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Congenital disorders of glycosylation: Rapidly enlarging group of (neuro)metabolic disorders

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Abstract

Only in the last couple of years, an ever-growing number of human genetic diseases in the synthesis of glycoproteins have been identified. Correct glycosylation of glycoproteins is essential for their biological function and the sugar chains act as biosignals for cell–cell communication, intracellular signalling, protein folding or targeting of proteins. Under-glycosylation of glycoproteins, functioning as hormones, enzymes or transporters, lead to impaired bioability, decreased activity and rapid degradation. Given the overall importance of glycosylation, it is not surprising, that a disruption of the glycosylation machinery can lead to multisystemic and severe diseases.

Up until now, mainly defects in the *N*-glycosylation pathway have been discovered and are grouped as Congenital Disorders of Glycosylation (CDG), formerly known as Carbohydrate-Deficient Glycoprotein syndromes. More recently, defects in the less well-defined *O*-glycosylation pathway were identified and combined glycosylation disorders in which both, the *N*- and *O*-glycosylation processes are affected.

1. Congenital disorders of glycosylation: an introduction

Glycosylation, the addition of sugar chains (glycans) to proteins, occurs in every human cell. Carbohydrates aid protein function by ensuring correct folding, providing solubility and protease resistance. By facilitating cell adhesion and migration, they help to mediate the process of development. In general, secreted, cell surface, and extracellular matrix proteins are glycosylated. Correct glycosylation of glycoconjugates, glycoproteins or glycolipids, is essential for their biological role. Not surprisingly, disruption of glycosylation leads to severe problems in man [1].

The recently delineated Congenital Disorders of Glycosylation (CDG), formerly known as Carbohydrate-Deficient Glycoprotein syndromes, are a group of inherited multisystemic diseases due to defective *N*- and/or *O*-glycosylation of proteins. The biosynthesis of N-linked oligosaccharides is elaborate and numerous glycosyltransferases are involved, attaching, stepwise, nucleotide-activated or dolichol phosphate-linked sugars to a growing lipid-linked oligosaccharide chain. Finally, as the very latest step in the endoplasmatic reticulum (ER), the completed structure ($Gluc_3Man_9GlcNAc_2$) is transposed en bloc in N-linkage to an asparagine residue of a nascent protein via the oligosaccharyltransferase complex. In the Golgi compartment, several glycosidases remodel the

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oligosaccharide chain into a more complex structure by removal of mannose residues and the addition of *N*-acetylglucosamine, galactose, fucose and sialic acid residues [2].

The biosynthesis of *O*-glycans is initiated after the folding and oligomerisation of proteins and can set off in the late ER or in one of the Golgi compartments. Whereas in *N*-glycosylation there is a common protein–glycan linkage with a common core structure, *O*-glycans have different protein–glycan linkages in which *N*-Acetylgalactosamine, mannose, fructose, xylose or other sugars can be attached to a serine or threonine residue, resulting in many different glycan types.

The synthesis of oligosaccharides, their transfer to the nascent polypeptide chain, and subsequent modification requires complex pathways comprising numerous steps. Considering the ubiquitous presence of glycosylation and the multiple functions of the glycans, it is obvious that defects in the biosynthesis of glycoproteins are detrimental and result in a broad spectrum of clinical signs and symptoms. The disorders of glycosylation are remarkable for their clinical diversity. In recent years over 20 *N*-glycosylation disorders have been characterized and whenever the gene responsible for the newly identified disorder has been described, the CDG subtype is included in the growing CDG alphabet: in 2007, 12 CDG-I disorders of the early assembling pathway (CDG-Ia through CDG-Il) and eight additional later defects of the later processing steps of the *N*-glycosylation pathway (CDG-IIa through CDG-IIh) are known [3,4].

Disease-causing defects in *O*-glycan biosynthesis have recently been identified in the *O*-mannose and *O*-xylose pathways. *O*-mannosylation disorders identified so far all cause muscular dystrophy whereas *O*-xylosylation defects primarily affect bone, cartilage and skin.

The discovery of *N*- and *O*-glycosylation defects has lately been of great interest, as they may affect the trafficking in the glycosylation machinery. The disruption of multiple glycosylation pathways is due to mutations of the so-called conserved oligomeric Golgi (COG) complex. This large, eight-subunit spanning complex plays a key role in protein transport between the ER and Golgi and within the Golgi [5].

