

Serial measurements of Circulating TTR **in Subjects with TTR Amyloidosis or Carriers of Mutant TTR**

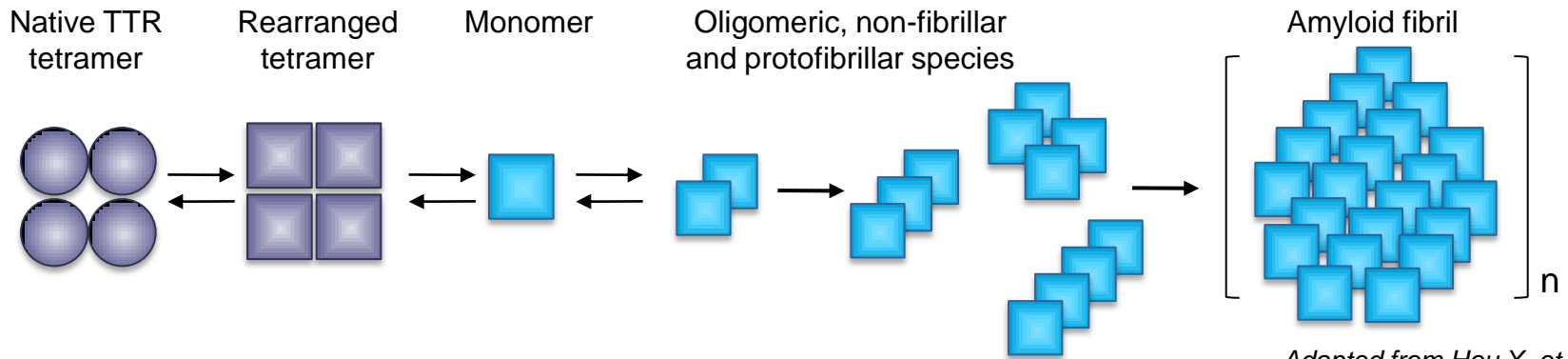
Peripheral Nerve Society
June 2011

Transthyretin (TTR) Amyloidosis (ATTR)

Background

- Autosomal dominant disease
- Caused by extracellular deposition of mutant and wild-type TTR in target organs such as the peripheral nervous system (PNS) and heart
 - » Deposition in the PNS leads to a polyneuropathy/autonomic disorder termed Familial Amyloidotic Polyneuropathy (FAP)
 - Val30Met (V30M) is most common mutation in FAP
 - Orthotopic liver transplant (OLT) is only current treatment for FAP
 - » Deposition in the heart leads to a cardiac disorder termed Familial Amyloidotic Cardiomyopathy (FAC)
 - Val122Ile (V122I) is most common mutation in FAC
 - OLT unproven in FAC

TTR Biology Background



- Wild-type TTR circulates as ~55 KDa tetramer
- Binds and transports serum retinol binding protein (RBP)/vitamin A and minor fraction of serum thyroxine (T4)
- Amyloid plaques contain both mutant and wt TTR

Objectives

Study Design

- To assess serial circulating levels of TTR in patients with TTR amyloidosis and in healthy carriers of a mutant TTR gene through:
 - » Measurement of total TTR by ELISA
 - » Exploratory analysis of wild type and mutant TTR using isoelectric focusing (IEF)
- To describe the intra-patient variability in mutant, wild-type, and total TTR over time



Key Inclusion Criteria

Study Design

Carriers:

- Male or female aged ≥ 18 years
- Documented carrier of a TTR mutation (heterozygote or homozygote)
- No clinical manifestations of amyloidosis
- No history of biopsy-proven amyloid deposits
- Subject is willing and able to comply with protocol schedule of assessments

Patients

- Male or female aged ≥ 18 years
- Biopsy-proven TTR amyloidosis
- Subject is willing and able to comply with protocol schedule of assessments

