Vascular Pharmacology 159 (2025) 107474

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

N-glycosylation signature and its relevance in cardiovascular immunometabolism

Monika Svecla [a], Ruifang Li-Gao [b], David Falck [c], Fabrizia Bonacina [d,*]

[a] Department of Neurosurgery, Charité Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
[b] Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands
[c] Leiden University Medical Center, Center for Proteomics and Metabolomics, Glycomics Group, Leiden, the Netherlands
[d] Department of Excellence of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan, Milan, Italy

ARTICLE INFO

Keywords:
Glycosylation
N-glycome
Immune cells
Metabolism
CVD
Multi-omics

ABSTRACT

Glycosylation is a post-translational modification in which complex, branched carbohydrates (glycans) are covalently attached to proteins or lipids. Asparagine-link protein (*N*-) glycosylation is among the most common types of glycosylation. This process is essential for many biological and cellular functions, and impaired *N*-glycosylation has been widely implicated in inflammation and cardiovascular diseases. Different technical approaches have been used to increase the coverage of the *N*-glycome, revealing a high level of complexity of glycans, regarding their structure and attachment site on a protein. In this context, new insights from genomic studies have revealed a genetic regulation of glycosylation, linking genetic variants to total plasma *N*-glycosylation and *N*-glycosylation of immunoglobulin G (IgG). In addition, RNAseq approaches have revealed a degree of transcriptional regulation for the glycoenzymes involved in glycan structure. However, our understanding of the association between cardiovascular risk and glycosylation, determined by a complex overlay of genetic and environmental factors, remains limited. Mostly, plasma *N*-glycosylation profiling in different human cohorts or experimental investigations of specific enzyme functions in models of atherosclerosis have been reported. Most of the uncovered glycosylation associations with pathological mechanisms revolve around the recruitment of inflammatory cells to the vessel wall and lipoprotein metabolism. This review aims to summarise insights from omics studies into the immune and metabolic regulation of *N*-glycosylation and its association with cardiovascular and metabolic disease risk and to provide mechanistic insights from experimental models.

The combination of emerging techniques for glycomics and glycoproteomics with already achieved omics approaches to map the transcriptomic, epigenomic, and metabolomic profile at single-cell resolution will deepen our understanding of the molecular regulation of glycosylation as well as identify novel biomarkers and targets for cardiovascular disease prevention and treatment.

1. Introduction

Glycosylation is the most frequent post-translational modification and consists in the enzymatic attachment of monosaccharides or oligosaccharides (called **glycan**) to a protein or lipid. The collection of glycans, whether free or covalently bound to a protein (glycoproteins) or a lipid (glycolipids), is usually referred to as **glycome** [1]. Normally, nine monosaccharides, which are present in diet, are used as building blocks for glycosylation [2]. The main types of protein glycosylation are (*i*) *N*-glycosylation, which starts in the endoplasmic reticulum (ER) with the transfer of an oligosaccharide to asparagine residue (Asn) of the NXT/S consensus sequon on proteins and continues with elaboration in Golgi apparatus, and (*ii*) O-glycosylation, which occurs mainly in the Golgi apparatus, with the biosynthesis of the glycan structure on a serine (Ser) or threonine (Thr) residue on a protein initiated by a family of *N*-acetylgalactosamine (GalNAc) transferases (GALNTs) [3]. However, O-glycosylation can occur also in the nucleus and cytoplasm, where a single *N*-acetylglucosamine (GlcNAc) is added to Ser or Thr [1].

Glycosylation is a complex and dynamic process that depends on the cell-specific enzymatic expression of glycosyltransferases, which synthesize glycan chains, and glycosidases, which hydrolyze the glycan linkage [4]. Nevertheless, glycosylation is also influenced by the availability of glycan nucleotides, which act as monosaccharide donors, as well as by the glycan substrate and its substrate affinity for the

* Corresponding author.
E-mail address: Fabrizia.bonacina@unimi.it (F. Bonacina).

https://doi.org/10.1016/j.vph.2025.107474
Received 31 October 2024; Received in revised form 21 January 2025; Accepted 20 February 2025
Available online 21 February 2025

glycosylation enzymes, which are driven by genetic, epigenetic, and environmental factors [2]. Glycosylation has distinct physicochemical properties by affecting protein synthesis, degradation, stability and solubility, protein trafficking, immune cell adhesion, cell signaling, and host-pathogen recognition, with different types of glycosylation also having distinct biological functions [4]. Even within the same cell and under the same physiological condition, glycosylation will differ greatly between proteins and site-driven protein structure [5]. This heterogeneity leads to multiple glycoforms, meaning that individual proteins or glycosylation sites may not always be efficiently glycosylated, as the process is affected by various cellular and environmental factors, resulting in a high heterogeneity that influences the turnover, secretion, and functional efficiency of glycoproteins [6].

Unlike glycosylation, which is the enzymatic attachment of a glycan to a protein or lipid, glycation happens as a chemical reaction without any enzyme involvement. This process leads to structural and functional changes in proteins, resulting in advanced glycation end-products (AGEs), which will not be covered in this review [7].

It is estimated that over 75 % of all human proteins have at least one *N*-linked glycan site, making *N*-glycosylation the most predominant form, affecting secreted, extracellular, and intracellular proteins [8]. Their biosynthesis starts with the assembly of oligosaccharides (two *N*-acetylglucosamine (GlcNac) and five mannose) on dolichol phosphate (Dol-P), a lipid precursor, in the cytoplasmic side of the endoplasmic reticulum (ER). Further, this structure is flipped to the ER lumen where four mannose and three glucose moieties are attached to form a 14-monosaccharide structure. Then, the preassembled oligosaccharide is transferred from the lipid precursor to the Asn residue on a nascent, unfolded protein [9]. The oligomannose structure moves then to the Golgi apparatus for trimming by glycosidases, and for elongation by glycosyltransferases to form the hybrid and complex *N*-glycans (Fig. 1) [5]. Thus, *N*-glycans are classified into oligomannose, 5 to 9 mannose oligomers found in mammals, resulting from the ER and hybrid, and complex *N*-glycans whose biosynthesis is in the Golgi apparatus.

N-glycans influence lipoprotein metabolism as well as all stages of the inflammatory response associated with cardiovascular diseases (CVDs) by modulating leukocyte trafficking and homing, innate and adaptive immune cell activation, immunoglobulin release, and acute phase proteins [10]. Therefore, given the increasing interest in *N*-glycosylation in the context of cardiovascular disease, the aim of this review is to provide a comprehensive overview of advances in *N*-glycosylation, including genomics as well as cellular mechanisms related to metabolism and inflammation, by reporting studies in humans and experimental models and their associations with CVD.

2. Technical consideration for glycans and glycoprotein analysis

2.1. Glycomics

Glycome analysis is typically limited to a specific type of glycosylation, as each type requires different analytical approaches and often an enrichment step to allow proper analysis of the glycosylation against the background of other biomolecules [11,12]. A prominent example of glycomics is the collection of *N*-glycans present on (major) plasma/serum glycoproteins, usually referred to as N-glycome [13,14,15]. Immunoglobulins (Igs), B-cell-produced antibodies, and especially IgG, are a prime example of such a glycoprotein [16,17].

N-glycome is most commonly studied by enzymatic release of *N*-glycans, for example using **PNGaseF**, which is a generic enzyme for all human *N*-glycans [12,13,18]. Released *N*-glycans are then typically labeled with a fluorescent tag and analyzed by separation techniques coupled with fluorescence detection (FLD). **Hydrophilic interaction liquid chromatography (HILIC)** is mostly applied, with **reversed-phase liquid chromatography (RP-LC)** and electrophoretic separations gaining use recently [13,15,19,20,21]. Retention is based either on the hydrophilicity of the glycans, or hydrophobicity of the label, or the charge of both, respectively. Its capability for molecular resolution has made mass spectrometry (MS) a coveted alternative either as a stand-alone, using for example **matrix-assisted laser desorption/ionization (MALDI-MS)** [22] or in combination with separation and/or FLD

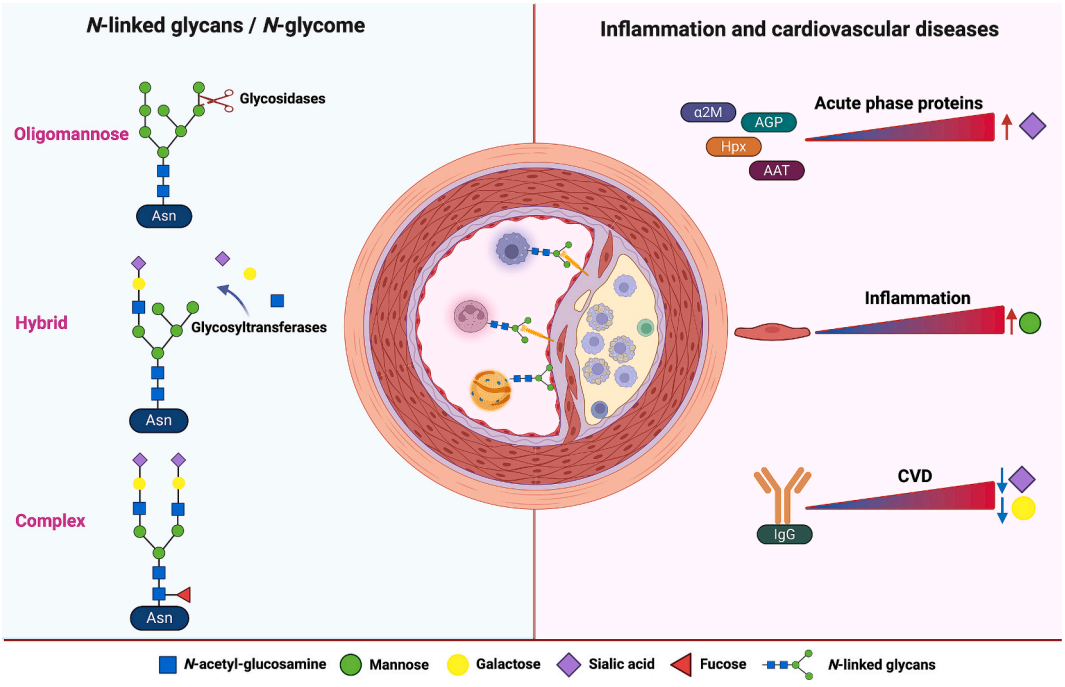

Fig. 1. The biology of N-linked glycans and their potential role in inflammation and cardiovascular disease.
N-linked glycans are classified into oligomannose, hybrid, and complex glycans that are formed from the former by the action of glycosidases, that catalyze the cleavage of mannose residues, followed by elongation from glycosyltransferases to form the hybrid and complex *N*-glycans by adding galactose, sialic acid or *N*-acetylglucosamine. Under inflammatory conditions, sialylation of circulating acute phase proteins is increased, while galactose and sialic acid moieties are increased on endothelial cells; in contrast, in subjects with cardiovascular an metabolic risk, a low presence of sialic acid and galactose on IgGs has been reported.

