0	11.3 ± 10.4 (3–23)

NIS-LL, neuropathy impairment score in the lower limb; TTR: transthyretin; UENS: Utah early neuropathy scale.

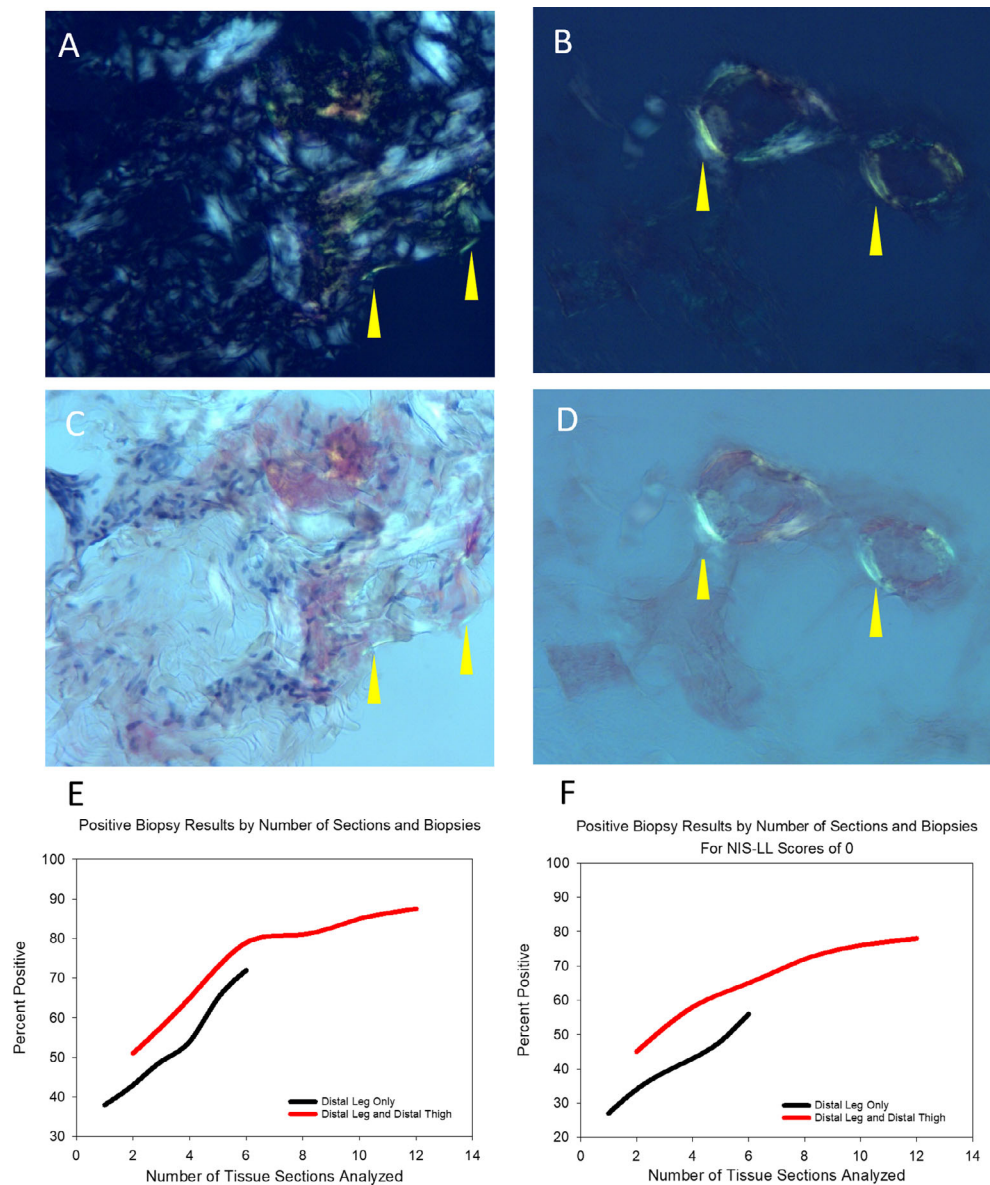


Figure 1. Amyloid deposition on skin biopsy. (A–D) Skin biopsies positive for amyloid deposition are shown in A and B. Yellow arrows denote amyloid deposits stained by Congo red appearing as apple green in color under polarized light. The same images are shown by light microscopy in C and D, where the deposition of amyloid is appreciated surrounding sweat glands. (E and F) The number of skin biopsies obtained and the number of tissue sections required for the detection of amyloid. At the distal leg biopsy site, there is a 37% chance that amyloid is detected within a single tissue section, this increases to 74% with six tissue sections (E). By adding a second biopsy with an additional six tissue sections, the positive detection rate of amyloid increases to 86%. In participants with no clinical signs of neuropathy (i.e., an NIS-LL score of 0), a single tissue section at the distal leg had a 28% amyloid detection rate (F). With six tissue sections from a single biopsy the amyloid detection rate was 52%, with the addition of a second skin biopsy, also with six tissue sections measured, the detection rate increased to 77% (F).

68% of the time, with a second skin biopsy the detection rate increased to 88%.

Thirteen individuals had no symptoms on any of the questionnaires and had a normal physical examination and were Coutinho stage 0. Amyloid was detected in 8/13 of these individuals (62%).

Patient-reported measures—painful and nonpainful sensory symptoms, autonomic symptoms, and QOL

Symptoms worsened significantly with ATTRv amyloidosis disease clinical progression measured by the NIS-LL

(NTSS-6 ($R = 0.66, p < 0.01$)), the BPI-SF ($R = 0.61, p < 0.01$), the Boston Autonomic Questionnaire ($R = 0.58, p < 0.05$), and QOL (as measured by the Norfolk-DN QoL ($R = 0.64, p < 0.01$)) worsened significantly with ATTRv amyloidosis disease clinical progression measured by the NIS-LL.

Similarly, when ATTRv participants were divided into those with no clinical evidence of neuropathy (NIS-LL of 0, $N = 41$), those with mild neuropathy (NIS-LL scores of 1–10, $N = 22$) and those with more severe neuropathy (NIS-LL scores $>10, N = 25$) significant worsening of symptom scores was observed across these divisions. No differences were evident across mutations although the study was not designed or powered to detect such differences.