Key Exclusion Criteria

Study Design

- Known or suspected active inflammatory disorder (e.g., chronic infection, active RA/OA)
- Taking medication that is known or suspected to affect circulating TTR level (e.g., oral contraceptives, systemic steroids)
- BMI < 19.0 kg/m² or clinical evidence of poor nutritional status
- Chronic renal disease (clinically significant elevation of serum creatinine)

Study Design

- Single-center, natural history, non-therapeutic study
 - » Boston University
- TTR amyloidosis patients and healthy carriers (≥ 18 years)
- Circulating levels of TTR assessed serially over 4 consecutive weeks



ALN-TTR-NT-001 Schedule of Assessments

Procedure	Screening/Visit 1 (W0)	Visit 2 (W1)	Visit 3 (W2)	Visit 4 (W3)
Informed Consent and Medical History	X			
Inclusion/Exclusion	X			
Enrollment	X			
Demographics	X			
Previous and Conmeds	X	X	X	X
Vital Signs	X	X*	X*	X*
Physical Exam	X			
Blood sampling for chem / hem	X			
Blood sampling for TTR	X	X	X	X
Interval Health Status Inquiry		X	X	X

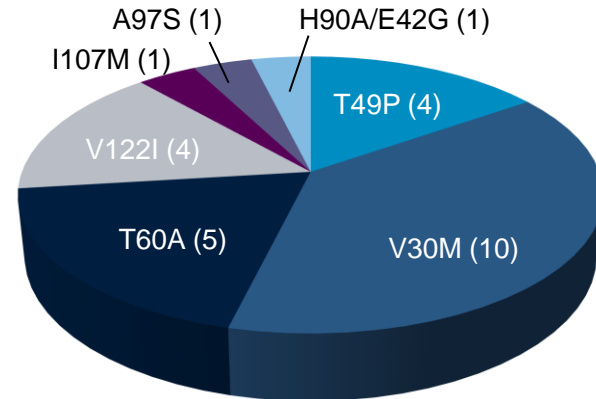
**Temperature only for visits 2 - 4*

ALN-TTR-NT-001 Study

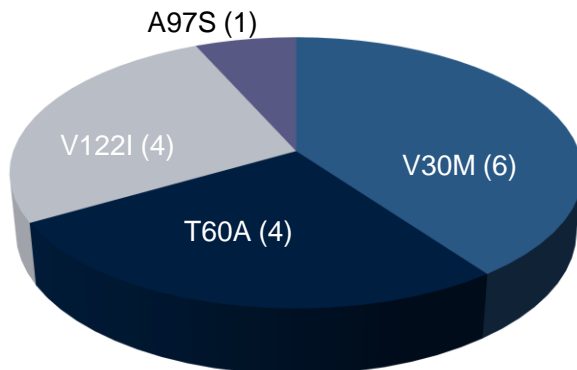
Demographics and Mutations

- 26 subjects enrolled
- 25 completers
- 15 patients / 11 carriers
- 13 males / 13 females

