SLGT2 Inhibitor Rescues Myelopoiesis in G6PC3 Deficiency

Prashant Hiwarkar (✉ drhprashant@gmail.com)

Bai Jerbai Wadia Hospital for Children <https://orcid.org/0000-0001-7504-3521>

Umair Bargir

ICMR-National Institute of Immunohaematology

Ambreen Pandrowala

Bai Jerbai Wadia Hospital for Children

Minnie Bodhanwala

Bai Jerbai Wadia Hospital for Children

Naresh Thakker

Bai Jerbai Wadia Hospital for Children

Prasad Taur

Anand Children's Nursing Home

Manisha Madkaikar

ICMR-National Institute of Immunohaematology

Mukesh Desai

Anand Children's Nursing Home

Research Article

Keywords: metabolism, myeloid, glycolysis, hexokinases, neutrophil

Posted Date: January 10th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1183329/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The energy metabolism of myeloid cells depends primarily on glycolysis. 1,5-anhydroglucitol (1,5AG), a natural monosaccharide is erroneously phosphorylated by glucose-phosphorylating enzymes to produce 1,5-anhydroglucitol-6-phosphate (1,5AG6P), a powerful inhibitor of hexokinases. The endoplasmic reticulum transporter (SLC37A4/G6PT) and the phosphatase G6PC3 co-operate to dephosphorylate 1,5AG6P. Failure to eliminate 1,5AG6P is the mechanism of neutrophil dysfunction and death in G6PC3-deficient mice. SLGT2-inhibitor reduces 1,5AG level in the blood and restores the neutrophil count in G6PC3-deficient mice. In the investigator-initiated study, a 30 year-old G6PC3-deficient woman with recurrent infections, distressing gastrointestinal symptoms and multi-lineage cytopenia was treated with an SLGT2-inhibitor. A significant increase in all the hematopoietic cell lineages and substantial improvement in the quality of life was observed.

Introduction

The enzyme glucose-6-phosphatase (G6PC1) hydrolyzes glucose-6-phosphate in the endoplasmic reticulum of liver, kidney and intestinal cells, a final step of glycogenolysis and neoglucogenesis. Glucose-6-phosphatase catalytic subunit 3 (G6PC3) is a ubiquitously expressed glucose-6-phosphatase homologue which has a role in differentiation and survival of myeloid cells.^{1,2} G6PC3-deficient mice have neutropenia with defective respiratory burst, chemotaxis, increased susceptibility to bacterial infection, elevated plasma glucagon and low serum cholesterol.³ Humans with G6PC3 deficiency have variable haematological and non-haematological manifestations. Non-haematological features include prominent superficial venous pattern, congenital cardiac anomalies, renal system malformations and genital anomalies.² Severe neutropenia is a consistent haematological feature of all patients with G6PC3 deficiency.⁴ Two-third of patients present with intermittent thrombocytopenia.⁴

Neutropenia causes significant morbidity.⁴ Patients with G6PC3 deficiency commonly present with recurrent bacterial infections in the first few months of life.⁵ Common infections are sino-pulmonary, otitis media, urinary tract infections, skin abscesses and sepsis. Oral ulcers, gingivitis, periodontitis, stomatitis, inflammatory bowel disease and fungal infections have also been reported. Although the neutropenia of G6PC3 deficiency is GCSF-responsive, inflammatory colitis secondary to G6PC3 deficiency does not respond to GCSF.⁶⁻⁸ Few cases of hematopoietic cell transplantation (HCT) have been successfully applied in patients with inflammatory colitis of G6PC3 deficiency.⁶⁻⁹ However, HCT is associated with risk of serious morbidity and mortality. Veiga-da-Cunha *et al.* have recently shown that in a G6PC3-deficient mouse model, neutropenia was explained by the accumulation of toxic amounts of 1,5AG6P in neutrophils. These authors have also shown that the administration of an SLGT2 inhibitor, lowered plasma 1,5AG, reduced the intracellular accumulation of 1,5AG6P and restored normal neutrophil counts in G6PC3-deficient mice¹⁰ as well as in patients with neutropenia-associated glycogen storage disease type 1b (GSD1b), deficient in SLC37A4/G6PT, the transporter that collaborates with G6PC3 to dephosphorylate 1,5AG6P in neutrophil's endoplasmic reticulum.¹¹ Now, we provide the first evidence for

the role of empagliflozin, an SGLT2 inhibitor, in rescuing neutropenia and neutrophil dysfunction in a patient with G6PC3-deficiency.

