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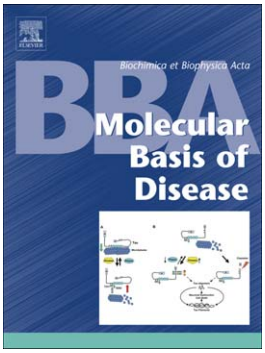

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PII: S0925-4439(11)00046-9
DOI: doi: 10.1016/j.bbadis.2011.02.011
Reference: BBADIS 63253

To appear in: *BBA - Molecular Basis of Disease*

Received date: 17 December 2010
Revised date: 18 February 2011
Accepted date: 22 February 2011

Please cite this article as: M. Mohamed, M. Guillard, S.B. Wortmann, S. Cirak, E. Marklova, H. Michelakakis, E. Korsch, M. Adamowicz, B. Koletzko, F.J. van Spronsen, K.E. Niezen-Koning, G. Matthijs, T. Gardeitchik, D. Kouwenberg, B. Chan Lim, R. Zeevaert, R.A. Wevers, D.J. Lefeber, E. Morava, Clinical and diagnostic approach in unsolved CDG patients with a type 2 transferrin pattern, *BBA - Molecular Basis of Disease* (2011), doi: 10.1016/j.bbadis.2011.02.011

Clinical and diagnostic approach in unsolved CDG patients with a type 2 transferrin pattern

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DISCLOSURE OF ALL AUTHORS

The study was not industry sponsored. The authors report no disclosures.

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Abstract: 224 words, Word count: 2803, Tables: 2, Figures: 3

Abstract

Objective: Dysmorphic features, multisystem disease and central nervous system involvement are common symptoms in congenital disorders of glycosylation, including several recently discovered Golgi-related glycosylation defects. In search for discriminative features, we assessed eleven children, suspected with a Golgi-related inborn error of glycosylation. **Methods:** We evaluated all genetically unsolved patients, diagnosed with a type 2 transferrin isofocusing pattern in the period of 1999-2009. By combining biochemical results with characteristic clinical symptoms we used a diagnostic flow chart to approach the underlying defect in patients with CDG-IIx. According to specific symptoms and laboratory results we initiated additional, targeted biochemical and genetic studies. **Results:** We found a distinctive spectrum of CDG type 2-associated anomalies including sudden hearing loss, brain malformations, wrinkled skin and epilepsy in combination with skeletal dysplasia, dilated cardiomyopathy, sudden cardiac arrest, abnormal copper and iron metabolism and endocrine abnormalities in our patients. One patient with severe cortical malformations and mild skin abnormalities was diagnosed with a known genetic syndrome, due to an *ATP6V0A2* defect. **Conclusion:** Here we present unique CDG type 2-associated anomalies, including both ATPase-related and unrelated cutis laxa, and sensorineural hearing loss; a recently recognized symptom of CDG. Based on our findings we recommend clinicians to consider CDG in patients with cardiac rhythm disorders, spondylodysplasia and biochemical abnormalities of the copper and iron metabolism even in absence of intellectual disability.

Key words:

CDG type 2, CDG-IIx, Golgi-system, hearing loss, seizures, TIEF, copper metabolism

Abbreviations

α_1-AT	alfa-1-antitrypsin
ALAT	Alanine aminotransferase
apo C-III	Apolipoprotein C-III
ASAT	Aspartate aminotransferase
AT-III	Anti-thrombin III
BEAP	Brain evoked acoustic potentials
CDG	Congenital disorders of glycosylation
CNS	Central nervous system
COG	Conserved oligomeric Golgi complex
ER	Endoplasmatic reticulum
LLO	Lipid-linked oligosaccharide
EEG	Electroencephalography
EMG	Electromyography
MALDI-MS	MALDI mass spectrometry
PMR	Psychomotor retardation
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TBG	Thyroxin binding globulin
TIEF	Transferrin isoelectric focusing
VEP	Visual evoked potentials

Database information

[#]EUROGLYCANET; Diagnosis and Research Management System; (www.euroglycanet.org/uz/Database)

Introduction

The congenital disorders of glycosylation (CDG) form a group of inborn errors of metabolism characterized by a defective biosynthesis of glycans, first described by *Jaeken et al* in 1980 [1]. A recent classification of CDGs distinguishes four major biochemical categories: three involving protein-glycosylation (disorders of N-linked glycosylation, O-linked glycosylation and combined N- and O-glycosylation) and one involving lipid-glycosylation [2]. Plasma transferrin isoelectric focusing (TIEF) is used as a simple and reliable biochemical screening tool for CDG associated with deficient sialylation [2] (Supplementary Figure 1). Compared to healthy individuals, in patients with N-glycosylation disorders hypoglycosylated isoforms are elevated and tetrasialotransferrin is decreased. The glycosylation route comprises the cytoplasm, the endoplasmatic reticulum and the Golgi apparatus. Depending on the localization of a defect two isofocusing patterns can be distinguished, the so called type 1 pattern demonstrates increases of even (2 and 0) sialotransferrin bands (previously CDG-I), and the type 2 pattern, showing additional uneven (3 and 1) sialotransferrin bands (previously CDG-II [2]). This classification, based on the TIEF pattern, relates to N-glycosylation diseases, found by the initial screening test in patients with a suspected CDG. According to preceding nomenclature however, not only patients with a CDG type 2 pattern were labeled as CDG-II defects, but a few additional CDG forms with normal TIEF as well, based on their underlying defect in Golgi or Golgi-associated proteins [3]. Unsolved patients with type 2 TIEF pattern were labeled as CDG-IIx (MIM 212067) [4].

