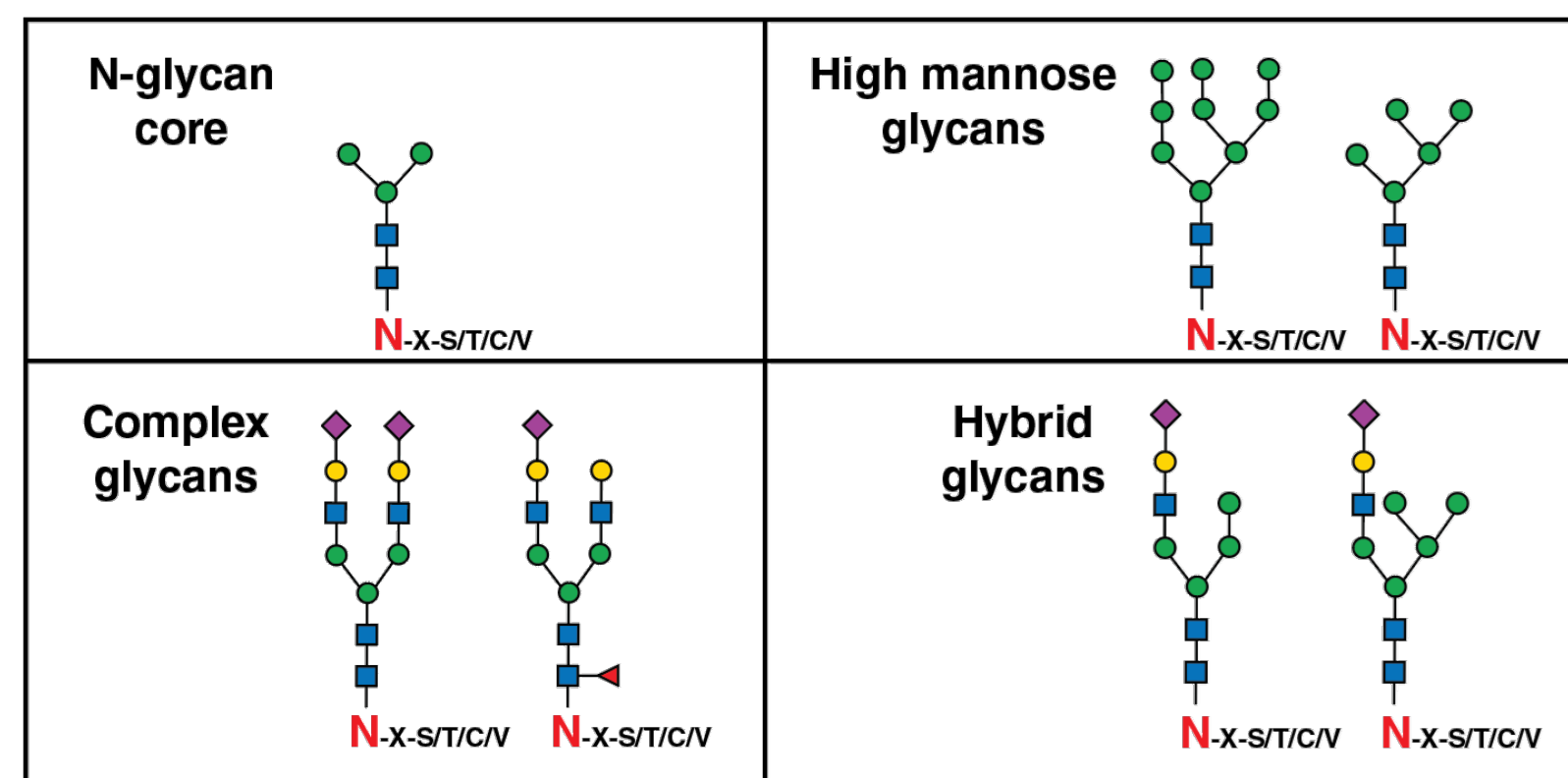


BACKGROUND

Limitations of current CCA diagnostics

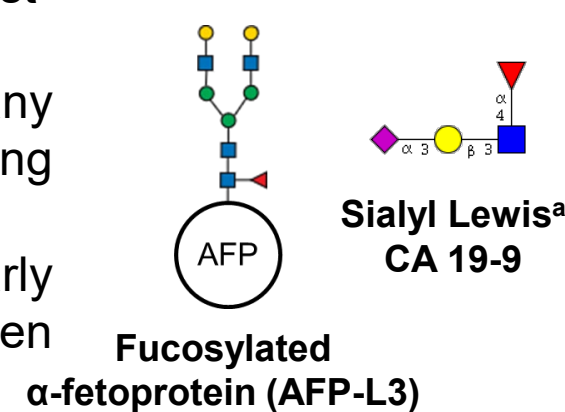
- CCA is an aggressive malignancy of the bile duct epithelium
- Despite advancements in diagnostic techniques, the diagnosis of CCA remains challenging
- Currently, CA19-9 is the best available blood-based marker for detection of CCA, but has limited sensitivity (~50-60%)
- Moreover, individuals who are Lewis-antigen-negative have undetectable CA 19-9 levels resulting in false negative diagnoses
- The levels of CA19-9 are also elevated in multiple cancers, limiting specificity
- Therefore, there is a critical need for developing novel blood-based diagnostic approach for improving the sensitivity of diagnosis of CCA to improve clinical outcomes
- We investigated the development of mass spectrometry-based methods to measure changes in glycosylation in patients with CCA

