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Review

The known unknowns of apolipoprotein glycosylation in health and disease

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SUMMARY

Apolipoproteins, the protein component of lipoproteins, play an important role in lipid transport, lipoprotein assembly, and receptor recognition. Apolipoproteins are glycosylated and the glycan moieties play an integral role in apolipoprotein function. Changes in apolipoprotein glycosylation correlate with several diseases manifesting in dyslipidemias. Despite their relevance in apolipoprotein function and diseases, the total glycan repertoire of most apolipoproteins remains undefined. This review summarizes the current knowledge and knowledge gaps regarding human apolipoprotein glycan composition, structure, glycosylation site, and functions. Given the relevance of glycosylation to apolipoprotein function, we expect that future studies of apolipoprotein glycosylation will contribute new understanding of disease processes and uncover relevant biomarkers and therapeutic targets. Considering these future efforts, we also provide a brief overview of current mass spectrometry based technologies that can be applied to define detailed glycan structures, site-specific compositions, and the role of emerging approaches for clinical applications in biomarker discovery and personalized medicine.

INTRODUCTION

Lipoproteins are lipid-protein complexes carrying lipids in circulation. Lipoproteins are the major determinants of lipid fractions in plasma (Feingold and Grunfeld, 2000). Apolipoproteins, the protein component of lipoprotein, are necessary for lipoprotein assembly, solubilizing lipid fractions into lipoproteins, interaction with receptors, and for the activity of lipid transport proteins and enzymes involved in lipoprotein metabolism. Apolipoproteins regulate the distribution of lipids between lipoproteins and peripheral tissues, thus, maintaining lipid levels in circulation (Dominiczak and Caslake, 2011). In addition, apolipoproteins mediate cell signaling and immune and inflammatory processes (Ren et al., 2019).

A key aspect of apolipoprotein physiology and function is glycosylation (Pirillo et al., 2021). Nearly all human apolipoproteins are glycosylated. Glycosylation of apolipoproteins occurs during synthesis and maturation in the ER-Golgi complex. *N*- and *O*-glycans are the predominant glycans observed on apolipoproteins. Altered glycosylation of apolipoproteins leads to dyslipidemias, a primary risk factor for vascular diseases (Koska et al., 2016). Aberrant glycosylation of apolipoproteins has been observed in several other diseases such as metabolic syndrome (Savinova et al., 2014), atherosclerosis (Tertov et al., 1992a), autoimmune diseases (Kondo et al., 2009), cancer (Kang et al., 2004), type 2 diabetes (Harvey et al., 2009), and neurological disorders (Qin et al., 2017). Genetic defects in the genes involved in the process of glycosylation (i.e., congenital disorders of glycosylation) result in defects in glycosylation of apolipoproteins, leading to hyperlipidemia, development delay, movement disorder, hepatic steatosis, and hepatosplenomegaly (Holleboom et al., 2011; Pirillo et al., 2021). Altogether, these studies suggest that changes in apolipoprotein glycosylation result in disease outcomes.

Although aberrant glycosylation is closely associated with the incidence of several diseases, underlying molecular mechanisms resulting in glycosylation changes in apolipoproteins leading to altered lipoprotein metabolism and disease progression remain poorly understood. This is due, in part, to the fact that classical approaches used to study lipoprotein physiology have focused on lipoprotein quantification (e.g., total LDL, HDL, VLDL), lipid profiling (e.g., triglyceride, cholesterol, phospholipids, and free fatty acids), and apolipoprotein quantitation. However, lipid levels alone do not fully explain residual cardiovascular risk despite lifestyle changes and lipid-lowering therapies (Renee Ruhaak et al., 2019). This emphasizes the

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need to investigate beyond quantitative levels to include qualitative changes in apolipoproteins to determine their role in disease onset and progression.

Besides oxidation, the contributions of post-translational modifications to apolipoprotein function are not currently well defined. Of the possible modifications, protein glycosylation plays multifaceted modulatory roles in apolipoprotein function, including ER-Golgi trafficking, lipoprotein assembly, receptor-binding, turnover, enzyme activity, and stability (Pirillo et al., 2021). Despite their varied roles, the full repertoire of protein glycosylation on human apolipoproteins has not yet been elucidated. Knowing the composition and structure of glycans present on each apolipoprotein and how each of these mediate apolipoprotein functions is the first step towards establishing molecular mechanisms between glycosylation and lipoprotein metabolism, advancing disease stratification, developing diagnostic markers, and identifying strategies to improve apolipoprotein function during hyperlipidemias. In this review, we summarize the current understanding of apolipoprotein glycosylation and highlight what is known and unknown regarding the specific *N*- and *O*-glycan structures and their impact on health and diseases. Given the current large knowledge gaps regarding apolipoprotein glycosylation, we also provide a brief overview of current and emerging technologies that can be applied to characterize apolipoprotein glycosylation in future studies. In addition, we highlight the value of mass spectrometry (MS) in determining detailed glycan structures and site-specific compositions and in emerging approaches for clinical applications for biomarker discovery and personalized medicine.

LIPOPROTEIN TYPES AND THEIR APOLIPOPROTEINS

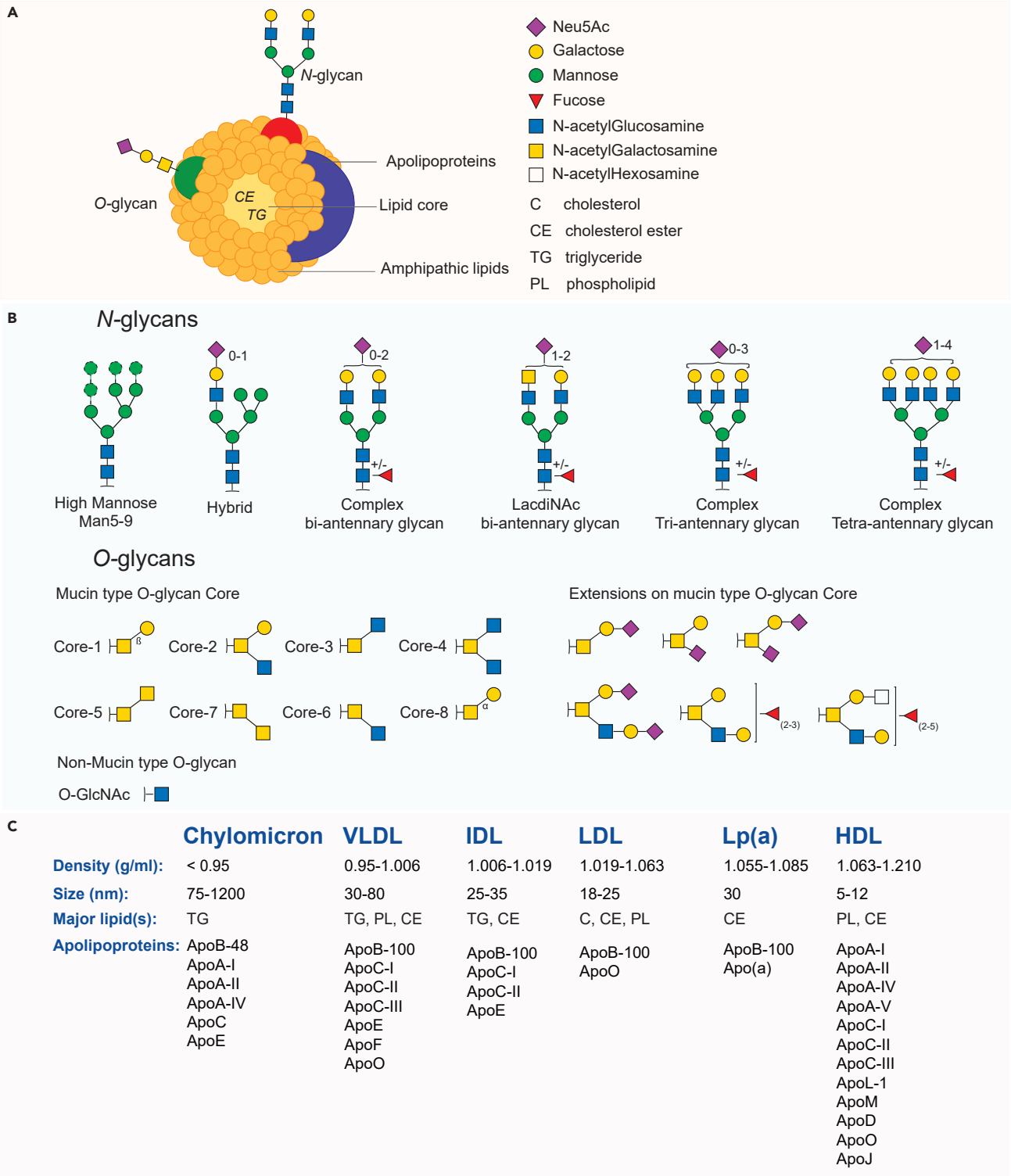
Human plasma lipoproteins are classified based on the apolipoprotein type and lipid composition into chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and lipoprotein(a) (Lp(a)). Lipoprotein structure, lipid composition, associated apolipoproteins found in human plasma lipoprotein, and overall *N*- and mucin-type *O*-glycan types found on apolipoproteins are summarized in Figure 1. The human apolipoprotein gene family consists of 12 apolipoprotein types, designated as A, B, (a), C, D, E, F, H, J, L, M, and O. Apolipoproteins A, B, C, and L contain multiple subtypes. Furthermore, human plasma apolipoproteins can be categorized into non-exchangeable and exchangeable apolipoproteins. ApoB-100, ApoB-48, and Apo(a) are the non-exchangeable apolipoproteins, other apolipoproteins can be exchanged between lipoprotein classes. An account of apolipoprotein types, their isoforms, genetic loci, plasma levels, and function is presented in Table 1.

