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ORIGINAL ARTICLE

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Successful use of empagliflozin to treat neutropenia in two G6PC3-deficient children: Impact of a mutation in SGLT5

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Abstract

Neutropenia and neutrophil dysfunction found in deficiencies in G6PC3 and in the glucose-6-phosphate transporter (G6PT/SLC37A4) are due to accumulation of 1,5-anhydroglucitol-6-phosphate (1,5-AG6P), an inhibitor of hexokinase made from 1,5-anhydroglucitol (1,5-AG), an abundant polyol present in blood. Lowering blood 1,5-AG with an SGLT2 inhibitor greatly improved neutrophil counts and function in G6PC3-deficient mice and in patients with G6PT-deficiency. We evaluate this treatment in two G6PC3-deficient children. While neutropenia was severe in one child (PT1), which was dependent on granulocyte cololony-stimulating factor (GCSF), it was significantly milder in the other one (PT2), which had low blood 1,5-AG levels and only required GCSF during severe infections. Treatment with the SGLT2-inhibitor empagliflozin decreased 1,5-AG in blood and 1,5-AG6P in neutrophils and improved (PT1) or normalized (PT2) neutrophil counts, allowing to stop GCSF. On empagliflozin, both children remained infection-free (>1 year – PT2; >2 years – PT1) and no side effects were reported. Remarkably, sequencing of SGLT5, the gene encoding the putative renal transporter for 1,5-AG, disclosed a rare heterozygous missense mutation in PT2, replacing the extremely conserved Arg401 by a histidine. The higher urinary clearance of 1,5-AG explains the more benign neutropenia and the outstanding response to empagliflozin treatment found in this child. Our data shows that SGLT2 inhibitors are an excellent alternative to treat the neutropenia present in G6PC3-deficiency.

Cécile Boulanger and Xavier Stephenne are joint first authors.

KEYWORDS
empagliflozin, G6PC3-deficiency, GSD1b, glycogen Storage Disease 1b, neutropenia, SGLT2-inhibitors, SGLT5, 1,5-anhydroglucitol

INTRODUCTION

Both G6PC3-deficiency present in severe congenital neutropenia type 4 (SCN4)[1] and G6PT/SLC37A4-deficiency found in glycogen storage disease type Ib (GSD1b)[2] cause neutropenia and neutrophil dysfunction, which may lead to life-threatening complications in patients. The mechanism of these neutropenias was recently elucidated showing that in neutrophils, G6PT collaborates with G6PC3 to dephosphorylate and prevent the accumulation of 1,5-anhydroglucitol-6-phosphate (1,5-AG6P), a powerful inhibitor of hexokinases.[3] 1,5-AG6P is made in a side reaction of the glucose phosphorylating enzymes on 1,5-anhydroglucitol (1,5-AG), a glucose analogue present in blood in concentrations ranging from 100 to 150 μM.[4] The failure to dephosphorylate 1,5-AG6P in G6PC3-deficient and GSD1b patients, inhibits glucose utilization in neutrophils and explains neutropenia and neutrophil dysfunction.[5]

1,5-AG is reabsorbed in the kidney tubules by a Na^+-dependent transporter of the SGLT family.[6] Yet, this reabsorption is inhibited by glucosuria either due to diabetes[7] or induced by the SGLT2 inhibitors that are commonly used to treat diabetes,[8] leading to a decrease in concentration of 1,5-AG in blood. Consequently, empagliflozin has been used, with success, for the treatment of neutropenia in mice deficient in G6PC3[3] and in GSD1b patients.[5,9–12] The goal of the present study was to evaluate this treatment in two patients with G6PC3-deficiency.

1 | METHODS

1.1 | Ethical approval and subjects enrolled

This study was conducted in accordance with the ethical protocol approved by the Cliniques Universitaires Saint-Luc Ethics Committee in Brussels, Belgium (2018-004191-35). Parents or legal guardians of the participants provided written informed consent. Proof is available upon request. The trial was registered at ClinicalTrials.gov—identifier NCT04138251.

Two molecularly diagnosed G6PC3-deficient children (7 years and 3 months and 12 years and 6 month old) were enrolled in this study which reports the successful repurposing of empagliflozin to treat their neutropenia during 420 (PT2) and 839 (PT1) days.

1.2 | Quantification of 1,5-anhydroglucitol (plasma and urine) and glucose (urine)

Quantification of 1,5-AG in plasma and urine was done by LC–MS analysis as previously described.[3,5] Practically, the metabolites in plasma and in 24 h urine collections (see below) were extracted by adding respectively 4 μl of plasma or 2 μl of urine to 91 or 93 μl of a solution containing 81% methanol, 10% H_2O and 9% chloroform and 5 μl of deuterated 30 μM 2-[D]-1,5AG ($m/z = 164.0674$), which was used as an internal standard to allow estimation of plasma or urinary 1,5-AG ($m/z = 163.0612$). Absolute concentrations were determined by comparing the integrated extracted ion chromatograms corresponding to 1,5-AG with those of the internal standard.[5]

