Molecular Genetics and Metabolism 133 (2021) 397–399

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Brief communication

Should patients with Phosphomannomutase 2-CDG (PMM2-CDG) be screened for adrenal insufficiency?

Anna Čechová [a], Tomáš Honzík [a], Andrew C. Edmondson [b], Can Ficicioglu [b], Mercedes Serrano [c,n], Rita Barone [d], Pascale De Lonlay [e,o], Manuel Schiff [e], Peter Witters [f], Christina Lam [g,p], Marc Patterson [h], Mirian C.H. Janssen [i], Joana Correia [j], Dulce Quelhas [j], Jolanta Sykut-Cegielska [k], Horacio Plotkin [l,q], Eva Morava [h,1,*], Kyriakie Sarafoglou [m,1]

[a] Department of Pediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic
[b] Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, USA
[c] Pediatric Neurology Department, Hospital Sant Joan de Déu, Institut de Recerca Sant Joan de Déu, Barcelona, Spain
[d] Child Neuropsychiatry Unit, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy
[e] Necker Hospital, APHP, Reference Center for Inborn Errors of Metabolism, University of Paris, Paris, France
[f] Metabolic Center, Department of Pediatrics, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium
[g] Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA 98101, USA
[h] Department of Clinical Genomics-Department of Laboratory Medicine and Pathology, Mayo Clinic, MN, USA
[i] Radboud University Medical Centre, Department of Internal Medicine, Nijmegen, the Netherlands
[j] Centro Hospitalar Universitário do Porto, Porto, Portugal
[k] Department of Inborn Errors of Metabolism and Paediatrics, the Institute of Mother and Child, Warsaw, Poland
[l] Glycomine, Inc, San Francisco, CA, USA
[m] Dept. of Pediatrics - Divisions of Endocrinology and Genetics & Metabolism, Dept. of Experimental & Clinical Pharmacology, University of Minnesota, USA
[n] U-703 Centre for Biomedical Research on Rare Diseases (CIBER-ER), Instituto de Salud Carlos III, Spain
[o] Inserm UMR_S1163, Institut Imagine, Paris, France
[p] Division of Genetic Medicine, Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA
[q] Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE, USA

ARTICLE INFO

Article history:
Received 8 April 2021
Received in revised form 21 May 2021
Accepted 7 June 2021
Available online 11 June 2021

Keywords:
PMM2-CDG
CDG
Glycosylation
Cortisol
ACTH
Inborn errors of metabolism
Phosphomannomutase 2-CDG

ABSTRACT

PMM2-CDG is the most common congenital disorder of glycosylation (CDG) accounting for almost 65% of known CDG cases affecting N-glycosylation. Abnormalities in N-glycosylation could have a negative impact on many endocrine axes. There is very little known on the effect of impaired N-glycosylation on the hypothalamic-pituitary-adrenal axis function and whether CDG patients are at risk of secondary adrenal insufficiency and decreased adrenal cortisol production.

Cortisol and ACTH concentrations were simultaneously measured between 7:44 am to 1 pm in forty-three subjects (20 female, median age 12.8 years, range 0.1 to 48.6 years) participating in an ongoing international, multicenter Natural History study for PMM2-CDG (ClinicalTrials.gov Identifier: NCT03173300). Of the 43 subjects, 11 (25.6%) had cortisol below 5 μg/dl and low to normal ACTH levels, suggestive of secondary adrenal insufficiency. Two of the 11 subjects have confirmed central adrenal insufficiency and are on hydrocortisone replacement and/or stress dosing during illness; 3 had normal and 1 had subnormal cortisol response to ACTH low-dose stimulation test but has not yet been started on therapy; the remaining 5 have upcoming stimulation testing planned.

Our findings suggest that patients with PMM2-CDG may be at risk for adrenal insufficiency. Monitoring of morning cortisol and ACTH levels should be part of the standard care in patients with PMM2-CDG.

1. Introduction

There are more than 130 known congenital disorders of glycosylation (CDG) [1,2]. CDG are inherited metabolic disorders caused by alterations to enzymatic processes of carbohydrate (glycan) formation, assembly and attachment to proteins and lipids. The most common subtype is PMM2-CDG (Phosphomannomutase 2-CDG, MIM# 212065).

