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Opportunities in Primary and Enteric Hyperoxaluria at the Cross-Roads Between the Clinic and Laboratory

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Hyperoxaluria is a condition in which there is a pathologic abundance of oxalate in the urine through either hepatic overproduction (primary hyperoxaluria [PH]) or excessive enteric absorption of dietary oxalate (enteric hyperoxaluria [EH]). Severity can vary with the most severe forms causing kidney failure and extrarenal manifestations. To address the current challenges and innovations in hyperoxaluria, the 14th International Hyperoxaluria Workshop convened in Perugia, Italy, bringing together international experts for focused presentation and discussion. The objective of the following report was to disseminate an overview of the proceedings and provide substrate for further thought. The format of this paper follows the format of the meeting, addressing, "PH type 1" (PH1) first, followed by "surgery, genetics, and ethics in PH", then "PH types 2 and 3," (PH2 and PH3) and, finally, "EH." Each session began with presentations of the current clinical challenges, followed by discussion of the latest advances in basic and translational research, and concluded with interactive discussions about prioritizing the future of research in the field to best serve the need of the patients.

H yperoxaluria is a pathologic condition characterized by increased urinary excretion of oxalate, an end-metabolic product in humans, leading to the formation of the poorly-soluble calcium oxalate (CaOx) that precipitates as stones and/or nephrocalcinosis in the kidneys.[1,2] The 2 main forms of hyperoxaluria, PH and EH, are characterized by a different origin of

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oxalate (Figure 1). In PH, increased endogenous production of oxalate occurs because of inherited mutations leading to the deficit of enzymes involved in the hepatic metabolism of glyoxylate, the direct precursor of oxalate through lactate dehydrogenase (LDH). The deficit of peroxisomal alanine:glyoxylate aminotransferase (AGT) causes PH1,[3–5] whereas the deficit of cytosolic/mitochondrial glyoxylate hydroxypyruvate reductase (GR/HPR) causes PH2.[6] The pathogenesis of PH3 is still puzzling because it is genetically caused by pathogenic variants leading to a functional deficit of 4-hydroxy-2-oxoglutarate aldolase (HOGA), an enzyme that generates glyoxylate from hydroxyproline.[7,8] Various hypotheses have been formulated to explain increased urinary oxalate excretion in PH3,

including the possible inhibitory effect of accumulating 4-hydroxy-2-oxoglutarate on GR/HPR, or the cleavage of 4-hydroxy-2-oxoglutarate by a still-unknown cytosolic aldolase.[9,10]

Because dietary oxalate accounts for a significant portion of urinary oxalate, excessive intake of oxalate-rich foods can lead to hyperoxaluria.[11] A distinct form of oxalate hyperabsorption by the large bowel known as EH is a frequent complication of intestinal fat malabsorption following small intestinal resection or gastric bypass surgery or medical conditions such as inflammatory bowel disease or cystic fibrosis.[12,13] Patients with EH are at increased risk of nephrolithiasis and nephrocalcinosis, possibly leading to chronic kidney disease and end-stage kidney disease (ESKD).[14]

Current therapies for all forms of hyperoxaluria aim at reducing CaOx supersaturation through high fluid intake and crystallization inhibitors, or, in the most severe forms, dialysis and kidney transplant.[15,16] For patients with PH1, other treatment options are available. A subset of them is responsive to vitamin B6 (pyridoxine), which can reduce oxalate production. Traditionally, combined liver-kidney transplantation was needed to address kidney failure and replace the oxalate-producing liver.[16,17] More recently, therapies based on RNA interference (RNAi) have been developed to reduce hepatic oxalate production in PH and,

though these biological drugs appear to be quite effective, they are not available for all patients and their long-term risks are not known.[18-21]

The 14th International Hyperoxaluria Workshop took place on June 23 and 24, 2023 in Perugia, Italy, organized by the Oxalosis and Hyperoxaluria Foundation, with participation of an international group of the primary experts in the field, as well as representatives from patient advocacy groups. The conference focused on the most critical clinical and ethical challenges currently facing patient management in PH and EH, and the latest innovations offered by basic and translational research. This narrative review summarizes the highlights of the lectures through the eyes of the chairs of each session of the workshop. The detailed meeting program can be accessed at https://ohf.org/14th_international_workshop.

