Detection of ATTR aggregates in plasma of polyneuropathic ATTR-V30M amyloidosis patients

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Abstract: ATTR amyloidosis is caused by the deposition of transthyretin amyloid fibrils in tissues often leading to organ failure and death. The clinical spectrum of this disease is highly diverse and dependent on many factors including the presence or absence of mutations within the transthyretin protein and/or an individual's ancestry. The phenotypic variability of ATTR amyloidosis makes it difficult to diagnose, delaying treatment and worsening patient prognosis. Our lab has recently developed a peptide probe that detects transthyretin aggregates in plasma of ATTR amyloidosis patients with cardiomyopathy but has not been tested in plasma from polyneuropathic patients. Here we evaluate our probe in a cohort of Portuguese patients carrying the ATTR-V30M mutation and having no cardiac phenotype. We found that we could indeed detect aggregates in their plasma, and there appeared to be no relationship between the presence of aggregates and patient age or gender. Our work has broad implications on the pathobiology of ATTR amyloidosis and contribute to the validation of our probe as a novel detection tool for this disease.

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Transthyretin amyloidosis is a disease caused by the systemic deposition of amyloidogenic transthyretin (ATTR) fibrils. The clinical spectrum of ATTR amyloidosis is highly diverse and may depend on factors such as the presence or absence of mutations within the transthyretin protein and an individual's ancestry. For example, ATTR amyloidosis resulting from the wild-type transthyretin protein is thought to result primarily in infiltrative cardiomyopathy (ATTR-CA) later in life, whereas the V30M mutation in the Portuguese population is associated with peripheral neuropathy (ATTR-PN) early in life[1,2]. Diagnosing ATTR amyloidosis is challenging due in part to the phenotypic variability of the disease.

Recent studies indicate that transthyretin may aggregate in the blood of ATTR amyloidosis patients[3-5]. The Kelly lab has developed a peptide probe after the β-strand B of transthyretin that detects non-native transthyretin (NNTTR) species in plasma of ATTR-PN patients with the V30M mutation[3]. These species are absent in those patients with cardiomyopathic or mixed phenotypes[3]. Our lab has also developed a Transthyretin Aggregation Detection (TAD1) probe targeting β-strands F and H that detects aggregated transthyretin species in plasma of ATTR-CA patients and patients with mixed phenotypes of both cardiomyopathy and polyneuropathy[4,5]. TAD1, however, has not been validated using plasma samples from ATTR-PN patients yet. Thus, here we assess whether the TAD1 probe can detect similar aggregated species in patients with strictly polyneuropathic phenotypes.

Raw data, patient data, and analytical methods can be made available upon request. Human tissues and peptide probes cannot be made available because of legal constraints. The Office of the Human Research Protection Program at UTSW granted exemption from Internal Board review
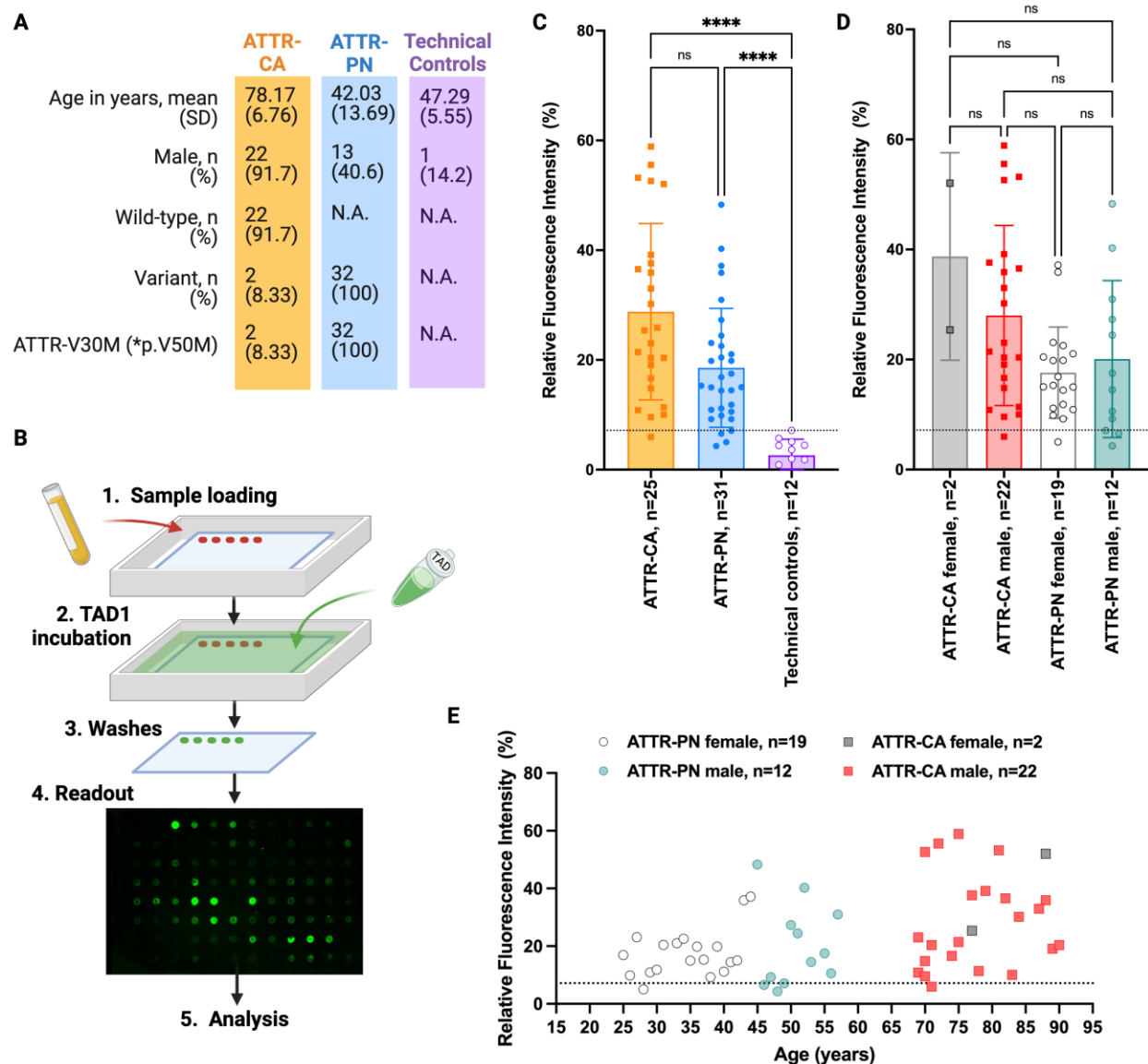
62   because specimens are anonymized. Experiments were conducted by the researchers blinded and