* Corresponding author.
E-mail addresses: hplotkin@glycomine.com (H. Plotkin), morava-kozicz.eva@mayo.edu (E. Morava).
[1] Senior authors.

https://doi.org/10.1016/j.ymgme.2021.06.003

PMM2-CDG is caused by a reduction of phosphomannomutase 2 (PMM2) enzyme activity, the enzyme responsible for conversion of mannose-6-phosphate (M6P) to mannose-1-phosphate (M1P) resulting in hypoglycosylation of N-linked glycoproteins. PMM2-CDG is a rare, predominantly pediatric, frequently lethal, inherited metabolic disease. Glycoproteins are involved in virtually every endocrine axis. Protein N-glycosylation can affect the stability, binding affinity and ligand specificity of polypeptide hormones, hormone binding proteins and hormone receptors or the downstream intracellular signal transductions. Thus, any abnormality in N-glycosylation could have a negative impact on many endocrine axes resulting in impaired growth, puberty onset and progression, thyroid function, glucose metabolism and bone health. [3]. There is very little known on the effect of impaired N-glycosylation on the hypothalamic-pituitary-adrenal axis function and whether CDG patients are at risk of adrenal insufficiency (AI) and decreased adrenal cortisol production.

2. Material and methods

Simultaneous baseline cortisol and ACTH levels were collected in a sub-cohort of CDG patients as part of an ongoing international, multicenter Natural History study for PMM2-CGD (ClinicalTrials.gov Identifier: NCT03173300). A total of 139 subjects with PMM2-CDG have been enrolled and are followed every 6 months for 4 years with collection of clinical and laboratory information appropriate for the clinical presentation and standard of care [4]. The subjects were in their usual state of health without signs of physical stress or illness (including fever) that could have impacted the HPA axis. To account for inter- and intra-assay variation and random times of morning collection [5–7] we classified subjects to be at-risk for central AI (CAI) and in need of confirmatory testing those with a cortisol below 5 μg/dL in combination with a normal or below normal ACTH level, dependent on the respective normative ranges of the assays of the local laboratories [8]. Subjects with cortisol below 5 μg/dL and ACTH >2-fold above the normative range were classified as at-risk for primary AI (PAI). Institutional Review Boards at each study site approved the protocols and informed consent forms, and all parents/caregivers/participants provided written consent before enrollment.

3. Results

Out of the 139 subjects enrolled, 43 subjects, 20 female, median age 13.2 years (range 0.1–48.6 years), had both cortisol and ACTH results. Of the 43 subjects, 11 (7 females, median age 7.8 years, range 0.1–48.6) were identified as at-risk for CAI and had cortisol below 5 μg/dL at baseline (median 3.1 μg/dL, range 1.0–4.8). Cortisol levels in 10 of 11 subjects were below normative range of the local laboratory assays and 1 was slightly above the lower limit of normal. Median ACTH level was 10.8 pg/mL (4.6–19.0) with 2 subjects below normative range and the rest ($n = 9$) in the normal range of the local laboratory assay (Fig. 1). Median time of sample collection was 10 am (range 7:45–11:50). Two of the 11 subjects (male, age 2.8 years, cortisol 1.7 μg/dL, ACTH 17.2 pg/mL, collected at 9:54 am; female, age 0.1 years, cortisol 1 μg/dL, ACTH 10.8 pg/mL, collected at 7:45 am) have confirmed CAI (peak cortisol 2.8 μg/dL and 2.0 μg/dL after low dose ACTH stimulation test, respectively) and are on hydrocortisone replacement and/or stress dosing during illness. The female infant had neonatal hypoglycemia, GH deficiency and central hypothyroidism. Of the remaining 9 subjects, 3 had normal response and 1 had subnormal peak cortisol (16 μg/dL) after low-dose ACTH stimulation test but has not yet been started on hydrocortisone therapy; 5 have upcoming low-dose ACTH stimulation tests planned. None of the 43 subjects were found to be at-risk for PAI.

