Pyridoxine Responsiveness in a Type 1 Primary Hyperoxaluria Patient With a Rare (Atypical) *AGXT* Gene Mutation

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INTRODUCTION

Primary hyperoxaluria (PH) refers to a group of genetic disorders that result from increased endogenous production of oxalic acid. Type 1 PH (PH1) is the most common and most severe form, and is caused by deficiency or loss of the liver-specific, vitamin-B6—dependent peroxisomal enzyme alanine: glyoxylate aminotransferase (AGT) encoded by the *AGXT* gene.[1] This results in overproduction and excessive urinary excretion of oxalate that can then cause recurrent kidney stones and nephrocalcinosis.[S1] Vitamin B6 (pyridoxine) responsiveness, as assessed by a decrease in urinary oxalate excretion by 30% or more while on pyridoxine in pharmacologic doses of 5-10 mg/kg/day, has been most clearly associated with 2 mutations in the *AGXT* gene: c.508G>A (p.Gly170Arg) which accounts for approximately 30% of cases in North America, and more rarely c.454T>A (p.Phe152Ile), but not for c.121G>A (p.Gly41Arg).[2,3] Here we present a case of PH1 homozygous for the p.Gly41Arg mutation that was found to be partially responsive to vitamin B6 therapy. These results reinforce the importance of an empirical trial of vitamin B6 in all PH1 patients, regardless of the underlying mutation(s).

CASE PRESENTATION

A 32-year-old man from Saudi Arabia with a frequent history of kidney stones presented to our clinic for further evaluation. His first kidney stone episode occurred at age 5 years, when he required urologic intervention for an obstructing stone. Since that time,

he had passed 1 to 2 kidney stones every month and required on average 1 or more shockwave lithotripsies and/or ureteroscopic stone procedures every year. When analyzed, passed or removed kidney stones were invariably composed of calcium oxalate monohydrate. At the time of a metabolic workup for his recurrent stones in 1993 he had normal kidney function with a serum creatinine of 0.6 mg/dl and a urinary oxalate excretion of 70 mg/d (normal, <40.5 mg/24 h). His maternal grandmother and paternal uncle had a history of kidney stone disease, whereas his parents and siblings (4 brothers) were stone free. There was a history of consanguinity. He reported a conscious effort toward high daily water intake and consumption of a diet relatively rich in oxalic acid (hummus and tahini) and low in calcium. However, after initial consultation at our center he began a lower-oxalate, higher-calcium diet. There was no history of gastrointestinal disease or chronic diarrhea.

Serum creatinine was 4.02 mg/dl on presentation with an estimated glomerular filtration rate of 18 ml/min per 1.73 m^2. A 24-hour urine collection revealed a volume of 4.1 L, oxalate of 134 mg (9.7—40.5 mg/24 h), citrate of 87 mg (235—1191 mg/24 h), and calcium of 83 mg (<250 mg/24 h). Plasma oxalate was also elevated at 13.7 μmol/ (<1.6 μmol/l). A random urinary hyperoxaluria metabolite panel revealed normal urinary glycolate-, glycerate-, and 4-hydroxy-2-oxoglutarate (HOG)—to-creatinine ratios, with elevated urinary oxalate-to-creatinine ratio. A noncontrast computed tomogram of the abdomen revealed more than 10 stones in each kidney (largest, 1.2 cm on the right and 1.0 cm on the left), with a 0.9-cm calculus in the distal left

ureter, for which he underwent ureteroscopy with stent placement.

With the overall picture suggestive of PH, his dose of pyridoxine was empirically increased from the 50 mg daily he had been taking for 1 month to 500 mg twice daily (5 mg/kg per day), and potassium citrate 20 mEq twice daily was initiated. He underwent genetic testing, which ultimately determined a diagnosis of PH1 with a homozygous *AGXT* gene mutation c.121G>A (p.Gly41Arg). After results of the genetic testing were known, he was advised to stop pyridoxine. Four months later, his 24-hour urine oxalate excretion increased from 110 mg to 297.5 mg (Figure 1), and plasma oxalate increased from 9.4 to 28.1 µmol/l despite a stable serum creatinine (3.25—3.42 mg/dl). Based upon these results, he was advised to resume pyridoxine (500 mg twice daily). One week later, urine oxalate excretion returned to his previous baseline of 102.1 mg/d and plasma oxalate to 6.5 µmol/l (Figure 1). Additional testing determined that he did *not* have the minor *AGXT* allele-p.Pro11Leu, which is associated with mistargeting of AGT to the mitochondria, and most often implicated in cases of pyridoxine responsiveness (Figure 2).