[20,23]. O-glycomes can be assessed with chemical release techniques, but their harshness can lead to artifacts and misassignment [24,25].

Glycomes are also studied with more classical affinity approaches, utilizing mainly lectins and antibodies [26]. For these approaches, the inherently limited specificity of lectins and the great difficulty in obtaining high-affinity anti-carbohydrate antibodies pose a major challenge, particularly for data interpretation [27] [28]. Glycan arrays, engineered lectins, and engineered glycosidases can address these challenges in part [29,30]. When comparing results to other glycomics techniques, it is important to realize that they are often performed on native glycoconjugates, thus measuring only 'accessible' glycans. As especially instrumental glycomics methods are still far less widely available than genomics methods, tackling glycomes by studying the transcriptional level of glycoenzymes has gained popularity [31]. While this promises important insight into the regulation and pathological dysregulation of glycosylation, there are many factors, such as the availability of sugar nucleotides, site accessibility, and intracellular enzyme distribution, that this approach does not capture.

2.2. Glycoproteomics

Glycoproteomics involves the identification and characterization of glycoproteins to understand their structure, function, and role in various biological processes [32]. Glycoproteomics approaches are based on proteolytic cleavage of the glycoproteins into peptides and glycopeptides and consecutive detection with RP-LC-MS. As opposed to glycan release methods, it allows simultaneous detection of protein N- and O-glycans. Importantly, glycoproteomics allows to assign and quantify the glycan to/on a specific protein and even separately to/on multiple attachment sites within a protein [33]. This is essential, as glycan structure and function can vary dramatically from protein to protein and even from site to site within a protein [34]. However, the complexity of the obtained data in combination with immature computational tools provides a large hurdle, which currently limits applications to few samples or simple glycomes, like that of IgG [35]. IgG glycoproteomics has been performed on many (patho-) physiological states, including various autoimmune, alloimmune, and infectious diseases [17]. Broader glycoproteomics studies into human pathologies are rarer and often include significant method development [34,36,37]. In CVD, a few large IgG glycoproteomics studies have already been conducted [38,39]. More complex glycoproteomes have rarely been investigated in CVD. These studies either used glycoproteomics only to extend protein coverage and did not investigate glycosylation changes [40] or were limited to animal models [41].

3. Fine-mapping of the human blood plasma N-glycome

N-glycosylation is the most abundant type of glycosylation found in human biofluids and biopsies and it is characterized by irregular branched glycans composed of mannose, galactose, fucose, sialic acid, and *N*-acetylglucosamine (GlcNAc) residues, whose combination results in a wide variety of protein glycoforms. Despite the technological advances in qualitative and quantitative detection and the possibility to develop novel biomarkers, interpretation of glycosylation signature (as the total plasma *N*-glycosylation) is limited by the analysis of a complex protein mixture, that would mask whether the differences detected are the consequence of changes in the relative protein abundance, in the relative glycoforms of a specific protein, or whether it reflects a general regulatory effect influencing the glycosylation of many different glycoproteins [42].

Immunoglobulins and proteins produced by the liver constitute the majority of the plasma glycoproteins [43]. Therefore, total plasma *N*-glycome has been proposed also as an indicator of liver and B-cell function [14,17,44]. Thanks to the relative stability over time in healthy individuals, *N*-glycans are considered biomarkers of diseased conditions as their modification would reflect pathological changes in an individual [45–48]. This allows glycoprotein heterogeneity to be used to identify personalized markers for disease risk or specific pathological mechanisms when the products of a particular glycosylation pattern are known to be associated with disease characteristics [49].

3.1. Genetic regulation of human N-glycome from genome-wide association studies

The process of protein glycosylation is complex and genetic factors play an important role. Heritability is a measure used in genetics to quantify the proportion of variation in a particular trait within a population that can be attributed to genetic differences among individuals. Twin studies offer a unique possibility to disentangle the genetic and environmental contributions to a trait; TwinsUK studies have demonstrated that the majority of traits in human plasma *N*-glycome are highly heritable (>50 %), especially for glycans mostly attached to immunoglobulins (Ig) [50,51]. To identify specific genetic variants underlying heritability, **genome-wide association studies (GWAS)** are commonly used as a hypothesis-free approach [52]. In the past decade, protein glycosylation has been utilized as phenotypes in numerous GWAS to investigate the genetic regulations of protein glycosylation. Based on the measurement domain, these GWAS can be categorized into studies of the human total plasma *N*-glycome, the IgG *N*-glycome, and other protein-specific *N*-glycomes.

3.1.1. Human total plasma N-glycome

To date, four large GWAS on plasma *N*-glycome have been conducted with, typically, 15-33 *N*-glycan structures directly quantified through mass spectrometry [53–56]. These *N*-glycans were subsequently grouped according to shared structural features (e.g., fucosylation, sialylation with linkage specificity, and bisection), resulting in what are known as glycosylation traits [12]. Although any subdivision of *N*-glycan features could theoretically lead to a glycosylation trait, only the most general traits were included in the studies, with the number of traits ranging from 13 to 81. Both *N*-glycans and glycosylation traits were used as outcomes in GWAS. The four GWAS were sequential efforts that iteratively replicated previous findings. In total, 16 loci have been identified across these studies, and except for the *KREMEN1* locus, all other 15 loci have been replicated at least once after their initial discovery. Among these replicated loci, *B3GAT1, B4GALT1, MGAT3, MGAT5, FUT6, FUT8, ST3GAL4, ST6GAL1*, and *RUNX3* are genes encoding glycosyltransferases. Additionally, *HNF1A* and *IKZF1* have been implicated as regulators of glycosylation based on in vitro experiments (Table 1).

3.1.2. IgG N-glycome

Glycosylation of IgG plays a crucial role in modulating IgG's effector functions by influencing its binding to Fc receptors. Previous studies have shown exceptionally high inter-individual variability in glycosylation of IgG, with a large variation explained by genetics [57]. Six GWAS have been conducted, primarily in populations of European ancestry. Similar to the studies on human total plasma *N*-glycome, both IgG *N*-glycans and glycosylation traits were treated as phenotypic outcomes in these GWAS. These six GWAS represented sequential, iterative efforts that built upon prior findings, employing diverse IgG glycomics measurement methods, progressively larger sample sizes, and both univariate and multivariate analytical approaches. The first GWAS, conducted by Lauc et al. in 2013, identified nine genetic loci, with four genetic loci encoding glycosyltransferases, namely *ST6GAL1*, *B4GALT1*, *FUT8,* and *MGAT3* [58] (Table 2). In 2017, Shen et al. conducted another GWAS with a larger sample size, improved reference genome for imputation, and a multivariate model. This study uncovered five additional loci, *IGH*, *ELL2*, *HLA-B-C*, *AZI1*, and *FUT3-FUT6*, associated with various IgG glycome traits [59] (Table 2). In 2018, another GWAS was performed using **LC–ESI-MS** [60]. Compared to released glycan analysis by **LC with fluorescence detection**, glycopeptide analysis by LC–ESI-MS allowed

Table 1
Genetic loci that are associated with human total plasma N-glycome.

	Lauc et al. 2010	Huffman et al. 2011	Sharapov et al. 2019	Sharapov et al. 2021	Function	Associated cardiometabolic traits*
beta-1,3-glucuronyltransferase 1 (B3GAT1)		X	X	X	enz	ALP, VAT, metabolic syndrome, whole body fat mass, GGT
beta-1,4-galactosyltransferase 1 (B4GALT1)			X	X	enz	AST, urate levels, eGFR
derlin 3/coiled-coil-helix-coiled-coil-helix domain containing 10 (DERL3/ CHCHD10)			X	X		Hypertrophic cardiomyopathy, PR interval, ejection fraction, heart failure, left ventricular internal dimension in systole, ECG latent space
HNF1 homeobox A (HNF1A)	X	X	X	X	in vitro	CRP, GGT, TC, LDL-C, ALP, non-HDL cholesterol levels, apoB, T2DM, CAD, apoA1, MI
Immunoglobulin heavy locus/ transmembrane protein 121 (IGH/ TMEM121)			X	X		albumin levels
IKAROS family zinc finger 1 (IKZF1)			X	X	in vitro	HDL-C, AST, TC, apoA1 levels, mean platelet volume, platelet distribution width
kringle containing transmembrane protein 1 (KREMEN1)			X			Waist-to-hip ratio, platelet distribution width, metabolic syndrome
beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase (MGAT3)			X	X	enz	Factor VIII levels or vWF, waist-to-hip ratio
alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase (MGAT5)		X		X	enz	ALP, LDL-C, waist circumference, TC
fucosyltransferase 3/fucosyltransferase 6 (FUT3-FUT6)	X	X	X	X	enz	LCL-C, TC, non-HDL cholesterol levels, GGT, AST, *E*-selectin levels
proline rich coiled-coil 2 A (PRRC2A)			X	X		ApoM, eGFR, whole body fat free mass, SBP, GGT, BMI, body fat percentage, T2D or CAD
solute carrier family 9 member A9 (SLC9A9)		X		X		Mean platelet volume, BMI
ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (ST3GAL4)			X	X	enz	ALP, LDL-C, non-HDL cholesterol levels, mean platelet volume, TC, apoB, HDL-C, platelet count, E-selectin levels, VTE, vWF, factor VIII levels, platelet distribution width, apoA1, SBP, hypertension, CAD
ST6 beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)			X	X	enz	T2DM, HbA1c, adiponectin levels, platelet count, LDL-C
mannosidase alpha class 1C member 1 (MAN1C1): AL445471.2 (MAN1C1/ RUNX3)			X	X	enz	Mean platelet volume, apoB
fucosyltransferase 8 (FUT8)	X	X	X	X	enz	Metabolic syndrome, HDL-C, BMI, SBP