Glycosylation

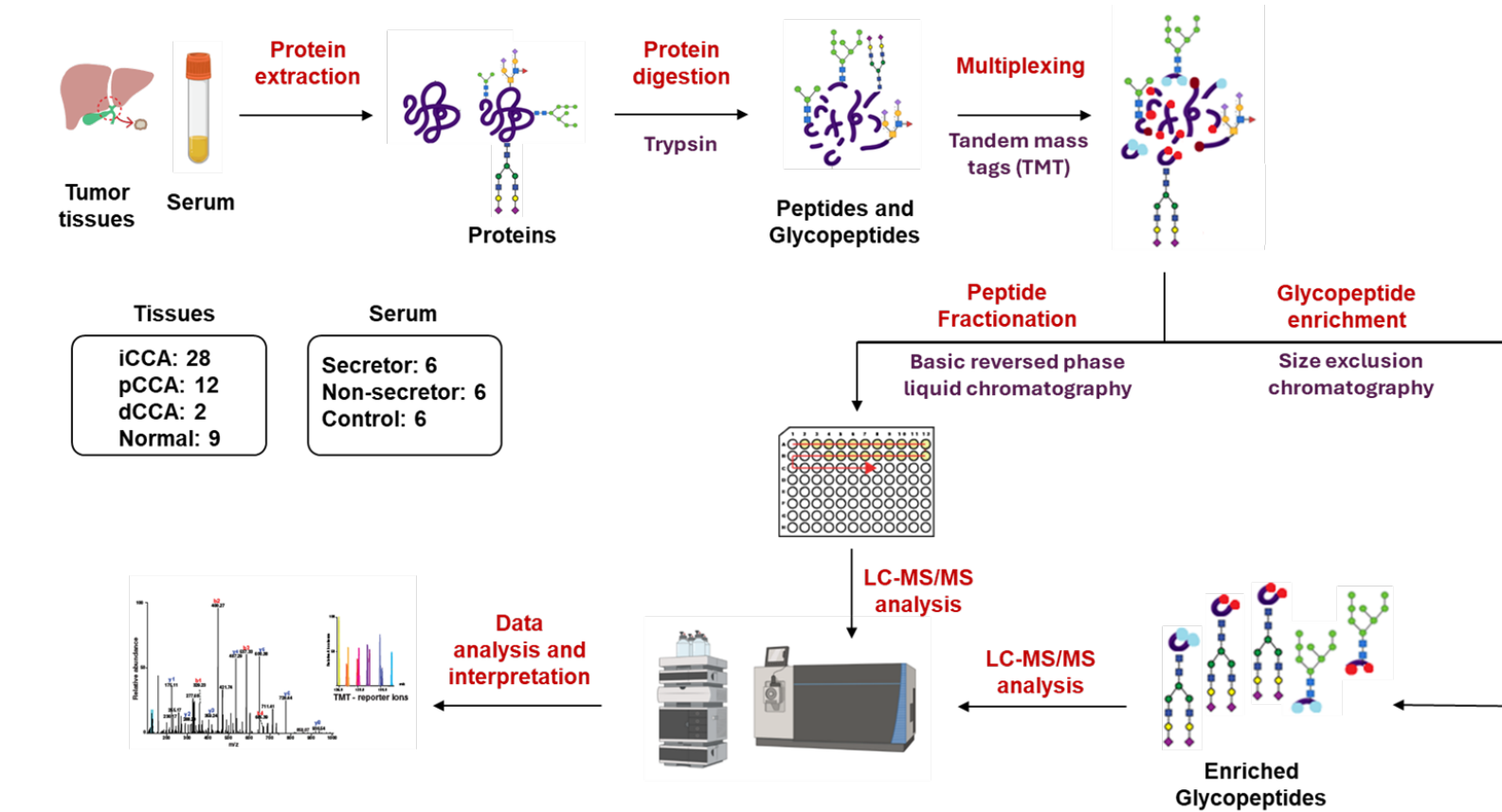


● Mannose (Man) ● Galactose (Gal) ■ N-acetylglucosamine (GlcNAc) ◆ Sialic acid (NeuAc) ▲ Fucose (Fuc)

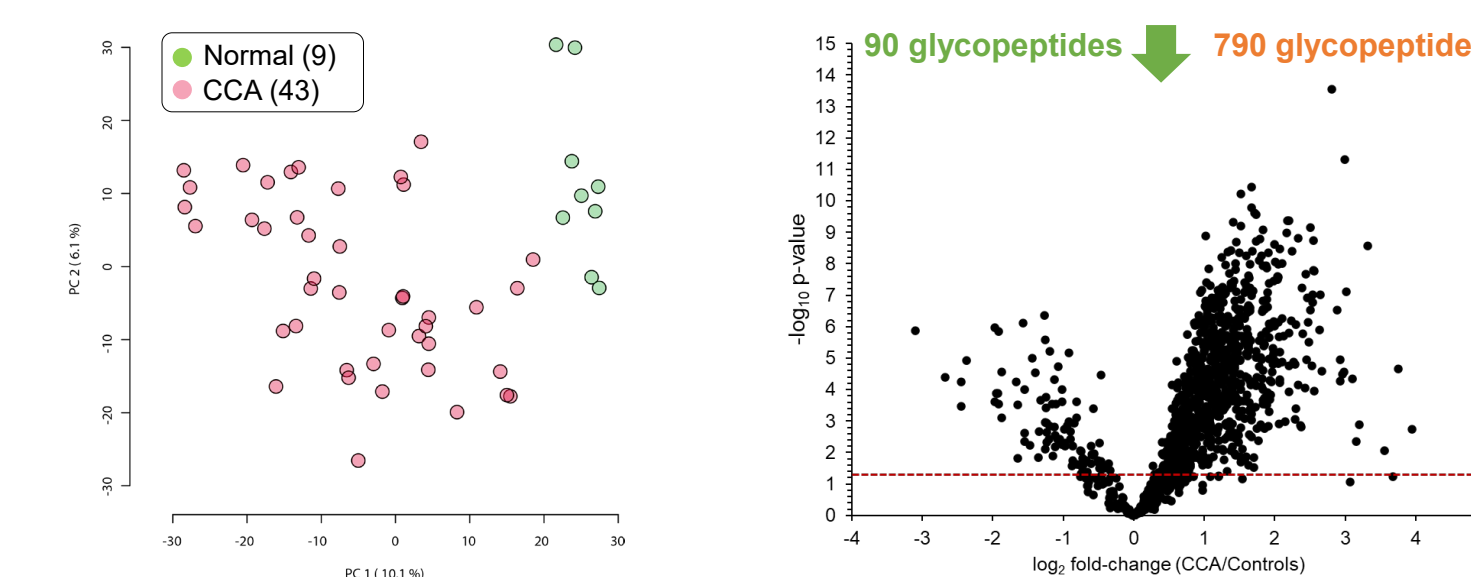
- Glycosylation is the most abundant post-translational modification
- Aberrant glycosylation is a hallmark of many cancers with most biomarkers being glycoproteins
- For example, AFP alone lacks sensitivity for early HCC detection, whereas AFP-L3 has been proposed as a biomarker



