Defining the Phenotype and Assessing Severity in Phosphoglucomutase-1 Deficiency

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Objective To define phenotypic groups and identify predictors of disease severity in patients with phosphoglucomutase-1 deficiency (PGM1-CDG).

Study design We evaluated 27 patients with PGM1-CDG who were divided into 3 phenotypic groups, and group assignment was validated by a scoring system, the Tulane PGM1-CDG Rating Scale (TPCRS). This scale evaluates measurable clinical features of PGM1-CDG. We examined the relationship between genotype, enzyme activity, and TPCRS score by using regression analysis. Associations between the most common clinical features and disease severity were evaluated by principal component analysis.

Results We found a statistically significant stratification of the TPCRS scores among the phenotypic groups ($P < .001$). Regression analysis showed that there is no significant correlation between genotype, enzyme activity, and TPCRS score. Principal component analysis identified 5 variables that contributed to 54% variance in the cohort and are predictive of disease severity: congenital malformation, cardiac involvement, endocrine deficiency, myopathy, and growth.

Conclusions We established a scoring algorithm to reliably evaluate disease severity in patients with PGM1-CDG on the basis of their clinical history and presentation. We also identified 5 clinical features that are predictors of disease severity; 2 of these features can be evaluated by physical examination, without the need for specific diagnostic testing and thus allow for rapid assessment and initiation of therapy. *(J Pediatr 2016;175:130-6)*.

Congenital disorders of glycosylation (CDGs) are a group of genetic metabolic diseases that are caused by defects in protein glycosylation.[1,2] Recently, phosphoglucomutase-1 deficiency (PGM1-CDG) was recognized as a special CDG that offers new insights into the highly complex relationship among protein glycosylation, other metabolic pathways, and organ system development.[3,4] PGM1-CDG is caused by a deficiency of phosphoglucomutase-1 (PGM1),[5] which catalyzes the bidirectional conversion of glucose 1-phosphate and glucose 6-phosphate and is thus an important carbohydrate trafficking point that participates in both glycogenolysis and glyconeogenesis.[6,7]

In addition to maintaining glucose homeostasis, PGM1 also appears to play a pivotal role in protein N–linked glycosylation, an important posttranslational modification.[3] Patients with PGMI-CDG demonstrate glycosylation defects that affect transport proteins, hormones, coagulation factors, and other proteins. PGM1-CDG is one of the few CDGs that are treatable. Indeed, both in vitro

CDG	Congenital disorder of glycosylation
HSD	Honest significant difference
NPCRS	Nijmegen Paediatric CDG Rating Scale
PC	Principal component
PCA	Principal component analysis
PGM1	Phosphoglucomutase-1
PGM1-CDG	Phosphoglucomutase-1 deficiency
PGM2	Phosphoglucomutase-2
TPCRS	Tulane PGM1-CDG Rating Scale

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and in vivo data have shown that supplementation with D-galactose alone can improve protein N-glycosylation and mitigate many clinical features.[4,6]

The phenotype of PGM1-CDG comprises a wide range of clinical manifestations, including hypoglycemia, congenital malformations, early-onset dilated cardiomyopathy, growth retardation, hormonal deficiencies, hepatopathy, hemostatic anomalies, and myopathy. The etiology of hypoglycemia is complex and could be caused by insufficient glucose release from glycogen, or in some cases, by functional hyperinsulinemia secondary to abnormal protein glycosylation. Typically, younger patients experience severe and frequent hypoketotic episodes, and older patients experience milder, normoketotic hypoglycemia.[6]

A variety of congenital malformations have been observed in PGM1-CDG: cleft palate, bifid uvula, anal atresia, and Pierre Robin syndrome.[4] Hepatopathy in PGM1-CDG involves elevated aspartate aminotransferase and alanine aminotransferase, but normal albumin and ammonia levels; however, hepatomegaly, cholestasis, fatty liver disease, fibrotic changes, and signs of liver failure have been reported in a few patients.[4] Patients can present with isolated muscle symptoms or in combination with any of the aforementioned features.[4,8] Many aspects of the pathophysiology of PGM1-CDG remain unclear, which presents challenges in the clinical practice, such as predicting course of the disease and establishing disease management and follow-up guidelines. Furthermore, residual PGM1 enzymatic activity does not seem to correlate with phenotypic presentation in these patients.[4,9] We evaluated patients with PGM1-CDG by assessing the relationships between the types of mutation at the PGM1 locus, PGM1 enzyme activity assayed in patient cells or in vitro, clinical features, and severity of disease.

