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Review

Galactose in human metabolism, glycosylation and congenital metabolic diseases: Time for a closer look

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ABSTRACT

Galactose is an essential carbohydrate for cellular metabolism, as it contributes to energy production and storage in several human tissues while also being a precursor for glycosylation. Galactosylated glycoconjugates, such as glycoproteins, keratan sulfate-containing proteoglycans and glycolipids, exert a plethora of biological functions, including structural support, cellular adhesion, intracellular signaling and many more. The biological relevance of galactose is further entailed by the number of pathogenic conditions consequent to defects in galactosylation and galactose homeostasis. The growing number of rare congenital disorders involving galactose along with its recent therapeutical applications are drawing increasing attention to galactose metabolism.

In this review, we aim to draw a comprehensive overview of the biological functions of galactose in human cells, including its metabolism and its role in glycosylation, and to provide a systematic description of all known congenital metabolic disorders resulting from alterations of its homeostasis.

1. Introduction

Galactose is an important carbohydrate for cellular metabolism and one of the most abundant sugar in the human diet [1]. Historically and panculturally, human diets have been primarily based on carbohydrates, accounting alone for 40–80% of the total daily calories [2,3]. The term carbohydrates derives from the definition “hydrates of carbon” which refers to polyhydroxylated compounds with a carbonyl group (either an aldehyde or a ketone). The most simple form (or basic unit) of carbohydrates is called monosaccharide (derived from the Greek word *sákkharon*, meaning sweetness) [4]. Two monosaccharides can be covalently linked together via a glycosidic bond to generate a disaccharide, while a chain of multiple monosaccharide units is defined as oligosaccharide (2–12 units) or polysaccharide (>12 units).

Despite being merely considered as primary source of energy and structural materials for a long time, over the past 50 years the awareness of the biochemical importance of carbohydrates has been growing exponentially, fueled by the incremental understanding of their biological functions, chemical properties and pharmaceutical relevance. Aside from energy production and storage, galactose and other carbohydrates are in fact involved in other essential biological processes, summarized in Fig. 1 [2,5,6]. Most of these functions are exerted by carbohydrates in the form of glycoconjugates. Glycoconjugates are hybrid macromolecules resulting from the covalent attachment of long branched oligo−/polysaccharides, named glycans, to a protein or lipid, via a sequence of finely regulated and compartmentalized enzymatic reactions that are collectively called glycosylation. Due to the highly versatile nature and unsurpassed high-density information-coding capacity of complex carbohydrates, glycans represent an ideal platform to encode information in the shape of oligomers. For this reason, glycans have been defined as the third alphabet of life (a.k.a. sugar code), alongside nucleic acids and amino acids [7–9]. In humans, this sugar code is for the most part written based on less than ten monosaccharides, of which galactose represents one of the most abundant [1].

In this review, we aim to draw a comprehensive overview of the biological functions of galactose in human cells, including its metabolism and its role in glycosylation, and to provide a systematic description of all congenital metabolic disorders resulting from alterations of its homeostasis.

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2. The 'lactic glucose': galactose

Galactose is a reducing monosaccharide containing six carbon atoms ($C_6H_{12}O_6$), making it an hexose. Galactose is a carbon 4 (C-4) epimer of glucose, which represents the main carbon source for anaerobic energy production and glycan biosynthesis in most living organisms [10]. Being a monosaccharide, galactose can directly be absorbed after digestion and enter the bloodstream to reach the target tissues where it fuels energy production, glycosylation and other important metabolic functions.

Thanks to its chemical structure, solubility and dedicated transport system that allows diffusion across the membranes, galactose is an interesting candidate for new biotechnological and pharmacological applications. For example, it has been used to generate galactose-containing conjugates to improve drug delivery and absorption, like successfully attempted for dopamine supplementation in Parkinson's patients [11].

Galactose, like all other aldohexoses, has two enantiomers: D-galactose (from Latin *dexter*, right) or L-galactose (from Latin *laevusor*, left), the latter not naturally occurring in higher living organisms as it cannot be further metabolized within the cell (Fig. 2). Henceforward, the term 'galactose' will be used throughout the manuscript to indicate D-galactose, unless otherwise specified. Galactose can occur in linear form or in cyclic form (Fig. 2). The six-carbon cyclic form, named galactopyranose, is the only cyclic form found in mammals, while the five-carbon ring, named galactofuranose, is frequently found in lower organisms such as bacteria, fungi, protozoa, sponges and green algae. Both these cyclic forms have two anomeric configurations depending on the position of the hydroxyl group on C-4: in the α the -OH group is located below the equatorial plan, while in the β anomer is located above (Fig. 2) [12].

Galactose was first isolated in the 1850s by L. Pasteur [13] from milk, but the characterization of its structural configuration was achieved only 30 years later by E. Fischer and R. Morrell [14]. The etymology of the name 'galactose' itself emphasizes the profound relation between

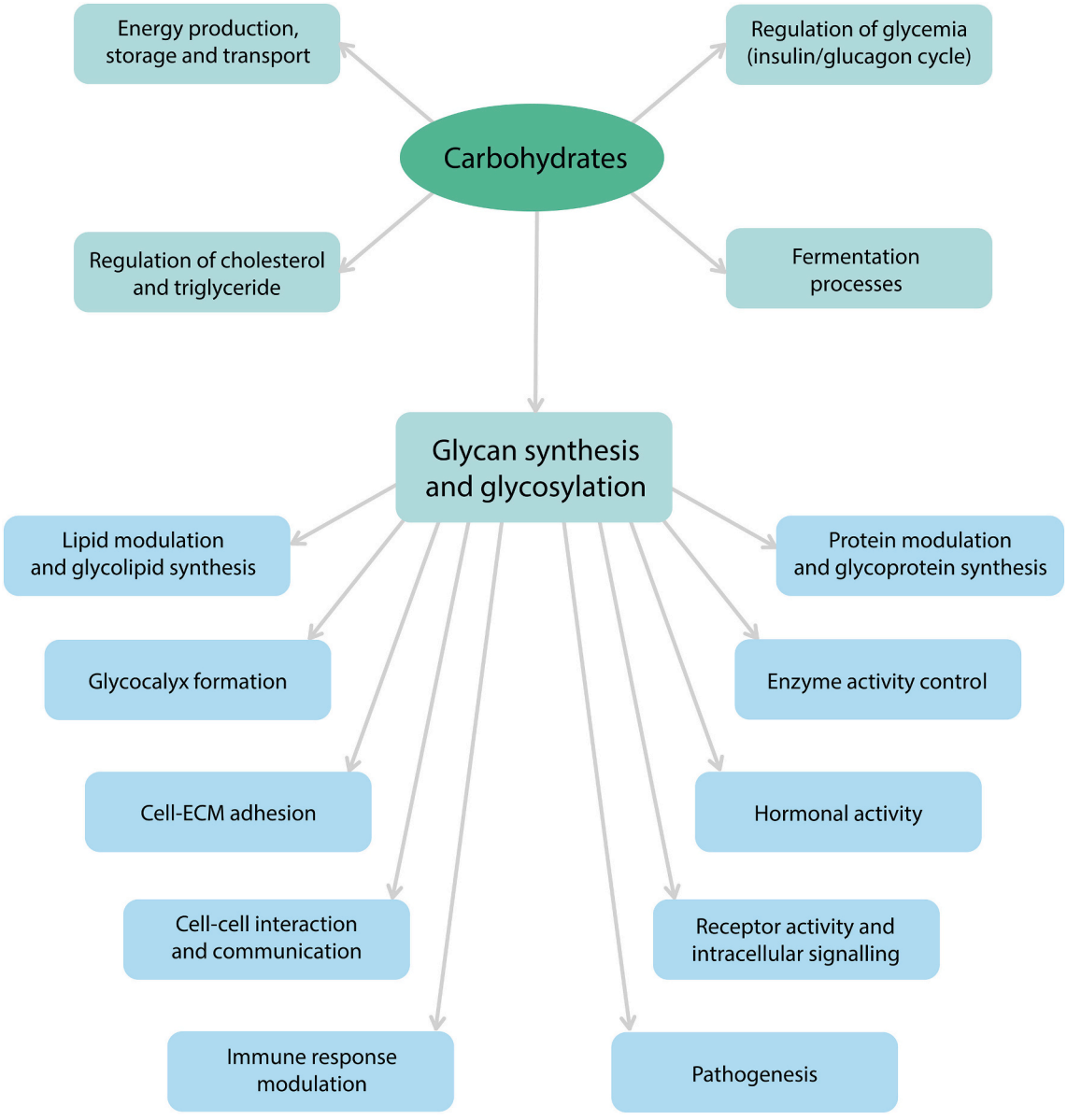

Fig. 1. Summary of the main biological functions of carbohydrates. The green boxes indicate the main biochemical functions of carbohydrates in eukaryotic cells, specifically human cells. The blue boxes indicate the main biological roles of glycans and glycoconjugates. The box 'pathogenesis' refers to the roles played by glycoconjugates in the pathogenesis of a wide array of human pathological conditions including inflammation, cancer, degenerative neuromuscular disorders, and diabetes.

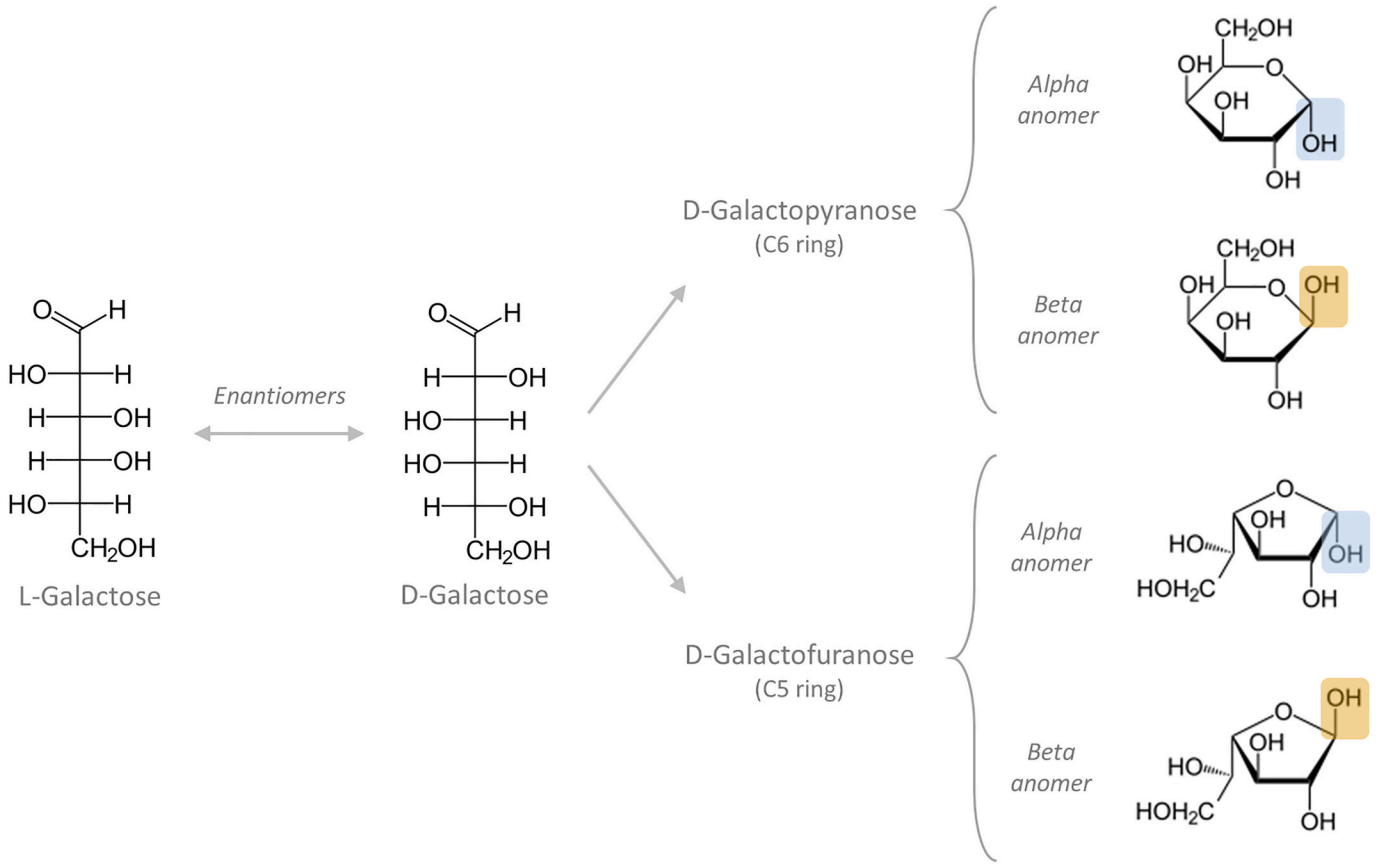

Fig. 2. Cyclic and linear configurations of the enantiomeric pairs of galactose. The hydroxy group on the hemiacetal carbon (C-1) is indicated in blue in the alpha (α) anomer and in yellow in the beta (β) anomer.

this carbohydrate and milk. In 1860s P.E.M. Berthelot [15] named this sugar as 'lactic glucose' or galactose (from the Greek word *galaktos*, meaning milk, followed by the chemical suffix for sugars *-ose*). In fact, although galactose is naturally present in our everyday diet as found in a variety of foods such as cereals, fruits, vegetables and honey [16–20], the largest amount of galactose in the human diet is provided by consumption of milk and dairy products. In milk, galactose is present in the form of lactose, a disaccharide formed by a β-1 → 4 glycosidic linkage between one molecule of galactose and one of glucose. This glycosidic bond can be hydrolyzed by the enzyme lactase, which is a 150 kDa β-D-galactosidase found in the apical surface of the intestinal microvilli. Lactase mediates the absorption of lactose by hydrolyzing it in glucose and galactose, which are then absorbed through the enterocyte membrane [*paragraph 4*].

3. Galactose in human anabolism and catabolism: over a century long puzzle

As galactose enters the body after ingestion, it starts its metabolic journey from the intestinal lumen, where it is absorbed by the endothelial cell of the villi via the sodium-glucose linked transporter type I (SGLT1) [21]. Next, galactose is released into the bloodstream via glucose transporter type 2 (GLUT2) located on the membrane on the opposite side of the enterocytes [21]. Galactose is then transported to the liver via the portal vein, entering the organ via other GLUTs. Most of the ingested galactose is retained in the liver, while a minor amount reaches other organs, such as the brain or the mammary glands where it is used for the synthesis of amino acids or for the production of lactose, respectively [10]. In the liver, skeletal muscles and other target tissues, most of the galactose is metabolized via three main routes: (i) Leloir pathway, from which galactose flows into glycolysis or glycosylation, (ii) conversion to galactonate that is further metabolized to enter the pentose phosphate pathway, and (iii) reduction to galactitol that is then excreted with urine.

3.1. Leloir pathway, the crossroad between glycolysis and glycosylation

From simple organisms like bacteria all the way to plants and animals, the metabolism of galactose has been highly conserved and generally follows the so called Leloir pathway. The Leloir pathway, firstly described by L.F. Leloir in 1948, is composed of four sequential enzymatic reactions that ultimately change the stereochemical configuration of a single carbon (C-1) of galactose (Figs. 3, 4) [22–24]. Via this route, galactose can either be activated to UDP-α-D-galactose (UDP-Gal) and be used as precursor for glycosylation, or can be converted to UDP-α-D-glucose (UDP-Glc) which then flows into glycogen synthesis or glycolysis, depending on the type of tissue and its energy demand.

First, β-D-galactose is converted to its stereoisomer α-D-galactose by the galactose mutarotase enzyme (GALM) (Fig. 3) [22,25]. GALM was first discovered in *Escherichia coli* by Wallenfels and colleagues in 1965 [26], but only in 2003 its human homolog gene and protein were characterized [27]. The human GALM is a 342 aa protein structured as a β-sheet "sandwich" with its active site lying in a rather open cleft, which unlike its bacterial counterpart acts like a monomer [22,27,28]. Aside from galactose, GALM is also able (with variable efficiency) to convert the anomeric configuration of D-glucose, L-arabinose, D-xylulose, maltose and lactose [28].

