

# A new ELISA to measure human Progranulin mutations in Frontotemporal Diseases

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## Introduction

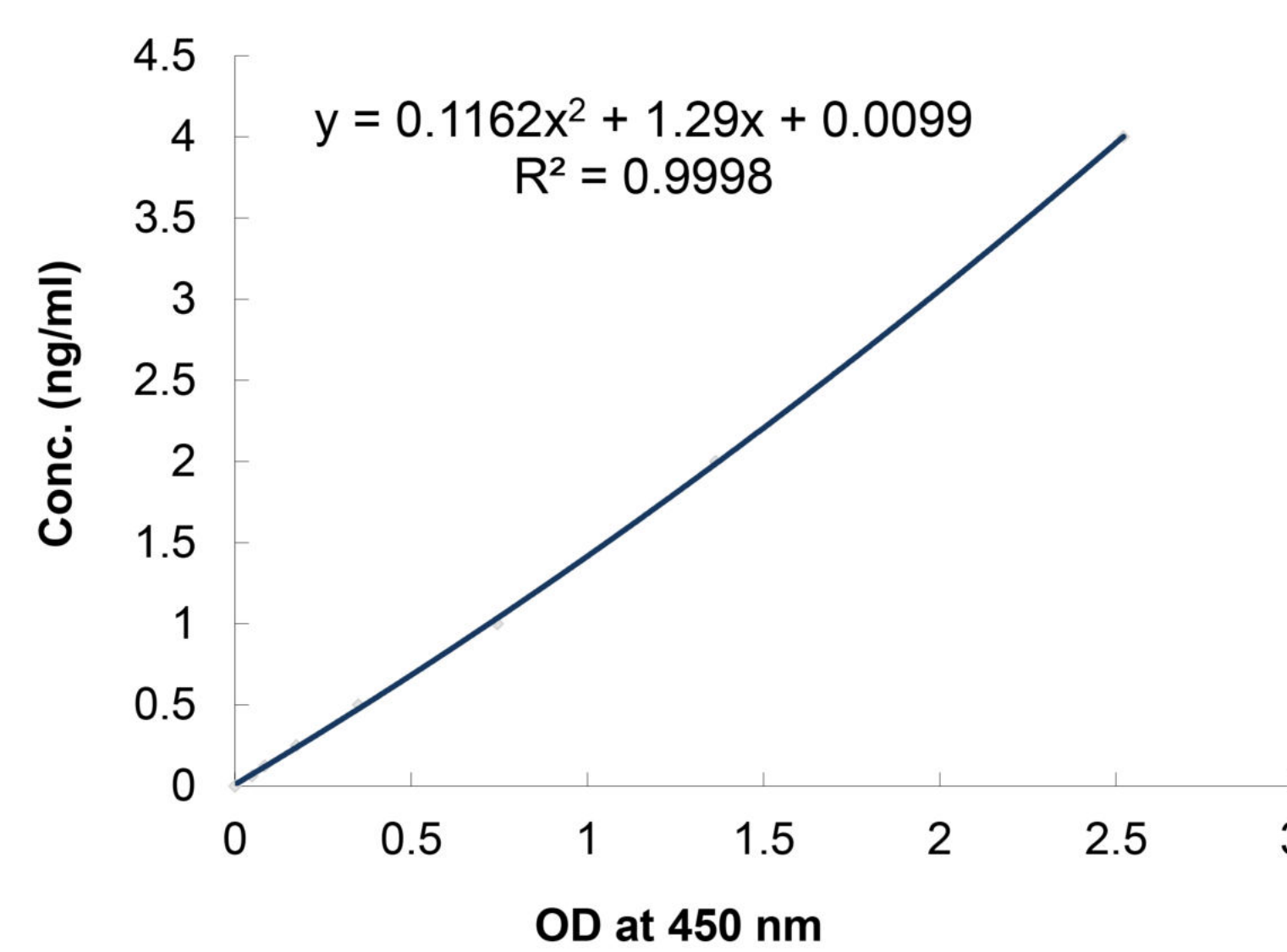
**Frontotemporal lobar degeneration (FTLD)** is a progressive neurodegenerative disease with an age at onset generally below 65 years. Mutations in Progranulin (*GRN*) have been reported to cause FTLT through haploinsufficiency. Mutations in *GRN* account for 5-10% of all the FTLT cases, leading to an approximately 50% reduction of the precursor protein Progranulin. Decreased levels of Progranulin protein predict *GRN* mutations, even in pre-symptomatic carriers more than four decades before disease onset. Screening of individuals with *GRN* mutations can be made by Progranulin measurement in serum and plasma using ELISAs and confirmed by molecular analysis of the *GRN* gene. We present here a comparison between two ELISA Kits developed at AdipoGen Life Sciences: a Progranulin (human) ELISA Kit based on polyclonal antibodies (AG-45A-0018Y, the Reference kit in the measurement of Progranulin levels for FTLT samples), and a new Progranulin (human) ELISA Kit (AG-45B-0027) based on monoclonal antibodies.