PH1

Clinical Update

PH1 is the most prevalent and severe type of PH. Over 200 pathogenic variants have been found in the AGT gene, most of them leading to an overall AGT-deficiency, but some only to AGT mistargeting to mitochondria where the enzyme does not detoxify glyoxylate.[22] PH1 may cause recurrent kidney stones, nephrocalcinosis, and kidney failure in over 60% of patients.[23] As kidney function declines, oxalate
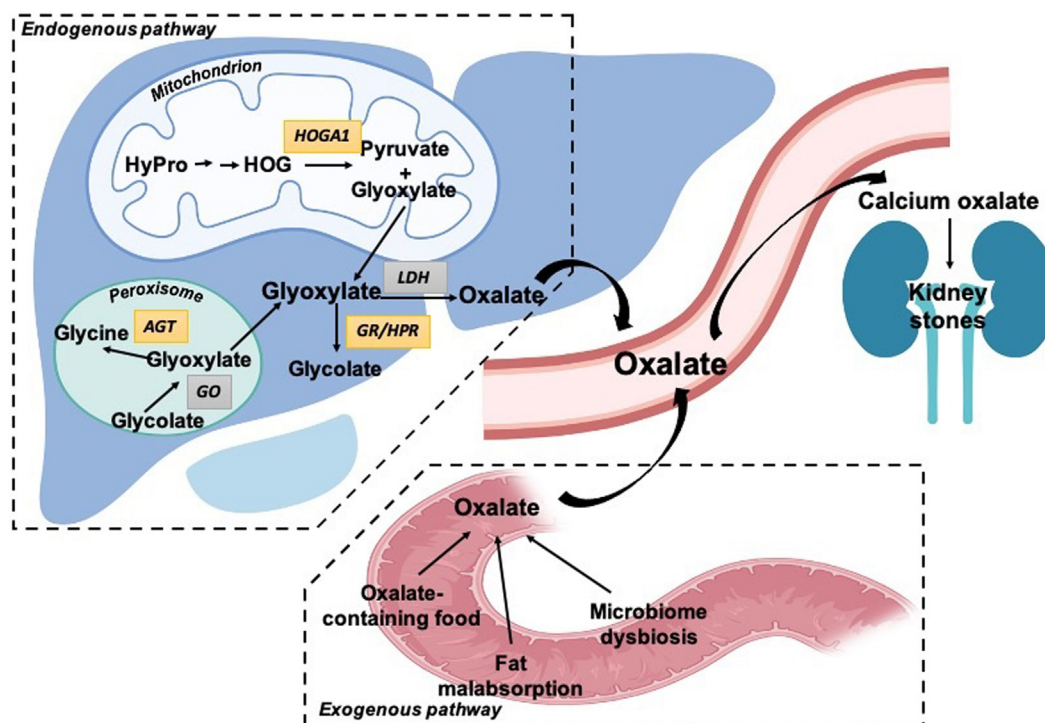

Figure 1. Overview of the pathways controlling oxalate homeostasis. AGT, alanine:glyoxylate aminotransferase; GO, glycolate oxidase; GR/HPR, glyoxylate hydroxypyruvate reductase; HOG, 4-hydroxy-2-oxoglutarate; HOGA1, HOG aldolase; HyPro, hydroxyproline; LDH, lactate dehydrogenase.

accumulates in the blood and is deposited in various tissues, leading to life-threatening condition referred to as systemic oxalosis. Unfortunately, even aggressive dialysis cannot keep up with oxalate generation, often resulting in ongoing systemic oxalosis.[24] For non-pyridoxine responsive patients with kidney failure, combined liver-kidney transplantation was the only therapeutic option before RNAi therapies.[25-27]

Two new drugs based on RNAi, lumasiran and nedosiran, have been shown to be safe and effective in clinical trials. In 2020, lumasiran was approved by both the US Food and Drug Administration and the European Medicines Agency as therapy for PH1.[28] Nedosiran was approved by the US Food and Drug Administration for patients with PH1 aged >9 years with estimated glomerular filtration rate (GFR) >30 ml/min per 1.73 m^2 in Fall 2023.[20] Both drugs aim to reduce endogenous oxalate production by gene silencing of enzymes involved in glyoxylate metabolism. Both are administrated subcutaneously at prescribed intervals and require lifelong treatment. Lumasiran blocks glycolate oxidase (GO), which promotes the conversion of glycolate into the oxalate precursor, glyoxylate. This effect results in an average reduction of about 65% in urine oxalate excretion from baseline.[26,28,29] The 12-month outcome studies have shown promising results with decreased kidney stone events and nephrocalcinosis, along with preservation of GFR over time,[30] with no significant side effects. Several case reports have described decrease in dialysis time or number of days, ability to stop dialysis in some patients, and ability to perform isolated renal transplant in patients due to significant reduction in plasma oxalate levels with lumasiran treatment.[31-33]