The second step of the Leloir pathway is represented by the phosphorylation of α-D-galactose into α-D-galactose 1-phosphate (Gal—1P) by galactokinase (GALK) (Fig. 3). GALK is 400 aa protein belonging to the GHMP (Galacto-, Homoserine, Mevalonate and Phosphomevalonate) kinase superfamily. Although the three-dimensional structure of human

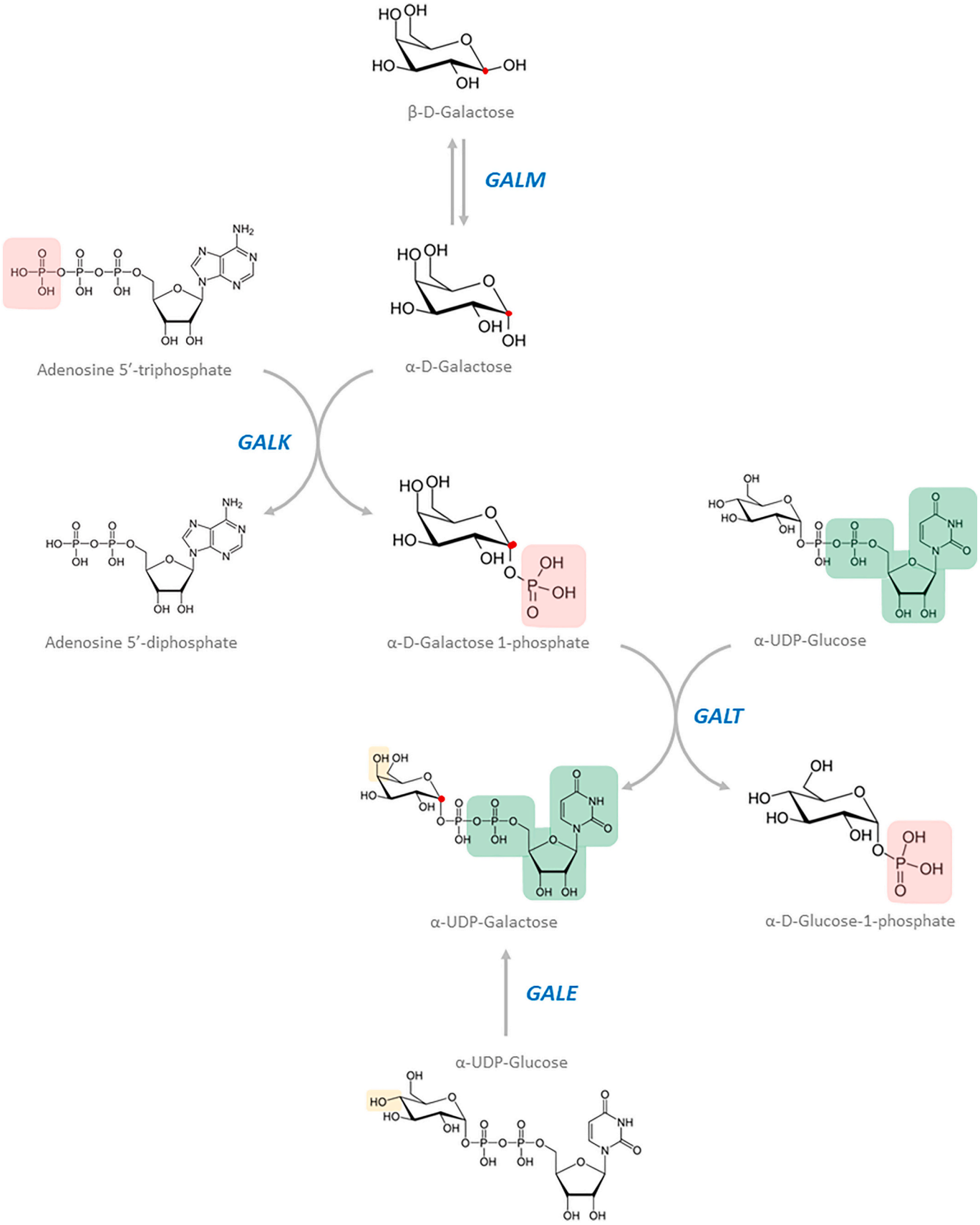

Fig. 3. Leloir pathway. The four steps of the Leloir pathway are shown in order of activity, and they are mediated by (1) GALM, (2) GALK, (3) GALT and (4) GALE. Legend: the anomeric carbon 1 (C-1) of galactose is indicated by a red dot; the UMP part of the molecules is indicated in green; the phosphate group is indicated in pink; the hydroxy group on carbon 4 (C-4) is indicated in yellow.

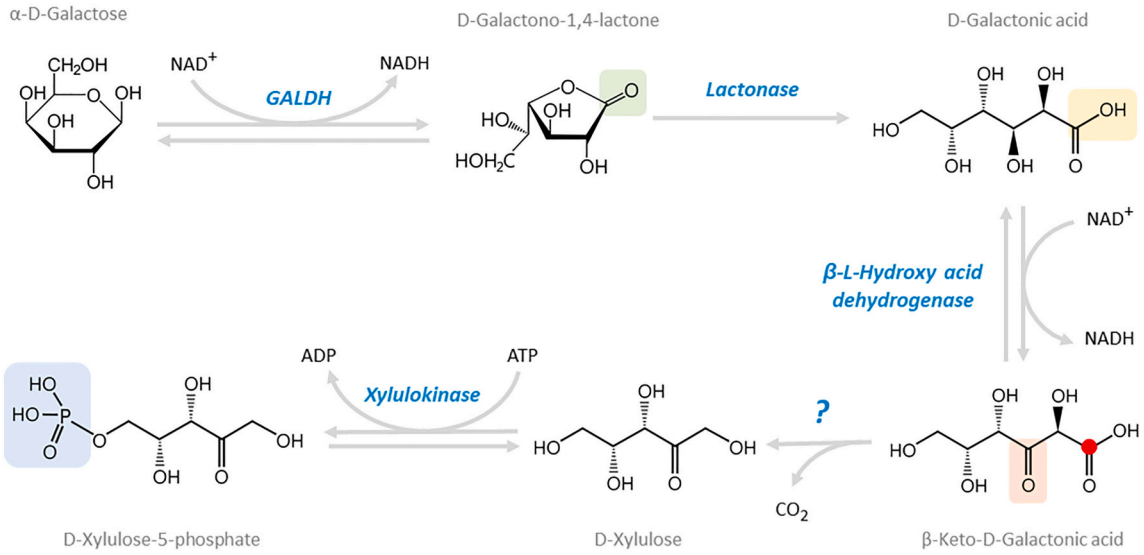

Fig. 4. Alternative route for metabolism of galactose to the PPP via galactonate. The names of the human enzymes that catalyze each reaction are reported in blue color. The name of the synthesized compound is reported in grey color. The colored blocks indicate the chemical groups modified in each reaction. The red dot in β-keto-D-galactonic acid indicates the anomeric carbon atom (C-1) that is removed via decarboxylation. This step has been proposed to be enzymatically achieved, although the specific mammalian decarboxylase has not been identified yet.

GALK has not yet been fully unravel, a crystal structure of GALK of L. *lactis* in complex with α-D-galactose and inorganic phosphate was described in 2003. This bacterial GALK (24% identity and 47% similarity with the human ortholog) has two domains of roughly equal size, with the catalytic site lying between the N- and C-terminal domains [22,29]. Unlike in bacteria, in humans this ATP-dependent enzyme first binds to ATP and later to α-D-galactose [22], and it is able to successfully phosphorylate 2-deoxy-D-galactose as well [30,31].

Galactose 1-phosphate uridylyltransferase (GALT) mediates the third step of the Leloir pathway to produce UDP–Gal (Fig. 3). GALT catalyzes the reaction through which Gal-1P and UDP-Glc are converted to α-D-glucose 1-phosphate (Glc-1P) and UDP-Gal [25]. Human GALT is a homodimer containing 379 aa residues per monomer, each containing a structural zinc-binding site and an active site at position 168. Although highly conserved, human GALT shows significant differences with bacterial GALT in metal ligation and dimer interactions [32,33].

The last step of the Leloir pathway is catalyzed by UDP-galactose 4′-epimerase (GALE). GALE is a 348 aa protein that mediates the interconversion of UDP-Gal and UDP-Glc (Fig. 3) [25,34]. Structurally, this enzyme is a member of the short chain dehydrogenase/reductase enzyme class. In solution, GALE is a dimer with a substrate binding site in each subunit, which requires NAD^+ for catalytic activity. Unlike the highly specific bacterial enzyme, human GALE can also catalyze the interconversion of UDP-*N*-acetyl-α-D-galactosamine (UDP-GalNAc) and UDP-*N*-acetyl-α-D-glucosamine (UDP-GlcNAC) [35–37].

Although the Leloir pathway is traditionally described as the metabolism from galactose and UDP-Glc towards UDP-Gal synthesis, the enzymatic reactions of this pathway (with the exception of GALK) can flow in both directions (Fig. 3), depending on substrate levels and biochemical demands of a tissue. In most tissues, UDP-Gal is used as building block for glycosylation. In skeletal muscle and liver, UDP-Gal can be converted to UDP-Glc, a precursor of glycogen synthesis, for energy storage when the energetic demand is low. Conversely, when the energy demand is high, UDP-Gal can participate to energy production by being converted to UDP-Glc, which is then transformed into Glc-1P by UDP-glucose pyrophosphorylase 2 (UGP2), and after converted to glucose-6-phosphate (Glc-6P) by phosphoglucomutase-1 (PGM1) upon entering glycolysis.

Lastly, in mammary glands of lactating mammals UDP-Gal in not only used for glycosylation but also to generate lactose thanks to the lactose synthase (LS) complex. The LS is an enzymatic complex resident in the GA lumen, and is formed by a catalytic subunit, β-1,4-galactosyltransferase (a.ka. *N*-acetyl-lactosamine synthase), and a non-catalytic regulatory subunit, α-lactalbumin. When α-lactalbumin is not expressed, β-1,4-galactosyltransferase synthesizes *N*-acetyl-lactosamine from UDP-Gal and GlcNAc. After birth, the reduction of the hormone progesterone leads to increased luteotropic hormone (a.k.a. prolactin), stimulating the production of α-lactalbumin. When increased, α-lactalbumin combines with β-1,4-galactosyltransferase to form the LS complex, in which the catalytic subunit undergoes conformational changes in the catalytic region. As a result, the LS complex acquires the capability of catalyzing a different biosynthetic reaction in which one UDP-Gal molecule and one glucose molecule are used as substrates to produce lactose.

3.2. *Galactonate and the pentose phosphate pathway*

In the presence of excessive levels of galactose, alternative pathways that usually play marginal roles in cellular biochemistry can be substantially fueled. This is the case for D-galactonate and galactitol biosynthesis [*paragraph 3.3*] (Figs. 4, 5).

The galactonate pathway was first discovered in mammals in 1966 from in vitro studies conducted on healthy rat liver [38]. Later, accumulation of D-galactonate (or D-galactonic acid) was detected also in other tissues, including rat intestine, heart and kidney when rats were maintained on a prolonged high-galactose diet [39–41]. In Guinea pigs high-galactose diet led to accumulation of D-galactono-1,4-lactone in lens, testis, circulation and muscles when galactitol production was suppressed [42,43]. In humans, D-galactonate has been detected in urine, plasma and red blood cells in patients affected by galactosemia [*paragraph 5.1*] [42,44].

D-Galactonate is an aldonic acid sugar derived from galactose, which can be excreted in urine or further metabolized to D-xylulose 5-phosphate, linking galactose to the pentose phosphate pathway (PPP) (Figs. 4, 5). In detail, galactose is oxidized to D-galactono-1,4-lactone by galactose dehydrogenase (GALDH), while producing NADH. Next, the lactone bond is hydrolyzed by a lactonase to form D-galactonate, which can be excreted or further converted to β-keto-D-galactonate by β-L-hydroxy acid dehydrogenase, producing more NADH. β-Keto-D-galactonate is then decarboxylated to D-xylulose and CO_2. Despite the

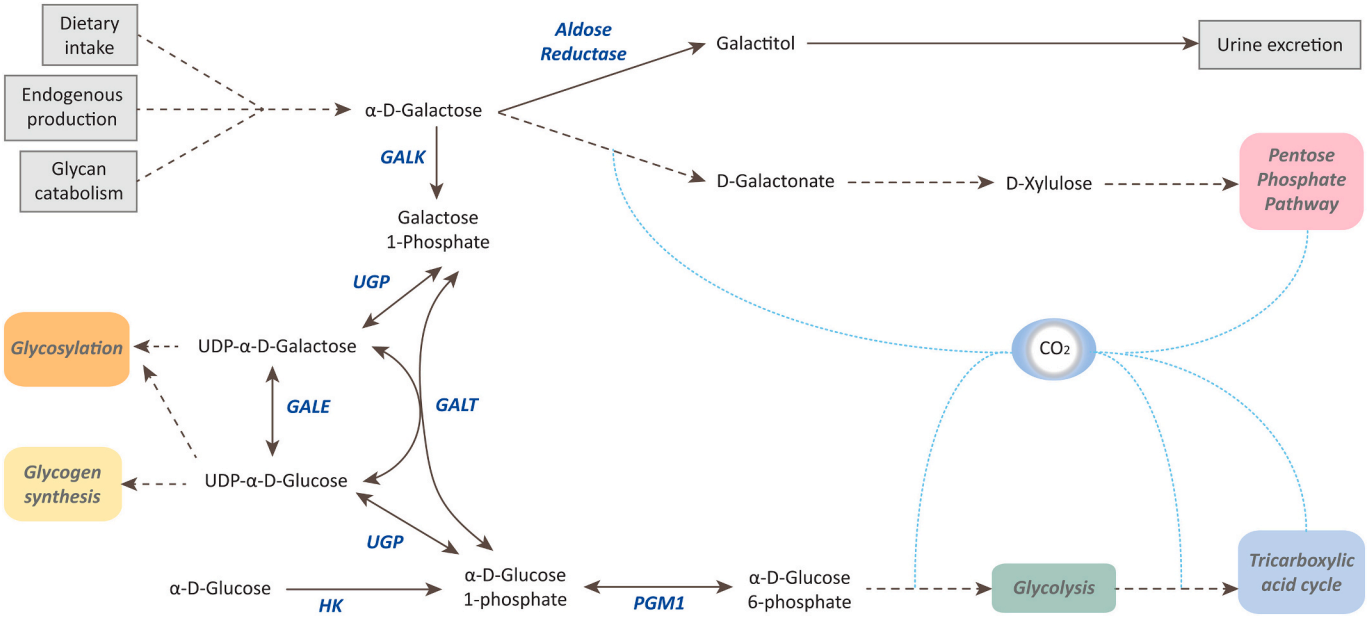

Fig. 5. Scheme of galactose metabolism. Abbreviations: CO_2, carbon dioxide; GALE, UDP-α-D-galactose 4-epimerase; GALK, galactokinase; GALT, galactose 1-phosphate uridylyltransferase; HK, Hexokinase; PGM1, phosphoglucomutase isoform I; UGP, UDP-α-D-glucose pyrophosphorylase. Solid arrows indicate single enzymatic reactions, dashed arrows indicate a sequence of enzymatic reactions (omitted) and the blue dotted arrows indicate the contribution of different biochemical pathways to the production of CO_2.

number of studies performed in animal models, the identity of the enzyme catalyzing this decarboxylation reaction remains to be unraveled [38]. Lastly, D-Xylulose can enter the non-oxidative part of the PPP as D-xylulose 5-phosphate after phosphorylation by ATP:D-xylulose 5-phosphotransferase (or xylulokinase) [45,46].

3.3. Galactitol and oxidative stress

In addition to the previous pathways, galactose can be metabolized to galactitol via its reduction by aldose reductase (also known as Aldehyde Reductase, AR, or Aldo-Keto Reductase Family 1 Member B, AKR1B1) (Fig. 5), which requires energy in the form of NADPH [45]. Galactitol is a sugar alcohol, or alditol, generated by hydrogenation of galactose. It is poorly metabolized and does not diffuse across the cell membranes due to poor liposolubility, increasing the intracellular osmotic pressure and resulting in membrane rupture [47,48].

