Molecular Genetics and Metabolism 120 (2017) 235–242

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Regular Article

Phenotypic and genotypic spectrum of congenital disorders of glycosylation type I and type II

Amal Al Teneiji [a], Theodora U.J. Bruun [a,b,c], Sarah Sidky [a], Dawn Cordeiro [a], Ronald D Cohn [a,b], Roberto Mendoza-Londono [a,b], Mahendranath Moharir [d], Julian Raiman [e], Komudi Siriwardena [f], Lianna Kyriakopoulou [g], Saadet Mercimek-Mahmutoglu [a,b,h,*]

[a] Division of Clinical and Metabolic Genetics, Department of Paediatrics, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada
[b] Genetics and Genome Biology Program, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada
[c] Department of Biochemistry, University of Oxford, Oxford, United Kingdom
[d] Division of Neurology, Department of Paediatrics, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada
[e] Birmingham's Children Hospital, Birmingham, England
[f] Department of Medical Genetics, University of Alberta, Edmonton, Canada
[g] Division of Genome Diagnostics, Department of Paediatric Laboratory Medicine, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada
[h] Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 28 December 2016
Accepted 29 December 2016
Available online 3 January 2017

Keywords:
Congenital disorders of glycosylation
N-glycosylation
Combined N- and O-glycosylation
Transferrin isoelectric focusing

ABSTRACT

Background: Congenital disorders of glycosylation (CDG) are inborn defects of glycan metabolism. They are multisystem disorders. Analysis of transferrin isoforms is applied as a screening test for CDG type I (CDG-I) and type II (CDG-II). We performed a retrospective cohort study to determine spectrum of phenotype and genotype and prevalence of the different subtypes of CDG-I and CDG-II.

Material and methods: All patients with CDG-I and CDG-II evaluated in our institution's Metabolic Genetics Clinics were included. Electronic and paper patient charts were reviewed. We set-up a high performance liquid chromatography transferrin isoelectric focusing (TIEF) method to measure transferrin isoforms in our Institution. We reviewed the literature for the rare CDG-I and CDG-II subtypes seen in our Institution.

Results: Fifteen patients were included: 9 with *PMM2*-CDG and 6 with non-*PMM2*-CDG (one *ALG3*-CDG, one *ALG9*-CDG, two *ALG11*-CDG, one *MPDU1*-CDG and one *ATP6V0A2*-CDG). All patients with *PMM2*-CDG and 5 patients with non-*PMM2*-CDG showed abnormal TIEF suggestive of CDG-I or CDG-II pattern. In all patients, molecular diagnosis was confirmed either by single gene testing, targeted next generation sequencing for CDG genes, or by whole exome sequencing.

Conclusion: We report 15 new patients with CDG-I and CDG-II. Whole exome sequencing will likely identify more patients with normal TIEF and expand the phenotypic spectrum of CDG-I and CDG-II.

1. Introduction

Congenital disorders of glycosylation (CDG) are a heterogeneous group of inherited metabolic disorders characterized by defective N- and O-glycosylation of proteins and lipids [15,16,37]. They are caused by inborn defects of glycan metabolism resulting in hypoglycosylation of proteins and lipids. N-glycosylation attaches N-glycans to the amino group of asparagine of proteins and occurs in the endoplasmic reticulum and Golgi. The steps necessary for the glycosylation involve assembly and processing components. N-glycosylation defects involving the assembly of protein glycosylation in the cytoplasm and endoplasmic reticulum are called CDG type I defects (CDG-I). Processing defects of protein glycosylation in the endoplasmic reticulum and Golgi are called CDG type II defects (CDG-II) [15,34]. O-glycosylation attaches O-glycans to the hydroxyl group of threonine or serine of proteins and their defects are only assembly defects. Combined N- and O-glycosylation defects are usually classified in the group of CDG-II [15,34,42,45].