Nedosiran blocks LDHA, which converts glyoxylate to oxalate. Studies in patients with PH1 show similar effectiveness and safety as lumasiran. In the PHYOX2 randomized controlled study, nearly 65% had normal or nearly normal urinary oxalate levels ($<1.3 \times$ upper limit of normal) after 6 months of treatment with nedosiran.[34,35] A case report demonstrated response of plasma oxalate in a patient with PH1 on dialysis, and successful kidney transplantation while receiving nedosiran.[36]

The following unanswered questions with respect to RNAi therapy in PH1 were discussed:

1. Should RNAi treatments be given to all patients with PH1 or only to those who do not fully respond to pyridoxine and have clinically severe disease? There are geographical differences on this point. In Europe, the level of recommendation differs based on severity of disease and pyridoxine-responsiveness,[25] whereas in the US, RNAi treatment is prescribed for any patient with PH1 who is not fully pyridoxine-sensitive.[27] The scientific community agrees that in case of full pyridoxine responsiveness with normalization of urine oxalate, there is likely limited benefit of RNAi. There is debate on the indication in patients who do not fully respond to pyridoxine, but still have milder disease. The arguments for restrictive application are cost, unknown long-term effects, and unnecessary addition of a drug in fully pyridoxine-responsive patients or patients with minimal or no clinical symptoms. Arguments supporting a low threshold for RNAi prescription, also in patients with minimal or no clinical symptoms, include the unpredictable course of PH1 and the risk for sudden deterioration resulting in ESKD, as well as the prevention of life-long treatment of ESKD such as dialysis and/or renal transplantation. In addition, long-term outcome data are warranted to fully appreciate the exact impact of RNAi on clinical outcome with respect to kidney function preservation and reduction of stone events.

2. Are RNAi treatments safe in pregnancy? Animal studies with high dose lumasiran showed no fetal abnormalities but no human pregnant patients with lumasiran maintenance therapy have been reported so far. Data on all pregnant patients with PH should be reported to determine outcomes. Indeed, some women may choose to discontinue therapy during pregnancy versus continue RNAi treatment; and these outcomes and safety information should also be documented.

3. Can RNAi therapy replace liver transplantation? The prospects for isolated kidney transplant in future patients on RNAi seem promising, but thus far only case reports are available.[31,36,37] Most centers are performing isolated renal transplant in patients who have demonstrated significant reduction in urine or plasma oxalate levels on lumasiran.[31-33] There is optimism that avoiding liver transplantation may be a viable option in those who have sufficient reduction in urine or plasma oxalate and long-term data are being collected.

4. What would be an acceptable plasma oxalate concentration in patients with PH1 on dialysis for isolated kidney transplantation? There is insufficient evidence to answer that question; however, there are some indications from case reports of kidney transplantation performed in patients treated with RNAi, as well as from data reported on plasma oxalate levels in non-PH patients on dialysis. Future data on kidney transplant in patients receiving RNAi treatment are required to answer emerging questions, including determination of the target plasma oxalate concentration at which this approach is safe.

5. Should RNAi therapy be individualized based on extent of enzyme deficit? A study with stable isotopes showed interpatient variability from 55% to over 90% in GO blockade from lumasiran.[38] No data exist on increasing dosage or dosing frequency in patients to improve response to lumasiran. Potential individualized combination therapy (lumasiran and nedosiran, or other potential future treatments) could be considered for patients who do not respond sufficiently to a single agent. This requires further investigation.

6. Is there a need for native nephrectomy prior to renal transplant? For patients with long-term PH1, kidneys may be a source of deposited oxalate that can be released back into the bloodstream, causing ongoing oxalate burden to the newly transplanted kidney, though there are no published data on this. Until investigation is performed to determine if there is a benefit to performing native nephrectomy, practice will continue to vary center to center.

Basic Science Research Update

The interest of the scientific community in PH1 has increased in the past decade related to interpretation of genetic data for a correct diagnosis, the design of new therapeutic strategies, and the assessment of patients' responsiveness using appropriate endpoints. Indeed, the reduced costs and turn-around times for genetic sequencing have brought out a new aspect of the diagnosis of rare diseases, that is, the assessment of the pathogenicity of newly identified variants. This aspect is of fundamental importance in PH1, because most disease-causing variants are missense and can lead to the AGT-deficit through molecular mechanisms spanning from a merely functional or structural defect to a combination of the 2.[3,22,39] In addition, as recently reported, the assessment of the minor or major haplotype in which a variant is inherited could remarkably affect disease manifestations and responsiveness to treatments.[25,40,41] Indeed, the AGT minor haplotype characterized by p.Pro11Leu and p.Ile340Met substitutions, can have broad effects on AGT fitness. Interestingly, the 2 variants act in different directions, where the introduction of Leu11 was destabilizing but Met340 stabilizing. Some variants can be pathogenic on both the major and minor haplotype, such as p.Ile56Asn, but the specific haplotype may further influence protein fitness and therefore be a major modifier of the clinical presentation or progression.[42] It follows that the AGT haplotype should be taken into consideration when assessing pathogenicity of likely penetrance of variants.