* Cardiometabolic traits associated with the gene were retrieved from the GWAS Catalog on October 30, 2024, using the gene name as the search keyword. Only traits with a *p*-value $<5 \times 10^{-8}$ were selected.
enz: genes encoding glycosyltransferases.
in vitro: genes implicated as regulators of glycosylation based on in vitro experiments.
Alkaline phosphatase levels: ALP; visceral adipose tissue: VAT; gamma glutamyl transferase levels: GGT; aspartate aminotransferase levels: AST; estimated glomerular filtration rate: eGFR; C-reactive protein levels: CRP; total cholesterol levels: TC; low-density lipoprotein cholesterol levels: LDL-C; high-density lipoprotein cholesterol levels: HDL-C; apolipoprotein B levels: apoB, type 2 diabetes mellitus: T2DM, coronary artery disease: CAD, apolipoprotein A1 levels: apoA1, myocardial infarction: MI; apolipoprotein M levels: ApoM; systolic blood pressure: SBP; body mass index: BMI; venous thromboembolism: VTE; von Willebrand factor levels: vWF; hemoglobin A1c levels: HbA1c.

for subclass-specific IgG glycan quantification, as the tryptic glycopeptides retain the information of the subclass origin in their peptide portion. This facilitates analysis of within-subclass ratios that represent single pathway steps in glycan synthesis, as well as between-subclass ratios and glycan proportions. This study confirmed six previously identified genetic loci and discovered a novel locus, *RUNX3*, which is a transcription factor (TF) that may drive regulatory networks in B lymphocytes, influencing the expression of key glycosyltransferase enzymes. In 2020, Klarić et al. conducted the largest GWAS to date, with a discovery sample size of 8090 individuals. This study identified 27 loci that reached genome-wide significance [53]. Beyond in silico evidence, this study also demonstrated that knockdown of *IKZF1* reduces the expression of the fucosyltransferase *FUT8*, leading to increased levels of fucosylated glycans. In 2021, Shadrina et al. applied a multivariate model to the same discovery population [61], identifying six novel genetic loci, all of which were also replicated in independent cohorts.

3.1.3. Other protein specific N-glycome

In addition to GWAS on the *N*-glycome of human total plasma and IgG glycosylation, a recent study by Landini et al. investigated the genetic regulation of transferrin *N*-glycosylation and compared these findings with the genetic regulation of IgG glycosylation [62]. The study identified 10 genetic loci associated with at least one of the 35 transferrin *N*-glycan traits. When comparing these results with IgG glycosylation-associated genes, some protein-specific associations were found with genes encoding glycosylated enzymes. For example, transferrin was associated with *MGAT5*, *ST3GAL4*, and *B3GAT1*, while IgG was linked to *MGAT3* and *ST6GAL1* (Table 2). Additionally, shared associations were observed with *FUT6* and *FUT8*, indicating common regulatory pathways across different glycoproteins.

3.2. N-glycome associated with human genetic loci and cardiometabolic disease risks

Some genetic loci associated with the human total plasma *N*-glycome has been also linked to subclinical or clinical cardiometabolic conditions (Table 1). The most frequently associated traits include liver enzymes, such as alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST), as well as lipid markers like total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein B (apoB), and apolipoprotein A1 (apoA1). Additionally, numerous loci are associated with cardiometabolic conditions; for example, *HNF1A, PRRC2A,* and *ST6GAL1* have been linked with condition of type 2 diabetes [63], while *MGAT3* [64], *IGH* and *ST3GAL4* have been associated with cardiac arrest, coronary heart disease, and hypertension. On the other hand,

Table 2
Genetic loci that are associated with human IgG N-glycome.

	Lauc et al. 2013	Shen et al. 2017	Wahl et al. 2018	Klarić et al. 2020	Shadrina et al. 2021	Landini et al. 2022	Function
ST6GAL1	x	x	x	x	x	x	enz
B4GALT1	x	x	x	x	x		enz
FUT8	x	x	x	x	x	x	enz
MGAT3	x	x	x	x	x	x	enz
IKZF1	x		x	x	x	x	Invitro
IL6ST-ANKRD55	x						
ABCF2-SMARCD3	x						
SUV420H1-CHKA	x						
SMARCB1-DERL3	x	x	x	x	x	x	
IGH		x					
ELL2		x		x	x		
HLA-B-C		x		x	x	x	
AZI1		x					
FUT6-FUT3		x		x	x	x	
RUNX3			x	x	x	x	TF
RUNX1				x	x		TF
CHCHD10-VPREB3				x	x	x	
ABCF2				x	x	x	
TMEM121				x	x	x	
SLC38A10-CEP131-TEPSIN				x	x		
IRF1-SLC22A4				x	x		
TXLNB				x	x	x	
HIVEP2				x	x		
DAGLB				x	x		
ODF1				x	x		
SPINK4				x	x		
NXPE1				x	x		
GGA2-COG7				x	x		
ORMDL3-GSDMBIKZF3-ZPBP2				x	x		
CRHR1-SPPL2C-MAPT-ARHGAP27				x	x		
TBX21				x	x		
RFXANK				x	x		
MGME1				x	x		
SMYD3					x		
ASXL2					x		
CHST2/SLC9A9					x		
RNF168					x		
OVOL1					x		
TNFRSF13B					x		
DELR3						x	
VPREB3						x	

enz: genes encoding glycosyltransferases.
in vitro: genes implicated as regulators of glycosylation based on in vitro experiments.
TF: TF-driven regulatory network acting in B lymphocytes to affect the expression of key glycosyltransferase enzymes.

several genetic loci associated with IgG glycome are linked to inflammatory conditions, including inflammatory bowel disease (IBD), ulcerative colitis (UC), and rheumatoid arthritis (RA) and might be also associated with traits of cardiovascular risk,

However, evidence are still limited, and further studies are essential to elucidate the connections between these genetic loci and cardiovascular and metabolic diseases. This can be achieved through comprehensive post-GWAS analyses, including approaches such as genetic correlation [65], colocalization, and Mendelian randomization [66]. Genetic correlation studies can help reveal shared genetic underpinnings between *N*-glycome traits and cardiometabolic diseases. Colocalization analyses can pinpoint specific loci where genetic variants influence both traits through shared causal mechanisms. Mendelian randomization approaches can then be used to assess causal relationships, providing insights into whether *N*-glycome traits may directly contribute to cardiometabolic disease risk. Together, these methods offer a robust framework for understanding the genetic links between *N*-glycosylation patterns and cardiometabolic health.

3.3. *N-glycome as biomarkers of cardiovascular risk*

Several studies have explored the association between plasma *N*-glycome and cardiovascular diseases aiming to identify novel biomarkers for early diagnosis and patient stratification. As an example, sialylation, that is the presence of sialic acid as terminal glycan moiety in a protein, is a general marker of inflammation, and changes in sialylation levels are reported in various inflammatory conditions, including CVD [14,67]. Indeed, sialylated acute phase proteins, such as fibrinogen and ceruloplasmin, have been associated with cardiovascular disease risk [68]. In the future, it would be important to potentially narrow down and connect directly these associations to individual sites on specific proteins and link them to interactions and function of these sites. In contrast, low sialylation of IgG is associated with cardiovascular risk in primary and secondary prevention patients [39,69], an observation in line with the negative correlation between IgG sialylation and inflammatory responses (Fig. 1) [70]. IgG glycosylation studies have thus been interpreted in the context of the established function of the conserved Fc-glycosylation site, but a role of variable domain glycosylation should be investigated/excluded, as released glycan-based assays do not distinguish these two functionally distinct types. To note, sialic acid in humans is mainly present as *N*-acetylneuraminic acid (Neu5Ac) while in mice and other mammals, sialic acid is primarily present as *N*-glycolylneuraminic acid (Neu5Gc). This difference is due to a loss-of-function mutation in the *CMAH* gene in humans, which converts Neu5Ac into Neu5Gc. Interestingly, deficiency of *CMAH* in dyslipidemic mice increases nearly 2-fold atherosclerotic plaque despite similar plasma lipid levels [71]. In addition, circulating sialic acid concentration is increased in patients with cardiovascular disease reporting high levels of

inflammation [67]. The elevated levels of sialic acid have been attributed to the circulating inflammatory glycoproteins such as alpha-1-acid glycoprotein, alpha-1-antitrypsin, fibrinogen, alpha-2-macroglobulin, and hemopexin where alpha-1-acid glycoprotein has been reported to account for the majority of all highly sialylated *N*-glycoproteins in circulation (Fig. 1) [42,67,72]. Interestingly, also the type of sialic acid linkage and branching would differently affect cardiovascular risk, showing a positive association with α2,6-sialylation in triantennary and tetra-antennary glycans, and a negative association with α2,3-sialylation in diantennary glycans [73]. In addition to being a widely researched biomarker in health and disease, IgG has been identified as a glycoprotein candidate to identify CVD risk as *N*-glycans account for the biological diversity of IgG effector function, reflecting varying states of systemic inflammation and immune activation [74]. IgG *N*-glycosylation profiles, especially reduced galactosylation and sialylation levels, are consistently associated with the risk of future CVD, independent of known CVD risk factors [39], and also associated with coronary artery disease and subclinical atherosclerosis [38,75]. In addition, bisecting *N*-acetylglucosamine of immunoglobulins is associated with CVD risk in diabetic patients [69,73]. However, it is yet unclear whether these differences reflect an indirect effect of general systemic inflammation in atherosclerotic CVD on IgG glycosylation, rather than mark the potential pathogenic role of these glycosylation states, that would be genetically driven. In the latter case, potential mechanistic involvement could be via a lower threshold for inflammatory events or via more potent effector functions of suspected autoantibodies, such as antiphospholipid antibodies [76,77].