63   patient information was revealed for analysis at the conclusion of the study.

64   We sought to test the TAD1 probe's ability to bind aggregates in plasma of ATTR

65   amyloidosis patients of varying clinical phenotype. We tested this using three distinct cohorts:

66   ATTR-PN patients, ATTR-CA patients, and technical control plasma samples that do not bind

67   TAD1 (Figure 1A). We subjected these cohorts to our TAD1 assay pipeline, where TAD1 is

68   incubated with plasma fixed on nitrocellulose membrane, followed by washing steps and

69   quantifying aggregates in the samples through TAD1 fluorescence intensity (Figure 1B). We found

70   that TAD1 detects aggregates in ATTR amyloidosis plasma regardless of whether the patient

71   suffers from a predominantly cardiac or neuropathic phenotype (Figure 1C). We found no

72   significant correlation between TAD1 signal and the patients' gender (Figure 1D) or age (Figure

73   1E). Even though the ATTR-PN cohort is much younger than the ATTR-CA cohort, their TAD1

74   signals are comparable.

75   Our findings may have important implications on the biology of ATTR amyloidosis as well

76   as the development of clinical tools to detect and treat disease. Combined with previous studies,

77   our results suggest that in ATTR-CA patients, there is only one type of prefibrillar aggregated

78   species in plasma, recognized by TAD1. However, it appears that ATTR-PN patients may have

79   multiple types of aggregated ATTR species, those that are detected by the NNTTR detection assay[3]

80   and those detected by TAD1[4,5]. This may or may not relate to the phenotypic variability observed

81   in ATTR amyloidosis patients and warrants further study. In combination, the probes developed

82   by our lab and the Kelly lab could have potential to be used as a blood-based diagnostic tool to

83   differentiate between ATTR-CA and ATTR-PN.

84   There are limitations to this study that are of importance to note. These limitations include

85   a small sample size that does not have the power to adjust for confounding variables. These

86   samples were obtained through convenience sampling and needs independent validation with a

87   larger sample size and a targeted experimental design.

88   In summary, the present study evaluates the presence of aggregated transthyretin species

89   in plasma of neuropathic ATTR amyloidosis patients, similar to those found in cardiac ATTR

90   amyloidosis patients[4,5]. We observed that patients with a predominantly neuropathic phenotype

91   contain similar ATTR species in their plasma, which are detected by our novel TAD1 probe. These

92   findings may have important implications on the biological and clinical aspects of ATTR

93   amyloidosis.

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Figure 1. TAD1 detects ATTR aggregates in plasma of neuropathic ATTR-V30M patients.

A. Characteristics of plasma samples used for the study. **B.** Schematic of the protocol used for

analyzing aggregates in plasma. 30 µL of plasma samples are loaded onto nitrocellulose

membrane then incubated with 5 µM of our Transthyretin Aggregation Detector 1 (TAD1) probe

overnight. Excess unbound TAD1 is washed off the membrane through three ten-minute washes

with 10% tris buffered saline, then the level of ATTR aggregates in samples are measured

through excitation of TAD1 at 472 nm and reading emission at 513 nm. Fluorescence intensity is

102 quantified using ImageJ (**C-E**). Signal is normalized to membrane as 0% and 0.5 µg *ex vivo*

103 ATTRwt fibrils as 100%. **C.** TAD1 detects ATTR aggregates in both cardiomyopathic (ATTR-

104 CA) and polyneuropathic (ATTR-PN) ATTR amyloidosis patients. Outliers were removed using

105 a Grubbs test of the normalized dataset. A non-parametric Mann-Whitney *t*-test was used to

106 establish significant differences between each pair of groups (ns, not significant; **** p

107 <0.0001). Dashed line denotes the highest value of technical controls that do not bind TAD1. **D.**

108 There is no correlation between the gender of the patient and TAD1 signal. Statistical analysis

109 was performed as in **C**. **E.** There appears to be no relationship between age and TAD1 signal for

110 both cohorts assessed.

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