Improved understanding of PH1 pathogenesis has offered great opportunities for the design of new therapeutic approaches. Utilizing a small-molecule approach, which may be of lower cost and more easily administered, progress has been made in reducing plasma oxalate by targeting LDH or GO, as well as by molecules leading to a combined inhibition of both proteins.[43-47] Some of these molecules have been already tested in clinical trials (www.clinicaltrials.gov, identifier NCT05367661). Moreover, efficient pharmacological chaperones to better rescue misfolded AGT and inhibitors of CaOx crystallization have been described.[48-50] Regarding biologicals, studies in AGT gene $1^{-/-}$ mice show targeting to hepatocytes, expression of AGT, reduction of oxalate levels, and prevention of nephrocalcinosis with specific doses.[48] Gene editing approaches are also on the horizon for PH1, aimed at reducing substrate load, by specific knock-out of LDH or GO.[51-53] A gene editing approach to correct the AGT gene pathogenic variant in PH1 patient-derived fibroblasts has been also proposed.[54] Patient fibroblasts were differentiated to hepatocytes by targeted reprograming, and using CRISPR-Cas9, the point defect within the gene was either corrected or a normal copy of the AGT gene was knocked into the endogenous locus. AGT expression was documented in the corrected cells, showing the proof of principle of this approach.

Another very active point of discussion in PH1 is the setup of suitable methods to monitor disease progression as well as treatment responsiveness. Until now, most of the studies relied on 24-hour urinary oxalate excretion.[55] However, the latter does not represent an accurate measure for endogenous oxalate production in patients with PH and it is not applicable in those with anuria.[56] Therefore, a gas chromatography tandem mass spectrometry analysis method following a stable isotope infusion protocol of $^{13}C_2$-oxalate and $^{13}C_1$-glycolate has been implemented. Using this method, the efficacy of lumasiran, which silences GO, was shown to vary between patients, likely reflecting different levels of drug effectiveness.[57]

Overall, the field of translational research in PH1 is living a particularly exciting moment, especially regarding approaches to test variant pathogenicity and modifying effects, monitoring the efficacy of existing treatments, and potential new treatments in the form of small molecules, transgenic expression, and pathogenic variant correction by gene therapy. Over the next few years these novel approaches are likely to be finding their way into the PH clinic.

Surgery, Genetics, and Ethics in PH
Surgery

Endourological techniques for kidney stone removal continue to advance. Current laser and ultrasonic lithotripsy devices allow many stones to be fragmented

and removed through ureteroscopic techniques.[58] Other larger stones may require percutaneous access for stone fragmentation and removal. Nevertheless, this is still much less invasive than open surgery which is now very rarely required. In addition, modern endourological techniques for the most part do not damage the kidneys; and chronic kidney disease strictly related to repeated surgery has become less of a concern. One major patient concern is, however, pain related to ureteral stents which are typically placed either before or after surgery or both to prevent ureteral obstruction secondary to stones or fragments of stones. To date, there are no consistently effective strategies to prevent pain and lower genitourinary tract symptoms when stents are in place.

One major surgery-related concern for patients with PH is the increased risk for acute oxalate nephropathy resulting from the procedure. To some extent, all patients can be susceptible to acute kidney injury secondary to oxalate during times of volume depletion or other factors that result in decreased renal perfusion. Thus, even during a routine kidney stone surgery, a patient with PH can develop acute kidney injury that is variably reversible if the surgery is complicated by hypotension, even when the hypotension is subtle. Patients with PH and diminished GFR are at even greater risk of acute kidney injury in the perioperative period.

Similar risk for acute kidney injury from acute oxalate nephropathy exists after kidney transplant, which can occur with either a liver or kidney transplant or combined liver-kidney transplant, as oxalate either already in the bloodstream or from tissue stores is excreted through the new allograft.[59] Thus, centers must be aware of this possibility and often aggressive dialysis before and after transplant is performed. It is very important that these procedures be performed in centers that are aware of these concerns and prepared to have access to appropriate dialysis and laboratory support. Patients who have had kidney failure for a significant period often have massive oxalate deposition in the native kidneys, prompting some centers to perform native nephrectomy at the time of transplant to remove this potential source of ongoing oxalate excretion. As discussed earlier, because there is only anecdotal evidence for this, controversy persists.

