

Work Flow

❖ With Discovery Cohort

- Finding differentially expressed proteins
- Identifying potential biomarkers using **normalized protein expression** data
- Correlation analysis between normalized protein expressions and disease severity in PD



❖ With Validation Cohort

- Validating differentially expressed proteins
- Validating potential biomarkers calculated by **absolute protein concentrations**
- Establishing diagnostic cut-offs using absolute protein concentrations
- Correlation analysis between absolute protein concentrations and disease severity in PD

Datasets

❖ Discovery Cohort

- Normalized protein expressions (log2 scale)**

Olink Target 96 Inflammation

Healthy Control	Parkinson's Disease
15 (55.6%)	12 (44.4%)

❖ Validation Cohort

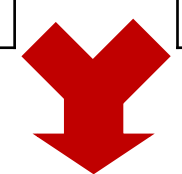
- Absolute protein concentrations**

Luminex

Healthy Control	Parkinson's Disease
40 (50.0%)	40 (50.0%)

OlinkFlex

Healthy Control	Parkinson's Disease
33 (41.8%)	46 (58.2%)



Samples analyzed by both platforms

Healthy Control	Parkinson's Disease
15 (55.6%)	12 (44.4%)

Statistical Analysis Methods

❖ For Group Comparison & Biomarker Discovery

- Welch's t-test & Mann-Whitney U test**
: To identify differentially expressed proteins (DEPs) between PD and HC groups.
- Analysis of Covariance (ANCOVA)**
: To assess mean differences while adjusting for age.

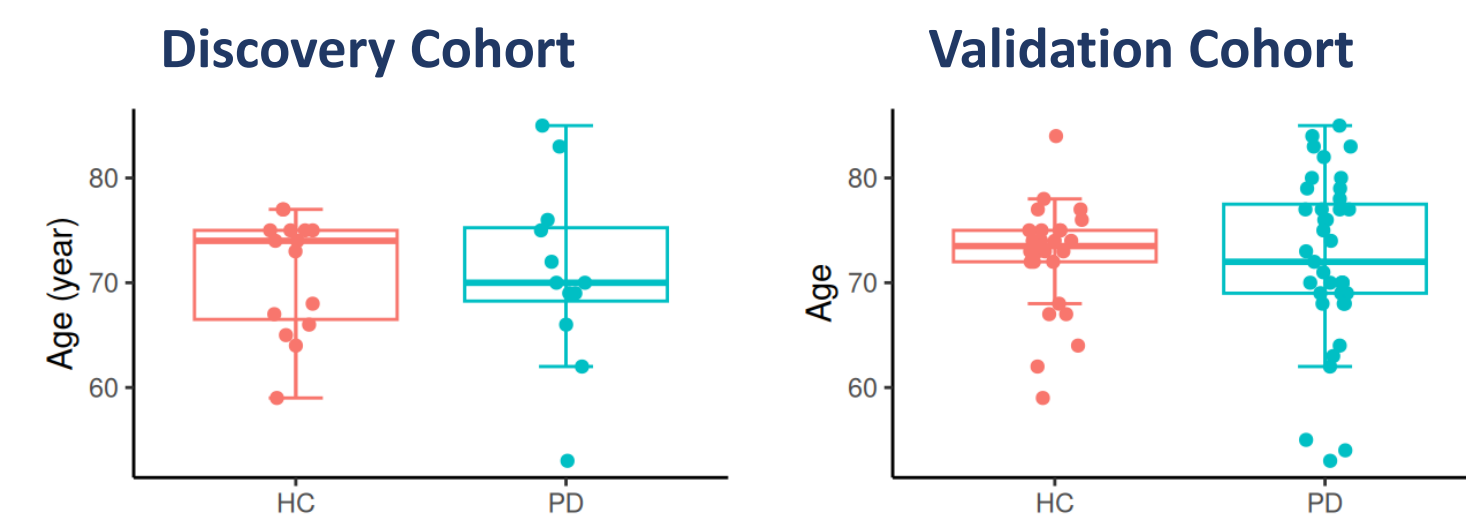
❖ For Diagnostic Model Development & Evaluation

- Logistic Regression**
: To build a classification model for PD diagnosis.
- ROC-AUC Analysis**
: To evaluate the diagnostic performance of biomarkers and models.
- Leave-one-out Cross-validation (LOOCV)**
: To verify the stability and generalizability of the classification model.

