Primary hyperoxaluria type 1: pathophysiology and genetics

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ABSTRACT

Primary hyperoxaluria type 1 (PH1) is a rare genetic form of calcium oxalate kidney stone disease. It is caused by a deficiency in the liver-specific enzyme, alanine:glyoxylate aminotransferase (AGT), a pyridoxal-5′-phosphate (PLP)-dependent enzyme involved in the metabolism of glyoxylate. The excessive endogenous synthesis of oxalate that ensues leads to hyperoxaluria, and the crystallization of the poorly soluble calcium salt of oxalate is responsible for a severe kidney stone disease, which can progress to end-stage renal disease, systemic deposition of oxalate and death. Knowledge about metabolic precursors of glyoxylate and oxalate, molecular pathology of AGT and analytical methods for diagnosis and clinical assessment have allowed a better understanding of the mechanisms underlying PH1 and opened the door to new therapeutic strategies.

Keywords: alanine:glyoxylate aminotransferase, glyoxylate, glycolate, kidney stones, oxalate, primary hyperoxaluria, urolithiasis
including the Agxt KO mouse, inhibiting the activity of AGT, in PH1. AGT, alanine-glyoxylate aminotransferase (AGT is also SPT, serine-pyruvate aminotransferase); GO, glycolate oxidase; PLP/PMP, pyridoxal phosphate/pyridoxamine phosphate, the two B6 vitamers interacting with AGT as coenzymes; GR, glyoxylate reductase; LDH, lactate dehydrogenase. The metabolism of hydroxyproline involves multiple steps, finishing with 4-hydroxy-2-oxoglutarate aldolase (HOGA1). PH1 is caused by a deficiency in AGT, PH2 by a deficiency in GR and PH3 by a deficiency in HOGA1.

PH1 is caused by a deficiency in AGT, PH2 by a deficiency in GR and PH3 by a deficiency in HOGA1. Oxalate is both synthesized endogenously and absorbed to a varying extent through the digestive tract from the diet, depending on the intake of nutrients (calcium, magnesium and fiber) and gut barrier permeability [7]. The role of dietary absorption in PH is considered as minor compared with the high rates of endogenous synthesis, but dietary guidelines do exclude oxalate-rich foods to minimize this contribution [1, 9]. Several sources of endogenous synthesis of oxalate have been identified over the last 60 years (Figure 1). Mouse models of PH, including the Agxt knock-out (KO) mouse PH1 model, have been instrumental in our understanding of pathways, although their relative importance appears to be different from that in humans [13]. Glycolate has been identified as a direct precursor to oxalate via oxidation, catalyzed by lactate dehydrogenase (LDH). All three types of primary hyperoxaluria involve glyoxylate metabolism [2, 14]. In PH1, the deficiency in alanine-glyoxylate aminotransferase (AGT), a pyridoxal-5′-phosphate (PLP), peroxisomal, liver specific enzyme, results in the lack of transamination of glyoxylate and alanine to glycine and pyruvate [15]. The excess peroxisomal glyoxylate formed by oxidation of glycolate catalyzed by glycolate oxidase (GO) is presumably transported to the cytosol, where it is either oxidized to oxalate by LDH or reduced back to glycolate by glyoxylate reductase (GR) (Figure 1). Both oxalate and glycolate are transported out of the hepatocyte by undefined mechanisms. Other sources of oxalate have been identified, some, but not all, involving glyoxylate as an intermediate: ascorbic acid, hydroxyproline and, to a lesser extent, glycolate and glycine (Figure 1) [2, 7, 16–18]. The different pathways of oxalate formation are compartmentalized within the cells and also between tissues and organs, as some of the key enzymes, such as AGT, GO and GR, have specific subcellular localizations and tissue-specific expressions. The excess oxalate synthesis in PH1 is driven by the liver [1, 2, 14]. In healthy subjects, studies using 13C and 14C tracers have shown that hydroxyproline contributes 15% to endogenous oxalate synthesis (18% in PH1), ascorbic acid 40–50% and glycine <5% [16, 18, 19]. Preliminary studies using glycolate indicate some other source(s) may yet need to be identified [20]. Older studies using 14C-glycolate had demonstrated that glycolate was a major precursor to oxalate in PH1 and the normalization of urinary oxalate in the double Agxt Go KO mouse, inhibition of GO by RNA interference and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated (Cas)9-mediated genome editing technology), have supported the idea of a major role of glycine in PH [7, 21–23]. More recently, clinical trials using small interfering RNA (siRNA) against GO have shown near-normalization of urinary oxalate in PH1, further solidifying the critical role of AGT in detoxifying the glyoxylate produced from the oxidation of glycolate [24].
Table 1. AGT mutations and molecular phenotypes associated with them; a subset of mutations studied in more detail is listed

