Supporting Information for

Title:

A Circulating, Disease-Specific, Mechanism-Linked Biomarker for ATTR Polyneuropathy Diagnosis and Response to Therapy Prediction

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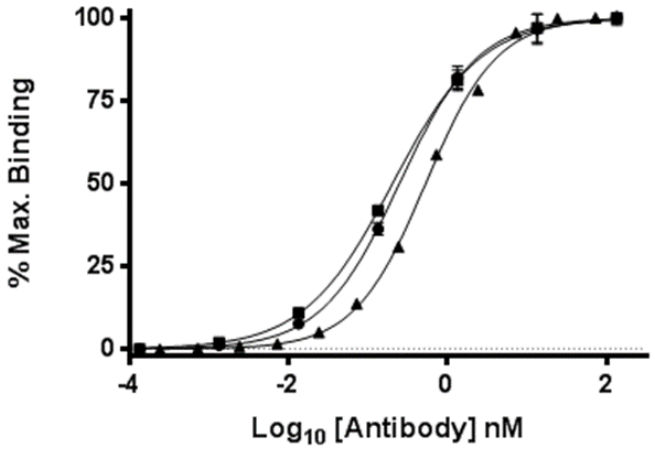


GPTGTGESKC PLMVKVLDAV RGSPAINVAV HVFRKAADDT WEPFASGKTS
ESGELHGLTT EEEFVEGIYK VEIDTKSYWK ALGISPFHEH AEVVFTANDS
GPRRYTIAAL LSPYSYSTTA VVTNPKE

Figure S1. Binding affinity of the anti-NNTTR antibodies

Illustrates the binding affinity of the mouse and rabbit antibodies to the respective peptides containing the corresponding epitopes. Peptide ADDTWEPFASGKT for 4C1 (square, K_D = 0.203 nM), peptide TSESGELHGLTTE for 5D4 (circle, K_D = 0.247 nM), and peptide AALLSPYSYSTTAV for 2504A (triangle, K_D = 0.529 nM). Schematic diagram of the sequence around the epitope region of the antibodies are shown in color.

NeutrAvidin (Pierce, USA; 5 µg/mL) in 50 mM sodium carbonate/bicarbonate buffer pH 9.6 was used to coat 96-well Corning high-binding EIA/RIA plates. The plates were washed three times with TBST buffer and blocked with Superblock (Pierce, USA). Biotinylated peptides were diluted with PBS to 2 µg/mL and 50–100 µL were added to each well and incubated at ambient temperature for one hour. The plates were then washed again with TBST and purified antibody (2 µg/mL) was added to the plates. After a one-hour incubation at ambient temperature and washing steps, HRP-conjugated secondary antibody was applied. Goat-anti-mouse IgG or Fc HRP conjugates were used in the case of mouse monoclonal antibodies, whereas goat-anti-rabbit IgG HRP-conjugated antibody was used for rabbit polyclonal detection. TMB was used as the HRP substrate for the detections.

