

Attenuation of Hemolysis Due to Glucose-6-Phosphate Isomerase Deficiency With Ketogenic Diet – A Case Report

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This case report describes the clinical course of a patient with glucose phosphate isomerase (GPI) deficiency, chronic non-spherocytic hemolytic anemia (CNSHA) and intractable seizures who demonstrated a dramatic resolution of seizures and reduction in hemolysis and transfusion dependence following initiation of a ketogenic diet. GPI is a glycolytic enzyme, catalyzing the interconversion of glucose-6-phosphate to fructose-6-phosphate in the glycolytic pathway, ultimately resulting in the conversion of glucose to pyruvate and generation of adenosine triphosphate (ATP).¹ GPI deficiency occurs as a result of mutations in the *GPI* gene and can present with wide phenotypic variation including various degrees of CNSHA and occasionally, severe neurodevelopmental delay and epilepsy. It is a very rare condition, with just over 50 case reports in medical literature. The case highlights some interesting insights into likely pathogenic mechanisms of the enzyme deficiency.

The proband is a South Asian female child homozygous for the novel p.Leu425Phe (sequence accession number NM_000175, transcript variant 2) missense mutation in the *GPI* gene (Enzyme Nomenclature EC. 5.3.1.9) with progressive neuromuscular deficit, intractable seizures, short-lived transfusion dependence and preferential hepatic iron overload.

She was born at term by emergency Caesarean section for fetal distress. Birth weight was 1.89 kg (0.4th percentile) indicating intra-uterine growth restriction. Investigations at birth showed anemia with hemoglobin (Hb) concentration of 60 g/L and jaundice (to a maximum serum bilirubin of 338 μ mol/L on day one of life) necessitating red cell exchange transfusion and phototherapy.

Persistent anemia (Hb 50–60 g/L) necessitated 4 to 6 weekly transfusions. Blood smear showed polychromasia, nucleated red cells, irregularly contracted cells, and basophilic stippling. Eosin-5-maleamide binding assay, Hb electrophoresis, glucose-6-phosphate dehydrogenase and pyruvate kinase enzyme assays were normal, and investigations for unstable Hb were negative. An extensive metabolic screen was normal. Following a parental red blood cell enzyme assay which revealed heterozygous levels of GPI (mother, 23 U/g of Hb; father, 19.5 U/g of Hb- normal adult reference range 38–53), a provisional diagnosis of transfusion dependent chronic non-spherocytic hemolytic anemia (CNSHA) due to GPI deficiency was made. For further diagnostic confirmation, GPI enzyme assay of whole cell homogenates from a normal skin fibroblast cell line CRL 1498 and skin fibroblasts generated from the patient was performed. The patient's residual GPI activity was 8% compared to normal (normal 687 ± 124 nmol/min/mg; patient 55 ± 36 nmol/min/mg) (Fig. 1).² The subject's tracing showed a brief burst of activity followed by a plateau after 30 seconds. In addition, Western blot analysis showed a reduction in GPI compared to normal skin fibroblasts (Fig. 2). Although this could simply reflect normal variation between individuals, it is of note that despite the presence of significant levels of the protein in the patient fibroblasts, it has shown very low levels of activity, suggesting destabilization of the mutated protein, likely due to increased susceptibility to proteolytic degradation.

RNA extraction from the fibroblasts, complementary DNA synthesis, polymerase chain reaction and sequencing revealed a previously unreported variant in homozygosity: Leu425Phe, which was likely deleterious, given that it is situated in the sugar isomerase domain, a highly conserved domain. Phylogenetic comparison of the mutated sequence did not retrieve any matches in any of the 13 species analyzed. Leu425 was conserved in all species analyzed (data not shown). Findings were confirmed by sequencing of DNA extracted from patient's peripheral blood mononuclear cells. Both parents were heterozygous for the same