❖ For Correlation Analysis

- Spearman's / Pearson's Correlation**
: To assess the association between biomarker levels and PD disease severity.

Distributions of Age



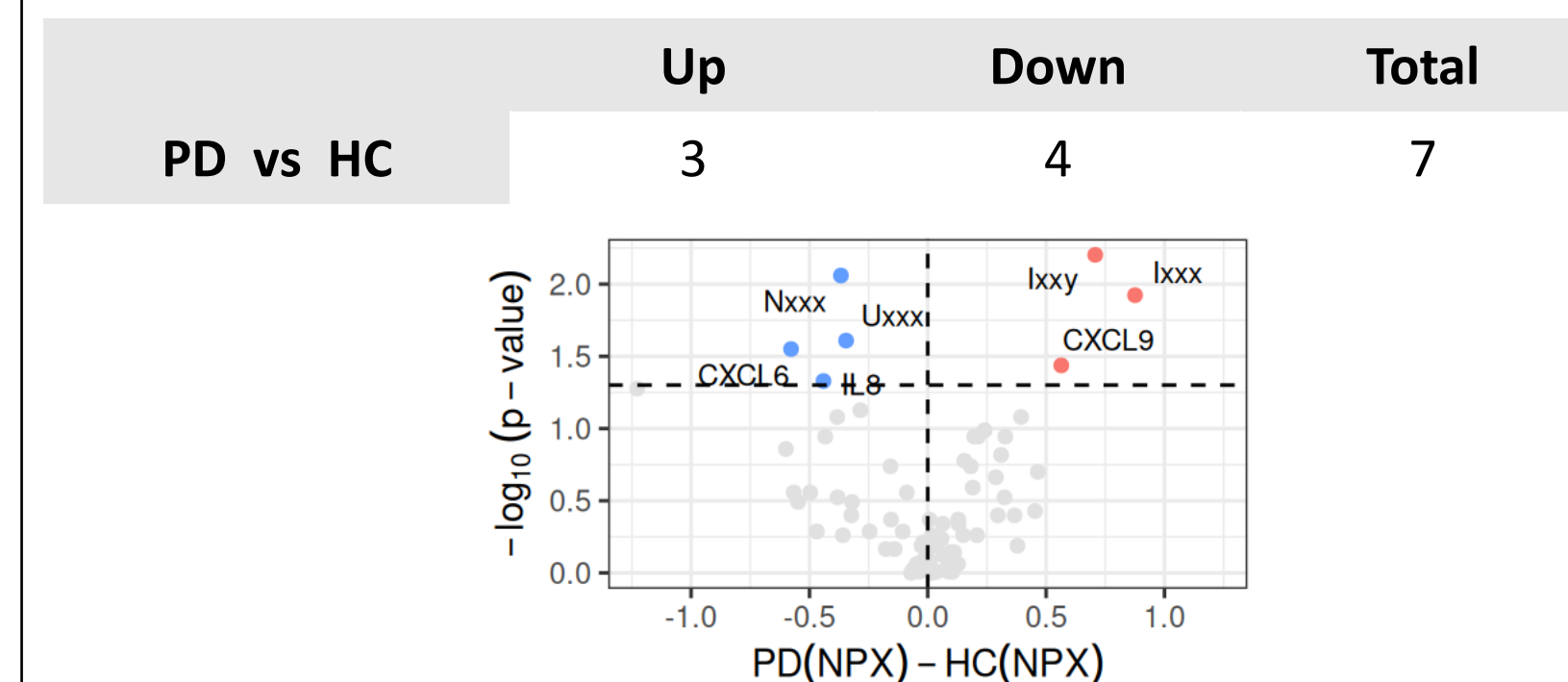
Known Functions

- Ixxx**
: modulating inflammation
maintaining cell homeostasis
- Ixxy**
: Autoimmune, chronic inflammatory diseases
- Nxxx**
: Neuroprotective growth factor
- Uxxx**
: Broad spectrum (fibrinolysis, proteolysis, inflammation, atherogenesis, plaque stabilization)