In addition to parts of the N-glycan biosynthesis pathway, the Golgi apparatus houses the full mucin type O-glycan biosynthetic pathway. Plasma apolipoprotein C-III (apo C-III) isofocusing has been developed to evaluate O-glycan biosynthesis [3] (Supplementary Figure 1), a useful tool also in *combined* N- and O-glycan biosynthesis defects [3].

Some of the CDG patients show not only abnormal N-linked, but also abnormal O-

glycosylation. Other, Golgi-related glycosylation defects however, like muscle-eye-brain disease, Schneckenbecken dysplasia, or multiple cartilaginous exostoses, do not show abnormal TIEF. Due to the emerging number of combined, and isolated O-glycosylation defects a consensus for a novel nomenclature, based on genetic etiology, became essential [2].

In the past couple of years, several novel CDG defects have been described with a type 2 TIEF pattern, some of them historically carrying the name CDG-II (COG7-CDG/CDG IIe, COG1-CDG/CDG IIg, etc), while the most recently discovered types were labeled as COG5-CDG and COG6-CDG [2, 5-11]. Neurologic involvement is frequent in both CDG-I and CDG-II defects [2,3,5-11]. COG7-CDG, typically presents with profound development delay, microcephaly, swallowing difficulties, failure to thrive and distal arthrogryposis [8,9] (Table 1). Another interesting example with distinctive CNS symptoms is ATP6V0A2-CDG; causing autosomal recessive cutis laxa syndrome type II (ARCL-type-II, MIM 219200), a disorder of sagging skin, dysmorphic features, developmental delay and unique cortex malformations [5,6], and a combined N- and O-glycosylation defect [12-14].

Recently the number of defects associated with a CDG type 2 pattern has been more than doubled [15,16]. Apart from central nervous system, hematologic and liver involvement in both types, the phenotype of the newly discovered defects is quite different from the well known clinical pattern in CDG-I (Table 1). Classic CDG-I features, like inverted nipples, hypothyroidism or strabismus are less common in patients with a type 2 TIEF pattern.

In the European database EUROGLYCANET[#] for glycosylation disorders approximately 80% of patients show a type 1 pattern. With advancing biochemical techniques, the primary molecular genetic defect has been elucidated in the majority of CDG-I cases. In contrast, no comprehensive analytical or biochemical techniques are available to identify and delineate the primary defects in patients with a TIEF type 2 pattern and suspected Golgi-related glycosylation defects. Thereby, the vast majority of these cases (80% of CDG-II patients

based on EUROGLYCANET[#], 2010) is still classified as unsolved; CDG-IIx (MIM 212067).

Patients and methods

All patients were evaluated according to standard diagnostic procedures for a suspected disorder of protein glycosylation.

Patient 1 was not fully evaluated due to an early lethal course.

Clinical patient' characteristics (Table 2, for extended description see Supplementary files)

Patients 1 and 2 were brothers. They presented with recurrent infections, anemia, hepatosplenomegaly, severe thrombocytopenia, disturbed coagulation and liver function tests and increased activity of lysosomal enzymes in plasma. Patient 1 had severe hypotonia, strabismus and developmental delay, the letter also present in patient 1. The female patient 3 showed generalized hypotonia, macrocephaly with large fontanel, psychomotor retardation, joint-hyperlaxity, intermittently elevated serum transaminases and leucopenia, without a history of infections. The male patient 4 showed normal development until the age of 13 years when he experienced a sudden cardiac arrest. He developed epilepsy and became physically and mentally disabled. Hemostasis and liver function were abnormal. Albuminuria and hypoglycemia were noted (Table 2). The male patient 5 showed multiple dysmorphic features and congenital genital abnormalities. He had hypothyroidism, abnormal coagulation, liver dysfunction, decreased serum ceruloplasmin and copper levels and elevated creatine kinase (CK). Subsequently he developed myopia and retinitis pigmentosa. Prepubertally his bilateral hearing loss required hearing aids. Right-sided sudden deafness occurred at the age of 13 years. Hearing was restored by cochlear implant and he attends a regular school. The patients 6 and 7 (sister and brother) showed cholestasis after birth. Patient 6 had elevated CK, alanine aminotransaminase and aspartate aminotransaminase (ASAT and ALAT) levels and disturbed clotting parameters. She was diagnosed at the age of 10 years with chronic liver disease, also

showing mild dysmorphic features, relative microcephaly (Figure 2), generalized muscular hypotonia, and a severe sleeping and behavioral disorder.

