Published in final edited form as:

Mol Genet Metab. 2007 June ; 91(2): 201–204.

Molecular and Clinical Characterization of a Moroccan Cog7 Deficient Patient

Bobby G. Ng¹, Christian Kranz¹, EEO Hagebeuk², M Duran³, NGGM Abeling², B Wuyts⁴, Daniel Ungar⁶, Vladimir Lupashin⁷, CM Hartdorff⁵, BT Poll-The², and Hudson H. Freeze^{1,*}

¹Department of Glycobiology and Carbohydrate Chemistry, Burnham Institute for Medical Research, La Jolla, California, 92037 USA ²Department of Pediatrics Neurology, University of Amsterdam/Academical Medical Centre, Amsterdam, The Netherlands ³Laboratory Genetic Metabolic Diseases, University of Amsterdam/Academical Medical Centre, Amsterdam, The Netherlands ⁴University Hospital Gent, Gent, Belgium ⁵Department of Pediatrics, OLVG, Amsterdam, The Netherlands ⁶Department of Molecular Biology, Princeton University, Princeton, New Jersey, 08544 USA ⁷Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, 72205 USA

Abstract

Mutations in the N-linked glycosylation pathway cause rare autosomal recessive defects known as Congenital Disorders of Glycosylation (CDG). A previously reported mutation in the Conserved Oligomeric Golgi complex gene, *COG7*, defined a new subtype of CDG in a Tunisian family. The mutation disrupted the hetero-octomeric COG complex and altered both N- and O- linked glycosylation. Here we present clinical and biochemical data from a second family with the same mutation.

Keywords

N-glycosylation; Cog7; Congenital Disorders of Glycosylation

Introduction

Congenital Disorders of Glycosylation are rare genetic disorders that alter the biosynthesis and processing of N-glycans. These disorders occur as two groups. The more common Type I involves the biosynthesis and assembly of dolichol-linked glycan Glc₃Man₉GlcNAc₂ precursor in the cytosol and endoplasmic reticulum and its transfer to proteins. The less common Type II involves the processing of the protein bound glycans mostly in the Golgi apparatus [1,2]. Common clinical appearances include; hypotonia, mental retardation, liver malfunction, dysmorphism and seizures.

Patients are typically identified first by clinical phenotype and by isoelectric focussing (IEF) of serum glycoproteins, such as transferrin. Defective glycosylation produces an abnormal

*Corresponding author: Hudson H. Freeze, Glycobiology and Carbohydrate Chemistry, Burnham Institute for Medical Research, 10901 N. Torrey Pines Rd, La Jolla, CA 92037, phone: 858-646-3142, fax: 858-713-6281, E-mail: hudson@burnham.org

Conflict of Interest Statement

The authors have no conflict of interest associated with this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

transferrin pattern due to absence of sialic acids. CDG-II patients have been reported with mutations in glycosyltransferases (MGAT2 and B4GALT1), Golgi based sugar transporters (SLC35A1 and FUCT1) and in a processing glucosidase [2]. More recently, a new family of CDG defects was identified with mutations in Conserved Oligomeric Golgi complex genes, COG7 and COG1 [3,4].

The Conserved Oligomeric Golgi complex is composed of 8 subunits and organized into two substructures; lobe A contains Cog 2-4 and lobe B Cog 5-7 [5,6,7]. The two structures are bridged by interactions between Cog1 and Cog8, with Cog1 having a higher affinity for lobe A and Cog 8 for lobe B [5]. An alternative model has been proposed by Loh *et al.* This model suggests that two sub complexes are formed and that Cog4 drives the formation of these complexes.[8] This complex facilitates transport within the Golgi and retrograde transport from the Golgi to ER. Mutations of different subunits disrupt Golgi integrity and retrograde trafficking of the glycosylation machinery [3].

Here we present data on a COG7 deficient Moroccan patient who carries the same mutation previously reported in a Tunisian family [3,9]. This mutation disrupts glycosylation and alters protein expression and localization of several other Cog subunits.

Materials and Methods

Molecular and Biochemical Analysis

Sequence analysis of Cog7 and Peanut lectin binding was performed as previously described by Wu *et al.* [3] Western Blotting has been performed as previously described by Oka *et al.* [10] Brefeldin A retrograde trafficking was carried out as previously described by Steet *et al.* [11]

Results

Clinical Description

The female patient was the second child of consanguineous Moroccan parents. Pregnancy was unremarkable, but delivery at 40 weeks was complicated by maternal fever. Apgar scores were 8/8 after 1 and 5 minutes respectively. Birth weight (2435 gram), head circumference (30.5 cm) were both below – 2.5 sd. Dysmorphic features included a flat face, full lips, protruding tongue and inverted nipples. Physical examination demonstrated hepatomegaly, severe hypotonia with the absence of deep tendon reflexes and distal arthrogryposis.