GLYCANS ON APOLIPOPROTEINS AND THEIR BIOLOGICAL ROLE

Apolipoprotein A (ApoA)

ApoA is a major component of HDL and is primarily synthesized in the liver and intestine. There are four types of ApoA: A-I, A-II, A-IV, and A-V (Dominiczak and Caslake, 2011). ApoA-I, A-II, and A-IV are glycosylated proteins (Figure 2). ApoA-I forms 70% of apolipoprotein in HDL and is essential for the assembly of HDL. ApoA-I stabilizes ATP-binding cassette transporter-1 to mediate efflux of phospholipid and free-cholesterol to nascent HDL and is a modulator of lecithin cholesterol acyltransferase (LCAT) (Cooke et al., 2018). ApoA-I is atheroprotective in function and plasma ApoA-I levels are a predictor of myocardial infarction, cardiovascular diseases (CVD), inflammation, and cancer (Georgila et al., 2019). ApoA-I contains *O*-GalNAc glycans (i.e., mucin-type glycans) at Thr-221, and Ser-228 (Steentoft et al., 2013), but their composition remains unknown. In addition, the presence of *O*-GlcNAc has also been reported in ApoA-I (Cubedo et al., 2014). *O*-GlcNAcylation is a metabolic sensor and master regulator of cellular processes (Hart et al., 2011). Nonetheless, the physiological role of *O*-GlcNAc modification on ApoA-I is unclear. Desialylation of ApoA-I reduces LCAT activity *in vitro*. Desialylation diminishes the electronegative charges on ApoA-I which prevents ApoA-I from interacting with lipases and lipid transfer proteins. Desialylation of ApoA-I impairs the reverse cholesterol transport property of HDL and enhances tissue deposition of cholesterol leading to foam cell formation (Sukhorukov et al., 2019). Nonetheless, the extent of ApoA-I desialylation and its impact in disrupting lipid homeostasis *in vivo* or during clinical conditions need to be established.

ApoA-II constitutes 20% of apolipoprotein present in HDL and is an inhibitor of triglyceride metabolism enzymes (hepatic lipase and lipoprotein lipase) (Boucher et al., 2004; Julve et al., 2010). ApoA-II contains sialylated Core-1 *O*-glycan at Thr-42 (Halim et al., 2013; Hussain and Zannis, 1990). *O*-glycans on ApoA-II



	Chylomicron	VLDL	IDL	LDL	Lp(a)	HDL
Density (g/ml):	< 0.95	0.95-1.006	1.006-1.019	1.019-1.063	1.055-1.085	1.063-1.210
Size (nm):	75-1200	30-80	25-35	18-25	30	5-12
Major lipid(s):	TG	TG, PL, CE	TG, CE	C, CE, PL	CE	PL, CE
Apolipoproteins:	ApoB-48 ApoA-I ApoA-II ApoA-IV ApoC ApoE	ApoB-100 ApoC-I ApoC-II ApoC-III ApoE ApoF ApoO	ApoB-100 ApoC-I ApoC-II ApoE	ApoB-100 ApoO	ApoB-100 Apo(a)	ApoA-I ApoA-II ApoA-IV ApoA-V ApoC-I ApoC-II ApoC-III ApoL-1 ApoM ApoD ApoO ApoJ

Figure 1. Overview of lipoprotein structure, glycan compositions, and associated apolipoproteins of lipoproteins in humans

(A) Generic cartoon representation of human plasma lipoprotein highlighting the different major components. CE and TG make the lipid core, amphipathic lipids C and PL form the surface of the lipoprotein.

(B) Representative structures of *N*-glycans, mucin-type *O*-glycans, and non-mucin-type O-glycan (O-GlcNAc) observed on apolipoproteins.

(C) Major lipoprotein classes in human plasma. Density, particle size, lipid composition, and the associated apolipoproteins are indicated.

Table 1. Summary of human apolipoprotein characteristics

Apolipoprotein	UniProt Accession	Gene Loci	Molecular Weight (KDa)	Plasma levels (mg/dL)	Function[a]
ApoA-I	P02647	11q23.3	31.7	90–130	Cofactor for LCAT
ApoA-II	P02652	1q23.3	11.1	30–50	Inhibit LPL Displace ApoA-I
ApoA-IV	P06727	11q23.3	45.4	15–40	Modulate LPL Activate LCAT
ApoA-V	Q6Q788	11q23.3	41.2	0.025–0.040	Modulate LPL Activate LCAT
ApoB-100	P04114	2p24.1	515.6	90–100	Ligand for LDL receptor
Apo(a)	P08519	6q25.3–26	250–800	~2–10	Anti-angiogenic
ApoC-I	P02654	19q13.32	9.3	~6	Activate LCAT Inhibitor of LPL, CETP
ApoC-II	P02655	19q13.32	11.2	2–6	Activate LPL
ApoC-III	P02656	11q23.3	10.8	~12	Inhibitor of LPL, displace ApoE from LDL receptor
ApoC-IV	P55056	19q13.32	14.5	0.1–2	unclear
ApoD	P05090	13q29	21.3	5–25	Cofactor for LCAT, LPL
ApoE	P02649	19q13.32	36.2	3–8	Modulate LCAT, CETP, LPL. Ligand for LDL receptor
ApoF	Q13790	12q13.3	35.4	8–13	Inhibit CETP
ApoH	P02749	17q24.2	38.3	5–60	unclear
ApoJ	P10909	8p21.1	52.5	~9	unclear
ApoL-1	O14791	22q12.3	43.9	0.85	unclear
ApoM	O95445	6p21.33	21.2	2–5	HDL metabolism
ApoO	Q9BUR5	Xp22.11	22.5	0.22	unclear

[a]LCAT = Lecithin Cholesterol Acyl Transferase; LPL = Lipoprotein lipase; CETP = Cholesterol ester transfer protein; LDL-R = Low Density lipoprotein receptor.

are required for secretion, association with HDL particles, and clearance of HDL. Glycosylation increases the Golgi residence time and aids in the folding and secretion of ApoA-II. Sialylated *O*-glycans regulate the association of ApoA-II with HDL subtypes. Sialylated *O*-glycans alter the structure of ApoA-II by changing the conformation of β-turns and amphipathic helices and this selectively decreases the affinity of ApoA-II towards lipid-rich HDL2 and increases the affinity for lipid-poor fraction HDL3 (Remaley et al., 1993). The differential affinity of ApoA-II towards HDL subtypes modulates the activity and half-life of HDL. Although high plasma levels of ApoA-II have been associated with increased body fat, insulin resistance, and atherosclerosis (Corella et al., 2011), little is known about the role of glycans of ApoA-II in diseases. Although ApoA-IV is suggested to be glycosylated (Berthier et al., 2018; Weinberg and Scanu, 1983), detailed site-specific structures on ApoA-IV are not yet defined.

Apolipoprotein B (ApoB-100 and ApoB-48)

ApoB-100 is a major apolipoprotein component of LDL, IDL, VLDL, and Lp(a), and ApoB-48, a splice variant of ApoB-100, is present only in chylomicrons. ApoB-100 is synthesized in the liver, and whereas ApoB-48, the truncated version of ApoB-100 is formed by alternative splicing of RNA, containing 48% molecular weight of ApoB-100 (hence named ApoB-48) is synthesized in the small intestine. ApoB-100 and B-48 play important roles in the assembly and secretion of VLDL and chylomicrons respectively (Dominiczak and Caslake, 2011). ApoB-100 in LDL is a ligand for the LDL-receptor and is important for endocytosis of cholesterol-rich LDL. ApoB-100 is an *N*-glycosylated protein and glycans constitute 5–8% by protein weight. There are 19 potential *N*-glycosylation sites on ApoB-100, of which 17 sites are glycosylated