Her brother (patient 7) is additionally known with skeletal dysplasia, ptosis (Figure 2) and a more severe psychomotor retardation. The male patient 8 has previously been reported [17]. In the following years wrinkled skin on his abdomen and neck became more apparent (Figure 2). He developed a behavioral disorder with aggression and self mutilation. Subsequently he showed severe myopia, sensorineural hearing loss, severe mental retardation and an expressive language disorder. The female patient 9 had a Pierre-Robin sequence (Figure 2), generalized muscular hypotonia with elevated CK, dilated cardiomyopathy and delayed motor development. The hypotonia improved significantly over the years. At 15 years she has short stature, hypogonadotrophic hypogonadism and a high normal intelligence. The male patient 10 had dysmorphic features, short stature and kyphoscoliosis. Vision was impaired due to glaucoma. His motor development was delayed. Severe anemia required transfusion. Currently he is 17 years old and attends regular school. Patient 11 had wrinkled skin of the abdominal region (Figure 1d) and other dysmorphic features. He had a motor and speech developmental delay, and showed complex partial seizures. At the age of 14 years he was mentally retarded.

Additional clinical evaluation

Hand X-rays were performed in three patients to determine bone age, in accordance with the Greulich & Pyle atlas. All investigations including brain imaging (MRI and ultrasound), visual evoked potentials (VEP), electromyography (EMG) and electroencephalography (EEG), liver biopsies and skeletal imaging were performed upon clinical indication.

Laboratory investigations

Standard laboratory investigations included full blood count, transaminases (ALAT, ASAT), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GT), infection parameters, hemoglobin, creatine kinase, lactate dehydrogenase (LDH) and urine reduction.

Investigations performed upon indication were serum bilirubin, copper, ceruloplasmin, iron, cholesterol, triglycerides, coagulation factors, coagulation tests, ceruloplasmin, hormones, amino acids and activity of lysosomal enzymes in plasma. Upon indication organic acids and oligosaccharides were evaluated in urine. Also upon indication hepatitis A, B and C antibodies were evaluated.

Diagnostic approach to CDG (Supplementary methods)

All patients were included with a TIEF showing a type 2 pattern. Secondary causes of hypoglycosylation were evaluated using clinical and dietary anamnesis, urine analysis, and if indicated by blood culture, genetic studies and plasma protein mass spectrometry. Lipid-linked oligosaccharides (LLO) analysis used in three inconclusive initial TIEF profile cases confirmed CDG type 1. To evaluate Golgi-related mucin-type O-glycosylation defects, isoelectric focusing of apoC-III was performed as described by Wopereis et al [3] MALDI mass spectrometry of plasma N-glycans was performed to exclude MGAT2-CDG, SLC35C1-CDG and B4GALT1-CDG [18], tetrasaccharides in urine were measured to screen for GLS1-CDG [19] and neutrophil surface sialyl-Lewis-X ligand for SLC35C1-CDG and SLC35A1-CDG [20]. Direct sequencing was used to evaluate COG defects (COG1-CDG - COG8-CDG) and ATP6V0A2-CDG.

Results

Table 2 provides an overview of clinical, biochemical and metabolic findings.

Additional clinical investigations

BEAP showed severe bilateral sensorineural hearing loss in patient 8 (55/65 dB). In patient 5

the audiogram showed sensorineural deafness of the right ear and a hypoacusis (70 dB) of the left ear. In three patients with muscle weakness EMG showed normal results. An EEG was performed in four cases, one was normal, two showed encephalopathy with generalized epileptic activity (patient 4 and 8) and one with bilateral frontotemporal focal activity, (secondary generalized) in patient 11. VEP was severely abnormal in patient 5 and showed a normal result in patient 10.

MRI was performed in ten patients. Head ultrasound was performed in patient 1.

In five patients no abnormalities where detected (patients 1, 3, 7, 9 and 10). Patient 2 and 8 showed partial agenesis of the corpus callosum and patient 6 decreased myelinisation around the occipital horn of the ventricle. In patient 4 widened ventricles and atrophy on the left fronto-temporal cerebral quadrant were seen. Patient 5 showed brain atrophy and small areas of increased paraventricular occipital signal intensity. Patient 11 had bilateral frontal cobblestone like cortical dysgenesis (Figure 1c).

Histology

In patients 1 and 2 a liver biopsy showed chronic hepatitis, micro nodular changes and necrosis. (In this phase the hepatitis serology did not support active infection).

Biochemical investigations

Liver function was abnormal in nine patients (Table 2). Patients 5 (increased TSH and decreased free T4) and 9 (decreased LH, FSH and oestradiol) had endocrine abnormalities. Full blood count abnormalities were found in patients 1, 2, 3 and 10 (thrombopenia, leucopenia and anemia). Serum copper was diminished and iron level fluctuated in patient 5. Coagulation was disturbed in eight patients (decreased clotting factors, abnormal coagulation tests or thrombotic events). Ceruloplasmin was decreased in patients 5, 6 and 7. Activity of lysosomal enzymes in serum were measured in four patients, of which two patients showed elevated levels (patient 1 and 2) and two showed normal levels (patient 3 and 6).