2. *N*-Glycosylation defects

2.1. The most frequently diagnosed CDG Subtype: CDG-Ia

The first CDG-Ia patients were described in 1980 [6]. In 2007, about 700 patients are known worldwide presenting with this most frequent type of CDG. Often patients can be diagnosed in the neonatal or early infantile period, on the basis of typical clinical features (inverted nipples and fat pads) in addition to strabismus, muscular hypotonia and failure to thrive. A very common feature is cerebellar hypoplasia, which can usually be documented at or shortly after birth. There is substantial childhood mortality due to organ failure and/or severe infections.

At a later age, the impairment of the neurological system becomes more evident with variable degree of mental retardation, cerebellar dysfunction and retinitis pigmentosa. Some children develop seizures or present stroke-like episodes. In adults, non-progressive ataxia, stable mental retardation and peripheral neuropathy mainly characterize the disease. The large majority of patients are wheelchair bound. Adult female patients present as a rule with hypergonadotropic hypogonadism. With broadening screening for CDG, the number of patients identified with a less typical presentation is increasing, including children with nearly normal psychomotor development and/or normal cerebellum. CDG-Ia is a result of a deficiency of phosphomannomutase (PMM 2) and a large variety of disease causing mutations of the PMM 2 gene have been identified.

2.2. Clinical features of other *N*-glycosylation defects

The majority of CDG disorders present with multiorgan involvement and literately all organs can be affected. For the vast majority of CDG subtypes, patients present with central nervous symptoms. Exceptions are CDG-Ib and CDG-Ih, which are mainly hepatic-intestinal diseases: their classical clinical presentation is protein-losing enteropathy, congenital hepatic fibrosis and coagulopathy without overt neurological manifestations [7,8]. Other presentations are persistent vomiting and hyperinsulinaemic hypoglycaemia. Early diagnosis of CDG-Ib is essential, because patients can be successfully treated with oral mannose (see "Treatment").

ALG 6 deficiency (CDG-Ic) causes mainly a neurological disorder that is in general milder than CDG-Ia and is probably next to CDG-Ia the most common CDG subtype. Features of CDG-Ia, such as cerebellar hypoplasia, fat pads and inverted nipples are missing. In two of 20 patients the disease had an early fatal outcome because of severe coagulopathy and hormonal disturbances, combined with untreatable seizures (unpublished data). CDG-Ic is probably underdiagnosed because of the absence of typical morphological features [9].

As for many of the other CDG subtypes only very few patients have been identified so far, it is not possible yet to give a decent description of their "typical presentation". However, Table 1 summarises more common clinical signs and symptoms recognised in CDG patients, reflecting the broad clinical variability.

2.3. From clinical symptoms to diagnosis

2.3.1. IEF of serum transferrin

The vast majority of patients suffering from CDG are first identified by studying changes in transferrin. Transferrin, one of the predominant serum glycoproteins, has two *N*-glycosylation sites. Hence, most transferrin molecules carry two biantennary chains with terminal sialic acid residues, and tetrasialotransferrin is the main serum sialotransferrin. Deficient synthesis of *N*-glycans results in a deficient incorporation of sialic acid, the terminal negatively charged sugar. The molecules acquire a more positive charge which causes a cathodal shift in the isoelectric focusing (IEF) pattern of transferrin. In the so-called type I pattern (the most frequent) there is an