Urinary glucose was measured in a 5% perchloric acid extract from an aliquot of a 24 h urine collection, which was neutralized by careful addition of the required volume of a 3 M $KOH/KHCO_3$ solution. After removing the insoluble potassium perchlorate by centrifugation (5 min at 12000*g* at 4°C) the glucose in this extract was measured spectrophotometrically using an hexokinase/glucose-6-phosphate dehydrogenase coupled assay run at 37°C. The assay mixture (1 ml in an assay cuvette) contained 100 mM Hepes buffer at pH 7.2, 10 mM $MgCl_2$, 40 mM KCl, 1 mg/mL BSA, 2 mM ATP-Mg^{2+}, 0.6 mM NAD^+, 6 μl of neutralized urine extract and a nonlimiting amount of glucose-6-phosphate dehydrogenase. After 10 min, we added a nonlimiting amount of yeast hexokinase. The consequent increase in absorbance at 340 nm is directly proportional to the concentration of glucose.

1.3 | Isolation and preparation of PMN and PBMC extracts for 1,5-AG6P determination and for analysis of LAMP2 glycosylation

PMNs and PBMCs to determine 1,5-AG6P and to prepare protein extracts for analysis of LAMP2 glycosylation pattern were isolated from 5 ml of EDTA blood from PT1 and PT2 using the Lymphoprep™ gradient from Alere Technologies AS (Oslo Norway) following the

manufacturer's instructions (https://cdn.stemcell.com/media/files/pis/29283-PIS_1_3_0.pdf). Practically, we used 5 ml of freshly collected EDTA blood that we diluted 2-fold with PBS with 2% fetal bovine serum (FBS). A 2 × 5 ml of the diluted blood was layered on 2 × 2.5 ml of Lymphoprep™ in two 15 ml tubes and centrifuged at 800*g* for 20 min at 20°C with the break off. After discarding the upper plasma layer, the PBMC layer in each tube was collected and washed in 10 ml of PBS + 1% FBS. To isolate the PMNs, the remaining plasma was removed from each tube without disturbing the erythrocyte/PMN pellet before adding 13 ml of filtered red blood cell lysis solution (0.15 M ammonium chloride, 10 mM potassium bicarbonate, 0.1 mM EDTA) per tube and leaving at 20°C for approximately 15 min with occasional mixing until red blood cells have lysed. Next, all four tubes (2 containing the PBMC suspension and 2 containing the lysed red blood cells and PMN suspensions) were centrifuged for 10 min at 800*g* to recover the pellets containing the white blood cells.

Each PBMC pellet was recovered and resuspended in 6 ml of 0.9% NaCl +1% FBS. After cell counting to check the purity of these preparations (see below), both tubes were centrifuged (10 min at 500*g*) to recover the isolated PBMCs in the pellets. One of the tubes (Tube 1) was used to extract the metabolites for subsequent detection of 1,5-AG6P by LC–MS analysis and the other one (Tube 2) was used to prepare a protein extract for LAMP2 analysis. The extract from the pellet of PBMCs in Tube 1 was prepared by addition of 1 ml of ice-cold 50% methanol/H_2O and 1 ml of chloroform as previously described.[5] The PBMC pellet in Tube 2 was used to prepare a protein extract for LAMP2 analysis by adding 150 µl of RIPA buffer as previously described.[5]

To prepare similar extracts from PMNs, each of the two PMN pellets was first washed to remove the red blood cell lysis solute by resuspending the cells in PBS + 1% FBS (10 ml for each tube) and recovering the cells by centrifugation (10 min at 500*g*). Each pellet was resuspended in 5 ml of 0.9% NaCl +1% FBS and after cell counting, both tubes were finally centrifuged (10 min at 500*g*) and the pellets recovered in 0.5 ml of 50% methanol/H_2O ice-cold solution and 0.5 ml chloroform (Tube 1) and 100 µl of RIPA buffer (Tube 2). Cells were counted to check the purity of the preparations using a Sysmex hematology analyzer in the body fluid cellular analysis mode. This indicated that the PBMC preparations were > 95% pure and the PMN ones around 85% with approximately 15% of PBMCs.

Quantification of 1,5-AG6P and analysis of LAMP2 glycosylation in the PMNs and PBMCs extracts described above were done by LC–MS or western blot analysis of the respective extracts as previously described.[5]

1.4 | Quantification of 1,5-anhydroglucitol-6-phosphate in leukocytes

Determination of the content of 1,5-AG6P in granulocytes was obtained by LC–MS analysis of 1,5-AG6P in leukocytes present in two types of samples. (1) In an extract prepared from a buffy coat plus the red blood cell pellet (after plasma removal) resulting from a centrifugation of 0.4 ml of EDTA blood to collect plasma; (2) from a leukocyte extract (prepared after red blood cell lysis) also derived from 0.4 ml of blood as already described in detail in a previous publication.[5]

1.5 | Urinary clearance for 1,5-AG and glucose

To calculate urinary glucose and 1,5-AG clearances, we performed 24-h urine collections which were kept at 4°C during the collection period to prevent glucose and 1,5-AG degradation. Once the 24-h period ended, care was taken to mix the collections, measure the total volume and immediately freeze an aliquot at −20°C for 1,5-AG and glucose analysis. 1,5-AG clearance was calculated in L/day based on the relevant plasma and urine concentrations of 1,5-AG that were determined as described above. Glucose clearance (L/day), was calculated based on the glucose concentration measured in the urine samples while the plasma concentration during the 24-h collection period was assumed to be 5 mM.

