Minireview

Deficiencies in subunits of the Conserved Oligomeric Golgi (COG) complex define a novel group of Congenital Disorders of Glycosylation

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Abstract

Processing of the glycan structures on glycoproteins by different glycosylation enzymes depends on, among other, the non-uniform distribution of these enzymes within the Golgi stacks. This compartmentalization is achieved by a balance between anterograde and retrograde vesicular trafficking. If the balance is disturbed, the glycosylation machinery is mislocalized, which can cause Congenital Disorders of Glycosylation type II (CDG-II), as illustrated by the identification of congenital defects in the Conserved Oligomeric Golgi (COG) complex in humans. We collected findings from different COG deficient cell types, such as CHO, yeast and human fibroblasts to hypothesize about structure and function of the COG complex, and compared the phenotypes and genotypes of the patients known with a COG deficiency. Among 35 CDG-II patients we found 5 patients with a COG defect. COG defects are a novel group of CDG-II with deficient *N*- as well as *O*-glycosylation.
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Introduction

The endoplasmic reticulum (ER) and Golgi apparatus are crucial organelles in the secretory pathway of proteins. Newly synthesized proteins enter the ER through docking of the ribosome onto a protein pore of the ER membrane [1]. Proteins are then transported from ER to Golgi and from Golgi to endosomes, lysosomes or the plasma membrane by transport vesicles [2]. During this process, a set of post-translational modifications like glycosylation, tyrosine sulfation, lysosomal targeting of enzymes and multimer assembly occur in the Golgi apparatus [3–5].

The covalent attachment of a glycan onto a protein constitutes the glycosylation process, an important post-translational modification involved in folding, stability and interactions of glycoproteins [6–8]. Glycosylation can be subdivided into *N*-, *O*- and *C*-glycosylation: *N*-glycans are attached to an amino-group of asparagines [9], *O*-glycans to the hydroxyl group of serine and/or threonine [6] and *C*-glycans to the C-2 atom of the indole moiety of tryptophan [10].

N-Glycosylation starts in the endoplasmic reticulum (ER) with the assembly of a dolichol-linked oligosaccharide consisting of 14 monosaccharides (3 glucoses, 9 mannoses and 2 *N*-acetylglucosamines). During assembly, the growing oligosaccharide is "flipped" from the cytoplasmic side of the ER to its luminal side where the last four mannoses and three glucoses are added. The resulting oligosaccharide is then transferred from its lipid carrier to a nascent protein in the ER lumen. Further processing in ER and Golgi by glycosidases and glycosyltransferases results in a mature complex type *N*-glycan [9].

O-Glycosylation on the other hand, mostly takes place in the Golgi apparatus and consists only of assembly without processing. A monosaccharide is transferred directly onto a serine or a threonine. The first monosaccharide can be *N*-acetylgalactosamine in case of a mucin type

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O-glycan, but it can also be fucose, galactose, glucose, mannose, *N*-acetylglucosamine or xylose. Eight core structures can be distinguished, depending on the attached monosaccharide(s) and/or its (their) binding. These core structures are further elongated or modified by sialylation, fucosylation, sulfatation, acetylation, and polylactosamine-extension [6]. For the *N*- as for the *O*-glycosylation, these sequential modifications by different glycosyltransferases depend on their non-uniform distribution within the Golgi stack [9].

The importance of glycosylation is dramatically illustrated by the fact that congenital defects in this process lead to, mostly severe, diseases: Congenital Disorders of Glycosylation (CDG). The first patients were described in 1980 by Jaeken et al. [11] . Since then 18 defects have been described in *N*- or *N*- and *O*-glycosylation. Twelve defects are known in the assembly of the *N*-glycan (CDG-Ia to CDG-IL) and six in the processing of the glycan (CDG-IIa to CDG-IIf) (overview in [12–15]). Isolated *O*-glycosylation defects have also been reported like Walker–Warburg syndrome and muscle–eye–brain disease (*O*-mannosylation), multiple hereditary exostoses and a progeria-like syndrome (*O*-xylosylation) and familial tumoral calcinosis (*O*-*N*-acetylgalactosaminylation) [16–18]. There is a rapidly expanding group of patients with a putative CDG-I or CDG-II in whom the known defects have been excluded (CDG-Ix and CDG-IIx).

What do we know about the Conserved Oligomeric Golgi (COG) complex?

The compartmentalization within the Golgi is achieved by a balance between anterograde and retrograde trafficking of the resident Golgi proteins [19]. If the balance is disturbed by a defect in vesicular trafficking, the glycosylation machinery is mislocalized and can cause glycosylation defects. This has originally been illustrated by the identification of the ldlB and ldlC mutant CHO cells [20,21].