4. Molecular insights of glycosylation: from immunometabolism to atherosclerosis

4.1. Crosstalk between glycosylation and metabolism from systemic to cellular level

Glycosylation is linked to systemic and cellular metabolism. It affects the function of proteins and enzymes involved in lipid homeostasis, including apolipoproteins, lipoprotein receptors, and other proteins involved in lipoprotein metabolism [78]. This suggests a critical role for glycosylation in maintaining plasma lipid levels [79]. Indeed, human congenital disorders of glycosylation (CDGs), a group of rare glycan genetic disorders, affect protein and lipid glycosylation and are associated with various forms of dyslipidaemia [78,79]. Conversely, glycosylation itself is affected by cellular metabolic fluxes and the availability of sugar phosphate intermediates and nucleotide sugars [6], as well as the levels of glycosyltransferase enzyme and activity in the secretory pathway (ER and Golgi), nucleus, cytoplasm, and mitochondria [80]. As glycans are constituted of diverse types of sugar molecules, monosaccharides availability - through the diet as well as the cellular flux of glucose -, limits the availability of hexoses and pentoses, such as mannose, fucose, and galactose derived from fructose-6-phosphate [80]. Indeed, glucose, beyond a fuel for glycolysis to produce energy (ATP production), is also diverted, in different proportions depending on cell type and environmental conditions [81], to the hexosamine biosynthetic pathway (HBP) for de novo production of uridine diphosphate-GlcNAc [67] (UDP-GlcNAc), an essential precursor for all types of

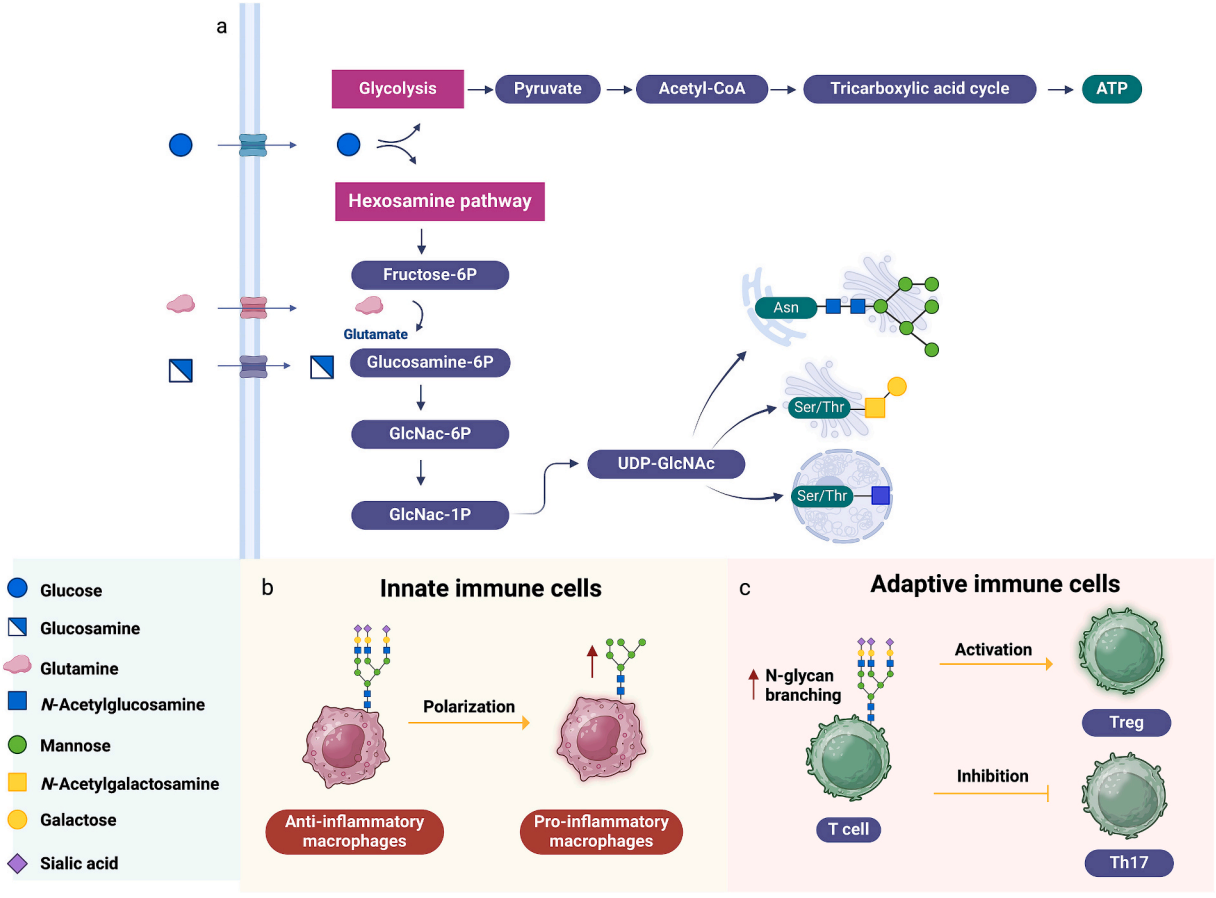

Fig. 2. *N*-glycan branching modulates innate and adaptive immune cell function.
a) UDP-GalNac is the first building block required for *N*-glycan synthesis, that can be generated from glucose through the hexosamine biosynthesis pathway (HBP), or by the external uptake of glutamate or glucosamine.
b-c) Loss of complex triantennary *N*-glycans in macrophages contributes to the phenotypic switch of anti-inflammatory macrophages to pro-inflammatory macrophages (b), while in T cells, increased N-glycan branching has been shown to favor Treg polarization over Th17 (c).

glycosylation. The HBP is a six-step pathway that converts glucose to UDP-GlcNAc. It shares the first two steps with glycolysis and requires the presence of glutamine, acetyl-CoA, and UTP [80,82] (Fig. 2), posing HBP as a nutrient-sensing process at the crossroads of different metabolic pathways. Accordingly, the rate of glutaminolysis, where extracellular glutamine is converted to glutamate to fuel α-ketoglutarate in the tricarboxylic acid cycle (TCA) [83], fatty acid oxidation, TCA cycle, and amino acid catabolism, that supply acetyl-CoA [84] or pyrimidine biosynthetic pathway [85] providing the UTP nucleotide could have a significant impact on the flux of glycosylation. In parallel, when de novo synthesis of GlcNAc is limited, the salvage pathway recycles precursors from glucosamine (GlcN) or *N*-acetylglucosamine (GlcNAc) from degraded glycoproteins and glycolipids, thus maintaining a steady supply of UDP-GlcNAc [82]. In addition to specific metabolic cellular preferences that could differentially affect the rate of UDP-GlcNAc production, cell signaling pathways that are mediated by extracellular signals, such as nutrient availability, cytokines, and growth factors, similarly control the rate of energy production. This, in turn, modulates the HBP pathway and therefore glycosylation.

4.2. Glycosylation and immuno-metabolic response

It is well established that glycosylation, and particularly *N*-glycans, influence all stages of the inflammatory response by modulating leukocyte trafficking and homing, innate and adaptive immune cell activation, immunoglobulin release, and acute phase proteins [6,86]. However, as immune cells modulate energetic metabolism to accomplish specific cell functions, interest is growing around the possibility that the immuno-metabolic cell machinery could, in concert, modulate the rate and type of glycosylation and, in turn, the immune response [87].

For instance, macrophages, one of the major cell types of the innate immune response, present a broad range of phenotypes that are associated with a specific metabolic preference. For simplicity, macrophages are typically classified into pro- and anti-inflammatory subsets. For energy production, the first relies primarily on glycolysis, whereas the second on oxidative phosphorylation [88]. Mouse data have shown that all macrophage subsets possess *N*-glycan structures, predominantly in the form of either high mannose or complex types, with a smaller proportion being of the hybrid type [89]. Interestingly, in mouse models, anti-inflammatory macrophages exhibit a higher relative abundance of triantennary *N*-glycans compared to pro-inflammatory macrophages (Fig. 2) [89]. While the mechanisms driving this reprogramming of glycosylation are not yet fully understood, local injury may trigger the release of metabolic products like intracellular UDP-GlcNAc and glucose, which enhance glycan precursors availability, thus promoting *N*-glycan branching through cellular uptake. Additionally, the increased *N*-glycan branching in peritoneal resident macrophages could support their proliferation, activation, and role in tissue repair [89].