Result I (Discovery Cohort)

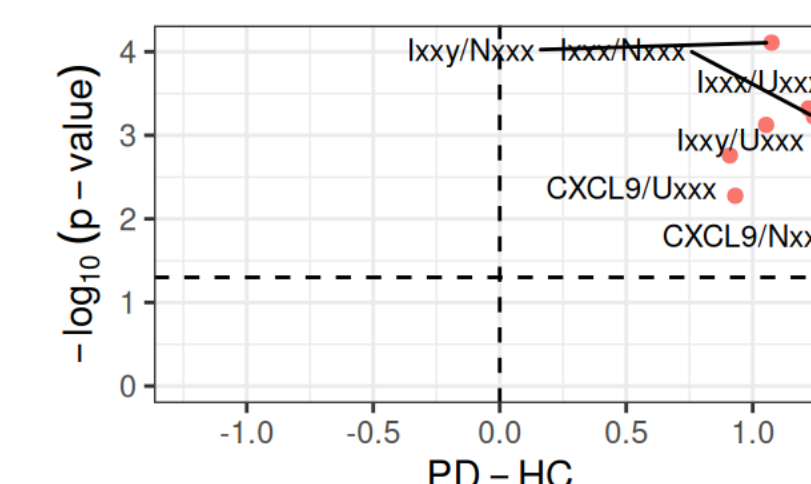
❖ DEPs (Differentially Expressed Proteins)

- Mann-Whitney U test
- P value < 0.05



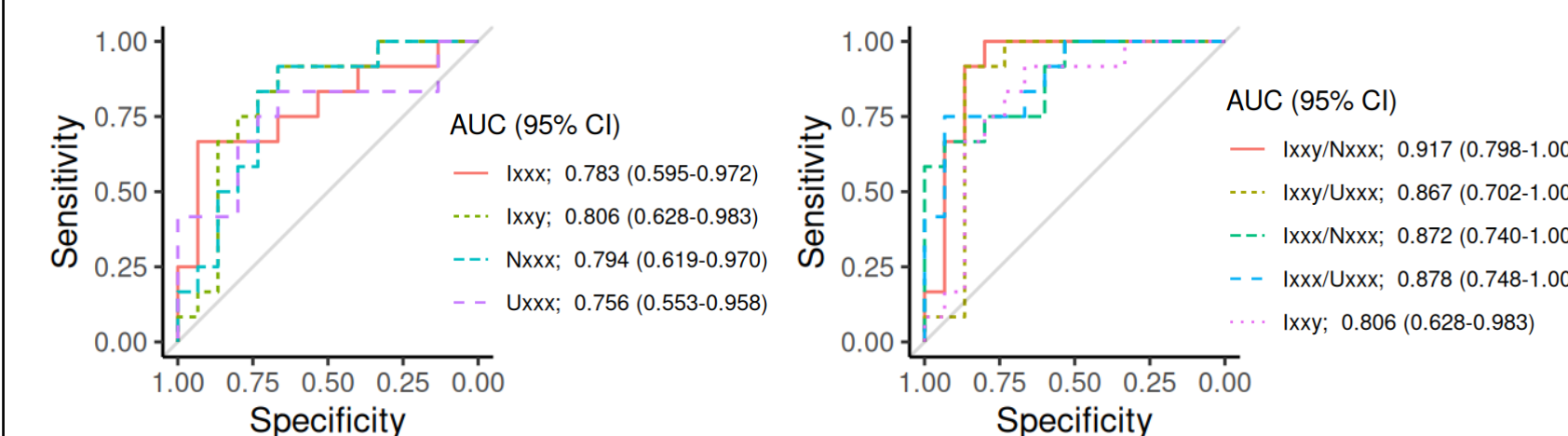
❖ New Biomarkers (Ratios of Protein Expressions)