4. Discussion

To our knowledge, low cortisol concentrations with inadequate hypothalamus, pituitary, adrenal (HPA) axis response as evidenced by the low to normal ACTH concentrations have not previously been described in patients with PMM2-CDG and is not currently recommended for routine screening in international consensus management guidelines [4]. Secondary adrenal insufficiency has a prevalence of 1.5 to 2.8 in 10,000 and is more common in women than men [9]. Our results suggest that PMM2-CDG patients are at risk of CAI and may be unable to mount an appropriate cortisol response to stress. The potential reasons for this are multifactorial. Corticotropin- releasing hormone receptor 1 (CRHR1) and melanocortin 2 receptor (MC_2R; aka ACTHR), prohormone convertase 1/3 (PC1/3) enzyme, and corticosteroid-binding globulin are all N-glycosylated suggesting that impaired N-glycosylation could lead to abnormal HPA axis function and regulation. It has been shown that the presence of highly conserved N-linked glycosylation sites in the CRH receptor family plays an important role in receptor functions and deletion of three or more N-glycosylated chains severely impairs ligand binding and signal transduction and could therefore lead to decreased ACTH production by the pituitary and subsequently decreased cortisol production [10]. Also, abnormal N-glycosylation of the ACTHR can influence receptor activity [11].

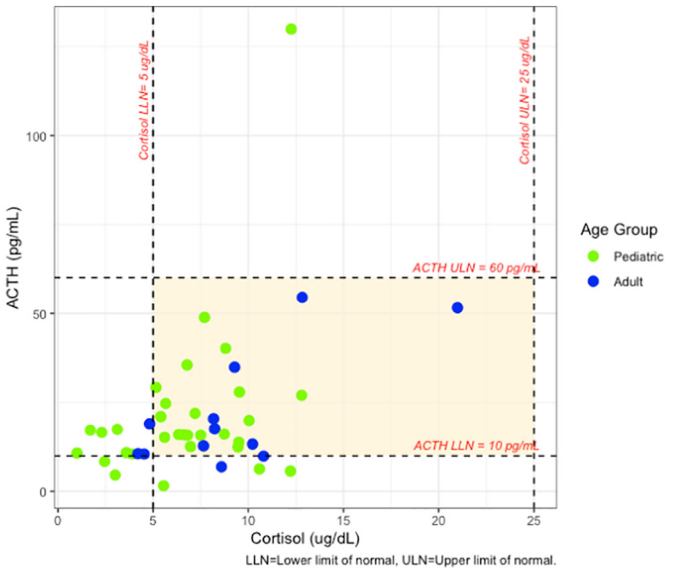

Fig. 1. Corresponding cortisol and ACTH Results (samples obtained before 1:00 PM).

In patients with CDG abnormal N-glycosylation of the PC1/3 enzyme could lead to impaired processing of preproopiomelanocortin (POMC) to ACTH [12] and thus decreased adrenal cortisol production as well as dysregulation of other endocrine axes. The enzyme PC1/3, encoded by *PCSK1 gene*, is essential for processing and conversion of a variety of prohormones into their bioactive forms. PC1/3 efficiently catalyzes the first three cleavages of POMC to produce β-lipotropic hormone (β-LPH) and ACTH. Abnormal PC1/3 function has been associated with impaired growth, puberty development, obesity, glucose metabolism and secondary adrenal insufficiency [13]. Lastly, differences in N-glycosylation can decrease the steroid –binding of CBG [14], resulting in decreased total cortisol and low to normal free cortisol and possibly contribute to hypofunction of the HPA axis.

Based on our findings, morning cortisol and ACTH levels should be evaluated at least annually on all patients with PMM2-CDG. If abnormal, a low dose ACTH stimulation test should follow to evaluate the HPA axis for CAI, which can be insidious at the early stages, as hypoglycemia may not be a presenting sign, or if present can be attributed to co-existent comorbidities and endocrinopathies such as growth hormone deficiency, poor feeding, or impaired enteral absorption. Early recognition of AI and initiation of glucocorticoid replacement therapy and stress dosing could be life-saving.

5. Conclusion

Our findings suggest that patients with PMM2-CDG may be at risk for CAI. Longitudinal studies are needed to identify the prevalence and time of onset of CAI.

Funding information

Glycomine, Inc. was the sponsor of this study, and was involved in the study design and in the and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Several authors of this publication are members of the European Reference Network for Rare Hereditary Metabolic Disorders (MetabERN) - Project ID No 739543.

Several authors of this publication are also supported by the NIH U54 NS115198-01 grant.

AC and TH were supported by RVO-VFN 64165 project of the Ministry of Health of the Czech Republic, TH was supported by EUROGLYCANomics, Ministry of Education Youth and Sports of Czech Republic No. 8F19002, under the frame of *E*-Rare-3, the ERA-Net for Research on Rare Diseases.

