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EDITORIAL

How to find and diagnose a CDG due to defective N-glycosylation

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The group of Congenital Disorders of Glycosylation (CDG) is expanding rapidly since the first clinical description of the N-glycosylation defect PMM2-CDG (CDG-Ia) in 1980 (Jaeken et al. 1980). Since then, more than 50 defects have been identified in protein N-glycosylation (some of them also associated with an O-glycosylation defect), in protein O-glycosylation only and in lipid glycosylation (GPI anchor and glycosphingolipid synthesis). Here, we provide a simple approach to the clinical, biochemical and genetic diagnosis of CDG due to a N-glycosylation defect (including combined N- and O-glycosylation defects).

When to consider a CDG due to a N-glycosylation defect

Most CDG with a N-glycosylation defect are multi-organ diseases with neurological involvement. Only few of these CDG are known as non-neurological disorders, such as MPI-CDG (de Lonlay and Seta 2009), DPM3-CDG (Lefeber et al. 2009) and SEC23B-CDG (Schwarz et al. 2009). Nearly all organs have been reported to be affected in CDG (Jaeken 2010). One should thus screen for CDG (i) in any unexplained neurological syndrome, particularly when associated with other organ disease, (ii) and also in any unexplained syndrome even without neurological involvement.

Experience gained in the last decade has shown that a number of clinical clues might alert for a CDG and that some of these clues might also provide a hint for a specific CDG subtype, either in the CDG-I group (Morava et al. 2008) or in the CDG-II group (Mohamed et al. 2011). These specific features include also the well known abnormality in fat distribution or cutis laxa. A brief overview of alerting clinical features is presented in Table 1 and currently known CDG subtypes are tabulated in Table 2. A laboratory finding to be added to this list is syndromic factor XI deficiency in CDG-I, and in an unknown number of CDG-II defects.

How to screen for CDG due to a N-glycosylation defect

Since its introduction in 1984, isoelectric focusing (IEF) of serum transferrin is still the method of choice for the diagnosis of N-glycosylation disorders associated with sialic acid deficiency (Jaeken et al. 1984). More recently, HPLC (Helander et al. 2001) and capillary zone electrophoresis (Carchon et al. 2004) have been introduced as well as mass spectrometry (Babovic-Vuksanovic and O'Brien 2007). In the absence of a protein polymorphism, the isoelectric point of transferrin is determined only by its glycosylation status. A protein polymorphism can be identified by incubation of the sample with commercial

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Table 1 Overview of suggestive clinical features

Clinical features suggestive for a distinct CDG type	Suspected gene defect	Suggested analysis after transferrin screening
Syndromic fat pads and/or inverted nipples	PMM2	PMM enzyme measurement
Chronic diarrhea	MPI	MPI enzyme measurement
	ALG6	LLO analysis in fibroblasts
	ALG8	"
Liver fibrosis	MPI	MPI enzyme measurement
Ichthyosis syndrome	MPDU1	LLO analysis in fibroblasts
	DOLK	Mutation analysis
	SRD5A3	Plasma polyprenols
Neurosyndromatic cataract and/or coloboma	SRD5A3	Plasma polyprenols
	ALG2	Mutation analysis
Neurosyndromatic sensorineural deafness	ALG11	LLO analysis in fibroblasts
	RFT1	Mutation analysis
Neurosyndromatic radio-ulnar synostosis	MGAT2	Serum glycan profiling
Neurosyndromatic recurrent infections with unusually high leukocytosis	SLC35C1	Bombay blood group
		Serum glycan profiling
Neurological syndrome with Bombay bloodgroup	SLC35C1	Serum glycan profiling
		Mutation analysis
Cerebrocostomandibular syndrome	COG1	Serum ApoC3 IEF
		Mutation analysis
Neurological syndromes with episodic hyperthermia	COG7	Serum ApoC3 IEF
		Mutation analysis
Cutis laxa syndrome	ATP6V0A2	Serum ApoC3 IEF
		Mutation analysis
Congenital dyserythropoietic anemia type II or HEMPAS	SEC23B	Mutation analysis
Syndromic cardiomyopathy	PMM2	PMM enzyme measurement
	ALG1	LLO analysis in fibroblasts
	DOLK	Mutation analysis
	DPM1	
	DPM3	

sialidase, by analysis of different proteins such as thyroxine-binding globulin (TBG) or by analysis of parental serum samples. In human plasma, the tetrasialotransferrin fraction is the most abundant form. Children younger than 1 month can show mildly elevated underglycosylated transferrin isoforms as compared to older children. Also, the diagnosis of CDG might be missed in very young children, in whom the profile becomes abnormal after 1–2 months of age. A few CDG patients have been described with a normal profile particularly in adolescent and adult age (Vermeer et al. 2007). Before proceeding with further diagnostics, it is important to rule out secondary causes of abnormal transferrin profiles, such as fructosemia, galactosemia, alcohol abuse and bacterial sialidase.