2 | RESULTS

2.1 | Case descriptions

PT1 is a boy born to healthy consanguineous Turkish parents. He was hospitalized when he was 10 days old in a neonatal intensive care unit for pulmonary hypertension, and chronic severe neutropenia was quickly detected. This resulted in granulocyte cololony-stimulating factor (GCSF) treatment (3 times/week) at the age of 2 months but despite this, recurrent infections, sometimes requiring hospitalization, kept on occurring during infancy and childhood. Genetic analysis revealed G6PC3-deficiency (Table 1). The empagliflozin treatment started when he was 7 years and 3 months.

PT2 is a girl born at 36 weeks gestation with mild growth retardation from healthy consanguineous Romanian parents. Severe neutropenia was identified at

birth following a generalized infection that required hospitalization. During infancy, she suffered from numerous infections which were mainly treated with intravenous antibiotics and leading to a failure to thrive. Her follow-up started in Belgium at the age of 6 when genetic G6PC3-deficiency was established (Table 1). Unlike PT1, she was only treated with GCSF on request, depending on the severity and the need to control infections. She enrolled the study at 12 years and 6 months.

Relevant demographic and clinical data for both children before empagliflozin treatment are assembled in Table 1.

2.2 | Response to empagliflozin treatment and adjustment of the dose based on blood biomarkers and neutrophil counts

Before empagliflozin treatment, both patients suffered from recurrent infections; PT1 was continuously treated with daily injections of GCSF, which increased but did not normalize neutrophil counts (mean = 1.05×10^6/ml; median = 0.92×10^6/ml; range: 0.19–2.98 $\times 10^6$/ml; $n = 8$), while PT2, despite low neutrophil counts (mean = 0.28×10^6/ml; median = 0.22×10^6/ml; range: 0.10–0.59 $\times 10^6$/ml; $n = 23$), was treated with GCSF only during infection episodes (Figure S1).

Since empagliflozin acts by lowering 1,5-AG in blood and 1,5-AG6P in neutrophils, we carefully assessed these two biomarkers during treatment. PT1 had before treatment a concentration of 1,5-AG in blood (120 μM) in the range of values reported for a normal population (mean = 76 μM; range: 47–106 μM),[4] but this level was less than half for PT2 (50 μM) (Figure 1A, B). A similar difference between PT1 and PT2 was observed for the level of 1,5-AG6P in the leukocytes (Figure 1C-D) (Figure S2), presumably as a consequence of the difference in plasma 1,5-AG concentration.

In both cases, treatment with empagliflozin resulted in ≈ 6-fold reduction in 1,5-AG in blood (Figure 1A, B) and 1,5-AG6P in leukocytes (Figure 1C, D and l Figure S1). Of note, both biomarkers reached a lower base-line in PT2 than in PT1. Empagliflozin caused a rapid normalization of the neutrophil counts (ANC) in PT2 (Figure 1B and Figure S1 – panel PT2 ANC). Following this remarkable response, the empagliflozin dose was lowered to 10 mg every second day (i.e., 0.12 mg/kg/day) from day 119 onwards. At day 420 (the last day of treatment reported here), ANC values were normal (4.2×10^6/ml; Figure 1B; Table 1) and plasma 1,5-AG (5.2 μM) and neutrophil 1,5-AG6P were remarkably low (Figure 1B and D).

The response to empagliflozin in terms of increase of ANC was less remarkable in PT1, presumably because of his higher levels of 1,5-AG and 1,5-AG6P. In this case, GCSF injections were stopped just before empagliflozin treatment began. ANC quickly fell to worryingly low levels (0.05×10^6/ml) requiring a new short period of GCSF injections until plasma 1,5-AG was sufficiently low. At this stage (day 45) an attempt to stop GCSF was successful, and ANC stabilized at about 10^6/ml (day 56–84), that is, ≈ 20-fold higher than during the first GCSF-free period (Figure 1A and Figure S1—panel PT1 ANC). A transient period of higher empagliflozin dosage (≈0.35 mg/kg/day), was accompanied by a further decrease in the 1,5-AG6P levels (Figure 1C) and increase in ANC ($2.3 \pm 0.8 \times 10^6$/ml). Despite this, ANC in PT1 failed to reach the numbers in PT2 ($4 \pm 1 \times 10^6$/ml) who was taking an approximate 3-fold lower dosage of empagliflozin (see above).

As PT1 was clinically stable and infection-free, we lowered his empagliflozin dose to 10 mg every second day (i.e., 0.15 mg/kg/day,) from day 406. This resulted in an increase in plasma 1,5-AG concentration (≈9 to 16 μM), a progressive rise of 1,5-AG6P in blood and leukocytes (Figure 1C and Figure S2), and a progressive decrease in ANC to 0.42×10^6/ml on day 775 (Figure 1A and Figure S1), although PT1 remained clinically stable and free of infections. Because of the decrease in the neutrophil counts and of his weight gain, empagliflozin was increased back to 10 mg every day (0.28 mg/kg/day) on day 811. This resulted in the expected decreases in plasma 1,5-AG (Figure 1A) and cellular 1,5-AG6P (Figure 1C; Figure S2) and a rise in ANC (to 1.55×10^6/ml).