| Amino acid change | Nucleotide change | Polymorphic allele background | Allelic frequency in PH1 | Molecular and cellular effects on AGT | B6 responsiveness, if known |
|-------------------|------------------|------------------------------|--------------------------|--------------------------------------|-----------------------------|
| c.33dup | c.33_34insC | Major | 13–15% | Truncated polypeptide, undetectable AGT catalytic activity | — |
| p.Arg36Gly | c.106C>T | Minor | <1% | Reduced catalytic activity | — |
| p.Pro11Leu | c.33G>A | Minor/major | 1% | Aggregation, accelerated degradation, partial mistargeting if minor allele | No or partial (case report) |
| p.Arg289His | c.866C>G | Major | <1% | Altered coenzyme binding, reduced catalytic activity | — |
| p.Asp201Glu | c.603T>G | Major | 1% | No catalytic activity (PLP binding site affected) | No |
| p.Gly170Arg | c.508G>A | Minor | 2% | Low affinity for PMP, reduced catalytic activity and mitochondrial mistargeting | B6 responsive |
| p.Gly170Arg | c.508G>A | Minor | 25–49% (Caucasians) | Normal to lower normal range of catalytic activity, mitochondrial mistargeting | B6 responsive |
| p.Met195Arg | c.584T>G | Minor | 2–3% | Reduced expression levels | — |
| p.Asp201Glu | c.603C>A | Major | 2% | Reduced catalytic activity | — |
| p.Ile244Thr | c.731T>C | Major | 9% | Aggregation, accelerated degradation and mitochondrial mistargeting | Partial (case reports) |
| p.Arg289His | c.866G>A | Major | (33% of AA mutant alleles) | Not tested | — |
| p.Pro11Leu/ p.Ile340Met | c.32C>T, c.1020A>G | (Minor allele) | 50% | (Minor allele): P11L responsible for slower dimerization, partial mitochondrial mistargeting and reduced catalytic activity | In vitro |

MOLECULAR PATHOLOGY IN PH1

Since the late 1980s, the molecular pathology in PH1 has been investigated in detail. The crystal structure and biochemical characteristics of normal and pathological variants of AGT, as well as genetic mutations causing them, have been determined and yielded key information in understanding the molecular defects underlying AGT deficiency [2, 14, 25]. AGT forms a homodimer, with each monomer’s N-terminal domain wrapping around the surface of the opposing monomer, stabilizing the dimer. A non-canonical peroxisomal targeting sequence (KKL) is located at each C-terminus and the binding of the cofactor, PLP, occurs via the formation of a Schiff base with the lysine at position 209. The mutations responsible for PH1 can cause a variety of molecular defects, such as protein destabilization, aggregation and elimination, lack of binding or low affinity for the co-factor and mis-localization of AGT to mitochondria. These, in turn, result in decreased enzyme activity, in vivo and in vitro, or metabolic inefficiency with residual catalytic activity for mislocalized AGT [2, 25–27].

GENETICS AND GENOTYPE–PHENOTYPE CORRELATIONS IN PH1

To date, more than 200 different pathogenic or likely pathogenic variants in the AGXT gene (NM_000030, located on 2q37.3) have been identified [28] with mostly missense variants (54%), but also frameshifts (16%), splice junction loss (11%), nonsense (13%), inframe indel (3%) and start loss (1%), located throughout the gene. The functional consequences of these changes can be loss of enzyme catalytic activity with conservation of AGT immunoreactivity, loss of catalytic activity and immunoreactivity, or mistargeting of AGT to mitochondria instead of peroxisomes (Table 1) [2, 27].