LdlB and ldlC mutant CHO cells did not express low density lipoprotein (LDL) receptors on their surface in spite of the absence of a gene defect in the LDL receptor [20]. This suggested a problem of trafficking/secretion and incorporation of the receptor in the plasma membrane. Furthermore, ldlB and ldlC mutant CHO cells showed pleiotropic defects in glycosylation and virtually all N- and O-linked oligosaccharides on glycoproteins and most glycolipids were not processed properly [21]. It was concluded that ldlB and ldlC are required for normal medial- and trans-Golgi associated processing of glycoconjugates. The ldlB and ldlC genes were cloned in 1999 and 1994, respectively [22,23]. They were later shown to belong to the Conserved Oligomeric Golgi (COG) complex. This is a heteromeric protein complex in the Golgi membrane that contains eight subunits (Cog1–8) and is composed of two lobes: lobe A (subunits 1–4) and lobe B (subunits 5–8). This complex was previously partially characterized as the Sec34/35 complex [24], the GTC-90 complex [25] and the

ldlCp complex [22,26]. GTC-90 corresponds to Cog5, ldlB and ldlC to Cog1 and Cog2 , and Sec34 to Cog3 [26].

What have we learned from COG deficiencies in humans?

COG function

While extensively studied, the cellular role of the COG complex in vesicular Golgi trafficking has not yet been fully elucidated. Interaction studies in yeast suggested a primary role for the COG complex in retrograde trafficking from Golgi to ER and between Golgi cisternae [27]. This was confirmed by the observation that fragmented Golgi membranes in Cog3 depleted HeLa cells were able to support anterograde trafficking, while retrograde trafficking was impaired [28]. These cells showed an accumulation of COG-complex-dependent (CCD) vesicles, carrying retrograde cargo molecules, but no anterograde cargo molecules [29].

After the description of the first CDG patients with a COG7 deficiency by Wu et al. [30], the COG7 deficient cells have become a tool to further study the role of the complex in vesicular trafficking. Support for a role in retrograde trafficking was provided by the results of Brefeldin A (BFA) treatment of COG7 deficient fibroblasts. BFA is a selective inhibitor of the GTP-exchange factor for ADP-ribosylation factor 1 (Arf1) which causes redistribution of Golgi proteins to the ER via retrograde transport. BFA treatment of COG7 deficient fibroblasts showed a delay in this retrograde trafficking. Golgi collapse was roughly 2.5 times slower compared with controls. After BFA wash-out—which allows one to check for defects in anterograde trafficking—only a small delay in return of proteins to the Golgi was observed [31]. This suggests that the retrograde transport is more significantly delayed than the anterograde transport. The same technique was used with fibroblasts from the patient with a homozygous COG8 mutation: mannosidase II (Man II) redistribution in the ER was significantly delayed [32].

The COG complex seems to be important to maintain the Golgi structure. Ultrastructural analysis of ldlB cells revealed that a COG defect can cause dilation of Golgi cisternae [26], while in COG3-depleted yeast cells fragmentation of the Golgi complex into multiple mini Golgi stacks with a retained juxtanuclear localization was observed [28].

Oka et al. have identified a set of COG sensitive, integral membrane Golgi type II membrane proteins called GEARs [33]. The steady state levels of Man II, GS-28, GS-15, GPP130, CASP, giantin and golgin-84 were decreased in Cog1 and Cog2 deficient CHO mutants. Possibly due to a mislocalization and/or abnormally rapid degradation, this can lead to further disturbance of the trafficking and glycosylation processes. Recently, Steet et al. have shown that the steady state levels of ERGIC-53 and GS-15 were decreased in COG7 deficient cells [31], while a significant reduction in perinuclear Golgi region staining of Man II and β 1,4-galactosyltransferase 1 (β4GALT1) compared

to controls was seen in the patient with COG1 deficiency [34]. In the COG8 deficient cells only β4GALT1 staining was reduced [32].

Although a deficiency of GEARs acting as glycosidases (e.g. Man II) or glycosyltransferases (e.g. β4GALT1) can explain the glycosylation defect in patients deficient in COG, it is not yet proven if this is due to a reduced stability or to a mislocalization of the enzymes.

If the COG complex is important in vesicular trafficking and for maintaining Golgi structure and function, it is likely that not only glycosylation but also other types of post-translational modification like tyrosine sulfatation, lysosomal targeting of enzymes and multimer assembly are disturbed in patients with a COG deficiency. This is an important topic for investigation.