At this stage (day 839—PT1; day 420—PT2) we measured neutrophil function in PT1 and PT2 and found it to be in the normal range, in agreement with their excellent (infection-free) clinical state. Of note, before treatment the neutrophil function for PT2 had been shown to be defective. Furthermore, hemoglobin increased in both patients, which could be related to ending GCSF injections in PT1, while there was no major change for the other blood cells counted, except for ANC (Figure S1).

2.3 | Analysis of glycosylation in neutrophils

A consequence of hexokinase inhibition is that glycosylation in neutrophils is pathologically decreased.[5,13] This could be shown by western blot analysis for LAMP2, a highly glycosylated protein, which migrated with an apparent molecular mass of ≈115 kDa in control samples, but as a smear with lower apparent molecular mass

TABLE 1 Clinical data for PT1 and PT2 before and on empagliflozin treatment

	PT1	PT2
Demographic and clinical data		
Genetic variant in *G6PC3*	c.765_766delAG, p.Ala257fs Homozygous	c.960del, p.Trp320fs Homozygous
Sex	Male	Female
Country of origin	Turkey	Romania
Gestational age at birth	38 weeks	36 weeks
Weight at birth	2.56 kg	2.30 kg
Neonatal infections[a]	Sepsis, pneumonia	Bilateral pneumonia, omphalitis, conjunctivitis
Other infections[a]	3–5 severe infections/year mainly nose throat and ear	Bronchitis, pneumonia, furunculosis
Other findings and malformations	Transient pulmonary arterial hypertension, atrial septal defect, specific peripheral venous laci, cryptorchidism	Atrial septal defect, specific peripheral venous laci, kidney cyst
Cardiac surgery	Yes	Yes
Other	Mild peripheral hypotonia, slight delay in fine motor skills	Skin aspect of lipodystrophy, failure to thrive
GCSF treatment	Yes, continuous	Yes, on request
Hospitalizations[b]	Turkey: ≥3 between 1 and 6 year old[a]: Mastoiditis, sinusitis, gastro-enteritis None from 6 year old onwards	Romania: Several from birth to 8 year old[a] 5 between the ages of 8 to 11 year old
Days in hospital[b]	data not available	4.8
At the start of empagliflozin		
Age	7 year and 3 month old	12 year and 6 month old
Weight and (SDS)	26 (0.43) kg	42.2 (−0.39) kg
Height and (SDS)	1.22 (−0.6) m	1.52 (−0.72) m
BMI and (SDS)	17.5 (1.04) kg/m^2	18.4 (−0.05) kg/m^2
Neutrophils	110 ANC/μl[c]	390 ANC/μl
Hemoglobin	10.3 g/dl	14.2 g/dl
Initial empaglifozin dosage	0.19 mg/kg/day	0.24 mg/kg/day
On optimized empagliflozin treatment		
Days of treatment	839	420
Weight and (SD)	37 (0.98) kg	44.3 (−0.71) kg
Height and (SD)	1.39 (0.31) m	1.58 (−0.47) m
BMI and (SD)	19.15 (1.1) kg/m^2	17.75 (−0.61) kg/m^2
Neutrophils	1550 ANC/μl	4200 ANC/μl
Hemoglobin	13.8 g/dL	15.0 g/dL
Optimized empagliflozin dosage	0.28 mg/kg/day	0.11 mg/kg/day
No. of hospitalizations	0	0
No. of days in hospital	0	0
GCSF treatment	Stop	No

Abbreviations: ANC, absolute neutrophil counts; BMI, body mass index; SDS, standard deviation score.

[a]Retrospective data collected from parents.

[b]For infection or fever. Mean per year, calculated over 5 years.

[c]GCSF stopped 2 days before starting empagliflozin.