Two days after birth she developed an indirect physiological hyperbilirubinaemia, that was treated with phototherapy. Hepatomegaly persisted. ASAT (230–325 U/l), AF (593–1064 U/L) and γ GT (164–231 U/L) were consistently elevated, but ALAT was normal. Coagulation studies (PT, APTT, factor V leiden, factor VIII, trombocytes) were all normal. The abdominal ultrasound showed no abnormalities of liver and hepatic bile duct.

Respiratory depression occurred during feeding and crying with severe laryngeal spasms that required intubation and admittance to the intensive care. Besides a minimal larynx malacia no anatomical disorders were found. These incidents were most likely caused by severe gastro-oesophageal reflux and ceased after omeprazol, cisapride and feeding by duodenum tube was started.

Generalized seizures began at one week, which were treated with phenobarbital, and at 2 month of age she developed a status epilepticus with respiratory depression that responded to benzodiazepines. Repeated electroencephalograms (EEG) showed no epileptic activity, but irregular and slowed background pattern.

Her psychomotor development was minimal at 2.5 months. She showed no ocular fixation, had a normal ophthalmologic examination and didn't respond to sound. Visual evokes responses (VEP) and brainstem auditory evokes responses (BAEP) were both abnormal. Polyneuropathy was confirmed by electrophysiological studies (EMG), without signs of myopathy or elevated CK. She regularly developed fever up to 39.5 °C, without signs of infection. Blood, urine and CSF were normal.

MRI of the brain at birth demonstrated mild enlargement of peripheral and ventricular spaces, which progressed by 3 months. Delayed myelination, without cerebellar hypoplasia occurred and MR spectroscopy showed low N-acetylaspartate, creatine and choline.

Despite continuous tube feeding, she had minimal weight gain. At 3 months, she was stable, on continuous feeding, required no respiratory support, and received phenobarbital and omeprazol. Respiratory depression during feeding continued and at the age of 3.5 months she experienced a severe laryngeal spasm accompanied by seizures, resulting in respiratory insufficiency with no response to maximal oxygen therapy and she died. Hepatomegaly, severe hypotonia and seizures suggested a CDG and isoelectric focusing (IEF) of serum transferrin showed increase in multiple undersialylated forms suggesting a CDG II.

Brefeldin A Induced Retrograde Transport

Cog7 deficient fibroblasts, treated with Brefeldin A, have been shown to have a delay in retrograde transport into the ER [10]. We compared these patient cells against control cells for impaired retrograde transport kinetics by incubating cells with 0.25ug/ml of BFA for different times. Indeed, patient cells have significantly slower retrograde transport kinetics when compared to control cells. FIGURE 1.

Western Blotting

Mutations with in COG subunits typically give rise to an unstable hetero-octomeric complex resulting in degradation or mislocalization of certain subunits within the complex. Mutations, like those presented within Cog7, resulted in nearly complete losses of Cog7, Cog5, Cog6 and partial loss of Cog1 and Cog8. FIGURE 2.

Other Golgi protein markers called GEAR proteins were also affected. GS15 and GPP130 were clearly reduced in patient fibroblasts when compared to control fibroblasts.

Sequence Analysis of Cog7

Sequencing of Cog7 was based largely on two observations. First, we observed a defect in retrograde trafficking upon Brefeldin A treatment. Second, western blotting showed severe decrease in Cog7 protein expression. Sequencing of the patient's Cog7 genomic DNA showed a homozygous intronic IVS1+4 A→C mutation in the 5' splice motif of the first intron similar to that presented previously. FIGURE 3. This mutation results in the use of an alternative splice site ultimately leading to a premature stop codon [3]. A similar biochemical and clinical phenotype is seen between the previous North African Cog7 patients [3,8] and this patient. No material was unavailable for further testing from any of the patients or their families.

Defective sialylation of cell surface O-linked glycans

Loss of either Cog1 or Cog7 can dramatically reduce sialylation of surface O-linked glycans, as measured by increased specific binding of fluorescent Peanut Lectin to inappropriately exposed terminal galactose epitopes. When sialic acid is present, as in control cells, PNA binding is minimal. In patient cells, sialic acid is reduced and PNA binds to the exposed galactose residues. Treating cells with neuraminidase prior to PNA staining gave equal binding which indicates a normal level of the underlying glycans in both cells. FIGURE 4.

Discussion

The recent discovery of mutations in the COG complex highlights the diversity and causes of Type II CDG. Using the Brefeldin A trafficking method, we identified a Cog7 patient who displays multisystem dysfunction, defects in N- and O- Linked glycosylation, loss of several key Cog subunits and altered Golgi trafficking. These lethal characteristics are due to a single homozygous splice site point mutation within the Cog7 gene. Biochemical and cell based experiments on the octomeric COG complex reveals that interactions between the 8 subunits are required to function properly [3,5]. Roles of individual Cogs and loss of a single subunit should also disrupt the function of the entire complex. In deed, Loss of the Cog7 subunit was seen to disrupt Lobe B, alter both N-and O-Linked glycosylation and alter retrograde Golgi trafficking. Recently mutations in Cog1 subunit have yielded similar biochemical results, but the patient had a much milder phenotype [4].

