IMPACT OF PROTEIN GLYCOSYLATION ON LIPOPROTEIN METABOLISM AND ATHEROSCLEROSIS

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Abstract

Protein glycosylation is a post-translational modification consisting of the enzymatic attachment of carbohydrate chains to specific residues of the protein sequence. Several types of glycosylation have been described, with N-glycosylation and O-glycosylation being the most common types impacting on crucial biological processes, such as protein synthesis, trafficking, localization, and function. Genetic defects in genes involved in protein glycosylation may result in altered production and activity of crucial proteins, with a broad range of clinical manifestations, including dyslipidemia and atherosclerosis. A large number of apolipoproteins, lipoprotein receptors, and other proteins involved in lipoprotein metabolism are glycosylated, and alterations in their glycosylation profile are associated with changes in their expression and/or function. Rare genetic diseases and population genetics have provided additional information linking protein glycosylation to the regulation of lipoprotein metabolism.

Keywords: Glycosylation; dyslipidaemias; atherosclerosis

Introduction

Biological systems rely upon post-translational modifications (PTMs) to widen the structural heterogeneity of proteins. Glycosylation is one of the major protein PTMs, which consists in the covalent attachment of glycan moieties to proteins. This process affects a wide range of biological processes, including protein folding, processing, and secretion as well as protein function, by regulating key processes, such as protein intracellular/cell surface localization, cell adhesion, self/non-self-recognition, molecular trafficking, receptor activation, and endocytosis.[1, 2]

Protein glycosylation includes i) N-glycosylation, which occurs on an asparagine residue (Asn) when present in the motif Asn-X-Ser/Thr (where X is any aminoacid, except proline); ii) *O*-glycosylation, which occurs on serine (Ser) or threonine (Thr) residues; iii) addition of long chain sugars to a core protein forming glycosaminoglycans (also known as proteoglycans).[3] Although the interaction of lipoproteins with proteoglycans plays a key role in lipoprotein retention in the arterial wall, the description of glycosaminoglycan biology goes beyond the scope of this review and will not be addressed here. Also C-mannosylation, which usually occurs at the first tryptophan (Trp) in the consensus aminoacid motif Trp-X-X-Trp and glypiation, the attachment of glycosylphosphatidylinositol (GPI) to proteins where the glycan core structure has a phospholipid tail, exist but will not be covered in this review.

The most abundant type of glycosylation is N-glycosylation, which is present in secreted and membrane-bound glycoproteins (Figure 1). This process starts in the endoplasmic reticulum (ER), where the glycan core, composed of 14 glycan residues is transferred from a dolichol-linked oligosaccharide to the nascent protein.[4] This is followed in the ER by the sequential trimming of glycans to result in the desired protein folding and by quality control by interaction with ER chaperones (calreticulin/calnexin), that recognize specific features of the trimmed N-glycans. During this step, mature ER glycoproteins with either eight- or nine-mannose residues are transferred from ER to Golgi apparatus, where a series of glycosidases and glycosyltransferases remodel N-glycoproteins generating glycoproteins in the oligomannose form (where terminal glycan is mannose), complex form (where the terminal glycan is sialic

acid, also known as N-acetylneuraminic acid), and hybrid form (combination of the first two types) (Figure 1).[3, 4]

The second common form of glycosylation is *O*-glycosylation, which occurs mainly in the Golgi apparatus through the action of N-acetylgalactosamine transferases (GALNTs).[5] This pattern of glycosylation is present in mucous secretions and transmembrane glycoproteins of the cell surface with the glycan exposed to the external environment. O-glycosylated proteins are involved in cell recruitment, cell interaction and activation of immune response.

Alterations in glycosylation patterns associate with a number of pathological conditions, which may include dyslipidemias and, as a consequence, atherosclerosis. This has been mainly appreciated when plasma lipid profile and cardiovascular outcomes were investigated in subjects with genetic defects of glycosylation.[6] Furthermore, genome wide association studies (GWAS) identified several loci in glyco-genes[7-9] that impact plasma lipids and lipoproteins, raising the question of how alterations in apolipoprotein and lipoprotein receptor glycosylation, by affecting their expression and/or function, might impact on lipid metabolism and, perhaps, cardiovascular disease risk. It is also noteworthy mentioning that, among plasma proteins, glycosylation pattern of immunoglobulins G changes with age, with some traits being associated with an increased cardiovascular risk.[10] Whether ageing might play a role also in lipoprotein (as well as lipoprotein-related proteins) glycosignature remains to be addressed. Nevertheless, increased plasma total sialic acid (TSA, bound and free) was suggested as possible marker of atherosclerosis and CVD in the general population: two large studies with long follow-up have shown that high serum levels of sialic acid are an independent risk factor for CAD.[11, 12] However, this aspect needs to be fully elucidated, as conflicting results have been reported in patients with CAD: some studies have proposed a positive relationship between plasma TSA levels and coronary artery disease severity,[13, 14] or, as alternative, a relation with complications of CAD rather than with CAD severity,[15] while other studies have failed to find such a relationship.[16, 17]

Although the importance of glycosylation in modulation lipoprotein functions is recognized, a comprehensive discussion on how and whether changes in their glycosylation pattern play a

role in atherosclerosis and cardiovascular disease is lacking. Aim of this review is to discuss genetic evidences linking changes in protein glycosylation with alterations in plasma lipid profile and atherosclerosis, and discuss protein glycosylation processes that affect proteins/enzymes involved in lipoprotein metabolism and/or function, lipoprotein receptors and, whenever known, their potential relevance in atherosclerosis.

Insights from inherited disorders of protein glycosylation and dyslipidaemias

Congenital disorders of glycosylation (CDGs) are a group of rare genetic disorders that affect protein and lipid glycosylation, and are usually clustered in two main groups: CDG type 1 (CDG-I), encompassing 22 defects in genes associated with assembly (DLO synthesis and glycan transfer) and CDG type 2 (CDG-II), which includes defects of the protein-bound oligosaccharide chain processing [18] (Figure 1, Table 1). Due to the high impact of N-glycosylation on protein maturation, folding, and function, carriers of CDG present with a wide spectrum of symptoms and disease severity, with mostly neuromuscular deficits. The most frequent protein N-glycosylation disorders are PMM2-CDG, MPI-CDG, ALG6-CDG and ALG1-CDG.

Of note, CDG-I patients also present hypocholesterolemia as an associated phenotypic trait.[19-23] Indeed, CDG-I patients with mutations in phosphomannomutase 2 (PMM2-CDG) or in asparagine-linked glycosylation protein 6 (ALG6-CDG) present with LDL-C and apoB plasma levels below the 5th percentile of the general population (Table 1).[24] Their clinically unaffected heterozygous relatives also present with reduced LDL-C and apoB compared to age- and sex-matched controls.[24] *In vitro* studies with hepatocytes and fibroblasts from PMM2-CDG or ALG6-CDG patients showed an increased LDLR expression due to an increased SREBP2 (sterol receptor element binding protein 2) expression independently of cellular ER stress,[24] thus suggesting a possible mechanism for the observed hypocholesterolemia. In another cohort of eight PMM2-CDG patients, defective N-glycosylation impacted PCSK9 N-glycosylation status and reduced PCSK9 plasma levels, thus potentially increasing LDLR expression and contributing to lower LDL-C levels in CDG-I patients (Table 1).[25] The latter observation is controversial, as in another group of CDG-I patients, PCSK9 plasma levels were similar to those of matched controls.[24] Further studies are required to clarify the molecular mechanisms underlying hypobetalipoproteinaemia in CDG-I patients.

Among CDG-II diseases (Table 1), patients with loss-of-function mutations in ß-1,4-galactosyl transferase (B4GALT1-CDG) present with lower non-HDL-cholesterol and larger HDL particles and, as described later, hypoglycosylated cholesteryl ester transfer protein (CETP), which may affect HDL metabolism.[26]

Another important group of glycosylation defects impacting on metabolic diseases are those influencing Golgi homeostasis and intracellular trafficking.[27] Mutations in CCDC115 and TMEM199 proteins have been associated with *N*- and mucin-type *O*-glycosylation disorders (Table 1). A storage-disease like phenotype with hepatosplenomegaly, steatohepatitis with fibrosis potentially leading to liver failure and marked hypercholesterolemia is present in patients with CCDC115 mutations.[28] Affected patients showed abnormal *N*- and *O*-glycosylation of plasma proteins, which was confirmed in patient fibroblasts and rescued by lentiviral transfection with wild-type CCDC115.[28] Similarly, individuals with mutations in TMEM199 present with mild hepatic steatosis and hypercholesterolemia. Their serum glycosylation pattern was similar to that observed in other Golgi homeostasis defects,[29, 30] and metabolic labelling of glycans in patient fibroblasts confirmed Golgi glycosylation disorders, which were restored by complementation with wild-type TMEM199.[31]

Also deglycosylation diseases, such as *NGLY1* deficiency, are included in the CDG disease spectrum. Interestingly, *NGLY* deficiency results in a dyslipidemic profile[32] (Table 1). *NGLY1* encodes *N*-glycanase 1, an enzyme involved in the degradation of misfolded *N*-glycoproteins.[32] Subjects with *N*-glycanase 1 deficiency present with marked hypocholesterolemia, as well as hepatic steatosis and elevated transaminases.[33] The molecular mechanisms linking deglycosylation defects to lipid metabolism are not yet fully understood and are under intensive investigation.[24, 26]

Beyond CDGs affecting lipid metabolism, GWAS have identified variants in genes related to glycobiology, which associate to changes in plasma lipid profile (Table 1). Among them, variants in GALNT2 (*N*-acetylgalactosaminyltransferase 2), which transfers an *N*-acetylgalactosamine (GalNAc) to specific Ser or Thr residues (the first step of *O*-linked glycosylation process), were shown to impact HDL-C and triglycerides (TG) plasma levels. Although contrasting findings have been reported, a detailed analysis of loss-of-function variants in humans and experimental models of GALNT2 deficiency in mice reported that impairment of GALNT2 lowers HDL-C levels.[34-36] As described below, this phenotype appears to be the consequence of altered *O*-glycosylation of plasma phospholipid transfer protein (PLTP), ANGPTL3 (angiopoietin-like 3) and apolipoprotein C-III (apoC-III).[35] Of note carriers

had normal blood tests including protein spectrum, platelet aggregation, and plasma coagulation assays; as well as no signs of preclinical atherosclerotic lesions as determined by the intima-media thickness of the carotid.[34]

Another key protein for glycobiology, which emerged through genetic studies, is the asialoglycoprotein receptor (ASGPR) (Table 1). This is a highly conserved hepatic receptor that facilitates the uptake of desialylated glycoproteins (with a terminal *N*-acetylgalactosamine or galactose).[6] Desialylation of glycoproteins occurs through the action of plasma or membranous sialidases and makes them prone to removal from circulation by the ASGPR. A genetic variant (12-base-pair (bp) deletion (del12) in intron 4) in ASGR1, the major subunit of the ASGPR, was reported to be associated with a significant reduction in non-HDL-C levels (9%) and a reduced risk of coronary artery disease (34%).[37] The del12 mutation is predicted to cause a frameshift and premature stop codon and indeed, when the mutated form was overexpressed in HeLa cells, no protein product was found.[37] Alkaline phosphatase and haptocorrin, a vitamin B12 transporter, are asialylated glycoproteins that are cleared from the circulation through binding to ASGPR; heterozygous carriers of ASGR1 del12 mutation have higher levels of circulating alkaline phosphatase and vitamin B12, confirming that the clearance of desialylated molecules from the circulation is compromised.[37]

The reduction of non-HDL-C levels might be due to a more stable LDLR expression on liver cells of patients with reduced hepatic ASGPR abundance,[37] thus suggesting that lowering ASGPR expression or inhibiting its function may be an approach to reduce LDL-C and cardiovascular risk. This mechanism has been suggested since LDLR and ASGPR are both located in clathrin-coated pits and ASGPR may promote the internalization of the LDLR after desialylation. However, in the study by Nioi, the beneficial effect of ASGR1 del12 variant on the risk of coronary artery disease exceeds that expected by its effect on non-HDL-C levels (6%); this suggested that the observed atheroprotective effect goes beyond the lowering of circulating cholesterol levels and might also be related to other processes impacting atherosclerotic plaque development.[37] Since ASGR1 has also been implicated in platelet homeostasis, a recent study evaluated platelet function in heterozygous ASGR1 del12 carriers and controls and found no difference.[38] In addition, compilation of a genetic risk score with three common ASGR1 variants

in the UK Biobank found the ASCVD risk reduction to be proportionate to the LDL-C plasma level reduction.[38]

Taken together, insights from rare genetic diseases and population genetics have recently provided valuable new information linking protein glycosylation to lipoprotein metabolism. This appears to occur at different levels as a consequence of the key role of glycosylation on the function of apolipoproteins, lipoprotein receptors and remodelling enzymes.