## Results

### Specifications of AG-45B-0027

- Sandwich ELISA (mAb/mAb)
- Sensitivity, Limit of Detection: 0.06 ng/ml
- Specificity: Validated on Mutated and Null *GRN* plasma samples
- Detects human Progranulin in cell culture supernatant, serum and plasma samples
- Range: 0.063 ng/ml - 4 ng/ml
- Intra-assays and inter-assays: <9%

### Standard Curve



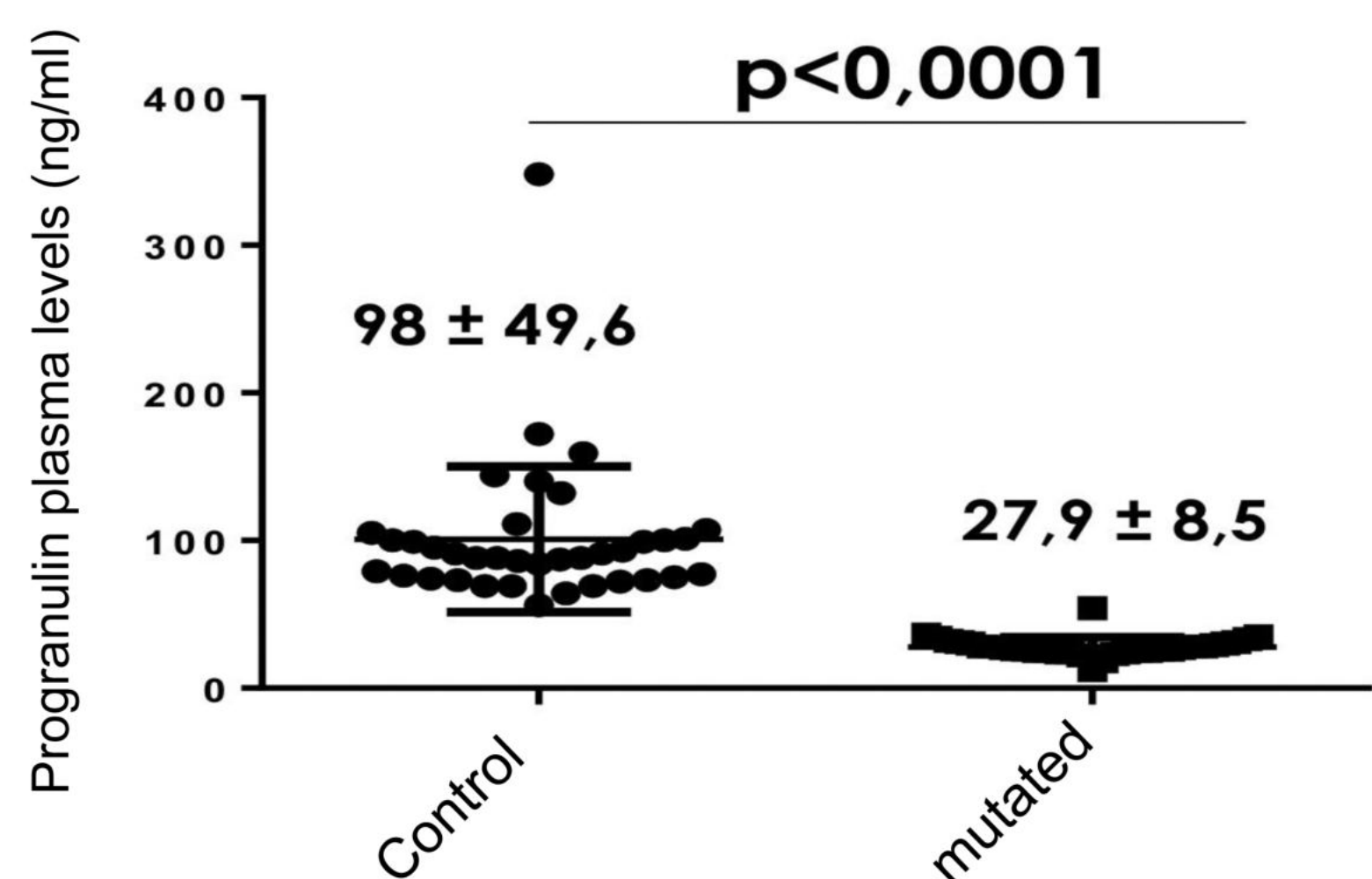
### Samples analyzed in this study

#### 191 samples have been analyzed using