Under physiological conditions, the affinity of AR for galactose is relatively low and galactitol is present only in traces. However, in case of galactose accumulation, such as in case of galactosemia [*paragraph 5.1*], its production increases [49]. When galactitol accumulates, it leads to depletion of NADPH and decreased glutathione reductase activity, acting as a metabotoxin, neurotoxin and hepatotoxin [50,51]. Moreover, galactitol acts as an inhibitor of GALM, raising the hypothesis that galactose accumulation could partly block the first step of the Leloir pathway and thus worsening the accumulation of galactose in loop [27].

Galactitol has also been proposed as one of the links between galactose overexposure and cell aging. In 2010 Uddin et al. [52] first reported how the continuous exposure to galactose via daily injections triggers oxidative stress and cell aging in young adult mice. According to their hypothesis, the inhibitory effect on the antioxidant enzymatic activity caused by galactitol accumulation contributes to the build-up of free radicals, and this, along with the osmotic effect caused by the hypertonic nature of galactitol, ultimately damages the integrity of cell membranes, proteins and mitochondrial DNA [47,53,54]. In addition, excessive levels of galactose are oxidized by oxidases into reactive aldehydes and hydrogen peroxide, causing a further decrease of antioxidant enzymes.

In light of these findings, it has also been proposed that galactose might accelerate the natural decline of the mitochondrial function during aging [55]. Nonetheless, the effects of galactose exposure seem to be neutralized when a physiological amount of glucose is present as main carbon source, as shown by Radenkovic et al. [56] in in vitro experiments on human primary dermal fibroblasts.

4. Galactose in human glycosylation

Protein glycosylation is a post-translational covalent modification essential for functional maturation of a wide array of macromolecules, like proteins and lipids. Through covalent attachment to glycans, these macromolecules acquire structural folding, stability and solubility, so that they can mature into their functional three dimensional configuration. Glycoconjugates play a plethora of effects in- and outside the cell, including modulation of lipid and protein functions, cell-cell communication, cell-matrix adhesion, intracellular signaling, glycocalyx formation, and immune response modulation (Fig. 1) [5,6].

In the next paragraphs, we will discuss the galactosylation of known glycoconjugates (Fig. 6), including protein N- and O-glycosylation [*paragraph 4.1*], proteoglycans [*paragraph 4.2*], collagen [*paragraph 4.3*] and glycolipids [*paragraph 4.4*].

4.1. Galactose and protein glycosylation

Among all post-translational modifications, glycosylation is the one with the largest contribution to proteome complexity. A given protein can have multiple sites of glycosylation, whose accessibility can influence the glycan formation, and its glycoforms can differ by site occupancy (macroheterogeneity) and by the type of occupying glycans (microheterogeneity) [57–61]. It has been estimated that more than 50% of human proteins are glycosylated, which entails why glycosylation is essential for a tremendous amount of biological events [6,62]. Galactose is an essential precursor of glycan biosynthesis via activation to a high energy donor, UDP-Gal (Figs. 3, 5) [*paragraph 3.1*]. UDP-Gal is transported into the Golgi Apparatus (GA) lumen by a specific transporter, namely the solute carrier family 35 member A2 (SLC35A2), and into the endoplasmic reticulum (ER) lumen by the spliced isoform of SLC35A2 [63]. Here, UDP-Gal acts as a donor of galactose, which is

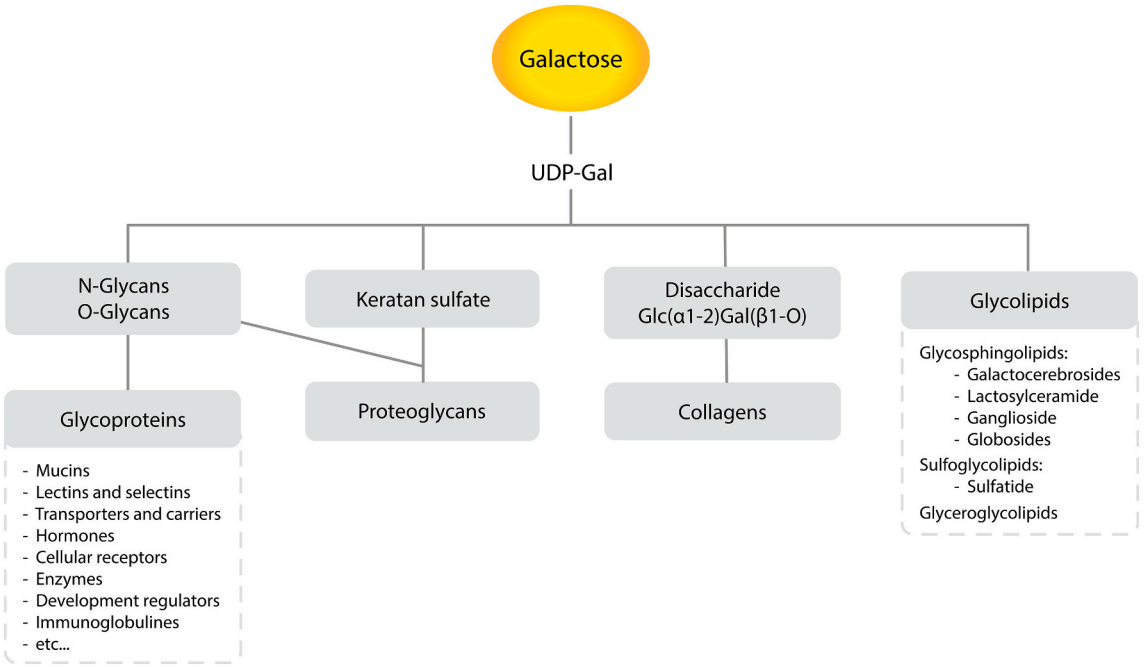

Fig. 6. Schematic representation of the glycosylation pathways involving galactose. Galactose is metabolized into UDP-Gal which is used as a precursor for glycosylation of different macromolecules. UDP-Gal can be used as building block for synthesis of N-glycans and O-glycans. Moreover, UDP-Gal can be used to synthesize keratan sulfate, a glycosaminoglycan that, along with N- and O-glycans, is an essential component of several extracellular proteoglycans. Additionally, UDP-Gal is used to glycosylate collagens. Lastly, UDP-Gal can be used to glycosylate lipids alone or in combination with other monosaccharides, generating glycolipids. Among glycolipids, several types of glyceroglycolipids, sulfoglycolipids and glycerolipids contain galactose in their sugar moieties.

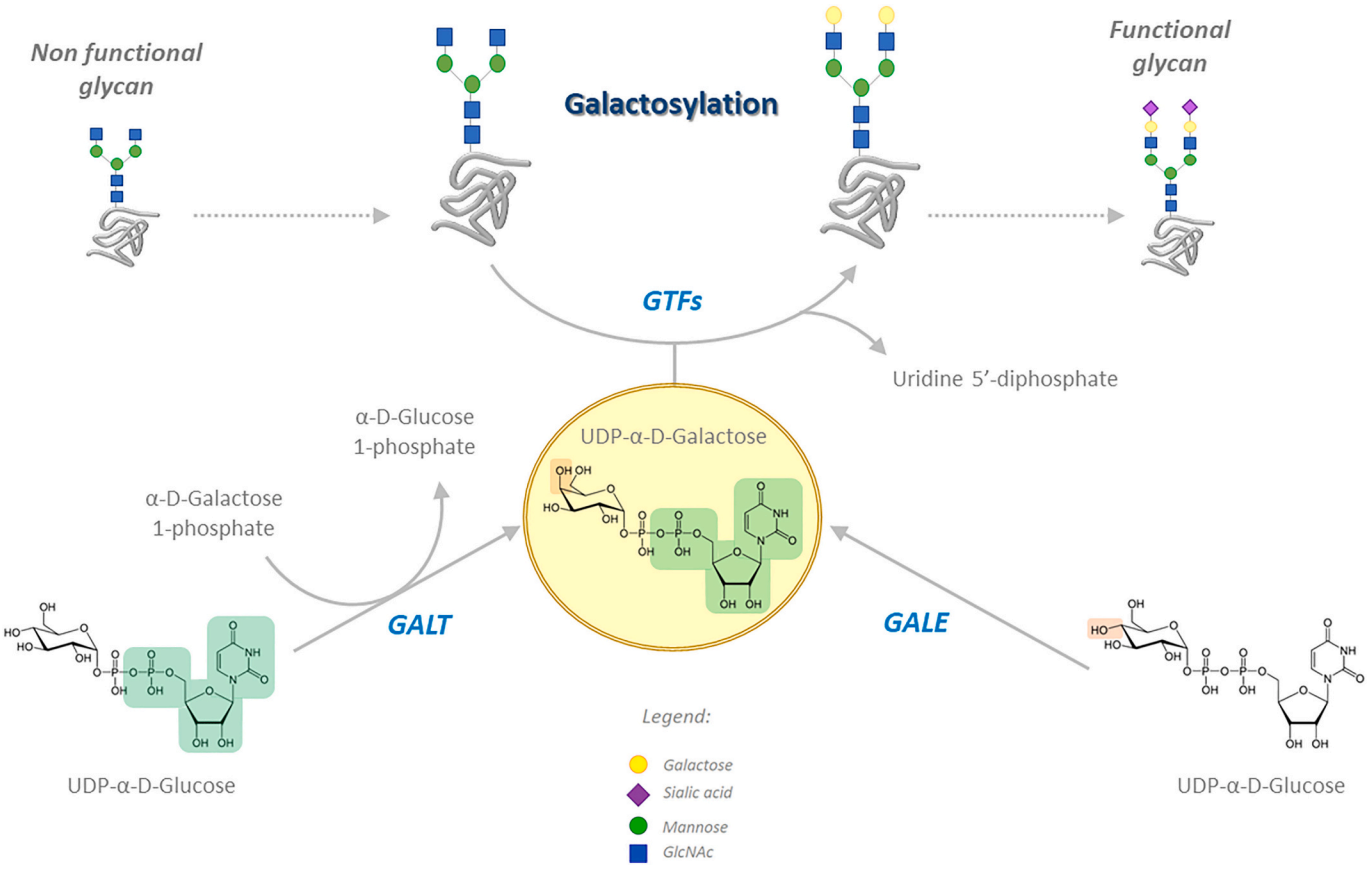

Fig. 7. UDP-Galactose synthesis and function in glycosylation. Solid line arrows indicate direct enzymatic reactions, while dashed line arrows indicates a series of sequential reactions.

covalently linked via glycosidic bond to the growing glycan on proteins or lipids, by galactosyltransferases (Fig. 7). About 20 galactosyltransferases have been described in human, with a high degree of specificity for their acceptor substrates and the type of glycosidic linkage generated [64,65].

The attachment of galactose molecules to a glycan chain is called galactosylation, and typically occurs at the (semi)terminal positions of several N-linked glycans or in the core structure of O-linked glycans (Fig. 8) [66].

After a glycoconjugate has fulfilled its functions, it undergoes degradation inside the lysosomes, where glycans are exposed to the activity of glycosidases. Lysosomal glycosidases are a super-family of enzymes that hydrolyze glycosidic bonds in complex sugars and mostly require low pH for optimal cleavage. This enzymatic superfamily includes the subfamily of galactosidases, which is further divided in (i) exogalactosidases, which cleave the glycosidic linkage of terminal galactoses from the nonreducing end of a glycan chain, and (ii) endogalactosidases, which instead cleave the glycosidic linkages of internal galactoses present in larger glycans, yielding more substrate (fragments) for the exogalactosidases [67].

The lysosomal degradation of glycans ultimately results in monosaccharides, like galactose, that exit the lysosome by diffusion or carrier-mediated transmembrane transport and, once in the cytosol, can either be metabolized or be reactivated for de novo synthesis of glycans [68]. This recycling process of galactose from the lysosome is conventionally called the salvage pathway.

The two main types of protein glycosylation in human cells are N-glycosylation and O-glycosylation, and both require the presence of galactose.

In N-linked protein glycosylation (Fig. 8), the glycans are covalently attached to asparagine (Asn) residues by an N-glycosidic bond. All eukaryotic N-glycans have a uniform base of GlcNAcβ1–Asn, and their synthesis starts on a dolichol-phosphate carrier. Hereafter the glycan is transferred to proteins and modified. Ultimately, three types of N-linked chain can be synthesized: oligomannose forms, complex forms and a hybrid forms [69].

Mucin O-linked glycans (Fig. 8) are initiated by GalNAc and are attached to the hydroxyl of serine (Ser) or threonine (Thr) residues by polypeptide GalNAc transferases. The length of O-linked glycans varies greatly, from a single GalNAc to over 20 residues. Mucin O-glycans are the largest group of O-linked glycans. The monosaccharides found in O-GalNAc linked glycans include GalNAc, galactose, GlcNAc, fucose, and sialic acid, whereas mannose, glucose, or xylose residues are not represented. O-linked glycans are greatly variable in sugar residues but there are four major core structures [70].

4.2. Galactose, keratan sulfate and proteoglycans

Glycosaminoglycans (GAGs), also known as mucopolysaccharides, are negatively-charged polysaccharides composed of repeating disaccharide units. They are present in every mammalian tissue and covered a wide range of functions determined by their molecular structure [71].

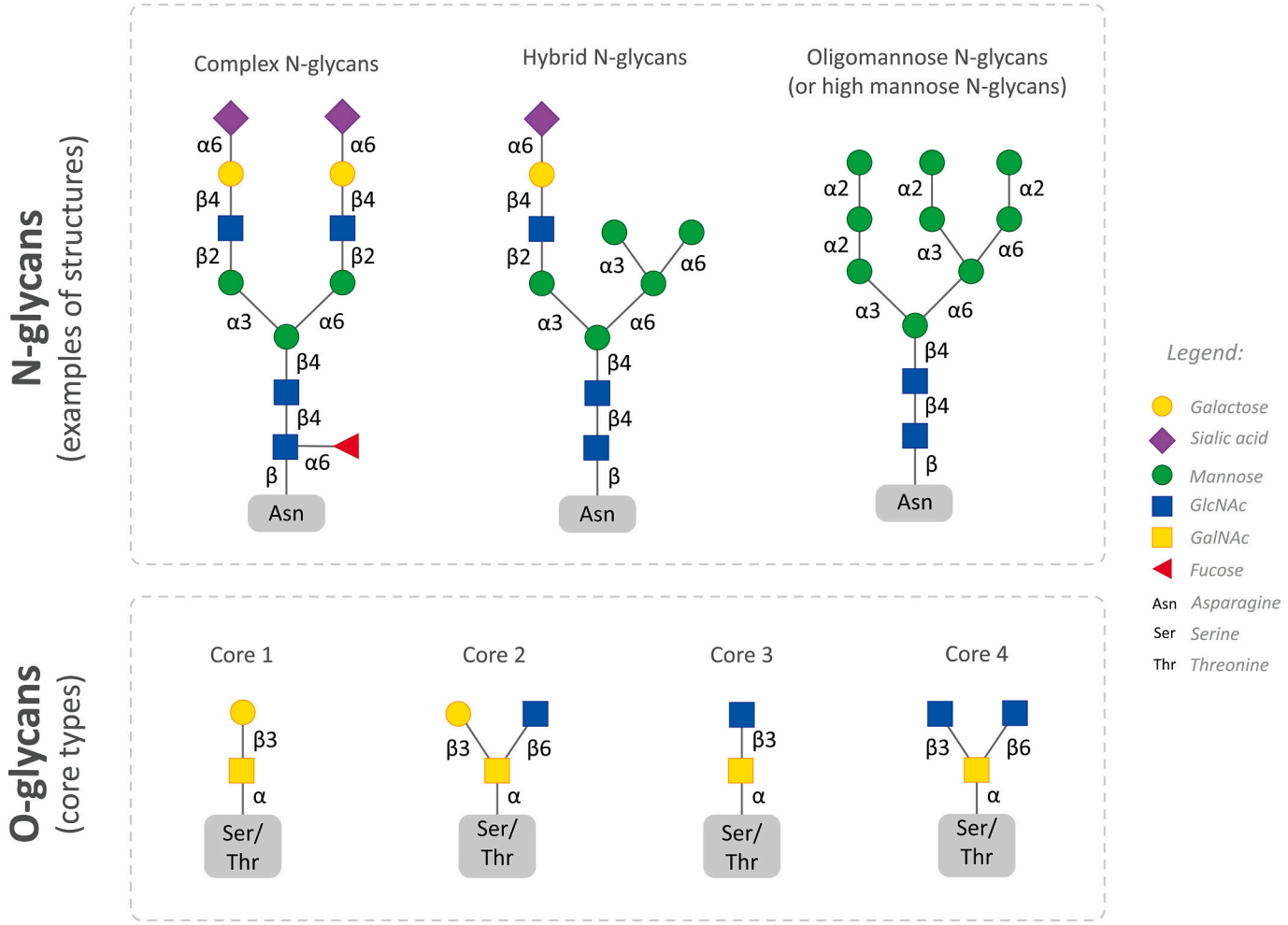

Fig. 8. Examples of N-glycan structures and the four types of human mucin O-glycans, namely: [core 1] Galβ1–3GalNAcαSer/Thr; [core 2] GlcNAcβ1–6(Galβ1–3) GalNAcαSer/Thr; [core 3] GlcNAcβ1–3GalNAcαSer/Thr; [core 4] GlcNAcβ1–6(GlcNAcβ1–3)GalNAcαSer/Thr.