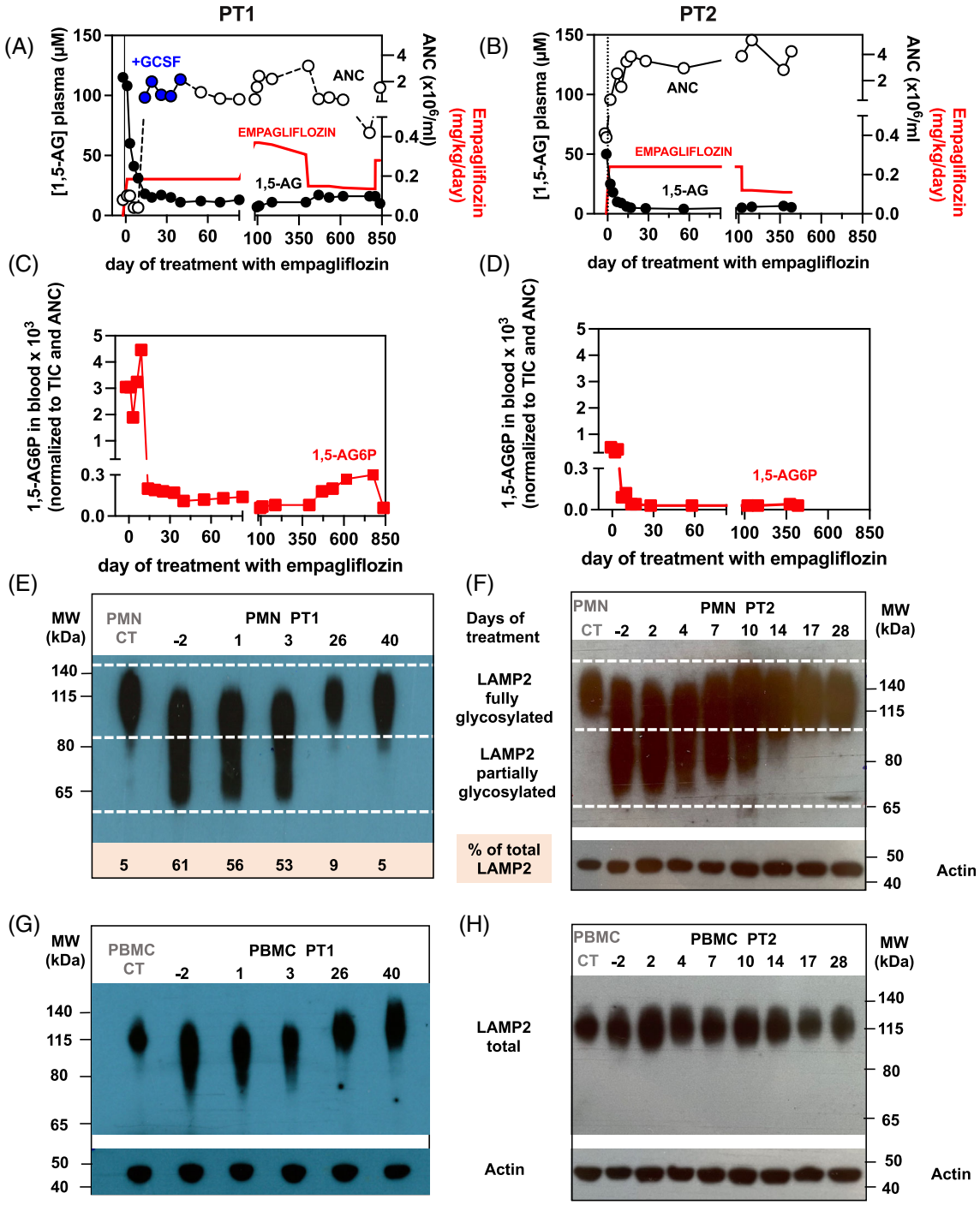

FIGURE 1 Empagliflozin improves neutropenia by lowering plasma 1,5-AG and intracellular 1,5-AG6P, and corrects protein glycosylation in PMNs and PBMCs from two G6PC3-deficient patients. (A, C, E, and G) show results for individual 1 (PT1) treated for 839 days and (B, D, F and H) results for individual 2 (PT2) treated for 420 days. (A and B) Time course of absolute neutrophil counts (ANC) and 1,5-AG concentration in plasma before and during treatment with empagliflozin (dosage indicated by the red line). (C and D) 1,5-AG6P in neutrophils determined during treatment by liquid-chromatography-mass spectrometry (LC–MS) in blood cells after plasma removal (for 1,5-AG determination) and normalized to total metabolite content (total ion current, TIC)[3] and ANC. (E-H) Western blots illustrating the almost complete correction of glycosylation of the protein LAMP2 in granulocytes (PMNs—E and F) and peripheral blood mononuclear cells (PBMCs—G and H) isolated before and during treatment with empagliflozin and compared with a healthy control (CT).

in extracts of granulocytes (polymorphonuclear cells—PMNs) from PT1 and PT2 before empagliflozin treatment (Figure 1E-F). Empagliflozin progressively normalized the migration of LAMP2 in both individuals, indicating that hexokinase inhibition was suppressed. Of note, the glycosylation of LAMP2 in isolated peripheral blood mononuclear cells (PBMCs) was slightly abnormal in PT1 before treatment and normalized by empagliflozin (Figure 1G). By contrast, even before treatment, no abnormal migration of LAMP2 was observed in PBMCs from PT2 (Figure 1H), further indicating that the toxic effects of 1,5-AG6P were less apparent in PT2 than in PT1.

Interestingly, results reported by others have shown, in G6PC3-deficient and GSD1b patients, a subtle lymphopenia affecting certain types of T lymphocytes and impairment in their cell function. This was linked to an increased autoimmune risk in GSD1b patients[14] and G6PC3-deficiency.[15] Our findings suggest that the observations reported by these authors could be related to the accumulation of 1,5-AG6P and inhibition of hexokinase in PBMCs of these patients, though in a less marked fashion than in neutrophils. This is supported by the finding that the content of 1,5-AG6P in PBMC from PT2 was about 10-fold lower than in his neutrophils (Figure S3).