Painful and nonpainful sensory symptoms and amyloid deposition

The NTSS-6 sensory total symptom score increased with a worsening NIS-LL score. All scores were worse

in participants with amyloid deposition detected on skin biopsy even in patients without clinically evident neuropathy (NIS-LL = 0) (see Fig. 2 and Table 2). Of the NIS-LL = 0 patients, 48% of participants with amyloid deposition reported sensory symptoms on the NTSS-6. In contrast, only 11% of patients without amyloid deposition reported sensory symptoms on this questionnaire.

All individual symptoms (e.g., pricking and tingling, numbness) were more prominent and more severe and occurred more frequently with disease progression based on the NIS-LL score. All questionnaire sensory symptoms were present in those patients who had amyloid detected on skin biopsy, even in the NIS-LL = 0 group. All items were worse if amyloid deposition was present (see Fig. 2). Numbness was the most prevalent symptom overall, however, in the NIS = LL = 0 patients with amyloid deposition, pricking and tingling were most prevalent. Only deep pain was present in patients without amyloid on skin biopsy.

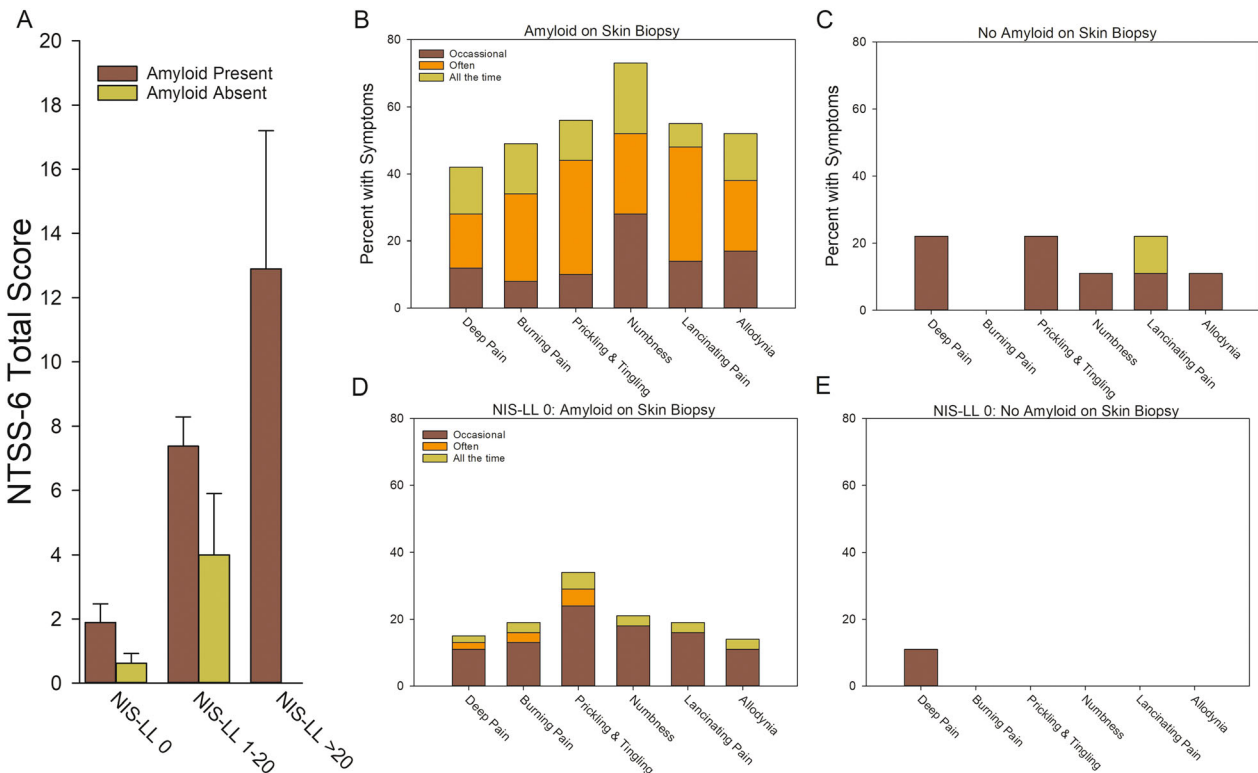


Figure 2. Neuropathy Total Symptom Score-6. The Neuropathy Total Symptom Score-6 (NTSS-6) evaluates the frequency and intensity of individual neuropathy painful and nonpainful sensory symptoms. Participants are grouped by NIS-LL = 0, NIS-LL 1–20, and NIS-LL >20. (A) Symptoms increase with the progression of the peripheral neuropathy as assessed by the Neuropathy Impairment Score of the Lower Limbs (NIS-LL). In all three groups, symptoms are worse in those participants with amyloid detected in the skin biopsy, even in those participants without clinically evident neuropathy (NIS-LL = 0). (B and C) In all participants with amyloid on skin biopsy ($N = 76$), all sensory symptoms are present and more severe in those participants with amyloid detected on skin biopsy. Numbness is the most prevalent symptom in participants with amyloid detected on skin biopsy. (D and E) All symptoms were present in the NIS-LL = 0 group with detected amyloid ($N = 32$). In this group, pricking and tingling were the most prevalent symptoms. In the NIS-LL = 0 without amyloid detection on skin biopsy, only occasional deep pain was present ($N = 9$).

Pain, pain interference, and amyloid deposition

The BPI-SF total score increased with the progression of the peripheral neuropathy as assessed by the Neuropathy Impairment Score of the Lower Limbs (NIS-LL). In all three groups, symptoms are worse in those patients with amyloid detected in the skin biopsy, even in those patients without a clinically evident neuropathy (NIS-LL = 0) (see Fig. 3A). Of the NIS-LL = 0 patients, 38% of participants with amyloid deposition reported pain on the BPI-SF. In contrast, only 11% of patients without amyloid deposition reported pain.

The individual items of the BPI-SF, the “worst,” “least,” “average,” and “pain right now scores” are shown in Figure 3B. All individual pain item scores increased with disease progression and were worse in those participants with amyloid deposition at every disease stage. The “do you have pain right now” item showed the greatest difference between those with and those without amyloid deposition. The percent of patients reporting interference and interference scores increased with disease progression and was significantly higher in patients with evidence of amyloid deposition (see Fig. 3C).