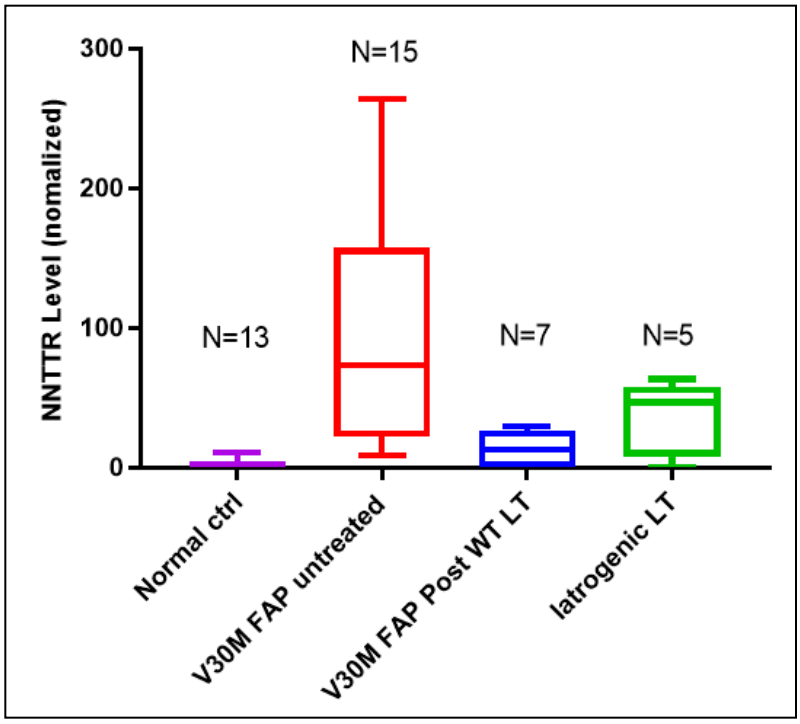
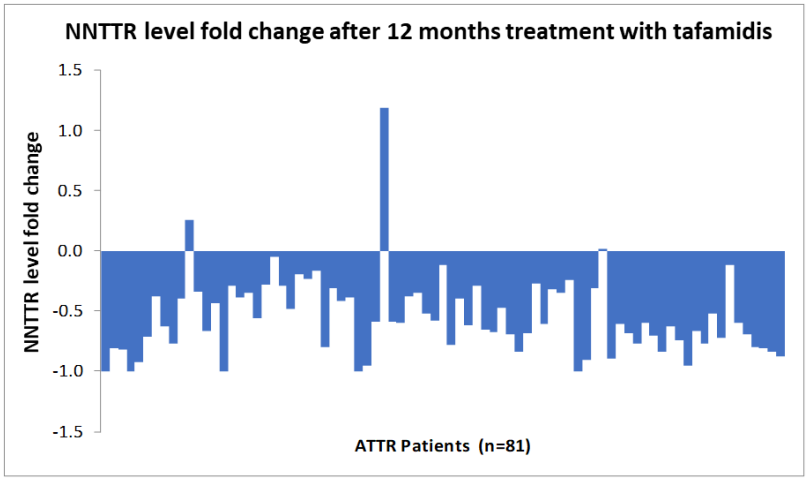


Figure S2. NNTTR levels in post-liver transplant (LT) patients

Patient plasma samples were kindly provided by Dr. Yoshi Sekijima at Shinshu University in Japan, in the Department of Medicine (Neurology & Rheumatology). None of the patients was treated with a TTR kinetic stabilizer. Normalized to the V30M TTR FAP pre-treatment level (average set to 100 based on a pooled sample from pre-treatment V30M TTR FAP patients, see Methods section). Comparing to the mean NNTTR level of V30M FAP untreated patients, normal controls have an average NNTTR level of 3.0 ($p<0.0001$); V30M FAP patients post-wild type (TTR) liver transplant have an average NNTTR level of 13.5 ($p=0.0028$) and iatrogenic LT patients who have received a WT/V30M heterozygous liver have an average NNTTR level of 35.8 ($P=0.064$). P values were calculated by 1-way ANOVA Dunnett's multiple comparison test. Note: two outliers were identified in the normal control group automatically using the ROUT method with Q=0.1% (99.9% chance of being true outliers) and were excluded from the comparison. These outliers are likely to be associated with damaged samples caused by repeated freeze/thaw.

A



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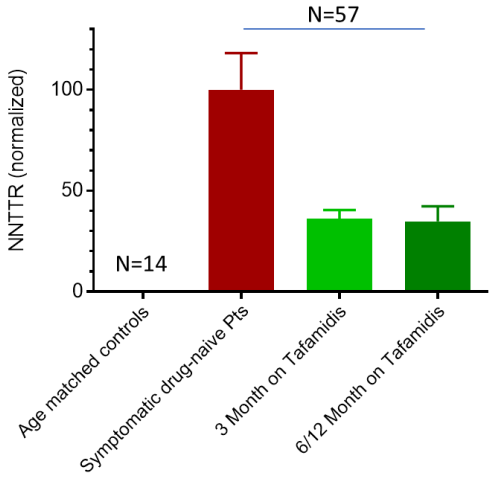


Figure S3. NNTTR levels reduced after tafamidis treatment

(a) NNTTR level reduction is seen in the majority of the individual patients.
(b) NNTTR levels in age-matched controls (N=14) and ATTR-FAP patients (N=57) pre- and 3- and 6/12 months post tafamidis treatment. Error bars represent SEM. Plasma samples from the Amyloidosis Reference Center in Porto, Portugal.