Impact of glycosylation on apolipoproteins, lipoprotein receptors and remodelling enzymes

In this section we discuss how glycosylation influences the expression and/or function of lipoproteins; this will involve not only structural apoproteins, but also protein/enzymes and receptors playing a crucial role in lipoprotein metabolism and function. Data linking these alterations with abnormal plasma lipid profile and atherosclerosis are also discussed.

Proteins within lipoproteins, indeed, are highly glycosylated, with sialic acid being the predominant terminal residue in several molecules, including apoB,[39, 40] apoC-III,[41] apoA-I,[42] and apoE.[43] The removal of sialic acid from the protein moiety of lipoproteins, a process known as desialylation, may occur under pathological conditions, leading to altered lipoprotein metabolism and function.[44-46] For instance, the treatment with neuraminidase (a sialidase responsible for sialic acid removal) generates a "desialylated"–LDL particle, which is taken up both by scavenger receptors and galactose-specific lectin receptors recognizing terminal galactose residues that are exposed after desialylation, thus resulting in intracellular cholesteryl ester accumulation.[46-48] Similarly, the treatment of HDL with neuraminidase may result in the impairment of reverse cholesterol transport[49] and reduced cholesterol esterification rate mediated by LCAT.[48] In addition, lipoprotein desialylation may induce a faster CETP-mediated cholesteryl ester transfer from HDL to LDL, resulting in increased cholesterol content in LDL.[49] These observations have suggested that the apolipoprotein glycome, as well as lipoprotein receptor glycome, may play a relevant role in the metabolism and biological properties of LDL, Lp(a), HDL, and TG-rich lipoproteins (Figure 2).

LDL, apolipoprotein B and LDLR

Apolipoprotein B100 (apoB), the main structural apoprotein of VLDL and LDL, contains several potential *N*-linked glycosylation motifs (Asn-X-Ser/Thr), 16 of which are glycosylated. The amount of carbohydrates present on apoB ranges from 5% to 9% by protein weight;[50] all carbohydrate chains contain complex *N*-linked glycans with sialic acid as terminal residue linked to galactose, *N*-acetylglucosamine and mannose (Table 2).[50] ApoB glycosylation is required for the proper folding of the nascent peptide, the stabilization of mature protein

conformation, and, as a consequence, plays an important role in the assembly and secretion of VLDL particles.[51]

Of note, among glycosyltransferases, GLT8D2 was found to increase apoB *N*-glycosylation and reduce apoB misfolding and proteasomal degradation, thus increasing apoB levels in hepatocytes,[52]; on the contrary, the inhibition of *N*-linked glycosylation results in a faster protein degradation.[51, 53]

Seven of the *N*-glycosylation sites on apoB are predicted to be close to the region involved in the binding with LDLR, which may suggest a relevant functional role; on the other hand, the composition of carbohydrates, and in particular the sialylation degree, contributes to determining the atherogenicity of LDL:[54] indeed, desialylation of LDL increases particle internalization by several cell types, and also hepatic clearance via ASGR1.[54] Overall, desialylated-LDL have a smaller size, an increased electronegative charge, and a higher propensity to undergo oxidative modifications, which may result in altered physico-chemical properties and metabolism.[55, 56] This might contribute to increase the cellular uptake of these particles and lipid accumulation,[44-46, 57, 58] likely due to the interaction of desialylated-LDL with receptors other than LDLR, such as scavenger receptors, and also with cell surface proteoglycans in cells other than hepatocytes.[46, 55]

Although the protein moiety of LDL isolated from blood of patients with coronary or carotid atherosclerosis contained similar amounts of glucosamine, galactose, and mannose, sialic acid ratio per apo B was reduced as compared to healthy donors,[59], particularly in very dense LDL subfraction.[60] An increased activity of sialidases may be responsible for the lower sialic acid content in LDL particles from CAD patients, which appears related to the severity of CAD.[61] In agreement with these observations, neuraminidase-1 knockdown protects cardiomyocytes from ischemic injury *in vitro* and *in vivo*[61] and hypomorphic sialidase expression reduces serum cholesterol levels, inflammation and atherosclerosis in apoE knockout mice.[62] In humans, however, ASGR1 deficiency, which results in increased circulating levels of asialylated proteins, is associated with lower levels of circulating apoB-containing lipoproteins and reduced risk of coronary artery disease.[37] The reason for this discrepancy still needs to be explored.

LDLR, by binding LDL, is the major regulator of circulating LDL-C levels; it is a glycoprotein, and glycosylation plays an essential role in determining its activity. The protein is initially synthesized as an *N*-glycosylated precursor then converted to its mature form by sequential *O*-glycosylation. Several *N*-glycosylation sites have been identified on LDLR, with the Asn[272] site, localized in the linker region between LA (LDLR class A) repeats 6 and 7 (where no *O*-glycosylation was observed) being highly conserved (Table 1).[63] *N*-glycosylation is essential for the normal production of LDLR; the inhibition of this process results in a significant reduction of the number of LDLR cell surface molecules, likely due to an increased degradation of non-glycosylated receptors.[64] Some loss-of-function mutations in the *LDLR* gene result in a severe familial hypercholesterolemia (FH) phenotype as a consequence of LDLR retention in ER and proteasome-mediated degradation due to altered glycosylation profile and protein misfolding.[65] On the other hand, *O*-glycosylation (Table 2) is critical to achieve a stable LDLR cell surface expression,[66, 67] and to enhance the binding affinity and uptake of LDL and VLDL.[68] Sialylation of LDLR plays a role in the stability of receptors expression,[69] and, accordingly, hypomorphic sialidase mice have more stable LDLR expression accompanied by more LDL uptake.[70]

The mechanism by which *O*-glycosylation regulates LDLR binding and uptake capacity is still unclear; it has been proposed that sialylated *O*-glycans, by increasing the negative charges of the conserved aspartic acids in the LA repeats or by inducing a conformational change, may result in a more favourable 3D binding structure.[68] In line with this, loss of *O*-glycosylation in the *N*-terminal region of LDLR results in reduced LDL binding and uptake.[71, 72]

Lp(a)

Lp(a) is a lipoprotein composed of an LDL particle in which apoB is covalently linked by a single disulfide bond to apolipoprotein (a) (apo(a)), a multikringle domain glycoprotein. Lp(a) levels are genetically determined and represent an independent and causal risk factor for atherosclerosis.[73] Apo(a) is a highly polymorphic glycoprotein, due to variations in kringle IV type 2 repeats;[74] one major post-translational modification of nascent apo(a) is glycosylation, resulting in a 28% of protein weight determined by carbohydrates.[75] Both *N*-linked and *O*-

linked glycosylation sites have been identified (Table 2), with sialic acid being the predominant carbohydrate (~38%), followed by galactose (~27%), mannose (~10%), galactosamine (~17%), and glucosamine (~13%). Overall, in Lp(a) the carbohydrate content of apo(a) is 3 to 6-fold higher than that of apoB.[75, 76] As the addition of *N*-linked glycans occurs in the ER, it is likely that alterations in the glycosylation might affect the kinetic rate at which apo(a) is processed through the secretory pathway. Thus, despite non-glycosylated apo(a) still appears competent to associate with LDL-apoB, *N*-linked glycosylation of apo(a) is necessary for normal intracellular processing and its inhibition results in the inhibition of Lp(a) assembly.[77]

Apo(a) is also *O*-glycosylated (Table 2) (mostly with monosialylated core type 1 structure) in the kringle IV linker domains,[78] which are enriched in Ser and Thr residues; thus, every additional kringle IV type 2 repeat in apo(a) contributes one branched *N*-glycan and six *O*-glycan chains.[79] *O*-glycans seem to play an important role in maintaining the stability of apo(a) by conferring resistance to proteolysis, thus potentially limiting the extent of proteolytic fragmentation of apo(a) and its accumulation in atherosclerotic lesions, where it may promote thrombogenesis.[79]

High-density lipoproteins and related proteins

Many of the proteins carried by HDL particles, including apoA-I, apoA-II, and apoCs, are potentially glycosylated.[80] Both *N*- and *O*-linked glycosylation have been detected in HDL-associated glycoproteins.[43, 81] Overall, proteins found in HDL present a different glycosylation pattern compared to that found in bulk plasma, suggesting that a differential glycan specificity might control HDL protein recruitment and function.[81]

HDL isolated from patients with cardiovascular disorders exhibit not only a different protein composition compared with those isolated from healthy subjects (being enriched in apoC-III, serum amyloid A (SAA) while depleted of paraoxonase and apoA-I),[82] but also differ for protein glycosylation profile. HDL from CAD subjects have lower apolipoprotein content (including ApoA-I, ApoA-II, and apoE) than non-CAD subjects, but sialylated glycans are significantly higher.[83] It is unclear whether different characteristics (CAD subjects were older and had higher TG and BMI) are responsible for difference in HDL proteome and glycome between the two

populations.[83] Compared to healthy controls, individuals with metabolic syndrome and diabetic patients on hemodialysis exhibit different HDL protein glycosylation patterns, with apoC-III, apoE and SAA4 glycosylation status being reduced.[84, 85]

ApoA-I, the major apoprotein of HDL, is both *N*- and *O*-glycosylated (Table 2), with increased levels of glycosylated forms observed in the first three days after acute myocardial infarction.[42] Protein O-GlcNAc transiently increases under different diseased conditions to improve tolerance to stress; accordingly O-GlcNAc formation improves cardiac cell survival and function after acute myocardial stress conditions, whereas inhibition of O-GlcNAc generation decreases myocardial cell survival.[86] Patients with acute myocardial infarction present increased levels of *O*-GlcNAc apoA-I forms associated with decreased troponin T and C-reactive protein levels, thus potentially suggesting a protective role.[42] However, the causal link between the apoA-I glycosylation profile and post-acute disease evolution of AMI-patients is still lacking and deserves further investigation.