Historically, the function of GAGs was thought to be limited to cell hydration and structural scaffolding. However, recent evidences entail that GAGs also play a key role in cell signaling, which modulates a vast amount of biochemical processes [72].

The four primary groups of GAGs are classified based on their core disaccharide units and include heparin/heparan sulfate, chondroitin sulfate, dermatan sulfate, hyaluronic acid and keratan sulfate [73]. Of these, keratan sulfate (KS) is the only type of GAG containing galactose as structural component.

Keratan sulfate is the most recently discovered GAG, and although eighty years have passed from its discovery in the cornea [74], it still remains the least understood. KS is a sulfated polylactosamine chain composed of repeating disaccharides of galactose and *N*-acetylglucosamine (GlcNAc), which can both be subjected to C-6 sulfation, and its nonreducing termini can be capped with various types of monosaccharides. Three classes of KS have been defined on the basis of tissue specificity and, most importantly, on the chemical structure, which varies depending on the extent of sulfation, the sulfation patterns, and the type of linkage oligosaccharide that connects keratan sulfate to the core protein [75]. KS type I is a N-linked KS chain with variable degrees of sulfation (non-, mono- and di-sulfation) and it is predominant in the cornea [76]. KS type II is an almost completely sulfated KS chain, O-linked to the core protein through GalNAc, mostly present in cartilaginous tissues [76]. KS type III is also highly sulfated but is attached to its core protein through a 2-O-mannose linkage, and it is mostly abundant in the central nervous system [76].

The biosynthesis of KS type I begins in the ER where dolichol phosphate in the ER membrane acts as a glycosyl receptor for the formation of high mannose N-linked oligosaccharide. The complex is then transferred to the GA [75]. Instead, for KS type II and III the addition of the first monosaccharide and the extension of the chain occur once the polymer has entered the GA. Dedicated glycosyltransferases resident in the GA catalyze the elongation of the KS chains, and in general this occurs by alternative action of a β1,4-galactosyltransferase (β4GalT) and a ß1,3-N- acetylglucosaminyltransferase (β3GnT). Lastly, the KS chains are covalently attached as modules to core proteins to form proteoglycans (PGs), which are then transported to the plasma membrane via vesicle trafficking.

KS-containing proteoglycans (KSPGs) play essential roles in ECM formation and connective tissue organization, mechanical support, and resistance to compression while maintaining tissue elasticity. Besides, KSPGs are involved in bone homeostasis, mucous composition and mucinous microenvironment, fertilization and oocyte implantation, development of heart tissue and in the valve leaflets, neuronal cell development, dendritic synaptic contact area protection and neurotransmitter transport, transferrin and prostaglandin biosynthesis [76]. Among the KS-containing PGs, several members can be found of the small leucine repeat proteoglycan (SLRP) family, such as fibromodulin, lumican, keratocan, mimecan, and osteoadherin that contain short N-linked KS chains, and aggrecan, a large PG containing both KS and CS that contributes to the structural scaffolding, water-retaining properties and compression resistance in cartilaginous tissues [77].

4.3. Galactose and collagen glycosylation

Although the majority of proteins undergo glycosylation as a process spanning from ER to GA, there are exceptions to this role. The most relevant exception in the context of this review are collagens, a family of proteins that are glycosylated entirely in the ER with a unique disaccharide that is involved in correct folding of collagen fibers.

Collagens, the most abundant class of proteins in the human body, provide structural support, tensile strength and elasticity to connective tissue, skin, bones, muscles, tendons and ligaments. To date, 29 types of collagen encoded by at least 46 genes have been reported [78]. The structure of collagens is rather diverse but generally characterized by repeats of a triplet, Gly-X-Y, where proline and lysine are often found at positions X and Y, respectively. A typical collagen polypeptide includes hundreds of this motif, which confers a left handed helical conformation to the single strand collagen domains [79]. The presence of glycans on collagens has been first described in 1935, when Grassmann and Schleich assigned up to 1% of the mass of collagen to carbohydrates [79,80].

While still in the ER, these collagen domains undergo different post-translational modifications, of which the most important are then hydroxylation on proline and lysine residues, followed by glycosylation on selected hydroxylysine residues (the extent of which varies according to the different types of collagen). Whereas the hydroxylation of proline increases the thermal stability of collagen, which would otherwise already partially denature at body temperature [81], the hydroxylation of lysine enable glycosylation. These hydroxylysine residues carry variable numbers of the monosaccharide Gal(β1-*O*) or the disaccharide Glc(α1–2)Gal(β1-*O*) through O-glycosidic linkages (Fig. 5) [78,79,82,83]. Although the galactosyltransferases specific for collagen glycosylation are still under investigation, the ER-resident galactosyltransferases COLGALT2 and COLGALT1 have been identified, and mutations in the latter have been linked to cerebral small vessel abnormalities and porencephaly [*paragraph 5.5*, 78, 79, 84].

After undergoing glycosylation and other post-translational modifications, three collagen polypeptides associate to form a right-handed triple helical coil stabilized through hydrogen bonds between the three subunits, ultimately forming collagen fibrils [79]. Next, the fibrils migrate outside the ER lumen and via vesicle transport they are secreted in the extracellular space. Here, the procollagen pro-peptide is cleaved so that the fibrils reach mature state and gain the ability to cross-link with other fibers, thus building the structural matrix that provides support and elasticity to tissues.

4.4. Lipid glycosylation

Glycosylation also occurs on lipids, generating glycolipids, which are formed by a lipid moiety covalently bound through glycosidic bonds to a mono- or oligosaccharide [85]. Glycolipids are mostly known for their role as bilayer membrane constituents. Lipid membranes are essential structural elements for the evolution of life, as they provide separation from the extracellular environment and allow compartmentalization by generating organelles within the cells. Glycolipids contribute to membrane structural stability, orientation and organization, while enabling signal transmission and interactions between the two sides (e.g. extracellular microenvironment and intracellular cytoplasm).

The biological functions of glycolipids are intertwined with their chemical structure that grants them an ambivalent nature: the lipidic chain is hydrophobic and constitutes the core of the membranal bilayer separating different aqueous environments and controlling their interactions, while the carbohydrate moieties are hydrophilic and can extend into the aqueous media on the opposite sides of the membrane creating polarization and encoding information [9]. Since their discovery towards the end of the 19th century [9], several classifications have been proposed, although the most simple divides glycolipids in (i) glycoglycerolipids, which contain a glycerol backbone and are absent in human but very common in plants and microbes, and (ii) glycosphingolipids, which instead contain a sphingosine backbone.

Glycosphingolipids typically account for about 5–10% of lipid in plasma membranes, and their structural diversity and strategic localization in the outer leaflet of the plasma membrane render them ideally suited for their functions in mediating intercellular adhesion, interactions, recognition, receptor function and signaling [9,86].

The majority of glycosphingolipids belong to the subclass of the cerebrosides, which can be further divided into different subtypes. One of these are the galactosylceramides (a.k.a. galactocerebrosides). Galactosylceramides are mostly found in brain where they act as major components of brain myelin [87,88] and alone accounts for 16% of the total lipid content in the CNS [9]. Sulfation of galactosylceramide results

in 3-O-sulfated-galactosylceramide, also called sulfatide. Sulfatide is an essential component of myelin in the nervous system, but it has also been detected in the pancreas where it is involved in insulin secretion, in the gastrointestinal tract and in the kidney [89]. Moreover, it has been identified on the membrane of various blood cells, such as leukocytes, platelets and erythrocytes, where it seems to be involved in immune system, hemostasis and thrombosis, bacterial infection, and virus infection [89,90].

Another subclass of glycosphingolipids includes the globosides, which are to glycosylated with more than one monosaccharide, usually galactose, glucose and GalNAc. Globosides can be mostly found on the membrane of erythrocytes, immune cells, stem cells and kidney cells, where they act as a membrane receptor [91,92].

Lastly, the most complex type of glycosphingolipids is represented by gangliosides, as they contain up to seven carbohydrate residues in a branched chain and include sialic acid (NeuAc) residues [86]. Gangliosides are ubiquitous membrane-bound components in vertebrates and are most abundant in the CNS, where they support its development and mediate protection and repair mechanisms [93].

The de novo biosynthesis of most of these glycosphingolipids starts in the ER with the biosynthesis of the ceramide moiety. The ceramide is a long-chain aminoalcohol sphingosine originating from palmitoyl-CoA and serine in amide linkage to a fatty acid thanks to the ceramidase synthase [9,94]. Next, the ceramide core is glycosylated, but in a peculiar fashion: the galactosylation of ceramide takes place inside the ER lumen, while additional glycosylation reactions occur in the GA [94,95]. The attachment of the galactose molecules is mediated by the UDP-galactose/ceramide galactosyltransferase (UGT8) [95]. Afterwards, the galactosylceramide traffics to the GA lumen, where galactose is 3′-O-sulfated by the cerebroside sulfotransferase to form sulfatides, or its glycan chain is elongated by glycosyltransferases to form glycosphingolipids with core structure Galα(1,4)Galβ-Cer (a.k.a. gala-series glycosphingolipids) [9,96,97].

5. Galactose-related congenital disorders of glycosylation

As glycosylation plays an important role in enabling and regulating a variety of biological processes, defects of this post-translational modification have been related to both acquired pathologic conditions, such as neurodegenerative disorders, diabetes and cancer [98], and to some congenital inborn errors of metabolism, specifically congenital disorders of glycosylation (CDG) and lysosomal storage disorders (LSDs).

CDG are mainly autosomal recessive monogenic disorders with multisystem symptoms, including hepatic, neurologic, muscular and skeletal involvement. Traditionally, CDG used to be divided into two classes based on the glycosylation steps affected by the pathogenic mutation: CDG type I, which includes the defects in the assembly of the immature chain and its transfer to the protein, resulting in missing glycan chains, and CDG type II, which beholds the defects in the processing of the protein-bound glycan and results in incomplete glycans [99]. Yet, in the past decade more and more 'mixed-type' CDG have been discovered.

Out of 137 CDG described to date [100–104], about ten have been linked to galactose via genetic mutations that disrupt different steps of galactose metabolism, transport, glycosylation or indirectly via the alteration of the ion homeostasis in the GA, which will be described in the following paragraphs.

5.1. Galactosemia

Galactosemia was originally described as an autosomal recessive defect for the first time in 1908 by A. von Reuss [105]. Over a century later, it was discovered that the term "galactosemia" was used to describe several congenital disorders affecting different enzymes of the Leloir pathway, and thus it was divided in four types: galactosemia type I due to GALT deficiency, type II due to GALK deficiency, type III due to GALE deficiency, and type IV due to GALM deficiency [35,36].

Galactosemia can result indirectly results in the impairment of both glycan assembly and processing and thus it is classified as a secondary CDG [106]. The accumulation of galactose fuels the activation of alternative metabolic pathways, such as the synthesis of galactonate [*paragraph 3.2*] and of galactitol [*paragraph 3.3*], which both have been proposed to contribute to the increased oxidative stress in the most affected tissues. The accumulation of these two metabolites can be detected in patients' plasma, red blood cells and urine, and thus it has been proposed as biomarker for defects in GALT, GALK and GALE [45].

To date, only a limited therapy is available for galactosemia. In clinical practice, as soon as galactosemia is suspected neonatally, the infant is promptly switched to a galactose-restricted diet, usually replacing milk with a galactose-free formulation. This dietary regimen stops the morbidity of the disease but cannot resolve the long-term consequences of some of the symptoms which accumulate over time.

On the other hand, it has been suggested that a galactose-restricted diet might diminish bone mineral density, resulting in decreased growth and height. Besides, over 80% of female galactosemic patients suffer from primary ovarian insufficiency and they often need puberty induction to reach maturity. Little research has been conducted about male fertility in galactosemia, but increased cryptorchidism has been reported [16,107].

5.1.1. Galactosemia type I: GALT deficiency

GALT deficiency is the most common form of galactosemia, also known as classical galactosemia [MIM#230400, 108], affecting 1 in 16,000:60,000 live births [16]. To date, over 200 GALT mutations have already been described [109], although over 70% of classic galactosemia cases are due to a few common mutations (Q188R, S135L, K285N, and L195P) [110].

Infants with classical galactosemia, often do not present any symptoms at birth but, once the consumption of milk is commenced, the symptoms appear due to exposure to galactose [111]. The first symptoms to manifest include vomiting, diarrhea, jaundice, lethargy, hypotonia, hepatomegaly and liver dysfunction (Table 1) [16,112,113]. The progression of the galactose toxicity leads to the development of cataracts, liver damage, abnormal bleeding, gram negative sepsis, and bulging fontanel due to pseudotumor cerebri [16,114]. Along with endocrine abnormalities, the cognitive symptoms are the most severe and persistent and can manifest motor disturbances, vision impairment, memory disruption, speech delay, language problems and below average IQ (85–100) [16].

From a molecular point of view, the increased Gal-1P level is typical to classic galactosemia, and it has been proposed that the accumulation of this intermediate could be responsible for the clinical features, especially the ophthalmological defects and the neurological symptoms [115]. Due to the severity of the disease, galactosemia has been included in newborn screening for over fifty years, based on the quantification of total galactose (TGAL) and GALT enzymatic activity [116,117].

IgG N-glycans were found as a potential biomarker for determining galactose tolerance in classical galactosemia [118]. This tolerance of galactose is important as increased galactose intake, when tolerated, may improve glycosylation. In 2015, a study with 13 galactosemic children already indicated that moderate increase in galactose intake may well be tolerated and indeed may improve glycosylation [119].

As alternative strategy, GALK inhibitors, which are typically used to treat GALK deficiency or galactosemia type II [*paragraph 5.1.2*], have been studied as potential treatment in patients with classic galactosemia [120].

5.1.2. Galactosemia type II: GALK deficiency

Deficiency of GALK, mediating the second step of the Leloir pathway, is the cause of galactosemia type II [MIM# 230200]. This type of galactosemia is more rare than type I, with a prevalence of less than 1:1,000,000 [35] and approximately 40 pathogenic mutations reported

Table 1
Summary of the details and clinical presentation of galactosemia and other galactose-related CDG.