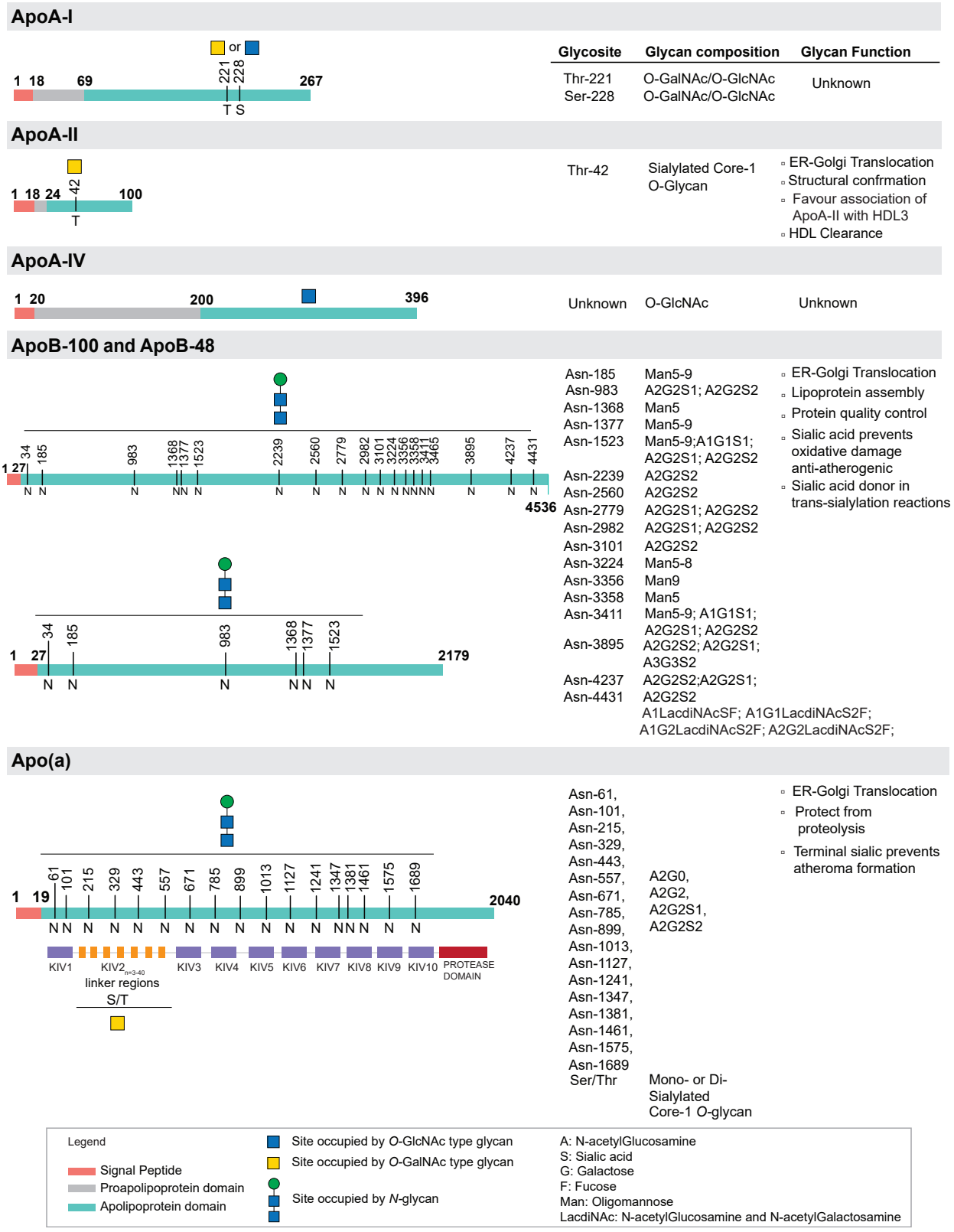


Figure 2. Summary of glycosylation site, glycan composition, and function of glycans observed on apolipoproteins A-I, A-II, A-IV, B-100, B-48, and Apo(a)

Protein sequences and glycosylation sites are based on UniProt annotations.

(Figure 2). Mono- and di-sialylated complex bi-antennary structures make up 60% of the *N*-glycans. Tri-antennary, bi-antennary with sialylated LacdiNAc, hybrid, truncated complex bi-antennary and high mannose type (Man 5–9) (Figure 1) structures make up the remainder (Garner et al., 2001a; Harazono et al., 2005; Sukhorukov et al., 2019). In ApoB-48, five out of six predicted *N*-glycosylation sites are glycosylated and contain sialylated complex bi-antennary type (78%) and high mannose type structures (Sasak et al., 1991).

N-glycans occupy distinct regions of ApoB, which include microsomal triglyceride transfer binding region, lipid-binding domain, and LDL receptor-binding domain. *N*-Glycans on the N-terminal microsomal triglyceride transfer binding region/lipid-binding domain are required for assembly, translocation through ER-Golgi apparatus, and secretion of VLDL (Vukmirica et al., 2002), in contrast, the presence of glycans near LDL receptor-binding domain had no impact on ApoB binding to LDL receptor (Harazono et al., 2005). Inhibition of *N*-glycosylation with tunicamycin delays ER exit and promotes proteasome-mediated degradation of ApoB (Liao et al., 1998). Thus, *N*-glycans are important for quality control for the proper folding of apoB. Glycosylation of intracellular ApoB has been studied in abetalipoproteinemia and Anderson's disease. In abetalipoproteinemia, intracellular ApoB-100 contains high mannose type *N*-glycans as the major structures, which suggests a defect in lipoprotein assembly at the endoplasmic reticulum. In contrast, in Anderson's disease, intracellular ApoB-48 contains complex-type glycans as the major structure, indicating defects in the post-Golgi secretion of lipoproteins. Thus, the glycosylation pattern of intracellular ApoB serves as a marker to assess intracellular defects in lipoprotein assembly and secretion observed in hereditary disorders (Berriot-Varoqueaux et al., 2001). Overexpression of GnT-III (N-acetylglucosaminyltransferase-III), an enzyme involved in the formation of bisecting GlcNAc in *N*-glycans, disrupts ApoB secretion and causes aberrant cellular morphology due to lipid accumulation (Ihara et al., 1998). This process is implicated in the progression of hepatocellular carcinoma. Similar mechanisms have been reported in hepatitis B-induced hepatocellular carcinoma. Viral protein X produced by the hepatitis B virus increases the expression of GnT-III to decrease the synthesis of ApoB and lipid accumulation leading to fatty liver and cancer (Kang et al., 2004).

The extent of sialylation of ApoB determines the atherogenic nature of LDL. Low ratio of sialic acid to ApoB content is a strong determinant of atheroma formation (Mezentsev et al., 2021). Although desialylation can favor clearance of LDL via ASGPR-mediated endocytosis (Demina et al., 2021), desialylation initiates physico-chemical changes such as lipid loss and reduced LDL particle size, acquisition of negative charge, reduced anti-oxidant levels in LDL, leading to oxidation and misfolding of ApoB (Orekhov et al., 2017). This causes aggregation of LDL particles that are not cleared by CD36 or scavenger receptors, leading to atheroma formation. In addition, desialylation exposes penultimate galactose-N-acetylglucosamine moieties, which are otherwise cryptic, to galactose-specific lectins on cell surfaces. Such interactions lead to intracellular lipid deposition in smooth muscle cells and macrophages (Tertov et al., 1992b). Thus, sialic acid plays a key role in maintaining the conformational stability of ApoB by preventing aggregation, subsequent modification of LDL, and atheroma formation. High plasma levels of neuraminidase/sialidase have been observed in CVD, stroke, diabetes, and inflammation, therefore, together with high plasma levels of LDL, an increase in neuraminidase adds to the severity of diseases (Demina et al., 2021). Likewise, silencing neuraminidase-1 exhibits cardioprotective action during ischemic injury (Zhang et al., 2018). Furthermore, LDL can serve as a donor and acceptor of sialic acids through the activity of trans-sialidases (Tertov et al., 2001). Among plasma lipoproteins, LDL has the highest affinity for trans-sialidases. The molecular dynamics of sialic acid exchange between the donor and acceptor glycoconjugates (trans-sialylation reaction) and its bearing in physiology and disease states are not completely understood. However, reports suggest that desialylation is one of the earliest modifications occurring on LDL, which initiates further modifications leading to vascular complications. Thus, the glycosylation status of ApoB can be deemed as one of the early markers for atherogenesis. Future efforts to define the specific interactions of hypo-glycosylated ApoB with cell surface receptors or lectins that are mediators of cell migration, signaling, and inflammatory process will be key to understanding the pathologies associated with LDL. It is expected that strategies to inhibit such interactions could be exploited as potential targets for therapeutic interventions.

