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Warning Signs From The Crypt: Circulating Aberrant Glycosylation Signatures Detect Advanced Adenoma And Colorectal Cancer

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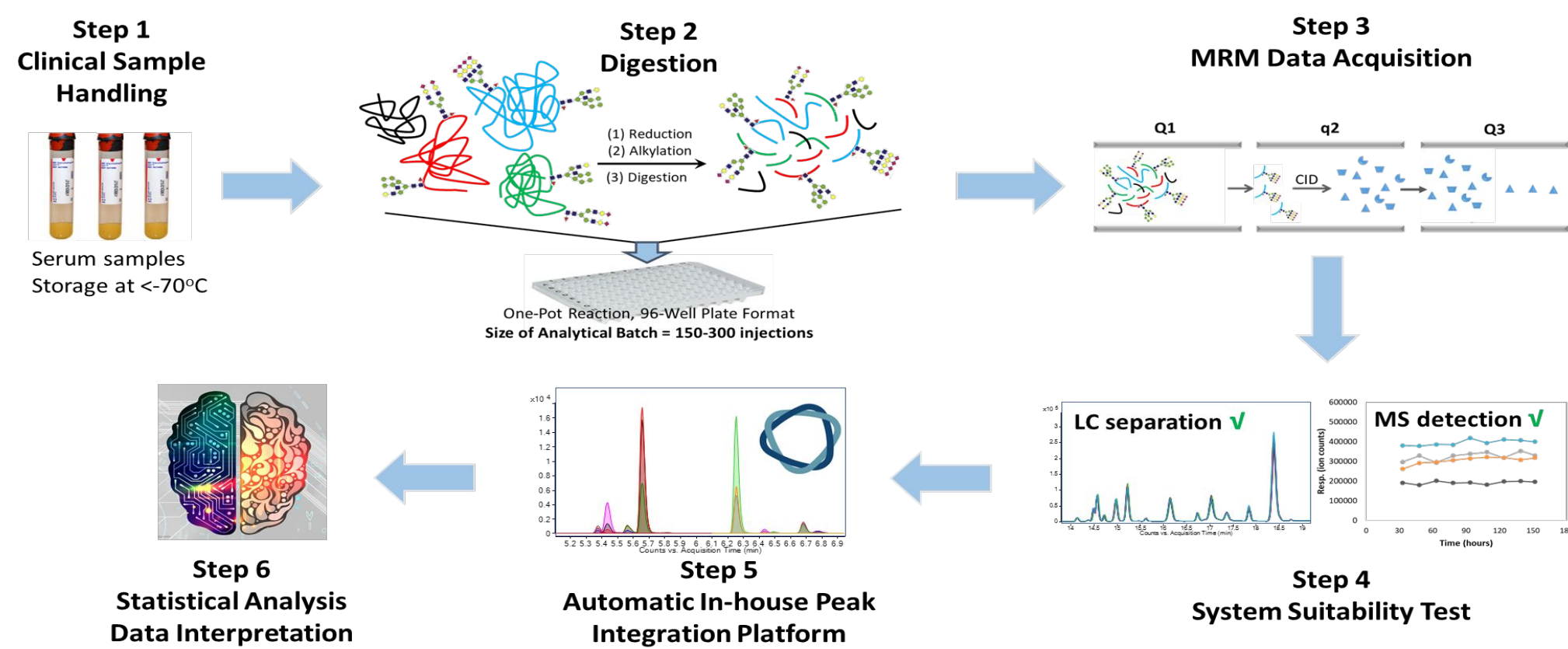[1]InterVenn Biosciences, 2 Tower Place, South San Francisco, CA

INTRODUCTION

Colorectal cancer (CRC) remains prevalent despite current screening modalities. Precancerous lesions, or advanced adenomas (AA), commonly precede invasive cancer development by a number of years.

Glycosylation is one of the most ubiquitous and functionally important forms of post-translational modification. InterVenn has built a novel platform that combines liquid chromatography-mass spectrometry (LC-MS) with a proprietary, artificial-intelligence-based data processing engine, allowing for highly scalable and reproducible quantification of glycoproteins with site- and glycan-specificity.

