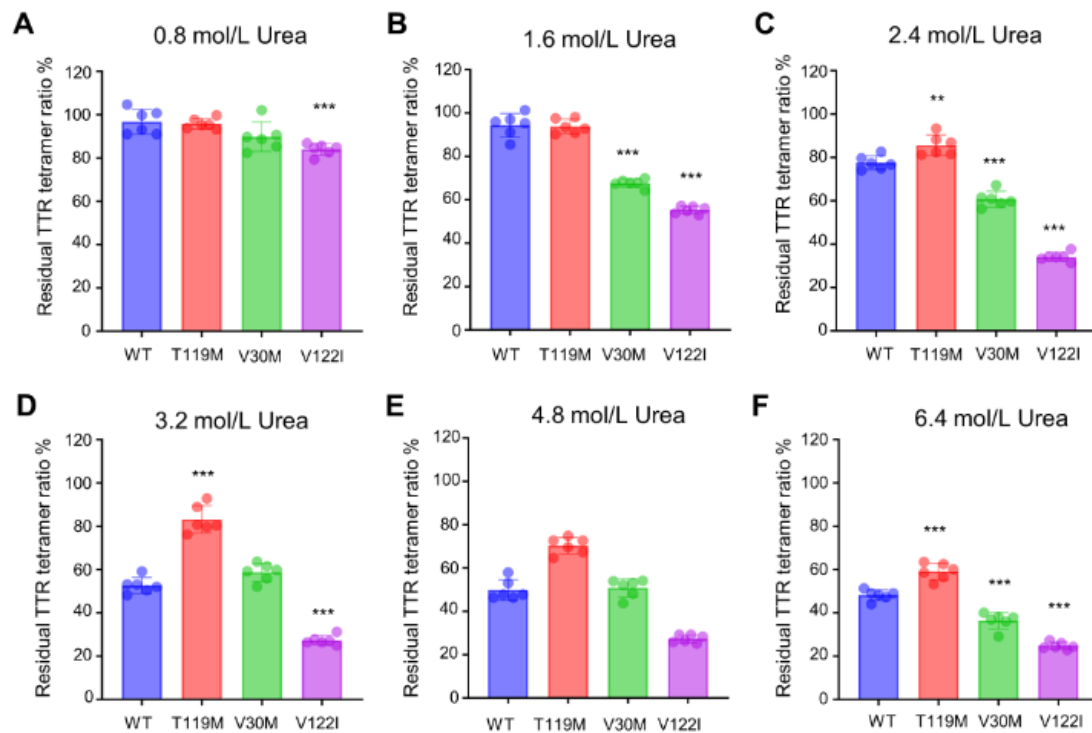


Supplementary Information

Assessment of transthyretin instability in patients with wild-type transthyretin amyloid cardiomyopathy