The first CDG-I defect, *PMM2*-CDG, caused by phosphomannomutase 2 deficiency (EC 5.4.2.8) (OMIM#212065), was reported by Jaeken in 1984 [16]. So far about 50 different protein N-glycosylation defects have been identified [34]. Patients with CDG-I N-glycosylation defects present with multisystem involvement including neurological, hematological, gastrointestinal, renal, cardiovascular, ophthalmological and skeletal systems and skin and connective tissue. The phenotype ranges from prenatal onset hydrops foetalis with intrauterine growth retardation or dilated

* Corresponding author at: Division of Clinical and Metabolic Genetics, Department of Pediatrics, University of Toronto, Genetics and Genome Biology, Research Institute, The Hospital for Sick Children, 555 University Avenue, M5G 1X8 Toronto, Ontario, Canada.
E-mail address: saadet.mahmutoglu@sickkids.ca (S. Mercimek-Mahmutoglu).

http://dx.doi.org/10.1016/j.ymgme.2016.12.014
1096-7192/

cardiomyopathy, to mild global developmental delay, cognitive dysfunction and ataxia [15,35,36]. Patients with combined N- and O-glycosylation defects have additional clinical features such as episodic hyperthermia in *COG7*-CDG and cutis laxa syndrome in *ATP6V0A2*-CDG [19].

Analysis of transferrin isoelectric focusing (TIEF) using capillary zone electrophoresis [6], high performance liquid chromatography (HPLC) [13] or mass spectrometry [2] is applied as a screening test for N-glycosylation CDG-I and CDG-II defects. The normal transferrin isoform pattern shows tetrasialotransferrin. In CDG-I, there is an increase in asilo- and disialotransferrin and a decrease in tetrasialotransferrin, whereas in CDG-II there is an increase in trisialo- and monosialotransferrin while tetrasialotransferrin can be normal or decreased [35,36]. Normal transferrin isoform pattern has been reported in various CDG subtypes [47]. The diagnosis for specific subtypes is confirmed by targeted single gene testing or targeted next generation sequencing for CDG genes, or whole exome sequencing. The majority of CDG-I and CDG–II subtypes are treated symptomatically [32].

To evaluate the outcome of patients with CDG-I and CDG-II, we performed a retrospective cohort study. We determined phenotypic spectrum, the genotype and prevalence of the different subtypes of CDG-I and CDG-II. In 2012, our biochemical genetics laboratory developed an HPLC TIEF method for the investigations of CDG, which has been used as a clinical screening test since January 2013. We also briefly describe our method in this study. Additionally, we summarized patients with rare CDG-I and CDG-II subtypes, seen in our institution, as a literature review.

2. Materials and methods

2.1. Patients

Institutional Research Ethics Board approved this retrospective cohort study (Approval# 1000050441). All patients with CDG-I and CDG-II seen in the Metabolic Genetics Clinics were included. Electronic and paper patient charts were reviewed for clinical features, biochemical investigations, molecular genetic testing, brain magnetic resonance imaging (MRI) and outcome by two independent research team members. All information was entered into an Excel database. Molecular genetic testing using direct sequencing of single genes, targeted next generation sequencing for CDG genes or whole exome sequencing was applied according to clinically certified molecular genetic laboratories' methods. We used the recommendations for mutation nomenclature (www.hgvs.org/mutnomen) to name gene variations.

Pubmed database was searched using rare non-*PMM2*-CDG diagnosed and followed in our Institution as the key words. Additionally, references of all published articles regarding those genes were reviewed for case reports.

2.2. Measurement of TIEF using HPLC

The serum samples used for technical evaluation of the HPLC method were obtained. Anonymized left over serum samples were used. Control sera from 21 healthy children for establishing reference intervals were obtained through the CALIPER project [7]. The method is a modification of the method of Helander et al. [13].