2.4 | Urinary excretion and renal absorption of 1,5-anhydroglucitol

Investigation of urinary excretion of glucose and 1,5-AG indicated that empagliflozin caused a striking increase in glucose and 1,5-AG excretion (Figure 2A, B) in both patients. While glucosuria remained constantly elevated with time, 1,5-AG excretion peaked soon after empagliflozin treatment was started, then lowered to reach a steady-state value after a few weeks. The peak of 1,5-AG excretion corresponds to the loss of most of the body pool of 1,5-AG, while the subsequent steady-state excretion value corresponds to the sum of the rates of 1,5-AG dietary intake and its metabolic formation. Remarkably, total daily urinary excretion of 1,5-AG was similar for PT1 and PT2 (55 ± 7 and 49 ± 2 μmol/day, respectively; Figure 2A, B), indicating similar dietary intake, but 1,5-AG clearance was about 3-fold higher in PT2 compared to PT1 (Figure 2C, D), while glucose clearance on empagliflozin treatment was similar.

As this suggested that the reabsorption of 1,5-AG in the kidney tubule is less efficient in PT2 than in PT1, we searched for mutations in the Na^+-dependent 1,5-AG transporter. Although this transporter was proposed to be SGLT4/SLC5A9,[16] recent results indicate that SGLT5/SLC5A10 is a more likely candidate, as single nucleotide polymorphisms and rare inactivating mutations in SLC5A10 correlate with the 1,5-AG levels in blood.[17–19] Sequencing of the SLC5A10 exons from PT2 disclosed a missense mutation (c.1243G>A; NM_001042450) replacing Arg401 by a histidine (Figure 2E). Arg401 is a highly conserved residue in SGLT5 and in many other members of the SLC5 family (Figure 2E). Inspection of the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org) indicates that the frequency of this variant is quite low (2.4×10^{-5}). Of note, in the gnomAD data base, the p.Arg401His variant present in the canonical sequence of SLC5A10 (NP_001035915.1) is named p.Arg417His, which relates to a longer but non-conserved isoform (NP_689564.3). In the calculated structure of SGLT5 (https://alphafold.ebi.ac.uk),[20] Arg401 is present in a loop where its side chain makes a salt bridge with a highly conserved glutamate (Glu408) in a neighboring alpha helix (Figure S4). It is plausible that replacement of Arg401 with a histidine weakens this salt bridge, leading to decreased activity of SGLT5, lower 1,5-AG renal reabsorption, and the outstanding response to empagliflozin treatment (Figure 2F).

3 | DISCUSSION

This is the first report showing that empagliflozin can be successfully repurposed to treat neutropenia and neutrophil dysfunction in G6PC3 deficiency. Our results indicate that it might be more efficient in treating the neutropenia in G6PC3-deficient compared to the one in GSDIb patients: (1) the dose of empagliflozin required to treat the patients (0.11 and 0.28 mg/kg/day – Table 1 – compared to 0.4–0.7 mg/kg/day[5]) was lower; (2) this lower dose led to a lower concentration of 1,5-AG in plasma (PT2: 5 μM; PT1: 10 μM compared to 34–90 μM in GSD1b);[5] (3) this improved the neutrophil count and, without necessarily normalizing it, ended infection episodes and the need for the administration of GCSF, which is not only expensive, but also known to have several side effects.[21] Of note, no side effects of the empagliflozin treatment were noted in this study.

As the effect of gliflozins to enhance renal 1,5-AG excretion is due to the indirect effect of glucosuria caused by SGLT2 inhibition, whereby elevation of glucose in the renal filtrate leads to a competitive inhibition of 1,5-AG reabsorption by a different transporter,[22] part of the difference in empagliflozin efficiency is probably explained by the lower blood glucose levels found in GSDIb patients compared to the G6PC3 ones. In the case of PT2, the presence of a heterozygous mutation in SGLT5 most