Autonomic symptoms

The orthostatic and vasomotor summary score of the Boston Autonomic Questionnaire increased with neuropathy progression and was significantly worse in those patients with amyloid deposition in all disease stages including those patients without clinically evident neuropathy (NIS-LL = 0) (see Fig. 4). The individual items of the summary score, lightheadedness, dizziness, presyncope, syncope, orthostatic symptoms on standing and limb color change were all worse in those patients with amyloid deposition. Of the NIS-LL = 0 patients, 58% of participants with amyloid deposition reported orthostatic or vasomotor autonomic symptoms on the Boston Autonomic Questionnaire. In contrast, only 11% of patients without amyloid deposition reported orthostatic or vasomotor autonomic symptoms. Gastrointestinal symptoms, genitourinary symptoms, and sudomotor symptoms worsened with neuropathy progression but amyloid deposition was not a differentiating factor.

Quality of life

The Norfolk QOL Total Score sensory symptom score increased with a worsening NIS-LL score. All scores were worse in participants with amyloid deposition detected on skin biopsy including those without clinically evident neuropathy (NIS-LL = 0) (see Fig. 3D). All QOL domain subscores (small fiber, large fiber, pain, activities of daily

life, functioning, and interference) were more severe with disease progression based on the NIS-LL score and all items were significantly worse if the amyloid deposition was present, including NIS-LL = 0 (see Fig. 3D). Of the NIS-LL = 0 patients, items in the small fiber domain (including, pricking/tingling or electric shocks) were present in 56% of individuals with amyloid deposition, but only 22% of those without amyloid deposition.

Clinician-reported scales—structured, quantified neurological examination

Across all mutation types, the severity of neuropathy, measured by a structured, quantified examination (UENS and NIS-LL) increased across the Coutinho stage (Table 1). The NIS-LL ranged from 0 to 73 and the UENS ranged from 0 to 42. The severity of neuropathy, as defined by NIS-LL and UENS scores, is reported in Table 1. A strong correlation was seen between the NIS-LL scores and the UENS scores. There was 98% concordance between NIS-LL score of 0 and UENS scores of 0.

Skin biopsy analysis

Intraepidermal and dermal nerve fiber density on skin biopsy

The data for IENFD grouped by NIS-LL score are reported in Table 2. IENFD declined significantly at the distal leg with an increase in NIS-LL scores ($p < 0.0001$, all comparisons). Similar results were observed when grouped by Coutinho staging and UENS scores. ATTRv participants had lower IENFD compared with control participants. Individuals with NIS-LL score = 0 (and those reported as Coutinho Stage 0) had significantly lower nerve fiber densities than control subjects across all mutation types (Table 1). Nearly identical results were seen when classifying by UENS scores of 0 ($N = 40$), UENS of 1–15 ($N = 25$), and NIS-LL scores of >15 ($N = 23$). None of the patients without amyloid deposition met the criteria for a small fiber neuropathy by skin biopsy, whereas, 44% of individuals with amyloid deposition on skin biopsy fulfilled the criteria for a small fiber neuropathy.

Amyloid deposition index

The deposition of amyloid increased with worsening neuropathy assessed by the NIS-LL (see Table 2). Similar results were seen when grouped by Coutinho stage and UENS: the deposition index at Coutinho stage 0 was 50 ± 108 , Coutinho stage 1 was 184 ± 257 , and at Coutinho stage 2–3 was 277 ± 244 , and for the UENS, similar results were noted.

Table 2. Participant characteristics subdivided by NIS-LL score.

	NIS-LL = 0 (N = 41)	NIS-LL range 1–10 (N = 22)	NIS-LL range 11–74 (N = 25)	ANOVA p Values
Examination scores				
NIS-LL	0	5.58 ± 3.09	34 ± 20.91	<0.01
UENS	0	7.33 ± 5.77	25.56 ± 12.17	<0.01
Coutinho (median [range])	0 [0–1]	1[0–1]	1 [0–3]	
Sensory symptoms and pain				
NTSS-6	1.33 ± 2.61	4.26 ± 5.61	11.2 ± 4.7	<0.01
BPI - Pain	1.3 ± 1.98	2.94 ± 2.79	5.17 ± 2.76	<0.01
BPI - Interference	0.88 ± 2.20	2.25 ± 3.27	4.26 ± 2.66	<0.01
Autonomic symptoms				
OHQ Score	4.72 ± 8.59	20.21 ± 22.89	32.65 ± 27.79	<0.01
BAQ OI	3.17 ± 9.19	6.84 ± 10.87	6.39 ± 9.49	
BAQ GI	6.06 ± 9.09	15 ± 16.53	22.74 ± 18.52	<0.01
BAQ GU	7.44 ± 7.89	6.58 ± 8.81	13.22 ± 12.49	
BAQ sweat	1.36 ± 2.67	6.47 ± 8.07	5.74 ± 6.74	<0.01
BAQ total	18.03 ± 21.12	34.89 ± 36.76	48.09 ± 32.26	<0.05
Quality of life				
Norfolk total	4 ± 7.8	19.83 ± 20.68	46.17 ± 27.5	<0.01
Norfolk small fiber	0.81 ± 1.24	2.04 ± 1.87	3.21 ± 1.79	<0.01
Norfolk large fiber	0.28 ± 0.81	1.52 ± 0.78	2.96 ± 1.83	<0.01
Norfolk pain	0.22 ± 0.81	0.78 ± 1.17	1.71 ± 2.14	<0.05
Norfolk ADL	1.58 ± 2.96	9.17 ± 10.51	26.83 ± 17.92	<0.01
Norfolk functioning	0.83 ± 2.8	3.7 ± 4.69	6.04 ± 5.67	<0.05
Norfolk interference	0.28 ± 0.94	2.61 ± 3.06	5.42 ± 3.45	<0.01
Nerve Fiber Density				
IENFD DL	16.8 ± 6.11	6.39 ± 4.63	1.43 ± 2.53	<0.01
IENFD DT	23.94 ± 7.01	13.57 ± 9.1	6.92 ± 7.42	<0.01
Amyloid deposition				
Amyloid counts	48 ± 106	186 ± 253	280 ± 246	<0.05
Amyloid % positive	77%	96%	92%	

ADL, activities of daily living; ANOVA, analysis of variance; BAQ, Boston autonomic questionnaire; BPI, Brief Pain Symptom; DL, distal leg; DT, distal thigh; GI, gastrointestinal; GU, genitourinary; gastrointestinal, GU: genitourinary IENFD, intraepidermal nerve fiber density; NIS-LL, Neuropathy Impairment Score in the lower limbs; NTSS6, neuropathy total symptom score-6 item; OHQ, orthostatic hypotension questionnaire; OI, orthostatic intolerance; UENS, Utah Early Neuropathy Score.