Briefly for materials, dextran sulfate sodium salt and CNBr-activated Sepharose 6B were obtained from Amersham Biosciences. Nitrilotriacetic acid trisodium salt monohydrate (NTA) was obtained from Fluka. Bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane (Bis-Tris), calcium chloride dihydrate ($CaCl_2$ 2 H_2O), and iron(III) chloride hexahydrate ($FeCl_3$ 6 H_2O) were obtained from Sigma-Aldrich. Ferric nitrilotriacetic acid (FeNTA) solution (10 mmol/L) was used for iron saturation of serum transferrin. The pH was adjusted to 7.0 with 1.0 mol/L NaOH. Water was added to give a final volume of 100 mL. The FeNTA solution was stored at 4 °C (stable for >1 year). Dextran sulfate (10 g/L) in 0.5 M $CaCl_2$ was used for lipid precipitation.

Briefly for sample preparation, patient and control serum (100 μL) was mixed with 20 μL FeNTA solution and incubated at room temperature for 5 s. Lipids were precipitated by addition of 20 μL of the dextran sulfate–$CaCl_2$ solution and incubated at 2–8 °C for 30 min followed by centrifugation at 18000× rcf at room temperature for 5 min. 100 μL of the supernatant was transferred to HPLC grade vials and 200 μL of deionised water was added.

Briefly for HPLC analysis, HPLC separation was performed on an Agilent Technologies 1200 series system using gradient chromatography on a GE HealthCare, Source15Q 4.6/100 column. The HPLC flow rate was set to 800 μL/min and the mobile phase consisted of phase A: 100% 10 mM Bis-Tris, pH 7.5 and phase B: 10 mM Bis-Tris/0.5 M NaCl, pH 6.2, phase C: 10 mM Bis-tris, pH 6.2 and phase D 2 mM NaCl. Quantification of the transferrin isoforms was performed by selective absorbance of the iron–transferrin complex at 470 nm. Peak integrations were reviewed and manually integrated when automated peak integration feature incorrectly or partially integrated peaks.

3. Results

Fifteen patients (5 males and 10 females) from 14 unrelated families were included in this retrospective cohort study. The clinical features were first noted at average age of 4months (range birth to 11 months). The current average age was 8.4 years (range 1 to 18 years). One patient was transferred to the adult metabolic clinic after 18 years of age: his follow-up was reported until the age of 18 years and his chronological age was not taken into account. Fourteen patients had CDG-I including 9 patients with *PMM2*-CDG (60%), one patient with *ALG3*-CDG, one patient with *ALG9*-CDG, two patients with *ALG11*-CDG and one patient with *MPDU1*-CDG. One patient had CDG-II, *ATPV0A2*-CDG. Patients 3 and 4 were siblings. All *PMM2*-CDG patients are summarized in Table 1 for their clinical, biochemical, molecular genetics and neuroimaging features. All patients with non-*PMM2*-CDG subtypes are summarized in Table 2 for their clinical, biochemical, molecular genetics and neuroimaging features.

3.1. PMM2-CDG: clinical, neuroimaging, biochemical and molecular genetic features

There were nine patients with *PMM2*-CDG including five males and four females (Table 1). The average age of initial presentation was 5.6 months (range birth-11 months). The current average age was 8.3 years (range 1 – 18 years). The average time between age of onset and the diagnosis of *PMM2*-CDG was 18 months (ranged from 1 month to 4 years).

Presenting symptom was early infantile onset global developmental delay in 7 patients, respiratory distress in one patient due to tetralogy of Fallot and failure to thrive and pericardial effusion in one patient. All patients had various degrees of global developmental delay and cognitive dysfunction. All patients had axial hypotonia. Seizures were present in three patients, only one patient was on anti-epileptic medications with seizure freedom for more than two years. Sensorineural hearing loss was present in two patients.

Dysmorphic features included strabismus (seven patients), hypoplastic inverted nipples (4 patients), abnormal fat pads over the buttocks and the suprapubic region (one patient) and microcephaly (one patient). Failure to thrive was present in four patients.

Two patients had cardiac features including tetralogy of Fallot in one and pericardial effusions in another patient. The patient with pericardial effusion was identified at the age of 2 months by echocardiography. She was treated with pericardiocentesis due to large amount of pericardial effusion. The similar amount of pericardial effusion re-occurred within 2 weeks. She underwent pericardial window and drainage procedure. She was treated with diuretic medications and albumin infusions for