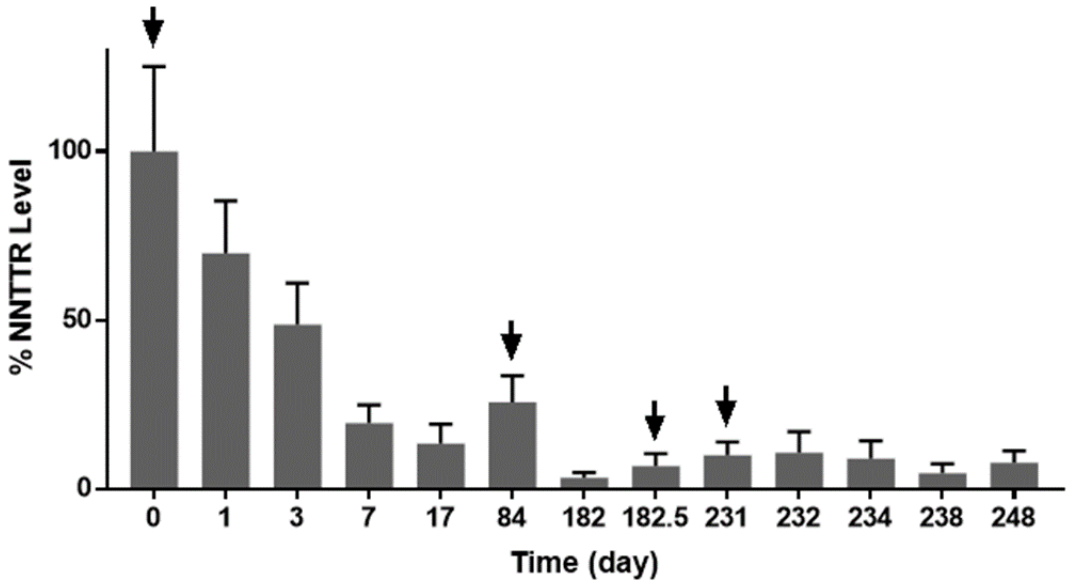


Figure S4. Patisiran results in NNTTR reduction

Patisiran patient samples were collected during the ALN-TTR02 clinical trial (ClinicalTrials.gov identifier: NCT01617967) and were provided by Alnylam Pharmaceuticals under a research agreement. Arrows indicate administration of patisiran. Error bars represent SEM.

Also note that none of the 80 plasma samples from 17 healthy controls from the ALN-TTR02 clinical trial (ClinicalTrials.gov identifier: NCT01559077) has any detectable NNTTR levels (data not shown).

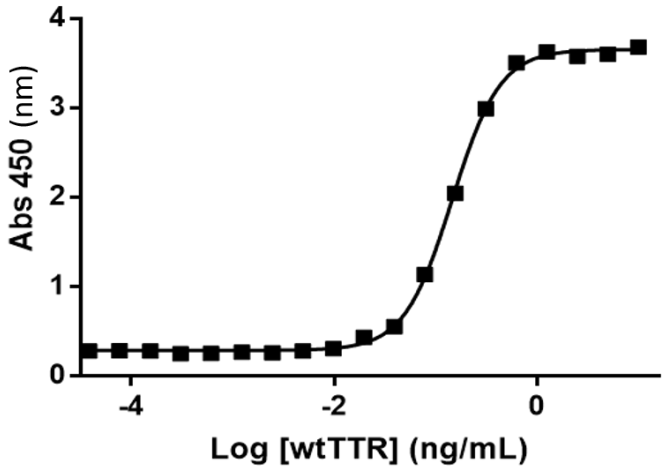


Figure S5. Total TTR ELISA using mouse monoclonal antibody 6H3 as the capture antibody and biotinylated 6H3 as the detection antibody, showing a standard curve, K_D=0.145 ng/mL