Study participants were divided into those with low levels of amyloid detected (ADI of <15, $N = 30$), those with moderate levels of amyloid (ADI of 15–150, $N = 31$) and those with high levels of amyloid detected (ADI >150, $N = 27$). Results are reported in Table 3. In all cases, the groups with greater levels of detected amyloid had more severe neuropathy, that is, worse sensory symptom scores, worse pain, worse autonomic symptoms, worse QOL, more abnormalities on the quantified neurological examination, more abnormalities to electrophysiological function, and lower nerve fiber densities.

Relationships among symptoms, signs, and cutaneous neuropathology

The ADI correlated with the symptoms scores: the NTSS-6 ($R = 0.46$, $p < 0.01$); the BPI-SF ($R = 0.44$, $p < 0.05$), autonomic symptoms, measured with the Boston

Autonomic Symptom Questionnaire ($R = 0.38$, $p < 0.05$); and QOL measured with the Norfolk Diabetic Neuropathy QOL Questionnaire ($R = 0.44$, $p < 0.01$). Similar correlations were observed with the examination scores (UENS [$R = 0.53$, $p < 0.01$] and the NIS-LL [$R = 0.48$, $p < 0.01$]) and the pathological findings (intraepidermal nerve fiber density at the distal leg [$R = 0.52$, $p < 0.01$]).

Discussion

We report a clinical case series of ATTRv amyloidosis with skin biopsy testing for detection of cutaneous amyloid. In this study, which focuses on patients in the earliest disease stages, the major findings are as follows: First, amyloid is detected frequently using Congo Red staining of skin biopsies in patients with ATTRv amyloidosis; second, amyloid can be detected in the earliest stages of the disease, before symptom onset, and before clinically evident changes in the