- Mann-Whitney U test

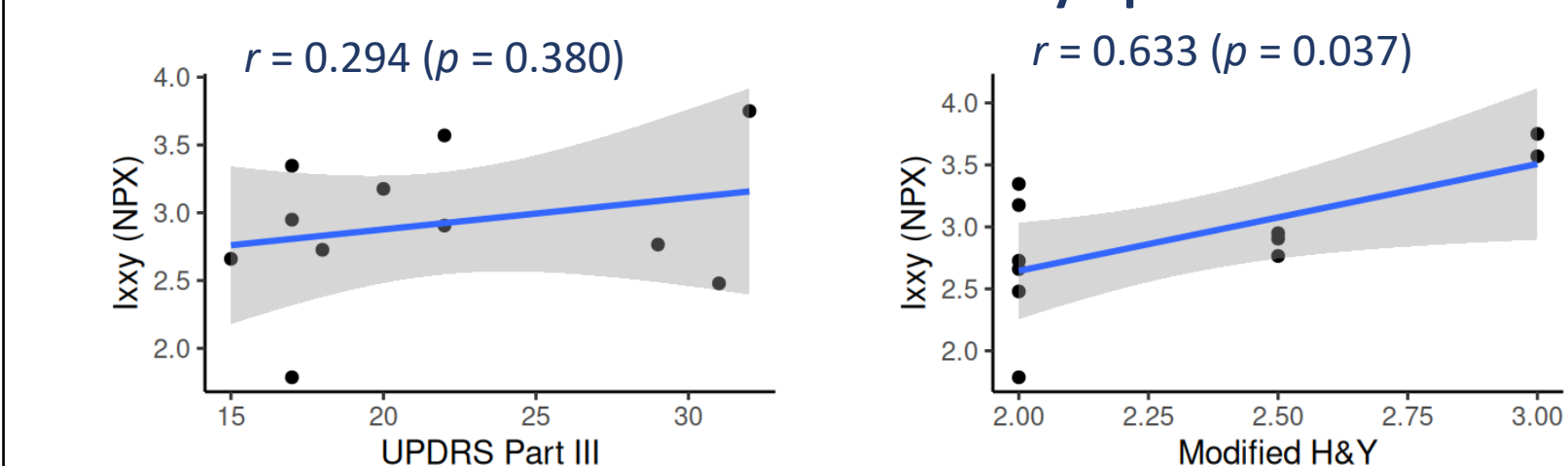


❖ ROC-AUC

- Logistic regression



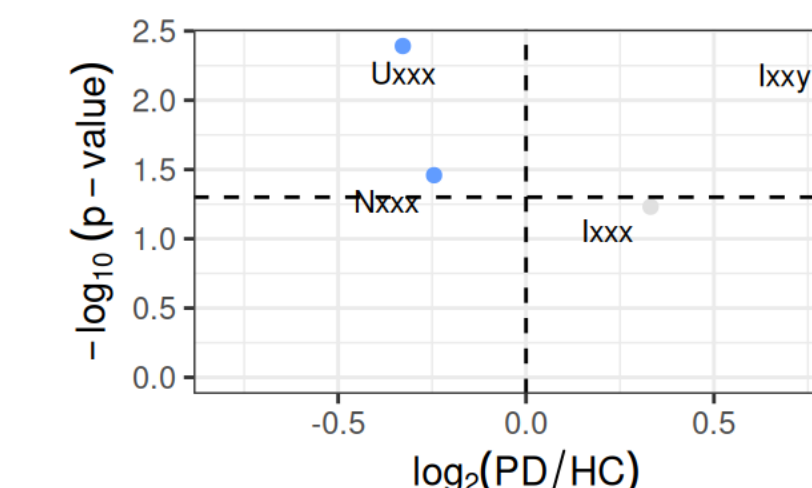
❖ Correlation between Biomarkers and Symptoms



Result II (Validation Cohort)