Organic acids and oligosaccharides in urine were measured in five patients: results were normal in four patients (3, 5, 8 and 9) but abnormal in patient 2 with elevated galactose levels. Sialyl-Lewis X expression in patient 8 was normal, ruling out the probability of SLC35A1-CDG .

Glycosylation analysis

Abnormal TIEF profiles led to exclusion of GLS1-CDG and SLC35C1-CDG, since these subtypes give normal TIEF profiles. The diasialo TIEF pattern was seen in eight patients and two patients showed an asialo pattern. Of the eight patients with a disialo pattern, one was not evaluated due to early death, and two had an apoC-III_0 profile and six an apoC-III_1 profile, which indicates a combined defect in N- and O-glycosylation [16]. Of the two patients with the asialo- TIEF pattern, one showed a normal apo C-III-profile and the second showed an apoC-III_0 profile (Supplementary Figure 1). Lipid-linked oligosaccharides (LLO) analysis was normal. Mass spectrometry analysis of N-glycans revealed a Golgi-related defect in 8 of the 9 analyzed patients (increase in truncated glycans associated with decreased triantennary structures), making MGAT2-CDG, SLC35C1-CDG and B4GALT1-CDG unlikely. Western Blot analysis for COG subunits was normal.

Genetic analysis

High resolution karyotype and/or array CGH was normal. The results of sequencing for the eight COG subunit genes were normal. In patient 5 genetic investigations ruled out Pendred syndrome (suspected based on hearing loss in combination with hypothyreoidism). Genome wide linkage analysis (homozygosity mapping) was performed in patients 6 and 7. This hasn't resulted in a candidate gene due to abundance of large homozygous regions. We also ruled out all genes known to be involved in the glycosylation pathway in the homozygotic regions. *ATP6V0A2* mutation analysis was noncontributory in patient 8, and showed two novel mutations in a compound heterozygous form (c1101delC/c1562_1563delins9) in patient 11 .

Discussion

The surprising discovery of the *ATP6V0A2* defect in autosomal recessive cutis laxa type II presenting as a glycosylation defect led to a rapid increase in the number of patients diagnosed with a type 2 TIEF pattern. This taught us that once a distinct phenotype is associated with CDG, systematic screening leads to large additional numbers of patients. To date eight patients with CDG-IIx have been published as case-reports [21-25], (one of these patients was diagnosed since the original report with COG4-CDG) [22]. This is in great contrast to the rapidly expanding CDG-IIx group. These genetically unsolved patients have been reported with a mild psychomotor retardation, infections (haemolytic uraemic syndrome), failure to thrive and facial dysmorphy [21], chronic diarrhea, progressive liver cirrhosis and recurrent infections [22], a lethal case with psychomotor retardation and bleeding tendency [23], an adult case with unexplained chronic hypertransaminasaemia with decreased clotting factors [24], and a cohort of four patients with hypertransaminasaemia, coagulopathy and steatosis [25].

The combination of liver and CNS involvement is common in CDG. In our own cohort 9 out of 11 patients showed liver involvement. Liver problems varied from slightly elevated transaminases without clinical consequence to hepatic coma leading to death. Central nervous symptoms are characteristic features of CDG. Interestingly two children in our study group showed no mental retardation. Of the two children with clinical epilepsy one presented with a seizures only after cardiac arrest. MRI changes were diagnostic in patient 11 showing cobble-stone like brain dysgenesis (frontal polymicrogyria, Figure 1). Eye involvement is well known in CDG-I, but unusual in CDG-II. We found ophthalmologic abnormalities including retinitis pigmentosa (patient 5), glaucoma (patient 10) and severe myopia (patient 8). A unique neurological presentation was observed in the most complex patient (patient 5). He suffered

from hypoacusis which suddenly developed to acute unilateral deafness. Sensorineural hearing loss has been recently associated with CDG-I in RFT1-CDG [26]. In CDG-II, sudden deafness had never been described before. Patient 8 and patient 11 had abdominal wrinkled skin/cutis laxa and CNS malformations. Compared to the *ATP6V0A2*-related phenotype, confirmed in patient 11, but not in patient 8, the latter developed progressive skin wrinkling through the course of the disease, in association with sever hypermetropia, inguinal hernias, joint deformations and hyperthrichosis.

Cardiomyopathy and rhythm disorders have not previously been associated with CDG-II. Patient 4 suffered a sudden cardiac arrest, most likely due to the combination of an underlying rhythm disorder (ventricular extrasystoles). Patient 9 showed a dilated cardiomyopathy with left sided heart failure and Wolff-Parkinson-White syndrome. Patient 10 had several skeletal and joint abnormalities (hypoplasia fifth ray hands, pectus excavatum, kyphoscoliosis, abnormal ossification/ lysis of cervical vertebrae), in combination with hernias. The features were not comparable with a known skeletal dysplasia [29]. Another three patients had delayed bone maturation, platyspondyly and/or decreased bone density, demonstrating common skeletal involvement in CDG-IIx.