There is evidence that the COG complex plays a role in male fertility and gonadal morphogenesis. A Cog5 deficiency in Drosophila was shown to cause failure of spermatogenesis and male sterility [35]. New arguments were provided by Kubota et al. [36,37]. In *Caenorhabditis elegans* MIG-17 glycosylation and gonadal localization are affected by two spontaneous mutations in Cogc3 and Cogc1. This results in misdirected migration of gonadal distal tip cells (DTC). Instead of migration into two U-shaped gonadal arms, the DTC cells show a meandering migration. RNAi knockdown of any of the four components of lobe A, also resulted in this meandering DTC phenotype. Other pathophysiological data of COG are not available.

COG structure

The subunit interaction map of the COG complex is still controversial and three models of interaction have been proposed. In the first one the Cog4 subunit is the central subunit [38]. In the second model, Cog1 plays a central role in the organization of the yeast COG complex [19]. In the third model, Cog1 and Cog8 together play the central role in linking the two lobes [39].

The fact that absence of one subunit affects the stability of the whole complex, mostly of the subunits that interact with the one that is absent, was already shown by Chatterton et al. [22]. Depletion of Cog3, the most evolutionary conserved COG subunit in HeLa cells, leads to reduced expression of Cog1 and Cog4 [28]. Moreover, in fibroblasts of patients with COG7 deficiency and in COG5 deficient HeLa cells, a severe reduction in both COG5 and COG7 together with a smaller decrease in the other subunits of the lobe B was observed [39].

The recent identification of mutations in the Cog1 and Cog8 subunits of CDG-II patients, led us to favor the third model [32,34]. Indeed, by using *in vitro* co-immunoprecipitation studies, we confirmed that there is direct interaction between Cog1 and Cog8 and we also showed that this interaction was abolished in both patients with the C-terminal truncations of Cog1 and Cog8, respectively [32]. In the patient with the truncating mutation in Cog1, the

steady state levels of all the lobe A subunits (Cog1–4) and of lobe B subunit Cog8 were significantly reduced. On the other hand, the truncated Cog8 dramatically affected the steady state level of the lobe A component Cog1. Cog 6 was the only other subunit from lobe B that was slightly but significantly reduced in the patient with Cog8 deficiency.

COG-phenotypes and genotypes in humans

The identification of COG defects in patients opened a new avenue for research in CDG. We were interested to know how many of our CDG-IIx cases would be due to a deficiency in the COG complex. Fibroblasts from 35 CDG-IIx patients have been checked by Western blot for the expression levels of COG subunits. Because antibodies were only available for Cog1, Cog3, Cog4, Cog6, Cog7 and Cog8, we only used these for the screening. However, given that the absence of one COG subunit would affect the stability or expression levels of the others, this approach should be dependable. We identified 5 patients with a deficiency in one of the COG subunits: 2 Cog7 [40], 1 Cog1 [34], 1 Cog8 [32] and 1 Cog4 [Reynders et al., in preparation]. To identify mutations that would not result in a decreased expression, the remaining 30 patients were checked by direct sequencing of the coding sequence of all 8 subunits. In 5 patients a missense variant was found, but only in the heterozygous state. In two patients, a homozygous intronic mutation caused a splice variant in the presence of the normal transcript. Further investigations will be necessary to prove whether these variants do cause disease. Thus the frequency of COG defects among our CDG-IIx cases is 5 in 35 or 1 in 7.

COG7

This deficiency is currently the most frequent. Six patients from four families were identified with a deficiency in the COG7 subunit. All children had consanguineous parents and died between the age of 5 weeks and 9 months. They originated from North African countries: two from Tunisia and four from Morocco. Wu et al. [30] described the first two patients in 2004. These siblings shared a phenotype of prenatal growth retardation, microcephaly, dysmorphic features with loose, wrinkled skin, hypotonia, seizures, episodes of hyperthermia and cholestatic liver disease [41]. Three additional patients from two different families were recently described by Morava, Zeevaert et al. [40], with a clinical phenotype consistent with the first patients. Another patient was recently described by Ng et al. [42]. Common clinical features included prenatal growth retardation, dysmorphic features of hands and face (progressive), microcephaly, failure to thrive, hypotonia and episodes of hyperthermia. Signs and symptoms seen in at least 3 of the 6 patients were wrinkled, loose skin especially around the neck, seizures, cortical and/or cerebellar atro-

phy, hypoplasia of the corpus callosum and a ventricular septum defect.

Laboratory findings included elevated transaminases in all 6 patients (range 200–890 U/L, nl < 40) and elevated creatine kinase in 4 of them, especially during hyperthermia (range 144–45.900 U/L, nl < 170).