Table 1 Clinical features of CDG patients

Neurology	Axial hypotonia; hyporeflexia; developmental delay; seizures; stroke-like events; micro- and macrocephaly; myopathy
Gastroenterology/ hepatology	Failure to thrive; vomiting; protein-losing enteropathy; liver dysfunction; hepatomegaly; cholangitis; chronic diarrhoea
Neonatology	Hydrops; ascites; multiorgan failure; failure to thrive; floppy baby
Haematology	Thrombocytosis; thrombocytopenia; coagulopathy; thrombosis; anaemia; leukocytosis, thrombocytopenia
Endocrinology	Hyperinsulinaemic hypoglycaemia; hypothyroidism; hypergonadotropic hypogonadism; growth retardation
Clinical genetics	Dysmorphic features
Orthopaedics	Osteopenia; joint contractures; kyphosis/scoliosis; short limbs; arthrogryposis
Ophthalmology	Abnormal eye movements; squint; cataract; retinitis pigmentosa; nystagmus; iris coloboma; cortical blindness
Radiology	Cerebellar hypoplasia; calcification of white matter; delayed myelinisation; micropolygyria; renal hyperechogenicity
Histology	Liver fibrosis; liver cirrhosis; lamellar inclusions in hepatocytes; intestinal villus atrophy
Dermatology	Ichthyosis; abnormal fat distribution
Nephrology	Nephrotic syndrome; tubulopathy; cystic kidneys
Immunology	Recurrent infections; hypogammaglobulinaemia
Cardiology	Cardiomyopathy; pericardial effusions
Biochemistry	Hypoalbuminaemia; elevated transaminases; low cholesterol, triglycerides; decreased antithrombin III; decreased factor VIII and XI; decreased protein C and S; elevated FSH, LH and prolactin; elevated TSH, low free T4

increase of di- and asialotransferrin and a decrease of tetrasialotransferrin, whereas the type II pattern is a combination of the type I pattern and an increase of trisialotransferrin and, but not always, monosialotransferrin. It is essential to exclude that the abnormal pattern is not due to a transferrin protein variant. Performing IEF of the parents' transferrin and/or pre-incubating the patient's sample with neuraminidase can do this. It might be helpful to study an additional glycoprotein to confirm a generalized glycosylation disorder.

In view of the extremely broad clinical spectrum of known CDG patients, it is recommended to consider CDG in any unexplained multisystem disorder. The IEF of transferrin is widely used as a screening test, although not all CDG types can be detected with this assay (eg CDG-IIb, -IIc, -IIf). For any abnormal result of the IEF of transferrin, secondary glycosylation defects suchlike galactosaemia and fructosaemia need to be excluded.

2.3.2. Enzymatic measurements and mutational studies

Enzyme activities are most readily measured in fibroblasts or leukocytes. Regarding PMM activity, leukocytes seem to be more reliable than fibroblasts, because a high residual activity has been observed in the fibroblasts of some CDG-Ia patients, whereas the leukocyte values were always in the clearly abnormal range. Thus, patients with slightly decreased or low normal values of PMM in fibroblasts might still harbour mutations in PMM2. Especially in the case of a clinical picture that strongly suggests CDG-Ia, it is worthwhile to look for PMM2 mutations [10]. The PMM2 gene has been cloned in 1997 and more than 50 different mutations have been identified. There is a clear predominance of missense mutations. The most common R141H mutation is found in approximately 40% of all patients. F119L is frequent in the Northern European countries due to a founder effect, whereas V231M and P113L are frequent all over Europe. Most patients are compound heterozygous, and homozygosity is not observed for R141H or other mutations that severely impair the protein.

2.3.3. LLO analysis

The rapid delineation of additional CDGs – especially for CDG-I defects – have been facilitated by using yeast as a model system: the *N*-glycosylation assembly pathway is highly conserved between yeast and man. *Saccharomyces cerevesiae* mutants with defective asparagine linked glycosylation (*alg*) had already been created in the early eighties and their metabolic defects had been characterized by the accumulation of specific lipid-linked oligosaccharide (LLO) patterns. Comparing these specific LLO patterns of different yeast mutants with those being found in patients' fibroblasts, helped to identify the candidate genes for several human glycosylation defects, primarily of the group of CDG-I defects [11].

2.3.4. Glycan structure analysis

The precise determination of the glycan structures by mass spectrum analysis accumulating in affected patients has helped to identify candidate genes for new CDG defects. Structural studies of the complex Asp-linked glycans have mainly been performed on serum transferrin. Electrospray mass spectrometry (ES-MS) of protein bound oligosaccharide chains (glycoproteins) can separate the different glycoforms and magnetic resonance spectroscopy analysis determines the glycan structures and molecular mass of the glycovariants. These glycan structure analysis are instrumental for the elucidation of CDG-x cases, by pinpointing candidate enzymes and genes responsible for the abnormal *N*-glycan synthesis [12].

2.3.5. Prenatal diagnosis

Prenatal diagnosis has become reliable for CDG-Ia since the mapping of the disease locus on chromosome 16p13, and the subsequent identification of the enzymatic defect and the cloning of the PMM2 gene. Early attempts for prenatal diagnosis on the basis of transferrin isoforms in

foetal blood have failed and thus revealed, that this method is not reliable. Enzymatic measurements of PMM activities in cultured amniocytes or trophoblasts are useful but may give inconclusive data. As a result, preference is given to the direct mutation analysis in the foetus.