Congenital disorder	Gene affected	Gene location (GRCh38/hg38)	Enzymatic reaction	Known mutations	Incidence	Endocrine symptoms	Skeletal symptoms	Hepatic symptoms	Kidney symptoms	Neurologic symptoms	Developmental delay	Muscle symptoms	Cardiac symptoms	Oral and/or GI symptoms	Respiratory symptoms	Ophthalmological symptoms	Hematological anomalies
Galactosemia type I	*GALT*	chr9:34,638,133-34,651,035	α-D-Gal-1P + UDP-α-D-Glc ⇄ α-D-Glc-1P + UDP-α-D-Gal	>200	1:16-60K	■		■		■		■		■		■	
Galactosemia type II	*GALK*	chr17:75,751,594-75,765,236	α-D-Gal + ATP ⇄ ADP + α-D-Gal-1P + H^+	~40	<1:1M					■						■	
Galactosemia type III	*GALE*	chr1:23,795,599-23,800,804	UDP-α-D-Glc ⇄ UDP-α-D-Gal *AND:* UDP-GlcNAc ⇄ UDP-GalNAc	>20	N/A			■	■	■						■	■
Galactosemia type IV	*GALM*	chr2:38,665,910-38,741,237	β-D-Hexose ⇄ α-D-Hexose *Including:* β-D-Gal ⇄ α-D-Gal	30	N/A											■	
PGM1 deficiency	*PGM1*	chr1:63,593,276-63,660,245	α-D-Glc-1P ⇄ α-D-Glc-6P	43	N/A	■	■	■	■	?	■	■	■	■			■
SLC35A2 deficiency	*SLC35A2*	chr5:140,564,565-140,569,104	Nucleotide monosaccharide /proton symporter	59	N/A		■			■	■	■		■		■	
B4GALT1 deficiency	*B4GALT1*	chr9:33,104,082-33,167,358	D-Glc + UDP-α-D-Gal ⇄ H^+ + lactose + UDP	2	N/A		■	■			■	■	■			■	
COLGALT1 deficiency	*COLGALT1*	chr19:17,555,649-17,583,162	(5R)-5-hydroxy-L-lysyl-[proCOL] + UDP-α-D-Gal ⇄ (5R)-5-O-(β-D-galactosyl)-5-hydroxy-L-lysyl-[proCOL] + H^+ + UDP	2	N/A					■	■	?					?
SLC39A8 deficiency	*SLC39A8*	chr4:102,251,041-102,431,258	Zinc/magnesium transporter	6	N/A		■			■			■				■
TMEM165 deficiency	*TMEM165*	chr4:55,395,913-55,453,397	Calcium/proton transporter	3	N/A	■	■			■				■	■		■

Red color, reported symptom; grey color, absent symptom; the symbol '?' indicates symptoms unclearly correlated with the disease. Abbreviations: chr, chromosome; Gal, galactose; Glc, glucose; GalNAc, N-acetyl-α-D-galactosamine; GlcNAc, N-acetyl-α-D-glucosamine; 1P, phosphate on carbon 1; UDP, uridine diphosphate; proCOL, procollagen; M, million; K, thousand.

[108]. Cataracts and pseudotumor cerebri are considered the only consistent abnormalities in this type of galactosemia (Table 1) [120]. Long-term follow-up of patients with this disorder has shown that, in spite of a severely galactose-restricted diet, most patients develop abnormalities such as a disturbed mental and/or motor development, dyspraxia and hypergonadotropic hypogonadism [120]. Nonetheless, the phenotype of galactosemia type II is generally considered less severe than that of classic galactosemia and of galactosemia type III [51].

Both the cataracts and the neurological symptoms have been proposed to be caused by the accumulation of galactose and galactitol that, in this type of galactosemia, reaches the highest levels. Based on this hypothesis, starting from 2014 high-throughput screenings have been performed to identify small molecules that could inhibit human GALK to prevent the accumulation of toxic collateral bioproducts. As results, multiple functional inhibitors have been identified, such as the so called Analog 36, but they have yet to be tested on patients [121–123]. GALK inhibitors are considered the most promising treatment option also for galactosemia type I, especially considering that gene therapy is currently not a feasible option for this CDG [123]. Furthermore, chaperone or co-factor therapies are unlikely to be beneficial as few if any cause protein misfolding [123].

5.1.3. *Galactosemia type III: GALE deficiency*

Galactosemia type III [MIM#230350] is caused by mutations affecting the gene encoding GALE enzyme, which catalyzes the last step of the Leloir pathway by converting UDP-Gal to UDP-Glc and vice versa. Over 20 disease-associated mutations have been reported and only a small number of patients has been diagnosed thus far, making this rarest type of galactosemia.

Galactosemia type III used to be divided into two groups: a severe generalized form, and a mild peripheral form, but more recently intermediate forms have become evident [36,124]. The peripheral form is mostly asymptomatic except for altered blood galactose levels. In contrast, the generalized form with very low or totally absent GALE activity presents with cataracts in the first few months of life, followed by liver, kidney and neurological damage largely due to the accumulation of Gal—1P. In these patients, galactose restriction is less effective than in other types of galactosemia, possibly because GALE is also involved in the conversion of UDP-GalNAc and UDP-GlcNAc, a problem which is not resolved by galactose restriction [36].

5.1.4. *Galactosemia type IV: GALM deficiency*

In 2018, Wada and colleagues investigated the causes of eight unexplained galactosemia cases and found biallelic variants of *GALM* gene in all patients. This new deficiency was called galactosemia type IV [MIM#618881, 125].

All eight patients were younger than one year of age and presented elevated blood galactose levels. Their Gal-1P levels appeared initially elevated during the perinatal period, but gradually decreased to normal levels in early infancy. Two patients showed bilateral cataracts [125], making GALM deficiency similar to galactosemia type II. Concerning the *decursus* of this disease, very little is known as no patients have yet reached adulthood, and are currently maintained on a galactose restricted diet [125]. Iwasawa and colleagues identified 30 *GALM* variants that cause low or absent enzyme activity, thus providing a first panel that can be used as reference for patient diagnosis [126]. As this type of galactosemia has been only recently described [125], much still remains to be discovered about its phenotypes, their evolution over time and the long term effects of galactose restriction during patients' life.

5.2. *PGM1 deficiency*

Aside from galactosemia, also other CDG are known to involve galactose metabolism. This is the case for phosphoglucomutase-1 (PGM1) deficiency (or PGM1-CDG) [MIM#614921], which was first reported in 2009 [127]. PGM1 enzyme is an heart-shaped phosphoglucomutase of 562 aa that catalyzes the interconversion of Glc-1P and Glc-6P (Fig. 4). Glc-6P can flow into glycolysis, while Glc-1P can be transformed to UDP-Glc, which can be used as substrate for UDP-Gal synthesis for glycosylation, or can fuel into glycogen synthesis. Therefore,

PGM1-CDG affects both energy production and storage, as well as glycosylation.

Being a ubiquitarian enzyme, PGM1-CDG affects a wide number of organs and systems and presents with a highly heterogenous clinical spectrum that includes hypoglycemia, cleft palate or bifid uvula, liver dysfunction, growth delay, endocrine and skeletal abnormalities (e.g. short limbs), cardiac defects (mostly dilated cardiomyopathy and ventricular defects), myopathy with or without rhabdomyolysis, and lastly coagulation disturbance (Table 1) [128–135]. Although the CNS was initially considered unaffected [132], recent studies suggest an association between PGM1-CDG and neurological symptoms, including seizure and intellectual disability, which do not appear to be secondary to hypoglycemia [135–137]. Thus far, 57 patients affected by PGM-CDG have been reported in the literature [135,138].

Aside from routine therapies targeting single symptoms (e.g. palatal surgery to repair the cleft palate), oral D-galactose supplementation is used as metabolic treatment to treat these patients. D-Galactose supplementation acts by replenishing the scarce pool of UDP-Gal in PGM1-deficient tissues, hence ameliorating the defective glycosylation by resolving hypogalactosylation and contributing to rescue missing glycans [128,129]. Thanks to this compensatory mechanism, hypoglycemic episodes are significantly reduced (and in some cases disappear), liver dysfunction is ameliorated, coagulation improves, while growth delay and puberty delay are reduced.

Although there is no standard protocol for such supplementation, although Wong and colleagues proposed a dose of 1 g/kg/day galactose for adult patients (Wong protocol), while infantile patients required dose adjustments to meet the higher requirements of this sugar [128,139]. In several pilot studies that made use of the Wong protocol, this dose was sufficient to successfully restore protein N-glycosylation within 3–4 weeks of treatment, without glycogen accumulation. This resulted in improvement of the hepatic and endocrine functions, normalization of transaminases, and prevented puberty delay when administered in younger patients [128,129]. Yet, no evident effects have been reported on the cardiac and muscular function, hinting that the currently used doses might be too low to have any effects on such symptoms or that galactose plays a minor role in the pathomechanisms taking place in these tissues [139].

5.3. SLC35A2 deficiency

The solute carrier (SLC) gene superfamily consists of around 400 putative genes including the SLC35 subfamily, which mainly encodes transporters involved in the translocation of nucleotide monosaccharides across membranes [63]. SLC35A2 encodes a 324 aa translocator of pyrimidine nucleotide monosaccharides, specifically UDP-GlcNAc and UDP-Gal, from which its name UDP-Gal transporter (UGT). Two UGT isoforms due to alternative splicing have been reported in human, UGT1 and UGT2, the latter differing from the former by eight amino acids in the C-terminal eight amino acids [140]. UGT1 (the main isoform) transports its ligand by creating heterologous complexes in the GA with the UDP-GlcNAc transporter (SLC35A3), which further interacts with mannosyl acetylglucosaminyltransferases (MGATs), ultimately generating a glycosylation-related transmembrane complex [141]. By concentrating UDP-Gal and UDP-GlcNAc molecules from the cytosol into the GA lumen, UGT enables proper protein and lipid galactosylation that results in the synthesis of functional N-/O-glycans (Fig. 6). Therefore, mutations affecting the functionality of this transporter result in a CDG with X-linked inheritance and severe neurologic involvement [MIM#300896]. These patients present with an heterogeneous phenotypic spectrum with predominant neurologic symptoms, including central nervous system malformations and early-onset encephalopathy with epilepsy, developmental delay, drug-resistant hypsarrhythmia, facial dysmorphism, skeletal abnormalities (e.g. short limbs), growth deficiency and ophthalmological defects. More rarely, patients may present hepatic, cardiac, respiratory and immunological symptoms.

Thus far, 77 SCL35A2-CDG patients have been identified, accounting for a total of 59 mutations (Table 1) [142–156]. Although in about half of the patients the transferrin glycoprofile spectrum revealed truncated glycans lacking terminal galactose (hypogalactosylation) and sialic acids (hyposialylation) [157], other patients present with normal glycosylation [149].

Oral D-galactose supplementation was attempted in ten SLC35A2-CDG patients, with escalating doses up to 1.5 g/kg/day over a period of 18 weeks [158]. Although results were rather variable among patients, overall the supplementation seemed to ameliorate growth and developmental delays, improved the gastrointestinal symptoms and epilepsy, and restored the levels of fully N-glycosylated glycans.

5.4. B4GALT1 deficiency

Structural glycan changes dominated by galactose loss are present also in B4GALT1-CDG [MIM#607091]. Beta-1,4-galactosyltransferase 1 (B4GalT1) is a 398 aa glycosyltransferase residing in the trans GA that, in the presence of manganese, transfers a galactose moiety from UDP-Gal to GlcNAc residues in a β1–4 linkage [159–161]. Besides, B4GalT1 can interact with α-lactalbumin to form the lactose synthase complex that leads to the synthesis of lactose in the lactating mammary gland [162,163].

B4GALT1-CDG has thus far been diagnosed only in four pediatric patients [142,164–166]. The clinical spectrum includes Dandy-Walker malformation, hydrocephalus, facial dysmorphisms, muscular symptoms (e.g. pronounced hypotonia, elevated creatin-kinase levels), transient cholestatic syndrome and liver defects, pulmonary symptoms, coagulation abnormalities (e.g. thrombocytopenia) (Table 1). Less frequently, heart failure, splenomegaly and developmental disability also occur. Transferrin glycoprofiling showed truncated glycans due to hypogalactosylation in patients. A recent study demonstrated hypogalactosylation and reduced activity of lipoproteins such as cholesteryl ester transfer protein, leads to anomalies in plasma HDL cholesterol levels [166]. To date, only symptomatic therapies are available in clinical practice for B4GALT1-CDG, although manganese and galactose supplementation are under investigations [167].

5.5. COLGALT1 deficiency

To reach its mature form, collagens require galactosylation, which is catalyzed by two collagen β(1-O) galactosyltransferases: COLGALT1 and COLGAT2 [*paragraph 4.3*]. While no patients with compound heterozygous mutations in *COLGALT2* have been reported, a recent case report [78,84] described two unrelated Japanese patients affected by missense mutations in highly conserved domains of *COLGALT1*. COLGALT1 is a 622 aa glycosyltransferase resident in the ER lumen, and its loss of function lead to an autosomal recessive condition named brain small vessel disease 3 (BSVD3) [MIM# 618360]. On a molecular level, in vitro knock-out cell models for COLGALT1 showed a dramatic decrease of intracellular and extracellular collagen type IV alpha 1 chain (COL4A1) secretion, inability to restore proper COL4A1 production and collagen accumulation in the ER lumen. And these molecular events ultimately disrupts the protein-protein interactions with other extracellular molecules and compromised structural integrity of the vascular basement membranes [84]. This experimental evidence also explained the phenotypic similarity with collagen type IV-related cerebral small vessel diseases, which is a class of disorders clinically heterogeneous with a broad range of manifestations of variable severity [78]. BSVD3 presented with early onset, slowly-progressive disease characterized by psychomotor delay and intellectual impairment, which however differ in terms of severity in the two reported cases (Table 1). The most severe of these two patients was affected by severe spastic quadriplegia and severe intellectual disability, while the least severe patient died at 9 years of age due to intracranial hemorrhages following an infection

[78,84]. At present, no specific metabolic management has been defined for BSVD3 but only symptomatic therapies.

5.6. SLC39A8 deficiency

A recently classified CDG type II affecting manganese metabolism, SLC39A8 deficiency (or SLC39A8-CDG) [MIM#616721], is also linked to galactosylation. SLC39A8 encodes for a metal cation transporter of 460 aa commonly known as zinc- and iron-related protein type 8 (ZIP8), which transports manganese across the membranes of GA, mitochondria, lysosomes and cytoplasm [168–171]. SLC39A8-CDG patients suffer from cranial asymmetry, infantile spasms with hypsarrhythmia, intellectual disability and cerebellar atrophy, dystonia, seizures, bilateral basal ganglia hyperintensities, disproportionate dwarfism and failure to thrive (Table 1) [168,170]. To date, 14 patients have been described [168,172,173], all manifesting severe glycosylation disruption with truncated N-glycans due to hypogalactosylation and secondary hyposialylation, and blood levels of manganese below the detection limit. Thus, oral D-galactose supplementation was also tested on SLC39A8-deficient patients [168,170,172]. The supplementation improved the glycosylation up to the normal range [168]. However, as galactose supplementation is only able to rescue the glycosylation anomalies and not the manganese related problems, in 2018 Park and colleagues tested a complementary oral manganese supplementation [172]. Manganese treatment resulted in clinical improvement in motor abilities, hearing symptoms and other neurological manifestations [172], officially entering the metabolic management protocol for this CDG.

5.7. TMEM165 deficiency

In 2012 another CDG characterized by reduced galactosylation caused by defects in an ion transporter, TMEM165, was described in five patients [174,175]. TMEM165 is a 324 aa transmembrane protein localized in the lysosome, endosome and GA membranes and it has been proposed to function as a calcium/proton transporter involved in calcium and lysosomal pH homeostasis [176].

Similarly to SLC39A8-CDG, TMEM165-CDG [MIM# 614727] is characterized by neurologic, pulmonary, endocrine, hematological, gastrointestinal and skeletal involvement [175] (Table 1). Furthermore, these patients show abnormally truncated N-glycans due to hyposialylation and hypogalactosylation and disrupted Golgi manganese homeostasis. The latter symptom can be resolved upon manganese supplementation, which however does not rescue all symptoms [174]. In 2017, due to the successful treatment of PGM1-CDG patients using only oral galactose supplementation [170], this supplementation was also investigated in TMEM165-CDG patients. This first trial reduced hypogalactosylation and had a positive effect on several parameters such as APTT, antithrombin, IGF1, IGFBP3, alanine aminotransferase levels and endocrine function [174].

6. Galactose-related lysosomal disorders

The turnover of glycoconjugates takes place in the lysosomes, where glycosidases digest the glycans, releasing monosaccharides that are recycled for biosynthesis of glycans or for fueling other metabolic processes.