We concluded that a deficiency in COG7 causes a lethal, multisystem disorder with growth retardation, encephalopathy, dysmorphy and hyperthermia but also involvement of other organs like heart (ASD, VSD), kidneys (obstructive uropathy), liver (intrahepatic cholestasis) and gastrointestinal system (intestinal (pseudo)-obstruction).

Molecular analysis showed that the same mutation in COG7 was present in the homozygous state in all 6 patients (IVS1+4A>C, c.169+4A>C). It is an intronic splice site mutation that disrupts a splice donor site and activates at least two different cryptic splice sites, leading to at least two different transcripts: one with a deletion of 19 base pairs in exon 2 and one with an insertion of 83 base pairs between exon 1 and exon 2 of COG7. Haplotyping showed that at least three families shared the same haplotype around the COG7 gene [40], which strongly suggests that they share a common ancestral mutation. Hence, we expect that this disease may be more common in Morocco and Tunisia, and may be other North African countries.

COG1

The only patient described with COG1 deficiency [34], is a girl born by caesarean section, after an uneventful pregnancy of 35 weeks. She is the second child of consanguineous parents. At birth, weight was 2237 g, length 40 cm and head circumference 31.5 cm. Feeding problems were present since birth and she developed failure to thrive by the end of the first month. She presented with generalized hypotonia but normal muscular strength at the age of 2.5 months. She had small hands, small feet, antimongoloid eyelids and hypoplasia of the temporal region. Cardiac ultrasound showed slight left ventricular hypertrophy without obstruction. Growth retardation with a rhizomelic short stature, progressive microcephaly and mild hepatosplenomegaly developed between 7 and 15 months of age. Growth and head circumference followed the 5th percentile. She showed only mild psychomotor retardation. Transaminases were slightly elevated. Brain MRI at the age of 21 months showed slight cerebellar and cerebral atrophy.

The mutation found in COG1 in this patient was a homozygous, single base pair insertion c.2666insC leading to a premature stop codon and a truncated protein that was 80 amino acids shorter than expected [34].

COG8

This patient was first described by Briones et al. in 2001 [43] and clinical updates were given by Wopereis et al. in 2003 [44] and Foulquier et al. in 2007 [32]. The patient is a girl born from consanguineous parents in a highly consanguineous family with several mentally retarded relatives. Dysmorphic features included epicanthus, small hands and feet and hypoplasia of the first phalanx of some fingers and toes. During infancy she had several episodes of encephalopathy with lethargy and hypotonia, mostly during infections. She lost her head control and the ability to sit without support during the first episode. The second episode was accompanied by a status epilepticus. She developed a progressive cerebellar syndrome with ataxia and action myoclonus. Other features were progressive microcephaly, alternating esotropia and eyelid pseudoptosis.

MRI of the brain showed brainstem and cerebellar atrophy at the age of 22 months. At the age of 3 years she presented with myopathic face, axial hypotonia, slight ataxia and action myoclonus. Clinical assessment at the age of 6 years showed cerebellar syndrome and failure to thrive. The clinical history revealed frequent stomatitis.

Laboratory findings showed elevated transaminases, CK, disturbed PT and aPTT, protein C and protein S deficiency, and low blood clotting factors II, V, VII, IX, X and XI.

At 8 years of age, cerebellar ataxia had worsened, but MRI investigation showed no further alterations. She showed minor developmental progress, developing simple language and understanding, oculomotor apraxia with dyssynergia oculocephalica and polyneuropathy of the lower limbs.

The mutation found in COG8 in this patient was the nonsense mutation c.1611C>G (p.Y536X), again homozygous [32].

The second patient with a COG8 deficiency has no dysmorphic features, which is in contrast with all other COG patients described so far [45]. The clinical features that overlap with the other COG8 patient are growth retardation, failure to thrive, hypotonia, epilepsy, psychomotor retardation, polyneuropathy, strabism and feeding problems. Both patients showed atrophy on brain MRI. We have no information on head circumference or liver volume in the second patient.

This patient carries two heterozygous mutations including a splice mutation IVS3+1G>A (c.560+1G>A) and a two basepairs deletion 1687–1688delTT in exon 5 leading to a truncated protein lacking the last 47 amino acids.

Comparison of COG-phenotypes

Contrary to patients with COG7 deficiency, the clinical phenotypes of patients with COG1 and COG8 deficiencies were non-lethal. The patient deficient in COG1 is mildly retarded, while the patients deficient in COG8 are moderately to severely retarded. Progressive microcephaly, encephalopathy with hypotonia, failure to thrive, dysmorphic features of hands and face are noticed in the patients with COG1, COG7 and COG8 deficiencies (Fig. 1).