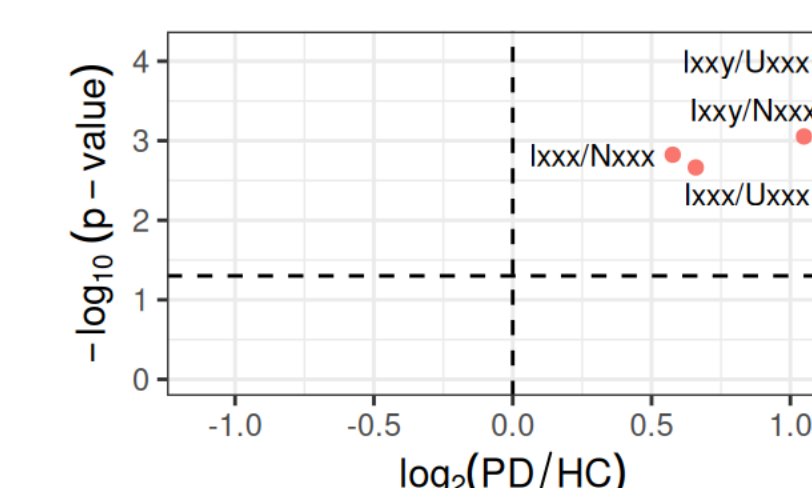
❖ Validation of DEPs

- Mann-Whitney U test
- P value < 0.05



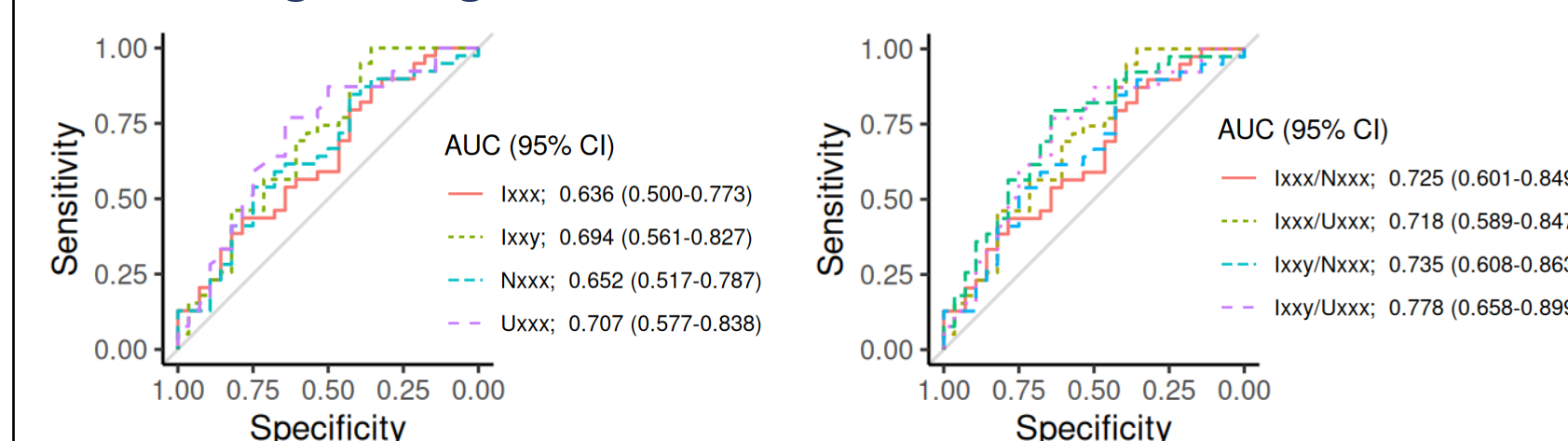
❖ New Biomarkers (Ratios of Protein Concentrations)