Genetics

Knowledge regarding the true prevalence of the 3 forms of PH is evolving as more genetic data are being generated worldwide, and as more novel variants have been confirmed in individual families and patients. Confirming pathogenicity remains potentially challenging, especially for missense changes and when large informative families with strong phenotypic data are not available. Newer laboratory techniques to look at expressed protein in cultured cells and enzyme activity assays *in vitro* can help to demonstrate likely pathogenicity of a novel variant. Based on these evolving data, current estimates suggest that the frequency of biallelic cases worldwide could approximate 1:150,000 for PH1, 1:300,000 for PH2, and 1:135,000 for PH3, with estimated carrier frequencies of ∼1:200, ∼1:280, and ∼1:185, respectively.[23] Thus, the prevalence of PH3 may exceed PH1 and PH2 remains the least common. Often patients present with risk factors for monogenic causes of kidney stone disease, including frequent stones at an early age, nephrocalcinosis, and kidney failure. However, reliably measuring biochemical data may be challenging where access to advanced laboratory testing is limited. Genetic testing with kidney stone or nephrocalcinosis candidate gene panels provide a method for rapid identification of monogenic causes, and current evidence indicates >40 monogenic causes of kidney stone disease, including PH1, PH2, and PH3.[60-62] Due to overlapping clinical features, panel screening is recommended even when the diagnosis seems obvious. For example, 10% of those with a strong PH phenotype do not have biallelic variants in a PH gene.[60]

Because PH is a biallelic disorder, worldwide prevalence varies widely among geographic and ethnic groupings. For example, whereas worldwide rates of consanguinity are estimated to be 10%, rates of consanguinity are highest in the Middle East, ranging from 35% to 80%, and less than 4% in Europe and the USA.[63] A large national study in the UK showed that the risk of baby being born with a recessive disorder doubled for consanguineous couples.[64] Thus, consanguinity can be regarded as a modifiable risk factor for autosomal recessive diseases including PH. Additional challenges identifying cases of PH arise in countries that do not include PH in their disease-tracking databases. Finally, Deesker and colleagues highlight that underlying genetic causes are not the only factor in PH disease severity, showing that younger siblings of index cases often have milder courses despite having the same genetic variants for PH.[65]

Ethics

Access to genetic testing and reliable urinary and plasma oxalate measurement to make an accurate diagnosis is quite variable around the world. Similar disparities to access exist for state-of-the-art treatments such as intensive dialysis, organ transplant, and RNAi therapies. These disparities will only increase with the development of innovative and very expensive treatment strategies involving small molecules and gene

editing. Access to RNAi treatment is limited in many countries by the governing agencies due to its high price. Most low- or middle-income, and even some European countries, have no access at all. Beyond cost, limited access to RNAi treatments are also exacerbated by the requirement for ultra-low temperatures during shipping and storage. Strategies to overcome these health disparities remain an important challenge in the hyperoxaluria community. Patient organizations may be in a good position to shine a light on these issues and mobilize caregiver and industry partners.

PH2 and PH3
Clinical Update

PH2 is caused by a deficiency of the enzyme GR/HPR[66,67] encoded by GRHPR.[68] GR/HPR activity is highest in the liver but also present in leukocytes and kidneys.[69] Lack of GR/HPR leads to an accumulation of glyoxylate and hydroxypyruvate, both metabolized by LDH to oxalate and L-glycerate, respectively. A retrospective review of genetically confirmed PH2 cases in the OxalEurope registry was recently published and included 112 patients from 11 countries, the majority residing in the UK.[70] Clinical characteristics of PH2 are like those of PH1. The median age of first symptom and diagnosis duplicate those reported for PH1 (3.2/9 years vs. 3.9/8.1 years), with urolithiasis noted in the majority.[23] Nephrocalcinosis is less common than in PH1 but can occur in all age groups.[71] Presentation of symptoms in infancy is as common as in PH1. In contrast, patients with PH2 are less likely to present in kidney failure at diagnosis, with no progression to ESKD recorded prior to the age of 15 years, unlike PH1. Renal survival and the overall long-term prognosis for patients with PH2 appears similar in outcome to the pyridoxine-sensitive PH1 genotypes. Genetic analysis revealed 18 novel mutations in the GRHPR gene.[70] Chronic Kidney Disease stage 2 or worse was recorded in 45 of 89 (50.6%), and 22 patients (24.7%) had reached stage 5. Median renal survival was 43.3 years and 15 transplants in 11 patients were recorded. Renal outcome did not correlate with genotype, biochemical parameters, or with the presence of nephrocalcinosis at presentation. Thus, PH2 is a disease with significant morbidity and accurate diagnosis by 24-hour urine analysis and careful follow-up is required.