Case Description

A 30-year-old female patient, born to non-consanguineous parents developed recurrent infections since birth. There was a history of a male sibling death due to an infection in infancy (Figure 1a).

The patient presented to us at the age of 27 years. Her height was 152 cm and weight was 58 kg. She had recurrent infections since birth. Infection profile is shown in Figure 1b and the frequency of the visits to a physician for infections and/or abdominal symptoms are shown in the supplemental figure 1. She had multiple episodes of upper and lower respiratory tract infection, skin abscesses, otitis media and urinary tract infections (supplemental table 1).

Recurrent tongue ulcers were common since she was 12 years old. These often lasted for 10 to 20 days (Figure 1c). Bone marrow test on four occasions confirmed left shifted myelopoiesis, indicating an increasing number of promyelocytes and myelocytes (Figure 1d).

Episodes of abdominal pain and diarrhea developed since the age of 17 years. She had one to two episodes every 3 months and each episode lasted for 15 to 20 days. She had 11 severe episodes of abdominal pain and diarrhea requiring visits to a physician but was never investigated for it. She also had mild thrombocytopenia and a small patent foramen ovale with left to right flow as often seen in G6PC3-deficient patients.⁵

Clinical exome sequencing was performed to identify the cause of congenital neutropenia - a novel homozygous missense variant in the gene coding for G6PC3 (LRG_182t1:c482G>C; pArg161Pro) was identified. The parents were heterozygous carriers for the same variant. A variant affecting the same amino acid (Arg161Gln) has been described as a part of compound heterozygous mutation in a patient with severe congenital neutropenia.¹² An *in vitro* study showed that Arg161Gln variant abolishes the catalytic activity of G6PC3.¹³ Therefore, Arg161Pro variant was reported as pathogenic mutation.

Methods

The patient was prospectively enrolled. Informed consent was taken for treatment with empagliflozin. Whole blood was obtained in EDTA and lithium heparin tubes under institutional research board approved protocols in accordance with the Declaration of Helsinki. Blood samples were obtained weekly for 7 weeks and then at every out-patient visit. Blood counts were monitored at every clinic visit. 1,5AG was monitored every week for 7 weeks and then on further two clinic visits. Flow cytometry for CD14, CD15, CD66B, CD18 and dihydrorhodamine-123 (DHR) assay was monitored for 4 weeks. The dose of empagliflozin was titrated to the neutrophil count.

Quantification of 1,5-anhydroglucitol in human plasma by LC-MS

Plasma was isolated after centrifugation (5 min at 500 x g; 22°C) of 0.4 ml of freshly collected EDTA blood and heated for 5 mins at 90°C and immediately frozen at -80°C. The samples were shipped from Mumbai to Brussels at room temperature. Plasma samples were centrifuged and the supernatant was used for 1,5AG measurement by LCMS as previously described.¹⁰ Plasma was collected before starting empagliflozin and weekly during treatment for 7 weeks and for two subsequent clinic visits on day 52 and day 58. Plasma samples were also collected at a later time-point on day 585, day 586, day 609 and day 610.

Dihydrorhodamine (DHR) assay

Neutrophil function test was performed on whole blood using dihydrorhodamine-123 (DHR) and phorbol myristate acetate (PMA) (a protein-kinase C activator) in the laboratory (Sigma-Aldrich) [NIIH, Mumbai] as previously described.¹⁴ Flow cytometry based DHR assays can detect, upon PMA stimulation, reduced production of ROS, especially hydrogen peroxide and abnormal levels indicate defective NADPH complex activity. A stimulation index (SI) was calculated for gated neutrophils by estimating the ratio of median fluorescent intensity (MFI) of PMA-stimulated and PMA-unstimulated cells in the DHR assay. SI value for patient and healthy control sample were evaluated simultaneously.