Methods

We evaluated 27 patients (18 children and 9 adults) with confirmed diagnosis of PGM1-CDG. The diagnosis was based on enzyme and molecular analysis. Patient age ranged from 2 to 43 years at the time of assessment, with 17 male and 10 female patients in the cohort. None of the patients exhibited severe motor or cognitive impairment. We obtained patient data by using a specific survey on informed consent (institutional review board reference #13-533377) and collected data on clinical features, laboratory results, mutation analysis, and enzyme activity assayed in extracts from patient cells (skin fibroblasts, leukocytes, or muscle biopsies). Data were collected before eventual oral galactose therapy. We also assayed the enzyme activity of recombinant versions of PGM1 mutant proteins in vitro (**Tables I** and **II**; available at www.jpeds.com).

Biochemical Studies of PGM1 Proteins

To remove potential complications of assaying enzyme activity in fibroblast extracts, which include heterozygosity and the presence of cellular homologs with PGM activity,[10] recombinant versions of PGM1 mutant proteins were prepared and purified. New missense mutants characterized for this study were G230E, P336R, T337M, W422R, R503Q, and R515L (**Figure 1**). Construction of missense mutations, recombinant expression, and purification were done as previously described[10] (**Appendix 1**; available at www.jpeds.com).

Phenotypic Groups

To assist physicians with providing early prognosis counseling for patients with PGM1-CDG, we developed a simple disease severity grading method that is based on clinical symptoms that present early in the disease course: congenital malformation and dilated cardiomyopathy. Congenital anomalies in patients with PGM1 deficiency suggest an intrauterine effect of the genetic defect. Dilated cardiomyopathy is an early-onset, life-threatening condition, and all deceased patients with PGM1-CDG succumbed as the result of complications arising from dilated cardiomyopathy. We divided our patient cohort into 3 phenotypic groups: severe, moderate, and mild. The groups were established according to the presence or absence of congenital malformation and dilated cardiomyopathy. The presence of congenital malformation, in addition to dilated cardiomyopathy, is the criterion for the "severe" phenotypic group. The presence of congenital malformation, but no dilated cardiomyopathy, is the criterion for the "moderate" phenotypic group. Patients with neither congenital anomalies nor dilated cardiomyopathy were sorted into the "mild" phenotypic group.

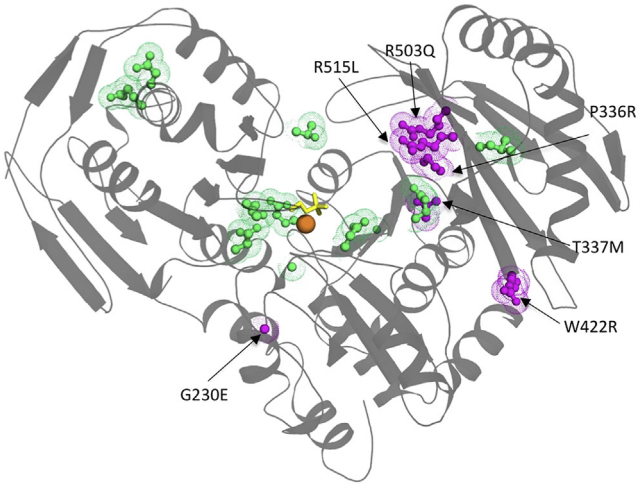

Figure 1. The novel missense mutants (*magenta*) are located in varying structural regions of the protein: G230E (helix in domain 2); P336R and T337M (near active site in domain 3); W422R (near the interface of domains 3-4); and R503Q and R515L (in an active site loop of domain 4). Previously characterized missense mutants associated with disease are highlighted in *green*; the active phosphoserine is shown in *yellow* and the metal ion in *orange*.