DISCUSSION

More than 200 causative mutations in the *AGXT* have been reported.[S2] The majority of them (63%) are point mutations, whereas the remaining 37% include spicing substitutions, small deletions, insertions, and nonsense mutations.[S2,S3] The most commonly identified mutation to date is the c.508G→A missense mutation, which leads to the p.Gly170Arg amino acid replacement. This has an estimated allelic frequency of about 30% in the European and North American PH1 population.[1] This
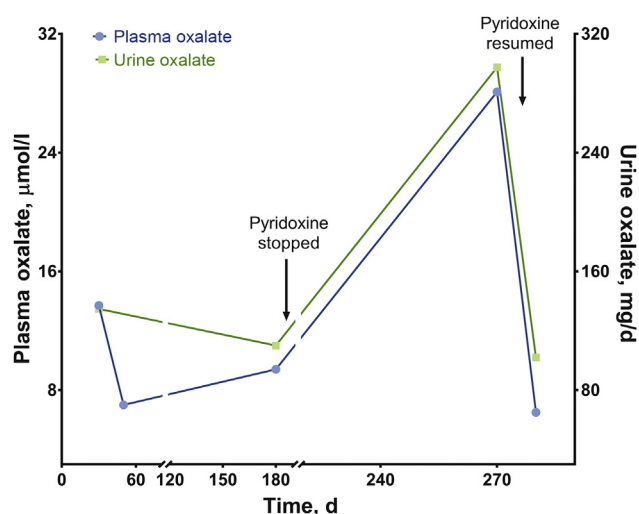

Figure 1. Plasma oxalate and urine oxalate levels with and without pyridoxine therapy.

mutation appears to have little or no effect on properties of the AGT enzyme when present on its own, but when located on the minor allele, that is with the p.Pro11 Leu variant, it acts synergistically to delay AGT dimerization.[1,2] When the p.Gly170Arg and p.Pro11Leu amino acid changes are present in combination, they are predicted to cause major structural alterations at the N-terminus of AGT and generate an amphiphilic α-helix characteristic of a mitochondrial target sequencing (MTS).[2,S4] When present in isolation, the p.Pro11Leu MTS is usually functionally ineffective, as it is tightly bound to the neighboring subunit of dimeric protein.[4] As a result, only a small proportion (∼5%) of AGT encoded by the minor allele finds its way to the mitochondria.[S4] However, when p.Gly170Arg is also present, p.Pro11Leu is exposed and the p.Pro11Leu-generated MTS is greatly enhanced, resulting in about 90% of the enzyme being rerouted to mitochondria.[2,4,5] It is possible that other genetic changes could also act in concert with p.Pro11Lu to increase mitochondrial targeting.[5,6]

In general, it has been speculated that patients with missense AGT mutations and significant levels of residual enzyme activity are most likely to be pyridoxine responsive.[1,3] In particular, AGT with the p.Gly170Arg mutation retains significant activity but is mislocalized to the mitochondria instead of to peroxisomes, where it is normally localized.[3,S4] A published case series confirmed that PH1 patients homozygous for p.Gly170Arg normalized urinary oxalate excretion on pharmacologic doses of pyridoxine (5—10 mg/kg), whereas heterozygotes demonstrated a partial reduction.[S5] Patients in this series without the p.Gly170Arg mutation were unresponsive to pyridoxine. More recently, Hoyer-Kuhn *et al.* confirmed that mean urinary oxalate excretion was lower in homozygous (1.80 mmol/d per 1.73 m^2) and heterozygous (1.53 mmol/d per 1.73 m^2) p.Gly170Arg patients compared to other confirmed mutations (2.42 mmol/d per 1.73 m^2), with an average maximal response to 5 to 20 mg/kg pyridoxine of 62%, 43%, and 24% in the 3 groups, respectively.[S6] The variability of individual patient response across the 28 weeks on increasing doses highlights the difficulty in clinically assessing pyridoxine response, and the reason to use genotype to try to predict likelihood of response.