Similarly, *N*-glycan branching is a critical regulator of T cell immunity. Following antigen-specific stimulation, T lymphocytes rapidly switch their metabolism to glycolysis. This metabolic adaptation is similar to that observed in proliferating cancer cells, which also preferentially utilize glucose and its fermentation to lactate in the presence of oxygen, as first described by Otto Warburg [91]. Indeed, rather than producing merely energy, the increased glucose uptake may provide the biomass required for rapid cell division thanks to the generation of metabolites that are precursors for nucleotide, amino acid, and lipid biosynthetic pathways [92]. However, these biosynthetic pathways do not account for the increased uptake of glucose, suggesting instead that glucose could be diverted to other, not energetic related pathways, such as glycosylation. Indeed, it has been shown that *N*-glycosylation regulates T cell polarization and function, influencing T cell receptor signaling and the clustering and surface retention of several T cell receptors, such as CD4, CD8, CD45, and CTLA-4 [93,94]. In more detail, increased glucose and glutamine flux reduces N-branching, thereby facilitating pro-inflammatory Th17 differentiation over anti-inflammatory Treg differentiation. By contrast, increased *N*-glycan branching, due to increased GlcNAc availability, such as through oral supplementation, or with age [95], has been shown to suppress T cell receptor signaling, proliferation, CTLA-4 endocytosis, and T helper 1 differentiation in an autoimmune encephalomyelitis mouse model [90]. This suggests that metabolite flux through the hexosamine and *N*-glycan pathways regulates autoimmunity by modulating T cell function via *N*-glycan branching. In addition, sufficient hexosamine biosynthesis is required to meet the increased demands for cell survival, supporting the expansion and maturation of αβ-thymocytes [96]. In this setting, decreased UDP-GlcNAc was accompanied by defective *N*-glycosylation, enhanced T-cell receptor misprocessing, and unfolding protein response [96].

Restoring N-branching and UDP-GlcNAc levels via the salvage pathway blocks the endocytic loss of IL-2 receptor-α (CD25), which is key to Treg differentiation, by FoxP3 transcriptional regulation [97]. In line with this, fatty acid oxidation induced by PPARγ activation has been shown to fuel acetyl-CoA-derived *N*-glycan branching of CD25, thus supporting Treg stability [98]. These findings suggest that, upon T cell activation, glycolysis and glutaminolysis may compete with oxidative metabolism to control the rate of the glycosylation process, suggesting that immuno-metabolic adaptations may have broader implications beyond energy requirements.

4.3. Glycosylation in vascular inflammation and atherosclerosis

Immune cell recruitment to the activated endothelial layer is one of the initial steps of vascular inflammation leading to atherosclerotic plaque formation and progression [99]. Immune cell migration, capture, and adhesion to the endothelium are coordinated by soluble cytokines, chemokines and corresponding ligand-receptors as well as adhesion molecules expressed on leukocytes and endothelial cell membranes [86,100]. Glycosylation of these proteins is crucial for leukocyte trafficking and endothelial adhesion, and it includes both glycans and glycan-binding proteins such as C-type lectins, and the selectin family of *E*-selectin, P-selectin, and L-selectin, which mediate all stages of leukocytes adhesion (extensively reviewed previously [101]). As an example, mice deficient for both α-1,3-fucosyltransferase (*FUT*) *IV* and *VII* show a significant decrease in leukocyte adhesion and smaller atherosclerotic lesions as well as macrophage accumulation. This effect is due to the impaired glycosylation, leading to decreased expression of P-selectin, which is essential for monocytes recruitment [102,103]. Besides, several glycosyltransferases can affect the leukocyte rolling and the progression of atherosclerosis. For instance, α2,3-sialyltransferase IV (*ST3Gal-IV*), which transfers sialic acid to the terminal galactose residue in α2,3-linkage, is critical for the sialylation of CCL5-mediated myeloid cells recruitment. Mice deficient for *ST3Gal-IV* show a reduction in the atherosclerotic plaque size as well as in macrophages [104].

Impaired immuno-metabolic response and endothelial barrier function observed under atherosclerotic conditions [105] could alter cell *N*-glycome thus promoting vascular inflammation. Indeed, several reports provide evidence that inflammatory responses, as well as impaired laminar sheared stress and dyslipidemia change the expression or activity of genes for glycan biosynthesis in endothelial cells, resulting in dysregulated glycosylation of adhesion molecules [79,106]. For instance, both hypoglycosylated *N*-glycans (high-mannose structures) and fucosylated/ sialylated *N*-glycans (complex structures) have been shown to be upregulated in the endothelial cells exposed to pro-inflammatory stimuli and in mouse and human atherosclerosis [107]. Increased expression of high-mannose *N*-glycans on the endothelium is important for the recruiting pro-inflammatory ($CD16^+$) monocytes [108,109], that ultimately become foam cells, thus contributing significantly to the initiation and growth of atherosclerotic lesions [110]. Macrophages, besides glycans, also express several glycan-binding proteins, including mannose receptor C-type 1 (*MRC1* or *CD206*), which is

involved in several immune processes, including in immunometabolic diseases, such as obesity [111], and in the uptake of modified lipoproteins, which in turn contribute to the plaque formation and progression [112]. Interestingly *MRC1* deficient mice did not show alteration in high mannose *N*-glycans rather than reduction in fucosylation [113]. On the other hand, naïve and modified lipoprotein uptake, especially by macrophages, could be affected by glycosylation of receptors involved [112]. Indeed, LDLR, CD36, and SRBI receptors are highly glycosylated, and the amount of glycan residues affects protein stability and interaction [79]. For example, the presence of sialic acids leads to enhanced stability and prolonged half-life of the LDLR which causes an increased uptake of LDL particles, thus lowering the circulating LDL in the plasma, leading to an atheroprotective effect [114].

5. Targeting glycosylation and its receptor as a therapeutic strategy

N-glycans have unique properties influencing protein stability, efficacy, and function which makes them valuable targets for drug development [115]. Many protein-based drugs, including antibodies and enzymes, rely on *N*-glycosylation for their proper function and delivery to the desired site of action [116] [117]. Different glycoengineering approaches are in use for improving drug delivery, efficacy and the pharmacokinetics of therapeutic proteins. Additionally, the presence of *N*-glycans reduces immunogenicity and protects the proteins against proteolytic degradation, aggregation, and thermal denaturation by maintaining optimal conformations of the structure [117] [118] [119]. As an example, the proteins that are highly α2,3-sialylated with complex *N*-glycans have a longer serum half-life since they cannot be taken up by hepatic Asialoglycoprotein receptor 1 (*ASGR1*) or *MRC1* for lysosomal degradation [117]. Therefore, increasing sialic acid residues is commonly used in pharmacokinetic optimisation to extend the plasma half-life of therapeutics, by direct sialylation of recombinant therapeutic proteins or introduction of additional glycosylation sites [120]. For similar purposes, glycosylation of endogenous proteins may be manipulated in the future to achieve health benefits. This may already be achievable with non- or minimally invasive theraphy, such as dietary interventions, and thus could play a largely unexplored role in existing theraphies.

In contrast, asialo-orosomucoid (ASOR) structures, which contain proteins or nucleic acids with galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues, are delivered preferentially to the liver [121]. Small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) used in clinic are engineered to express a triantennary GalNAc conjugate that enhances the endocytosis on hepatocytes via the binding to the ASGR1 [122]. For example Inclisiran, the first drug used to reduce dyslipidemia by inhibiting heaptic PCSK9 production, that has been approved for patients with heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease who require additional lowering of LDL-C [123]. Whereas ASGR1 is the first lectin receptor to be investigated for its glycan affinity and use as a therapeutic delivery system, it should be noted that ASGR1 per se has a causal role in cardiovascular disease, as patients with loss-of-function variants for ASGR1 have lower LDL-cholesterol and a 34 % reduction in CVD burden [124]. Subsequent studies in mice have shown that ASGR1 deficiency lowers cholesterol levels and reduces atherosclerosis by modulating lipid metabolism in the liver, suggesting a dual role for ASGR1 in both therapeutic delivery and lipid metabolism [125,126,127].

All together these evidence suggest that, beyond ASGR1, exploring other lectin-binding receptors holds great potential for optimizing delivery strategies in clinical applications, as these receptors have highly specific and selective binding regardless of the protein cargo.

6. Conclusions: when glycomics meets multi-omics

Protein glycosylation is an intricate process involving the coordinated activity of multiple enzymes responsible for glycan transfer, glycosidic linkage hydrolysis, and glycan biosynthesis [2]. This complex process is regulated by a combination of genetic, epigenetic, metabolic and environmental factors. To deepen our understanding of these processes, glycomics has been increasingly linked to other omics layers such as epigenomics, proteomics, and metabolomics, as previously reviewed [128–130]. To date, controversial results have been obtained on the role of epigenetic regulation of both IgG glycosylation [131–133] and the overall plasma *N*-glycome [133], but it is expected that with the increasing availability of cohort studies that incorporate both glycomics and epigenomics data, larger investigations will provide a more comprehensive understanding of the epigenetic regulation of protein glycosylation.

On the other hand, linking glycomics with proteomics in population-based studies is crucial for understanding how protein glycosylation affects protein levels and, in turn, contributes to disease risk. To date, only a limited number of studies have explored the relationship between glycomics and proteomics [134]. Larger studies with more diverse populations are needed to establish a complete atlas of protein glycosylation-protein level and its impact on disease, which would provide an initial comprehensive overview of the associations between the circulating proteome and its corresponding *N*-glycome that ultimately will have to be confirmed and refined by glycoproteomics.

Finally, metabolomics can provide insights into the biosynthesis of glycan structures by identifying substrates involved in glycosylation processes [135]. The combination of glycomics and lipidomics has identified strong and consistent associations between specific glycans and lipids, providing valuable insights into the interplay between these biomolecules [136]. This field offers a fertile ground for research as most of the consequences of impaired glycosylation are associated with immuno-inflammatory response and several evidence have shown a key role of metabolism and energetic reprogramming on immune cell polarization and function [105]. Therefore, the combination of glycomics and metabolomics would pave the way to identify a new level of metabolic regulation of immune cell response beyond energetic metabolism. Finally, the impact of glycosylation on lipid metabolism is emerging as a driving regulator of the plasma lipid profile; indeed, lipoproteins and especially apolipoproteins are highly glycosylated, such as apolipoprotein B and apolipoprotein (a), suggesting that glycosylation would profoundly affect atherogenic lipoprotein catabolism and associated cardiovascular risk.