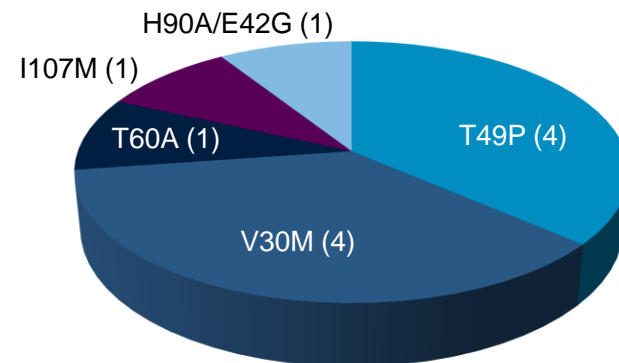
All Mutations



Patients Mutations

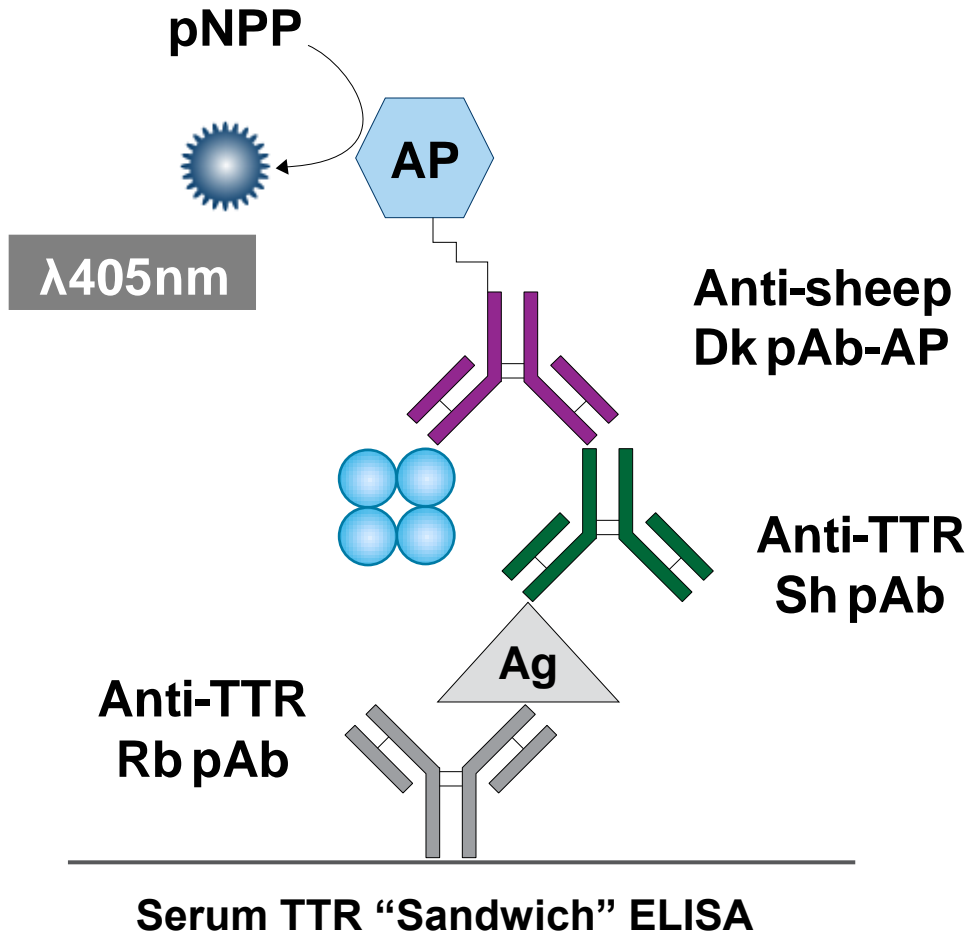


Carriers Mutations



TTR Sandwich ELISA

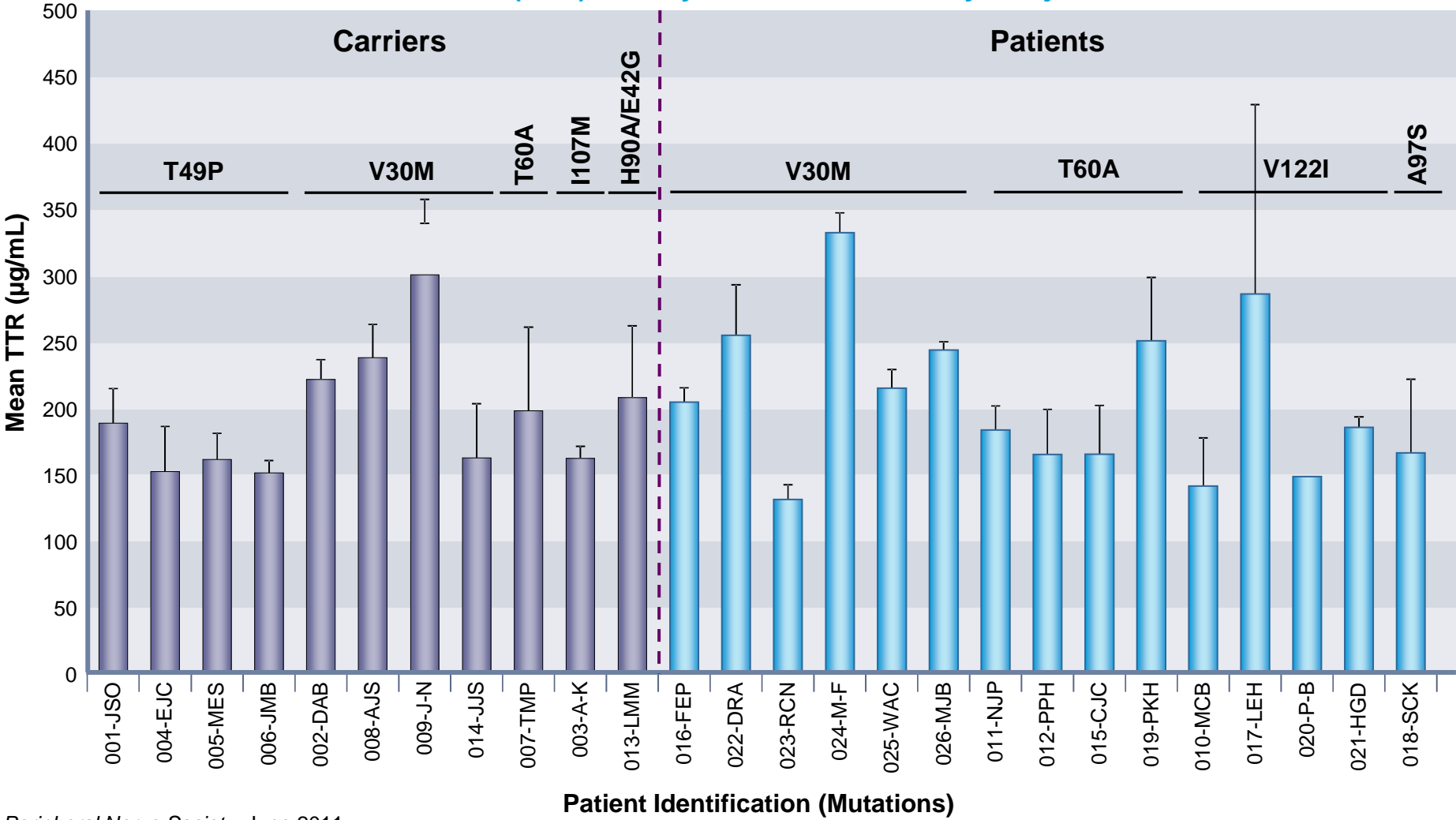
Methods



- "Sandwich" ELISA
- Developed internally
- GLP validated assay for wt human and cyno TTR (Charles River Labs)
- Measures TOTAL (TTR)

TTR Sandwich ELISA Results

Mean (\pm SD) Weekly Total Serum TTR by Subject



Peripheral Nerve Society, June 2011

Summary Statistics of TTR Levels Over Time

Group included in analysis	Visits included in analysis	Number of Subjects	Number of Observations	Mean (µg/mL)	Intrasubject Variance* (µg/mL)	Intersubject Variance* (µg/mL)
Carrier (All)	All visits	11	44	194.9	33.2 (17%)	42.6 (22%)
Patient (All)	All visits	15	57	207.9	47.8 (23%)	52.3 (25%)
Overall (All)	All visits	26	101	202.2	42.0 (21%)	47.7 (24%)
Carrier (V30M)	All visits	4	16	230.7	26.5 (11%)	55.1 (24%)
Patient (V30M)	All visits	6	24	230.2	19.0 (8%)	65.5 (28%)
Overall (V30M)	All visits	10	40	230.4	22.3 (10%)	58.2 (25%)