The supportive management is as for PH1, but with no subset responsive to pyridoxine. High fluid intake and citrate are the mainstay. Although the risk for systemic oxalosis has traditionally been considered lower in patients with PH2, the increasing documentation of renal impairment places this group of patients in a category like PH1 where risks for systemic oxalosis increase as GFR falls.

The clinical practice recommendations for PH2 suggest that liver transplantation may be advocated in those with advanced disease (estimated GFR <30 ml/min/SA).[25] The 1-year and 5-year cumulative kidney allograft survival were 43% and 29%, respectively; and 3 patients underwent a repeat kidney transplant (at 5, 6, and 22 years). Successful liver-kidney transplantation in patients with PH2 has been reported in several cases.[72-74]

Nedosiran treatment reported from the PHYOX2 trial demonstrated no statistically relevant reduction in urine oxalate in the small number of patients with PH2 enrolled,[34] though investigations into its potential effectiveness in patients with PH2 and PH3 are ongoing. Future therapeutic strategies may require a personalized approach, consideration of other medications (e.g., stiripentol as an LDH inhibitor[32]), and development of better biomarkers to inform disease progression.

PH3 was described in 2010 and appears to be the mildest form of PH in terms of risk for ESKD, though patients tend to present at a young age and can have abundant stone formation.[75] It is caused by pathogenic variants in HOGA1 encoding the mitochondrial enzyme HOGA. The exact mechanism through which this defect leads to increased oxalate production has not been clearly elucidated. It is likely that PH3 is more prevalent than PH2 but is newer and perhaps underdiagnosed due to the milder phenotype. Like PH2, because there is no effective targeted therapy, treatment consists of hyperhydration and crystal inhibition. In 1 report, 97% of patients with PH3 had intact GFR at age 40 years.[76] Few cases of PH3 resulting in ESKD exist and involve patients who required multiple surgical interventions, which could have contributed to their GFR decline.[23,77] For both PH2 and PH3, there is a significant unmet need for a safe and effective treatment.

Basic Science Research Update

The main research topics related to PH2 and PH3 dealt with the knowledge gaps in endogenous oxalate synthesis in humans and how it relates to the pathogenesis of the 2 diseases, particularly the role of hydroxyproline catabolism. Indeed, the liver is a major site of glyoxylate synthesis from hydroxyproline, glycolate, and glycine metabolism,[78-82] and is therefore an important organ to target to reduce endogenous oxalate synthesis in all types of PH.

Common to both PH2 and PH3 is the central role of hydroxyproline catabolism to oxalate synthesis. Fargue and colleagues continuously infused i.v. carbon-13 hydroxyproline in adults with PH2 and PH3 and demonstrated that hydroxyproline metabolism

contributes 47% (PH2) and 33% (PH3) to urinary oxalate excretion.[81] Bone turnover,[83] exercise,[84] dietary collagen intake,[79] and renal function[85] have all been shown to influence hydroxyproline levels in blood or urine. However, the impact of these factors on urinary oxalate excretion in those afflicted with PH2 or PH3 warrants further investigation. Nonetheless, inhibition of the hydroxyproline degradation pathway could have a significant impact on lowering urinary oxalate excretion in PH2 and PH3. In this regard, a key opportunity is offered by the possible use of hydroxyproline dehydrogenase inhibitors. Hydroxyproline dehydrogenase is the first enzyme in the hydroxyproline catabolic pathway and represents a promising target because humans[86] and mice[87] with hydroxyproline dehydrogenase deficiency is healthy. A recent report demonstrated that the inhibitor N-propargylglycine prevents 4-hydroxyproline catabolism in mouse liver and kidney.[88] However, this compound also interferes with proline metabolism, highlighting that agents targeting hydroxyproline catabolism must also be evaluated for potential side effects. Furthermore, hydroxyproline metabolism occurs in kidneys and thus blockage of hydroxyproline dehydrogenase activity at both sites may be needed for optimal reduction of urinary oxalate excretion.

Another strategy currently pursued to reduce oxalate synthesis in PH2 and PH3 is the inhibition of LDH. Approaches to reduce LDH function have focused on developing agents that only target this enzyme in the liver, because systemic LDH inhibition can result in serious side effects.[89] Nedosiran, a siRNA that specifically knocks down expression of hepatic LDHA, is a US Food and Drug Administration-approved drug to reduce urinary oxalate excretion in PH1. However, it demonstrated a mixed efficacy in reducing urinary oxalate excretion in patients affected by PH2 and PH3.[34,90] This further underscores the need for a better understanding of endogenous oxalate synthesis in PH2 and PH3.

