

Proceedings of the 8th International Primary Hyperoxaluria Workshop, UCL-Institute of Child Health, 29–30 June 2007

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The 8th International Primary Hyperoxaluria Workshop was held on 29–30 June 2007 at the Institute of Child Health, University College London. This meeting was the most recent in a series of highly-focussed interdisciplinary workshops, that have run since 1990, concentrating on the latest advances in the understanding and clinical management of primary hyperoxaluria. They have taken place every 2–3 years across Europe and, more recently, North America. So far they have been held in Brussels (1990), Cambridge (1992), Lyon (1994), Turin (1997), Zurich (1999), Hanover (2002), and Rochester MN (2004). Participation in these workshops is attractive to a wide range of researchers and clinicians, including molecular biologists, cell biologists, clinical and basic biochemists, clinical and molecular geneticists, chemists, adult and paediatric nephrologists, urologists, transplant surgeons, metabolic physicians, nurses and dieticians. In addition, this and the previous (7th) workshop have held sessions specifically aimed at the lay audience, in particular patients, families and friends.

Primary hyperoxaluria (PH) is a collective term which encompasses a group of rare, potentially lethal, hereditary metabolic diseases which result in the deposition of insoluble calcium oxalate in the kidney parenchyma (nephrocalcinosis) and/or urinary tract (urolithiasis/nephrolithiasis). Only two types of primary hyperoxaluria, namely type 1 (PH1) and type 2 (PH2), have been well characterized. Both are autosomal recessive conditions caused by mutations in the *AGXT* and *GRHPR* genes, respectively. *AGXT* encodes the liver-specific, pyridoxal-phosphate-dependent enzyme alanine:glyoxylate aminotransferase (AGT), whereas *GRHPR* encodes the more widely-distributed enzyme glyoxylate/hydroxypyruvate reductase (GRHPR). Dysfunction of either of these enzymes results in increased synthesis and excretion of the metabolic end-product oxalate, and hence renal deposition of calcium oxalate.

The 8th Workshop, which attracted approximately 100 scientific, clinical and lay delegates from across the world, was divided into five main sessions: (1) recent advances in the molecular aetiology and pathology of PH, (2) current approaches to diagnosis and treatment, (3) search for new treatments (part 1: chemical chaperones), (4) search for new treatments (part 2: novel use of the liver and gut), and finally (5) a lay session specifically designed for PH patients, families and friends. In addition, there were a number of poster and oral communication sessions.

The 8th Workshop was held in honour of two pioneers in the PH field—Richard Watts, who was formerly Head of the Division of Inherited Metabolic Diseases, at the Clinical Research Centre in Harrow, and Martin Barratt, who was Head of Paediatric Nephrology at the Institute of Child Health. In addition, the 8th Workshop followed the precedent set by the 7th Workshop by inviting two world-famous Guest Speakers, Alan Fersht and Sue Povey, whose research interests, although not directly in the area of PH, are in important fields of considerable relevance to PH researchers.

The session on “recent advances in the molecular aetiology and pathology of PH” (abstracts 1–8) concentrated on the new information provided by the recently solved crystal structures of AGT and GRHPR, and how this has allowed rational analysis of the relationships between genotype and molecular, if not clinical, phenotype. Of particular interest were the new insights into how specific missense mutations caused their untoward effects, such as enzyme aggregation, accelerated degradation, loss of catalytic activity, and mistargeting. It is clear that, as is the case in many genetic diseases, most missense mutations in PH cause their effects by decreasing protein stability. The long-recognized relationship between pyridoxine-responsiveness of some PH1 patients and AGT peroxisome-to-mitochondrion mistargeting has become much clearer, allowing pyridoxine supplementation to be used more rationally in the clinical management of PH1 patients. However, its exact mechanism of action in PH1 remains obscure. Within this session, one of the Guest Speakers, Sue Povey, gave a light-hearted talk on the work of the HUGO Gene Nomenclature Group and how problems with the naming of *AGXT* and *GRHPR* were resolved.

The session on “current approaches to diagnosis and treatment” (abstracts 9–15) dealt with the practical difficulties of differential diagnosis and the best treatment options for PH patients. Although a general consensus exists with regard to most areas of the diagnosis and treatment of PH1 and PH2, disagreements are still found with regard to the relative importance of enzyme and DNA analysis in diagnosis. For example, while it is apparent from the presentations that molecular analysis is very much in favour at the moment, issues have arisen regarding confirmation of pathogenicity of any missense mutations found. The relative value of isolated kidney, isolated liver, and combined liver-kidney transplantation in the treatment of PH1 is also much debated,

especially because the rationale behind kidney and liver transplantation in PH1 is completely different. Kidney transplantation replaces the organ whose function is impaired by calcium oxalate deposition, whereas liver transplantation is a specialized form of enzyme replacement therapy.