both kits

- 38 EDTA plasma samples from individuals carrying different mutations of the *GRN* gene (covering all 12 exons of the *GRN* gene)
- 153 plasma samples (control) from healthy individuals

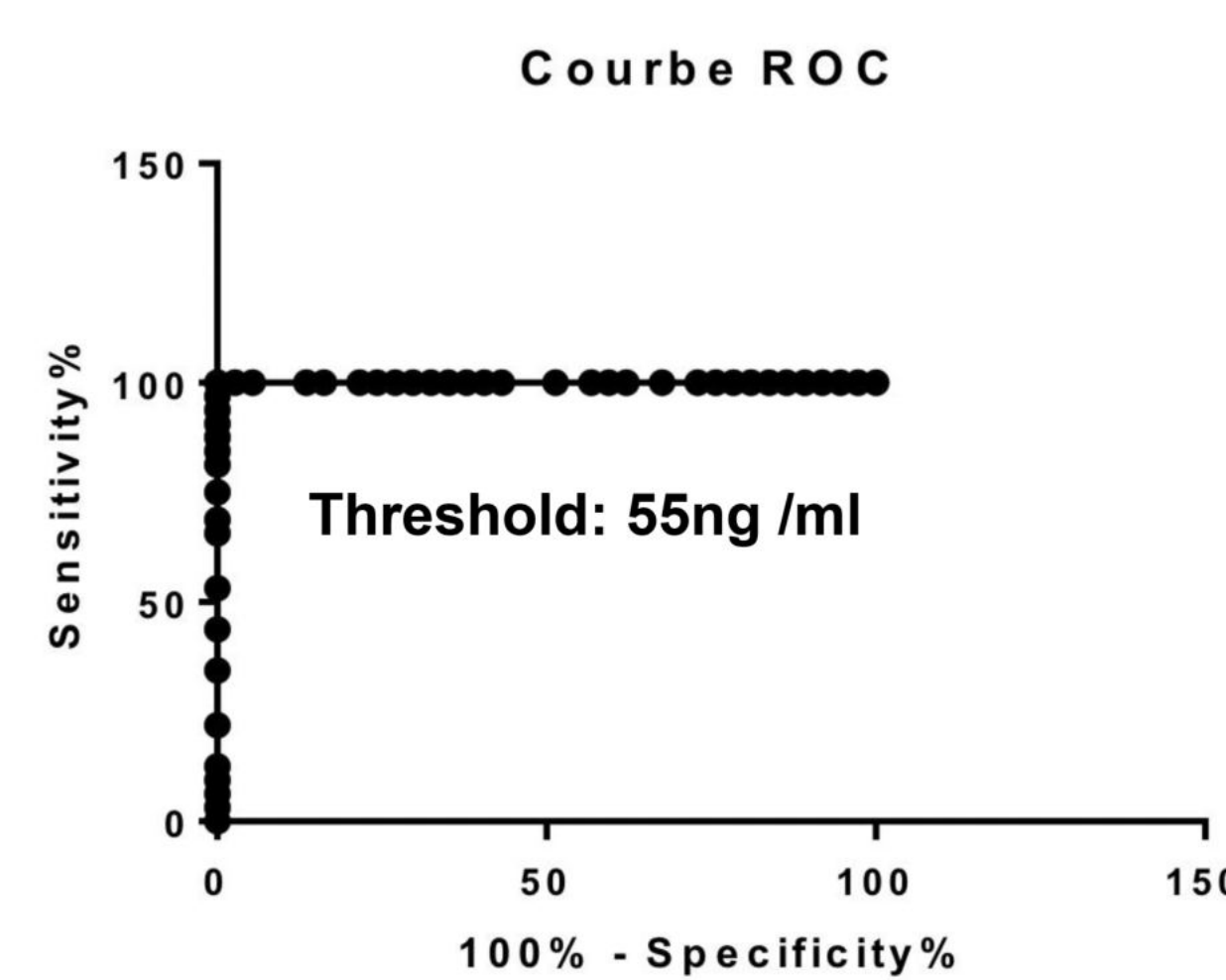
**Note:** Using 4 samples of patients with Neuronal ceroid lipofuscinosis-11 (*CLN11*), caused by homozygous mutations on *GRN* gene (= KO samples), the levels of *GRN* was below the detection limit with both kits (data not shown).