ApoA-II does not contain consensus sequences for *N*-linked glycosylation, and in plasma it is not significantly glycosylated. However, apoA-II can undergo *O*-glycosylation (Table 2) and the sialylation degree appears to determine the distribution of apoA-II among HDL subfractions: sialylated apoA-II appears to bind preferentially smaller HDL3 particles rather than larger HDL2.[87] HDL particles enriched in apoA-II are catabolized slower and are less effective in promoting cholesterol efflux, and thus, changes in apoA-II glycosylation status in response to various metabolic conditions may also impact HDL metabolism and function.[87]

ApoE plays an essential role in HDL biology and exists as multiple glycosylated and sialylated forms; newly synthesized apoE is highly sialylated, whereas 75-80% of plasma apoE is not. ApoE does not contain the consensus sequence required for *N*-linked glycosylation, and carbohydrate residues are initially attached to apoE via an *O*-linkage to Thr^{194} (Table 2).[88] Although this modification does not affect protein secretion,[88, 89] *O*-glycosylation critically contributes to an initial and essential conformational change for further apoE glycosylation at Ser^{290} at the C-terminus.[90] Since the C-terminal domain plays a relevant role in regulating apoE solubility, stability and lipid binding capacity, alterations of glycosylation at these sites might have important implications in apoE function.[90] More recently, it was reported that apoE has 4

additional mucin-type *O*-glycosylation sites, with glycans ranging from simple GlcNAc to biantennary structures containing both fucose and sialic acid.[91] Low molecular weight apoE present on the cell surface is modified to higher molecular weight apoE by the addition of sialic acid residues prior to secretion.[92] Once secreted, sialytated apoE is further desialylated by neuraminidase activity at the macrophage surface, thus generating low molecular weight apoE glycoforms.[90]

In plasma, apoE is associated with lipoproteins, mainly HDL (30-50%), followed by VLDL (25-50%) and LDL (10-25%) and mediates their catabolism through the interaction with hepatic and extrahepatic apoB/E receptors. ApoE in HDL plays a major role in the removal of cholesterol from peripheral tissues back to the liver for excretion and the modulation of immune cells function.[93-95] How apoE glycosylation affects protein structure and/or function and, as a consequence, lipoprotein function, is still unknown. However, desialylation of apoE decreases its affinity for HDL,[96] which appears to translate into an impaired ability of HDL to deliver esterified cholesterol to the liver, thus suggesting a functional alteration.

Proteins involved in HDL metabolism

LCAT. Lecithin:cholesterol acyltransferase (LCAT) is the enzyme responsible for the esterification of free cholesterol and plays a critical role in regulating the composition and levels of circulating HDL. Its carbohydrate content has been estimated to be ~25% of the total protein mass; four potential sites for *N*-linked glycosylation have been identified (Table 2).[97] Blocking *N*-glycosylation of LCAT was shown to affect its enzymatic activity but not protein secretion,[98] indeed, loss of carbohydrate at position Asn^{20}, Asn^{84} or Asn^{272} resulted in a reduced enzymatic activity (by 18 %, 82% and 62%, respectively); while lack of glycosylation at position Asn^{384} results in an increased enzymatic activity[97] (Figure 2). LCAT also contains two *O*-linked glycosylation sites at Thr^{407} and Ser^{409}, both containing sialic acid residues, whose functional relevance to date is unknown.[99, 100]

CETP. Cholesteryl ester transfer protein (CETP) is a plasma protein that mediates the transfer of cholesteryl esters (CE) and TG between HDL and VLDL/LDL. CETP is highly glycosylated and

has four predicted *N*-linked glycosylation sites (Table 2), to which sialic acid as terminal residues is attached.[101] Patients with B4GALT1-CDG, a congenital disorder affecting galactosylation of *N*-linked glycans, show hypoglycosylated CETP, accompanied by a significant reduction of CETP activity (26% lower than control subjects), which translated into lower levels of non-HDL-C and the presence of larger HDL particles[26] (Figure 2 and table 1).

In plasma, CETP is present in two forms, which differ for the presence or absence of *N*-linked glycosylation at Asn[341]; with the form lacking carbohydrate chain showing a 40% higher activity.[101, 102] Changes of the ratio between these two protein forms under pathological conditions, such as dyslipidaemias, might affect plasma CETP activity.[101, 102]

PLTP. PLTP is a plasma glycoprotein that facilitates the transfer of phospholipids from apoB-containing lipoproteins to HDL, thus playing a role in lipoprotein remodelling and HDL metabolism.[103] Several studies have reported a positive correlation between plasma PLTP activity and cardiovascular disease;[104] one potential mechanism linking PLTP to CVD is the positive association of plasma PLTP activity with TG and apoB levels,[104] suggesting that factors that can modulate PLTP levels and/or activity might also have clinical implications. PLTP contains six putative *N*-glycosylation sites and five *O*-glycosylation sites[105] (Table 2). The inhibition of *N*-glycosylation dramatically reduces PLTP secretion owing to the fact that the non-glycosylated form of PLTP is actively degraded intracellularly.[106, 107] Studies with PLTP mutants, in which each of the putative *N*-glycosylation site had been removed by replacing a serine with an alanine, showed that all 6 potential *N*-linked sites are utilized; however, no single N-linked carbohydrate chain is essential for secretion or activity and, instead, each one has a quantitative impact on PLTP secretion and function; the removal of carbohydrate at positions Asn[47] to Asn[77] resulted in the lowest PLTP activity, suggesting that glycosylation at these sites might be required for proper docking of PLTP on the lipoprotein surface, or, alternatively, affect PLTP stability.[108] Moreover, PLTP *O*-linked glycosylation by GALNT2, is required for facilitating PLTP function and the modulation of HDL-C levels. In fact, GALNT2 knock out animals display a reduced PLTP activity despite normal plasma levels, and similarly subjects

with homozygous *GALNT2* loss-of-function mutations present reduced PLTP activity[35] (Table 1).

SR-BI. Scavenger receptor class B type I (SR-BI) is an integral membrane cell surface glycoprotein belonging to the family of CD36. It is an HDL receptor playing a major role in cholesterol homeostasis, which is critical to modulate cholesterol homeostasis,[109, 110] by mediating the selective uptake of cholesteryl esters from HDL, and it is involved also in other biological processes, including the modulation of inflammation and apoptosis.[111] The large extracellular loop of SR-BI, which plays a critical role in ligand binding and lipid uptake, contains multiple sites for *N*-linked glycosylation (Table 2). A mutational study of each of these putative *N*-glycosylation sites showed that when Asn[108] or Asn[173] *N*-glycosylation was affected, the protein failed to localize to the plasma membrane and exhibited a reduced ability to transfer lipid from HDL to cells[112] (Figure 2). Two rare point mutations in the gene encoding SR-BI, occurring in the large extracellular loop of the receptor (S112F and T175A), have been associated with extremely high HDL-C levels (>90th percentile for age and gender)[113] due a reduced cell surface expression and/or impaired ability to bind HDL and mediate CE uptake from HDL.[114] Mutation of Ser[112] to Phe neither impacts *N*-glycosylation at Asn[108] nor reduces protein cell surface expression, suggesting that the significantly reduced uptake of CE from HDL observed in S112F subjects might be related to conformational changes in the mutated protein.[114] On the contrary, mutation of Thr[175] to Ala disrupts *N*-linked glycosylation at the evolutionary conserved Asn[173] residue, thus impacting SR-BI proper cell surface expression and efficient lipid uptake[112, 114] (Figure 2). Another variant, P376L, has been described as a loss-of-function mutation associated with significantly higher levels of circulating HDL-C and increased risk of CHD in homozygous carriers compared with noncarriers.[115] This variant alters *N*-glycosylation profile of SR-BI and reduces its cell surface expression, thus reducing selective CE uptake from HDL.[115]

Triglyceride-rich lipoprotein-associated proteins

ApoC-III

Apolipoprotein C-III (apoC-III) plays a crucial role in regulating TG metabolism and is mainly synthesized by the liver and, to a lesser extent, by the intestine.[116] It is carried mostly by VLDL and chylomicrons, but it is also present in LDL and HDL; apoC-III inhibits lipoprotein lipase and hepatic lipase, promotes hepatic VLDL secretion and reduces the hepatic clearance of TG-rich lipoproteins (TRL) by the LDLR and LRP1.[116] Several studies have established a direct correlation between circulating levels of apoC-III and TG, as well as between apoC-III levels and cardiovascular disease.[117-119] Normolipidemic subjects carry the majority of apoC-III in HDL, while in patients with high levels of plasma TG it is found predominantly in TG-rich lipoproteins.[120] ApoC-III is known to contain only O-linked glycopeptides at Thr^{74} (Table 2).[41] Two genetic variants of apoC-III have been described, one being oversialylated,[121] the other impacting Thr^{74} that prevents O-glycosylation;[122] carriers of these variants are normolipidemic, suggesting that the degree of apoC-III sialylation does not affect lipoprotein metabolism.

The sugar moiety is represented by galactose linked to *N*-acetyl-galactosamine (Gal-GalNAc) and a variable number of molecules of sialic acid, thus resulting in three major glycoforms referred to as apoC-III_{0b}, apoC-III_1 and apoC-III_2 containing 0, 1, and 2 molecules of sialic acid per molecule of protein, respectively. In plasma, the non-glycosylated form of apoC-III (apoC-III_{0a}) is present at very low levels, with glycosylated glycoforms (both non-sialylated and sialylated) representing approximately 99% of circulating apoC-III.[123] Physiologically, each glycoform contributes to ~10%, 55% and 35% of the total circulating apoC-III levels. It is still unclear whether apoC-III glycoforms interact differently with LPL thus modulating its enzymatic activity, yet alterations in the relative abundance of apoC-III glycoforms have been observed under pathological conditions (such as obesity, metabolic syndrome, diabetes, hyperlipidemia and CAD).[119, 123-125] Furthermore, differential glycosylation of apoC-III may affect secretion and/or metabolism of TRL: LDLR and LRP1 rapidly clear TRL containing apoC-III_1, whereas apoC-III_2 is preferentially and more slowly cleared by the heparan sulfate proteoglycan syndecan-1.[126]

Overweight and obese subjects have higher apoC-III_0/apoC-III_2 and apoC-III_1/apoC-III_2 ratios compared with lean subjects, which correlate directly with TG levels and inversely with insulin sensitivity.[123] Furthermore, apoC-III_{0a}, apoC-III_{ob} and apoC-III_1 levels, but not apoC-III_2, correlate

with fasting TG levels.[123] In agreement with this observation, increased apoC-III$_2$ levels and apoC-III$_2$/apoC-III$_1$ ratio were associated with lower plasma levels of TG and total cholesterol in two large cohorts of patients with glucose intolerance or type 2 diabetes (T2D), and with lower levels of the highly atherogenic small dense LDL particles in diabetics.[127] In patients with CAD, the relative abundance of apoC-III$_1$ increased, whereas that of apoC-III$_0$ decreased across apoC-III quartiles; the proportion of apoC-III$_2$ was unchanged, and higher levels of apoC-III$_1$ correlated with an overall unfavourable lipid profile (increase of total cholesterol, LDL-C, TG, and apoE, and decrease of apoA-I).[124] Unexpectedly, in this study, changes in the relative abundance of the three glycoforms did not impact LPL activity;[124] it is worth noting that the extent of variations in the relative abundance was relatively small (10-13%) and likely unable to translate into major differences in the ability to inhibit LPL. The treatment of hypertriglyceridemic patients with volanesorsen, an antisense oligonucleotide targeting *APOC3* mRNA, greatly reduced apoC-III and TG levels, but also resulted in the reduction of apoC-III$_1$ relative abundance paralleled by increased apoC-III$_2$ relative abundance.[126] Carriers of a loss-of-function mutation in the gene encoding GALNT2, an enzyme involved in the glycosylation of apoC-III, exhibited a 6.6-fold increase in the levels of apoC-III$_0$ and ~30% apoC-III$_1$ reduction and showed an improved postprandial TG clearance, likely due to a reduced glycosylation of apoC-III.[34] Recently, plasma from patients carrying other *GALNT2* loss-of-function mutations were shown to contain only non-glycosylated apoC-III (apoC-III$_0$), emphasizing apoC-III as a reliable biomarker for this disorder.[36]

ANGPTL3

ANGPTL3 is a protein of hepatic origin belonging to the subfamily of angiopoietin-like proteins that are involved in the regulation of lipid metabolism.[128] ANGPTL3 is an endogenous inhibitor of lipoprotein lipase and endothelial lipase, resulting in increased levels of TG and LDL-C; loss-of-function mutations in *Angptl3* gene are associated with low TG and LDL-C levels and a lower risk of coronary disease.[129] ANGPTL3 undergoes several post-translational modifications, including *N*-glycosylation at several sites and *O*-glycosylation mediated by GalNAc-T2 (Table 2), the latter being involved in regulating the activation of ANGPTL3 by proprotein convertases