The session on “search for new treatments (part 1: chemical chaperones)” (abstracts 16–20) concentrated on the development of model systems with potential in the screening for small molecule drugs (chemical chaperones) that might stabilize mutant AGT or GRHPR. Systems based on humanized transgenic KO mice, transformed mammalian cell lines, and yeast were discussed. All were shown to have possibilities at various stages of the screening process. In this session, the second of the Guest Speakers, Alan Fersht, gave a very informative talk on the destabilizing effects of mutations in the tumour suppressor p53 and how they can be counteracted by chemical chaperones. At the end of this session, PH1 was discussed in the wider context of peroxisomal diseases in general.

The session on “search for new treatments (part 2: novel use of the liver and gut)” (abstracts 21–24) concentrated on the development of alternative organ-orientated approaches to treatment that had not previously been considered to be a possibility in PH. One such approach was replacing liver transplantation by hepatocyte transplantation, using cells either from a normal liver or the patient’s own liver cells virally-transduced *ex vivo* to express AGT. Another approach discussed was to increase oxalate excretion into the gut, thereby decreasing the load on the kidney.

The lay session (abstracts 25–33) covered all aspects of PH of concern to PH patients, their families, and friends. The talks in this highly-interactive session were delivered mainly by practicing

clinicians and scientists, but also by representatives from North American and European patient support organizations. Almost all aspects of PH1 and PH2 were discussed, including causes, diagnosis, and treatment. The patients and their families benefited greatly from interaction with the professionals and each other.

In addition to the above session, there were two poster sessions (abstracts 34–61). Four of the posters were selected for oral communication (abstracts 36, 46, 49, 50). One oral communication (abstract 36) and two posters (abstracts 35 and 60) were awarded prizes for “best in show”.

Financial support for research into rare diseases, such as the primary hyperoxalurias, is difficult to obtain. Support for meetings to discuss the latest research discoveries and improvements in clinical management is even more difficult to find. Therefore, the organizers of the 8th International Primary Hyperoxaluria Workshop wish to express their sincere thanks and indebtedness to the following organizations who generously provided the financial support necessary for this meeting to take place: Oxalosis and Hyperoxaluria Foundation, National Institutes of Health (NIDDK, R13DK080513), Altus Pharmaceuticals, Oxthera, Promega, Astellas, Trinity Biotech, Wyeth, and PH-selbsthilfe.

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(Co-organizers of the 8th International Primary Hyperoxaluria Workshop)

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1. What have we learnt from the crystal structure of AGT?

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The solution of the crystal structure of human alanine:glyoxylate aminotransferase (AGT)¹ has advanced our understanding of its normal physicochemical, enzymological and cell biological properties, as well as how they are perturbed by the missense mutations and polymorphisms found in primary hyperoxaluria type 1 (PH1). For example, knowledge of the three-dimensional conformation of AGT explains: (1) how the Gly82Glu mutation abolishes AGT catalytic activity by preventing the binding of the cofactor pyridoxal phosphate, (2) how the Gly41Arg mutation prevents AGT dimerization thereby initiating its intraperoxisomal aggregation, and (3) how the synergistic interaction between the Pro11Leu polymorphism and Gly170Arg mutation leads to peroxisome-to-mitochondrion AGT mistargeting. In a more general sense, it allows the prediction to be made that many missense mutations, as well as the Pro11Leu polymorphism, cause their effects by destabilizing the AGT protein. This has opened up a new line of research: the design of pharmacological agents that might counteract the untoward destabilizing effects of these mutations and polymorphisms. Proof of principle that this approach might work has been obtained, using non-specific protein stabilization agents, for both the Pro11Leu–Gly170Arg combination that leads to AGT mistargeting² and the Pro11Leu–Ile244Thr combination that leads to AGT aggregation and accelerated degradation³. The crystal structure of AGT can now be used for computer-aided structure-based design of small, much more specific, high-affinity stabilizing molecules that might be of use as pharmacological treatments in the future.

1. Zhang et al. *J Mol Biol* 331, 643–652, 2003

2. Lumb et al. *Biochem J* 374, 79–87, 2003

3. Santana et al. *PNAS* 100, 7277–7282, 2003

2. What we can learn from the crystal structure of GRHPR

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Primary hyperoxaluria type 2 (PH2) diseases are associated with changes in the function of the enzyme glyoxylate reductase/hydroxypyruvate reductase (GRHPR). In order to explore the molecular basis of GRHPR activity, we have determined the crystal structure of human GRHPR. Fortunately, the crystal structure provides two views of the active site of the enzyme: one as a ternary complex (enzyme + NADPH + reduced substrate) form, and the other as a binary (enzyme + NADPH) form. Overall, GRHPR displays a typical D-2-hydroxy-acid dehydrogenase family structure. However, the active site arrangement exhibits some curious features that explain the mode of substrate binding, stereospecificity and likely catalytic mechanism for this enzyme. GRHPR utilises both glyoxylate and hydroxypyruvate as substrates, but not—despite its similarity—pyruvate. This unusual selectivity is explained through the composition of the active site cleft, including the protrusion of a tryptophan residue (Trp141) from a neighbouring subunit. GRHPR also differs substantially in structure from LDH, even though both can utilise similar substrates. Finally, the GRHPR crystal structure provides a basis on which to interpret the reduction in GRHPR activity associated with mis-sense mutations of the gene for this enzyme that lead to PH2.