It is reasonable to expect that, thanks to technological advances, the integration of these omic platforms combined with pre-clinical validations, would deepen our understanding of the role of glycosylation in immuno-inflammatory and metabolic response with the aim to identify novel targets for cardiovascular disease treatments.

CRediT authorship contribution statement

Monika Svecla: Writing – review & editing, Writing – original draft, Conceptualization. **Ruifang Li-Gao:** Writing – review & editing, Writing – original draft. **David Falck:** Writing – review & editing, Writing – original draft, Conceptualization. **Fabrizia Bonacina:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

Authors declare no competing interests.

Acknowledgements

F.B. is supported by PSR2022_DIP_022_AZIONE_A_FBONA and Progetti di Rilevante Interesse Nazionale (PRIN) 2022 2022NBKCWP, Roche Foundation (Grant 2022). R.L-G is supported by JPI HDHL NUTRIMMUNE DIYUFOOD project.

Figures of the manuscript were created in BioRender.com

Data availability

No data was used for the research described in the article.

References

[1] C. Reily, T.J. Stewart, M.B. Renfrow, J. Novak, Glycosylation in health and disease, Nat. Rev. Nephrol. 15 (2019) 346–366.
[2] K. Ohtsubo, J.D. Marth, Glycosylation in cellular mechanisms of health and disease, Cell 126 (2006) 855–867.
[3] K.W. Moremen, M. Tiemeyer, A.V. Nairn, Vertebrate protein glycosylation: diversity, synthesis and function, Nat. Rev. Mol. Cell Biol. 13 (2012) 448–462.
[4] A. Varki, Biological roles of glycans, Glycobiology 27 (2017) 3–49.
[5] M. Thaysen-Andersen, N.H. Packer, Site-specific glycoproteomics confirms that protein structure dictates formation of N-glycan type, core fucosylation and branching, Glycobiology 22 (2012) 1440–1452.
[6] K.T. Schjoldager, Y. Narimatsu, H.J. Joshi, H. Clausen, Global view of human protein glycosylation pathways and functions, Nat. Rev. Mol. Cell Biol. 21 (2020) 729–749.
[7] A.B. Uceda, L. Mariño, R. Casasnovas, M. Adrover, An overview on glycation: molecular mechanisms, impact on proteins, pathogenesis, and inhibition, Biophys. Rev. 16 (2024) 189–218.
[8] Y. Fan, et al., Loss and gain of N-linked glycosylation sequons due to single-nucleotide variation in cancer, Sci. Rep. 8 (2018) 4322.
[9] S. Esmail, M.F. Manolson, Advances in understanding N-glycosylation structure, function, and regulation in health and disease, Eur. J. Cell Biol. 100 (2021) 151186.
[10] B. Radovani, I. Gudelj, N-glycosylation and inflammation; the not-so-sweet relation, Front. Immunol. 13 (2022).
[11] P.M. Rudd, et al., Glycomics and Glycoproteomics, Preprint at, http://europepmc.org/abstract/MED/35536926, 2022.
[12] N. de Haan, et al., Developments and perspectives in high-throughput protein glycomics: enabling the analysis of thousands of samples, Glycobiology 32 (2022) 651–663.
[13] J. Beimdiek, et al., Serum N-glycomics of a novel CDG-IIb patient reveals aberrant IgG glycosylation, Glycobiology 32 (2022) 380–390.
[14] T. Pongracz, O.A. Mayboroda, M. Wuhrer, The human blood N-Glycome: unraveling disease glycosylation patterns, JACS Au 4 (2024) 1696–1708.
[15] M. Szigeti, A. Guttman, Sample preparation scale-up for deep N-glycomic analysis of human serum by capillary electrophoresis and CE-ESI-MS, Mol. Cell. Proteomics 18 (2019) 2524–2531.
[16] F. Nimmerjahn, G. Vidarsson, M.S. Cragg, Effect of posttranslational modifications and subclass on IgG activity: from immunity to immunotherapy, Nat. Immunol. 24 (2023) 1244–1255.
[17] N. de Haan, D. Falck, M. Wuhrer, Monitoring of immunoglobulin N- and O-glycosylation in health and disease, Glycobiology 30 (2020) 226–240.
[18] T. Damelang, et al., Impact of structural modifications of IgG antibodies on effector functions, Front. Immunol. 14 (2024).
[19] A. Knežević, et al., High throughput plasma N-glycome profiling using multiplexed labelling and UPLC with fluorescence detection, Analyst 136 (2011) 4670–4673.
[20] A.B. Moran, R.A. Gardner, M. Wuhrer, G.S.M. Lageveen-Kammeijer, D.I. R. Spencer, Sialic acid derivatization of fluorescently labeled N-glycans allows linkage differentiation by reversed-phase liquid chromatography–fluorescence detection–mass spectrometry, Anal. Chem. 94 (2022) 6639–6648.
[21] G.S.M. Lageveen-Kammeijer, et al., Highly sensitive CE-ESI-MS analysis of N-glycans from complex biological samples, Nat. Commun. 10 (2019) 2137.
[22] D.J. Harvey, Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: an update for 2021–2022, Mass Spectrom. Rev. 42 (1) (2023) 227–431.
[23] Y. Helali, et al., Development and validation of online SPE purification coupled to HILIC-fluorescence-MS analysis for the characterization of N-glycans, Talanta 270 (2024) 125541.
[24] M. Kotsias, R.P. Kozak, R.A. Gardner, M. Wuhrer, D.I.R. Spencer, Improved and semi-automated reductive β-elimination workflow for higher throughput protein O-glycosylation analysis, PLoS ONE 14 (2019) e0210759.
[25] N. de Haan, et al., In-depth profiling of O-glycan isomers in human cells using C18 nanoliquid chromatography–mass spectrometry and glycogenomics, Anal. Chem. 94 (2022) 4343–4351.
[26] L. Liu, J. Li, Y. Yang, C. Hu, X. Tian, Altered glycosylation profiles of serum IgG in Takayasu arteritis, Eur. J. Med. Res. 28 (2023) 69.
[27] O.D. Hendrickson, A.V. Zherdev, Analytical application of lectins, Crit. Rev. Anal. Chem. 48 (2018) 279–292.
[28] G. Lu, L.A. Holland, Profiling the N-glycan composition of IgG with lectins and capillary Nanogel electrophoresis, Anal. Chem. 91 (2019) 1375–1383.
[29] M.C. Cook, S.J. Kaldas, G. Muradia, M. Rosu-Myles, J.P. Kunkel, Comparison of orthogonal chromatographic and lectin-affinity microarray methods for glycan profiling of a therapeutic monoclonal antibody, J. Chromatogr. B 997 (2015) 162–178.
[30] J. Arnaud, A. Audfray, A. Imberty, Binding sugars: from natural lectins to synthetic receptors and engineered neolectins, Chem. Soc. Rev. 42 (2013) 4798–4813.
[31] Q. Zhang, C. Ma, L.-S. Chin, S. Pan, L. Li, Human brain glycoform coregulation network and glycan modification alterations in Alzheimer's disease, Sci. Adv. 10 (2024) eadk6911.
[32] S.A. Malaker, Glycoproteomics: charting new territory in mass spectrometry and glycobiology, J. Mass Spectrom. 59 (2024) e5034.
[33] J. Klein, L. Carvalho, J. Zaia, Expanding N-glycopeptide identifications by modeling fragmentation, elution, and glycome connectivity, Nat. Commun. 15 (2024) 6168.
[34] K.R. Reiding, Y.-H. Lin, F.P.J. van Alphen, A.B. Meijer, A.J.R. Heck, Neutrophil azurophilic granule glycoproteins are distinctively decorated by atypical pauci- and phosphomannose glycans, Commun. Biol. 4 (2021) 1012.
[35] D. Falck, M. Wuhrer, GlYcoLISA: antigen-specific and subclass-specific IgG fc glycosylation analysis based on an immunosorbent assay with an LC–MS readout, Nat. Protoc. 19 (2024) 1887–1909.
[36] N. de Haan, et al., Sensitive and specific global cell surface N-Glycoproteomics shows profound differences between glycosylation sites and subcellular components, Anal. Chem. 95 (2023) 17328–17336.
[37] D.E. Marrero Roche, K.B. Chandler, Clinical glycoprotein mass spectrometry: the future of disease detection and monitoring, J. Mass Spectrom. 59 (2024) e5083.
[38] B. Radovani, et al., IgG N-glycosylation is altered in coronary artery disease, Biomolecules 13 (2023).
[39] R.A. Hoshi, et al., N-glycosylation profiles of immunoglobulin G and future cardiovascular events, Circ. Res. 134 (2024) e3–e14.
[40] K.Y. Deleon-Pennell, et al., Glycoproteomic profiling provides candidate myocardial infarction predictors of later progression to heart failure, ACS Omega 4 (2019) 1272–1280.
[41] B.L. Parker, et al., Quantitative N-linked Glycoproteomics of myocardial ischemia and reperfusion injury reveals early remodeling in the extracellular environment, Mol. Cell. Proteomics 10 (2011).
[42] F. Clerc, et al., Human plasma protein N-glycosylation, Glycoconj. J. 33 (2016) 309–343.
[43] A. Timoshchuk, S. Sharapov, Y.S. Aulchenko, Twelve years of genome-wide association studies of human protein N-glycosylation, Engineering 26 (2023) 17–31.
[44] B. Blomme, et al., N-glycan based biomarker distinguishing non-alcoholic steatohepatitis from steatosis independently of fibrosis, Dig. Liver Dis. 44 (2012) 315–322.
[45] I. Gudelj, G. Lauc, M. Pezer, Immunoglobulin G glycosylation in aging and diseases, Cell. Immunol. 333 (2018) 65–79.
[46] O. Gornik, et al., Stability of N-glycan profiles in human plasma, Glycobiology 19 (2009) 1547–1553.
[47] R. Hennig, et al., Towards personalized diagnostics via longitudinal study of the human plasma N-glycome, Biochim. Biophys. Acta 1860 (2016) 1728–1738.
[48] G. Lauc, M. Pezer, I. Rudan, H. Campbell, Mechanisms of disease: the human N-glycome, Biochim. Biophys. Acta 1860 (2016) 1574–1582.
[49] Y.-L. Chiu, et al., Alternative complement pathway is activated and associated with galactose-deficient IgA1 antibody in IgA nephropathy patients, Front. Immunol. 12 (2021).
[50] O.O. Zaytseva, et al., Heritability of human plasma N-glycome, J. Proteome Res. 19 (2020) 85–91.
[51] A. Mijakovac, et al., Heritability of the glycan clock of biological age, Front. Cell Dev. Biol. 10 (2022).
[52] E. Uffelmann, et al., Genome-wide association studies, Nat. Rev. Methods Primers 1 (2021) 59.
[53] L. Klarić, et al., Glycosylation of immunoglobulin G is regulated by a large network of genes pleiotropic with inflammatory diseases, Sci. Adv. 6 (2024) eaax0301.
[54] S.Z. Sharapov, et al., Replication of 15 loci involved in human plasma protein N-glycosylation in 4802 samples from four cohorts, Glycobiology 31 (2021) 82–88.
[55] G. Lauc, et al., Genomics meets Glycomics—the first GWAS study of human N-Glycome identifies HNF1α as a master regulator of plasma protein fucosylation, PLoS Genet. 6 (2010) e1001256.
[56] J.E. Huffman, et al., Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults, Hum. Mol. Genet. 20 (2011) 5000–5011.
[57] M. Pučić, et al., High throughput isolation and glycosylation analysis of IgG variability and heritability of the IgG glycome in three isolated human populations, Mol. Cell. Proteomics 10 (2011).
[58] G. Lauc, et al., Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers, PLoS Genet. 9 (2013) e1003225.
[59] X. Shen, et al., Multivariate discovery and replication of five novel loci associated with immunoglobulin G N-glycosylation, Nat. Commun. 8 (2017) 447.
[60] A. Wahl, et al., Genome-wide association study on immunoglobulin G glycosylation patterns, Front. Immunol. 9 (2018).
[61] A.S. Shadrina, et al., Multivariate genome-wide analysis of immunoglobulin G N-glycosylation identifies new loci pleiotropic with immune function, Hum. Mol. Genet. 30 (2021) 1259–1270.
[62] A. Landini, et al., Genetic regulation of post-translational modification of two distinct proteins, Nat. Commun. 13 (2022) 1586.
[63] A. Mahajan, et al., Multi-ancestry genetic study of type 2 diabetes highlights the power of diverse populations for discovery and translation, Nat. Genet. 54 (2022) 560–572.
[64] B.E. Aouizerat, et al., GWAS for discovery and replication of genetic loci associated with sudden cardiac arrest in patients with coronary artery disease, BMC Cardiovasc. Disord. 11 (2011) 29.

