Glycomic markers of inflammatory bowel disease and IgA nephropathy

Clerc, F.

Chapter 7

Thesis Summary

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Protein glycosylation is a post-translational modification where carbohydrates (glycans) are attached to proteins, influencing their stability, function, and immune recognition as outlined in **chapter 1** of this thesis. This process plays a crucial role in cell signaling, inflammatory responses, and disease mechanisms. It offers great potential for disease biomarker discovery. By studying glycomic signatures in patient cohorts and comparing them with groups of healthy controls, the research presented in this thesis aims to enhance disease understanding, and provide leads for diagnostic tools and personalized treatment strategies. This thesis first provides an overview of the glycosylation of twenty-five highly abundant human plasma glycoproteins (**chapter 2**) and then investigates glycosylation changes in inflammatory bowel disease (IBD, **chapter 3** and **chapter 5**) and IgA nephropathy (IgAN, **chapter 4**).

The two major variants of IBD are Crohn's disease (CD) and ulcerative colitis (UC), both chronic inflammatory conditions of the intestinal tract. The exact causes of the diseases remain unknown, and despite available treatments, including medication and surgery, relapses are common, and early differential diagnosis between CD and UC remains challenging as their symptoms often overlap. The distinction between CD and UC is essential because the therapeutic approaches differ. UC is more localized, making surgical removal of the affected area more feasible, while CD can affect multiple areas of the intestine at the same time, limiting surgical treatment options. Current diagnostic techniques may take years to confirm a diagnosis, particularly in young patients, emphasizing the need for more precise and early biomarkers.

The research presented in this thesis focuses on plasma protein glycosylation (**chapter 3)** and immunoglobulin A (IgA) glycosylation (**chapter 5**) as potential biomarkers for distinguishing between these diseases and helping early patient stratification with non-invasive procedures. Lower galactosylation of immunoglobulin G (IgG) is a well-established marker of inflammation and has been strongly associated with both CD and UC. Moreover, alterations in fucosylation and sialylation patterns have been linked to disease severity and progression. Genome studies have also identified changes in genes related to glycosylation enzymes in IBD patients, further highlighting the connection between glycosylation and inflammation. The glycomic studies reported here reveal changes in sialylation, fucosylation, and branching patterns of plasma proteins in IBD patients, associating with disease severity and treatment response. By employing advanced mass spectrometry techniques and robust sample preparation methodology, the thesis

provides novel insights into how glycosylation patterns relate to disease pathophysiology, potentially leading to improved diagnostic tools.

Chapter 3 of the thesis employs high-throughput glycomic analysis using a robotic platform and MALDI-TOF-MS to analyze total plasma *N*-glycosylation in over 3,500 samples, identifying numerous associations with IBD. This large-scale study provides a robust dataset for future research and paves the way for personalized treatment strategies. The thesis then builds upon these findings by investigating glycosylation changes of IgA in IBD, due to its crucial role in mucosal immunity. **Chapter 5** reports new glycomic signatures that may serve as biomarkers for early disease detection and patient stratification.

Another disease approached in **chapter 4** of this thesis is IgA nephropathy. Also called Berger's disease, IgAN is a kidney disorder caused by abnormal glycosylation of IgA, leading to immune complex deposition in the kidneys' glomeruli and progressive renal damage. The disease progresses slowly, and patients may remain asymptomatic for years before experiencing kidney failure. Once kidney function deteriorates significantly, treatment options are limited to dialysis or transplantation. The underlying cause of IgAN remains poorly understood, but it is known that the immune system produces antibodies against abnormally glycosylated IgA, leading to immune complex formation and kidney inflammation. Current diagnostic tools, such as kidney biopsies, are invasive and not practical for routine screening, highlighting the need for non-invasive biomarkers. A major challenge for the clinicians is the lack of non-invasive biomarkers for early detection.

Glycosylation abnormalities in IgAN primarily involve under-galactosylation of IgA1, resulting in galactose-deficient IgA1 (Gd-IgA1), which promotes disease progression. Several lectin-based assays have been used to measure Gd-IgA1 levels, but they suffer from biases due to selective glycan affinities. This thesis addresses this limitation by developing a more precise and higher-resolution analytical method based on mass spectrometry. **Chapter 4** describes this approach, demonstrating that it shows better association with disease severity than the existing methods. This thesis investigates the previously reported glycosylation abnormalities in IgAN, particularly the reported under-galactosylation of *O*-glycans in IgA1, and reports novel *N*-glycosylation signatures of IgAN, with potential for improved IgAN diagnosis and prognosis.

Overall, the research conducted in this thesis employs high-end analytical techniques, including MALDI-TOF-MS (**chapter 3**) and LC-ESI-MS (**chapter 4 and 5**), to study glycosylation patterns in great detail and at a large scale. The automation of high-throughput glycan analysis enables the handling of thousands of patient samples, reducing bias and increasing reproducibility and statistical power, providing

deeper insights into disease mechanisms. A key innovation in this research is the automation of glycan sample preparation. Using robotic platforms, the study ensures consistent sample handling, minimizing technical variability and improving data reliability. For plasma *N*-glycan analysis, a chemical derivatization technique was applied to stabilize sialic acids, facilitating high-throughput analysis of up to 384 samples per MS run (**chapter 3)**. For IgA glycopeptide analysis, an optimized workflow using immobilized camelid antibody domains for IgA capture was developed (**chapter 4 and 5**). This method replaces manual lectin-based approaches, reducing bias and improving scalability.

The findings of this thesis have important biomedical and clinical implications as discussed in **chapter 6**. By mapping disease-specific glycosylation changes, the research contributes to the development of novel biomarkers for IBD and IgAN. The identification of specific glycosylation patterns associated with disease severity and treatment response could enable more precise patient stratification and personalized therapeutic approaches. Moreover, the high-throughput methodologies developed in this research set the stage for future large-scale glycomic studies, potentially leading to new discoveries in other immune-related diseases.

In conclusion, this thesis advances our understanding of glycosylation changes in IBD and IgAN, providing new insights into disease processes. The research highlights the potential of glycomic analysis for improved diagnosis and prognosis in immune-mediated diseases.