### Concentrations of Progranulin plasma levels in FTLT and healthy samples



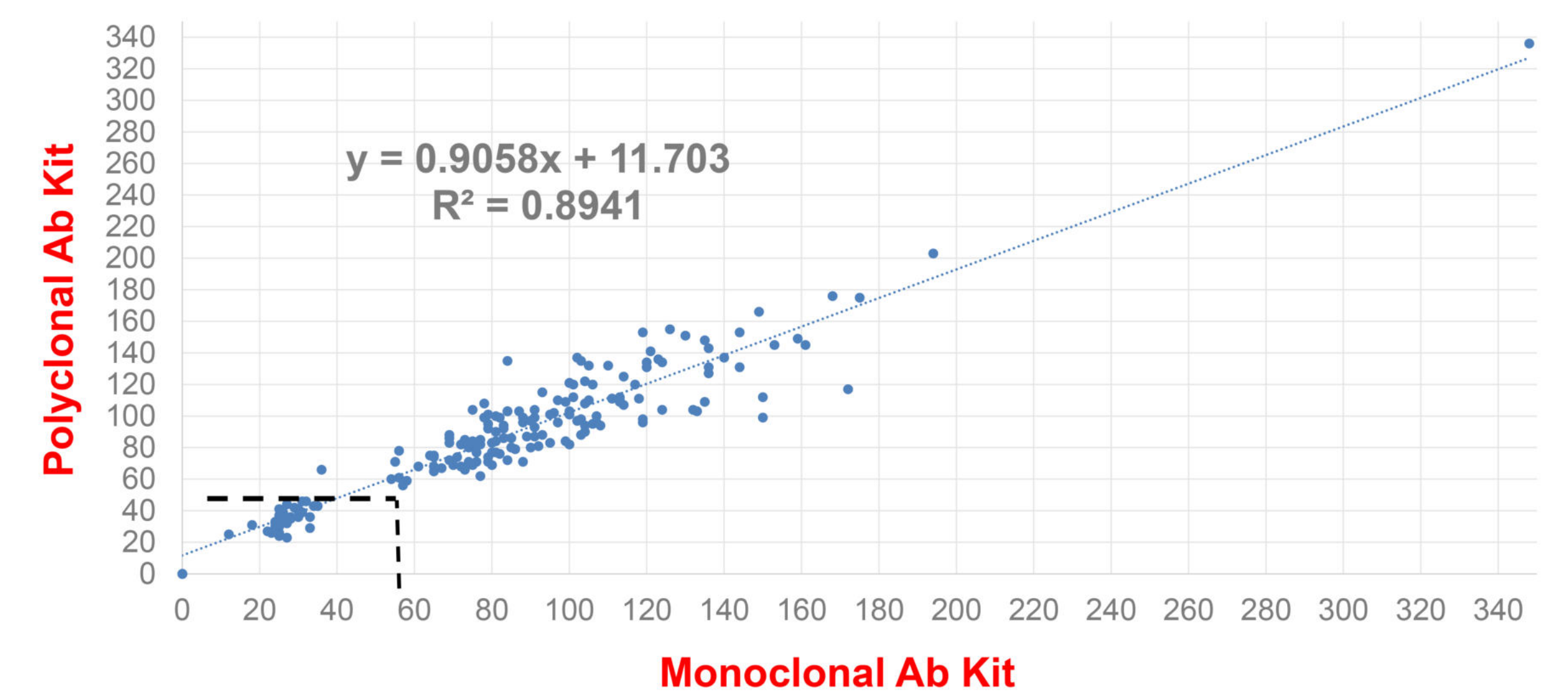
Using the new mAb-based ELISA kit (AG-45B-0027), plasma progranulin levels in FTLT-GRN are lower (~28 ng/ml) compared to controls (~98 ng/ml).