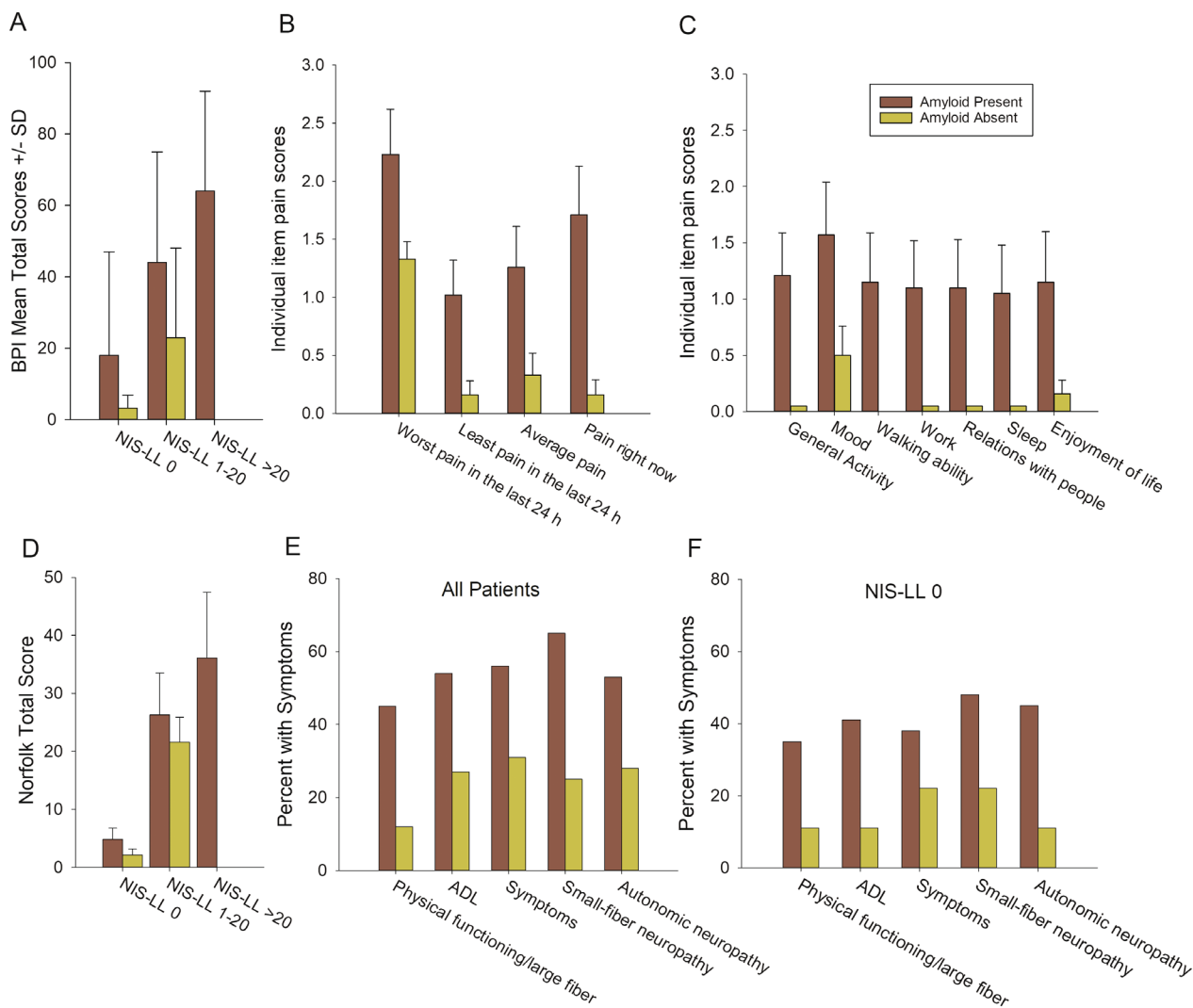


Figure 3. Brief Pain Symptom Inventory and Norfolk Quality of Life Questionnaire. The Brief Pain Symptom Inventory assesses the severity of pain and the impact of pain on daily functioning. Norfolk Diabetic Neuropathy Quality of Life Questionnaire (Norfolk-DN QoL) is a 47-item questionnaire that groups items according to small fiber, large fiber, and autonomic nerve function, symptoms, and activities of daily living. Participants are grouped by the Neuropathy Impairment Score of the Lower Limbs (NIS-LL) = 0, NIS-LL 1–20, and NIS-LL >20. (A) Pain total score increases with progression of the peripheral neuropathy as assessed by the NIS-LL. In all three groups, symptoms are worse in those participants with amyloid detected in the skin biopsy, even in those participants without clinically evident neuropathy (NIS-LL = 0). (B) All components of the pain total score are worse in those participants with amyloid detected on skin biopsy. (C) All interference items are worse in those participants with amyloid detected on skin biopsy. (D) The Norfolk QoL Total Score increased with disease progression based on the NIS-LL score. All scores were worse in participants with amyloid deposition detected on skin biopsy, even in the participants without clinically evident neuropathy (NIS-LL = 0). (E and F) The percentage of participants endorsing symptoms in QoL domains (physical functioning/large fiber, activities of daily life, symptoms, small fiber neuropathy domain, and autonomic neuropathy domain) was greater in participants with amyloid deposition, even in participants with NIS-LL = 0.

neurological examination; third, an association exists between the deposition of amyloid and the severity of neuropathy assessed using validated patient- and clinician-reported evaluation instruments; and fourth, symptoms, signs, and neuropathological changes to nerve fiber structure worsen with increasing amyloid deposition. These

findings have implications for our understanding of the earliest manifestations of the clinical phenotype, for the pathophysiological construct of disease staging, and for timing the introduction of disease-modifying therapy.

Even in genetically confirmed cases, tissue confirmation of amyloid is required for definitive diagnosis of

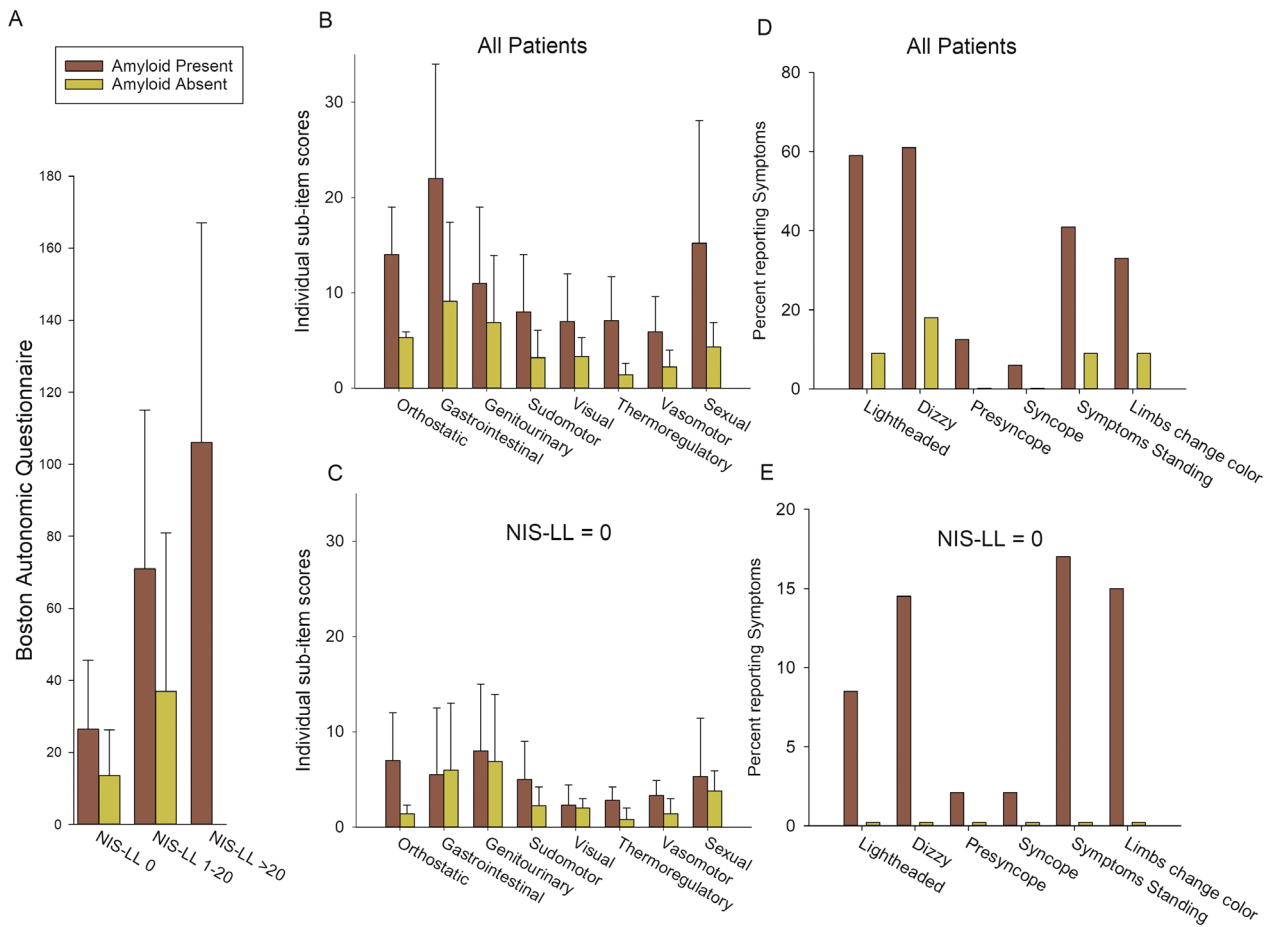


Figure 4. The Boston Autonomic Questionnaire. The Boston Autonomic Questionnaire assesses autonomic symptoms across multiple domains. Participants are grouped by the Neuropathy Impairment Score of the Lower Limbs (NIS-LL) = 0, NIS-LL 1–20, and NIS-LL >20. (A) The Boston Autonomic Questionnaire Total Score increased with disease progression based on the NIS-LL score. All scores were worse in participants with amyloid deposition detected on skin biopsy, even in the participants without clinically evident neuropathy (NIS-LL = 0). (B and C) Autonomic symptoms were more severe in participants with amyloid deposition, even in those without clinically evident neuropathy (NIS-LL = 0). (D and E) Individual items of the orthostatic and vasomotor domain were more severe in participants with amyloid deposition, even in those without clinically evident neuropathy (NIS-LL = 0).