(PCs).[130] *In vitro*, profurin overexpression, which inhibits PCs activity, reduces the cleavage, and thus the activation, of ANGPTL3, resulting in a reduced ANGPTL3-mediated inhibition of EL;[131] experimental data indicate that GalNAc-T2-mediated *O*-glycosylation plays a crucial role in the accessibility of the nearby cleavage site by PCs, resulting in reduced processing and activation of ANGPTL3 (Figure 2).[131] In agreement with this observation, O-glycosylation of ANGPTL3 (Thr[226]) was selectively lost in the human *GALNT2*-deficient O-glycoproteome,[35, 132] reduced activation of ANGPTL3, combined with the altered glycosylation of apoC-III due to *GALNT2* loss-of-function, can contribute to the improved plasma triglyceride clearance in carriers of this mutation.[34]

CD36

CD36 (cluster of differentiation 36) is a transmembrane glycoprotein belonging to the family of class B scavenger receptors; it is expressed on the surface of several cell types (including endothelial cells and macrophages) and binds a broad spectrum of ligands, including oxidized LDL, long-chain fatty acids and apoptotic cells.[133] CD36 has two transmembrane domains with a large extracellular loop containing 10 *N*-linked glycosylation sites[134] (Table 2). *N*-glycosylation appears to be required for proper protein folding and surface localization of CD36, while ligand recognition is not affected by alterations in the *N*-glycosylation pattern.[135] Despite being a classical modification occurring in the ER and Golgi, it appears that the overall *N*-glycosylation status of CD36 may be modulated, resulting in an increased expression of fully *N*-glycosylated CD36 and a more efficient translocation from intracellular membranes to the cell surface[136] (Figure 2). Besides *N*-glycosylation, CD36 is also a target for O-GlcNAcylation (Table 2), which, when induced in perfused hearts, stimulates fatty acid oxidation, likely by increasing the amount of CD36 associated with plasma membrane.[137] Also, alterations in CD36 sialylation contribute to the ability of mediating oxidized LDL uptake by macrophages.[138]

Conclusion

Specific defects in glycosylation enzymes often impact lipoprotein biosynthesis, suggesting that a proper apolipoprotein, lipoprotein receptor or lipid transfer enzyme glycosylation is critical in regulating their cellular biosynthesis but also their function. Novel insights into receptors recognizing specific glycan chains on glycoproteins also suggest that plasma lipid and lipoprotein metabolism might be affected through mechanisms independent of the classical lipoprotein/lipoprotein receptor axis. This sets the stage for further investigation of the molecular mechanisms associated with different carbohydrate configurations in lipoproteins and related proteins to develop novel therapeutic approaches to modulate lipid metabolism. As several enzymes involved in remodelling and recognition of carbohydrate structures are common to several other proteins, therapeutics targeting glycosylation in the context of dyslipidaemias should be tested not only for their efficacy but also for their safety and for potential off-target effects.

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Conflicts of interest

AP and MS have nothing to disclose; ALC reports grants from Amgen, Sanofi, Regeneron personal fees from Merck, Sanofi, Regeneron, AstraZeneca, Amgen, outside the submitted work; AGH reports support from Gilead and personal fees from Amgen and Gilead outside of the submitted work; GDN reports grants from Pfizer, Amgen, personal fees from Amgen outside the submitted work.

Legends to the Figures

Figure 1. Schematic view of protein N-glycosylation pathway and related genetic mutations impacting lipid and lipoprotein metabolism. N-linked glycosylation process starts in endoplasmic reticulum (ER), where a dolichol-phosphate (Dol-P) serves as a carrier of the forming oligosaccharide. As first, two molecules of GlcNAc-P are added to Dol-P. In the cytoplasm, phosphomannomutase 2 (PMM2) catalyses the conversion of mannose-6-phosphate (Man-6-P) into Man-1-P, the precursor for GDP-Man. GDP-Man is then added to Dol-P-$GlcNAc_2$ at the cytosolic side of the ER membrane. After the addition of 5 Man residues, the complex is translocated across the ER membrane making it accessible to enzymes in the ER lumen, and further extended by the action of sequential glycosyltransferases (ALGs) to form the oligosaccharide that is then transferred to selected asparagines (Asn) residues of the nascent polypeptide. Immediately after the oligosaccharide is transferred to a nascent polypeptide, all three glucose residues and a specific mannose residue are removed by three different enzymes. Through shuttle proteins, such as TMEM165 and CCDC115, the protein-oligosaccharide complex reaches the Golgi department, where modifications to N-linked oligosaccharides are then completed. The cis, medial, and trans cisternae of the Golgi contain distinct enzymes that introduce different modifications to the oligosaccaride linked to the protein. Congenital disorders of glycosylation (CDG) are defined as either assembly (CDG-I) or processing (CDG-II) glycosylation defects and rely on defects of specific enzymes involved in the glycosylation process. LOF mutations in PMM2, ALG6, TMEM165, CCDC115, B4GAL1 are associated with changes in plasma lipids and lipoprotein profile.

Figure 2. The role of glycosylation on lipoprotein metabolism and the effect of altered glycosylation status. (A) Glycosylation of apoproteins is required for the proper folding of nascent peptide, the stabilization of protein conformation, and function of mature protein for apoC-III, apoB, apoE, apo(a), apoA-I. Glycosylation is also essential for the proper membrane localization and activity of some receptors involved in lipoprotein metabolism, including LDLR,

SR-BI and CD36. Finally, proteins involved in lipoprotein metabolism, such as CETP, PLTP, LCAT, and, indirectly, ANGPTL3, present different glycosylation sites driving their activity. **(B)** Altered glycosylation status may negatively impact lipoprotein metabolism. Alterations in the glycosylation process of apoproteins may led to an increased degradation of protein due to misfolding (apoB), a reduced secretion (apo(a)), an increased affinity for pro-atherogenic lipoproteins such as VLDL(apoE); apoC-III glycoform changes is correlated with changes in the lipid profile. Alterations in the glycosylation of lipid/lipoprotein receptors may impact their proper localization at cell surface as well as change their affinity for the ligand thus resulting in reduced lipoprotein uptake (LDLR, SR-BI). Finally, alterations in the glycosylation profile of proteins involved in lipoprotein remodelling or lipid exchange can directly impact the physiological metabolism of lipoproteins. An exception may be represented by ANGPTL3, for which the inhibition of the glycosylation process results in a reduced activation of ANGPTL3 with a consequent increase of LPL activity.

References

1. Varki A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 1993;**3**:97-130.
2. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. *Cell* 2006;**126**:855-867.
3. Moremen KW, Tiemeyer M, Nairn AV. Vertebrate protein glycosylation: diversity, synthesis and function. *Nat Rev Mol Cell Biol* 2012;**13**:448-462.
4. Stanley P, Taniguchi N, Aebi M. In: Varki A CR, Esko JD, et al., editors., ed. Essentials of Glycobiology 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017, 2017.
5. Van den Steen P, Rudd PM, Dwek RA, Opdenakker G. Concepts and principles of O-linked glycosylation. *Crit Rev Biochem Mol Biol* 1998;**33**:151-208.
6. van den Boogert MAW, Rader DJ, Holleboom AG. New insights into the role of glycosylation in lipoprotein metabolism. *Curr Opin Lipidol* 2017;**28**:502-506.
7. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008;**40**:161-169.
8. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker PI, O'Donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL, Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009;**41**:56-65.
9. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, Johansen CT, Fouchier SW, Isaacs A, Peloso GM, Barbalic M, Ricketts SL, Bis JC, Aulchenko YS, Thorleifsson G, Feitosa MF, Chambers J, Orho-Melander M, Melander O, Johnson T, Li X, Guo X, Li M, Shin Cho Y, Jin Go M, Jin Kim Y, Lee JY, Park T, Kim K, Sim X, Twee-Hee Ong R, Croteau-Chonka DC, Lange LA, Smith JD, Song K, Hua Zhao J, Yuan X, Luan J, Lamina C, Ziegler A, Zhang W, Zee RY, Wright AF, Witteman JC, Wilson JF, Willemsen G, Wichmann HE, Whitfield JB, Waterworth DM, Wareham NJ, Waeber G, Vollenweider P, Voight BF, Vitart V, Uitterlinden AG, Uda M, Tuomilehto J, Thompson JR, Tanaka T, Surakka I, Stringham HM, Spector TD, Soranzo N, Smit JH, Sinisalo J, Silander K, Sijbrands EJ, Scuteri A, Scott J, Schlessinger D, Sanna S, Salomaa V, Saharinen J, Sabatti C, Ruokonen A, Rudan I, Rose LM, Roberts R, Rieder M, Psaty BM, Pramstaller PP, Pichler I, Perola M, Penninx BW, Pedersen NL, Pattaro C, Parker AN, Pare G, Oostra BA, O'Donnell CJ, Nieminen MS, Nickerson DA, Montgomery GW, Meitinger T, McPherson R, McCarthy MI, McArdle W, Masson D, Martin NG, Marroni F, Mangino M, Magnusson PK, Lucas G, Luben R, Loos RJ, Lokki ML, Lettre G, Langenberg C, Launer LJ, Lakatta EG, Laaksonen R, Kyvik KO, Kronenberg F, Konig IR, Khaw KT,

Kaprio J, Kaplan LM, Johansson A, Jarvelin MR, Janssens AC, Ingelsson E, Igl W, Kees Hovingh G, Hottenga JJ, Hofman A, Hicks AA, Hengstenberg C, Heid IM, Hayward C, Havulinna AS, Hastie ND, Harris TB, Haritunians T, Hall AS, Gyllensten U, Guiducci C, Groop LC, Gonzalez E, Gieger C, Freimer NB, Ferrucci L, Erdmann J, Elliott P, Ejebe KG, Doring A, Dominiczak AF, Demissie S, Deloukas P, de Geus EJ, de Faire U, Crawford G, Collins FS, Chen YD, Caulfield MJ, Campbell H, Burtt NP, Bonnycastle LL, Boomsma DI, Boekholdt SM, Bergman RN, Barroso I, Bandinelli S, Ballantyne CM, Assimes TL, Quertermous T, Altshuler D, Seielstad M, Wong TY, Tai ES, Feranil AB, Kuzawa CW, Adair LS, Taylor HA, Jr., Borecki IB, Gabriel SB, Wilson JG, Holm H, Thorsteinsdottir U, Gudnason V, Krauss RM, Mohlke KL, Ordovas JM, Munroe PB, Kooner JS, Tall AR, Hegele RA, Kastelein JJ, Schadt EE, Rotter JI, Boerwinkle E, Strachan DP, Mooser V, Stefansson K, Reilly MP, Samani NJ, Schunkert H, Cupples LA, Sandhu MS, Ridker PM, Rader DJ, van Duijn CM, Peltonen L, Abecasis GR, Boehnke M, Kathiresan S. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;**466**:707-713.

10. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol* 2018;**333**:65-79.
11. Lindberg G, Rastam L, Gullberg B, Eklund GA. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow-up. *Int J Epidemiol* 1992;**21**:253-257.
12. Khalili P, Sundstrom J, Franklin SS, Jendle J, Lundin F, Jungner I, Nilsson PM. Combined effects of brachial pulse pressure and sialic acid for risk of cardiovascular events during 40 years of follow-up in 37,843 individuals. *J Hypertens* 2012;**30**:1718-1724.
13. Serdar Z, Yesilbursa D, Dirican M, Sarandol E, Serdar A. Sialic acid and oxidizability of lipid and proteins and antioxidant status in patients with coronary artery disease. *Cell Biochem Funct* 2007;**25**:655-664.
14. Serdar Z, Serdar A, Altin A, Eryilmaz U, Albayrak S. The relation between oxidant and antioxidant parameters and severity of acute coronary syndromes. *Acta Cardiol* 2007;**62**:373-380.
15. Govindarajan S, Raghavan VM, Rao AC. Plasma Myeloperoxidase and Total Sialic Acid as Prognostic Indicators in Acute Coronary Syndrome. *J Clin Diagn Res* 2016;**10**:BC09-13.
16. Wu EB, Lumb P, Chambers JB, Crook MA. Plasma sialic acid and coronary artery atheromatous load in patients with stable chest pain. *Atherosclerosis* 1999;**145**:261-266.
17. Salomone OA, Crook JR, Hossein-Nia M, Holt D, Kaski JC. Serum sialic acid concentration is not associated with the extent or severity of coronary artery disease in patients with stable angina pectoris. *Am Heart J* 1998;**136**:620-623.
18. Jaeken J. Glycosylation and its disorders: general overview. *Ref Modul Biomed Sci* 2016:1-8.
19. Jaeken J, Eggermont E, Stibler H. An apparent homozygous X-linked disorder with carbohydrate-deficient serum glycoproteins. *Lancet* 1987;**2**:1398.
20. Ohno K, Yuasa I, Akaboshi S, Itoh M, Yoshida K, Ehara H, Ochiai Y, Takeshita K. The carbohydrate deficient glycoprotein syndrome in three Japanese children. *Brain Dev* 1992;**14**:30-35.
21. Pavone L, Fiumara A, Barone R, Rizzo R, Buttitta P, Dobyns WB, Jaeken J. Olivopontocerebellar atrophy leading to recognition of carbohydrate-deficient glycoprotein syndrome type I. *J Neurol* 1996;**243**:700-705.
22. de Lonlay P, Seta N, Barrot S, Chabrol B, Drouin V, Gabriel BM, Journel H, Kretz M, Laurent J, Le Merrer M, Leroy A, Pedespan D, Sarda P, Villeneuve N, Schmitz J, van Schaftingen E, Matthijs G, Jaeken J, Korner C, Munnich A, Saudubray JM, Cormier-Daire V. A broad spectrum of clinical presentations in congenital disorders of glycosylation I: a series of 26 cases. *J Med Genet* 2001;**38**:14-19.

23. Di Rocco M, Hennet T, Grubenmann CE, Pagliardini S, Allegri AE, Frank CG, Aebi M, Vignola S, Jaeken J. Congenital disorder of glycosylation (CDG) Ig: report on a patient and review of the literature. *J Inherit Metab Dis* 2005;**28**:1162-1164.
24. van den Boogert MAW, Larsen LE, Ali L, Kuil SD, Chong PLW, Loregger A, Kroon J, Schnitzler JG, Schimmel AWM, Peter J, Levels JHM, Steenbergen G, Morava E, Dallinga-Thie GM, Wevers RA, Kuivenhoven JA, Hand NJ, Zelcer N, Rader DJ, Stroes ESG, Lefeber DJ, Holleboom AG. N-Glycosylation Defects in Humans Lower Low-Density Lipoprotein Cholesterol Through Increased Low-Density Lipoprotein Receptor Expression. *Circulation* 2019;**140**:280-292.
25. Chong M, Yoon G, Susan-Resiga D, Chamberland A, Cheillan D, Pare G, Seidah NG. Hypolipidaemia among patients with PMM2-CDG is associated with low circulating PCSK9 levels: a case report followed by observational and experimental studies. *J Med Genet* 2020;**57**:11-17.
26. van den Boogert MAW, Crunelle CL, Ali L, Larsen LE, Kuil SD, Levels JHM, Schimmel AWM, Konstantopoulou V, Guerin M, Kuivenhoven JA, Dallinga-Thie GM, Stroes ESG, Lefeber DJ, Holleboom AG. Reduced CETP glycosylation and activity in patients with homozygous B4GALT1 mutations. *J Inherit Metab Dis* 2020;**43**:611-617.
27. Freeze HH, Chong JX, Bamshad MJ, Ng BG. Solving glycosylation disorders: fundamental approaches reveal complicated pathways. *Am J Hum Genet* 2014;**94**:161-175.
28. Jansen JC, Cirak S, van Scherpenzeel M, Timal S, Reunert J, Rust S, Perez B, Vicogne D, Krawitz P, Wada Y, Ashikov A, Perez-Cerda C, Medrano C, Arnoldy A, Hoischen A, Huijben K, Steenbergen G, Quelhas D, Diogo L, Rymen D, Jaeken J, Guffon N, Cheillan D, van den Heuvel LP, Maeda Y, Kaiser O, Schara U, Gerner P, van den Boogert MA, Holleboom AG, Nassogne MC, Sokal E, Salomon J, van den Bogaart G, Drenth JP, Huynen MA, Veltman JA, Wevers RA, Morava E, Matthijs G, Foulquier F, Marquardt T, Lefeber DJ. CCDC115 Deficiency Causes a Disorder of Golgi Homeostasis with Abnormal Protein Glycosylation. *Am J Hum Genet* 2016;**98**:310-321.
29. Kornak U, Reynders E, Dimopoulou A, van Reeuwijk J, Fischer B, Rajab A, Budde B, Nurnberg P, Foulquier F, Group AD-tS, Lefeber D, Urban Z, Gruenewald S, Annaert W, Brunner HG, van Bokhoven H, Wevers R, Morava E, Matthijs G, Van Maldergem L, Mundlos S. Impaired glycosylation and cutis laxa caused by mutations in the vesicular H+-ATPase subunit ATP6V0A2. *Nat Genet* 2008;**40**:32-34.
30. Dulary E, Potelle S, Legrand D, Foulquier F. TMEM165 deficiencies in Congenital Disorders of Glycosylation type II (CDG-II): Clues and evidences for roles of the protein in Golgi functions and ion homeostasis. *Tissue Cell* 2017;**49**:150-156.
31. Jansen JC, Timal S, van Scherpenzeel M, Michelakakis H, Vicogne D, Ashikov A, Moraitou M, Hoischen A, Huijben K, Steenbergen G, van den Boogert MA, Porta F, Calvo PL, Mavrikou M, Cenacchi G, van den Bogaart G, Salomon J, Holleboom AG, Rodenburg RJ, Drenth JP, Huynen MA, Wevers RA, Morava E, Foulquier F, Veltman JA, Lefeber DJ. TMEM199 Deficiency Is a Disorder of Golgi Homeostasis Characterized by Elevated Aminotransferases, Alkaline Phosphatase, and Cholesterol and Abnormal Glycosylation. *Am J Hum Genet* 2016;**98**:322-330.
32. Takahashi N. Demonstration of a new amidase acting on glycopeptides. *Biochem Biophys Res Commun* 1977;**76**:1194-1201.
33. Lam C, Wolfe L, Need A, Shashi V, Enns G. NGLY1-Related Congenital Disorder of Deglycosylation. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K*, et al.*, eds. GeneReviews((R)). Seattle (WA), 1993.
34. Holleboom AG, Karlsson H, Lin RS, Beres TM, Sierts JA, Herman DS, Stroes ES, Aerts JM, Kastelein JJ, Motazacker MM, Dallinga-Thie GM, Levels JH, Zwinderman AH, Seidman JG, Seidman CE, Ljunggren S, Lefeber DJ, Morava E, Wevers RA, Fritz TA, Tabak LA, Lindahl M, Hovingh GK,

Kuivenhoven JA. Heterozygosity for a loss-of-function mutation in GALNT2 improves plasma triglyceride clearance in man. *Cell Metab* 2011;**14**:811-818.
35. Khetarpal SA, Schjoldager KT, Christoffersen C, Raghavan A, Edmondson AC, Reutter HM, Ahmed B, Ouazzani R, Peloso GM, Vitali C, Zhao W, Somasundara AV, Millar JS, Park Y, Fernando G, Livanov V, Choi S, Noe E, Patel P, Ho SP, Myocardial Infarction Exome Sequencing S, Kirchgessner TG, Wandall HH, Hansen L, Bennett EP, Vakhrushev SY, Saleheen D, Kathiresan S, Brown CD, Abou Jamra R, LeGuern E, Clausen H, Rader DJ. Loss of Function of GALNT2 Lowers High-Density Lipoproteins in Humans, Nonhuman Primates, and Rodents. *Cell Metab* 2016;**24**:234-245.
36. Zilmer M, Edmondson AC, Khetarpal SA, Alesi V, Zaki MS, Rostasy K, Madsen CG, Lepri FR, Sinibaldi L, Cusmai R, Novelli A, Issa MY, Fenger CD, Abou Jamra R, Reutter H, Briuglia S, Agolini E, Hansen L, Petaja-Repo UE, Hintze J, Raymond KM, Liedtke K, Stanley V, Musaev D, Gleeson JG, Vitali C, O'Brien WT, Gardella E, Rubboli G, Rader DJ, Schjoldager KT, Moller RS. Novel congenital disorder of O-linked glycosylation caused by GALNT2 loss of function. *Brain* 2020;**143**:1114-1126.
37. Nioi P, Sigurdsson A, Thorleifsson G, Helgason H, Agustsdottir AB, Norddahl GL, Helgadottir A, Magnusdottir A, Jonasdottir A, Gretarsdottir S, Jonsdottir I, Steinthorsdottir V, Rafnar T, Swinkels DW, Galesloot TE, Grarup N, Jorgensen T, Vestergaard H, Hansen T, Lauritzen T, Linneberg A, Friedrich N, Krarup NT, Fenger M, Abildgaard U, Hansen PR, Galloe AM, Braund PS, Nelson CP, Hall AS, Williams MJ, van Rij AM, Jones GT, Patel RS, Levey AI, Hayek S, Shah SH, Reilly M, Eyjolfsson GI, Sigurdardottir O, Olafsson I, Kiemeney LA, Quyyumi AA, Rader DJ, Kraus WE, Samani NJ, Pedersen O, Thorgeirsson G, Masson G, Holm H, Gudbjartsson D, Sulem P, Thorsteinsdottir U, Stefansson K. Variant ASGR1 Associated with a Reduced Risk of Coronary Artery Disease. *N Engl J Med* 2016;**374**:2131-2141.
38. Ali L, Cupido AJ, Rijkers M, Hovingh GK, Holleboom AG, Dallinga-Thie GM, Stroes ESG, van den Boogert MAW. Common gene variants in ASGR1 gene locus associate with reduced cardiovascular risk in absence of pleiotropic effects. *Atherosclerosis* 2020;**306**:15-21.
39. Tertov VV, Orekhov AN, Sobenin IA, Morrisett JD, Gotto AM, Jr., Guevara JG, Jr. Carbohydrate composition of protein and lipid components in sialic acid-rich and -poor low density lipoproteins from subjects with and without coronary artery disease. *J Lipid Res* 1993;**34**:365-375.
40. Swaminathan N, Aladjem F. The monosaccharide composition and sequence of the carbohydrate moiety of human serum low density lipoproteins. *Biochemistry* 1976;**15**:1516-1522.
41. Roghani A, Zannis VI. Mutagenesis of the glycosylation site of human ApoCIII. O-linked glycosylation is not required for ApoCIII secretion and lipid binding. *J Biol Chem* 1988;**263**:17925-17932.
42. Cubedo J, Padro T, Badimon L. Glycoproteome of human apolipoprotein A-I: N- and O-glycosylated forms are increased in patients with acute myocardial infarction. *Transl Res* 2014;**164**:209-222.
43. Huang J, Lee H, Zivkovic AM, Smilowitz JT, Rivera N, German JB, Lebrilla CB. Glycomic analysis of high density lipoprotein shows a highly sialylated particle. *J Proteome Res* 2014;**13**:681-691.
44. Orekhov AN, Tertov VV, Mukhin DN, Mikhailenko IA. Modification of low density lipoprotein by desialylation causes lipid accumulation in cultured cells: discovery of desialylated lipoprotein with altered cellular metabolism in the blood of atherosclerotic patients. *Biochem Biophys Res Commun* 1989;**162**:206-211.
45. Tertov VV, Sobenin IA, Tonevitsky AG, Orekhov AN, Smirnov VN. Isolation of atherogenic modified (desialylated) low density lipoprotein from blood of atherosclerotic patients:

separation from native lipoprotein by affinity chromatography. *Biochem Biophys Res Commun* 1990;**167**:1122-1127.
46. Orekhov AN, Tertov VV, Sobenin IA, Smirnov VN, Via DP, Guevara J, Jr., Gotto AM, Jr., Morrisett JD. Sialic acid content of human low density lipoproteins affects their interaction with cell receptors and intracellular lipid accumulation. *J Lipid Res* 1992;**33**:805-817.
47. Grewal T, Bartlett A, Burgess JW, Packer NH, Stanley KK. Desialylated LDL uptake in human and mouse macrophages can be mediated by a lectin receptor. *Atherosclerosis* 1996;**121**:151-163.
48. Sukhorukov V, Gudelj I, Pucic-Bakovic M, Zakiev E, Orekhov A, Kontush A, Lauc G. Glycosylation of human plasma lipoproteins reveals a high level of diversity, which directly impacts their functional properties. *Biochim Biophys Acta Mol Cell Biol Lipids* 2019;**1864**:643-653.
49. Harada LM, Carvalho MD, Passarelli M, Quintao EC. Lipoprotein desialylation simultaneously enhances the cell cholesterol uptake and impairs the reverse cholesterol transport system: in vitro evidences utilizing neuraminidase-treated lipoproteins and mouse peritoneal macrophages. *Atherosclerosis* 1998;**139**:65-75.
50. Alipov VI, Sukhorukov VN, Karagodin VP, Grechko AV, Orekhov AN. Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference? *Vessel Plus* 2017;**1**:107-115.
51. Vukmirica J, Nishimaki-Mogami T, Tran K, Shan J, McLeod RS, Yuan J, Yao Z. The N-linked oligosaccharides at the amino terminus of human apoB are important for the assembly and secretion of VLDL. *J Lipid Res* 2002;**43**:1496-1507.
52. Wei HS, Wei HL, Zhao F, Zhong LP, Zhan YT. Glycosyltransferase GLT8D2 positively regulates ApoB100 protein expression in hepatocytes. *Int J Mol Sci* 2013;**14**:21435-21446.
53. Macri J, Adeli K. Conformational changes in apolipoprotein B modulate intracellular assembly and degradation of ApoB-containing lipoprotein particles in HepG2 cells. *Arterioscler Thromb Vasc Biol* 1997;**17**:2982-2994.
54. Harazono A, Kawasaki N, Kawanishi T, Hayakawa T. Site-specific glycosylation analysis of human apolipoprotein B100 using LC/ESI MS/MS. *Glycobiology* 2005;**15**:447-462.
55. Tertov VV, Orekhov AN. Metabolism of native and naturally occurring multiple modified low density lipoprotein in smooth muscle cells of human aortic intima. *Exp Mol Pathol* 1997;**64**:127-145.
56. Tertov VV, Sobenin IA, Gabbasov ZA, Popov EG, Jaakkola O, Solakivi T, Nikkari T, Smirnov VN, Orekhov AN. Multiple-modified desialylated low density lipoproteins that cause intracellular lipid accumulation. Isolation, fractionation and characterization. *Lab Invest* 1992;**67**:665-675.
57. Ruelland A, Gallou G, Legras B, Paillard F, Cloarec L. LDL sialic acid content in patients with coronary artery disease. *Clin Chim Acta* 1993;**221**:127-133.
58. Goodarzi MT, Rezaei M, Moadel E, Homayounfar S, Safari MR. Changes in sialylation of low-density lipoprotein in coronary artery disease. *Eur J Lipid Sci Technol* 2008;**110**:302-306.
59. Zakiev ER, Sobenin IA, Sukhorukov VN, Myasoedova VA, Ivanova EA, Orekhov AN. Carbohydrate composition of circulating multiple-modified low-density lipoprotein. *Vasc Health Risk Manag* 2016;**12**:379-385.
60. Lindbohm N, Gylling H, Miettinen TA. Sialic acid content of low density lipoprotein and its relation to lipid concentrations and metabolism of low density lipoprotein and cholesterol. *J Lipid Res* 2000;**41**:1110-1117.
61. Zhang L, Wei TT, Li Y, Li J, Fan Y, Huang FQ, Cai YY, Ma G, Liu JF, Chen QQ, Wang SL, Li H, Alolga RN, Liu B, Zhao DS, Shen JH, Wang XM, Zhu W, Li P, Qi LW. Functional metabolomics characterizes a key role for N-acetylneuraminic acid in coronary artery diseases. *Circulation* 2018;**137**:1374-1390.

62. White EJ, Gyulay G, Lhotak S, Szewczyk MM, Chong T, Fuller MT, Dadoo O, Fox-Robichaud AE, Austin RC, Trigatti BL, Igdoura SA. Sialidase down-regulation reduces non-HDL cholesterol, inhibits leukocyte transmigration, and attenuates atherosclerosis in ApoE knockout mice. *J Biol Chem* 2018;**293**:14689-14706.
63. Pedersen NB, Wang S, Narimatsu Y, Yang Z, Halim A, Schjoldager KT, Madsen TD, Seidah NG, Bennett EP, Levery SB, Clausen H. Low density lipoprotein receptor class A repeats are O-glycosylated in linker regions. *J Biol Chem* 2014;**289**:17312-17324.
64. Filipovic I. Effect of inhibiting N-glycosylation on the stability and binding activity of the low density lipoprotein receptor. *J Biol Chem* 1989;**264**:8815-8820.
65. Kizhakkedath P, John A, Al-Sawafi BK, Al-Gazali L, Ali BR. Endoplasmic reticulum quality control of LDLR variants associated with familial hypercholesterolemia. *FEBS Open Bio* 2019;**9**:1994-2005.
66. Kozarsky K, Kingsley D, Krieger M. Use of a mutant cell line to study the kinetics and function of O-linked glycosylation of low density lipoprotein receptors. *Proc Natl Acad Sci U S A* 1988;**85**:4335-4339.
67. Kingsley DM, Kozarsky KF, Hobbie L, Krieger M. Reversible defects in O-linked glycosylation and LDL receptor expression in a UDP-Gal/UDP-GalNAc 4-epimerase deficient mutant. *Cell* 1986;**44**:749-759.
68. Wang S, Mao Y, Narimatsu Y, Ye Z, Tian W, Goth CK, Lira-Navarrete E, Pedersen NB, Benito-Vicente A, Martin C, Uribe KB, Hurtado-Guerrero R, Christoffersen C, Seidah NG, Nielsen R, Christensen EI, Hansen L, Bennett EP, Vakhrushev SY, Schjoldager KT, Clausen H. Site-specific O-glycosylation of members of the low-density lipoprotein receptor superfamily enhances ligand interactions. *J Biol Chem* 2018;**293**:7408-7422.
69. Yang A, Gyulay G, Mitchell M, White E, Trigatti BL, Igdoura SA. Hypomorphic sialidase expression decreases serum cholesterol by downregulation of VLDL production in mice. *J Lipid Res* 2012;**53**:2573-2585.
70. Tozawa R, Ishibashi S, Osuga J, Yamamoto K, Yagyu H, Ohashi K, Tamura Y, Yahagi N, Iizuka Y, Okazaki H, Harada K, Gotoda T, Shimano H, Kimura S, Nagai R, Yamada N. Asialoglycoprotein receptor deficiency in mice lacking the major receptor subunit. Its obligate requirement for the stable expression of oligomeric receptor. *J Biol Chem* 2001;**276**:12624-12628.
71. Yoshimura A, Yoshida T, Seguchi T, Waki M, Ono M, Kuwano M. Low binding capacity and altered O-linked glycosylation of low density lipoprotein receptor in a monensin-resistant mutant of Chinese hamster ovary cells. *J Biol Chem* 1987;**262**:13299-13308.
72. Tomita K, Yoshida T, Yoshimura A, Ono M, Kuwano M. Chinese hamster ovary cell mutant with defective down-regulation of low density lipoprotein receptors. *J Biol Chem* 1987;**262**:1398-1404.
73. Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: insights from epidemiology, genetics, and biology. *J Lipid Res* 2016;**57**:1953-1975.
74. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987;**330**:132-137.
75. Fless GM, ZumMallen ME, Scanu AM. Physicochemical properties of apolipoprotein(a) and lipoprotein(a-) derived from the dissociation of human plasma lipoprotein (a). *J Biol Chem* 1986;**261**:8712-8718.
76. Seman LJ, Breckenridge WC. Isolation and partial characterization of apolipoprotein (a) from human lipoprotein (a). *Biochem Cell Biol* 1986;**64**:999-1009.
77. Bonen DK, Nassir F, Hausman AM, Davidson NO. Inhibition of N-linked glycosylation results in retention of intracellular apo[a] in hepatoma cells, although nonglycosylated and immature

forms of apolipoprotein[a] are competent to associate with apolipoprotein B-100 in vitro. *J Lipid Res* 1998;**39**:1629-1640.
78. Kratzin H, Armstrong VW, Niehaus M, Hilschmann N, Seidel D. Structural relationship of an apolipoprotein (a) phenotype (570 kDa) to plasminogen: homologous kringle domains are linked by carbohydrate-rich regions. *Biol Chem Hoppe Seyler* 1987;**368**:1533-1544.
79. Garner B, Merry AH, Royle L, Harvey DJ, Rudd PM, Thillet J. Structural elucidation of the N- and O-glycans of human apolipoprotein(a): role of o-glycans in conferring protease resistance. *J Biol Chem* 2001;**276**:22200-22208.
80. Jin Y, Manabe T. Direct targeting of human plasma for matrix-assisted laser desorption/ionization and analysis of plasma proteins by time of flight-mass spectrometry. *Electrophoresis* 2005;**26**:2823-2834.
81. Kailemia MJ, Wei W, Nguyen K, Beals E, Sawrey-Kubicek L, Rhodes C, Zhu C, Sacchi R, Zivkovic AM, Lebrilla CB. Targeted Measurements of O- and N-Glycopeptides Show That Proteins in High Density Lipoprotein Particles Are Enriched with Specific Glycosylation Compared to Plasma. *J Proteome Res* 2018;**17**:834-845.
82. Pirillo A, Catapano AL, Norata GD. Biological Consequences of Dysfunctional HDL. *Curr Med Chem* 2019;**26**:1644-1664.
83. Krishnan S, Huang J, Lee H, Guerrero A, Berglund L, Anuurad E, Lebrilla CB, Zivkovic AM. Combined High-Density Lipoprotein Proteomic and Glycomic Profiles in Patients at Risk for Coronary Artery Disease. *J Proteome Res* 2015;**14**:5109-5118.
84. Krishnan S, Shimoda M, Sacchi R, Kailemia MJ, Luxardi G, Kaysen GA, Parikh AN, Ngassam VN, Johansen K, Chertow GM, Grimes B, Smilowitz JT, Maverakis E, Lebrilla CB, Zivkovic AM. HDL Glycoprotein Composition and Site-Specific Glycosylation Differentiates Between Clinical Groups and Affects IL-6 Secretion in Lipopolysaccharide-Stimulated Monocytes. *Sci Rep* 2017;**13**:43728.
85. Savinova OV, Fillaus K, Jing L, Harris WS, Shearer GC. Reduced apolipoprotein glycosylation in patients with the metabolic syndrome. *PLoS One* 2014;**9**:e104833.
86. Lima VV, Spitler K, Choi H, Webb RC, Tostes RC. O-GlcNAcylation and oxidation of proteins: is signalling in the cardiovascular system becoming sweeter? *Clin Sci (Lond)* 2012;**123**:473-486.
87. Remaley AT, Wong AW, Schumacher UK, Meng MS, Brewer HB, Jr., Hoeg JM. O-linked glycosylation modifies the association of apolipoprotein A-II to high density lipoproteins. *J Biol Chem* 1993;**268**:6785-6790.
88. Wernette-Hammond ME, Lauer SJ, Corsini A, Walker D, Taylor JM, Rall SC, Jr. Glycosylation of human apolipoprotein E. The carbohydrate attachment site is threonine 194. *J Biol Chem* 1989;**264**:9094-9101.
89. Zanni EE, Kouvatsi A, Hadzopoulou-Cladaras M, Krieger M, Zannis VI. Expression of ApoE gene in Chinese hamster cells with a reversible defect in O-glycosylation. Glycosylation is not required for apoE secretion. *J Biol Chem* 1989;**264**:9137-9140.
90. Lee Y, Kockx M, Raftery MJ, Jessup W, Griffith R, Kritharides L. Glycosylation and sialylation of macrophage-derived human apolipoprotein E analyzed by SDS-PAGE and mass spectrometry: evidence for a novel site of glycosylation on Ser290. *Mol Cell Proteomics* 2010;**9**:1968-1981.
91. Zhu C, Wong M, Li Q, Sawrey-Kubicek L, Beals E, Rhodes CH, Sacchi R, Lebrilla CB, Zivkovic AM. Site-Specific Glycoprofiles of HDL-Associated ApoE are Correlated with HDL Functional Capacity and Unaffected by Short-Term Diet. *J Proteome Res* 2019;**18**:3977-3984.
92. Zhao Y, Mazzone T. Transport and processing of endogenously synthesized ApoE on the macrophage cell surface. *J Biol Chem* 2000;**275**:4759-4765.
93. Bonacina F, Coe D, Wang G, Longhi MP, Baragetti A, Moregola A, Garlaschelli K, Uboldi P, Pellegatta F, Grigore L, Da Dalt L, Annoni A, Gregori S, Xiao Q, Caruso D, Mitro N, Catapano AL,