Patient 11 was diagnosed with a so far undescribed mutation in *ATP6V0A2* based on phenotyping combined with apoC-III results, demonstrating the success of our approach. Interestingly, the other patient with cutis laxa and CDG-II did not carry a mutation in *ATP6V0A2*.

Endocrine abnormalities are common in CDG-I, especially with decreased TBG and total T4 levels. Patient 5 had hypothyroidism with goiter, not previously reported in CDG-II. Patient 9 interestingly showed a hypogonadotrophic hypogonadism in combination with growth delay and decreased IgF1 levels [34]. Coagulopathy defined as decreased clotting factors, thrombopenia and prolonged bleeding time was present in seven patients. Some of these

patients had bleedings after minimal invasive medical procedures, even with only slightly decreased clotting factors. CDG thus should be considered in unexplained bleeding disorders. The diminished serum copper and ceruloplasmin levels in two patients are unexplained, and might be a consequence of abnormal Golgi transport.

Increased activities of lysosomal enzymes in plasma are present in most CDG-I but not in CDG-II [27], except for COG7-CDG [28]. Both patients died shortly after birth and showed outspoken dysmorphic features and skeletal anomalies, in contrast with our patients 1 and 2 who also showed increased activity of lysosomal enzymes. The exact mechanism leading to increased activity of lysosomal enzymes remains unclear.

Mass spectrometry has a growing significance in CDG diagnostics, especially in MGAT2-CDG, SLC35C1-CDG and B4GALT1-CDG. Direct genetic approaches, like direct sequencing for known genes and homozygosity mapping, in combination with clinical/biochemical grouping, have been proven effective in elucidating underlying genetic defects (ATP6V0A2-CDG, DPM3-CDG and SRD5A3-CDG) [14,30,31]. Clinical phenotyping should be included early on to decide on the diagnostic approach in CDG patients with a type 2 pattern (Figure 3). Pathognomic features, such as cobble-stone like cortical dysgenesis with skin wrinkling [13,32,33], or severe microcephaly with adducted thumbs [9] are key to the diagnosis. The combination of neurological symptoms and liver dysfunction is highly suggestive for congenital disorders of glycosylation, but normal psychomotor development does not exclude CDG-II.

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Legends to Figures

Figure 1: T1 weighed sagittal MRI images demonstrating frontal cobblestone like cortical dysgenesis (a) and no significant cerebellar hypoplasia in ATP6V0A2-CDG (b). T2 weighed horizontal image showing bilateral frontal polymicrogyria, enlarged Virchow-Robin spaces (arrow) by symmetrical cobblestone like cortical dysgenesis (c) and wrinkled abdominal skin (d) in patient 11.

Figure 2: Fig 2a,b, e: Patient 7 presented with microcephaly, bilateral ptosis, blue sclerae, downslanting palpebral fissures, scapula alatae and platyspondyly.. **Fig 2c, d:** His sister, patient 6 showed mild dysmorphic features like a flat face, hypertelorism and relative microcephaly. **Fig 2f, g:** Patient 9 presented with a Pierre-Robin sequence, a flat face, hypertelorism, hypogonadotropic hypogonadism and cardiomyopathy. **Fig 2h-l:** A syndrome like presentation in patient 8 with marked hypotonia (h), cutis laxa (i), pes adductus with hypoplastic small toe (j), clinodactyly of the fifth finger, low insertion of the thumb, camptodactyly (k), and extended hypertrichosis of the back (l)

Fig. 3: Diagnostic flowchart for patients diagnosed with a CDG type 2 pattern. Once a type 2 TIEF profile has been confirmed, and secondary glycosylation defects are ruled out, the pattern is matched to known type 2 profiles. If these do not match, isoelectric focusing of apoC-III protein is performed for Golgi related secretory O-mucin type defects. Mass spectrometry (MS) of plasma glycans is used to exclude MGAT2-CDG or B4GALT1-CDG, because these two types have distinct MS profiles. Western Blot analysis and sequencing of COG genes can lead to diagnosing a COG defect. The use of homozygosity mapping, especially in consanguineous cases and whole genome analysis can be implemented simultaneously.

Table 1: Genetic, clinical, and laboratory features in the historically defined CDG-II group