Apolipoprotein (a) (Apo(a))

Apo(a) is a component of Lp(a). Lp(a) contains two apolipoprotein moieties ApoB-100 and Apo(a) covalently linked by a disulfide bond. Apo(a) is synthesized in the liver as two isoforms, low and high molecular weight protein (Utermann, 1989). The physiological roles of Apo(a) remain unclear; however, *in vitro* experiments suggest an anti-angiogenic function. Apo(a) inhibits ECM remodeling and signaling pathways (mitogen-activated protein kinase, p21-activated protein kinase, and α3β1integrin-mediated JNK pathway)

necessary for the proliferation and migration of endothelial cells (Kalaivani and Jaleel, 2020). Furthermore, Apo(a) inhibits activation of plasminogen (Romagnuolo et al., 2014) and is a ligand for megalin/Gp330 – endocytotic receptor involved in the reabsorption of glomerular filtrate (Niemeier et al., 1999). Apo(a) is a polymorphic protein made of a tri-loop structure 'kringle' and is a heavily glycosylated protein. *O*-glycans constitute 80% of total glycans and *N*-glycans constitute up to 20%. Mono-sialylated Core-1 *O*-glycan (NeuAcα2-3Galβ1-3GalNAc) account for 80% of *O*-glycans whereas non-sialylated and di-sialylated Core-1 *O*-glycan (Galβ1-3GalNAc and NeuAcα2-3Galβ1−3(NeuAcα2−6)GalNAc) make up 10–20% of total *O*-glycans in Apo(a) (Garner et al., 2001b). *N*-glycans in Apo(a) are mono- or di-sialylated complex bi-antennary type structures. However, the presence of truncated (non-sialylated complex bi-antennary and non-galactosylated complex bi-antennary glycans) structures have also been reported. The number of glycans on Apo(a) varies according to the molecular size of Apo(a) isoform. Altogether, the number of *N*-glycans can range from 14 to 40, whereas the number of *O*-glycans can range from 36 to 200, making Apo(a) the most heavily glycosylated apolipoprotein in human plasma (Figure 2).

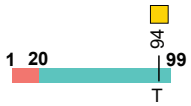
Glycans on Apo(a) are important for Lp(a) assembly and secretion, clearance, anti-angiogenic function, immune-complex formation, and foam cell formation. Glycosylation of Apo(a) determines the rate of synthesis and secretion of Lp(a) (Bonen et al., 1998). High molecular weight Apo(a) (heavily glycosylated) is retained longer in the ER-Golgi compartment, leading to lower levels of higher molecular weight Lp(a) isoform in circulation. *O*-glycans are essential for the stability of Apo(a) by preventing proteolysis and limiting the accumulation of Apo(a) fragments in atherosclerotic lesions and thrombus formation. High plasma levels of Lp(a) are an independent risk factor for cerebrovascular and cardiovascular diseases, pre-eclampsia, myocardial infarction, and ischemic stroke (Jones et al., 2007; Meekins et al., 1994). In addition to high plasma levels, desialylation increases the atherogenic potential of Lp(a). Desialylation exposes underlying galactose moieties in *N*- and *O*-glycans, making ApoA(a) a ligand for tissue lectin galectin-1 and circulating anti-glycan antibodies (Chellan et al., 2007; Kalaivani and Appukuttan, 2014; Mandagini et al., 2013; Sabarinath et al., 2014). Interactions with tissue galectin lead to foam cell formation in macrophages and anti-glycan antibody bound-Lp(a) immune complex increases the propensity of Lp(a) to be deposited on the vessel wall and trigger antibody-dependent cytotoxicity and atheroma formation (Sabarinath and Appukuttan, 2015). Similarly, glycans on Apo(a) are important for anti-angiogenic function of Lp(a). Deglycosylated and desialylated forms of Apo(a) fail to inhibit angiogenesis (Liu et al., 2013), impairing anti-angiogenic properties of Lp(a). Although several lines of evidence demonstrate a role for sialic acid in Lp(a)-related pathology, the exact mechanism of how terminal sialic acid or desialylated/truncated *N*- and *O*-glycans on kringle domains modulate Apo(a) mediated signaling cascades on endothelial cells, renal glomerular function, and lipid transfer is not fully defined.

Apolipoprotein C (ApoC)

ApoC is a component of chylomicrons, VLDL, and HDL. ApoC is mainly synthesized in the liver and to a small extent in the intestine, lung, and spleen (Jong Miek et al., 1999). There are four forms of ApoC (C-I, C-II, C-III, and C-IV). ApoC is an inhibitor of enzyme lipoprotein lipase which catalysis hydrolysis of triglycerides and regulates triglyceride levels in plasma (Larsson et al., 2013). ApoC is also an inhibitor of receptor-mediated uptake of lipoproteins, overexpression of ApoC is associated with hypertriglyceridemia and atherosclerosis. ApoC-I and ApoC-II are not glycosylated (Fojo et al., 1986). ApoC-III is *O*-glycosylated, containing sialylated Core-1 *O*-glycan at Thr-94. Three glycoforms of ApoC-III have been described, deglycosylated ApoC-III_{0a}, desialylated ApoC-III_{0b} (Galβ1−3GalNAc), mono-sialylated ApoC-III_1 (Galβ1−3[NeuAcα2−6]GalNAc) and di-sialylated ApoC-III_2 (NeuAcα2−3Galβ1−3[NeuAcα2−6]GalNAc) (Vaith et al., 1978). In addition, six new glycoforms of ApoC-III composed of multiple fucosylated *O*-glycan structures ($Hex_{2-3}HexNAc_{2-3}Fuc_{2-5}$) have been reported in the serum of healthy individuals and in urine of patients infected by *Schistosoma mansoni* (Balog et al., 2010; Nicolardi et al., 2013). ApoC-IV contains one sialylated complex-type *N*-glycan at Asn-63 (Kotite et al., 2003) (Figure 3).

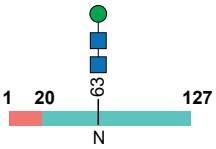
Sialylated *O*-glycans on ApoC-III mediate differential clearance of triglyceride-rich lipoproteins. Monosialylated ApoC-III containing lipoproteins are cleared via LDL receptor or LDL-receptor-like proteins, whereas the desialylated ApoC-III containing lipoproteins are cleared by binding to heparan sulfate proteoglycans (Kegulian Natalie et al., 2019). Physiologically, plasma levels of ApoC-III_{0a}, C-III_{0b}, and C-III_1 correlate with fasting plasma triglyceride levels, and ApoC-III_1 is the main regulator of plasma triglyceride levels (Yassine et al., 2015). *O*-glycosylation of ApoC-III is specifically initiated by the enzyme ppGalNAc-T2. Absence of *O*-GalNAc or the terminal sialic acid reduces the capacity of ApoC-III to inhibit LPL and cause

ApoC-III



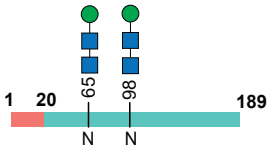
Glycosite	Glycan composition	Glycan Function
Thr-94	Mono- or Di-Sialylated Core-1 *O*-glycan Fuc2-5Hex2-3HexNAc2-3	▫ Modulate ApoC-III function and maintain TG levels ▫ Marker for CDGs

ApoC-IV



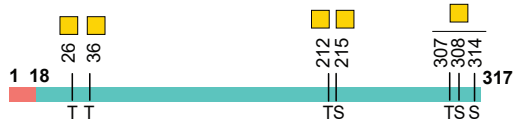
Asn-63	A2G2S2	Unknown

ApoD



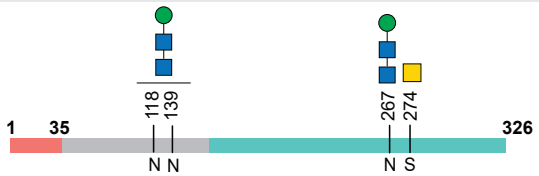
Asn-65	A3G3S2; A2G2S2; A3G3S3; A4G4S4;	▫ Modulate ApoD function
Asn-98	A2G2FS1; A2G2S2; A2G2S2F; A3G3S2F; A3G3S3F; A4FG4S3F	▫ Marker for neurological diesases and autism

ApoE



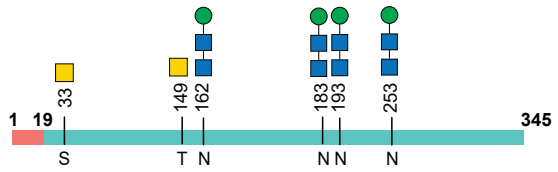
Thr-26 Thr-36 Thr-212 Ser-215 Thr-307 Ser-308 Ser-314	Mono- or Di-Sialylated Core-1 and Core-2 *O*-glycan Core-1/8 *O*-glycan	▫ ApoE receptor binding ▫ Modulate reverse cholesterol transport ▫ ApoE receptor binding ▫ Putative marker for Alzheimer's disease and dementia

ApoF



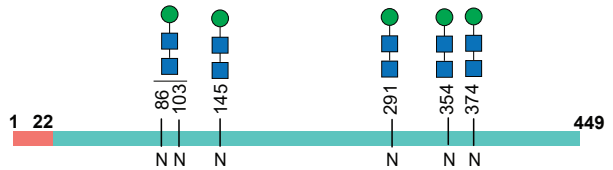
Asn-118 Asn-139 Asn-267 Thr-274	Man5-9; A2G2S1; A2G2S2 N.D Core-1/8 *O*-glycan	▫ Lipid transfer function

ApoH



Ser-33 Ser-149 Asn-162 Asn-183 Asn-193 Asn-253	*O*-GalNAc *O*-GalNAc A2G2S1; A2G2S2 N.D A3G3S1; A3G3S2 A3G3S3 N.D	▫ Structural conformation ▫ Involved in anti-phospholipid syndrome