amyloidosis, for prognostication and life planning, and prior to initiation of costly disease-modifying therapeutic interventions. A convenient, sensitive, and specific method of tissue confirmation would be a valuable tool in the diagnosis of patients with amyloidosis.²⁰ At present, several different biopsy sites are considered, including some that require invasive procedures, such as kidney, heart, gastrointestinal tract, salivary gland, and peripheral nerve, but skin biopsy is still not typically included among these. The sensitivity of these investigations varies widely.²⁰ Sural nerve biopsy, which is performed in some centers, has a sensitivity of up 83%,^{21,22} but may be complicated by pain, sensory loss, infection, and delayed wound healing.²³ Salivary gland biopsies are often performed, particularly in Portugal. This technique has a reported diagnostic sensitivity ranging

from 75%²⁴ to 91%.²⁵ Fat pad aspirate from abdominal tissue is widely used in the United States and may be the simplest approach to acquire tissue but has low sensitivity even for established disease.^{19,20} Several technical differences may underlie the increased sensitivity of skin biopsy over abdominal fat pad analysis in the detection of amyloid. First, the fat pad needle aspiration provides a smaller sample of tissue than a 3 mm skin punch biopsy; second, the tissue morphology is not maintained using a fat pad aspirate, and the physical integrity of amyloid deposits may be disrupted and difficult to identify; and third, the sensitivity is increased by the availability of additional sections and skin biopsy sites. Assessment of amyloid deposition in the abdominal fat pad obtained by skin biopsy may increase the sensitivity.²⁶

Table 3. Participant characteristics subdivided by ADI.

	ADI 0–15 (N = 30)	ADI >15–150 (N = 31)	ADI >150 (N = 27)	ANOVA
Examination scores				
NIS-LL	4.3 ± 11.28	5.98 ± 11.23	24.81 ± 23.7*	<0.01
UENS	3.5 ± 9.37	6.48 ± 8.98*	18.85 ± 14.83*	<0.01
Coutinho (median [range])	0 [0–1]	1[0–2]	1 [0–3]	
Sensory symptoms and pain				
NTSS6	2.14 ± 3.76	4.11 ± 5.58*	8.74 ± 6.19* [†]	<0.01
BPI - Pain	1.48 ± 2.40	2.63 ± 2.53	4.4 ± 3.1* [†]	<0.01
BPI - Interference	0.99 ± 2.36	2.22 ± 2.91*	3.40 ± 3.19* [†]	<0.01
Norfolk Total	6.62 ± 11.05	17.84 ± 21.49*	37.81 ± 31.39* [†]	<0.01
Norfolk Small fiber	0.88 ± 1.63	1.97 ± 1.58*	2.65 ± 2.06*	<0.05
Norfolk Large fiber	0.54 ± 1.03	1.32 ± 1.89*	2.35 ± 1.98*	<0.01
Norfolk Pain	0.12 ± 0.33	0.71 ± 1.16*	1.62 ± 2.08* [†]	<0.01
Norfolk ADL	3.08 ± 5.86	8.61 ± 11.55*	21.73 ± 19.75* [†]	<0.01
Norfolk functioning	0.92 ± 1.67	3.48 ± 5.08*	4.92 ± 5.8*	<0.05
Norfolk interference	1.08 ± 2.19	1.74 ± 2.78	4.54 ± 3.81* [†]	<0.01
Autonomic symptoms				
OHQ Score	7.23 ± 10.84	16.65 ± 22*	27 ± 28.55* [†]	<0.01
BAQ OI	1.96 ± 4.20	9.40 ± 13.73*	11.45 ± 16.76*	<0.01
BAQ GI	9.0 ± 10.27	11.34 ± 15.42	19.33 ± 18.96* [†]	<0.01
BAQ GU	7.11 ± 6.78	8.13 ± 9.63	13.19 ± 12.11	<0.05
BAQ sweat	3.22 ± 6.14	5.30 ± 6.98	6.7 ± 9.96*	<0.05
BAQ total	18.88 ± 17.67	33.84 ± 35.27*	40.77 ± 34.54*	<0.01
Nerve fiber density				
IENFD DL	12.79 ± 6.78	12.21 ± 8.24	2.9 ± 5.78* [†]	<0.001
IENFD DT	19.69 ± 8.1	20.51 ± 10.52 ± 9.1	7.47 ± 7.59* [†]	<0.001

Abbreviations: ADI, Amyloid Deposition Index; ADL, activities of daily living; ANOVA, analysis of variance; BAQ, Boston autonomic questionnaire; BPI, Brief Pain Symptom; DL, distal leg; DT, distal thigh; GI, gastrointestinal; GU, genitourinary; gastrointestinal, GU: genitourinary IENFD, intraepidermal nerve fiber density; NIS-LL, Neuropathy Impairment Score in the lower limbs; NTSS6, neuropathy total symptom score-6 item; OHQ, orthostatic hypotension questionnaire; OI, orthostatic intolerance; UENS, Utah Early Neuropathy Score.

* $p < 0.05$ vs ADI 0–15 group using Tamhane T2 post hoc test.

[†] $p < 0.05$ vs ADI 15–150 group using Tamhane T2 post hoc test.

Prior studies have reported that cutaneous amyloid can be detected in the skin of an individual with ATTRv mutations.^{5,6} Our approach increases the sensitivity and extends these findings to individuals *without* clinical evidence of peripheral neuropathy on examination. In the present study, the likelihood of amyloid detection increases from 37% with a single section to 74% with six sections from a single biopsy; and from 74% to 86% with an additional biopsy with six tissue sections. Most noteworthy is the detection of amyloid in 88% of asymptomatic patients (Coutinho Grade 0) and in 77% of patients without clinically evident neuropathy on examination. The majority of patients included in prior diagnostic discrimination studies had clinically evident disease. The increased sensitivity of amyloid detection in this study may be due to several reasons. First, a greater number of tissue sections studied resulted in a direct increase in the sensitivity of testing. Second, 50- μ m-thick tissue sections provide greater tissue sampling than thin paraffin-embedded specimens. Third, the modified

Highman's Congo Red protocol appears to provide greater differentiation of amyloid deposits from peripheral tissue than standard Congo Red staining. Finally, the types of TTR mutation studies may influence test results. Our results can be compared with two recent publications that used Congo red staining but with differing results: Ebenezer et al.⁵ examined three 50- μ m-thick sections from two biopsy sites, with lower sensitivity in TTR-positive cases with and without neuropathy; their results were similar to that shown in Figure 1 but with fewer samples tested, whereas Leonardi et al.⁷ examined 7- μ m-thick sections and had lower sensitivity for patients with and without neuropathy (the estimated volume of tissue analyzed appears <1/6 of that used in the present study).