Genetic defects affecting lysosomal glycosidases lead to congenital disorders called LSDs. LSDs represent a group of at least 41 genetically distinct, biochemically related, congenital diseases that are individually considered rare, although collectively they affect 1 in 7000–8000 live births [177,178]. Several classes of congenital diseases are included under the umbrella term LSDs, but here we will focus only on those directly related to the lysosomal catabolism of galactosylated conjugates, specifically: Krabbe disease (galactosylceramidase deficiency), Fabry disease (alpha-galactosidase deficiency), beta-galactosidase deficiency and galactosialidosis.

6.1. Galactosylceramide lipidosis (Krabbe disease)

Galactosylceramide lipidosis, also known as Krabbe disease [MIM#245200], is a neurodegenerative LSD with autosomal recessive inheritance and a worldwide incidence of 1:100,000–250,000 live births, caused by mutations in the galactosylceramidase gene *GALC* [179,180].

GALC is 669 aa lysosomal enzyme that catalyzes the hydrolysis of galactose from several glycosphingolipids, including galactosylceramide and galactosylsphingosine [*paragraph 4.4*] [181]. When it is defective, the accumulation of GALC substrates (of which psychosines are cytotoxic lipids) induces damage to the myelin-forming cells, thus compromising tissue integrity and functionality of the nervous system [182].

Since its first description in 1916 by K. Krabbe, over 130 pathogenic *GALC* mutations have been identified, and according to OMIM over a couple of hundreds of patients have been reported thus far [182].

Infantile Krabbe disease accounts for approximately 85–90% of cases and can be further divided into early-infantile disease, in which symptoms appear by 6 months of age, and late-infantile disease, in which symptoms typically appear between 7 and 12 months of age [179]. The infantile-onset clinical spectrum includes feeding difficulties, irritability, followed by rapid mental and motor deterioration, hyperactive reflexes, hypertonicity, loss of vision, and seizures. In the final stage of the disease, stiffness decreases, but the child develops blindness and deafness, while also losing control of voluntary movements (Table 2) [183,184]. The more rare, adult-onset Krabbe disease is instead mostly characterized by weakness, lower limb hypoesthesia and spastic paraparesis (Table 2) [183]. Although pharmacological chaperone therapy is currently under investigation for Krabbe disease [182], the recommended treatment is based on human stem cell transplantation, which can improve the clinical outcome in infantile-onset cases only if initiated within 30 days after birth. Therefore, Krabbe disease has been included for about a decade in newborn screening [185].

6.2. Alpha-galactosidase deficiency (Fabry disease)

Alpha-galactosidase deficiency, or Fabry disease [MIM#301500], is the second most common LSD, firstly reported in 1898 [186–188]. Despite being the only X-linked recessive sphingolipidosis, heterozygous women with Fabry disease experience significant life-threatening conditions requiring medical treatment and intervention. Although its prevalence greatly varies depending on the study considered, in recent European screening studies showed an incidence of up to 1:1250 newborns [189–191].

Fabry disease is caused by mutations in the gene *GLA*, which encodes a 429 aa lysosomal glycoside hydrolase (α-galactosidase A or α-Gal A) that hydrolyzes the terminal alpha-galactosyl moieties from glycoconjugates. Over 1000 mutations in the *GLA* gene have been identified in several thousands of patients [191,192]. Although heterogeneous, the clinical features and their severity appear to correlate with the residual activity of α-Gal A. The first symptoms can manifest in childhood and include episodic intermittent pain crises (acroparestesia), vascular lesions, cornea opacity and damage, proteinuria, and hypohidrosis or anhidrosis [186,187]. The disease progresses into adulthood with cardiac, renal, and cerebral complications including proteinuria, chronic kidney disease, left ventricular hypertrophy, cerebral white matter lesions and occlusive cerebrovascular events, ultimately leading to premature death due to organ failure (Table 2) [186]. At a molecular level, the symptoms are linked to accumulation of glycolipids, like globotriaosylceramide, in the lysosome of the vascular endothelium of skin, kidneys, nervous system, and heart. Over time this accumulation induces inflammation and fibrosis which ultimately develops into organ dysfunction [193]. Currently, the main therapy for Fabry disease is enzyme replacement therapy (ERT), which in some cases has been tested in concert with substrate reduction therapy (SRT) [186,191], which limits biosynthesis of the accumulating metabolites.

Table 2
Summary of the details and clinical presentation of galactose-related LSDs.

Congenital disorder	Gene affected	Gene location (GRCh38/hg38)	Enzymatic reaction [UniProt]	Known mutations	Incidence	Endocrine symptoms	Skeletal symptoms	Hepatic symptoms	Kidney symptoms	Neurologic symptoms	Developmental delay	Muscle symptoms	Cardiac symptoms	Oral and/or GI symptoms	Respiratory symptoms	Ophthalmological symptoms	Hematological anomalies	Others
Krabbe disease	*GALC*	chr14:87,837,820-87,993,665	a β-D-galactosyl-(1↔1′)-N-acylsphing-4-enine + H_2O = an N-acylsphing-4-enine + D-Gal	>130	1:100-250K													Hearing and vision loss
Fabry disease	*GLA*	chrX:101,393,273-101,408,012	globoside Gb3Cer (d18:1(4E)) + H_2O = a β-D-Gal-(1→4)-β-D-Glc-(1↔1)-Cer(d18:1(4E)) + D-Gal	<40	1:1.2-10													Acroparestesia, vascular lesions, hypohidrosis
GM1-gangliosidosis (infantile form)	*GLB1*	chr3:32,996,608-33,097,230	Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides	154	1:100-200K													Skin defects
GM1-gangliosidosis (juvenile form)				<15														
GM1-gangliosidosis (adult form)				<30														
Morquio disease type B	*GLB1*	chr3:32,996,608-33,097,230	Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides	<40	1:250K-1M													Joint defects
Galactosialidosis (early infantile)	*CTSA* (or PPCA)	chr20:45,890,144-45,898,820	Regulation of the lysosomal multienzyme complex	25	N/A													Visceromegaly, skin defects, early death
Galactosialidosis (late infantile)																		Visceromegaly
Galactosialidosis (juvenile/adult)																		Angiokeratoma

Legend: red color, reported symptom; pink color, more rare or suspected symptoms; grey color, absent symptom. Numbers of patients in red color indicate numbers calculated using references from OMIM and available literature. Abbreviations: chr, chromosome; Gal, galactose; Glc, glucose; Cer, ceramide; M, million; K, thousand.

6.3. LDSs related to beta-galactosidase deficiency

Other two autosomal recessive neurodegenerative LSDs linked to galactose homeostasis are GM1-gangliosidosis and Morquio disease type B. Both disorders are caused by mutations affecting the same gene, *GLB1*, that encodes the lysosomal β-galactosidase, an enzyme with catalytic effects on ganglioside GM1, keratan sulfate and several glycopeptides [194]. In general, when the activity of β-galactosidase is compromised, the glycoconjugates with terminal β-galactose residues accumulate in the body fluids and in several tissues of the patients, mostly in the central nervous system and visceral organs [195].

GM1-gangliosidosis has been known since the late 1960s, and it is the most common, with an incidence estimated around 1:100,000–200,000 live births [196]. This disorder has a variable clinical onset that varies according to age [195]. The infantile form (a.k.a. type I) [MIM#230500] shows rapid psychomotor deterioration within 6 months of birth, generalized central nervous system involvement, cardiomyopathy and valvular defects, hepatosplenomegaly, facial dysmorphism, macular cherry-red spots, skeletal dysplasia, and early death (Table 2). The juvenile form (a.k.a. type II) [MIM#230600], manifests between 7 months and 3 years, and shows similar but overall less sever clinical features than the infantile form (excluding hepatosplenomegaly and skin conditions), and usually the patient survives into childhood (Table 2). The adult (or chronic) form (a.k.a. type III) [MIM#230650], which is less severe than types I and II, manifests from 3 to 30 years and is characterized by localized skeletal involvement and localized central nervous system involvement, such as dystonia, gait or speech disturbance (Table 2) [195]. The onset and severity of these three types of GM1-gangliosidosis could be related to the differences in the residual enzyme activity, which accounts for less than 1.5% in type I, for 1–4% in type II, and 4–10% in type III. According to OMIM and current literature, to date 154 patients have been reported for GM1-gangliosidosis type I, less than 15 for type II and less than 30 for type III [196].

Morquio disease type B (a.k.a. mucopolysaccharidosistype IVB) [MIM#253010] has been estimated to affect 1:250,000-1,000,000 live births [197]. It differs from GM1-gangliosidosis as it mostly affects bone tissue and no neurologic alterations. Aside from skeletal defects (e.g. kyphoscoliosis, dysmorphism), joint laxity, progressive growth impairment and cornea opacity, other more rare symptoms have been reported, including cardiac valve pathology, tracheal stenosis, adenotonsillar hypertrophy, and hepatosplenomegaly (Table 2) [198]. For Morquio disease type B so far less than 40 patients have been reported in OMIM and in literature [197], and it becomes recognizable around late infantile or juvenile age.

In both GM1-gangliosidosis and Morquio disease type B disease high amounts of oligosaccharides derived from keratan sulphate or glycoproteins are detected in visceral organs and urine of the patients. For both diseases only symptomatic therapies are available.

6.4. Galactosialidosis

Galactosialidosis [MIM#256540] is an autosomal recessive LSD with a combined deficiency of two lysosomal enzymes, β-galactosidase [*paragraph 6.3*] and *N*-acetyl-α-D-neuraminidase (or sialidase) 1, due to homozygous or compound heterozygous mutations affecting the protective protein/cathepsin A (PPCA or CTSA) [199]. Approximately a hundred of patients and 25 *PPCA* mutations have reported in the literature, but the prevalence remains still to be determined [199].

PPCA is a multifunctional enzyme of 480 aa, formed by two subunits of 32 kDa and 20 kDa, with both protective and catalytic functions [199,200]. In its mature form, PPCA is found as constituent of the so-called lysosomal multienzyme complex (LMC), together with β-galactosidase and neuraminidase [201,202].

The close interaction with the β-galactosidase and the consequent

secondary deficiency of this enzyme explain the similarities with GM1-gangliosidosis. In fact, galactosialidosis was first reported in 1968 [203] and was initially defined as a new variant of GM1-gangliosidosis. However, in vitro studies detected a rather high residual activity of the β-galactosidase (15–20%) in the patients, which was not consistent with the other forms of GM1-gangliosidosis. Closely after, the secondary deficiency of the neuraminidase was detected, followed by the identification of the mutated protein, PPCA, in 1982 [202].

Similarly to GM1-gangliosidosis, also galactosialidosis presents with a wide spectrum of symptoms whose severity varies according to the onset age. Galactosialidosis can be divided in three clinical variants: early infantile form, late infantile form or juvenile/adult form.

The early infantile type is the most severe form and exhibits hydrops fetalis, macular cherry-red spots, visceromegaly, psychomotor disability, coarse facies, skeletal dysplasia, and early death (Table 2). Late infantile forms are characterized by corneal opacity, cardiac involvement, visceromegaly and rare occurrence of disability (Table 2). The milder juvenile/adult form presents with myoclonus, ataxia, neurological deterioration, angiokeratoma, and absence of visceromegaly (Table 2) [204,205].

Like several other LSDs, galactosialidosis is responsive to ERT and the first steps have been moved towards gene therapy, but no metabolic management is currently in place in clinical practice [206].

7. Conclusion

Galactose is an essential carbohydrate for cellular metabolism, being a building block for glycan biosynthesis and a carbon source alternative to glucose. Human cells are not programmed to be able to live on galactose alone, due to the fact that this molecule would fuel into glycolysis via the Leloir pathway at a much slower rate than glucose preventing a rapid ATP production. Nevertheless, galactose is critical to balance all carbohydrate-based pathways in the presence of glucose and other sugars.

The biological relevance of galactose is highlighted by the extent of deleterious consequences due to defects in its metabolism. In this review, we have illustrated how both accumulation and depletion of galactose, UDP-Gal and galactosylated glycoconjugates lead to a wide range of congenital metabolic disorders. Oral galactose supplementation has proven to ameliorate clinical features in some of these disorders and improve the patients' prognosis.

Thanks to its properties, versatility and key-role in human metabolism, galactose and galactose-containing molecules hold great but still mostly unexplored potential for nutritional, biotechnological and pharmacological applications.

Authors' contributions

- F. Conte: conceptualization; manuscript writing and editing; figures and tables creation.
- N. van Buuringen: contribution to initial literature search and early version draft writing.
- N. C. Voermans: manuscript revision.
- D. J. Lefeber: conceptualization, manuscript revision.

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Declaration of Competing Interest

The authors have no conflicts of interests to disclose.

References

[1] K.J. Newens, J. Walton, A review of sugar consumption from nationally representative dietary surveys across the world, J. Hum. Nutr. Diet. 29 (2) (2016) 225–240.
[2] E.J.A.S. Holesh, A. Martin, Physiology, Carbohydrates. [Updated 2020 Aug 25], in: StatPearls [Internet], StatPearls Publishing, Treasure Island (FL), 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459280/.
[3] D.D.J. Lineback, J. Slavin, A. Stephen, D. Gordon, L. Prosky, E.F. Scarborough, S. Lee, F. Clydesdale, Complex carbohydrates: the science and the label, Nutr. Rev. 53 (7) (1995) 186–193.
[4] Monosaccharide diversity, in: P.H. Seeberger, A. Varki (Eds.), Essentials of Glycobiology, Cold Spring Harbor Laboratory Press Copyright 2015–2017 by the Consortium of Glycobiology La Jolla, California. All rights reserved.: Cold Spring Harbor (NY), 2017.
[5] M. Kilcoyne, L. Joshi, Carbohydrates in therapeutics, Cardiovasc. Hematol. Agents Med. Chem. 5 (3) (2007) 186–197.
[6] A.V.N. Sharon, *Historical Background and Overview*, in *essentials of glycobiology*, 2019.
[7] H.J. Gabius, J. Roth, An introduction to the sugar code, Histochem. Cell Biol. 147 (2) (2017) 111–117.
[8] H.J. Gabius, Biological information transfer beyond the genetic code: the sugar code, Naturwissenschaften 87 (3) (2000) 108–121.
[9] J. Kopitz, Lipid glycosylation: a primer for histochemists and cell biologists, Histochem. Cell Biol. 147 (2) (2017) 175–198.
[10] A.I. Coelho, G.T. Berry, M.E. Rubio-Gozalbo, Galactose metabolism and health, Curr Opin Clin Nutr Metab Care 18 (4) (2015) 422–427.
[11] S.S. Yuan, et al., Application of mono-and disaccharides in drug targeting and efficacy, ChemMedChem 13 (8) (2018) 764–778.
[12] B. Tefsen, et al., Galactofuranose in eukaryotes: aspects of biosynthesis and functional impact, Glycobiology 22 (4) (2011) 456–469.
[13] L. Pasteur, Note sur le sucre de lait, 1856.
[14] E. Fischer, R.S. Morrell, Ueber die Configuration der Rhamnose und Galactose, Ber. Dtsch. Chem. Ges. 27 (1) (1894) 382–394.
[15] M. Berthelot, Chimie organique fondée sur la synthèse Vol. 1, 1860. Mallet-Bachelier.
[16] A.I. Coelho, et al., Sweet and sour: an update on classic galactosemia, J. Inherit. Metab. Dis. 40 (3) (2017) 325–342.
[17] K. Gross, P. Acosta, Fruits and vegetables are a source of galactose: implications in planning the diets of patients with galactosaemia, J. Inherit. Metab. Dis. 14 (2) (1991) 253–258.
[18] K.C. Gross, et al., Soluble galactose content of selected baby food cereals and juices, J. Food Compos. Anal. 8 (4) (1995) 319–323.
[19] S. Stepnick, et al., Free galactose content of fresh fruits and strained fruit and vegetable baby foods: more foods to consider for the galactose-restricted diet, J. Acad. Nutr. Diet. 100 (5) (2000) 573.
[20] S.C. Van Calcar, et al., Galactose content of legumes, caseinates, and some hard cheeses: implications for diet treatment of classic galactosemia, J. Agric. Food Chem. 62 (6) (2014) 1397–1402.
[21] L.A. Drozdowski, A.B. Thomson, Intestinal sugar transport, World J. Gastroenterol. 12 (11) (2006) 1657–1670.
[22] H.M. Holden, I. Rayment, J.B. Thoden, Structure and function of enzymes of the Leloir pathway for galactose metabolism, J. Biol. Chem. 278 (45) (2003) 43885–43888.
[23] B. Grossiord, et al., Genetics of galactose utilisation via the Leloir pathway in lactic acid bacteria, Lait 78 (1) (1998) 77–84.
[24] P.A. Frey, The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose, FASEB J. 10 (4) (1996) 461–470.
[25] M. Tang, E. Etokidem, K. Lai, The Leloir pathway of Galactose metabolism - a novel therapeutic target for hepatocellular carcinoma, Anticancer Res. 36 (12) (2016) 6265–6271.
[26] K. Wallenfels, F. Hucho, K. Herrmann, Enzymatically catalyzed mutarotation of aldoses. Studies on aldose-1-epimerase from E. coli, Biochem. Z. 343 (3) (1965) 307–325.
[27] J.B. Thoden, et al., Molecular structure of human galactose mutarotase, J. Biol. Chem. 279 (22) (2004) 23431–23437.
[28] Uniprot, UniProtKB - Q96C23 (GALM_HUMAN), 2019.
[29] Uniprot, *UniProtKB - U6EK71 (U6EK71_LACLL)*. 2019.
[30] D.G. Walker, H.H. Khan, Some properties of galactokinase in developing rat liver, Biochem. J. 108 (2) (1968) 169–175.
[31] D.J. Timson, R.J. Reece, Sugar recognition by human galactokinase, BMC Biochem. 4 (2003) 16.
[32] Uniprot, UniProtKB - P07902 (GALT_HUMAN), 2019.
[33] T.J. McCorvie, et al., Molecular basis of classic galactosemia from the structure of human galactose 1-phosphate uridylyltransferase, Hum. Mol. Genet. 25 (11) (2016) 2234–2244.
[34] Uniprot, UniProtKB - Q14376 (GALE_HUMAN), 2019.
[35] D. Demirbas, et al., Hereditary galactosemia, Metabolism 83 (2018) 188–196.
[36] D.J. Timson, The structural and molecular biology of type III galactosemia, IUBMB Life 58 (2) (2006) 83–89.
[37] R.G. Hansen, Hereditary galactosemia, Jama 208 (11) (1969) 2077–2082.
[38] P. Cuatrecasas, S. Segal, Galactose conversion to D-xylulose: an alternate route of galactose metabolism, Science 153 (3735) (1966) 549–551.
[39] N.J. Rancour, E.D. Hawkins, W.W. Wells, Galactose oxidation in liver, Arch. Biochem. Biophys. 193 (1) (1979) 232–241.