### ROC curve of the new ELISA Kit



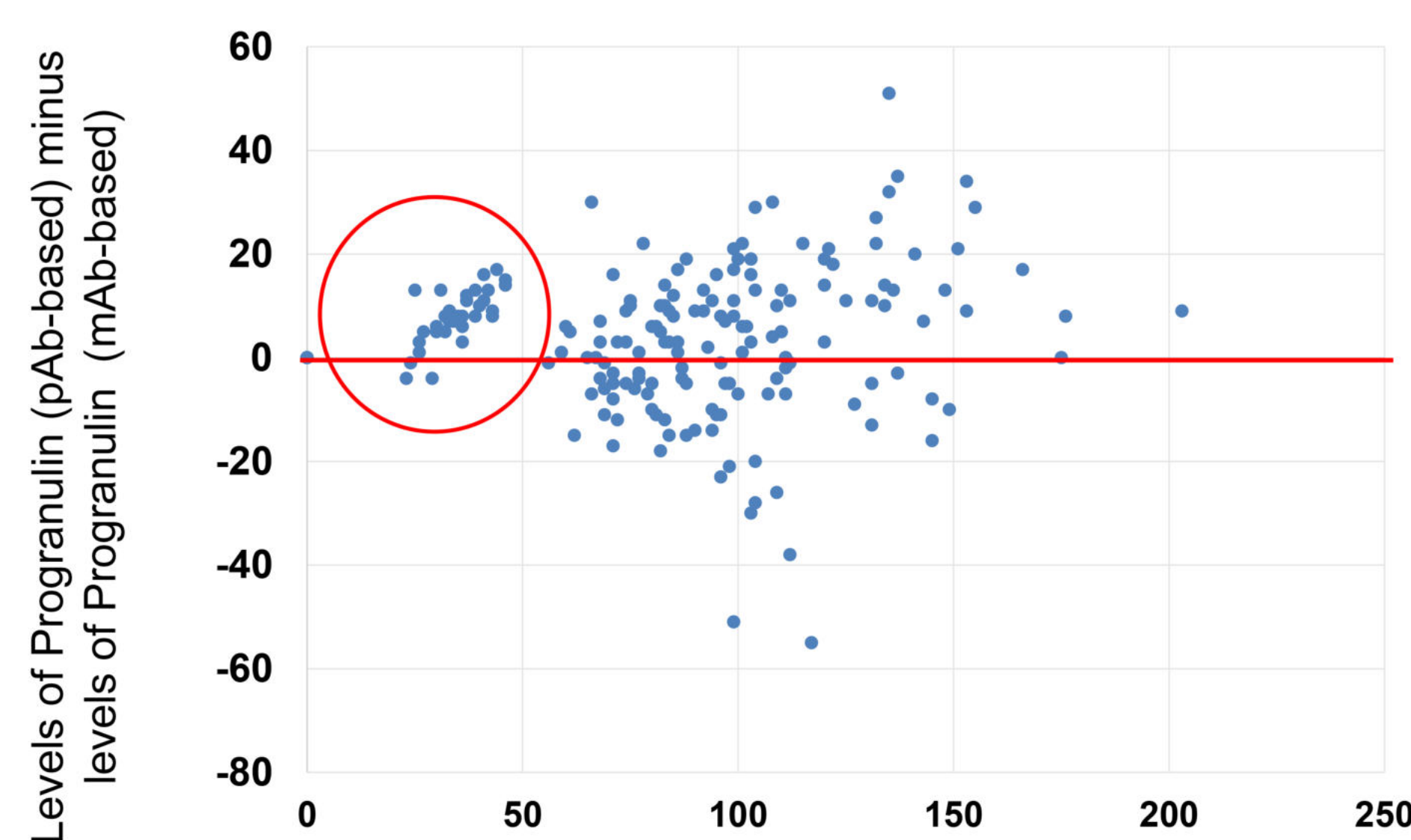
ROC analysis shows that at a threshold of 55 ng/ml plasma progranulin measured with ELISA Kit (AG-45B-0027) discriminates FTLT-GRN patients from controls with 100% sensitivity and 100% specificity.