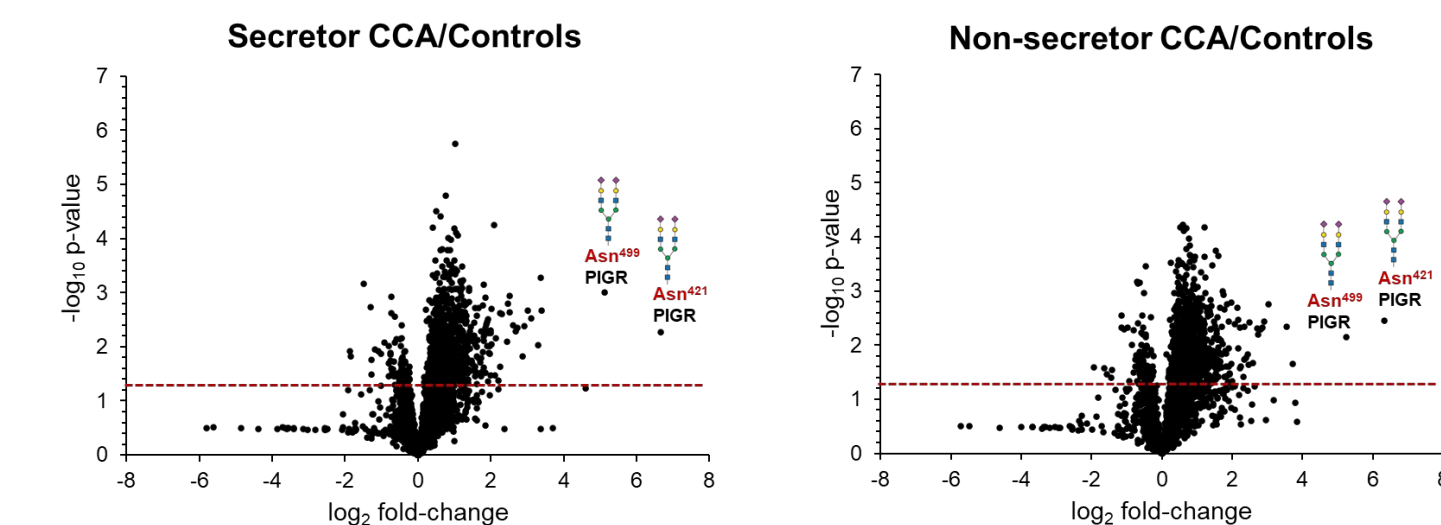
Glycoproteomics of tissue and serum samples



Identification of altered glycopeptides



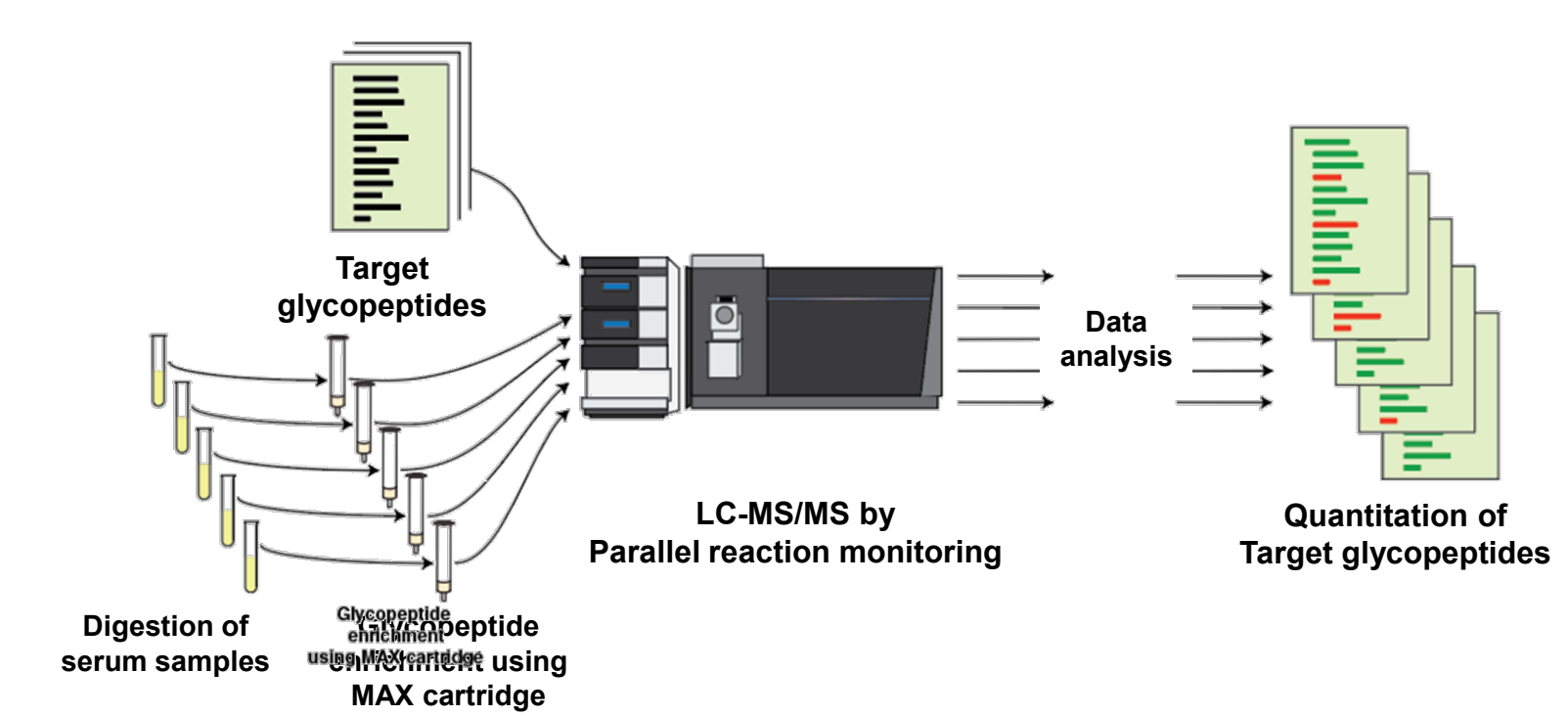
- Tumor tissues - Identification of 5,056 glycopeptides