[40] S. Rogers, et al., Accumulation of galactonate in liver of suckling rats perfused with galactose, Biochem. Biophys. Res. Commun. 118 (1) (1984) 304–309.
[41] C. Ning, et al., Galactose metabolism in mice with galactose-1-phosphate uridyltransferase deficiency: sucklings and 7-week-old animals fed a high-galactose diet, Mol. Genet. Metab. 72 (4) (2001) 306–315.
[42] S.L. Wehrli, et al., Urinary galactonate in patients with galactosemia: quantitation by nuclear magnetic resonance spectroscopy, Pediatr. Res. 42 (6) (1997) 855–861.
[43] E. Wada, R-Galactonolactone in experimental galactosemic animals, Arch. Biochem. Biophys. 251 (1) (1986) 215–221.
[44] W.R. Bergren, et al., Galactonic acid in galactosemia: identification in the urine, Science 176 (4035) (1972) 683–684.
[45] C. Ficicioglu, et al., Monitoring of biochemical status in children with Duarte galactosemia: utility of galactose, galactitol, galactonate, and galactose 1-phosphate, Clin. Chem. 56 (7) (2010) 1177–1182.
[46] R.D. Bunker, et al., Structure and function of human xylulokinase, an enzyme with important roles in carbohydrate metabolism, 2013 (1083-351X (Electronic)).
[47] J.H. Kinoshita, Mechanisms initiating cataract formation. *Proctor Lecture*, Investig. Ophthalmol. 13 (10) (1974) 713–724.
[48] C. Bo-Htay, et al., Effects of d-galactose-induced ageing on the heart and its potential interventions, J. Cell. Mol. Med. 22 (3) (2018) 1392–1410.
[49] C. Jakobs, S. Schweitzer, B. Dorland, Galactitol in galactosemia, Eur. J. Pediatr. 154 (2) (1995) S50–S52.
[50] HMDB, *metabocard for Galactitol (HMDB0000107)*. 2019.
[51] H. Holden, et al., Galactokinase: structure, function and role in type II galactosemia, Cell. Mol. Life Sci. CMLS 61 (19–20) (2004) 2471–2484.
[52] M.N. Uddin, et al., Toxic effects of D-galactose on thymus and spleen that resemble aging, J. Immunotoxicol. 7 (3) (2010) 165–173.
[53] C. Bo-Htay, et al., Effects of d-galactose-induced ageing on the heart and its potential interventions, J. Cell. Mol. Med. 22 (3) (2018) 1392–1410.
[54] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (1) (2009) 1–13.
[55] N. Sun, R.J. Youle, T. Finkel, The mitochondrial basis of aging, Mol. Cell 61 (5) (2016) 654–666.
[56] S. Radenkovic, et al., The metabolic map into the Pathomechanism and treatment of PGM1-CDG, Am. J. Hum. Genet. 104 (5) (2019) 835–846.
[57] F. Clerc, et al., Human plasma protein N-glycosylation, Glycoconj. J. 33 (3) (2016) 309–343.
[58] A. Harazono, et al., Site-specific glycosylation analysis of human apolipoprotein B100 using LC/ESI MS/MS, Glycobiology 15 (5) (2004) 447–462.
[59] A.J. Hülsmeier, P. Paesold-Burda, T. Hennet, N-glycosylation site occupancy in serum glycoproteins using multiple reaction monitoring liquid chromatography-mass spectrometry, Mol. Cell. Proteomics 6 (12) (2007) 2132–2138.
[60] M. Molinari, N-glycan structure dictates extension of protein folding or onset of disposal, Nat. Chem. Biol. 3 (6) (2007) 313.
[61] H.J. An, C.B. Froehlich, Jw Fau Lebrilla, C.B. Lebrilla, Determination of glycosylation sites and site-specific heterogeneity in glycoproteins, 2009 (1879–0402 (Electronic)).
[62] M. Dalziel, et al., Emerging principles for the therapeutic exploitation of glycosylation, Science 343 (6166) (2014) 1235681.
[63] B. Hadley, et al., Nucleotide sugar transporter SLC35 family structure and function, Comput Struct Biotechnol J 17 (2019) 1123–1134.
[64] J. M, Glycosyltransferases and glycan-processing enzymes, in: Essentials of Glycobiology, 2017.
[65] T. Hennet, The galactosyltransferase family, Cell. Mol. Life Sci. 59 (7) (2002) 1081–1095.
[66] A. Helenius, M. Aebi, Intracellular functions of N-linked glycans, Science 291 (5512) (2001) 2364–2369.
[67] H. Freeze, Schnaar, Glycosylation precursors, in: C.R. Varki, J.D. Esko (Eds.), Essentials of Glycobiology, 2017.
[68] B. Winchester, Lysosomal metabolism of glycoproteins, Glycobiology 15 (6) (2005), 1r-15r.
[69] P. Stanley, N. T, M. Aebi, in: A. Varki, J.D. Esko (Eds.), *N-glycans*, in *Essentials of Glycobiology*, 2017.
[70] I.B.P. Stanley, *O-GalNAc Glycans*, in *Essentials of Glycobiology*, 2017.
[71] Casale, J. and J.S. Crane. *Biochemistry, Glycosaminoglycans.* In: StatPearls [Internet] 2020 [Updated 2020 Jul 10]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK544295/.
[72] R. Raman, V. Sasisekharan, R. Sasisekharan, Structural insights into biological roles of protein-glycosaminoglycan interactions, Chem. Biol. 12 (3) (2005) 267–277.
[73] K. Prydz, Determinants of Glycosaminoglycan (GAG) structure, Biomolecules 5 (3) (2015) 2003–2022.
[74] M. Suzuki, Biochemical studies on carbohydrates. Prosthetic group of cornea mucoid, J. Biochem. 30 (2) (1939) 185–191.
[75] K. Uchimura, Keratan sulfate: biosynthesis, structures, and biological functions, Methods Mol. Biol. 1229 (2015) 389–400.
[76] B. Caterson, J. Melrose, Keratan sulfate, a complex glycosaminoglycan with unique functional capability, Glycobiology 28 (4) (2018) 182–206.
[77] C. Kiani, et al., Structure and function of aggrecan, Cell Res. 12 (1) (2002) 19–32.
[78] B. Schegg, et al., Core glycosylation of collagen is initiated by two beta(1-O) galactosyltransferases, Mol. Cell. Biol. 29 (4) (2009) 943–952.
[79] T. Hennet, Collagen glycosylation, Curr. Opin. Struct. Biol. 56 (2019) 131–138.
[80] W. Grassmann, H. Schleich, Uber den Kohlenhydratgehalt des Kollagens II, Biochem. Z. 277 (1935) 320–328.
[81] E. Leikina, et al., Type I collagen is thermally unstable at body temperature, Proc. Natl. Acad. Sci. U. S. A. 99 (3) (2002) 1314–1318.
[82] R.G. Spiro, Characterization and quantitative determination of the hydroxylysine-linked carbohydrate units of several collagens, J. Biol. Chem. 244 (4) (1969) 602–612.
[83] R.G. Spiro, F. Lucas, K.M. Rudall, Glycosylation of hydroxylysine in collagens, Nat. New Biol. 231 (19) (1971) 54–55.
[84] S. Miyatake, et al., Biallelic COLGALT1 variants are associated with cerebral small vessel disease, Ann. Neurol. 84 (6) (2018) 843–853.
[85] M.A. Chester, IUPAC-IUB joint commission on biochemical nomenclature (JCBN). Nomenclature of glycolipids–recommendations, Eur. J. Biochem. 257 (2) (1997) 293–298.
[86] L.R. Engelking, Chapter 20 - glycoproteins and glycolipids, in: Textbook of Veterinary Physiological Chemistry, E.S.P.C. Inc., 3rd edition, Academic Press, 2015, pp. 130–135.
[87] M.C. Raff, et al., Galactocerebroside is a specific cell-surface antigenic marker for oligodendrocytes in culture, Nature 274 (5673) (1978) 813–816.
[88] K.R. Jessen, et al., Galactocerebroside is expressed by non-myelin-forming Schwann cells in situ, J. Cell Biol. 101 (3) (1985) 1135–1143.
[89] T. Takahashi, T. Suzuki, Role of sulfatide in normal and pathological cells and tissues, J. Lipid Res. 53 (8) (2012) 1437–1450.
[90] M. Kyogashima, The role of sulfatide in thrombogenesis and haemostasis, Arch. Biochem. Biophys. 426 (2) (2004) 157–162.
[91] T. Zhang, et al., The role of Glycosphingolipids in immune cell functions, Front. Immunol. 10 (2019) 90.
[92] H. Suila, et al., Are globoseries glycosphingolipids SSEA-3 and -4 markers for stem cells derived from human umbilical cord blood? J. Mol. Cell Biol. 3 (2) (2011) 99–107.
[93] P.J. Magistretti, et al., Gangliosides: treatment avenues in neurodegenerative disease, Front. Neurol. 10 (2019) 859.
[94] D. Wigger, et al., Monitoring the Sphingolipid de novo synthesis by stable-isotope labeling and liquid chromatography-mass spectrometry, Front Cell Dev Biol 7 (2019) 210.
[95] K. Honke, UDP-Gal: ceramide galactosyltransferase (UGT8), in: N. Taniguchi, et al. (Eds.), Handbook of Glycosyltransferases and Related Genes, Springer: Springer, Tokyo, 2014, pp. 131–140.
[96] S. Degroote, J. Wolthoorn, G. van Meer, The cell biology of glycosphingolipids, Semin. Cell Dev. Biol. 15 (4) (2004) 375–387.
[97] R. Tidhar, A.H. Futerman, The complexity of sphingolipid biosynthesis in the endoplasmic reticulum, Biochim. Biophys. Acta 1833 (11) (2013) 2511–2518.
[98] F. Dall'Olio, *Glycobiology of Aging.* Subcell Biochem 90 (2018) 505–526.
[99] S. Grunewald, G. Matthijs, J. Jaeken, Congenital disorders of glycosylation: a review, Pediatr. Res. 52 (5) (2002) 618–624.
[100] J. Jaeken, R. Péanne, What is new in CDG? J. Inherit. Metab. Dis. 40 (4) (2017) 569–586.
[101] P. Witters, D. Cassiman, E. Morava, Nutritional therapies in congenital disorders of glycosylation (CDG), Nutrients 9 (11) (2017) 1222.
[102] R. Péanne, et al., Congenital disorders of glycosylation (CDG): quo vadis? Eur. J. Medical Genet. 61 (11) (2018) 643–663.
[103] C.R. Ferreira, et al., Recognizable phenotypes in CDG, J. Inherit. Metab. Dis. 41 (3) (2018) 541–553.
[104] N. Ondruskova, et al., Congenital disorders of glycosylation: still "hot" in 2020, Biochim. Biophys. Acta Gen. Subj. 2021 (1) (1865) 129751.
[105] A. Von Reuss, Zuckerausscheidung im Säuglingsalter, Wien. Med. Wochenschr. 58 (1908) 799–801.
[106] A. Maratha, et al., Classical galactosaemia and CDG, the N-glycosylation interface. A review, in: JIMD Reports *Volume 34*, Springer, 2016, pp. 33–42.
[107] M.E. Rubio-Gozalbo, et al., The endocrine system in treated patients with classical galactosemia, Mol. Genet. Metab. 89 (4) (2006) 316–322.
[108] D.J. Timson, The molecular basis of galactosemia - past, present and future, Gene 589 (2) (2016) 133–141.
[109] F.R. Calderon, et al., Mutation database for the galactose-1-phosphate uridyltransferase (GALT) gene, Hum. Mutat. 28 (10) (2007) 939–943.
[110] S.F. Dobrowolski, et al., Analysis of common mutations in the galactose-1-phosphate uridyl transferase gene: new assays to increase the sensitivity and specificity of newborn screening for galactosemia, The Journal of molecular diagnostics 5 (1) (2003) 42–47.
[111] G.T. Berry, Disorders of galactose metabolism, in: Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease, Elsevier, 2015, pp. 615–626.
[112] N. Karadag, et al., Literature review and outcome of classic galactosemia diagnosed in the neonatal period, Clin. Lab. 59 (9–10) (2013) 1139–1146.
[113] V. Lewis, et al., Galactosemia: clinical features, diagnosis and management. A case report, J. La State Med. Soc. 147 (6) (1995) 262–265.
[114] G.T. Berry, Galactosemia: when is it a newborn screening emergency? Mol. Genet. Metab. 106 (1) (2012) 7–11.
[115] K. Lai, M.B. Boxer, A. Marabotti, GALK inhibitors for classic galactosemia, Future Med. Chem. 6 (9) (2014) 1003–1015.
[116] L. Welling, et al., Nine years of newborn screening for classical galactosemia in the Netherlands: effectiveness of screening methods, and identification of patients with previously unreported phenotypes, Mol. Genet. Metab. 120 (3) (2017) 223–228.
[117] B.M. Pyhtila, et al., Newborn screening for galactosemia in the United States: looking back, looking around, and looking ahead, JIMD Rep 15 (2015) 79–93.
[118] K. Coss, et al., IgG N-glycans as potential biomarkers for determining galactose tolerance in classical Galactosaemia, Mol. Genet. Metab. 105 (2) (2012) 212–220.