Most outcomes in this manuscript are patient-centric, that is, address the patient experience and quality of life using validated PRO and QOL questionnaires. This approach is of growing importance in the delivery of high-quality healthcare. In the present study, the patients endorsed symptoms on questionnaires despite physician's

determination of Coutinho stage 0. These findings highlight the discordance that may occur when mild symptoms are present but not reported to or recognized by treating physicians. In providing symptom and QOL relationships with objective pathological findings, we reinforce the importance of subjective patient report in the patient with ATTRv amyloid neuropathy. Consistent with the notion that small nerve fibers are affected early and prominently in the course of the ATTRv neuropathy, the present data highlight the relationship between small fiber sensory symptoms (both painful and nonpainful), autonomic symptoms, and amyloid deposition. The strength and consistency of this relationship lend credence to the importance of these subjective manifestations even in the earliest disease stages. The NTSS-6, a sensory symptom questionnaire with both painful and nonpainful items, reveals the negative symptom, numbness is the most prevalent symptom across all disease stages. In contrast, in those individuals with amyloid deposition present in the skin, but without clinically evident disease, that is, the earliest stages of the disease, numbness is less prevalent while positive symptoms, such as prickling, tingling and lancinating pain are more prevalent, suggesting “irritability” or hypersensitivity of intact nociceptors. The Norfolk QOL small fiber questionnaire that includes items such as pricking/tingling or electric shocks corroborates these reported symptoms in the NIS-LL = 0 cohort.

While pain and sensory symptoms are recognized features of ATTRv neuropathy, although arguably underemphasized—they were not among the “potential red flags” suggesting ATTRv neuropathy in a diagnostic guidance²⁷—the present data emphasize not only the progressive severity of pain and other sensory symptoms across disease stages and their association with cutaneous amyloid deposition, but also the extent to which pain interferes with mood, work, sleep, and other activities of daily life throughout the disease.

As expected, autonomic symptoms were present across disease stages, in particular, symptoms of cardiovascular, gastrointestinal, and sexual autonomic impairment, which were the most prominent autonomic symptoms, and progressed in parallel with disease progression. Of note, symptoms of orthostatic intolerance and cutaneous vasomotor changes were prominent in patients without clinical evidence of neuropathy and significantly more severe in those individuals with cutaneous amyloid deposition. Throughout the disease stages, all autonomic symptoms were more severe in individuals with cutaneous amyloid deposition providing additional support for the measurement of cutaneous amyloid deposition as a biomarker for disease severity.

Our results show that amyloid deposition can be detected in the earliest disease stages—in a substantial

number of patients before significant symptoms and/or clinical signs are evident. In the major, recent large-scale clinical trials, clinically evident peripheral neuropathy has been an inclusion criterion. While neuropathy criteria currently frame the use of disease-modifying treatments, our data suggest that objective pathological evidence of the presence of disease is detectable at an even earlier disease stage, that is, at a stage that would not have met entry criteria in these trials, and raise the possibility of interventions earlier in the course of the disease.

Several lines of evidence support an approach to early treatment. First, although surgical gene therapy with liver transplantation improves prognosis, peripheral neuropathy and organ impairment do not usually reverse, and may, progress.²⁸ Second, despite unequivocal group benefits, the response to disease-modifying therapies with TTR tetramer stabilizers^{29,30} and gene silencing therapy,^{31,32} is not universal and some patients continue to progress despite therapy. Earlier intervention, consistent with strategies advocated in other neurodegenerative diseases, for example, mild cognitive impairment and asymptomatic AD,³³ and premotor Parkinson’s disease³⁴ may improve these numbers.

This manuscript introduces the notion that cutaneous amyloid deposition is a biomarker for the underlying disease pathology and pathophysiology. The detection of amyloid deposition in the earliest disease stages, even prior to clinically detectable manifestations, supports the utility of cutaneous amyloid deposition as a diagnostic biomarker that could enhance patient care, assist with life planning, and guide therapeutic interventions. Further, the increase in amyloid deposition with disease severity and the correlation with PRO measures, structured examinations, and neuropathology suggests that this biomarker has pathophysiological relevance and lends additional support to the potential utility of this measure as a predictive and prognostic biomarker. Importantly, these findings are consistent with a sural nerve biopsy study that showed earlier loss of small compared with large nerve fibers. In this study, amyloid deposition correlated negatively with the time to onset of symptoms in asymptomatic patients.³⁵ Longitudinal studies are required to confirm these findings in the skin.

This study has limitations and several questions remain. This is a cross-sectional study. It is not known whether amyloid deposition continues throughout the disease stages, whether deposition is continuous or has stepwise progression, and whether cutaneous amyloid will continue to correlate with the clinical manifestations of the disease. Serum neurofilament was not measured and the relationship between cutaneous amyloid deposition and serum neurofilament levels could not be determined.³⁶ We were unable to volumetrically quantify the

amount of amyloid within a given skin biopsy section. Despite these limitations, the present data provide a framework for the exploration of these topics in future studies.

Authors' Contribution

RF, AGD, FB, and CG contributed to the conception and design of the study; RF, AGD, FB, MC, SR, JG, JYK, NW, LO, and CG contributed to the acquisition and analysis of data; RF, AGD, FB, and CG contributed to drafting a significant portion of the manuscript or figures.

Conflict of Interest

RF received consultant fees and RF and AGD received a grant from Pfizer, which manufactures a drug to treat ATTRv cardiomyopathy. RF received a grant from Akcea, which manufactures a drug to treat ATTRv neuropathy. AGD received a grant from Alnylam, which manufactures a drug to treat ATTRv neuropathy. RF and CG have stock options in CND Life Sciences which is a skin biopsy company. The remaining authors have nothing to report.