Marelli-Berg FM, Norata GD. Myeloid apolipoprotein E controls dendritic cell antigen presentation and T cell activation. *Nat Commun* 2018;**9**:3083.
94. Pirillo A, Catapano AL, Norata GD. HDL in infectious diseases and sepsis. *Handb Exp Pharmacol* 2015;**224**:483-508.
95. Norata GD, Pirillo A, Catapano AL. HDLs, immunity, and atherosclerosis. *Curr Opin Lipidol* 2011;**22**:410-416.
96. Marmillot P, Rao MN, Liu QH, Lakshman MR. Desialylation of human apolipoprotein E decreases its binding to human high-density lipoprotein and its ability to deliver esterified cholesterol to the liver. *Metabolism* 1999;**48**:1184-1192.
97. O K, Hill JS, Wang X, McLeod R, Pritchard PH. Lecithin:cholesterol acyltransferase: role of N-linked glycosylation in enzyme function. *Biochem J* 1993;**294 (Pt 3)**:879-884.
98. Collet X, Fielding CJ. Effects of inhibitors of N-linked oligosaccharide processing on the secretion, stability, and activity of lecithin:cholesterol acyltransferase. *Biochemistry* 1991;**30**:3228-3234.
99. Schindler PA, Settineri CA, Collet X, Fielding CJ, Burlingame AL. Site-specific detection and structural characterization of the glycosylation of human plasma proteins lecithin:cholesterol acyltransferase and apolipoprotein D using HPLC/electrospray mass spectrometry and sequential glycosidase digestion. *Protein Sci* 1995;**4**:791-803.
100. Spahr C, Kim JJ, Deng S, Kodama P, Xia Z, Tang J, Zhang R, Siu S, Nuanmanee N, Estes B, Stevens J, Zhou M, Lu HS. Recombinant human lecithin-cholesterol acyltransferase Fc fusion: analysis of N- and O-linked glycans and identification and elimination of a xylose-based O-linked tetrasaccharide core in the linker region. *Protein Sci* 2013;**22**:1739-1753.
101. Stevenson SC, Wang S, Deng L, Tall AR. Human Plasma Cholesteryl Ester Transfer Protein Consists of a Mixture of two forms reflecting variable glycosylation at asparagine 341. *Biochemistry* 1993;**32**:5121-5126.
102. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res* 1993;**34**:1255-1274.
103. Albers JJ, Vuletic S, Cheung MC. Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolism. *Biochim Biophys Acta* 2012;**1821**:345-357.
104. Jiang XC. Phospholipid transfer protein: its impact on lipoprotein homeostasis and atherosclerosis. *J Lipid Res* 2018;**59**:764-771.
105. Day JR, Albers JJ, Lofton-Day CE, Gilbert TL, Ching AF, Grant FJ, O'Hara PJ, Marcovina SM, Adolphson JL. Complete cDNA encoding human phospholipid transfer protein from human endothelial cells. *J Biol Chem* 1994;**269**:9388-9391.
106. Huuskonen J, Jauhiainen M, Ehnholm C, Olkkonen VM. Biosynthesis and secretion of human plasma phospholipid transfer protein. *J Lipid Res* 1998;**39**:2021-2030.
107. Qu SJ, Fan HZ, Gillard BK, Pownall HJ. N-Glycosylation is required for secretion-competent human plasma phospholipid transfer protein. *Protein J* 2006;**25**:167-173.
108. Albers JJ, Day JR, Wolfbauer G, Kennedy H, Vuletic S, Cheung MC. Impact of site-specific N-glycosylation on cellular secretion, activity and specific activity of the plasma phospholipid transfer protein. *Biochim Biophys Acta* 2011;**1814**:908-911.
109. Yvan-Charvet L, Bonacina F, Guinamard RR, Norata GD. Immunometabolic function of cholesterol in cardiovascular disease and beyond. *Cardiovasc Res* 2019;**115**:1393-1407.
110. Gomaraschi M, Bonacina F, Norata GD. Lysosomal Acid Lipase: From Cellular Lipid Handler to Immunometabolic Target. *Trends Pharmacol Sci* 2019;**40**:104-115.
111. Sahebi R, Hassanian SM, Ghayour-Mobarhan M, Farrokhi E, Rezayi M, Samadi S, Bahramian S, Ferns GA, Avan A. Scavenger receptor Class B type I as a potential risk stratification biomarker and therapeutic target in cardiovascular disease. *J Cell Physiol* 2019;**234**:16925-16932.

112. Vinals M, Xu S, Vasile E, Krieger M. Identification of the N-linked glycosylation sites on the high density lipoprotein (HDL) receptor SR-BI and assessment of their effects on HDL binding and selective lipid uptake. *J Biol Chem* 2003;**278**:5325-5332.
113. Brunham LR, Tietjen I, Bochem AE, Singaraja RR, Franchini PL, Radomski C, Mattice M, Legendre A, Hovingh GK, Kastelein JJ, Hayden MR. Novel mutations in scavenger receptor BI associated with high HDL cholesterol in humans. *Clin Genet* 2011;**79**:575-581.
114. Chadwick AC, Sahoo D. Functional characterization of newly-discovered mutations in human SR-BI. *PLoS One* 2012;**7**:e45660.
115. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, DerOhannessian S, Kontush A, Surendran P, Saleheen D, Trompet S, Jukema JW, De Craen A, Deloukas P, Sattar N, Ford I, Packard C, Majumder A, Alam DS, Di Angelantonio E, Abecasis G, Chowdhury R, Erdmann J, Nordestgaard BG, Nielsen SF, Tybjaerg-Hansen A, Schmidt RF, Kuulasmaa K, Liu DJ, Perola M, Blankenberg S, Salomaa V, Mannisto S, Amouyel P, Arveiler D, Ferrieres J, Muller-Nurasyid M, Ferrario M, Kee F, Willer CJ, Samani N, Schunkert H, Butterworth AS, Howson JM, Peloso GM, Stitziel NO, Danesh J, Kathiresan S, Rader DJ, Consortium CHDE, Consortium CAE, Global Lipids Genetics C. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science* 2016;**351**:1166-1171.
116. Norata GD, Tsimikas S, Pirillo A, Catapano AL. Apolipoprotein C-III: From Pathophysiology to Pharmacology. *Trends Pharmacol Sci* 2015;**36**:675-687.
117. Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014;**371**:22-31.
118. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med* 2014;**371**:32-41.
119. van Capelleveen JC, Bernelot Moens SJ, Yang X, Kastelein JJP, Wareham NJ, Zwinderman AH, Stroes ESG, Witztum JL, Hovingh GK, Khaw KT, Boekholdt SM, Tsimikas S. Apolipoprotein C-III Levels and Incident Coronary Artery Disease Risk: The EPIC-Norfolk Prospective Population Study. *Arterioscler Thromb Vasc Biol* 2017;**37**:1206-1212.
120. Fredenrich A, Giroux LM, Tremblay M, Krimbou L, Davignon J, Cohn JS. Plasma lipoprotein distribution of apoC-III in normolipidemic and hypertriglyceridemic subjects: comparison of the apoC-III to apoE ratio in different lipoprotein fractions. *J Lipid Res* 1997;**38**:1421-1432.
121. Jabs HU, Assmann G. Characterization of an apolipoprotein C-III mutant by high-performance liquid chromatography and time-of-flight secondary ion mass spectrometry. *J Chromatogr* 1987;**414**:323-333.
122. Maeda H, Hashimoto RK, Ogura T, Hiraga S, Uzawa H. Molecular cloning of a human apoC-III variant: Thr 74----Ala 74 mutation prevents O-glycosylation. *J Lipid Res* 1987;**28**:1405-1409.