Genetic defect	Protein	Clinical features	Specific laboratory features
MGAT2-CDG (CDG-IIa)	N-acetylglucosaminyl-transferase II	CNS, L, S, H, C	-
GLS1-CDG (CDG-IIb)	Glucosidase I	CNS, L, S, R, GI	Abnormal levels of a tetrasaccharide in urine
SLC35C1/FUCT1-CDG (CDG-IIc)	Solute carrier family 35, member C1/GDP-fucose transporter 1	CNS, SS, B, I	-
B4GALT1-CDG (CDG-IId)	β-1,4-galactosyltransferase	CNS, L, C, M	Lack of SLeX expression in neutrophils
SLC35A1-CDG (CDG-IIf)	CMP-sialic acid transporter	I, C	Lack of SLeX expression in neutrophils
COG1-CDG (CDG-IIg)	COG 1	CNS, L	-
COG4-CDG	COG 4	CNS, L, R, I	-
COG5-CDG	COG 5	CNS, L	-
COG6-CDG	COG 6	CNS, L	Vitamine K deficiency
COG7-CDG (CDG-IIe)	COG 7	CNS, L, S, H, C, M I, GI, cutis laxa, hyperthermia	-
COG8-CDG (CDG-IIh)	COG 8	CNS, L, S, C, M	-
ATP6V0A2-CDG	ATP6V0A2	CNS,S, cutis laxa	-

Table 1: *According to the new nomenclature, the name of the gene followed by the CDG suffix is used. B= hematological, C=coagulopathy, CNS= Central nervous system, COG= conserved oligomeric Golgi complex subunit, GI=gastro-intestinal, H= heart, I=immunological, L=liver, M=muscle, R=respiratory, S=skeletal abnormalities, SLeX= Sialyl-Lewis X ligand.*

Table 2: Clinical features

	Patient 1 ♂	**Patient 2** ♂	**Patient 3** ♀	**Patient 4** ♂	**Patient 5** ♂
Age of onset	3 m (deceased 15m)	5 m	3 m	13 y	neonatal
Ethnicity/consanguinity	Turkish/+	Turkish/+	Greek/suspected	German/-	Polish/-
Dysmorphic features/ congenital anomalies	-	-	macrocephaly, large fontanel	-	upslanting palpebral fissures, epicanthal folds, thin nasal bridge, bilateral inguinal hernias, micropenis, testes migrans,
Skeletal/limb anomalies	-	-	hyperlaxity of the joints	-	clinodactyly
PMR/hypotonia	+/-	-/+	+/+	+/-	-/-
Hepatic involvement	HSM, liver cirrhosis, ↑TA, ↑AP, ↑bili	HSM, liver cirrhosis, ascites, ↑TA, ↑AP, ↑bili	↑TA, ↑AP	↑TA	HM, pancreatic steatosis, ↑neonatal icterus , ↑TA
Hematological involvement, coagulopathy	anemia/thrombopenia, ↑TT, ↓fibrinogen	thrombopenia, ↑APTT,↑TP, ↑TT. ↑INR	leucopenia	recurrent GI-bleedings/thrombosis. ↑APTT, ↓FII, ↓FV, ↓FVII, ↓FIX, ↓FXI, ↓protein C/S, ↓AT-III	↓AT-III, ↓protein C/S
Neurological /psychiatric involvement	-/-	-/-	-/ADHD	Focal motor seizures/-	bilateral sensorineural deafness, sudden unilateral deafness/-
Cerebral MRI	NA	agenesis CC	normal	slight ventriculomegaly/left temporal atrophy	widening of Virchow Robin spaces (occipital, parietal, frontal), hyperintens signal (T2) occipital horns of lateral ventricles
Cardiac involvement	-	-	-	sudden cardiac arrest, VES, normal echocardiography	-
Opthalmological involvement	-	divergent strabismus	-	-	hypermetropia, periferal retinal pigmentation
Recurrent infections	*S. typhii* GE, chronic hepatitis C infection	chronic hepatitis C infection	-	-	-
Other laboratory abnormalities	↑ lysosomal enzymes	↑ lysosomal enzymes		hypoglycemia,↓α1-AT, albuminuria	↑↓serum copper and ceruloplasmin ↑ CK, ↓T3, ↓FT4

	Patient 6 ♀/ Patient 7 ♂		**Patient 8 ♂**	**Patient 9 ♀**
Age of onset	neonatal		neonatal	neonatal
Ethnicity/consanguinity	Turkish/+		Czech/-	German/-
Dysmorphic features/ congenital anomalies	mild	microcephaly, ptosis, blue sclerae, downslanting palpebral fissures	microcephaly, dolichocephaly, gothic palate, blepharophimosis, cutis laxa, hypertrichosis, arachnodactyly, duodenal atresia and intestinal malrotation, bilateral inguinal hernias	Pierre-Robin sequence, hypotelorism, crowded teeth, short stature
Skeletal/limb anomalies	delayed bone maturation		talipes equinovarus adductus, brachymetatarsia, congenital hip dysplasia, subluxation thumbs/ hyperlaxity joints	delayed bone maturation
PMR/hypotonia	+/+		+/+	-/+
Hepatic involvement	cholestasis, ↓ceruloplasmin, ↑LDH/↑TA		↑TA, ↓LDH	↑TA
Hematological involvement, coagulopathy	↓protein S, ↓AT-III, ↓fibrinogen, ↓FIX		↑APTT, ↑PT, anemia	↓AT-III
Neurological /psychiatric involvement	physical contact aversion		hyperkinesia, dyslalia/aggression, self-mutilation	-/-
	sleep disorder	ataxia		
Cerebral MRI	↓ occipital myelinisation	normal	hypogenesis posterior CC	NA
Cardiac involvement	-		-	DCM, MI, TI (SF 22%)
Opthalmological involvement	-		↓vision, myopia, nystagmus	-
Recurrent infections	-		upper respiratory tract infections	-
Other laboratory abnormalities	↑CK		hypoglycemias, ↑CK, ↓LH, ↓FSH, ↓IGF-1, ↓TBG, ↓oestradiol	-

