

## Brief communication

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

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## 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, multi-center 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.

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## 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).

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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, multi-center Natural History study for PMM2-CDG (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

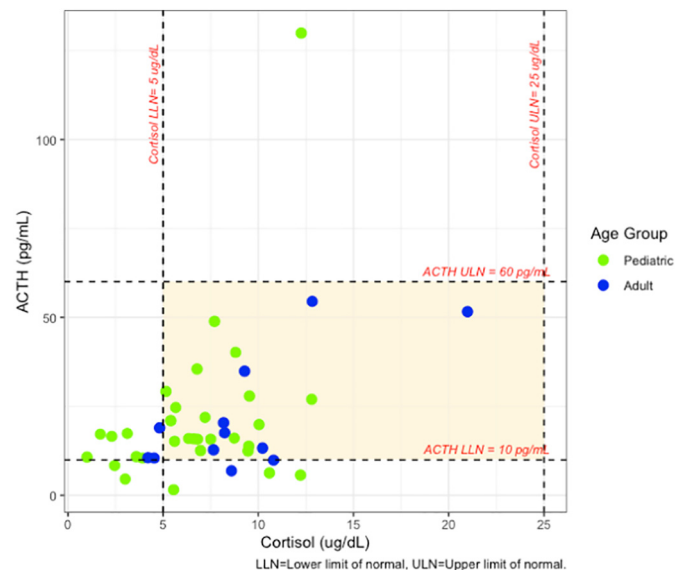


Fig. 1. Corresponding cortisol and ACTH Results (samples obtained before 1:00 PM).

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<sub>2</sub>R; 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].

In patients with CDG abnormal N-glycosylation of the PC1/3 enzyme could lead to impaired processing of proopiomelanocortin (POMC) to ACTH [12] and thus decreased adrenal cortisol production as well as dysregulation of other endocrine axes. The enzyme PC1/3, encoded by *PCKS1* 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-lipotrophic 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.

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