123. Yassine HN, Trenchevska O, Ramrakhiani A, Parekh A, Koska J, Walker RW, Billheimer D, Reaven PD, Yen FT, Nelson RW, Goran MI, Nedelkov D. The Association of Human Apolipoprotein C-III Sialylation Proteoforms with Plasma Triglycerides. *PLoS One* 2015;**10**:e0144138.
124. Olivieri O, Chiariello C, Martinelli N, Castagna A, Speziali G, Girelli D, Pizzolo F, Bassi A, Cecconi D, Robotti E, Manfredi M, Conte E, Marengo E. Sialylated isoforms of apolipoprotein C-III and plasma lipids in subjects with coronary artery disease. *Clin Chem Lab Med* 2018;**56**:1542-1550.
125. Catapano AL. The distribution of apo C-II and apo C-III in very low density lipoproteins of normal and type IV subjects. *Atherosclerosis* 1980;**35**:419-424.
126. Kegulian NC, Ramms B, Horton S, Trenchevska O, Nedelkov D, Graham MJ, Lee RG, Esko JD, Yassine HN, Gordts P. ApoC-III Glycoforms Are Differentially Cleared by Hepatic TRL (Triglyceride-Rich Lipoprotein) Receptors. *Arterioscler Thromb Vasc Biol* 2019;**39**:2145-2156.
127. Koska J, Yassine H, Trenchevska O, Sinari S, Schwenke DC, Yen FT, Billheimer D, Nelson RW, Nedelkov D, Reaven PD. Disialylated apolipoprotein C-III proteoform is associated with improved lipids in prediabetes and type 2 diabetes. *J Lipid Res* 2016;**57**:894-905.
128. Mattijssen F, Kersten S. Regulation of triglyceride metabolism by Angiopoietin-like proteins. *Biochim Biophys Acta* 2012;**1821**:782-789.
129. Stitziel NO, Khera AV, Wang X, Bierhals AJ, Vourakis AC, Sperry AE, Natarajan P, Klarin D, Emdin CA, Zekavat SM, Nomura A, Erdmann J, Schunkert H, Samani NJ, Kraus WE, Shah SH, Yu B, Boerwinkle E, Rader DJ, Gupta N, Frossard PM, Rasheed A, Danesh J, Lander ES, Gabriel S, Saleheen D, Musunuru K, Kathiresan S, Promis, Myocardial Infarction Genetics Consortium I. ANGPTL3 Deficiency and Protection Against Coronary Artery Disease. *J Am Coll Cardiol* 2017;**69**:2054-2063.
130. Schjoldager KT, Vester-Christensen MB, Bennett EP, Levery SB, Schwientek T, Yin W, Blixt O, Clausen H. O-glycosylation modulates proprotein convertase activation of angiopoietin-like protein 3: possible role of polypeptide GalNAc-transferase-2 in regulation of concentrations of plasma lipids. *J Biol Chem* 2010;**285**:36293-36303.
131. Jin W, Wang X, Millar JS, Quertermous T, Rothblat GH, Glick JM, Rader DJ. Hepatic proprotein convertases modulate HDL metabolism. *Cell Metab* 2007;**6**:129-136.
132. Schjoldager KT, Vakhrushev SY, Kong Y, Steentoft C, Nudelman AS, Pedersen NB, Wandall HH, Mandel U, Bennett EP, Levery SB, Clausen H. Probing isoform-specific functions of polypeptide GalNAc-transferases using zinc finger nuclease glycoengineered SimpleCells. *Proc Natl Acad Sci U S A* 2012;**109**:9893-9898.
133. Choromanska B, Mysliwiec P, Choromanska K, Dadan J, Chabowski A. The role of CD36 receptor in the pathogenesis of atherosclerosis. *Adv Clin Exp Med* 2017;**26**:717-722.
134. Luiken JJ, Chanda D, Nabben M, Neumann D, Glatz JF. Post-translational modifications of CD36 (SR-B2): Implications for regulation of myocellular fatty acid uptake. *Biochim Biophys Acta* 2016;**1862**:2253-2258.
135. Hoosdally SJ, Andress EJ, Wooding C, Martin CA, Linton KJ. The Human Scavenger Receptor CD36: glycosylation status and its role in trafficking and function. *J Biol Chem* 2009;**284**:16277-16288.
136. Hsieh J, Longuet C, Maida A, Bahrami J, Xu E, Baker CL, Brubaker PL, Drucker DJ, Adeli K. Glucagon-like peptide-2 increases intestinal lipid absorption and chylomicron production via CD36. *Gastroenterology* 2009;**137**:997-1005, 1005 e1001-1004.
137. Laczy B, Fulop N, Onay-Besikci A, Des Rosiers C, Chatham JC. Acute regulation of cardiac metabolism by the hexosamine biosynthesis pathway and protein O-GlcNAcylation. *PLoS One* 2011;**6**:e18417.

138. Kawecki C, Bocquet O, Schmelzer CEH, Heinz A, Ihling C, Wahart A, Romier B, Bennasroune A, Blaise S, Terryn C, Linton KJ, Martiny L, Duca L, Maurice P. Identification of CD36 as a new interaction partner of membrane NEU1: potential implication in the pro-atherogenic effects of the elastin receptor complex. *Cell Mol Life Sci* 2019;**76**:791-807.

Table(s)

Table 1. Impact of loss-of-function mutations in glyco-genes on lipid/lipoprotein phenotype

Glyco-gene	Name	Location	Clinical manifestations	Effect of plasma lipid/lipoprotein profile
PMM2	Phosphomannomutase 2	16p13.2	Cerebellar hypoplasia, abnormal subcutaneous fat distribution, dysmorphism	↓LDL-C , apoB, HDL-C
ALG6	Alpha-1,3-glucosyltransferase	1p31.3	Hypotonia, developmental delay, ataxia and epilepsy	↓LDL-C , apoB, HDL-C
B4GALT1	Beta-1,4-galactosyltransfrase 1	9p21.1	Mild developmental delay, Dandy-Walker malformation, coagulation abnormalities	↓non-HDL-C, TC/HDL-C
CCDC115	Coiled-coil domain-containing protein 115	2q21.1	Hepatosplenomegaly, steatohepatitis with fibrosis	↑ total cholesterol, LDL-C
TMEM199	Transmembrane protein 199	17q11.2	Hepatosplenomegaly, coagulation factor deficiencies, low ceruloplasmin, elevated AST, ALT	↑ total cholesterol, LDL-C
NGLY1	N-glycanase 1	3p24.2	Developmental delay, hypotonia, movement disorder, hepatic steatosis	↑ total cholesterol, LDL-C, TG
GALNT2	N-acetylgalactosaminyl transferase 2	1q42.13	Developmental delay, dysmorphic features, epilepsy, chronic insomnia	↓HDL-C
ASGR1	Asialoglycoprotein receptor 1	17p13.1	Increased levels of alkaline phosphatase and vitamin B12	↓non-HDL-C

Table 2. Glycosylation sites of proteins involved in lipoprotein metabolism

Protein	N-glycosylation sites*	O-glycosylation sites*
ApoB	Asn^{7}, Asn^{158}, Asn^{956}, Asn^{1341}, Asn^{1350}, Asn^{1496}, Asn^{2212}, Asn^{2533}, Asn^{2752}, Asn^{2955}, Asn^{3074}, Asn^{3197}, Asn^{3309}, Asn^{3331}, Asn^{3384}, Asn^{3438}, Asn^{3868}, Asn^{4210}, Asn^{4324}, Asn^{4404}	Thr^{3193}
LDLR	Asn^{76}, Asn^{135}, Asn^{251}, Asn^{494}, Asn^{636}, Asn^{644}	Thr^{700}, Ser^{711}, Ser^{712}, Thr^{703}, Thr^{713}, Ser^{704}, Thr^{705}, Thr^{717}, Thr^{720}, Thr^{721}, Thr^{722}, Ser^{729}
Apo(a)	Asn^{42}, Asn^{82}, Asn^{196}, Asn^{310}, Asn^{424}, Asn^{538}, Asn^{652}, Asn^{766}, Asn^{880}, Asn^{994}, Asn^{1108}, Asn^{1222}, Asn^{1336}, Asn^{1450}, Asn^{1564}, Asn^{1678}, Asn^{1792}, Asn^{1906}, Asn^{2020}, Asn^{2134}, Asn^{2248}, Asn^{2362}, Asn^{2476}, Asn^{2590}, Asn^{2704}, Asn^{2818}, Asn^{2932}, Asn^{3046}, Asn^{3160}, Asn^{3274}, Asn^{3388}, Asn^{3502}, Asn^{3614}, Asn^{3728}, Asn^{3836}, Asn^{3870}, Asn^{3950}, Asn^{4064}, Asn^{4178}	Yes, unknown sites (80% represented by the monosialylated core type I structure)
ApoA-I	Yes, unknown sites	Thr^{203}, Ser^{210}
ApoA-II	-	Thr^{42}
ApoE	-	Thr^{8}, Thr^{18}, Thr^{194}, Ser^{197}, Thr^{289}, Ser^{290}, Ser^{296}
LCAT	Asn^{20}, Asn^{84}, Asn^{272}, Asn^{384}	Thr^{407}, Ser^{409}
CETP	Asn^{88}, Asn^{240}, Asn^{341}, Asn^{396}	-
PLTP	Asn^{47}, Asn^{77}, Asn^{100}, Asn^{126}, Asn^{228}, Asn^{381}	Ser^{466}, Thr^{467}, Thr^{470}, Ser^{472}, Thr^{473}
SR-BI	Asn^{102}, Asn^{108}, Asn^{173}, Asn^{212}, Asn^{227}, Asn^{255}, Asn^{310}, Asn^{330}, Asn^{383}	-
ApoC-III	-	Thr^{74}
ANGPTL3	Asn^{7}, Asn^{99}, Asn^{280}, Asn^{341}	Thr^{210}
CD36	Asn^{79}, Asn^{102}, Asn^{134}, Asn^{163}, Asn^{205}, Asn^{220}, Asn^{235}, Asn^{247}, Asn^{321}, Asn^{417}	Yes, unknown sites

* numbering based on mature protein without signal peptide

Figure 1

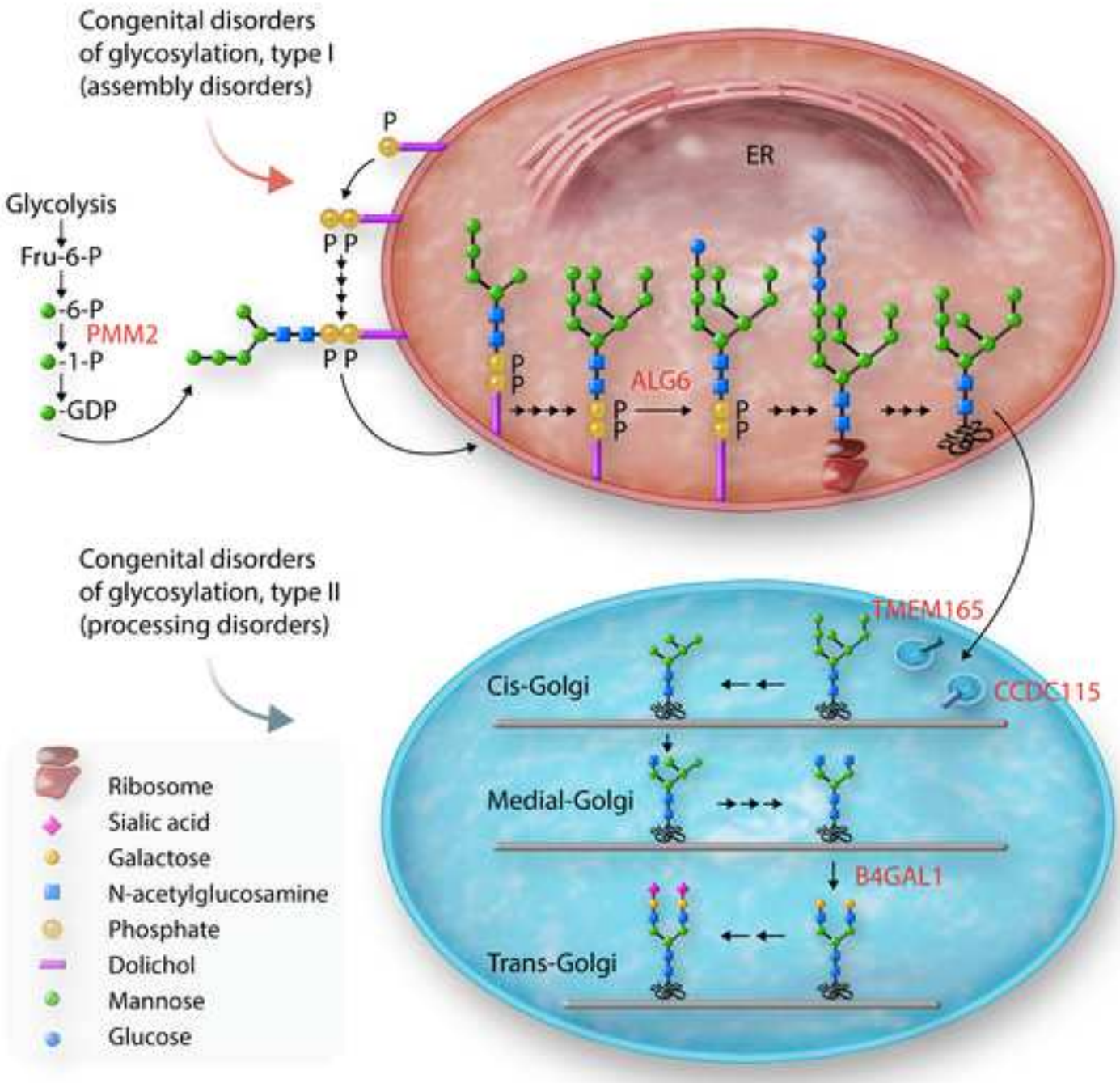

Figure 2