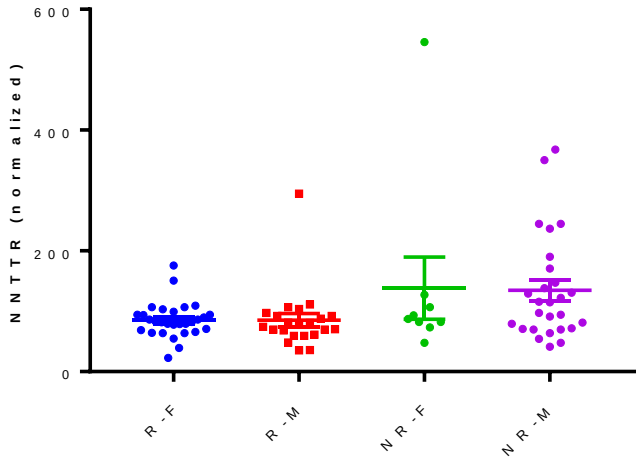


Figure S6. Male and female baseline NNTTR levels in the R vs NR groups. Error bars represent SEM.

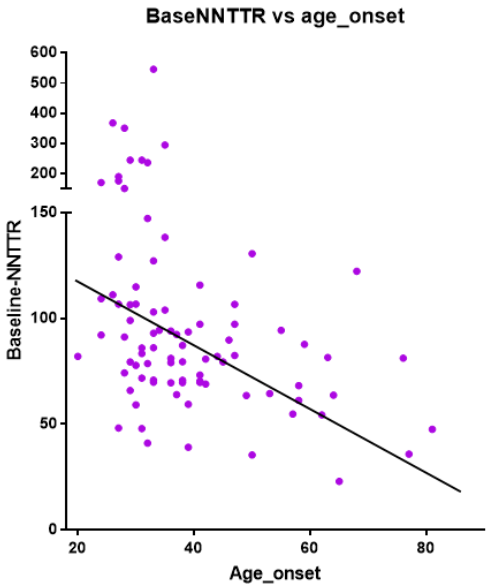
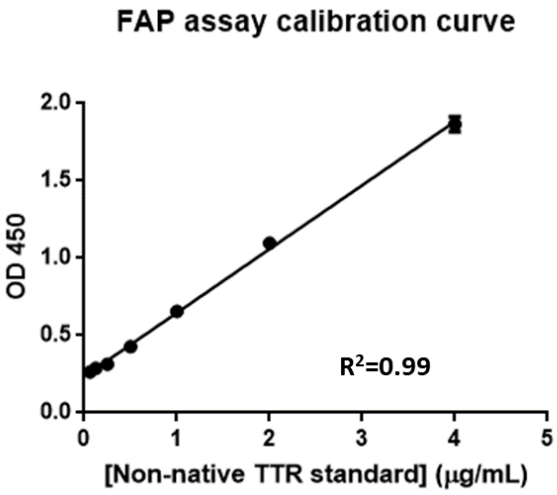


Figure S7. Baseline NNTTR vs age plot. An arbitrary age of 50 is used as the cutoff for the "old (>50)" and "young (≤50)" groups.

Figure S8. Assay analytical performance (LLOQ, accuracy, precision)



Accuracy

Four samples were tested with 5 replicates in three separate assays to assess accuracy.

Actual Value (µg/mL)	Calculated Mean Value			% Accuracy Range
	Assay 1	Assay 2	Assay 3	
5	4.44	4.07	4.33	81.4 - 88.9
10	8.74	9.67	11.05	87.4 - 110.5
40	34.63	39.18	37.51	86.6 - 97.9
60	59.08	53.44	58.13	89.1 - 98.5

Precision

Intra-assay Precision

Three FAP patient plasma samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision

Four samples were tested with 5 replicates in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision			
Sample	Patient 1	Patient 2	Patient 3	Sample 1	Sample 2	Sample 3	Sample 4
n	20	20	20	3	3	3	3
Mean (µg/mL)	5.85	19.46	55.55	4.28	9.82	37.11	56.88
Std. deviation	0.396	1.522	4.146	0.19	1.16	2.30	3.02
CV (%)	6.8%	7.8%	7.5%	4.5%	11.9%	6.2%	5.3%