Two main polymorphic variants have been identified in the AGXT gene based on nucleotide substitution at position 32 and 1020 on the cDNA. The minor allele differs from the major (wild type) allele by a c.32C>T (p.Pro11Leu) substitution, c.1020A>G (p.Ile340Met) and insertion of 74 bp in intron 1. The p.Pro11Leu variant acts synergistically with some pathogenic variants (Table 1) by revealing a mitochondrial targeting sequence that competes with the peroxisomal targeting sequence and results in a mitochondrial AGT targeting dependent on the speed at which protein folding and dimerization occurs [2, 25]. Although most mutations are restricted to individual families, some of them are more frequent. The p.Gly170Arg pathogenic variant is responsible for AGT mitochondrial mistargeting and accounts for ~30% of mutant alleles [3, 14]. The p.Ile244Thr variant, present in about 9% of all PH1 patients, is significantly higher in patients of North African descent, with frequencies as high as 84% in patients from Morocco and 92% in patients from the Canaries [29, 30]. Approximately one-third of PH1 patients are responsive to pharmacological doses of pyridoxine, a precursor to PLP, the AGT coenzyme [31]. In vitro studies have shown that pyridoxine can act as a chemical chaperone, promote dimerization, stabilize the folded protein and increase
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peroxisome targeting. It also has a prostatic group effect and can increase enzyme catalytic activity [26, 32, 33]. Two missense pathogenic mutations, in particular, have been shown to be B6 responsive in patients with PH1, with a significant reduction of oxalate excretion (~30% reduction to full normalization) and clinical improvement: the p.Gly170Arg and p.Phe152Ile [34, 35]. Some case reports mention partial pyridoxine responsiveness in patients carrying the p.Ile244Thr and p.Gly41Arg mutations [36].

Differences in clinical outcome, mainly onset of ESRD, are influenced by genotype, emphasizing the importance of performing AGXT molecular analysis. Several cohort studies have shown that the p.Gly170Arg mutation is associated with a better outcome, defined as delayed progression to ESRD, especially when present in the homozygous state [37]. In contrast, null mutations are generally associated with a poor prognosis. The great inter-individual and intra-familial heterogeneity of the clinical course of the disease suggests that gene modifiers may play a role. The fact that patients with null mutations may reach ESRD midway during childhood, whereas other patients reach ESRD in infancy, highlights how much still remains to be understood in PH [37]. After ruling out classical causes of secondary hyperoxaluria, the definitive diagnosis of PH1 requires AGXT genetic testing even if metabolic investigations are normal. Identification of bi-allelic pathogenic or probably pathogenic variants in AGXT gene confirms the diagnosis. The implementation of massive parallel sequencing improved the diagnosis performance in PH by allowing simultaneous study of AGXT and the genes involved in PH2 and PH3.

It is important to be sure that the method used allows the detection of large intragenic deletion and/or duplication. Parental testing is necessary to verify bi-allelic segregation of the variants identified for an index case and is mandatory in the case of prenatal or pre-implantatory testing requirement. Extending mutation analysis in siblings is recommended because of interfamilial phenotypic variability [9, 14].

**BIOCHEMICAL ANALYSES IN PH1**

The basic clinical biochemistry work-up in PH1 patients relies on 24-h urinary oxalate, corrected for creatinine or body surface area. Random spot urine collections are sometimes necessary in children but require age-related reference ranges as there is a physiologic rapid fall in oxalate:creatinine ratio in the first years of life [14, 38]. Although urinary oxalate excretion is high in PH1 when urine output is preserved, there is great variability in 24-h urinary oxalate between PH1 patients and even between collections in the same patient. Urinary values within the upper normal range in confirmed PH1 patients have been reported. The cause for such variability is not entirely known and may include biological variations in endogenous synthesis, differences in dietary absorption of oxalate, analytical variability or a combination of the three [8, 14, 38]. Repeat measures in patients with high clinical suspicion of PH are recommended and a single normal result is not sufficient to exclude PH. Urinary glycolate excretion is often elevated in PH1 and its assessment can be helpful but has a low diagnosis sensitivity and specificity, as glycolate:creatinine ratio is within the reference range in about one-third of PH1 patients. Plasma glycolate can increase in PH1 and has been reported to be as high as 30 times values found in healthy subjects [1, 14]. Plasma oxalate increases in patients with PH even at early stages of CKD. A recent study showed a moderate but significant inverse correlation between eGFR and plasma oxalate [8]. Concerns with plasma oxalate measurements are the analytical difficulties and the risk of overestimation due to ascorbic acid breakdown during sample handling and processing. The rapid release of oxalate in tissue stores into the circulation at late disease stages makes interpretation of plasma oxalate variations difficult [8, 17]. Plasma oxalate in PH1 overlaps that of patients with ESRD due to other causes, but plasma oxalate >50 μmol/L (control range <5 μmol/L) is suggestive of PH [1]. Measurement of AGT catalytic activity and immunoreactivity on a liver biopsy has few indications nowadays but may retain a place to confirm or exclude the diagnosis of PH1 when genetic testing is not conclusive. Some difficulties of interpretation are possible between heterozygous carriers and patients homozygous for the c.508A>G mutation [14].

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**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflict of interest with the content of the article.

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