To detect glycoproteomic profiles associated with the occurrence of AA/CRC, we studied serum glycoproteins in 575 patient samples: 296 healthy controls, 21 high-grade dysplasia AAs, 11 low-grade AAs, and 247 CRC.
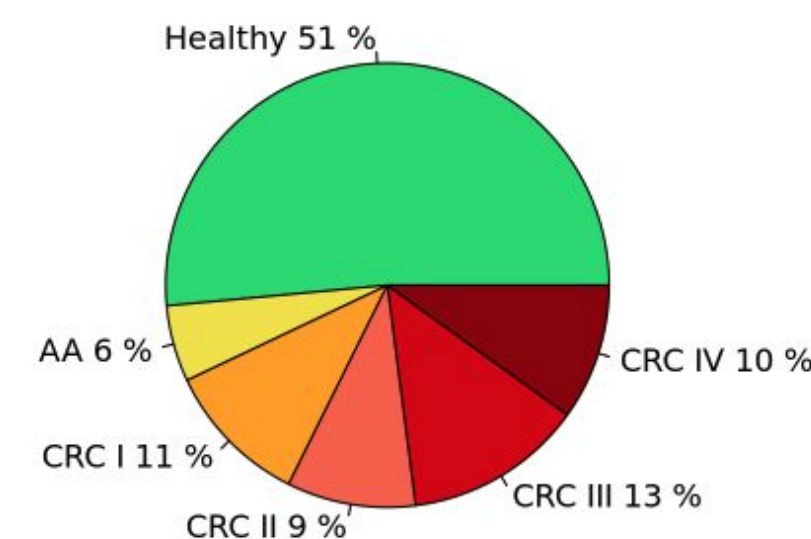
ANALYTICAL WORKFLOW

Assessed 519 glycopeptide (GP) biomarkers derived from 70 serum proteins

Step 1
Clinical Sample Handling

Serum samples Storage at <-70°C

Step 2
Digestion
(1) Reduction
(2) Alkylation
(3) Digestion

One-Pot Reaction, 96-Well Plate Format
Size of Analytical Batch = 150-300 injections