The patients with COG8 deficiency have predominant neurological signs and symptoms like hypotonia, seizures,
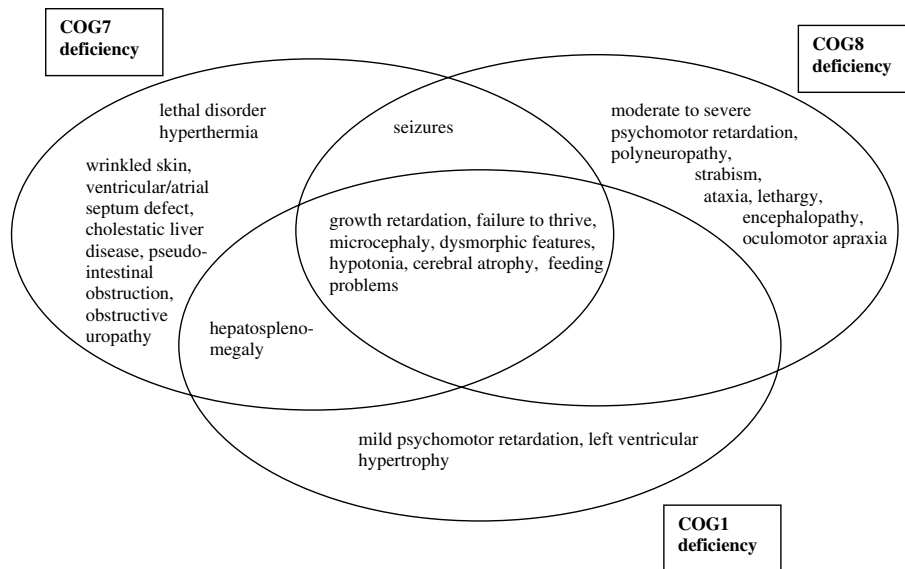
Fig. 1. Clinical features in different COG deficiencies.

polyneuropathy, ataxia and oculomotor apraxia whereas the COG1 and COG7 deficient phenotype is more dominated by growth retardation. This growth retardation is more severe in COG7 and already prenatally present. Possibly, cerebellar ataxia, oculomotor apraxia and peripheral neuropathy are not seen in patients deficient in COG7 because of their early death. In the first patient deficient in COG8 there are episodes of encephalopathy with neurological regression, growth retardation and microcephaly. The highly consanguineous familial background of this patient may have affected the phenotype. Dysmorphy of the face and hands is a common feature of all patients with COG defects, except for the second patient deficient in COG8, and the specific characteristics can differ. In COG7 deficiency it is described as low set, posterior rotated ears and micrognathia together with long, overlapping fingers and a simian crease, whereas in patients with COG1 and COG8 deficiencies small hands and small feet were noticed. Interestingly, the COG1 and COG8 defects are caused by truncating mutations or the combination of a truncating mutation and a splice mutation. Still, there is some truncated protein left in the patients' cells. The COG7 mutation is a splice defect. One has to wait for the identification of more and different patients and mutations to draw firm conclusions about the genotype–phenotype correlation. Still, the observed phenotypes could point to a more critical role of COG7 as compared to COG1 or COG8.

Conclusion

CDG-II due to defects in subunits of the COG complex represent a novel group of disorders. In our group of 35 CDG-IIx patients, we found 5 patients with mutations in one of the COG subunits. In total 10 patients with defects in four of the subunits of the COG complex have now been identified and it is highly anticipated that defects in the other COG subunits will eventually be found. In any case, COG should be analyzed in all unsolved CDG-II cases. This can be done by Western blot analysis. Thus far, in all the known cases, the expression level or stability of at least one of the subunits was affected. The alternative is to sequence the 8 *COG* genes in the patients.

Possibly, patients with a combined defect in *N*- and *O*-glycosylation are the better candidates for a defect in the COG complex. Hence, determining the *O*-glycosylation status is a first step in the diagnostic path, before COG screening. But screening for *O*-glycosylation is not yet well established and limited to core 1 mucin type *O*-glycans with isoelectric focusing of apo-CIII, a glycoprotein which is only *O*-glycosylated.

The clinical pictures lack specific features and the small number of patients prevents us from drawing firm conclusions, but a combination of features like growth retardation, microcephaly, hyperthermia and adducted thumbs is suggestive of a COG7 defect, since 6 patients from 4 families with the same mutation in COG7 share these characteristics. Identification of new patients with the same and other mutations will enlarge our knowledge on the phenotypes of the COG deficiencies, for which, unfortunately, no treatment is available.

Acknowledgments

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