Assays for granulocyte activation

Granulocytes were examined for abundance of CD14, CD15, CD18 and CD66B neutrophil antigens by flow cytometry analysis. Whole blood samples collected in EDTA were processed within three hours of collection. Fluorescence staining of granulocytes was carried out with CD14-PE, CD66B-FITC, CD18-PerCP and CD15-APC labelled antibodies (Becton Dickinson) directly on whole blood followed by lysis using Optilyse C (Beckman Coulter). 20000 events were acquired on Navioe EX (Beckman Coulter) and data analysis was done using Kaluza® software. Quantification of neutrophil antigens in the patient before and during treatment with empagliflozin was compared with normal untreated controls.

QoL – PROMIS scoring system

The patient was requested to complete a quality of life questionnaire for two time points - 1) before starting empagliflozin and 2) one year on treatment with empagliflozin. Three domains of quality of life were assessed 1) cognitive function, 2) emotional distress such as anger, depression and anxiety, 3) clinical symptoms such as pain interference, belly pain and diarrhea. T score of 50 is the average for the general population in PROMIS scoring system.^{15,16}

Results And Discussion

A significant increase in the absolute neutrophil counts (ANC) was observed during treatment with empagliflozin (Figure 2). Increase in ANC was associated with a drop in blood 1,5AG levels (Figure 2A).

When blood samples from before (n = 17) and during empagliflozin treatment (n = 20) were compared, a significant increase in the monocyte counts, platelet counts and hemoglobin were also observed during empagliflozin treatment (Figure 2b; $p < 0.0001$ unpaired t test).

To examine changes in neutrophil function, we used a DHR based assay that showed an improvement of the neutrophil SI from 9.46 (before treatment) to a median of 51.9 (during treatment on day 7, 14 and 21) i.e. similar to untreated normal controls (Figure 3A and B).

Neutrophils in patients with G6PC3 deficiency have been extensively studied by Goenka et al.⁸ who have reported an altered inflammatory activation status of neutrophils in patients with G6PC3 deficiency, possibly due to dependence on glycolysis as their energy source.¹⁷ Therefore, we measured surface expression of neutrophil markers^{18–20} (before and during empagliflozin treatment) to determine neutrophil activation status in our G6PC3-deficient patient. Before treatment, we found higher expression of CD14, CD15, CD18 and CD66B in neutrophils of the G6PC3 deficient patient, similar to the previous report.⁸ Interestingly, we observed prompt normalization of the mean fluorescence intensity during treatment with empagliflozin – an observation similar to G6PC3 deficient patients that underwent HCT (Figure 3b).⁸ Thus, there was correction of neutrophil activation during treatment with empagliflozin. Taken together, these results indicate a possible role of empagliflozin in normalizing the neutrophil phenotype by activating glycolysis due to the release of hexokinase inhibition.

The improvement in ANC and neutrophil function was associated with resolution of clinical symptoms. The patient has not developed new infections nor tongue ulcers since starting the treatment with empagliflozin. There has been no episode of abdominal pain or diarrhea during 521 days, since treatment was started. Moreover, our patient reported a significant improvement in the quality of life assessed by PROMIS questionnaire. Cognitive function, emotional distress and clinical symptom scores were below average before treatment. There was significant improvement (T score of $> \text{or} < 10$ i.e. > 1 standard deviation) in all 3 domains during empagliflozin treatment (supplemental table 2). It is noteworthy that the clinical symptoms scores improved to above average for the general population.

There was no evidence of hypoglycemic episodes or any other adverse event during the treatment. Empagliflozin is a frequently used drug in type 2 diabetes with good safety profile.²¹ The usual starting dose of empagliflozin in an adult with type 2 diabetes is 10 mg daily. To determine the dose of empagliflozin that would be sufficient to treat neutropenia and neutrophil function in G6PC3 deficiency, we started with the daily dose of 5 mg (0.09 mg/kg/day). The dose of empagliflozin was increased to 10 mg/day (0.2 mg/kg/day) on day 42 of treatment, to achieve a normal neutrophil count as shown in Fig. 2A and supplemental table 3. After achieving a normal neutrophil count, on day 52 we reduced the dose to 10 mg every second day (back to 0.09 mg/kg/day) to consistently maintain ANC above $500 \times 10^9/\text{L}$. Since day 182 our patient has been kept on a maintenance dose of 5 mg every 3rd day (0.03 mg/kg/day). Her neutrophil count has remained consistently above $700 \times 10^9/\text{L}$ on the maintenance therapy for 345 days (since day 182 of treatment) (see Fig. 2A). Patient is asymptomatic without any evidence of infection, tongue ulcers nor gastrointestinal symptoms. It is important to note, that the