Step 3
MRM Data Acquisition

Step 4
System Suitability Test

LC separation ✓   MS detection ✓

Step 5
Automatic In-house Peak Integration Platform
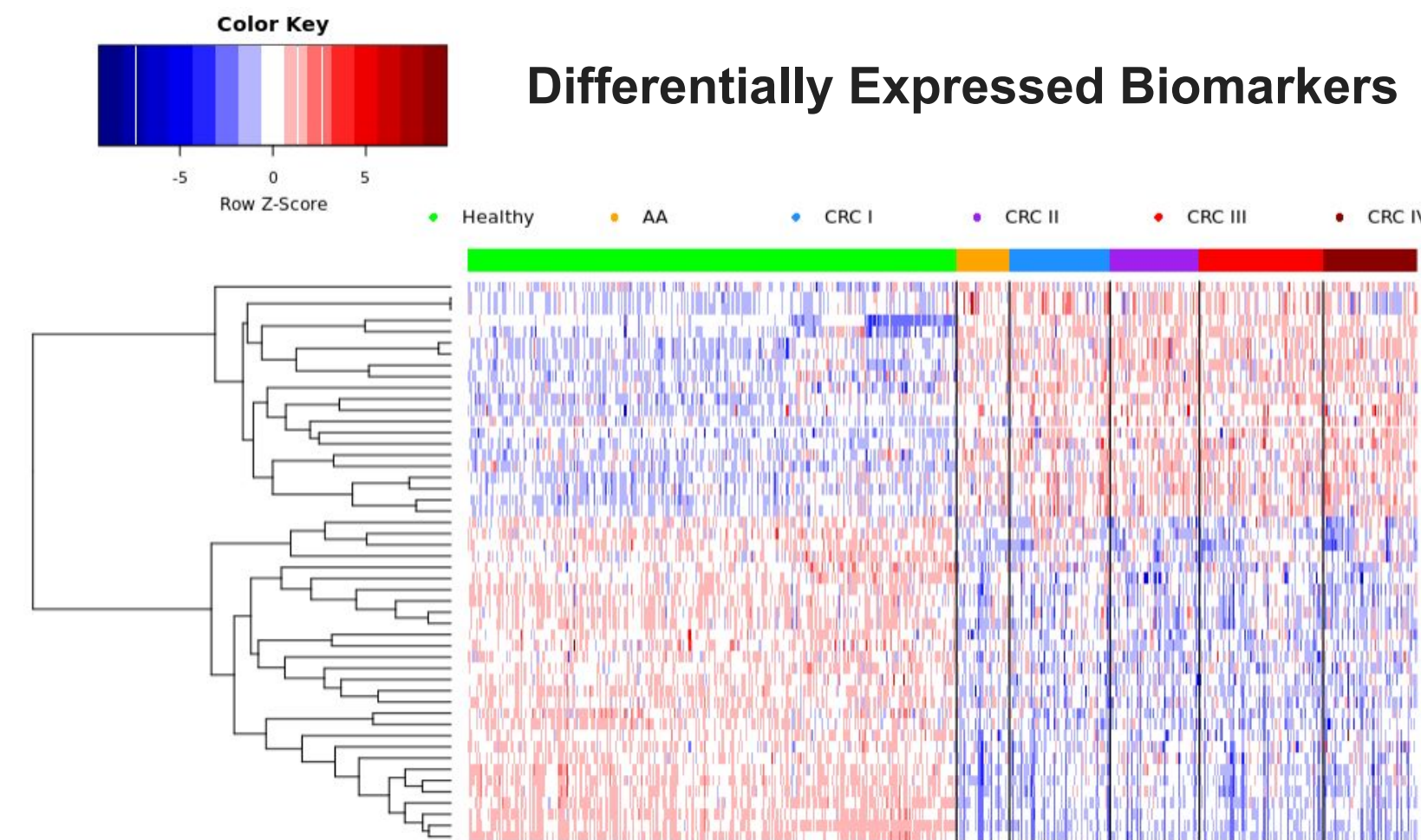
Step 6
Statistical Analysis Data Interpretation

DEMOGRAPHICS

- 475 retrospective samples sourced from biorepositories & 100 prospective samples
- Mean age: 61.2 years (range: 19-94)
- 50% female

Healthy 51 %
AA 6 %
CRC I 11 %
CRC II 9 %
CRC III 13 %
CRC IV 10 %

RESULTS

Differentially Expressed Biomarkers

Color Key
Row Z-Score
-5   0   5

Healthy   AA   CRC I   CRC II   CRC III   CRC IV

Clustered heatmap of patients (color-coded along the x-axis by their disease indication) for top 50 normalized abundance features that have an FDR<0.05. As indicated above, several potential biomarkers are differentially expressed between CRC/AA patients and healthy controls