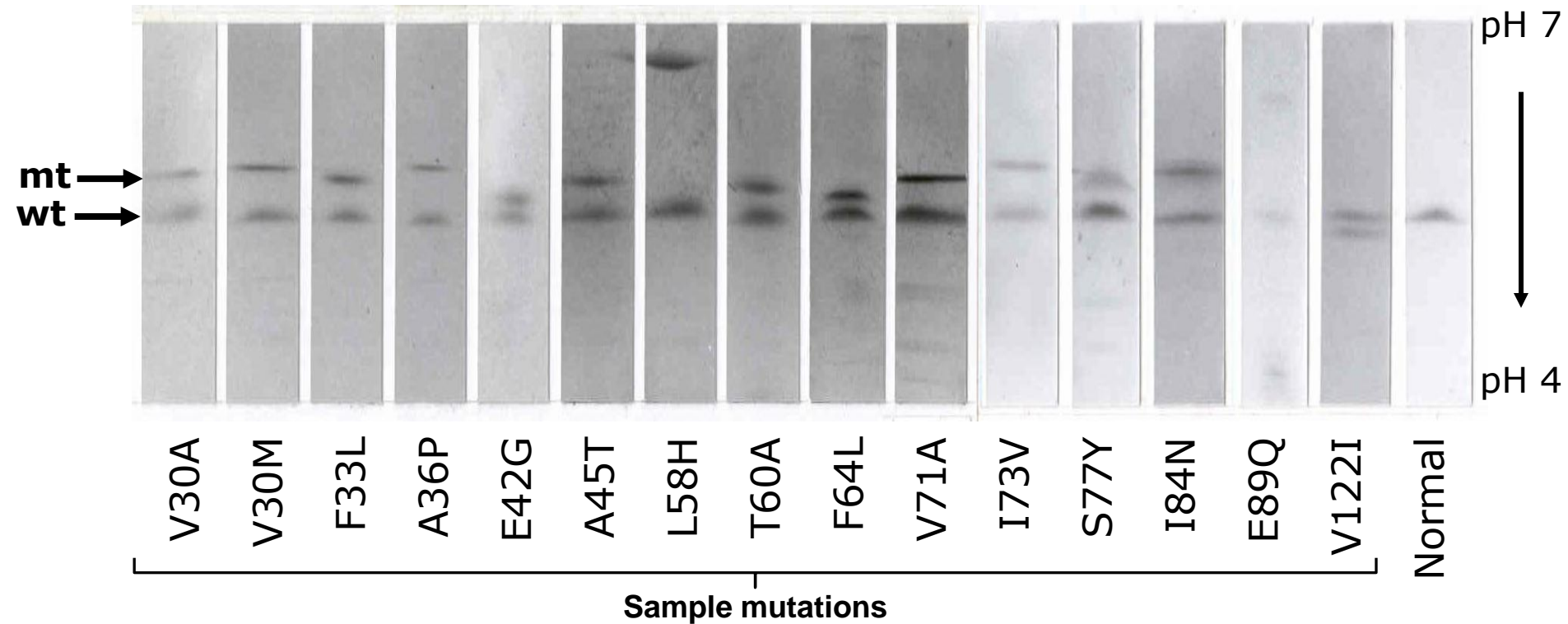
* Variance is estimated using a random effects model

2 Step Semi-Quantitative Method

- Native Polyacrylamide gel electrophoresis (7.5%)
 - » Non-denaturing gels
- IEF
 - » pH 4.0 – 7.0 gradient; partially denaturing conditions (2.5M Urea)
 - » Proteins separated based on charge; migrate through pH gradient until net charge of protein is zero
 - » Bands quantified using densitometry
 - » Isolates monomeric form of TTR protein