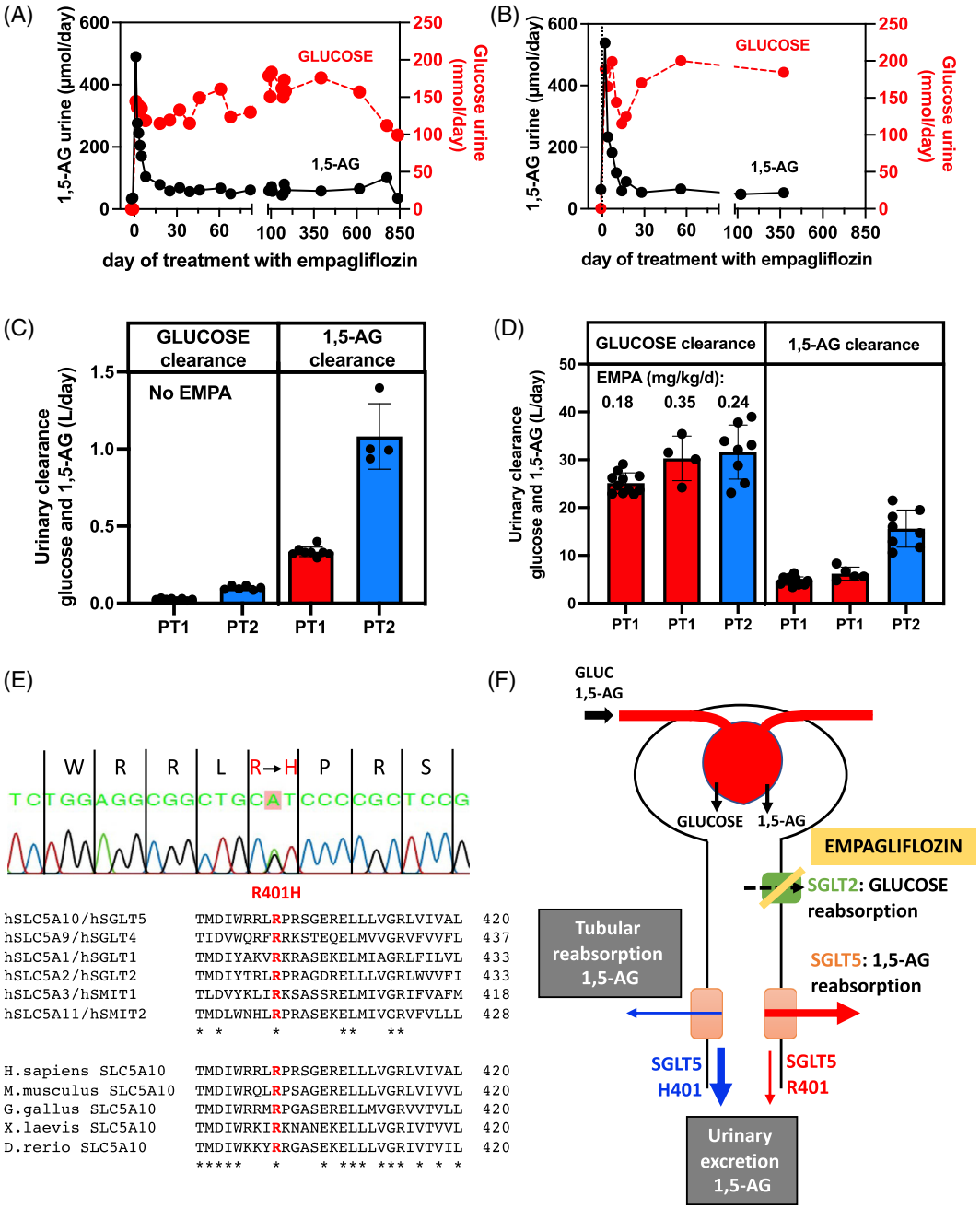

FIGURE 2 The replacement of the highly conserved arginine R401 by a histidine in SGLT5 is associated with a higher 1,5-anhydroglucitol urinary clearance. (A and B) Urinary excretion over 24 hours of glucose and 1,5-AG before and during empagliflozin treatment. (C) Glucose and 1,5-anhydroglucitol (1,5-AG) urinary clearance measured in 24-h urine collections before empagliflozin treatment; measurements were repeated in triplicate (or more) for two different collections for PT1 and one collection for PT2. (D) Glucose and 1,5-AG urinary clearance measured in 24-hr urine collections from PT1 and PT2 on empagliflozin treatment at the indicated dose; measurements shown are respectively for $n = 11$, $n = 5$ (PT1 taking 0.18 or 0.35 mg/kg/day EMPA) or $n = 8$ (PT2 taking 0.24 mg/kg/day EMPA) in different collections. Note the different scale used to represent clearance in C and D. (E) DNA sequence of exon11 in the SGLT5 gene of PT2 showing the heterozygous missense change c.1243G>A (NM_001042450) corresponding to p.R401H (shown in gnomAD as R417H). The protein alignments show strict conservation of the amino acid R401 in six members of the human sodium-glucose transport protein family (hSLC5A10: NP_001035915; hSLC5A9: NP_001011547; hSLC5A1: NP_000334; hSLC5A2: NP_003032; SMIT2: NP_001339171; SMIT1: NP_008864) as well as among the indicated SLC5A10 orthologs (NP_001035915; NP_001369197; XP_001233919; XP_041435094; XP_017211446). (E) Schematic representation of the impact of the heterozygous replacement of the arginine 401 by a histidine in SGLT5 (D). The R401H change is suggested to decrease the tubular reabsorption and increase the urinary excretion of 1,5-AG (blue arrows) in comparison to the wild-type SGLT5 (red arrows). The R401H replacement is likely to result in a lower renal reabsorption of 1,5-AG, explaining the observed increase in 1,5-AG clearance before (C) and during (D) empagliflozin treatment. During empagliflozin treatment, SGLT2, the renal glucose transporter is inhibited and the resulting glucosuria inhibits the tubular reabsorption of 1,5-AG. This lowers plasma 1,5-AG and intracellular 1,5-AG6P.

likely also has an important role. This must be equally so for subjects bearing other SGLT5 variants (p.Asn96Ile and p.Gly471Glu – p.Gly487Glu in gnomAD) known to be associated, in the heterozygous state, with a ≈50% decrease in the concentrations of plasma 1,5-AG.[19] The total frequency of these two variants in the heterozygous state amounts to ≈ 2% in the European population (0.6% for p.Asn96Ile and 1.2% for p.Gly471Glu).