ApoJ



Asn-86 Asn-103 Asn-145 Asn-291 Asn-354 Asn-374	A2G2S2; A2G2S1 A2G2S2; A2G2S2F A3G3S3; A3G3S3F A4G4S4	Unknown

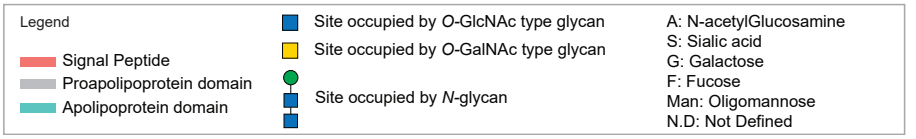


Figure 3. Summary of glycosylation site, glycan composition, and function of glycans observed on apolipoproteins C-III, C-IV, D, E, F, H, J

Protein sequences and glycosylation sites are based on UniProt annotations.

hypertriglyceridemia. The biological implications of poly-fucosylated *O*-glycan structures in ApoC-III function remain unknown. Plasma levels of ApoC-III glycoforms have been reported to vary in congenital disorders of glycosylation (CDG) of mucin-type *O*-glycans, mutations in sugar transporters, sialic acid metabolism, and Golgi traffic proteins. Genetic defects in *O*-glycosylation remarkably increase serum levels of ApoC-III_{0a} and decrease ApoC-$III_{1/2}$ glycoforms. High circulating levels of non-glycosylated ApoC-III_0 have been observed in GALNT2-CDG (Zilmer et al., 2020), glycogen storage disease III and IX (Ondruskova et al., 2018), and in the mutation of SLC35A1 (CMP-NeuAc transporter) (Ng et al., 2017) and Golgi traffic/Golgi maintenance proteins (Conserved Oligomeric Golgi complex subunits (COG subunits) (Foulquier et al., 2006), and vesicular H + -ATPase subunit (Kornak et al., 2008). On the contrary, hypersialylation of ApoC-III has been observed due to overproduction of CMP-NeuAc caused by loss of feedback control in the activity of enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine-kinase, the main enzyme in CMP-NeuAc biosynthesis. This results in elevated levels of α2-6NeuAc containing ApoC-III compared to α2-3 linked NeuAc (Wopereis et al., 2006). Similarly, by an unknown mechanism, increased ApoC-III_2 to ApoC-III_1 ratio was also observed in chronic renal dysfunction (Holdsworth et al., 1982). In addition to genetic defects related to *O*-glycosylation, desialylation of ApoC-III glycoforms has been observed in the acute phase of hemolytic uremic syndrome, where neuraminidase is released to circulation by *Streptococcus pneumonia* (Wopereis et al., 2003). Minor changes in ApoC-III have also been observed in Prader-Willi syndrome, PGM1 (phosphoglucomutase 1), and MAN1B1 (class 1B alpha-1,2-mannosidase) deficiency (Ondrušková et al., 2015). From these studies, it is evident that ApoC-III glycosylation is not only important in lipoprotein metabolism but also serves as a marker of diseases related to metabolic dysregulation of glycan biosynthesis, metabolic syndrome, and infection.

Apolipoprotein D (ApoD)

ApoD is a glycosylated protein associated mainly with HDL (Drayna et al., 1987). ApoD is synthesized in the brain, kidney, placenta, spleen, pancreas, and adrenal glands. ApoD is a cofactor for the enzyme LCAT and lipoprotein lipase (Steyrer and Kostner, 1988). ApoD is an *N*-glycosylated protein containing two *N*-glycan sites located at Asn-65 and Asn-98 (Halim et al., 2012; Schindler et al., 1995) (Figure 3). ApoD contains complex-type *N*-glycans of sialylated/fucosylated bi-, tri-, and tetra-antennary structures. Glycoproteomics analysis reveals sialylated tri-antennary structures as predominant structures occurring at Asn-65. However, sialylated di- and tetra-antennary species and tri-antennary species with N-acetyllactosamine (LacNAc) have also been observed at Asn-65. Both fucosylated and sialylated bi-antennary structures have been shown to occupy Asn-98. Other glycoforms, such as tri-antennary structures with or without LacNAc repeats have also been reported at Asn-98. Although earlier reports on the glycans of ApoD have indicated the presence of terminal fucose on tri-antennary structures (Schindler et al., 1995), recent studies indicate presence of core-fucosylated tri-antennary structures (Jia et al., 2009). The glycosylation pattern of ApoD varies among tissues depending on the site of biosynthesis. Compared to ApoD from plasma, ApoD in human brain is less glycosylated with respect to sialic acid content and modifications at the *N*-glycan core (Li et al., 2016). Likewise, ApoD produced in axillary gland cells contains high-mannose type glycans instead of tri-antennary structures at Asn-98 (Zeng et al., 1996). These observations suggest tissue-specific roles for ApoD glycoforms.

The absence of *N*-glycans on ApoD negatively affects the activity of LPL (Perdomo et al., 2010). ApoD levels positively correlates with signaling cascades associated with stress, inflammation, cancer, cardiovascular, and neurodegenerative conditions (Sanchez and Ganfornina, 2021). Sialylated ApoD glycoforms correlate with autism spectrum disorder (ASD). Sialylated ApoD (α2-3 Sialylated) is three-fold elevated in ASD when compared to age-matched controls (Qin et al., 2017). Furthermore, copy number variations in genes involved in glycosylation (B3GALT6, GCNT2, LARGE, GALNT9, B4GALT1, and GALNTL5) have been associated ASD. Thus, correlating copy number variations of glycogenes to glycosylation changes on ApoD could serve as a potential marker and target for diagnosis and studying the pathophysiology of ASD.

Although the glycan structures on ApoD have been investigated, their role in ApoD function involving lipid transport and homeostasis through interaction with small molecules, fatty acids, and other lipidic compounds, and immune and signaling cascade have not been clear. Based on differential glycosylation patterns observed in ApoD, it can be hypothesized that glycans on ApoD modulate physiology and pathological processes by interacting with cognate receptors. These mechanisms would have a profound influence on the progression of ASD and cancers, where glycosylation changes have been previously observed.

Thus, a detailed functional evaluation of differentially glycosylated ApoD is warranted to define its role more clearly in physiology and pathology.

Apolipoprotein E (ApoE)

ApoE is a component of several lipoproteins, including chylomicron remnants, VLDL, IDL, and HDL. ApoE is primarily synthesized in the liver and brain, and to some extent in macrophages, kidneys, and spleen. ApoE facilitates the binding of ApoB-containing lipoproteins to LDL receptors (Wilson et al., 1991). Three isoforms of ApoE differ by two amino acid residues at positions 112 and 158, ApoE-ε2 (Cys112, Cys158), ApoE-ε3 (Cys112, Arg158), and ApoE-ε4 (Arg112, Arg158). ApoE-ε3 is the most abundant isoform followed by ApoE-ε2 and ApoE-ε4. ApoE is an *O*-glycosylated protein containing seven *O*-glycan sites. Two sites (Thr-26 and Thr-36) are in the N-terminal region (receptor-binding region), two (Thr-212 and Ser-215) at the hinge region, and three sites (Thr-307, Ser-308, and Ser-314) at the C-terminus (lipid-binding region). Mono- and di-sialylated Core-1 *O*-glycans (Neu5Acα2–3Galβ1–3GalNAcα1 and Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAc) are the prominent structures observed (Flowers et al., 2020; Lee et al., 2010; Wernette-Hammond et al., 1989). In addition, Core-2 like structures comprising Hex(Hex-HexNAc)HexNAc at Thr-212 have also been reported (Halim et al., 2013) (Figure 3). Although glycosylation or sialylation status did not affect the rate of synthesis and extent of secretion of ApoE (Zanni et al., 1989), *O*-glycans at Ser-194 on ApoE are essential for the increased secretion of ApoE upon induction with oleic acid (Wernette-Hammond et al., 1989), but mechanisms involved are not yet defined.