Table 2: *Abbreviations and symbols: α1-AT= alpha1-antitrypsine, ADHD= attention deficit hyperactivity disorder, ALP= alkaline phosphatase, APTT= activated partial thromboplastin time, AT-III= antithrombin-III, ASD= atrial septum defect, Bili= bilirubine, CC= corpus callosum, CK= creatinine kinase, DCM= dilated cardiomyopathy, F= factor, FSH= follicle stimulating hormone, FTT= failure to thrive, Fe= iron, GE=gastroenteritis, GI= gastro-intestinal, HM= hepatomegaly, IGF-1= insulin like growth factor 1, INR= International Normalized*

Ratio, HSM= hepato-splenomegaly, LDH= lactate dehydrogenase , L= left, LH= luteinizing hormone , MI= mitral insufficiency, PMR= psychomotor retardation, R= right, SF= shortenings fraction, TA= transaminases, T3= triiodothyronine, T4= thyroxine, TBG= thyroxine binding globuline, TI= tricuspid insufficiency, TT= thrombin time, VES=ventricular extrasystole

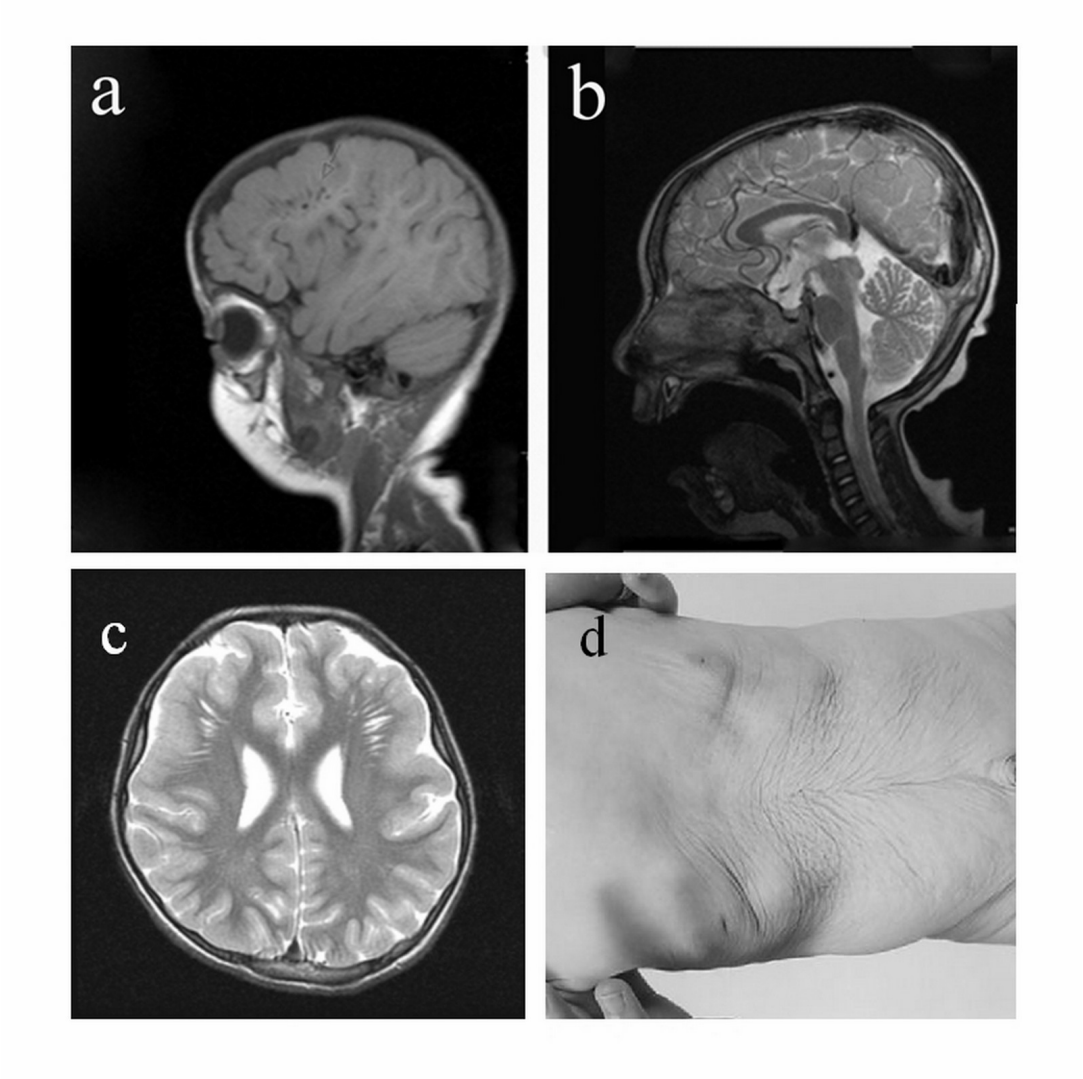

Fig. 1

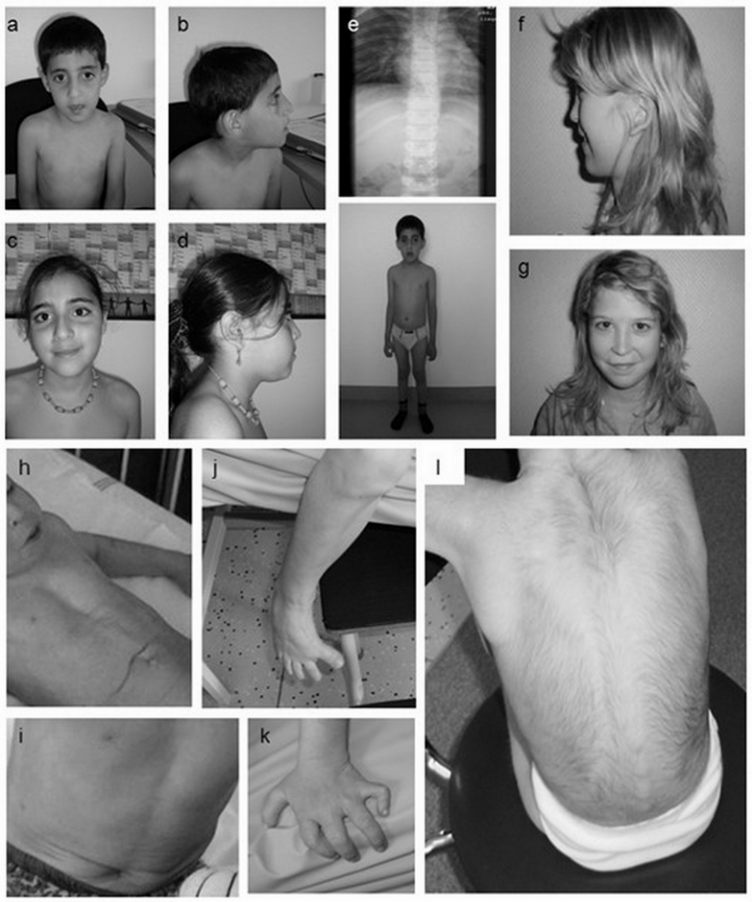

Fig. 2

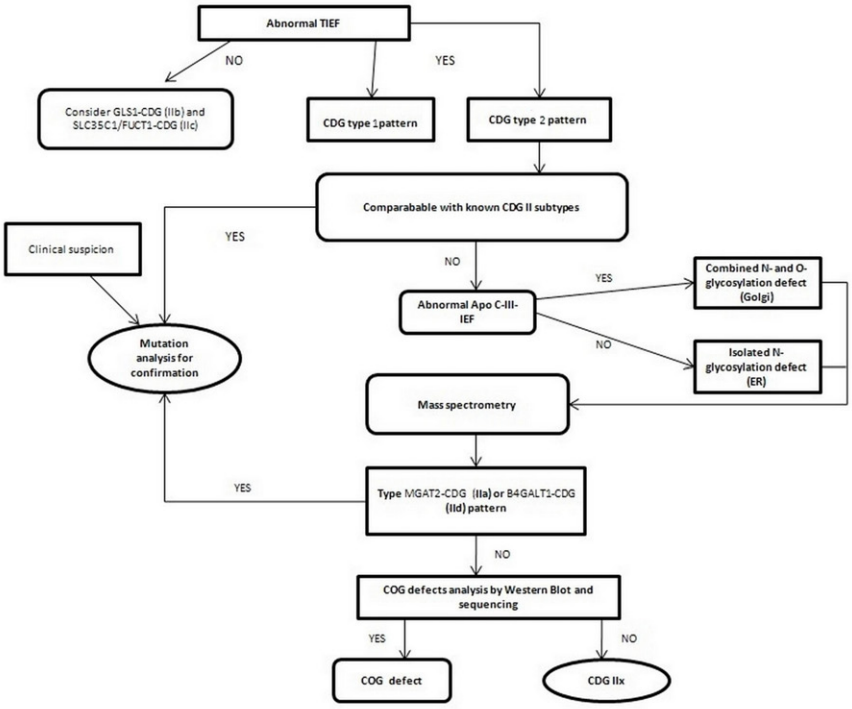

Fig. 3

Highlights

The genetic etiology in most CDG patients with type 2 TIEF pattern remains unknown>We systematically evaluate 11 unsolved patients and define discriminative clinical symptoms> We use an extended biochemical diagnostic "tool-kit" in CDG-IIx >We report on unique laboratory findings in lysosomal function, copper and iron metabolism> Deafness and non ATPase-related cutis laxa are also observed in CDG-IIx>