empagliflozin maintenance dosage (0.03 mg/kg/day) that is used in this patient is more than 10-fold lower in comparison to the previously reported dose (about 0.4 mg/kg/day) used to treat neutropenia in GSD1b patients.¹¹ As yet we do not have an explanation for this, but it is possible that for G6PC3-deficient patients who do not take cornstarch, which is likely an important source of 1,5-anhydroglucitol,²² once they have taken enough empagliflozin to lower the concentration of 1,5-anhydroglucitol in blood (as seen during the initial days of treatment – Figure 2a), a lower maintenance dose can be used to keep 1,5-anhydroglucitol in blood sufficiently low for neutrophils to remain functional and infections to be prevented, as is seen in this particular patient. Clinical trials enrolling more G6PC3-deficient patients treated with empagliflozin are necessary before recommending an empagliflozin dosage for the treatment of neutropenia in G6PC3-deficiency.

Currently, HCT is considered the only curative option for G6PC3 deficiency.⁹ Typically, HCT is performed in children and adults with G6PC3 deficiency and inflammatory colitis in this GCSF-responsive neutropenia.^{6–8} Although the neutrophils are GCSF-responsive, they exhibit an activated phenotype and accelerated apoptosis.⁸ Despite some response to GCSF, patients with G6PC3 deficiency often develop inflammatory colitis. This is not surprising since as shown by Veiga-da-Cunha *et al.* despite GCSF treatment neutrophils continue to accumulate toxic amounts of 1,5AG6P.^{10,11} The inflammatory colitis is attributed to abnormal myeloid biology due to G6PC3 deficiency.⁸ Our patient also had gastrointestinal symptoms, abnormal neutrophil function and counts, and neutrophils with an activated phenotype, before starting empagliflozin treatment. The neutrophil count, function of the neutrophils and their phenotype was normalized, and the gastrointestinal symptoms resolved on empagliflozin. This is an encouraging finding and suggests that reduction in 1,5AG with SLGT2-inhibitor improves the quality of myeloid cells and hence could have a therapeutic role in inflammatory colitis of G6PC3 deficiency. Accordingly, a very recent report, describing role of empagliflozin in the treatment of neutropenia associated with GSD1b, also demonstrated a clear improvement of inflammatory colitis.¹¹

This is the first-in-human report of an SLGT2 inhibitor in G6PC3 deficiency advancing the proof-of-concept demonstrated by G6PC3-deficient mouse models. Complex molecular and genetic techniques have been employed to develop therapeutic options for G6PC3 deficiency and other enzyme deficiencies.^{23,24} However, the approach of eliminating the toxic metabolite is simple and cost-effective. Similar approaches were tried in the past in a few patients with adenosine deaminase deficiency but the results were not encouraging.^{25,26} The success of empagliflozin in rescuing the myelopoiesis in G6PC3-deficiency indicates that such simple approaches should be investigated in the laboratory. More trials are now underway to test the definitive role of SLGT2 inhibitor in neutropenia of G6PC3 deficiency and GSD1b. However, our report suggests that patients with G6PC3 deficiency and inflammatory colitis or other end organ damage should be offered the treatment with an SLGT2 inhibitor before proceeding to high-risk procedure of HCT.

Declarations

Funding

No funding was received for this work

Declaration of competing interest

The authors have no conflicts of interest to declare

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Hospital Core Committee.

Availability of data and material

There is no data for repository

Code availability

Not applicable

Authors' contribution

P.H. designed the study, collected the data and wrote the manuscript; U.B. and M.M. performed the experiments; A.P., P.T., M.B., N.T., and M.D. were actively involved in patients care; all authors approved the manuscript.