ApoE is essential for the delivery of HDL cholesterol to the liver, this happens via interaction between ApoB/E receptors. Differential glycosylation at the N- and C-terminal regions of ApoE have been suggested to play an important role in the receptor-binding and lipid-binding property of ApoE (Flowers et al., 2020). In addition, desialylation of *O*-glycans in ApoE decreases ApoE binding to plasma HDL and impairs reverse cholesterol transport to the liver (Marmillot et al., 1999). A more recent study suggests that sialylated *O*-glycans at Ser-215 and Ser-308 of ApoE impart negative charge and hydrophilicity to the VLDL and facilitate interaction with positively charged amino acids at the ApoB/E receptor binding site (Ke et al., 2020). Desialylated *O*-glycans have been observed in ApoE Leiden, a variant of human ApoE associated with type III hyperlipoproteinemia. Lack of sialic acid affects VLDL clearance and causes hypercholesterolemia. Similarly, desialylated ApoE glycoforms have been observed in preeclampsia, metabolic syndrome, alcoholism, and diabetes (Atkinson et al., 2009; Savinova et al., 2014). Unlike ApoE secreted in plasma, ApoE produced in the brain through activated astrocytes and microglia exhibits a varied glycan distribution. ApoE found in cerebrospinal fluid (CSF) is ten times more glycosylated at the C-terminal lipid-binding domain and contains a larger proportion of di-sialylated Core-1 glycans, whereas plasma ApoE was predominantly glycosylated at the N-terminal region (Flowers et al., 2020). In patients with dementia, all ApoE isoforms were heavily glycosylated in CSF, compared to plasma. Percentage of glycosylation of ApoE4, a risk factor for Alzheimer's disease, in CSF was significantly greater than in other isoforms (Hu et al., 2020), suggesting a role for ApoE4 glycosylation in Alzheimer's disease. Moreover, ApoE isoforms have been shown to activate Akt/PKB phosphorylation and MAPK signaling neurons to induce amyloid-beta synthesis (Huang et al., 2019; Laffont et al., 2002). Based on these studies, a role for differentially glycosylated ApoE in HDL metabolism, brain function, and progression of Alzheimer's disease mediated via signaling cascades is suggested.

Apolipoprotein F (ApoF)

ApoF is synthesized in the liver and is a component of VLDL, LDL, and HDL. ApoF is an inhibitor of cholesterol ester transfer protein and increases the movement of cholesterol ester from HDL to VLDL and reduces cholesterol levels in circulation. ApoF in its pro-protein form contains three potential *N*-glycosylation sites located at Asn-118, Asn-139, and Asn-267, and one *O*-glycosylation site located at Thr-274. Mature ApoF protein contains only one *N*-glycan and one *O*-glycan site located at Asn-267 and Thr-274. Asn-118 and Asn-139 contain high-mannose structures and sialylated complex-type *N*-glycans at Asn-267 (Lagor et al., 2009; Morton et al., 2008). Glycoproteomic analysis of peptides from urine shows an additional *O*-glycan site at Thr-256 whose composition resembles Core-1 (Galβ1-3GalNAc) or Core-8 (Galα1-3GalNAc) like structures (Halim et al., 2012) (Figure 3). Desialylation of ApoF resulted in a significant reduction in lipid transfer inhibition of ApoF whereas de-*O*-glycosylation of ApoF resulted in the complete absence of lipid transfer inhibition activity (Morton et al., 2008). Although glycans are important for the lipid transfer property of ApoF, the precise mechanisms regarding how ApoF glycoforms modulate cholesterol homeostasis and hypercholesterolemia needs further investigation.

Apolipoprotein H (ApoH or Beta-2-glycoprotein 1)

ApoH or beta-2-glycoprotein 1 (B2GPI) is an apolipoprotein belonging to the complement control protein superfamily of proteins, found on chylomicrons, VLDL, and HDL. The physiological role of ApoH in lipoprotein metabolism is unclear. ApoH has four *N*-glycan sites and one *O*-glycan site located at Asn-162, Asn-183, Asn-193, Asn-253, and Thr-149 respectively (Liu et al., 2005; Lozier et al., 1984). Asn-162 contains α2-6 sialylated bi-antennary type structures and Asn-193 contains α2-6 sialylated tri-antennary type structures (Figure 3). Nevertheless, truncated bi- and tri-antennary structures constitute 5–10% of total *N*-glycans observed in ApoH (Kondo et al., 2009). ApoH is a target of anti-phospholipid antibodies and is implicated in diseases such as anti-phospholipid syndrome and systemic lupus erythematosus. Glycans are important in maintaining the closed circular conformation of ApoH. Deglycosylation favors hockey stick conformation which promotes its interaction with negatively charged phospholipid, making ApoH a target for anti-phospholipid antibodies (Hammel et al., 2002). In patients with antiphospholipid syndrome, ApoH had lower sialylated tri-antennary glycans and high levels of sialylated bi-antennary glycans at Asn-193 (Kondo et al., 2009). Taken together, both desialylation and deglycosylation make ApoH susceptible to recognition by anti-B2GPI antibodies, which are driving factors for inflammatory response occurring in anti-phospholipid syndrome and lupus. Inhibiting sialidases or glycosidases are therefore potential therapeutic strategies for the treatment of autoimmune diseases associated with ApoH glycosylation.

Apolipoprotein J (ApoJ)

ApoJ is a component of HDL and LDL and is produced mainly in the liver, brain, testis, and ovary, and to a lesser extent in the heart, lung, spleen, and mammary gland (DeSilva et al., 1990). The role of ApoJ in lipoprotein metabolism is unclear. ApoJ contains six *N*-glycans at Asn-86, Asn-103, Asn-145, Asn-291, Asn-354, and Asn-374. Seven distinct types of *N*-glycans have been reported which include mono or di-sialylated biantennary structures with and without fucose, sialylated tri-antennary structures with and without fucose, and sialylated tetra-antennary structures (Figure 3). Di-sialylated bi-antennary structures without fucose were the most predominant glycoform (Kapron et al., 1997). Overall, little is known about the biology of ApoJ and the functions of attached glycans.

Apolipoprotein L (ApoL)

ApoL-1 is associated with HDL. There are six human APOL genes (designated as APOL1-6) located on Chr 22q12. ApoL-1 is produced in the pancreas, kidney, brain, vascular endothelium, liver, heart, lung, and placenta. ApoL-1 confers protection against trypanosome infection (Pérez-Morga et al., 2005), but elevated levels of ApoL-1 in podocytes cause kidney failure (Bruggeman et al., 2021). ApoL-1 contains one potential *N*-glycan site at Asn-261 and *O*-glycan at Thr-59 (Figure 4), but their composition and function in lipid transfer or metabolism have not been determined. Delineating mechanisms involving glycans on ApoL-1 causing lysis of trypanosome and kidney damage will be crucial towards understanding the biological role of ApoL.

Apolipoprotein M (ApoM)

ApoM is a 26kDa protein found in HDL, LDL, and chylomicrons and is synthesized in the liver, kidney, and adipocytes. ApoM is a cofactor for the enzyme lipoprotein lipase and functions as an anchor between sphingosine-1-phosphate (S1P) and HDL. ApoM is suggested to modulate HDL metabolism and exhibit anti-atherosclerotic functions. HDL-ApoM-S1P complex activates signaling cascades that maintain vascular integrity and decrease inflammatory reactions (Christoffersen et al., 2011) and recent reports have described the role of ApoM in triglyceride turnover and renal diseases (Bisgaard and Christoffersen, 2021). ApoM has one *N*-glycosylation site at Asn-135. Five glycoforms of ApoM comprising the sialylated, desialylated, and non-glycosylated types have been reported (Chen et al., 2009; Karlsson et al., 2005; Liu et al., 2005; Xu and Dahlbäck, 1999) (Figure 4). Overall, relatively little is known about the biological role of ApoM and function of glycosylation on ApoM.

Apolipoprotein O (ApoO)

ApoO is a component of HDL, LDL, and VLDL. ApoO is the only apolipoprotein located on the X chromosome (Xp22.1) and is expressed in the heart, brain, and kidney. ApoO contains chondroitin sulfate and is the only apolipoprotein to contain proteoglycans (Lamant et al., 2006) (Figure 4). ApoO has been implicated to play a role in mitochondrial dysfunction and cardiomyopathy. Intracellular ApoO has been shown to cause accumulation of lipotoxic byproducts leading to mitochondrial damage, lipo-apoptosis, cardiomyopathy, and loss of cardiac cells, in type 2 diabetes (Turkieh et al., 2014). The presence of proteoglycan-like glycans among plasma apolipoproteins is unusual. Therefore, it is possible that glycosylation may act as a