With this and other considerations in mind, an important message of this report is the need to adapt the dose of empagliflozin individually to each patient based on biological (plasma 1,5AG concentration; ANC counts) and clinical criteria.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Maria Veiga-da-Cunha, Nathalie Chevalier, Jennifer Diederich, and Pierre Mounkoro collected the data. Maria Veiga-da-Cunha and Emile Van Schaftingen performed data analysis and wrote the manuscript; Maria Veiga-da-Cunha constructed the figures; Cécile Boulanger, Xavier Stephenne and Alina Ferster treated the patients. All authors reviewed, contributed and edited the manuscript.

ETHICAL APPROVAL

No animal models have been involved in the conduction of this study.

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REFERENCES

1. Boztug K, Appaswamy G, Ashikov A, et al. A syndrome with congenital neutropenia and mutations in G6PC3. *N Engl J Med*. 2009;360(1):32-43.
2. Veiga-da-Cunha M, Gerin I, Chen YT, et al. A gene on chromosome 11q23 coding for a putative glucose- 6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. *Am J Hum Genet*. 1998;63(4):976-983.
3. 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):1241-1250.
4. Ying L, He X, Ma X, et al. Serum 1,5-anhydroglucitol when used with fasting plasma glucose improves the efficiency of diabetes screening in a Chinese population. *Sci Rep*. 2017;7(1):11968.
5. 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;136(9):1033-1043.
6. Yamanouchi T, Tachibana Y, Akanuma H, et al. Origin and disposal of 1,5-anhydroglucitol, a major polyol in the human body. *Am J Physiol*. 1992;263(2 Pt 1):E268-E273.
7. Yamanouchi T, Minoda S, Yabuuchi M, et al. Plasma 1,5-anhydro-D-glucitol as new clinical marker of glycemic control in NIDDM patients. *Diabetes*. 1989;38(6):723-729.
8. Chao EC, Henry RR. SGLT2 inhibition — a novel strategy for diabetes treatment. *Nat Rev Drug Discov*. 2010;9(7):551-559.
9. Grunert SC, Elling R, Maag B, et al. Improved inflammatory bowel disease, wound healing and normal oxidative burst under treatment with empagliflozin in glycogen storage disease type Ib. *Orphanet J Rare Dis*. 2020;15(1):218.
10. Rossi A, Miele E, Fecarotta S, et al. Crohn disease-like enterocolitis remission after empagliflozin treatment in a child with glycogen storage disease type Ib: a case report. *Ital J Pediatr*. 2021;47(1):149.
11. Mikami M, Arai A, Mizumoto H. Empagliflozin ameliorated neutropenia in a girl with glycogen storage disease Ib. *Pediatr Int*. 2021;63:1394-1396.
12. Magdalena Kaczor MG, Kierus K, vel Emczyńska-Seliga EE, et al. Sodium-glucose cotransporter type 2 channel inhibitor: breakthrough in the treatment of neutropenia in patients with glycogen storage disease type 1b? *JIMD Rep*. 2022;63(3):199-206.
13. Hayee B, Antonopoulos A, Murphy EJ, et al. G6PC3 mutations are associated with a major defect of glycosylation: a novel mechanism for neutrophil dysfunction. *Glycobiology*. 2011;21(7):914-924.
14. Melis D, Carbone F, Minopoli G, et al. Cutting edge: increased autoimmunity risk in glycogen storage disease type 1b is associated with a reduced engagement of glycolysis in T cells and an impaired regulatory T cell function. *J Immunol*. 2017;198(10):3803-3808.
15. Begin P, Patey N, Mueller P, et al. Inflammatory bowel disease and T cell lymphopenia in G6PC3 deficiency. *J Clin Immunol*. 2013;33(3):520-525.
16. Tazawa S, Yamato T, Fujikura H, et al. SLC5A9/SGLT4, a new Na+−dependent glucose transporter, is an essential transporter for mannose, 1,5-anhydro-D-glucitol, and fructose. *Life Sci*. 2005;76(9):1039-1050.
17. Li M, Maruthur NM, Loomis SJ, et al. Genome-wide association study of 1,5-anhydroglucitol identifies novel genetic loci linked to glucose metabolism. *Sci Rep*. 2017;7(1):2812.

18. Long T, Hicks M, Yu HC, et al. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. *Nat Genet*. 2017;49(4):568-578.
19. Loomis SJ, Kottgen A, Li M, et al. Rare variants in SLC5A10 are associated with serum 1,5-anhydroglucitol (1,5-AG) in the atherosclerosis risk in communities (ARIC) study. *Sci Rep*. 2019;9(1):5941.
20. Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596(7873):583-589.
21. Beaupain B, Leblanc T, Reman O, et al. Is pegfilgrastim safe and effective in congenital neutropenia? An analysis of the French severe chronic neutropenia registry. *Pediatr Blood Cancer*. 2009;53(6):1068-1073.
22. Fortuna D, McCloskey LJ, Stickle DF. Model analysis of effect of canagliflozin (Invokana), a sodium-glucose cotransporter 2 inhibitor, to alter plasma 1,5-anhydroglucitol. *Clin Chim Acta*. 2016;452:138-141.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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