Acknowledgements – We acknowledge Dr Christopher Klein for supporting with the genetic diagnosis. We are thankful to Dr. Maria Veiga-da-Cunha from de Duve Institute, UCLouvain, Belgium for measuring 1,5AG in plasma samples and the helpful comments during the writing and editing of the manuscript.

Consent to participate and publication

Patient was consented for treatment and publication of the report

References

1. Gautam S, Kirschnek S, Gentle IE, et al. Survival and differentiation defects contribute to neutropenia in glucose-6-phosphatase- β (G6PC3) deficiency in a model of mouse neutrophil granulocyte differentiation. *Cell Death Differ.* 2013;20(8):1068–79.
2. Banka S, Newman WG. A clinical and molecular review of ubiquitous glucose-6-phosphatase deficiency caused by G6PC3 mutations. *Orphanet J Rare Dis.* 2013;8:84.
3. Cheung YY, Kim SY, Yiu WH, Pan CJ, Jun HS, Ruef RA, Lee EJ, Westphal H, Mansfield BC, Chou JY. Impaired neutrophil activity and increased susceptibility to bacterial infection in mice lacking

- glucose-6-phosphatase-beta. *J Clin Invest*. 2007;117:784–93.
4. Banka S. G6PC3 Deficiency. In: Adam MP, Ardinger HH, Pagon RA et al, editors. *GeneReviews®*. Seattle (WA). University of Washington, Seattle; 1993.
  5. Desplantes C, Fremond ML, Beaupain B, et al. Clinical spectrum and long-term follow-up of 14 cases with G6PC3 mutations from the French Severe Congenital Neutropenia Registry. *Orphanet J Rare Dis*. 2014;9:183.
  6. Bégin P, Patey N, Mueller P, et al. Inflammatory bowel disease and T cell lymphopenia in G6PC3 deficiency. *J Clin Immunol*. 2013;33(3):520–5.
  7. Bolton C, Burch N, Morgan J, et al. Remission of Inflammatory Bowel Disease in Glucose-6-Phosphatase 3 Deficiency by Allogeneic Haematopoietic Stem Cell Transplantation. *J Crohns Colitis*. 2020;14(1):142–7.
  8. Goenka A, Doherty JA, Al-Farsi T, et al. Neutrophil dysfunction triggers inflammatory bowel disease in G6PC3 deficiency. *J Leukoc Biol*. 2020 Sep 15.
  9. Bakhtiar S, Shadur B, Stepensky P. The Evidence for Allogeneic Hematopoietic Stem Cell Transplantation for Congenital Neutrophil Disorders: A Comprehensive Review by the Inborn Errors Working Party Group of the EBMT. *Front Pediatr*. 2019;7:436.
  10. Veiga-da-Cunha M, Chevalier N, Stephenne X, et al. Failure to eliminate a phosphorylated glucose analog leads to neutropenia in patients with G6PT and G6PC3 deficiency. *Proc Natl Acad Sci U S A*. 2019;116(4):1241–50.
  11. Wortmann SB, Van Hove JLK, Derks TGJ, et al. Treating neutropenia and neutrophil dysfunction in glycogen storage disease type Ib with an SGLT2 inhibitor. *Blood*. 2020 Aug 27;136(9):1033–1043.
  12. Boztug K, Rosenberg PS, Dorda M, et al. Extended spectrum of human glucose-6-phosphatase catalytic subunit 3 deficiency: novel genotypes and phenotypic variability in severe congenital neutropenia. *J Pediatr*. 2012;160:679–83.e2.
  13. Lin SR, Pan CJ, Mansfield BC, Chou JY. Functional analysis of mutations in a severe congenital neutropenia syndrome caused by glucose-6-phosphatase- β deficiency. *Mol Genet Metab*. 2015;114(1):41–5.
  14. Kulkarni M, Desai M, Gupta M, et al. Clinical, Immunological, and Molecular Findings of Patients with p47^{phox} Defect Chronic Granulomatous Disease (CGD) in Indian Families. *J Clin Immunol*. 2016 Nov;36(8):774–84.
  15. Cella D, Yount S, Rothrock N, et al. The Patient-Reported Outcomes Measurement Information System (PROMIS): progress of an NIH Roadmap cooperative group during its first two years. *Med Care*. 2007;45(5 Suppl 1):3–11.
  16. Rothrock NE, Amtmann D, Cook KF. Development and validation of an interpretive guide for PROMIS scores. *J Patient Rep Outcomes*. 2020;4(1):16.
  17. Chacko BK, Kramer PA, Ravi S, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. *Lab Invest*.