PCA by Patient's Indication


Healthy   AA   CRC

PC2 (6%)
PC1 (12.7%)
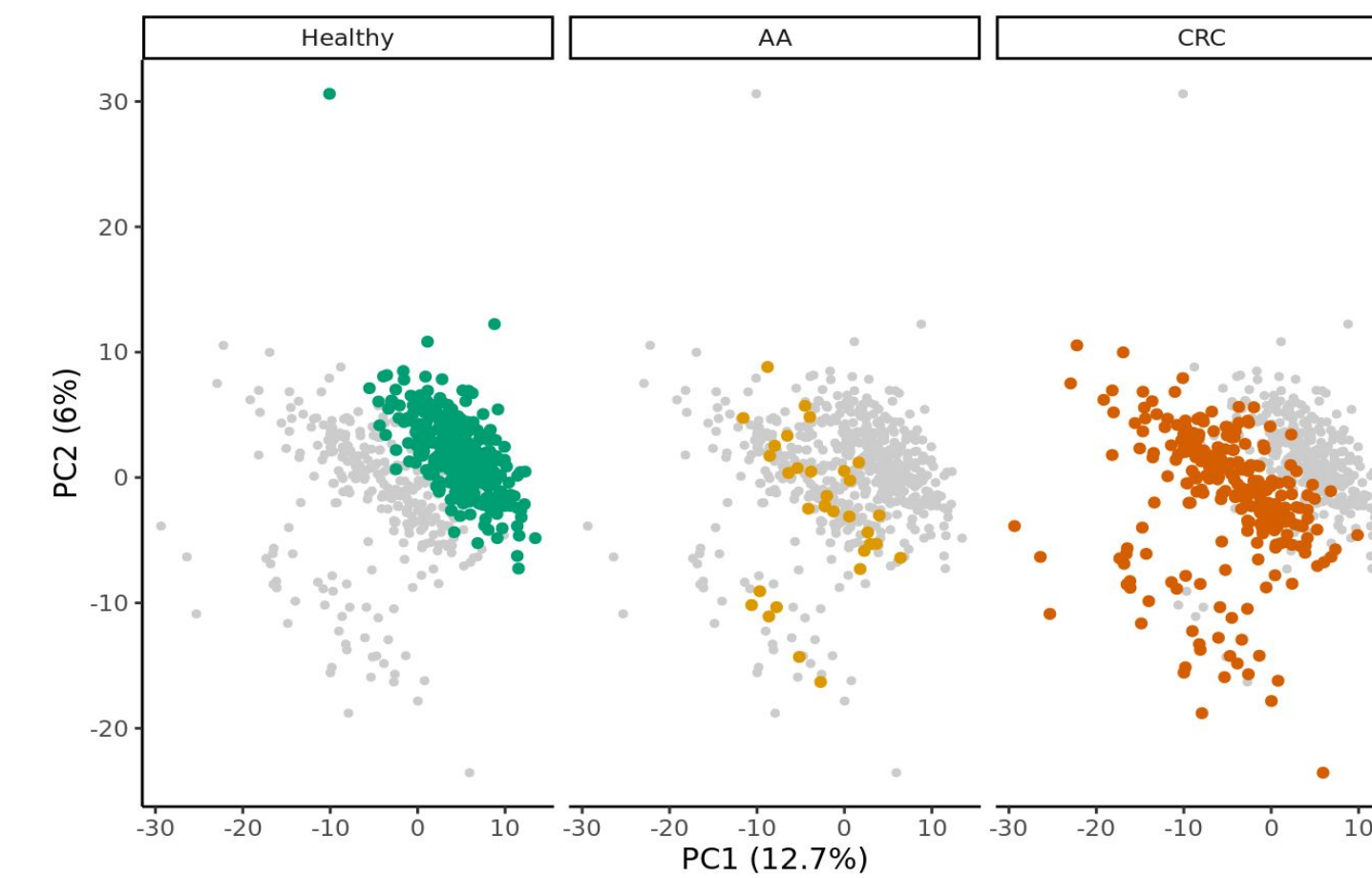
Principal component analysis (PCA) plot