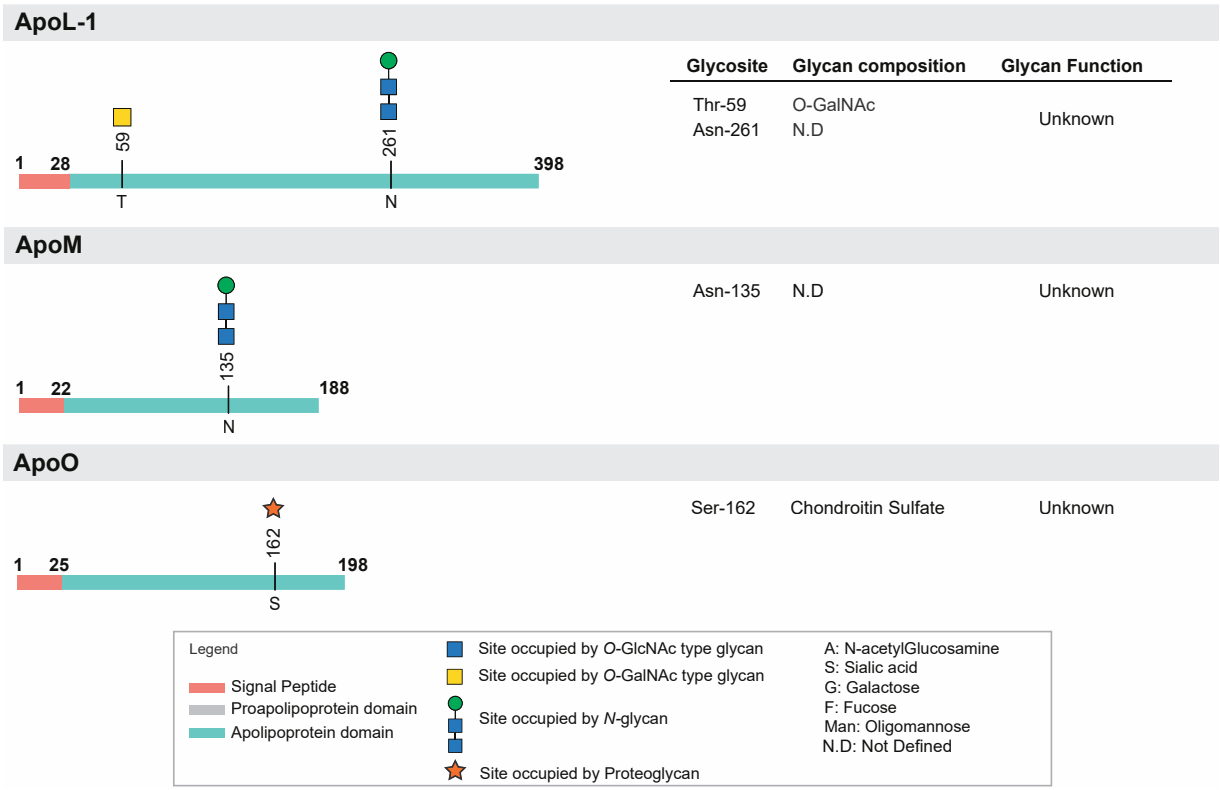


Figure 4. Summary of glycosylation site, glycan composition, and function of glycans observed on apolipoproteins L-1, M, O
Protein sequences and glycosylation sites are based on UniProt annotations.

regulatory switch in determining intracellular and extracellular functions of ApoO. Understanding the structure, biosynthesis, and function of chondroitin sulfate-like structures in ApoO will be crucial in delineating the intracellular and extracellular functions of ApoO.

APPROACHES TO STUDY APOLIPOPROTEIN GLYCOSYLATION

Overview of approaches

The previous studies reviewed here provide evidence that alterations in apolipoprotein glycosylation include hypo- and hyper-glycosylation and changes in the glycan class at specific glycosites. However, our current view of the detailed glycan structures present on human apolipoproteins and their molecular mechanisms leading to glycosylation changes observed in disease remains limited, emphasizing the need for additional studies. Biochemical approaches combining lipoprotein fractionation techniques and MS can be used to determine the composition, structure, and site-specific information of glycans on apolipoproteins. The choice of sample preparation and MS analysis method directly influences the depth of coverage that can be achieved.

Considerations for apolipoprotein isolation for glycomics and glycoproteomics

Lipoproteins are associated with redox enzymes and inflammatory proteins found in circulation (Krishnan et al., 2017). These associated proteins may be present as contaminants in isolated lipoproteins, complicating glycan analysis. Consequently, obtaining pure lipoproteins from complex biological matrices like plasma, CSF, lymph, or tissue is therefore the first challenge to be addressed when studying apolipoprotein glycosylation and function. Although density gradient ultracentrifugation (Hoofnagle and Heinecke, 2009) and methods of lipoprotein fractionation that include size exclusion chromatography and precipitation are broadly used for lipoprotein isolation (Burstein et al., 1970; Gordon et al., 2010), size and density-based separation alone cannot purify all lipoprotein moieties from extracellular vesicles because of their overlapping sizes (e.g., exosomes range 40–100 nm (Brennan et al., 2020; Yuana et al., 2014)). Consequently, glycans present on any of the proteins from these vesicles will contribute to the pool of released glycans.

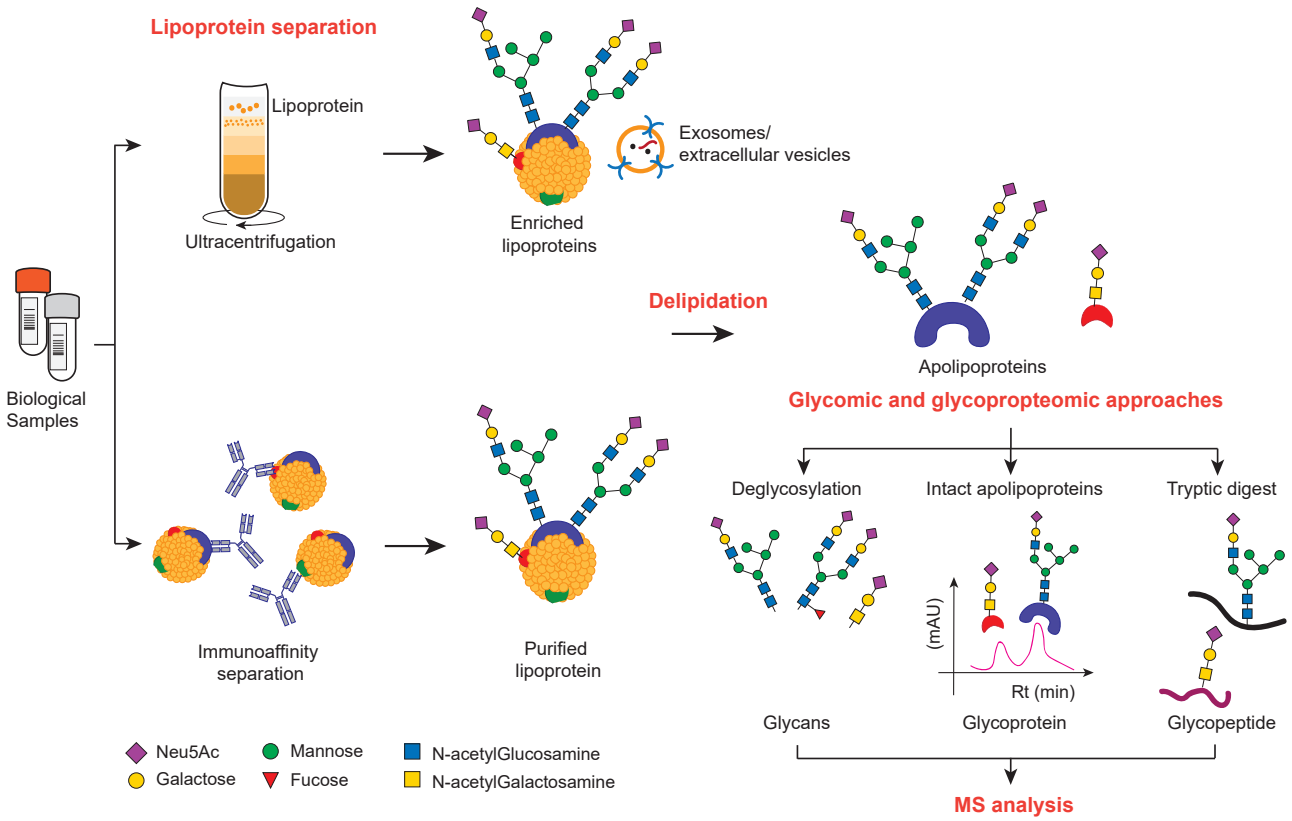


Figure 5. Overview of the sample preparation and MS-based analysis strategies for apolipoprotein glycan characterization

To overcome these challenges, immunoprecipitation, electrophoretic separation, and chromatography can be used to enhance purity of isolated lipoproteins (Krishnan et al., 2015; Rodríguez et al., 2022; Santos Seckler et al., 2021). Overall, a combinatorial approach utilizing density, immunoaffinity, and chromatographic separation is generally recommended for obtaining apolipoproteins of sufficient purity for glycomic and glycoproteomic analyses (Figure 5).

MS-based approaches to study apolipoprotein glycosylation

MS approaches can be broadly categorized based on the level of detail that can be achieved. The analysis of intact glycopeptides allows for the determination of glycan composition (i.e., identity of monosaccharides present in the glycan) and site-specific information. The determination of structural details (i.e., the order and linkages between monosaccharides in the glycan) requires analysis of glycans that have been chemically or enzymatically released from the protein backbone. MS analysis of released glycans includes MS1-level data (i.e., mass of the glycan) that can be used to inform glycan composition and MS/MS data (i.e., measurement of gas-phase fragment ions of the glycan) that provides information on degree of branching and elongation. Importantly, although liquid chromatography or capillary electrophoresis-based separation followed by MS analysis is applicable to glycan structure elucidation and quantification, structural elucidation of novel glycans requires validation by exoglycosidase or endoglycosidase digestion. A detailed overview of advances in glycomic strategies is summarized in recent reviews (Dong et al., 2018; Donohoo et al., 2022)

Determining the glycan compositions present at specific amino acid residues is key to identifying apolipoprotein glycoforms (Oliveira et al., 2021). There are two glycoproteomic approaches currently applied to study apolipoprotein glycoforms - namely protein-level and peptide-level analyses. Protein level analyses have been used to determine the relative distribution of ApoC-III glycoforms using mass spectrometric immunoassay (MISA) (Rodríguez et al., 2022). Other studies have performed top-down proteomic analysis of intact apolipoproteins to determine fatty acid-modified ApoA-I, ApoA-II, and ApoC-III glycoforms.