2013;93:690–700.

18. Ssemaganda A, Kindinger L, Bergin P, et al. Characterization of neutrophil subsets in healthy human pregnancies. *PLoS One*. 2014;9(2):e85696.
19. McAvoy EF, McDonald B, Parsons SA, et al. The role of CD14 in neutrophil recruitment within the liver microcirculation during endotoxemia. *J Immunol*. 2011;186(4):2592–601.
20. Fukuda S, Schmid-Schönbein GW. Regulation of CD18 expression on neutrophils in response to fluid shear stress. *Proc Natl Acad Sci U S A*. 2003;100(23):13152–7.
21. Kohler S, Zeller C, Iliev H, Kaspers S. Safety and Tolerability of Empagliflozin in Patients with Type 2 Diabetes: Pooled Analysis of Phase III Clinical Trials. *Adv Ther*. 2017;34(7):1707–26.
22. Yu S. The anhydrofructose pathway of glycogen catabolism. *IUBMB Life*. 2008 Dec;60(12):798–809.
23. Cicalese MP, Ferrua F, Castagnaro L, et al. Gene Therapy for Adenosine Deaminase Deficiency: A Comprehensive Evaluation of Short- and Medium-Term Safety. *Mol Ther*. 2018 Mar 7; 26(3): 917–931
24. Hoffmann D, Kuehle J, Lenz D, et al. Lentiviral gene therapy and vitamin B3 treatment enable granulocytic differentiation of G6PC3-deficient induced pluripotent stem cells. *Gene Ther*. 2020;27(6):297–306.
25. Cowan MJ, Martin DW Jr, Wara DW, Ammann AJ. Intravenous deoxycytidine therapy in a patient with adenosine deaminase deficiency. *Adv Exp Med Biol*. 1984;165 Pt A:39–45.
26. Ammann AJ, Cowan MJ, Martin DW, Wara DW. Dipyridamole and intravenous deoxycytidine therapy in a patient with adenosine deaminase deficiency. *Birth Defects Orig Artic Ser*. 1983;19(3):117–20.

Figures

Figure 1

Characteristics of neutropenia in the G6PC3-deficient patient. (A) Family pedigree. (B) Episodes of infections at different sites. (C) Tongue ulcer. (D) Bone marrow shows left-sided myelopoiesis.

Figure 2

Treatment with empagliflozin lowers plasma 1,5AG and increases neutrophil, platelets and monocytes counts as well as hemoglobin concentration in the blood of a G6PC3-deficient patient. (A) Time course of ANC and 1,5AG versus dosage of empagliflozin before and during treatment in a G6PC3-deficient patient. (B) ANC, monocytes and platelets counts and hemoglobin concentration are all significantly increased during empagliflozin treatment (**** $p < 0.0001$ student's unpaired t-test). Abbreviations – CT = control; EMPA = empagliflozin.

Figure 3

Neutrophil function and phenotype before and during treatment with empagliflozin. (A) DHR fluorescence of normal control versus G6PC3-deficient patient before and during treatment with empagliflozin. (B) Scatter plot of DHR stimulation index of normal controls versus G6PC3-deficient patient before and during treatment with empagliflozin. (C) Flow-cytometric assessment of CD14, CD66B, CD18 and CD15 antigens on neutrophils. CD14, CD66B, CD18 and CD15 were over expressed in the G6PC3-deficient patient compared to the normal controls. Rapid normalization of CD14, CD66B, CD18 and CD15 expression was observed during treatment with empagliflozin. Abbreviations – CT = control; EMPA = empagliflozin.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaldata.pdf](#)