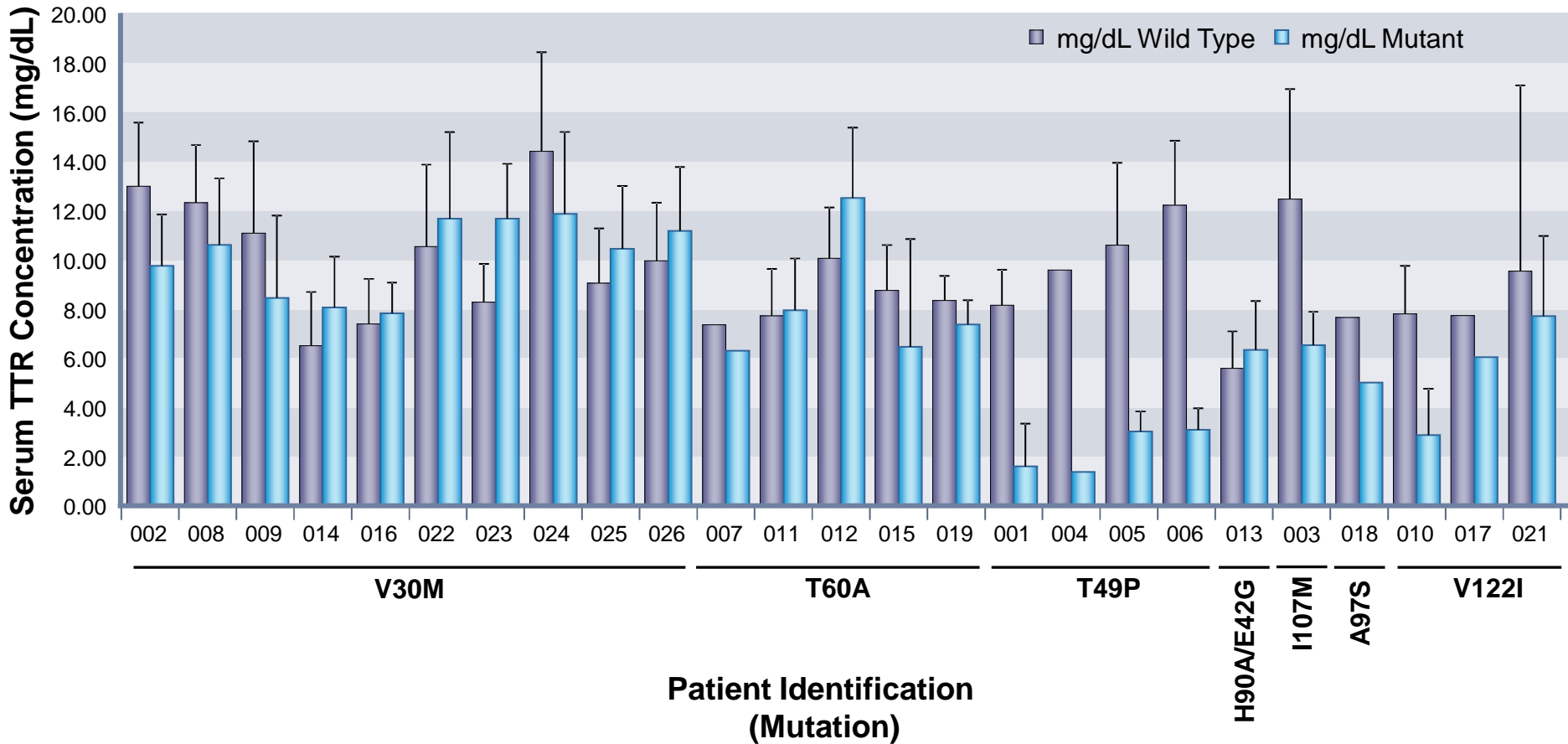
Isoelectric Focusing Gel (IEF) Methods



Connors LH *et al. BBA* (1998) 1407:185-92.

Isoelectric Focusing Gel (IEF) Results

Average 4-Week Serum TTR Concentration



TTR

- Assays developed for assessment of both mutant and WT TTR
- Over a 28 day observation period, mean TTR levels measured ~200 µg/mL with 10-20% intra-subject variability and approximately 25% inter-subject variability
 - » V30M variability lower compared to the overall study population
 - » TTR levels similar between patients and carriers
- Overall, IEF demonstrated an approximate 1:1 ratio of mutant to wild type TTR in V30M patients and carriers
- These data support further evaluation of RNAi therapeutics (ALN-TTR) for TTR Amyloidosis