- Serum- Identification of 3,421 glycopeptides

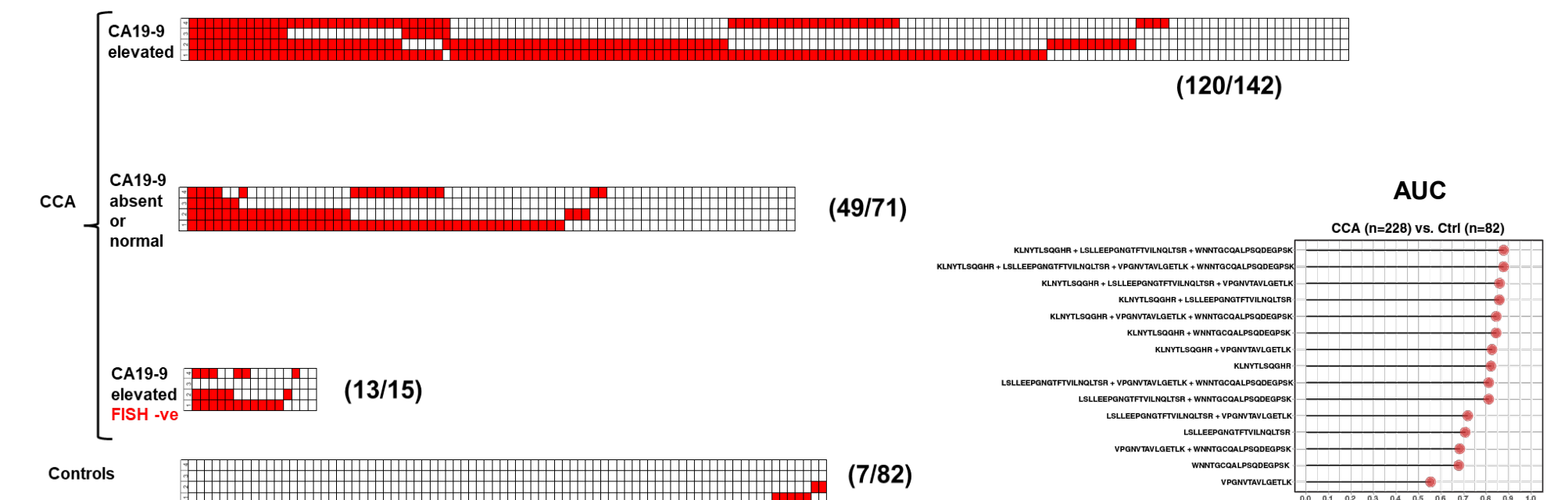
RESULTS

Targeted study for quantifying altered glycopeptides



- We developed a targeted method to monitor multiple glycopeptides in a single run

Validation of altered glycopeptides



- Targeted measurements of four glycopeptides were performed in 228 CCA and 82 control serum samples
- Altered glycopeptides were detected in 49 patients missed by CA19-9-based diagnosis
- Combination of glycopeptides resulted in sensitivity (0.80) and specificity (0.92)

CONCLUSIONS

- We discovered candidate glycopeptides with potential utility for the blood-based diagnosis of CCA
- We applied targeted mass spectrometry to monitor these altered glycopeptides in a large independent patient cohort
- Further studies are needed to evaluate integration with existing diagnostics to improve overall diagnostic performance.