[65] W. van Rheenen, W.J. Peyrot, A.J. Schork, S.H. Lee, N.R. Wray, Genetic correlations of polygenic disease traits: from theory to practice, Nat. Rev. Genet. 20 (2019) 567–581.
[66] V. Zuber, et al., Combining evidence from Mendelian randomization and colocalization: review and comparison of approaches, Am. J. Hum. Genet. 109 (2022) 767–782.
[67] J. Cheeseman, et al., Sialic acid as a potential biomarker for cardiovascular disease, diabetes and cancer, Biomark. Med 15 (2021) 911–928.
[68] M.S. Ahmed, A.B. Jadhav, A. Hassan, Q.H. Meng, Acute phase reactants as novel predictors of cardiovascular disease, Int. Sch. Res. Notices 2012 (2012) 953461.
[69] C. Wittenbecher, et al., Plasma N-Glycans as emerging biomarkers of Cardiometabolic risk: a prospective investigation in the EPIC-Potsdam cohort study, Diabetes Care 43 (2020) 661–668.
[70] R. Vattepu, S.L. Sneed, R.M. Anthony, Sialylation as an important regulator of antibody function, Front. Immunol. 13 (2022).
[71] K. Kawanishi, et al., Human species-specific loss of CMP-N-acetylneuraminic acid hydroxylase enhances atherosclerosis via intrinsic and extrinsic mechanisms, Proc. Natl. Acad. Sci. 116 (2019) 16036–16045.
[72] J. Cheeseman, et al., Elevated concentrations of Neu5Ac and Neu5,9Ac2 in human plasma: potential biomarkers of cardiovascular disease, Glycoconj. J. 40 (2023) 645–654.
[73] E. Memarian, et al., Plasma protein N-glycosylation is associated with cardiovascular disease, nephropathy, and retinopathy in type 2 diabetes, BMJ Open Diabetes Res. Care 9 (2021) e002345.
[74] P.R. Lawler, Glycomics and cardiovascular disease, Circ. Res. 122 (2018) 1488–1490.
[75] C. Menni, et al., Glycosylation profile of immunoglobulin G is cross-Sectionally associated with cardiovascular disease risk score and subclinical atherosclerosis in two independent cohorts, Circ. Res. 122 (2018) 1555–1564.
[76] Y. Wang, et al., Antiphospholipid antibodies modify the prognostic value of baseline platelet count for clinical outcomes after ischemic stroke, J. Am. Heart Assoc. 13 (2024) e035183.
[77] Y. Wang, et al., Changes in serum immunoglobulin G glycosylation patterns for antiphospholipid syndrome patients with lectin microarray, Scand. J. Immunol. 99 (2024) e13366.
[78] M.A.W. van den Boogert, et al., N-glycosylation defects in humans lower low-density lipoprotein cholesterol through increased low-density lipoprotein receptor expression, Circulation 140 (2019) 280–292.
[79] A. Pirillo, M. Svecla, A.L. Catapano, A.G. Holleboom, G.D. Norata, Impact of protein glycosylation on lipoprotein metabolism and atherosclerosis, Cardiovasc. Res. 117 (2021) 1033–1045.
[80] A.F. Scheper, J. Schofield, R. Bohara, T. Ritter, A. Pandit, Understanding glycosylation: regulation through the metabolic flux of precursor pathways, Biotechnol. Adv. 67 (2023) 108184.
[81] S. Marshall, V. Bacote, R.R. Traxinger, Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance, J. Biol. Chem. 266 (1991) 4706–4712.
[82] N.M. Akella, L. Ciraku, M.J. Reginato, Fueling the fire: emerging role of the hexosamine biosynthetic pathway in cancer, BMC Biol. 17 (2019) 52.
[83] D.R. Wise, C.B. Thompson, Glutamine addiction: a new therapeutic target in cancer, Trends Biochem. Sci. 35 (2010) 427–433.
[84] L. Shi, B.P. Tu, Acetyl-CoA and the regulation of metabolism: mechanisms and consequences, Curr. Opin. Cell Biol. 33 (2015) 125–131.
[85] N.J. Mullen, P.K. Singh, Nucleotide metabolism: a pan-cancer metabolic dependency, Nat. Rev. Cancer 23 (2023) 275–294.
[86] M. Sperandio, C.A. Gleissner, K. Ley, Glycosylation in immune cell trafficking, Immunol. Rev. 230 (2009) 97–113.
[87] S.S. Pinho, I. Alves, J. Gaifem, G.A. Rabinovich, Immune regulatory networks coordinated by glycans and glycan-binding proteins in autoimmunity and infection, Cell. Mol. Immunol. 20 (2023) 1101–1113.
[88] S.K. Wculek, G. Dunphy, I. Heras-Murillo, A. Mastrangelo, D. Sancho, Metabolism of tissue macrophages in homeostasis and pathology, Cell. Mol. Immunol. 19 (2022) 384–408.
[89] X. He, et al., Impaired bisecting GlcNAc reprogrammed M1 polarization of macrophage, Cell Commun. Signal. 22 (2024) 73.
[90] O. Warburg, F. Wind, E. Negelein, The metabolism of tumors in the body, J. Gen. Physiol. 8 (1927) 519–530.
[91] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 1979 (324) (2009) 1029–1033.
[92] L.A. Earl, L.G. Baum, CD45 glycosylation controls T-cell life and death, Immunol. Cell Biol. 86 (2008) 608–615.
[93] E. De Bousser, L. Meuris, N. Callewaert, N. Festjens, Human T cell glycosylation and implications on immune therapy for cancer, Hum. Vaccin. Immunother. 16 (2020) 2374–2388.
[94] H. Mkhikian, et al., Age-associated impairment of T cell immunity is linked to sex-dimorphic elevation of N-glycan branching, Nat. Aging 2 (2022) 231–242.
[95] M.C. Ryczko, et al., Metabolic reprogramming by Hexosamine biosynthetic and Golgi N-glycan branching pathways, Sci. Rep. 6 (2016) 23043.
[96] G. Werlen, et al., Dietary glucosamine overcomes the defects in αβ-T cell ontogeny caused by the loss of de novo hexosamine biosynthesis, Nat. Commun. 13 (2022) 7404.
[97] L. Araujo, P. Khim, H. Mkhikian, C.-L. Mortales, M. Demetriou, Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation, Elife 6 (2017) e21330.
[98] Y. Miao, et al., The activation of PPARγ enhances Treg responses through up-regulating CD36/CPT1-mediated fatty acid oxidation and subsequent N-glycan branching of TβRII/IL-2Rα, Cell Commun. Signal. 20 (2022) 48.
[99] Y. Döring, E.P.C. van der Vorst, C. Weber, Targeting immune cell recruitment in atherosclerosis, Nat. Rev. Cardiol. 21 (2024) 824–840.
[100] D.W. Scott, R.P. Patel, Endothelial heterogeneity and adhesion molecules N-glycosylation: implications in leukocyte trafficking in inflammation, Glycobiology 23 (2013) 622–633.
[101] D. Teng, et al., The effects of glycosylation modifications on monocyte recruitment and foam cell formation in atherosclerosis, Biochim. Biophys. Acta 1870 (2024) 167027.
[102] J.W. Homeister, A. Daugherty, J.B. Lowe, α(1,3)Fucosyltransferases FucT-IV and FucT-VII control susceptibility to atherosclerosis in apolipoprotein E−/− mice, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1897–1903.
[103] D. Dasagrandhi, A. Muthuswamy, J.K. Swaminathan, Atherosclerosis: nexus of vascular dynamics and cellular cross talks, Mol. Cell. Biochem. 477 (2022) 571–584.
[104] Y. Döring, et al., Deficiency of the sialyltransferase St3Gal4 reduces Ccl5-mediated myeloid cell recruitment and arrest, Circ. Res. 114 (2014) 976–981.
[105] F. Bonacina, L. Da Dalt, A.L. Catapano, G.D. Norata, Metabolic adaptations of cells at the vascular-immune interface during atherosclerosis, Mol. Asp. Med. 77 (2021) 100918.
[106] J.J. García-Vallejo, et al., Activation of human endothelial cells by tumor necrosis factor-α results in profound changes in the expression of glycosylation-related genes, J. Cell. Physiol. 206 (2006) 203–210.
[107] B.K. Chacko, D.W. Scott, R.T. Chandler, R.P. Patel, Endothelial surface N-Glycans mediate monocyte adhesion and are targets for anti-inflammatory effects of peroxisome proliferator-activated receptor γ ligands, J. Biol. Chem. 286 (2011) 38738–38747.
[108] D.W. Scott, et al., Role of endothelial N-glycan mannose residues in monocyte recruitment during Atherogenesis, Arterioscler. Thromb. Vasc. Biol. 32 (2012) e51–e59.
[109] K. Regal-McDonald, B. Xu, J.W. Barnes, R.P. Patel, High-mannose intercellular adhesion molecule-1 enhances CD16+ monocyte adhesion to the endothelium, Am. J. Phys. Heart Circ. Phys. 317 (2019) H1028–H1038.
[110] M. Nahrendorf, Myeloid cell contributions to cardiovascular health and disease, Nat. Med. 24 (2018) 711–720.
[111] J. Nour, et al., Mannose receptor deficiency impacts bone marrow and circulating immune cells during high fat diet induced obesity, Metabolites 12 (2022).
[112] G.J. Koelwyn, E.M. Corr, E. Erbay, K.J. Moore, Regulation of macrophage immunometabolism in atherosclerosis, Nat. Immunol. 19 (2018) 526–537.
[113] M. Svecla, et al., Impact of Asialoglycoprotein receptor and mannose receptor deficiency on murine plasma N-glycome profiles, Mol. Cell. Proteomics 22 (2023).
[114] A. Yang, et al., Hypomorphic sialidase expression decreases serum cholesterol by downregulation of VLDL production in mice, J. Lipid Res. 53 (2012) 2573–2585.
[115] R.J. Solá, K. Griebenow, Glycosylation of therapeutic proteins, BioDrugs 24 (2010) 9–21.
[116] F. Higel, A. Seidl, F. Sörgel, W. Friess, N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and fc fusion proteins, Eur. J. Pharm. Biopharm. 100 (2016) 94–100.
[117] Q. Zhou, H. Qiu, The mechanistic impact of N-glycosylation on stability, pharmacokinetics, and immunogenicity of therapeutic proteins, J. Pharm. Sci. 108 (2019) 1366–1377.
[118] P. Zhang, et al., Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs, Drug Discov. Today 21 (2016) 740–765.
[119] T. Nishiyama, et al., N-Glycans protect proteins from protease digestion through their binding affinities for aromatic amino acid Residues1, J. Biochem. 127 (2000) 427–433.
[120] S. Chia, et al., Enhancing pharmacokinetic and pharmacodynamic properties of recombinant therapeutic proteins by manipulation of sialic acid content, Biomed. Pharmacother. 163 (2023) 114757.
[121] O. Khorev, D. Stokmaier, O. Schwardt, B. Cutting, B. Ernst, Trivalent, Gal/GalNAc-containing ligands designed for the asialoglycoprotein receptor, Bioorg. Med. Chem. 16 (2008) 5216–5231.
[122] P. Kandasamy, et al., Metabolically stable anomeric linkages containing GalNAc–siRNA conjugates: an interplay among ASGPR, glycosidase, and RISC pathways, J. Med. Chem. 66 (2023) 2506–2523.
[123] F. Kevin, et al., A highly durable RNAi therapeutic inhibitor of PCSK9, N. Engl. J. Med. 376 (2025) 41–51.
[124] N. Paul, et al., Variant ASGR1 associated with a reduced risk of coronary artery disease, N. Engl. J. Med. 374 (2025) 2131–2141.
[125] J.-Q. Wang, et al., Inhibition of ASGR1 decreases lipid levels by promoting cholesterol excretion, Nature 608 (2022) 413–420.
[126] Y. Zhang, et al., ASGR1 deficiency inhibits atherosclerosis in western diet–fed ApoE−/− mice by regulating lipoprotein metabolism and promoting cholesterol efflux, Arterioscler. Thromb. Vasc. Biol. 44 (2024) 2428–2449.
[127] M. Svecla, et al., ASGR1 deficiency improves atherosclerosis but alters liver metabolism in ApoE-/- mice, Cardiovasc. Diabetol. 23 (2024) 428.
[128] V. Zoldoš, T. Horvat, G. Lauc, Glycomics meets genomics, epigenomics and other high throughput omics for system biology studies, Curr. Opin. Chem. Biol. 17 (2013) 34–40.
[129] G. Lauc, A. Vojta, V. Zoldoš, Epigenetic regulation of glycosylation is the quantum mechanics of biology, Biochim. Biophys. Acta 1840 (2014) 65–70.
[130] T. Štambuk, M. Klasić, V. Zoldoš, G. Lauc, N-glycans as functional effectors of genetic and epigenetic disease risk, Mol. Asp. Med. 79 (2021) 100891.