The role of non-hepatic tissues in endogenous oxalate synthesis is also an important topic of discussion within the scientific community. Indeed, the expanded tissue expression of GRHPR and HOGA1 suggests that the kidney may also contribute, although future work is needed to define its role as a premise to novel strategies for reducing urinary oxalate excretion in PH2/3.

Specific to PH3, challenges to our understanding include the involvement of HOGA in the catabolism of hydroxyproline, with this enzyme generating glyoxylate rather than detoxifying it.[7,91,92] Moreover, the enzyme exhibits oxaloacetate decarboxylase activity suggesting it may have a wider role in metabolism,[93] whose impact on glyoxylate detoxification and/or

oxalate synthesis remains unknown. Hypotheses to explain the mechanism(s) by which oxalate synthesis is increased in PH3 may include the inhibition of GR/HPR by 4-hydroxy-2-oxoglutarate,[9] breakdown of 4-hydroxy-2-oxoglutarate to glyoxylate by an alternative aldolase or aldolase(s),[10] and an alteration in cellular redox balance resulting in increased glyoxylate oxidation to oxalate by LDH.[93] However, the relative role of each of these potential mechanisms in oxalate synthesis in PH3 requires further investigation.

EH
Clinical Update
EH is the abnormally high excretion of oxalate in urine due to fat malabsorption by the small intestine, which leads to excess oxalate absorption by the colon. Normal healthy adults typically excrete <40 mg (<0.5 mmol) of oxalate daily. Idiopathic CaOx stone-formers may have excretion rates up to 50 mg/d (0.5 mmol/d), whereas urine oxalate can range between 50 and 100 mg/d (0.5–1.0 mmol/d) for patients with EH. Conditions frequently associated with EH include bariatric surgery, specifically Roux-en-Y gastric bypass, short gut syndrome, Crohn's disease, cystic fibrosis with pancreatic insufficiency, and more rarely, celiac disease.[94] Notably, restrictive gastric bypass procedures (i.e., gastric banding), have not been associated with the development of hyperoxaluria or kidney stones.[95,96] If possible, the first line of intervention for EH should be correction of the underlying bowel disease. Adjunctive treatments can seek to reduce oxalate absorption through dietary manipulations to decrease the bioavailability of oxalate or limit calcium sequestration by fatty acids within the large intestine. These include a low-oxalate diet, but this is challenging to sustain and with mixed evidence of efficacy.[13] Restricting dietary fat consumption and maintaining sufficient calcium intake to maximize the opportunity for calcium and oxalate to bind within the gut thereby limiting absorption has long been recognized to help reduce urinary oxalate.[97] Similarly, some (but not all) older studies demonstrated that bile acid sequestrants such as cholestyramine could also decrease urinary oxalate excretion.[12] There is, however, a paucity of contemporary data for these approaches with patients with EH who have undergone modern bariatric procedures.[98]

Surgical treatment options for patients with EH with symptomatic kidney stones are much the same as those for idiopathic kidney stone disease and thus have similar challenges. Recent advances in endourology equipment, including single use ureteroscopes and high-power lasers offer technologic advantages and increased efficiency.[99] In addition, the adoption of predictive models increasingly allows urologists to

select the appropriate intervention and power source to remove stones with greater effectiveness.[100]

Basic Science Research Update

Research efforts on oxalate handling by the gut have been focused along 2 main lines: oxalate degradation by commensal microbes and oxalate transport across the intestinal epithelium. Concerning the former, it is known that the gut microbiome has an essential role in human health, and its disruption has been implicated in the pathophysiology of a wide variety of diseases including kidney stones.[101-104] The presence of oxalate-degrading gut bacteria is associated with lower rates of urinary oxalate excretion which can help guard against hyperoxaluria and reduce the risk for CaOx stone formation.[105] Several of these bacteria have been identified although only a few are active *in vivo*, perhaps the best known of these is *Oxalobacter formigenes*.[106,107] Differences have been noted between the microbiome of stone formers compared to non-stone formers, including a lower prevalence of *O. formigenes*.[107,108] The rat Roux-en-Y gastric bypass model of EH could be robustly colonized with *O. formigenes* leading to normalized urinary oxalate excretion.[107] Oxalate-degrading probiotics such as *O. formigenes* hold promise as a potential approach for treating EH. Although clinical trials with patients with PH and *O. formigenes* yielded conflicting results,[109-112] no such trials have been attempted for patients with EH. An alternative strategy to probiotic administration is orally available exogenous oxalate-degrading enzymes of microbial origin, such as reloxaliase (Allena Pharmaceuticals) or OX-1 (Oxidien Pharmaceuticals), whose safety and efficacy in heathy subjects[113,114] and patients with EH [115] has been investigated. It is also worth mentioning that though many studies have focused on the most abundant bacterial components of the microbiota, other less abundant microbes may play a role in oxalate handling. In this regard, commensal fungi may reveal a previously unrecognized role within the microbiota in oxalate degradation.[116]