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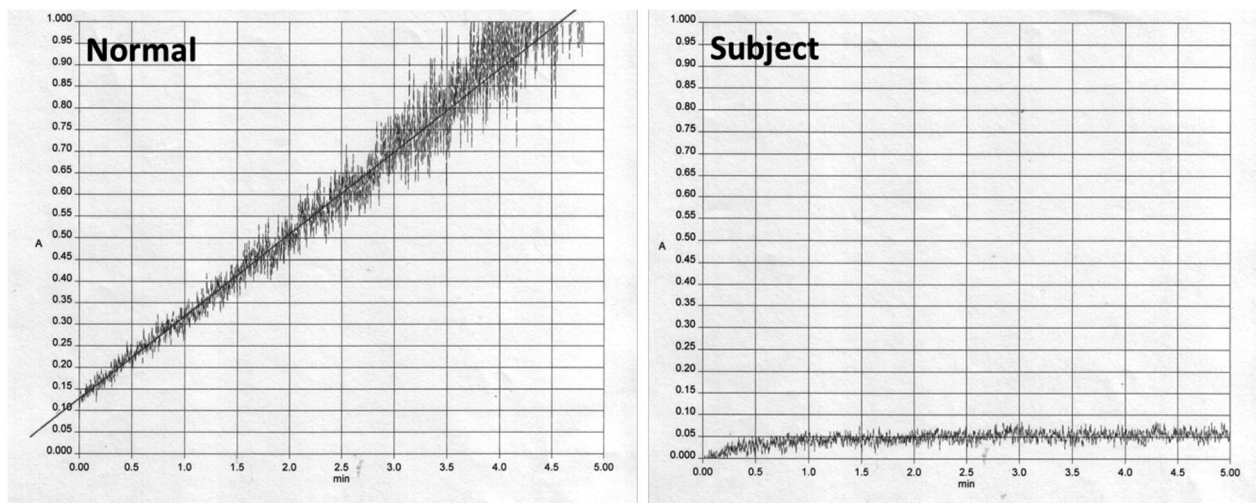


Figure 1. Quantitative GPI enzyme assays of whole cell homogenates from a normal skin fibroblast cell line CRL 1498 and patient's fibroblasts indicated 8% GPI activity in the patient. GPI = glucose phosphate isomerase.

variant. Hepatic R2 Magnetic Resonance imaging (MRI) (FerriScan[®]) at age 5 years showed significant iron overload with an estimated average liver iron concentration (LIC) of 43 mg/g/dry weight (770 mmol/kg dry tissue). However, there was no concurrent cardiac iron loading.

Although there was normal neurodevelopmental progress until 6 months of age, the patient subsequently had progressive motor delay and regression, including persistent head lag, inability to roll over or sit unsupported and lack of speech. From age 18 months, the patient developed mixed tonic-clonic and partial seizures, with several episodes of status epilepticus following minor childhood infections. Extensive neurological investigations were performed to exclude the possibility of an underlying cofactor. MRI brain at age 4 years showed no structural abnormality, although slight delayed myelination. Seizures, including status epilepticus, occurred daily, despite polytherapy with anti-epileptic drugs. Subsequently, at age 7 years a ketogenic diet (with a fat to carbohydrate ratio of 3:1, aiming for serum ketone quantification of 3.2–3.9 mmol/l) was adopted. This

resulted in significant reduction in seizures and an almost complete reduction of clusters.

Six months after commencement of ketogenic diet, at age 8 years, the patient achieved transfusion independence without the need for splenectomy, having commenced transfusions at birth. Her baseline hemoglobin remains at 80 g/l without transfusion and she suffers from occasional haemolytic crisis with intercurrent viral infections, requiring ad hoc transfusion therapy. Her iron chelation therapy was discontinued after serum ferritin level reached the threshold of <300 ug/l.

GPI deficiency is the third most common erythrocytic enzymopathy, with a wide spectrum of phenotypes. The most recognized clinical manifestation is CNSHA. In a limited number of cases, neurodevelopmental delay and neuromuscular deficit have also been found to be associative features.³ GPI is termed a 'moonlighting enzyme' because in addition to its intracellular glycolytic activity; extracellularly, it functions as the neurotropic growth factor neuroleukin, autocrine motility factor for cancer cells, and differentiation and maturation mediator for immune cells.⁴

This is the first known GPI deficient patient with the homozygous missense mutation p.Leu425Phe. The residual GPI activity in the patient was of 8% of normal. This is lower than reported 15% to 25% activity in previously reported cases.^{3,5} However, difficulty in determining genotype-phenotype correlations are well-established.¹ For instance, patients with residual GPI activity at 60% has been shown to suffer from severe hemolysis (Hb 88 g/L),¹ whereas GPI Homburg with a GPI activity of 4.4% maintained mild hemolysis, with a Hb of 133 g/L.³ Interestingly, phenotypic differences have also been observed in individuals of identical genotypes and residual GPI activity.¹ In view of this, it is likely that secondary genetic loci and environmental factors play a role in determining the disease severity. One such mechanism has been proposed by Haller *et al.*, who determined the pleiotropic effects of GPI deficiency (GroD1 cell line) in Mechanistic Target of Rapamycin (mTOR) activation via glucose-6-phosphate accumulation and cytosolic sequestration of lipin 1, which hinders lipogenic synthesis.⁶ The downstream effects of phosphatidic acid accumulation, a known mTOR activator, due to altered glycerolipid biosynthesis also contributes to the abnormal nutrient mTOR signaling pathway.⁶