[131] C. Menni, et al., Glycosylation of immunoglobulin G: role of genetic and epigenetic influences, PLoS ONE 8 (2013) e82558.
[132] A. Wahl, et al., IgG glycosylation and DNA methylation are interconnected with smoking, Biochim. Biophys. Acta Gen. Subj. 1862 (2018) 637–648.
[133] V. Zoldoš, et al., Epigenetic silencing of HNF1A associates with changes in the composition of the human plasma N-glycome, Epigenetics 7 (2012) 164–172.
[134] K. Suhre, et al., Fine-mapping of the human blood plasma N-glycome onto its proteome, Metabolites 9 (2019).
[135] E.C. Soo, J.P.M. Hui, Metabolomics in glycomics, in: J. Li (Ed.), Functional Glycomics: Methods and Protocols, Humana Press, Totowa, NJ, 2010, pp. 175–186, https://doi.org/10.1007/978-1-60761-454-8_12.
[136] W. Igl, et al., Glycomics meets lipidomics—associations of N-glycans with classical lipids, glycerophospholipids, and sphingolipids in three European populations, Mol. BioSyst. 7 (2011) 1852–1862.

Glossary

Glycan: a complex, often branched carbohydrate (polysaccharide) polymer composed of multiple sugar molecules (monosaccharides).
Glycolipid: lipid with glycan(s) attached.
Glycome: the entire set of glycans (complex carbohydrates) found in a specific sample, such as a biofluid, tissue, cell, or organism.
Glycoprotein: protein with glycan(s) attached.
Glycoproteomics: the study of protein glycosylation in its protein and attachment site context.
Glycosylation: enzymatic, co- and post-translational modification consisting of the attachment of monosaccharides or oligosaccharides to a protein or lipid.
GWAS: genome-wide association studies. GWAS exploits single nucleotide polymorphisms (SNPs), variations of a single nucleotide at a specific position in the genome, in a population. The principle of GWAS is based on linkage disequilibrium (LD) in the genome. LD is the non-random association of (SNP) alleles at separated chromosomal loci in a population. Thus, GWAS tests the hypothesis that a particular SNP either is the causative mutation itself or in the close vicinity (in LD) of the causative mutation that is associated with the trait.
Multi-omics: integration of various "omics" data types to understand biology at a system level. Each "omics" type represents a specific area of biology and includes but is not limited to genomics, epigenomics, transcriptomics, proteomics, glycomics, and metabolomics.
Hydrophilic interaction liquid chromatography (HILIC): a type of normal phase liquid chromatography that features a water layer around the stationary phase, essential for the separation mechanism. This technique is particularly useful for separating polar and hydrophilic compounds, including glycans.
Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS): combining the separation of liquid chromatography (LC) with the mass analysis of mass spectrometry (MS) using the soft conditions of electrospray ionization (ESI). This technique is particularly useful for analyzing complex mixtures.
Matrix-assisted laser desorption/ionization (MALDI-MS): a soft ionization technique used in mass spectrometry to analyze large biomolecules like proteins, peptides, and polymers, such as oligosaccharides, without causing significant fragmentation.
PNGase F (Peptide N-glycosidase F): an enzyme that cleaves *N*-acetylglucosamine (GlcNAc) moiety in *N*-linked glycans from the asparagine, resulting in a deaminated protein or peptide and a free glycan. This enzyme is widely used in glycoprotein analysis to study glycan structures and their roles in various biological processes.
Reversed-phase liquid chromatography (RP-LC): a widely used chromatographic technique where the stationary phase is non-polar (hydrophobic) and the mobile phase is polar in order to separate compounds based on their hydrophobicity.