In addition to the gut microbiome, the relatively recent discovery that the urinary tract is not sterile, even in the absence of infection,[117] has launched a new line of research into how this previously unrealized microbiome influences kidney stone formation and urine composition. Improvements in culture-based techniques, together with advances in "-omic" approaches, are allowing investigators to explore this "new" microbiome. However, given its low biomass, rigorous control of potential contamination during sample collection, processing, and analysis is paramount.[118] So far, sequencing studies indicate that distinct microbial communities exist in the urinary tract of CaOx stone-formers compared with controls.[119]

Over 3500 microbial genes were more highly expressed in non-stone formers and 19 % of these (including oxalate-degrading genes) bacteria such as Lactobacillus crispatus may therefore have important implications for hyperoxaluria and potential stone risk.

Oxalate transport in the intestine has also received considerable attention. Indeed, understanding how oxalate is absorbed across the intestinal epithelium is important for developing effective strategies to limit excess uptake by the colon in EH. The current model proposes that the absorption mechanism is entirely passive and paracellular along the length of the intestine.[120] There is, however, evidence from rodent models of a significant transcellular component to absorption by the large intestine involving the apical anion exchanger, Slc26a3.[121] Investigations of the mechanistic basis of EH with the Roux-en-Y gastric bypass rat model have emphasized how dietary fat increases luminal oxalate solubility and enhances epithelial permeability, which are predicted to drive paracellular absorption.[122] In this setting, any role for colonic Slc26a3 is likely to be insignificant. Studies on other Slc26a family members may provide further insights, including Slc26a1, recently characterized for its major role in sulfate homeostasis, but potentially able to contribute to oxalate influx.[123]

The progress in our understanding of oxalate handling in the intestine toward the development of novel therapeutic strategies relies on the parallel refinement of animal models. In this regard, after gastric bypass, another prevalent cause for EH is Crohn's disease; however, suitable animal models resembling the human pathology of spontaneous ileal inflammation are few. A promising candidate is the SAMP1/YitFc (SAMP1) mouse model.[124] SAMP1 mice displayed clear signs of fat malabsorption and urinary oxalate excretion increased markedly with dietary fat content. Expression levels of tight junction proteins were altered in the intestine of SAMP1 mice suggesting enhanced gut permeability, as demonstrated by the Roux-en-Y gastric bypass rat model.[107] In addition, the SAMP1 microbiome was enriched with several oxalate degraders, indicating a possible adaptation to increased oxalate levels within the large intestine. Continued study of these potential contributors to the pathophysiology of EH will be crucial for the development of effective therapies.

CONCLUSION

The Oxalosis and Hyperoxaluria Foundation-sponsored 14th International Hyperoxaluria Workshop hosted in Perugia, Italy in 2023 brought together the world's experts to discuss the current challenges in managing patients with PH and EH; and visualized a path

forward for investigators to answer the most pressing questions to facilitate innovation in research and improved clinical care. The format allowed for open, honest, and respectful discussion between physicians, clinical investigators, and basic scientists to work toward this common goal.

As our coauthors and session chairs have outlined above, great work continues to be performed expanding on the strong foundation of dedicated predecessors. For PH1, we celebrate the victory of game-changing treatment with RNAi medications but acknowledge that many new questions have arisen that must be answered, all while newer treatment modalities evolve through various stages of development. For PH2 and PH3, patients continue to struggle without definitive treatment, highlighting the crucial need to improve our understanding of the underlying mechanisms of oxalate production in these 2 conditions. Similarly, EH warrants further study because elucidating the details of oxalate transport in the gut and the role of the microbiome may lead to targeted therapies to reduce the risk of hyperoxaluria in these patients. Finally, we acknowledged that all the hard work being performed in the field must include the voices of the patients and include an ethical perspective to maximize equity and inclusion.

It is crucial for conferences such as this to continue to facilitate progress in the management and research of rare diseases. Dedicated, focused time together with diverse experts and stakeholders sharing physical space and ideas results in efficient communication in real time providing opportunities for rich discussions that cannot be replicated in any other way. We are grateful for the opportunity and express great appreciation for the participation of all attendees.

DISCLOSURE

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