### Values measured by the AG-45A-0018Y (pAb-based) & AG-45B-0027 (mAb-based) ELISA Kits



A strong positive correlation was observed between the pAb-based ELISA kit (Reference kit, AG-45A-0018Y) and the newly developed mAb-based ELISA kit (AG-45B-0027) in the measurement of Progranulin plasma levels.

### Diagram of differences between both ELISA Kits



- The Kit AG-45B-0027 (mAb-based) gives lower values than the Kit AG-45A-0018Y (pAb-based), especially for the concentrations <50 ng/ml.
- Difference is less obvious for higher concentrations.

### Evaluation of both ELISA Kits (pAb and mAb-based)

Kit Name	Threshold	Area Under the ROC Curve (AUC)	Sensitivity	Specificity
Kit AG-45A-0018Y Polyclonal based	67.5	0.982	97.5	94.59
Kit AG-45B-0027 Monoclonal based	55	1	100	100

Comparison of ROC curves of both kits (AG-45A-0018Y and AG-45B-0027) shows a better performance of Progranulin (human) ELISA Kit (mAb-based).

- Specificity: Monoclonal based Kit > Polyclonal based Kit
- Sensitivity: Monoclonal based Kit > Polyclonal based Kit
- Threshold values measured are lower with the mAb-based Kit compared to pAb-based Kit

## Conclusions

1. After comparison of both Progranulin (human) ELISA kits developed at Adipogen Life Sciences to measure Progranulin in biological fluids (serum or plasma), one based on polyclonal antibodies and routinely used in many laboratories worldwide (AG-45A-0018Y) and a new Kit based on monoclonal antibodies (AG-45B-0027), we can conclude that the new Kit based on monoclonal antibodies behaves better with specificity and sensitivity at 100% when the threshold level for *GRN* mutations is fixed at 55 ng/ml.
2. The new Progranulin (human) ELISA Kit (mAb/mAb) (AG-45B-0027) is a suggested alternative to the pAb-based Kit (AG-45A-0018Y) to improve the precision of the progranulin analysis in the screening of *GRN* mutations.