to visualize few important features that exhibit the intrinsic variation among different subgroups
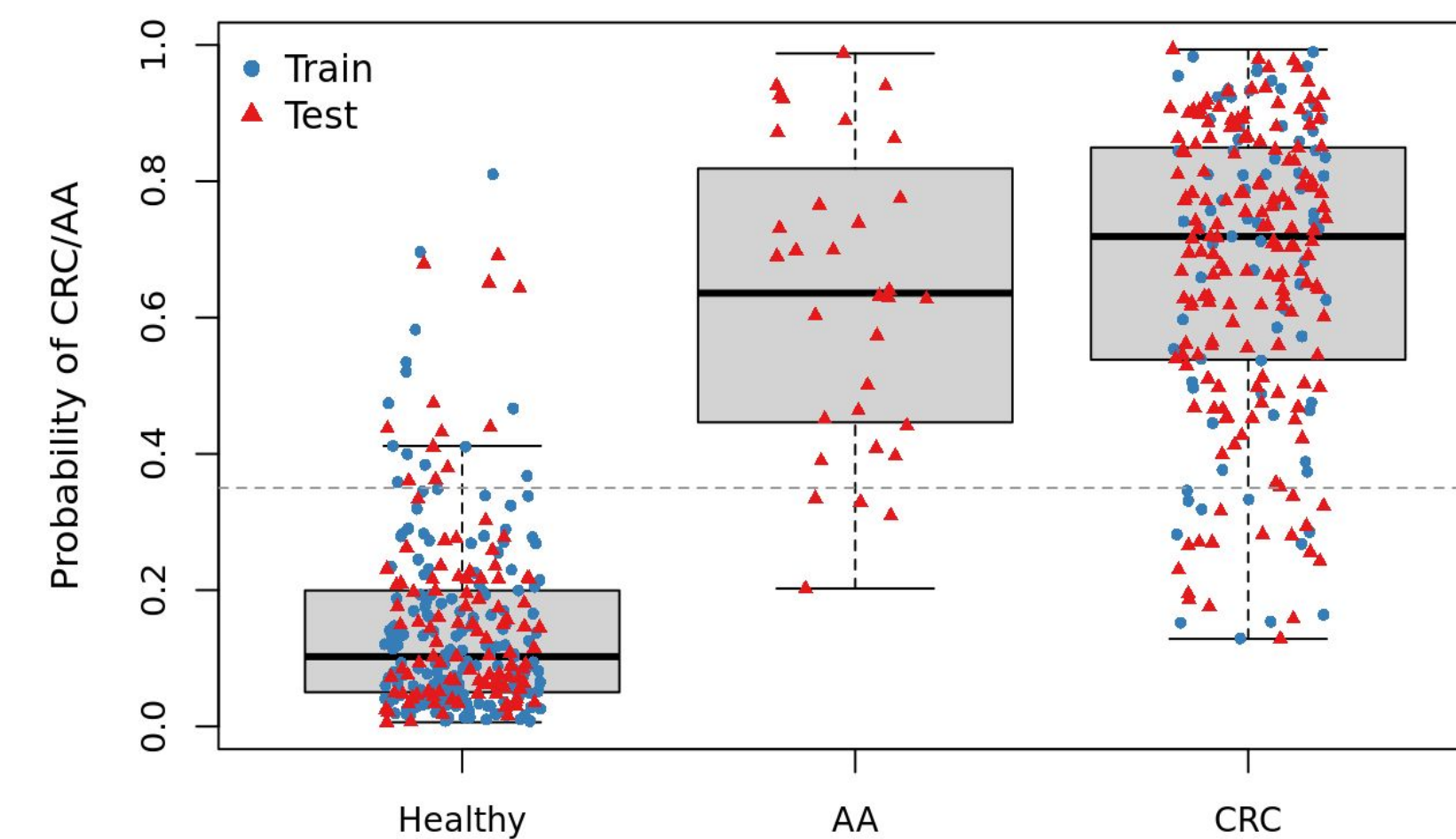
MODELING