Prenatal diagnosis is possible in all other types of CDG for which the molecular defect is known, on the condition that the diagnosis has been confirmed in the index patient or the mutations have been detected in the parents.

2.4. Treatment

There are only few options for treatment available for the minority of CDG subtypes.

2.4.1. CDG-Ib

In CDG-Ib, patients on treatment with oral mannose (4–6 doses per day × 100–150mg/kg per day) have shown significant improvement. The supplementation of mannose bypasses the enzymatic defect and reverses not only the abnormal glycosylation of the marker protein but has as well a favourable effect on clinical symptoms suchlike coagulapathy, hypoglycaemia, protein-losing enteropathy. Serum mannose levels should be greater than 200 μmol/l. Since mannose supplementation is probably a life-long therapy for phospomannose isomerase (MPI) deficient patients, side effects have to be monitored carefully. High mannose intake can cause osmotic diarrhoea. Also a slight increase of the glycosylated haemoglobin (HbA1c) has been observed.

2.4.2. CDG-IIc

In patients with a GDP-fucose transporter defect, fucose supplementation (25mg/kg per day in 3 doses) was reported to improve the fucosylation of glycoproteins and to control the recurring infections. However, no effect can be expected on the neurological complications of the disease.

2.4.3. CDG-Ia

Unfortunately, an efficient treatment is still not available for this vast majority of CDG patients. Although it was reported that incubation with mannose resulted in an increased incorporation of mannose in CDG-Ia patient's fibroblasts, mannose administration to CDG-Ia patients did not improve the clinical or biochemical features. Also fucose supplementation, with the aim to enhance the GDP-mannose pool, was not successful.

As to symptomatic treatment, we have obtained efficient prevention of stroke-like events by using 0.5mg/kg per day acetylsalicylic acid. In patients with recurrent fractures, biphosphonates should be considered and females with hypergonadotropic hypogonadism may need hormonal replacement therapy.

3. *O*-Glycosylation defects

Whereas *N*-glycosylation takes place in the cytoplasm, endoplasmatic reticulum and Golgi and includes assembly and processing steps, *O*-glycosylation lacks a processing pathway but is otherwise more complex: there is much more diversity in *O*-glycan structures, as there are many more core structures underlying the synthesis of *O*-glycoproteins. The most commonly observed *O*-glycans are the so-called mucin-type *O*-glycans with *N*-acetylgalactosamine (GalNAc) as the initiating sugar, comprising six core structures. *O*-glycosylation defects in man have so far only been described for *O*-glycans with mannose or xylose as first sugar attached in threonine or serine binding to its protein (Table 2).

Table 2 Update on *O*-glycosylation defects (September 2007)

	Defective gene	Enzymatic defect
Defects in the biosynthesis of O-mannosylated glycans		
Muscle–eye–brain disease (MEB)	*POMTGnt1*	*O*-linked mannose β-1, 2-*N*-acetylglucosaminyl-transferase
Walker–Warburg syndrome (WWS)	*POMT1/ POMT 2*	Protein-*O*-mannosyl transferase
Defects in the biosynthesis of O-xylolylated glycans		
Hereditary multiple exostoses syndrome	*EXT1, 2 or 3*	Glucuronyltransferase/ *N*-acetyl-d-hexosaminyl-transferase
Ehlers–Danlos syndrome – Progeria variant	*XGALT7/ XGAL-T1*	Xylosylprotein β-1, 4-galactosyltransferase