In general, two diagnostic types of abnormal profiles can be distinguished (Fig. 1a): (i) a type 1 pattern in CDG-I, characterized by an increase of di- and/or asialotransferrin; (ii) a type 2 pattern in CDG-II, characterized by an increase of tri-, di-, mono-and/or asialotransferrin.

The *type 1 pattern* (CDG-I) points to an assembly or transfer defect of the dolichol-linked glycan (in the cytosol or ER glycosylation pathway). Measurement of phosphomannomutase activity in fibroblasts or leukocytes is the next step for further diagnostics because PMM2-CDG (CDG-Ia) is by far the most frequent N-glycan assembly defect. In case of a purely hepato-intestinal clinical presentation, the activity of phosphomannose isomerase should be measured for diagnosis of MPI-CDG (CDG-Ib), which is a treatable disease. A normal activity of these enzymes necessitates analysis of the lipid-linked oligosaccharides (LLO; more specifically dolichol-linked oligosaccharides) in fibroblasts or other more specific assays to identify the known (or still unknown) CDG-I subtype. The recently identified SRD5A3-CDG could be diagnosed directly by analysis of plasma polyprenols (Morava et al. 2010).

Table 2 Overview of current CDG subtypes

CDG type#	*Gene*, Protein	TIEF	ApoCIII	Alternative assay
PMM2-CDG (Ia)	*PMM2*, Phosphomannomutase	Type I		
MPI-CDG (Ib)	*MPI*, Phosphomannose isomerase	Type I		
ALG6-CDG (Ic)	*ALG6*, Glucosyltransferase I	Type I		
ALG3-CDG (Id)	*ALG3*, Mannosyltransferase VI	Type I		
DPM1-CDG (Ie)	*DPM1*, Dol-P-Man synthase	Type I		
MPDU1-CDG (If)	*MPDU1*, Dol-P-Man utilization protein	Type I		
ALG12-CDG (Ig)	*ALG12*, Mannosyltransferase VIII	Type I		
ALG8-CDG (Ih)	*ALG8*, Glucosyltransferase II	Type I		
ALG2-CDG (Ii)	*ALG2*, Mannosyltransferase II	Type I		
DPAGT1-CDG (Ij)	*DPAGT1*, GlcNActransferase I	Type I		
ALG1-CDG (Ik)	*ALG1*, Mannosyltransferase I	Type I		
ALG9-CDG (IL)	*ALG9*, Mannosyltransferase VII	Type I		
DOLK-CDG (Im)	*DOLK*, Dolichol kinase	Type I		
RFT1-CDG (In)	*RFT1*, RFT1 protein	Type I		
DPM3-CDG (Io)	*DPM3*, Dol-P-Man synthase	Type I		
ALG11-CDG (Ip)	*ALG11*, Mannosyltransferase III	Type I		
SRD5A3-CDG (Iq)	*SRD5A3*, Steroid5αreductase3	Type I		
MGAT2-CDG (IIa)	*MGAT2*, GlcNActransferase II	Type II	normal	MS*
TUSC3-CDG	*TUSC3*, Oligosaccharyltransferase subunit	normal	normal	gene
MAGT1-CDG	*MAGT1*, Oligosaccharyltransferase subunit			
GCS1-CDG (IIb)	*MOGS*, Glucosidase I (GCS1)	normal	normal	Urine oligosaccharides
SLC35C1-CDG (IIc)	*SLC35C1*, GDP-fucose transporter	normal	normal	Bombay blood group, MS*
B4GALT1-CDG (IId)	*B4GALT1*, Galactosyltransferase	Type II	normal	MS*
SLC35A1-CDG (IIf)	*SLC35A1*, CMP-NeuAc transporter	normal	normal	CD15s on leukocytes
–	*COG1-8*, COG 1–8 subunits	Type II	abnormal	
–	*ATP6V0A2*, vesicular $H^{(+)}$-ATPase subunit a2	Type II	abnormal	

Official gene names were used (www.genenames.org) to indicate the new nomenclature. In parenthesis (e.g. Ia for CDG-Ia) the formerly used nomenclature.