- Samples were split into a training (47%) and a hold-out test set (53%)
- 399 differentially abundant glycopeptides/peptides between CRC/AA and healthy controls

- Repeated cross-validated Lasso regression yielded a multivariable classifier consisting of six glycopeptide/peptide biomarkers with non-zero coefficients

Sensitivity
Specificity

Cohort: AUC
Training: 0.966
Test: 0.964
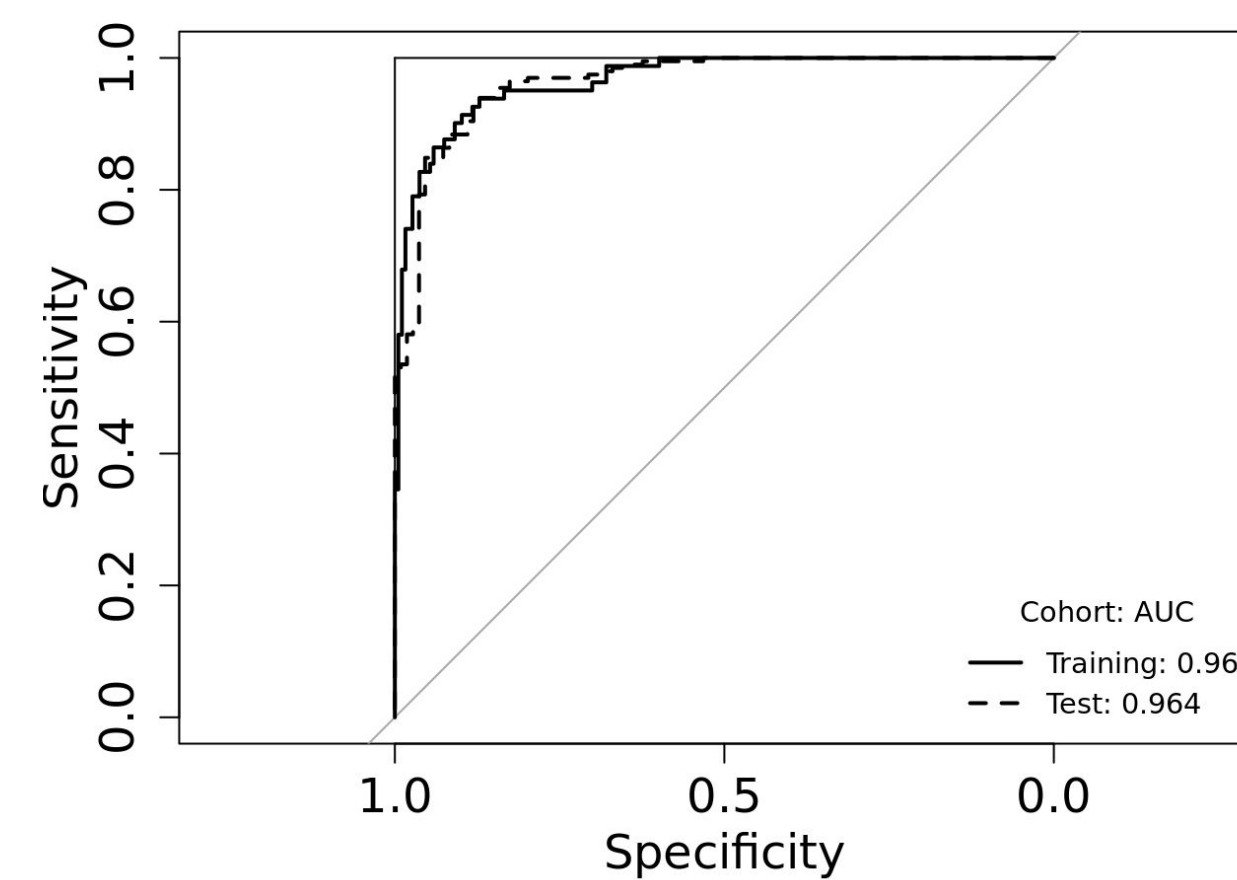
ROC for the comparison of CRC/AA vs. healthy controls

Train
Test

Probability of CRC/AA

Healthy   AA   CRC

Predicted Probabilities for CRC/AA in train and test set

DISCUSSION

Predicted probabilities for the training and test samples based on the classifier show increasing severity with disease progression for AAs and CRCs; this indicates a link to the biology of disease.

The model achieved excellent performance in the test set (accuracy=0.89, sensitivity=0.89, specificity=0.89), with AuROCs of 0.97 and 0.96 for training and test sets respectively.

Sensitivity for all stages of CRC was 89.4% (91.2% for stage 1/2, 89.4% for stage 3/4) and 87.5% for AAs.

FUTURE WORK

Our results indicate that peripheral blood glycoproteomic profiles of abundant proteins show statistically significant differences among individuals with CRC/AA, and healthy controls.

Identification of GPs/peptides patterns in blood serum may be a promising approach to develop an effective non-invasive screening tool for the early detection of AA and CRC.