3.1. Defects in the biosynthesis of *O*-mannosylated glycans

Two distinct disorders from the genetically heterogeneous group of congenital muscular dystrophies were recently included in the CDG family. Defective post-translational modification seems to be confined to dystroglycan which is heavily *N*- and *O*-glycosylated. Whereas *N*-glycosylation plays a minor role in alpha-dystroglycan, the *O*-glycans play a crucial role in the binding of alpha-dystroglycan to the extracellular matrix protein laminin. The underglycosylation of the dystroglycan, the key component of the dystroglycan complex, leads to a disruption of the integrity of the basal membrane in skeletal muscle and brain. Walker–Warburg Syndrome (WWS) and muscle–eye–brain disease (MEB) are caused by mutation in genes involved in *O*-mannosylation, POMT1/POMT 2 and POMGnT1, respectively [13,14]. The clinical presentations of WWS and MEB show a considerable overlap and might be undistinguishable on clinical grounds. Muscular dystrophy, ocular abnormalities, type II lissencephaly and cortical dysplasia has been described in both diseases. Skeletal muscle from affected patients, show hardly any α-dystroglycan on Western blotting or immunohistochemical analysis. The finding, that one of its ligands in skeletal muscle, the laminin α chain, is simultaneously reduced, supports the hypothesis, that aberrant α-dystroglycan glycosylation underlies the pathogenesis of these disorders. In parallel, basal lamina defects affecting the lamina propria of the central nervous system, is thought to be the underlying cause for uncontrolled neuronal growth resulting in a migration disorder [15].

3.2. Defects in the biosynthesis of *O*-xylolylated glycans

3.2.1. Hereditary multiple exostoses syndrome

Of all glycosylation defects, this is the most prevalent CDG and the only one to be inherited as an autosomal dominant trait. Osteochondromas, often already present postnatally, start growing with time with hazardous effects on the local tissue and vessels and a tendency for developing malignancy. The defective genes encoding for glycosyltransferases EXT1/EXT2/EXT3 are involved in heparin sulphate synthesis [16].

3.2.2. Ehlers—Danlos syndrome — Progeria variant

Premature ageing and hyperelastic skin in a patient with macrocephaly and joint hyperlaxicity should lead to further investigation of members of the β-1,4-galactosyltransferase family. Hypoglycosylation of small proteoglycans (decorin and biglycan) result in core protein carrying only one xylose *O*-linked to serine. Molecular studies and biochemical analysis showed markedly reduced activity of galactosyltransferase 1 activity and mutations were found in the β-GalT7 and XGal-T1 gene in a patient described by Seidler et al. [17].

4. Combined *N*- and *O*-glycosylation defects

Part of the already well-identified and known CDG defects involve actually not only the *N*-glycosylation but as well the *O*-glycosylation pathway: several nucleotide sugars and transporters play a role in both pathways. Defects such as CDG-Ia, -Ib, -Ie and -If for example reduce the pool of luminal dol-P-Man and therefore affect *N*- and *O*-glycosylation.

More combined defects are waiting to be discovered, however, screening methods for *O*-glycosylation still need to be established. The first successful attempt in detecting patients with an *O*-glycosylation defect has been introducing the IEF of Apolipoprotein C-III (apoC-III). It is a rapid and simple technique that may be used as a screening assay for *O*-glycosylation defects, but is limited to the detection of only abnormalities in core 1 mucin-type *O*-glycans. This glycan type is widely expressed throughout the human body and is abundant in the nervous system; it is considered the most common type of *O*-glycan.

Combining both, IEF of transferrin and ApoCIII led to the identification of a new clinical entity: three patients with a combined *N*- and *O*-glycosylation defect presented with cutis laxa and developmental delay. The underlying defect has yet to be established. Detailed biochemical hallmarks of these patients have been characterized and narrowed down the possible gene candidates [18].

5. Remarks and future directions

In the last couple of years, the field of human glycosylation defects has been rapidly expanding, mainly thanks to the increasing awareness of the clinical variability of CDG. The human genome contains as little as 20,000 to 25,000 protein coding genes and 2—3% of its transcripts are probably devoted to the synthesis and recognition of glycans. This underlines the importance of glycosylation in man, which has also received dramatic support from the recent elucidation of the biochemical and genetic causes of several subtypes of congenital disorders of glycosylation (CDG). Defects in glycosylation are becoming increasingly associated with a range of human diseases. The challenges for the clinician is to keep her/his mind open considering a glycosylation defect in any patient with an unexplained clinical presentation, especially if more than one organ system is involved. The diagnostic workup of a patient with possible congenital disorder of glycosylation might be very complex and close collaboration with biochemists, glycobiologists, and geneticists eg might be needed to characterize the underlying CDG subtype. The challenges for the researchers in the field of glycosylation defects are manifold and areas for future activity will be focused on, I. the development of new screening techniques, II. identification of new gene defects, III. extended studies for increasing the understanding of the pathomechanisms and IV. the development of treatment options.

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