References

- Adams D, Koike H, Slama M, Coelho T. Hereditary transthyretin amyloidosis: a model of medical progress for a fatal disease. *Nat Rev Neurol*. 2019;15(7):387-404.
- Gertz MA. Hereditary ATTR amyloidosis: burden of illness and diagnostic challenges. *Am J Manag Care*. 2017;23(7 Suppl):S107-S112.
- Yang NC, Lee MJ, Chao CC, et al. Clinical presentations and skin denervation in amyloid neuropathy due to transthyretin Ala97Ser. *Neurology*. 2010;75(6):532-538.
- Chao CC, Huang CM, Chiang HH, et al. Sudomotor innervation in transthyretin amyloid neuropathy: pathology and functional correlates. *Ann Neurol*. 2015;78:272-283.
- Ebenezer GJ, Liu Y, Judge DP, et al. Cutaneous nerve biomarkers in transthyretin familial amyloid polyneuropathy. *Ann Neurol*. 2017;82(1):44-56.
- Chao CC, Hsueh HW, Kan HW, et al. Skin nerve pathology: biomarkers of premanifest and manifest amyloid neuropathy. *Ann Neurol*. 2019;85(4):560-573.
- Leonardi L, Adam C, Beaudonnet G, et al. Skin amyloid deposits and nerve fiber loss as markers of neuropathy onset and progression in hereditary transthyretin amyloidosis. *Eur J Neurol*. 2022;29(5):1477-1487.
- Bastyr EJ 3rd, Price KL, Bril V. Development and validity testing of the neuropathy total symptom score-6: questionnaire for the study of sensory symptoms of diabetic peripheral neuropathy. *Clin Ther*. 2005;27(8):1278-1294.
- Cleeland CS, Ryan KM. Pain assessment: global use of the brief pain inventory. *Ann Acad Med Singap*. 1994;23(2):129-138.
- Gibbons CH, Freeman R. Treatment-induced neuropathy of diabetes: an acute, iatrogenic complication of diabetes. *Brain*. 2015;138(Pt 1):43-52.
- Vinik EJ, Hayes RP, Oglesby A, et al. The development and validation of the Norfolk QOL-DN, a new measure of patients' perception of the effects of diabetes and diabetic neuropathy. *Diabetes Technol Ther*. 2005;7(3):497-508.
- Dyck PJ, Davies JL, Litchy WJ, O'Brien PC. Longitudinal assessment of diabetic polyneuropathy using a composite score in the Rochester diabetic neuropathy study cohort. *Neurology*. 1997;49(1):229-239.
- Singleton JR, Bixby B, Russell JW, et al. The Utah early neuropathy scale: a sensitive clinical scale for early sensory predominant neuropathy. *J Peripher Nerv Syst*. 2008;13(3):218-227.
- Coutinho P, Martins da Silva A, Lopes Lima J, Resende BA. Forty years of experience with type I amyloid neuropathy. Review of 483 cases. In: Glenner G, Costa P, de Freitas A, eds. *Amyloid and Amyloidosis*. Amsterdam; 1980:88-98.
- England JD, Gronseth GS, Franklin G, et al. Practice parameter: evaluation of distal symmetric polyneuropathy: role of laboratory and genetic testing (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of physical medicine and rehabilitation. *Neurology*. 2009;72(2):185-192.
- McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency [see comments]. *Arch Neurol*. 1998;55(12):1513-1520.
- Provitiera V, Gibbons CH, Wendelschafer-Crabb G, et al. A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg. *Eur J Neurol*. 2016;23(2):333-338.
- Lauria G, Hsieh ST, Johansson O, et al. European Federation of Neurological Societies/peripheral nerve society guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the peripheral nerve society. *Eur J Neurol*. 2010;17(7):903-909.
- Westermarck P, Davey E, Lindbom K, Enqvist S. Subcutaneous fat tissue for diagnosis and studies of systemic amyloidosis. *Acta Histochem*. 2006;108(3):209-213.
- Adams D, Ando Y, Beirão JM, et al. Expert consensus recommendations to improve diagnosis of ATTR amyloidosis with polyneuropathy. *J Neurol*. 2021;268(6):2109-2122.

21. Luigetti M, Romozzi M, Bisogni G, et al. hATTR pathology: nerve biopsy results from Italian referral centers. *Brain Sci.* 2020;10(11):780. doi:10.3390/brainsci10110780
22. Plante-Bordeneuve V, Ferreira A, Lalu T, et al. Diagnostic pitfalls in sporadic transthyretin familial amyloid polyneuropathy (TTR-FAP). *Neurology.* 2007;69(7):693-698.
23. McLeod JG. Sural nerve biopsy. *J Neurol Neurosurg Psychiatry.* 2000;69(4):431.
24. de Paula EF, de Mello BL, de Carvalho DL, et al. Minor salivary gland biopsy for the diagnosis of familial amyloid polyneuropathy. *Neurol Sci.* 2017;38(2):311-318.
25. Do Amaral B, Coelho T, Sousa A, Guimaraes A. Usefulness of labial salivary gland biopsy in familial amyloid polyneuropathy Portuguese type. *Amyloid.* 2009;16(4):232-238.
26. Paulsson Rokke H, Sadat Gousheh N, Westermark P, et al. Abdominal fat pad biopsies exhibit good diagnostic accuracy in patients with suspected transthyretin amyloidosis. *Orphanet J Rare Dis.* 2020;15(1):278.
27. Conceicao I, Gonzalez-Duarte A, Obici L, et al. "Red-flag" symptom clusters in transthyretin familial amyloid polyneuropathy. *J Peripher Nerv Syst.* 2016;21(1):5-9.
28. Carvalho A, Rocha A, Lobato L. Liver transplantation in transthyretin amyloidosis: issues and challenges. *Liver Transpl.* 2015;21(3):282-292.
29. Coelho T, Maia LF, Martins da Silva A, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology.* 2012;79(8):785-792.
30. Berk JL, Suhr OB, Obici L, et al. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. *JAMA.* 2013;310(24):2658-2667.
31. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med.* 2018;379(1):11-21.
32. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *N Engl J Med.* 2018;379(1):22-31.
33. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 2018;14(4):535-562.
34. Lang AE, Espay AJ. Disease modification in Parkinson's disease: current approaches, challenges, and future considerations. *Mov Disord.* 2018;33(5):660-677.
35. Fernandes A, Coelho T, Rodrigues A, et al. Clinicopathological correlations of sural nerve biopsies in TTR Val30Met familial amyloid polyneuropathy. *Brain Commun.* 2019;1(1):fcz032.
36. Ticau S, Sridharan GV, Tsour S, et al. Neurofilament light chain as a biomarker of hereditary transthyretin-mediated amyloidosis. *Neurology.* 2021;96(3):e412-e422.