Although the precise function of the Cog complex is still being unraveled, we can clearly say that the complex plays a pivotal role in maintaining the functionality of the Golgi apparatus.

Although we were unable to obtain additional patient or family DNA for further analysis, finding the identical mutation and similar clinical phenotype between this Moroccan patient and the previously described Tunisian patients, suggest that the mutation may occur frequently in these populations. We propose that sequencing this region of genomic *COG7* DNA may be a useful diagnostic follow up in similar patients of North African descent who initially show multiple undersialylated forms of transferrin.

Acknowledgements

Monty Krieger provided the polyclonal antibodies to Cog1 and Cog2. This work was supported by the National Institute of Health (ROI DK55615), and a postdoctoral research fellowship from the Deutsche Forschungsgemeinschaft (KR 2916/1-1) to C.K

References

1. Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. *Eur J Pediatr* 2003;162:359–379. [PubMed: 12756558]
2. Freeze HH. Genetic defects in the human glycome. *Nat Rev Genet* 2006;77:537–551. [PubMed: 16755287]
3. Wu X, Steet RA, Bohorov O, Bakker J, Newell J, Krieger M, Spaapen L, Kornfeld S, Freeze HH. Mutation of the COG complex subunit gene *COG7* causes a lethal congenital disorder. *Nat Med* 2004;105:518–523. [PubMed: 15107842]
4. Foulquier F, Vasile E, Schollen E, Callewaert N, Raemaekers T, Quelhas D, Jaeken J, Mills P, Winchester B, Krieger M, Annaert W, Matthijs G. Conserved oligomeric Golgi complex subunit 1 deficiency reveals a previously uncharacterized congenital disorder of glycosylation type II. *Proc Natl Acad Sci U S A* 2006;10310:3764–3769. [PubMed: 16537452]
5. Ungar D, Oka T, Vasile E, Krieger M, Hughson FM. Subunit architecture of the conserved oligomeric Golgi complex. *J Biol Chem* 2005;28038:32729–32735. [PubMed: 16020545]
6. Oka T, Vasile E, Penman M, Novina CD, Dykxhoorn DM, Ungar D, Hughson FM, Krieger M. Genetic analysis of the subunit organization and function of the conserved oligomeric golgi (COG) complex: studies of COG5- and COG7-deficient mammalian cells. *J Biol Chem* 2005;28038:32736–32745. [PubMed: 16051600]
7. Ungar D, Oka T, Brittle EE, Vasile E, Lupashin VV, Chatterton JE, Heuser JE, Krieger M, Waters MG. Characterization of a mammalian Golgi-localized protein complex, COG that is required for normal Golgi morphology and function. *J Cell Biol* 2002;157:405–15. [PubMed: 11980916]
8. Loh E, Hong W. The binary interacting network of the conserved oligomeric Golgi tethering complex. *J Biol Chem* 2004;279:24640–8. [PubMed: 15047703]

9. Spaapen LJ, Bakker JA, van der Meer SB, Sijstermans HJ, Steet RA, Wevers RA, Jaeken J. Clinical and biochemical presentation of siblings with COG-7 deficiency, a lethal multiple O- and N-glycosylation disorder. *J Inherit Metab Dis* 2005;28:707–14. [PubMed: 16151902]
10. Oka T, Ungar D, Hughson FM, Krieger M. The COG and COPI complexes interact to control the abundance of GEARs, a subset of Golgi integral membrane proteins. *Mol Biol Cell* 2004;15:2423–35. [PubMed: 15004235]
11. Steet R, Kornfeld S. COG-7-deficient Human Fibroblasts Exhibit Altered Recycling of Golgi Proteins. *Mol Biol Cell* 2006;175:2312–2321. [PubMed: 16510524]

FIG 1.

BFA-induced retrograde transport kinetics of control and patient cells. (A) Control and patient fibroblasts were treated with 0.25 μ g/ml BFA. Cells were fixed as described at different time points and the localization of the Golgi matrix protein giantin was observed by using an Alexa-488 coupled anti giantin antibody. The percentage of cells without Golgi staining were shown at the given time points. Approximately 100 cells were counted in duplicate for each time point

FIG 2.

Western blot analysis of Cog 1-8 subunits, GS15, GPP130 and Actin in control and patient cells. Blots were first normalized for Actin loading and then protein densitometry was done using Scion Image.

FIG 3.
Comparison of Exon 1 sequence from Cog 7 genomic DNA. Control compared to patient.

FIG 4.

Control and Patient fibroblasts were treated with or without 50mU Neuraminidase for 1 hour at 37°C followed by fixing with 2% Paraformaldehyde. Cells were then stained with 5ug/ml of PNA-Alexa 488.