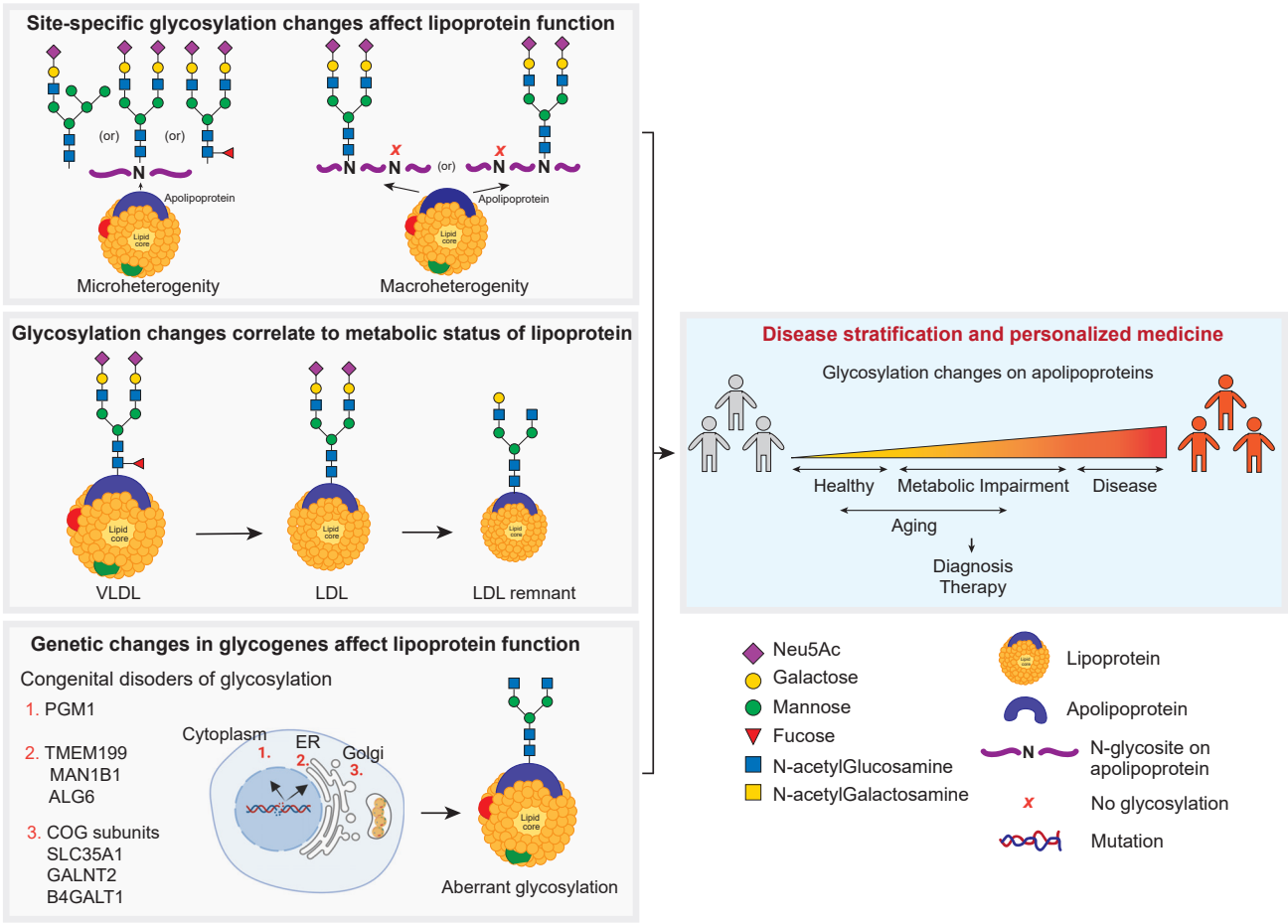


Figure 6. Summary of the unknown aspects of apolipoprotein glycosylation

Top left: Site-specific changes in glycosylation of various apolipoproteins and their impact on lipoprotein function. Middle left: Glycosylation changes on apolipoproteins during metabolism, Bottom left: Genetic changes leading to defective lipoprotein function. Right: The factors from the panels on the left can altogether impact apolipoprotein function and influence the transition from normal to disease states. Such changes may be exploited for the development of approaches toward disease stratification and new therapies. This figure was generated with BioRender.com and Adobe Illustrator.

Through these approaches, inter-individual proteoforms of apolipoproteins correlating with underlying diseases were identified (Demus et al., 2021; Nicolardi et al., 2013; Seckler et al., 2018; Wilkins et al., 2021). High-resolution top-down proteomics of intact high molecular weight apolipoproteins will be a promising approach for characterizing apolipoproteins >50kDa that exist in multiple glycoforms (McGee et al., 2021). For larger molecular weight and heavily glycosylated apolipoproteins (>200kDa), such as ApoB-100 and Apo(a), glycopeptide analysis remains the method of choice (Garner et al., 2001a,2001b).

PERSPECTIVES AND CONCLUSION

Implications of structural studies on apolipoproteins

Although aberrantly glycosylated apolipoproteins have been strongly correlated with lipoprotein-associated diseases, such as dyslipidemia, CVD, stroke, obesity, cancer, type 2 diabetes, and neurological disorders, several structural details remain unknown. Of the structures that have been characterized to date, most of these pertain to *N*-glycans, whereas *O*-glycans have not garnered much attention. Therefore, structure and type of extension in mucin-type *O*-glycans and information on the presence and site occupancy of non-mucin-type *O*-glycans, including *O*-GlcNAc, *O*-Man, and *O*-Fuc, are yet to be established. Among the changes that occur within the apolipoprotein glycome, the primary focus has been on loss of terminal sialic acid (desialylation). Little emphasis has been given to other monosaccharide moieties, such as mannose, galactose, core-fucose/terminal-fucose, and LacdiNAc, which are known to be altered during diseases processes. Another important aspect

for consideration is that glycosylation changes on apolipoproteins reported thus far are derived from small cohorts. So far, there is only one large-scale study focused on the glycans of ApoC-III (Demus et al., 2021). Considering the diversity in sample set and extent of glycosylation, studies on larger cohorts will be useful in correlating precise changes in apolipoprotein glycosylation to a specific disease with statistical power. Combining information on apolipoprotein glycosylation and expression of glycogenes will establish the role of specific glycan moieties in apolipoprotein function, enabling identification of disease markers for impaired lipoprotein metabolism. In addition, glycan profiles of apolipoproteins can serve as an index to design treatment modalities to restore lipoprotein function and prevent atherosclerosis. Glycomimetics and other inhibitors of deglycosylating enzymes (trans-sialidase/neuraminidase) have been proposed as a suitable therapy to mitigate the pro-atherogenic properties of desialylated LDL and inflammation (Sobenin et al., 2021). Alternatively, anti-glycan antibodies that can specifically scavenge desialylated lipoproteins can be administered as intravenous immunoglobulin (IVIG) therapy (Markina et al., 2020). The practical application of these potential therapies will require a close tracking of the glycosylation status of apolipoproteins. In addition, a direct readout based on glycan profiling could be a promising strategy in assessing the prognostic effects of glycomimetic drugs in prospective direct anti-atherosclerotic therapy.

Prospective directions

It is expected that having an in-depth understanding of the glycosylation patterns of apolipoproteins will be essential for informing qualitative and quantitative assessments of glycosylation changes occurring during metabolic and disease processes. Major unanswered questions are conceptually summarized in Figure 6 and include: (1) How does apolipoprotein glycosylation relate to lipid levels, age, sex, and diseases? (2) How does glycan composition and its diversity (micro- and macro-heterogeneity) regulate or fine-tune apolipoprotein function? (3) Is a select apolipoprotein class more vulnerable to glycosylation changes or are these changes global? (4) If so, how do these changes correlate with the expression or activity of glycosyltransferases and the metabolic status of cells? Studies that address one or more of these questions could aid the development of novel diagnostic tools, approaches to disease stratification, and new therapeutic interventions. Although these questions could be addressed through large-scale studies of clinical samples, such studies are currently challenging because of the limited availability of high-throughput methods for lipoprotein and glycan analysis. Continued development of advanced methodologies for high throughput lipoprotein isolation, glycan structure measurements, and glycan data analysis will benefit future studies to expand our understanding of apolipoprotein glycobiology.

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AUTHOR CONTRIBUTIONS

S.P.S. conceived the study and performed the literature search; S.P.S. and R.L.G. prepared and edited figures; S.P.S. wrote the first full draft of the manuscript and both authors contributed to editing and approved the final manuscript.

DECLARATION OF INTERESTS

Authors have no competing interest to declare.

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