*MS = serum N-glycan profiling

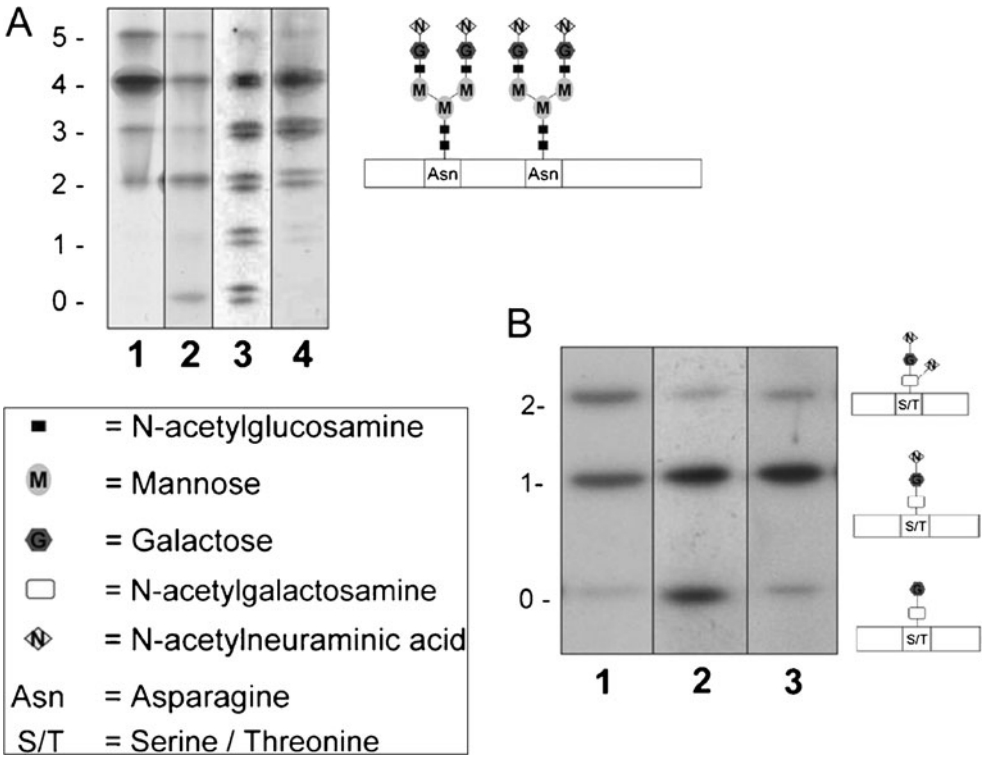

Fig. 1 Isofocusing techniques in the screening for CDG. **a**. Isofocusing of plasma transferrin for detection of N-glycosylation defects. Indicated is the most abundant glycan isoform of transferrin with two bi-antennary N-glycans, corresponding with tetrasialotransferrin. Lane 1: control, lane 2: CDG-I profile, lane 3: asialo type 2 profile, lane 4: disialo type 2 profile. **b**. Isofocusing of plasma apolipoprotein C3 for detection of mucin type O-glycosylation defects. Lane 1: control, lane 2: ApoC3-0 profile, lane 3: ApoC3-1 profile

The *type 2 pattern* (CDG-II) indicates a processing defect after glycan transfer in the ER or during Golgi glycosylation. Not all processing defects can be picked up by transferrin IEF. Patients with *GCS1*, *SLC35C1 (*fucose has no electric charge) and *SLC35A1* defects (formerly CDG-IIb, -IIc and CDG-IIf) have been described with a normal transferrin IEF profile. Type 2 patterns can show an asialo type 2 profile with increased asialo- to trisialotransferrin, or a disialo type 2 profile with increased disialo- and trisialotransferrin. The next step in the identification of the CDG-II subtypes is mass spectrometry of isolated serum N-glycans (Guillard et al. 2011). This will permit to identify isolated N-glycosylation defects as MGAT2-CDG (CDG-IIa) or B4GALT1-CDG (CDG-IId) and secondary causes, but most CDG-II types are associated with an aspecific glycan profile. In the latter situation, the possibility of an associated mucin type O-glycosylation defect should be investigated by isoelectrofocusing of serum apolipoprotein C-III (APOC3). This protein contains a single core 1 mucin type O-glycan on Thr-74. Abnormal profiles (Fig. 1b) include an increased monosialo APOC3 with decreased disialo APOC3 (a so-called APOC3-1 profile) or an increased asialo APOC3 (a so-called APOC3-0 profile) (Wopereis et al. 2005). Young children in the first months of life can present with an APOC3-2 profile in which an increase of disialo APOC3 is observed. In our experience, the latter pattern can also be observed in patients with severe liver pathology.

Currently, no genetic glycosylation disorders have been described with an isolated abnormality of the APOC3 profile. However, several defects are known with a combined N- and O-glycosylation defect. In general, these defects are characterized by abnormal functioning of the secretory pathway, such as abnormal retrograde trafficking or abnormal acidification. If the patient presents with clinical symptoms of cutis laxa, mutation analysis of the ATP6V0A2 gene is indicated. It should be noted that ATP6V0A2-CDG patients younger than 6 months of age can present with normal transferrin glycosylation, while apolipoprotein C-III isofocusing is abnormal. In all other cases, we recommend to look for a defect in one of the COG (conserved oligomeric Golgi complex) subunits by mutation analysis of the COG1-8 subunit genes.

The emerging innovative next-generation sequencing techniques like whole-exome-sequencing will provide new opportunities to unravel genetic causes for glycosylation disorders, such as the CDG-II trafficking defects, but also the many glycosylation defects that cannot be identified via transferrin screening.

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