p.Gly41Arg mutations may be associated with the minor allele (p.Pro11Leu) or major allele.[S4] When associated with the minor allele, p.Gly41Arg mutations tend to be more severe and cause reduced amounts of immunoreactive AGT with less localized to peroxisomes.[S4] Experimental evidence from cell free translation/transcription systems indicate that the p.Gly41Arg variant displays proteasomal degradation
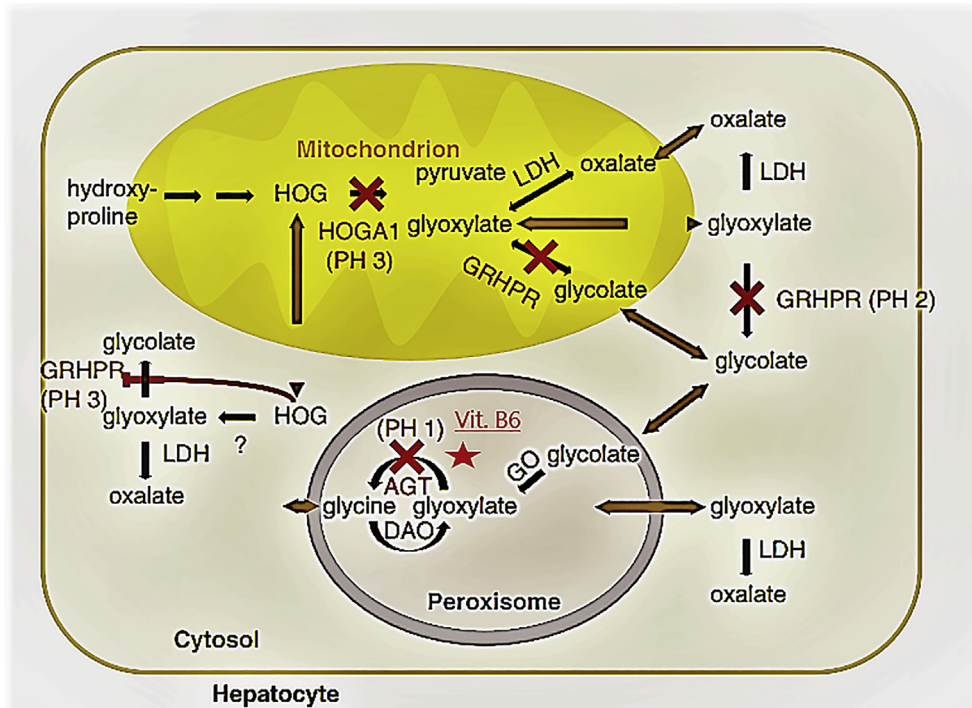
Figure 2. Diagram showing site of action of pyridoxine (vitamin [Vit.] B6) in the AGT enzyme pathway inside a hepatocyte. AGT, alanine: glyoxylate aminotransferase; DAO, D-amino acid oxidase; GRHPR, glyoxylate/hydroxypyruvate reductase; HOG, 4-hydroxy-2-oxoglutarate; HOGA1, 4-hydroxy-2-oxoglutarate aldolase; LDH, lactate dehydrogenase; PH1, type 1 primary hyperoxaluria; PH3, type 3 primary hyperoxaluria.

and a propensity to aggregate.[6] Earlier work from Coulter-Mackie *et al.* suggested that pharmacological concentrations of pyridoxal phosphate (PLP) could rescue *in vitro* AGT enzyme activity.[7] However it was speculated that administration of pyridoxine alone would not be sufficient to treat PH1 patients with the p.Gly41Arg mutation as the molecular defect is related to both apo and holo forms of the enzyme (AGT) prone to degradation and aggregation.[2] In a more recent *in vivo* cell culture study, Fargue *et al.* reported that the p.Pro11Leu change, when present with mutants other than p.Gly170Arg (p.Ile244Thr and p.Phe152Ile), could improve the activity of AGT with exogenous pyridoxine.[3] Although it was hypothesized that pyridoxine could potentially act as a prosthetic group to exert a chemical chaperone effect to aid in proper folding and/ or dimerization of the protein and thereby rescue activity, in that study, p.Gly41Arg appeared to aggregate in peroxisomes, and exogenous pyridoxine did not improve enzyme activity.

However, as the p.Gly41Arg mutation typically segregates with the minor allele haplotype (p.Pro11-Leu), Fargue *et al.* did not study p.Gly41Arg on the background of the major allele (as was present in our case).[3] The Gly41 residue sits in the center of a dimerization interface and when substituted, the large side-chain of the arginine moiety is predicted to alter protein dimerization.[2] Thus, by reducing resistance to

thermal inactivation, this mutation induces suscepti-bility to proteolytic degradation and self-aggrega-tion.[8,S4,S7] Two other *in vitro* studies (Lumb and Danpure and Coulter-Mackie *et al.*) did look at the p.Gly41Arg mutations on the major allele, and sug-gested some residual activity, with a Km for pyridoxine between that of wild-type AGT and AGT on the minor allele (p.Pro11Leu) background.[5,7] Results in our case study suggest that exogenous pyridoxine, indeed, rescued enzyme activity. However, unlike other pyridoxine-responsive mutations, this was most likely