MS is supported by the Generalitat de Catalunya (PERIS SLT008/18/00194) and National Grant PI17/00101 from the National R&D&I Plan, cofinanced by the Instituto de Salud Carlos III (Subdirectorate-General for Evaluation and Promotion of Health Research) and FEDER (European Regional Development Fund).

PW was partially funded by FWO Flanders, Belgium (G049220N). P.W. is supported by the clinical research fund, University Hospitals Leuven, Leuven, Belgium.

HP is a full-time employee of Glycomine, Inc.

Acknowledgements

The authors would like to thank the patients and families for their participation in the study, and Frederique Vernhes for the statistical analysis of the data.

References

[1] C.R. Ferreira, R. Altassan, D. Marques-Da-Silva, R. Francisco, J. Jaeken, E. Morava, Recognizable phenotypes in CDG, J. Inherit. Metab. Dis. 41 (3) (2018) 541–553.

[2] N. Ondruskova, A. Cechova, H. Hansikova, T. Honzik, J. Jaeken, Congenital disorders of glycosylation: still “hot” in 2020, Biochim. Biophys. Acta Gen. Subj. 2021 (1) (1865) 129751.

[3] B. Miller, H.H. Freeze, New disorders in carbohydrate metabolism: congenital disorders of glycosylation and their impact on the endocrine system, Rev. Endocr. Metab. Disord. 4 (2003) 103–113.

[4] R. Altassan, R. Peanne, J. Jaeken, et al., International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: diagnosis, treatment and follow up, J. Inherit. Metab. Dis. 42 (1) (2019) 5–28.

[5] F. Ceccato, C. Scaroni, Central adrenal insufficiency: open issues regarding diagnosis and glucocorticoid treatment, Clin. Chem. Lab. Med. 26 (8) (2019) 1125–1135 57.

[6] J.M. Hawley, L.J. Owen, S.J. Lockhart, P.J. Monaghan, A. Armston, C.A. Chadwick, H. Wilshaw, M. Freire, L. Perry, B.G. Keevil, Serum cortisol: an up-to-date assessment of routine assay performance, Clin. Chem. 62 (9) (2016) 1220–1229.

[7] Mackenzie SD, Gifford RM, Boyle LD, Crane MS, Strachan MWJ, Gibb FW. Validated criteria for the interpretation of a single measurement of serum cortisol in the investigation of suspected adrenal insufficiency. Clin. Endocrinol.;91(5):608–615. doi: https://doi.org/10.1111/cen.14071. Epub 2019 Aug 14. PMID: 31380575 2019.

[8] S.R. Bornstein, B. Allolio, W. Arlt, A. Barthel, A. Don-Wauchope, G.D. Hammer, E.S. Husebye, D.P. Merke, M.H. Murad, C.A. Stratakis, D.J. Torpy, Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 101 (2) (2016) 364–389.

[9] O. Chabre, B. Goichot, D. Zenaty, J. Bertherat, Group 1. Epidemiology of primary and secondary adrenal insufficiency: Prevalence and incidence, acute adrenal insufficiency, long-term morbidity and mortality, Ann. Endocrinol. (Paris). 78 (6) (2017) 490–494.

[10] I.Q. Assil, A.B. Abou-Samra, N-glycosylation of CRF receptor type 1 is important for its ligand-specific interaction, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E1015–E1021.

[11] S. Roy, et al., Role of Asparagine-Linked Glycosylation in Cell Surface Expression and Function of the Human Adrenocorticotropin Receptor (Melanocortin 2 Receptor) in 293/FRT Cells Endocrinology, 151, 2010 660–670.

[12] W.F. Zandberg, et al., N-Glycosylation controls trafficking, zymogen activation and substrate processing of proprotein convertases PC1/3 and subtilisin kexin isozyme-1, Glycobiology 21 (10) (2011) 1290–1300.

[13] M.G. Martín, et al., Congenital proprotein convertase 1/3 deficiency causes malabsorptive diarrhea and other endocrinopathies in a pediatric cohort, Gastroenterology 145 (1) (2013) 138–148.

[14] L.A. Hill, et al., N-glycosylation influences human corticosteroid binding globulin measurements, Endocrine Conn. 8 (2019) 1136–1148.