- Mann-Whitney U test

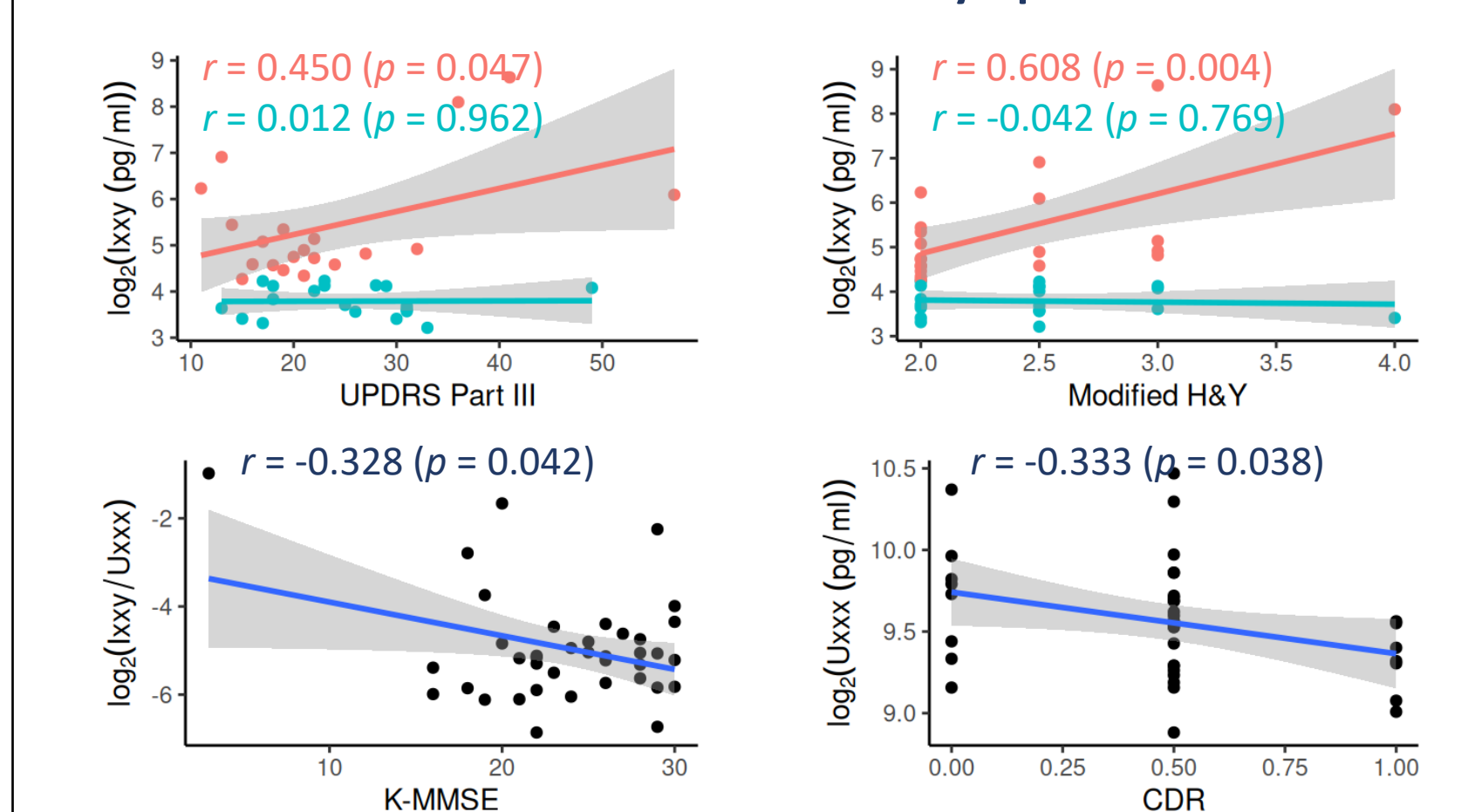


❖ ROC-AUC

- Logistic regression



❖ Correlation between Biomarkers and Symptoms



Concluding Remarks

- Absolute protein concentrations, despite their inherent variability, are sufficient for drawing **results consistent** with those from NPX (normalized protein expression)
- While the relative scale of NPX data poses challenges for direct diagnostic application, **absolute protein concentrations** provide a quantitative foundation for developing **clinical assays**.
- Furthermore, we observed that utilizing **ratios** of specific absolute protein concentrations **enhances** diagnostic accuracy compared to relying on single protein levels.