Table 1. Teaching points

PH1 is a genetic disorder resulting from mutations in liver-specific peroxisomal enzyme AGT encoded by the *AGXT* gene.
PH1 results in marked overproduction of endogenous oxalate, leading to excessive urinary excretion resulting in early onset recurrent nephrolithiasis, nephrocalcinosis and in some cases ESKD.
The most commonly identified mutation in European and North American population is a missense mutation c.508G>A (p.Gly170Arg), which almost always segregates with the p.Pro11 Leu variant and causes mistargeting of AGT from peroxisome-to-mitochondria, thus rendering the enzyme inactive.
Pyridoxine (vitamin B6) responsiveness has been shown in human and cell line studies to be associated with the more commonly seen p.Gly170Arg mutations when present with p.Pro11leu variant (minor allele).
However, as shown in our report, pyridoxine responsiveness was also seen in a non-mistargeting *AGXT* mutation-p.Gly41Arg not associated with the p.Pro11Leu variant (major allele).
An empirical trial of pyridoxine therapy may therefore be tried in PH1 patients who also show less common *AGXT* missense pathologic variants (such as p.Gly41Arg).

AGT, alanine:glyoxylate aminotransferase; ESKD, end-stage kidney disease; PH1, type 1 primary hyperoxaluria.

not due to reduced mistargeting of a functional enzyme to mitochondria and away from its normal peroxisome location, as our patient lacked the p.Pro11LeuMTS.[6] Instead it seems likely that exogenous pharmacological doses of pyridoxine stabilized the protein, improved dimerization, decreased aggregation, and increased catalytic activity. It is also possible that pyridoxine could rescue a small but significant amount of AGT enzyme activity of the p.Gly41Arg mutation (reported at 7% in a single case report) via allosteric mechanisms when paired with the major allele.[9]

Thus, mutations (p.Gly41Arg) that are not predicted to result in mistargeting of AGT to mitochondria might benefit from pharmacologic doses of pyridoxine. Hence, we suggest that all individuals with PH1 with at least 1 missense mutation should undergo a trial of pharmacologic-dose pyridoxine at the time of diagnosis, to assess responsiveness including pathologic variants of less common *AGXT* gene mutations (Table 1).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)
Supplementary References.

REFERENCES

1. Danpure CJ. Molecular etiology of primary hyperoxaluria type 1: new directions for treatment. *Am J Nephrol*. 2005;25:303–310.

2. Cellini B, Oppici E, Paiardini A, Montioli R. Molecular insights into primary hyperoxaluria type 1 pathogenesis. *Front Biosci (Landmark Ed)*. 2012;17:621–634.

3. Fargue S, Rumsby G, Danpure CJ. Multiple mechanisms of action of pyridoxine in primary hyperoxaluria type 1. *Biochim Biophys Acta*. 2013;1832:1776–1783.

4. Fargue S, Lewin J, Rumsby G, Danpure CJ. Four of the most common mutations in primary hyperoxaluria type 1 unmask the cryptic mitochondrial targeting sequence of alanine: glyoxylate aminotransferase encoded by the polymorphic minor allele. *J Biol Chem*. 2013;288:2475–2484.

5. Lumb MJ, Danpure CJ. Functional synergism between the most common polymorphism in human alanine: glyoxylate aminotransferase and four of the most common disease-causing mutations. *J Biol Chem*. 2000;275: 36415–36422.

6. Cellini B, Montioli R, Voltattorni CB. Human liver peroxisomal alanine:glyoxylate aminotransferase: characterization of the two allelic forms and their pathogenic variants. *Biochim Biophys Acta*. 2011;1814:1577–1584.

7. Coulter-Mackie MB, Lian Q, Wong SG. Overexpression of human alanine:glyoxylate aminotransferase in Escherichia coli: renaturation from guanidine-HCl and affinity for pyridoxal phosphate co-factor. *Protein Expr Purif*. 2005;41:18–26.

8. Cellini B, Montioli R, Paiardini A, et al. Molecular defects of the glycine 41 variants of alanine glyoxylate aminotransferase associated with primary hyperoxaluria type I. *Proc Natl Acad Sci U S A*. 2010;107:2896–2901.

9. Amoroso A, Pirulli D, Florian F, et al. AGXT gene mutations and their influence on clinical heterogeneity of type 1 primary hyperoxaluria. *J Am Soc Nephrol*. 2001;12:2072–2079.