[119] I. Knerr, et al., Effects of temporary low-dose galactose supplements in children aged 5–12 y with classical galactosemia: a pilot study, Pediatr. Res. 78 (3) (2015) 272.
[120] A.M. Bosch, et al., Clinical features of galactokinase deficiency: a review of the literature, J. Inherit. Metab. Dis. 25 (8) (2002) 629–634.
[121] K.J. Wierenga, et al., High-throughput screening for human galactokinase inhibitors, J. Biomol. Screen. 13 (5) (2008) 415–423.
[122] L. Liu, et al., Structure activity relationships of human galactokinase inhibitors, Bioorg. Med. Chem. Lett. 25 (3) (2015) 721–727.
[123] M. Tang, et al., Innovative therapy for classic Galactosemia - tale of two HTS, Mol. Genet. Metab. 105 (1) (2012) 44–55.
[124] J. Chhay, et al., Analysis of UDP-galactose 4′-epimerase mutations associated with the intermediate form of type III galactosaemia, J. Inherit. Metab. Dis. 31 (1) (2008) 108–116.
[125] Y. Wada, et al., Biallelic GALM pathogenic variants cause a novel type of galactosemia, Genet. Med. 21 (6) (2018) 1286.
[126] S. Iwasawa, et al., The prevalence of GALM mutations that cause galactosemia: a database of functionally evaluated variants, Mol. Genet. Metab. 126 (4) (2019) 362–367, https://doi.org/10.1016/j.ymgme.2019.01.018 (Epub 2019 Mar 18. PMID: 30910422).
[127] T. Stojkovic, et al., Muscle glycogenosis due to phosphoglucomutase 1 deficiency, N. Engl. J. Med. 361 (4) (2009) 425–427.
[128] S.Y. Wong, et al., Oral D-galactose supplementation in PGM1-CDG, Genet Med 19 (11) (2017) 1226–1235.
[129] L.C. Tegtmeyer, et al., Multiple phenotypes in phosphoglucomutase 1 deficiency, N. Engl. J. Med. 370 (6) (2014) 533–542.
[130] C. Mandato, et al., Cryptogenic liver disease in four children: a novel congenital disorder of glycosylation, Pediatr. Res. 59 (2) (2006) 293.
[131] M. Mohamed, et al., Clinical and diagnostic approach in unsolved CDG patients with a type 2 transferrin pattern, Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1812 (6) (2011) 691–698.
[132] B. Pérez, et al., A novel congenital disorder of glycosylation type without central nervous system involvement caused by mutations in the phosphoglucomutase 1 gene, J. Inherit. Metab. Dis. 36 (3) (2013) 535–542.
[133] S. Timal, et al., Gene identification in the congenital disorders of glycosylation type I by whole-exome sequencing, Hum. Mol. Genet. 21 (19) (2012) 4151–4161.
[134] S.Y.-W. Wong, et al., Defining the phenotype and assessing severity in phosphoglucomutase-1 deficiency, J. Pediatr. 175 (2016) 130–136, e8.
[135] F. Conte, et al., Phosphoglucomutase-1 deficiency: early presentation, metabolic management and detection in neonatal blood spots, Mol. Genet. Metab. 131 (1–2) (2020) 135–146.
[136] N. Ondruskova, et al., Glycogen storage disease-like phenotype with central nervous system involvement in a PGM1-CDG patient, Neuroendocrinol. Lett. (2014) 35(2).
[137] S. Radenkovic, P. Witters, E. Morava, Central nervous involvement is common in PGM1-CDG, Mol. Genet. Metab. 125 (3) (2018) 200–204.
[138] R. Altassan, et al., International consensus guidelines for phosphoglucomutase 1 deficiency (PGM1-CDG): diagnosis, follow-up, and management, J. Inherit. Metab. Dis. 44 (1) (2021) 148–163.
[139] K. Nolting, et al., Limitations of galactose therapy in phosphoglucomutase 1 deficiency, Mol Genet Metab Rep 13 (2017) 33–40.
[140] H. Sprong, et al., Association of the Golgi UDP-galactose transporter with UDP-galactose:ceramide galactosyltransferase allows UDP-galactose import in the endoplasmic reticulum, Mol. Biol. Cell 14 (8) (2003) 3482–3493.
[141] D. Maszczak-Seneczko, et al., UDP-galactose (SLC35A2) and UDP-N-acetylglucosamine (SLC35A3) transporters form glycosylation-related complexes with Mannoside Acetylglucosaminyltransferases (Mgats), J. Biol. Chem. 290 (25) (2015) 15475–15486.
[142] C. Medrano, et al., Clinical and molecular diagnosis of non-phosphomannomutase 2 N-linked congenital disorders of glycosylation in Spain, Clin. Genet. 95 (5) (2019) 615–626.
[143] H. Kodera, et al., De novo mutations in SLC35A2 encoding a UDP-galactose transporter cause early-onset epileptic encephalopathy, Hum. Mutat. 34 (12) (2013) 1708–1714.
[144] K. Dörre, et al., A new case of UDP-galactose transporter deficiency (SLC35A2-CDG): molecular basis, clinical phenotype, and therapeutic approach, J. Inherit. Metab. Dis. 38 (5) (2015) 931–940.
[145] T. Kimizu, et al., A case of early onset epileptic encephalopathy with de novo mutation in SLC35A2: clinical features and treatment for epilepsy, Brain and Development 39 (3) (2017) 256–260.
[146] K. Westenfield, et al., Mosaicism of the UDP-Galactose transporter SLC35A2 in a female causing a congenital disorder of glycosylation: a case report, BMC Med. Genet. 19 (1) (2018) 100.
[147] T.M. Yates, et al., SLC35A2-related congenital disorder of glycosylation: defining the phenotype, Eur. J. Paediatr. Neurol. 22 (6) (2018) 1095–1102.
[148] M.A. Vals, et al., Clinical, neuroradiological, and biochemical features of SLC35A2-CDG patients, J. Inherit. Metab. Dis. 42 (3) (2019) 553–564.
[149] B.G. Ng, et al., SLC35A2-CDG: functional characterization, expanded molecular, clinical, and biochemical phenotypes of 30 unreported individuals, Hum. Mutat. 40 (7) (2019) 908–925.
[150] S. Miyamoto, et al., A case of de novo splice site variant in SLC35A2 showing developmental delays, spastic paraplegia, and delayed myelination, Mol Genet Genomic Med 7 (8) (2019), e814.
[151] F. Lopes, et al., Identification of novel genetic causes of Rett syndrome-like phenotypes, J. Med. Genet. 53 (3) (2016) 190–199.
[152] A.M. Muir, et al., Genetic heterogeneity in infantile spasms, Epilepsy Res. 156 (2019) 106181.
[153] S. Baldassari, et al., Dissecting the genetic basis of focal cortical dysplasia: a large cohort study, Acta Neuropathol. 138 (6) (2019) 885–900.
[154] A. Magalhães, et al., Transferrin isoelectric focusing for the investigation of congenital disorders of glycosylation: analysis of a ten-year experience in a Brazilian center, J. Pediatr. 96 (6) (2019) 710–716, https://doi.org/10.1016/j.jped.2019.05.008 (Epub 2019 Oct 31. PMID: 31677975).
[155] M.R. Winawer, et al., Somatic SLC35A2 variants in the brain are associated with intractable neocortical epilepsy, Ann. Neurol. 83 (6) (2018) 1133–1146.
[156] N.S. Sim, et al., Brain somatic mutations in SLC35A2 cause intractable epilepsy with aberrant N-glycosylation, Neurol Genet 4 (6) (2018), e294.
[157] M. van Scherpenzeel, et al., High-resolution mass spectrometry glycoprofiling of intact transferrin for diagnosis and subtype identification in the congenital disorders of glycosylation, Transl. Res. 166 (6) (2015) 639–649, e1.
[158] P. Witters, et al., Clinical and biochemical improvement with galactose supplementation in SLC35A2-CDG, Genet Med 22 (6) (2020) 1102–1107.
[159] D. Harrus, et al., The dimeric structure of wild-type human glycosyltransferase B4GalT1, PLoS One 13 (10) (2018), e0205571.
[160] B. Ramakrishnan, et al., Structure and catalytic cycle of beta-1,4-galactosyltransferase, Curr. Opin. Struct. Biol. 14 (5) (2004) 593–600.
[161] B. Ramakrishnan, V. Ramasamy, P.K. Qasba, Structural snapshots of beta-1,4-galactosyltransferase-I along the kinetic pathway, J. Mol. Biol. 357 (5) (2006) 1619–1633.
[162] U. Brodbeck, et al., The isolation and identification of the B protein of lactose synthetase as alpha-lactalbumin, J. Biol. Chem. 242 (7) (1967) 1391–1397.
[163] B. Ramakrishnan, P.K. Qasba, Crystal structure of lactose synthase reveals a large conformational change in its catalytic component, the beta1,4-galactosyltransferase-I, J. Mol. Biol. 310 (1) (2001) 205–218.
[164] V. Peters, et al., Congenital disorder of glycosylation IId (CDG-IId) – a new entity: clinical presentation with Dandy-Walker malformation and myopathy, Neuropediatrics 33 (1) (2002) 27–32.
[165] M. Guillard, et al., B4GALT1-congenital disorders of glycosylation presents as a non-neurologic glycosylation disorder with hepatointestinal involvement, J. Pediatr. 159 (6) (2011) 1041–1043, e2.
[166] M.A.W. van den Boogert, et al., Reduced CETP glycosylation and activity in patients with homozygous B4GALT1 mutations, J. Inherit. Metab. Dis. 43 (3) (2020) 611–617.
[167] P. Witters, D. Cassiman, E. Morava, Nutritional therapies in congenital disorders of glycosylation (CDG), Nutrients (2017) 9(11).
[168] J.H. Park, et al., SLC39A8 deficiency: a disorder of manganese transport and glycosylation, Am. J. Hum. Genet. 97 (6) (2015) 894–903.
[169] E.-K. Choi, et al., Functional analysis of SLC39A8 mutations and their implications for manganese deficiency and mitochondrial disorders, Sci. Rep. 8 (1) (2018) 3163.
[170] L.G. Riley, et al., A SLC39A8 variant causes manganese deficiency, and glycosylation and mitochondrial disorders, J. Inherit. Metab. Dis. 40 (2) (2017) 261–269.
[171] D.W. Nebert, Z. Liu, SLC39A8 gene encoding a metal ion transporter: discovery and bench to bedside, Hum Genomics 13 (Suppl. 1) (2019) 51.
[172] J.H. Park, et al., SLC39A8 deficiency: biochemical correction and major clinical improvement by manganese therapy, Genetics in Medicine 20 (2) (2018) 259.
[173] K.M. Boycott, et al., Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene SLC39A8, Am. J. Hum. Genet. 97 (6) (2015) 886–893.
[174] W. Morelle, et al., Galactose supplementation in patients with TMEM165-CDG rescues the glycosylation defects, J. Clin. Endocrinol. Metab. 102 (4) (2017) 1375–1386.
[175] F. Foulquier, et al., TMEM165 deficiency causes a congenital disorder of glycosylation, Am. J. Hum. Genet. 91 (1) (2012) 15–26.
[176] Uniprot, UniProtKB - Q9HC07 (TM165_HUMAN), 2019. Available from, https://www.uniprot.org/uniprot/Q9HC07.
[177] P.J. Meikle, et al., Prevalence of lysosomal storage disorders, Jama 281 (3) (1999) 249–254.
[178] V. Nair, E.C. Belanger, J.P. Veinot, Lysosomal storage disorders affecting the heart: a review, Cardiovasc. Pathol. 39 (2019) 12–24.
[179] D.A. Wenger, et al., Krabbe disease: genetic aspects and progress toward therapy, Mol. Genet. Metab. 70 (1) (2000) 1–9.
[180] H.W. Moser, Peripheral nerve involvement in Krabbe disease: a guide to therapy selection and evaluation, Neurology 67 (2) (2006) 201–202.
[181] I. Visigalli, et al., The galactocerebrosidase enzyme contributes to the maintenance of a functional hematopoietic stem cell niche, Blood 116 (11) (2010) 1857–1866.
[182] T.K. Dv, et al., Molecular dynamics simulations to decipher the structural and functional consequences of pathogenic missense mutations in the galactosylceramidase (GALC) protein causing Krabbe's disease, J. Biomol. Struct. Dyn. (2020) 1–16.
[183] M.L. Escolar, et al., Clinical management of Krabbe disease, J. Neurosci. Res. 94 (11) (2016) 1118–1125.
[184] K. Suzuki, Globoid cell leukodystrophy (Krabbe's disease): update, J. Child Neurol. 18 (9) (2003) 595–603.
[185] J.M. Kwon, et al., Consensus guidelines for newborn screening, diagnosis and treatment of infantile Krabbe disease, Orphanet J Rare Dis 13 (1) (2018) 30.
[186] K. Turkmen, I. Baloglu, Fabry disease: where are we now? Int. Urol. Nephrol. 52 (11) (2020) 2113–2122.
[187] R. Schiffmann, Fabry disease, Pharmacol. Ther. 122 (1) (2009) 65–77.

[188] W. Anderson, A case of angiokeratoma, Br. J. Dermatol. 10 (1898) 113–117.
[189] M. Spada, et al., High incidence of later-onset fabry disease revealed by newborn screening, Am. J. Hum. Genet. 79 (1) (2006) 31–40.
[190] T.R. Hsu, D.M. Niu, Fabry disease: review and experience during newborn screening, Trends Cardiovasc Med 28 (4) (2018) 274–281.
[191] M. Lenders, E. Brand, Fabry Disease, The Current Treatment Landscape. Drugs, 2021.
[192] A. Gal, E. Schäfer, I. Rohard, The genetic basis of Fabry disease, in: B.M. Mehta, G. Sunder-Plassmann (Eds.), Fabry Disease: Perspectives from 5 Years of FOS, Oxford PharmaGenesis: Oxford, 2006.
[193] A. Tuttolomondo, et al., Anderson-Fabry disease: a multiorgan disease, Curr. Pharm. Des. 19 (33) (2013) 5974–5996.
[194] R. Santamaria, et al., Twenty-one novel mutations in the GLB1 gene identified in a large group of GM1-gangliosidosis and Morquio B patients: possible common origin for the prevalent p.R59H mutation among gypsies, Hum. Mutat. 27 (10) (2006) 1060.
[195] Y. Suzuki, A. Oshima, E. Nanba, β-Galactosidase deficiency (β-galactosidosis) GM1 gangliosidosis and Morquio B disease, in: C.R. Scriver, et al. (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill Professional: New York (NY), 2001, pp. 3775–3809.
[196] F.M. Lang, et al., The natural history of Type 1 infantile GM1 gangliosidosis: a literature-based meta-analysis, Mol. Genet. Metab. 129 (3) (2020) 228–235.
[197] M. Bleier, et al., Morquio B patient/caregiver survey: first insight into the natural course of a rare GLB1 related condition, Mol Genet Metab Rep 16 (2018) 57–63.
[198] N. Yuskiv, K. Higaki, S. Stockler-Ipsiroglu, Morquio B disease. disease characteristics and treatment options of a distinct GLB1-related dysostosis multiplex, Int. J. Mol. Sci. (2020) 21(23).
[199] A. Caciotti, et al., Galactosialidosis: review and analysis of CTSA gene mutations, Orphanet J Rare Dis 8 (2013) 114.
[200] N.J. Galjart, et al., Human lysosomal protective protein has cathepsin A-like activity distinct from its protective function, J. Biol. Chem. 266 (22) (1991) 14754–14762.
[201] A. d'Azzo, E. Bonten, Molecular mechanisms of pathogenesis in a glycosphingolipid and a glycoprotein storage disease, Biochem. Soc. Trans. 38 (6) (2010) 1453–1457.
[202] A. d'Azzo, et al., Molecular defect in combined beta-galactosidase and neuraminidase deficiency in man, Proc. Natl. Acad. Sci. 79 (15) (1982) 4535.
[203] D.M. Derry, et al., Late infantile systemic lipidosis. Major monosialogangliosidosis. Delineation of two types, Neurology 18 (4) (1968) 340–348.
[204] N. Darin, et al., Juvenile galactosialidosis with attacks of neuropathic pain and absence of sialyloligosaccharidurıa, Eur. J. Paediatr. Neurol. 13 (6) (2009) 553–555.
[205] A. Lehman, et al., Emphysema in an adult with galactosialidosis linked to a defect in primary elastic fiber assembly, Mol. Genet. Metab. 106 (1) (2012) 99–103.
[206] I. Annunziata, A. d'Azzo, Galactosialidosis: historic aspects and overview of investigated and emerging treatment options, Expert Opin Orphan Drugs 5 (2) (2017) 131–141.