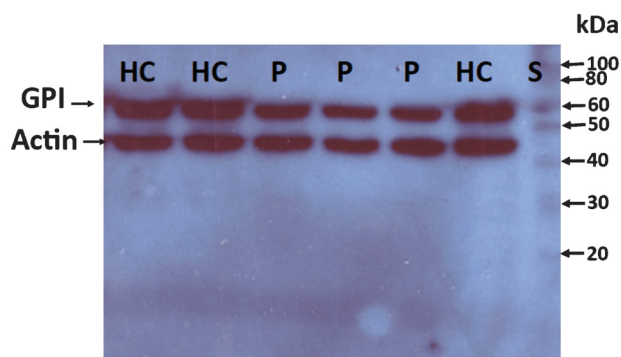


Figure 2. Western blot study using a normal skin fibroblast cell line CRL 1498 and the subject's cultured fibroblasts. A 10% SDS gel and a biotinylated protein standard ladder (cell signalling) to determine the molecular weights (not shown). Quantitation of the Western Blot was undertaken by using GelAnalyzer. The density ratio of GPI/actin was 1.297 (SD=0.052) for normal fibroblasts and 0.630 (SD=0.166) for the patient's fibroblasts. The actin and GPI bands corresponded to the correct molecular weights. HC = Healthy control skin primary fibroblast; P = subject's skin fibroblasts; S = Standard.

Given the function of lipid composition on the integrity of erythrocytic permeability and viability,⁷ it is plausible that such effects would be modulators of disease severity.

Haller et al also reported that phenotypes associated with the GPI-deficient cell line GroD1 were influenced by glucose levels in the medium; reduction of medium glucose levels resulted in recovered growth of the cells at non-permissive temperature (40°C), recovered translocation of lipin 1 α to the nucleus and partial restoration of phospholipid biosynthesis.⁶ These findings may help to explain the improvement of the patient's symptoms upon the initiation of a ketogenic diet which has been shown to lower fasting and postprandial blood glucose levels in patients with type 2 diabetes.⁸

There remains a paucity of published data pertaining to neurological involvement in GPI deficiency. The putative pathogenic mechanism involves the attenuated neurotrophic properties of monomeric GPI (as neuroleukin).^{9,10} Of the 6 published reports available that describe neurological involvement, only GPI Homburg¹¹ and GPI Mount Scopus¹² have been characterized at the molecular level. Kugler et al postulated that mutations that target the structural integrity of the monomeric form are most likely to lead to neurological complications alongside a loss of catalytic activity, in contrast to mutations that influence the active site in close proximity of the subunit interface, which may preserve neurotrophic function.¹¹ However, an alternative hypothesis for the neurological phenotype and the success of the ketogenic diet could be that the GPI deficiency may result in an energy deficit in the brain, which may be improved by providing non carbohydrate energy in the ketogenic diet. Elucidation of these pathways through further in vivo investigation will be critical to yield potential therapeutic applications beyond the current standard of blood transfusion and splenectomy that is reserved for severe hemolytic cases.

Despite the well-established relationship between secondary iron overload and altered iron homeostasis in hemolytic states, our knowledge of its relevance in GPI deficiency remains limited.⁵ Inappropriate hepcidin suppression with dysregulated erythropoiesis has been demonstrated in three Czech cases of GPI deficiency.¹³ Interestingly, the patient developed hepatic hemosiderosis despite normal cardiac iron levels. Additionally, the finding by Baronciani et al, that a case of GPI deficiency was found to have a two-fold increase in iron stores post-splenectomy during a 10 year period despite maintaining transfusion independence and normal erythropoiesis highlights the complexity of iron regulation and indicates the need for close surveillance in these patients.⁵

In summary, this report describes the clinical features of a patient with unexplained CNSHA, complicated by neurodevelopmental impairment and selective hepatic iron-mediated toxicity induced by the p.Leu425Phe pathogenic variant and attenuation of hemolysis on a ketogenic diet. GPI deficiency remains rare, and the continued elucidation of the patterns of clinical characteristics will be critical in aiding the prompt recognition and treatment of this disorder.

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