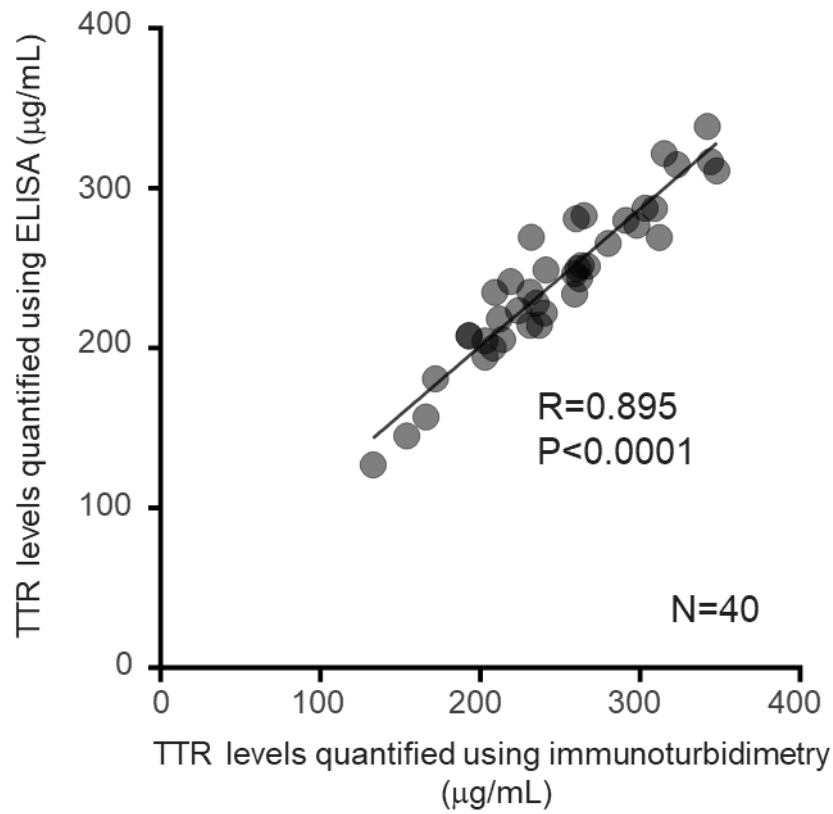
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Supplementary Figure S1. Effects of Each Concentrations of Urea for TTR Stability

The residual TTR tetramer formation ratio after urea-induced denaturation of recombinant wild-type TTR, recombinant T119M TTR, recombinant V30M TTR, and recombinant V122I TTR. The urea concentrations used were 0.8 M (A), 1.6 M (B), 2.4 M (C), 3.2 M (D), 4.8 M (E), and 6.4 M (F). Values are expressed as the means \pm SDs ($n = 6$). ** $p < 0.01$, *** $p < 0.001$ compared to the WT group. Data were analyzed by one-way ANOVA with Dunnett's multiple comparisons test (A-D, F) or by Kruskal-Wallis test, followed by Dunn's post-test (E). TTR, transthyretin; WT, wild-type;



Supplementary Figure S2. Correlation Between ELISA and Immunoturbidimetry

The correlation between the quantitative results of TTR in plasma samples measured by ELISA and immunoturbidimetry(n=40).

Supplementary Table 1. Detailed ELISA data

TTR (ng/mL)	Luminescence intensity		
	TTR purified from plasma	Recombinant Wild type TTR	Recombinant F87M/L110M TTR
10	18874.5	17017.5	1479.0
5	9744.5	9461.5	964.0
2.5	5248.5	5055.5	661.0
1.25	2959.0	2901.0	470.0
0.625	1751.0	1733.0	400.0
0.3125	1086.5	1218.0	402.0
0.15625	807.0	858.0	383.5
0.078125	712.0	672.0	405.0
0.0390625	424.5	410.0	406.0
0.0195313	432.0	397.5	358.0
0.0097656	372.5	383.5	398.0
0	335.5	329.0	351.0

Supplementary Table 2. Calibration curve analysis.

TTR (ng/mL)	%CV	Accuracy %
10	4.1	87.8
5	2.4	99.0
2.5	2.3	107.3
1.25	4.9	101.8
0.625	4.8	98.9
0.3125	10.5	89.6

Supplementary Table 3. Dilution linearity of serum

Dilution factor	Measured concentration (ng/mL)	Dilution-corrected concentration (ng/mL)	% Change
40000	6.39	-	-
80000	3.53	3.19	110.6
160000	1.82	1.77	103.0
320000	0.86	0.91	94.4
640000	0.40	0.43	92.9

Supplementary Table 4. Intra- and inter-assay precision

	<u>Intra-assay (n = 6)</u>			<u>Inter-assay (n = 6)</u>		
	Mean, ng/mL	SD	%CV	Mean, ng/mL	SD	%CV
High	8.3	0.1	1.1	8.0	0.6	7.8
Medium	4.8	0.1	2.2	4.5	0.3	7.0
Low	2.4	0.1	2.5	2.3	0.1	5.2