3. Effects of mutations on the dimerization and stability of AGT

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In genetic enzyme deficiencies, few missense mutations have a direct effect on catalysis but more frequently lead to mis-folding and subsequent increased susceptibility to degradation and/or inappropriate oligomerization. Approximately 50% of the PH1 mutations in the AGT gene are missense. The best characterized of these is gly170arg which is not only the most common mutation in PH1, but also has been demonstrated to exert its effect by hindering dimerization, thereby potentiating mis-targeting of AGT to mitochondria rather than peroxisomes. We have used bacterial and rabbit reticulocyte expression systems to examine the impact of a spectrum of missense mutation on enzymatic activity, dimerization, aggregation and biological stability of AGT. Partial or complete dimerization failure was observed for 13 of 15 mutant AGTs examined. Varying degrees of ATP-dependent proteasome-mediated degradation were observed for most of the mutants although there were significant exceptions. In a few cases, addition of PLP increased stability to proteasome degradation and enhanced dimerization. We also observed a specific and limited non-proteasomal proteolytic cleavage in the absence of ATP. Mimicking this activity with trypsin suggested a specific susceptible site. These results provide a starting point for possible future testing of potential pharmacological chaperones.

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4. New insights into pyridoxine (VB6) response in type 1 primary hyperoxaluria (PH1): possible association of glycine to arginine substitutions at alanine: glyoxylate aminotransferase (AGT) residues other than 170

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To date, VB6 response has been observed in PHI patients harboring 1 or 2 alleles of AGT peroxisome-to-mitochondrion mistargeting mutations (G170R, F152I). Partial VB6 response has been characteristic with 1, and complete response with 2 alleles. While a VB6 chaperone effect that corrects AGT mistargeting has been postulated, in vitro work has failed to confirm this explanation. During genotyping of PHI patients, we observed a few unexpected patterns of VB6 response.

We detected 3 G170R compound heterozygotes with normal post-VB6 urine oxalate (Uox), 2 of whom appeared to show a dose response:

Documented segregation of G41R with the major AGXT allele in patient 1, along with her observed complete VB6 response (in contrast to patient 4), supports a milder pathogenic effect for G41R when inherited on this haplotype, as shown in vitro. The observed association of different mutations (G170R, F152I, G41R, G190R) with VB6 response suggests the effect is not

*AGXT genotyping in “atypical” VB6 responders:

PT	Polymorphisms		Mutations		Uox (mmol/ 1.73 m ² / 24 h)	Dose VB6 (mg/kg/ day)
	IVS1 + 74 bp	c. 32 C > T (P11L)	Allele 1	Allele 2		
1	Dd [†]	CT	G170R	G41R	1.33 1.05 0.46	0 2.2 7.4
2	Dd	CT	G170R	G190R	2.54 1.04 0.24	0 1.7 5.7
3	Dd	CT	G170R	Pending	1.71 0.94 0.46–0.53	0 7.8 5.9
4	Dd	TT	G170R	G41R	3.30 2.25	0 5

* Results confirmed in separately amplified products

[†] mother, Dd + G170R heterozygous; father, DD

specific to mistargeting phenotypes. A VB6-specific conformational effect on AGT dimerization or glycine to arginine substitutions is a potential explanation.

5. Clinical implications of mutation analysis in primary hyperoxaluria type 1

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Background: Primary hyperoxaluria type 1 (PH1) has an extensive clinical and genetic heterogeneity. Although over 50 disease-causing mutations have been identified, the relationship between genotype and clinical outcome remains unclear. The aim of this study was to determine this association in order to find clues for improvement of patient care.

Methods: AGXT mutation analysis and assessment of biochemical characteristics and clinical outcome of patients from a Dutch PH1 cohort.

Results: 33 of a cohort of 57 PH1 patients, identified in The Netherlands over 30 years, were analyzed. Ten different mutations were found. The most common mutations were the Gly170Arg, Phe152Ile and the 33InsC mutations with an allele frequency of 43, 19 and 15%, respectively. Homozygous Gly170Arg and Phe152Ile mutations were associated with pyridoxine responsiveness and a preserved renal function over time when treatment was timely initiated. All patients homozygous for the 33InsC mutation had end-stage renal disease before the first year of age. In two unrelated patients, a new Val336Asp mutation was found coupled with the Gly170Arg mutation on the minor allele. We also found 3 patients homozygous for a novel Gly82Arg mutation with adverse outcome in two of them.

Conclusion: Early detection of Gly170Arg and Phe152Ile mutations in PH1 has important clinical implications because of their

association with pyridoxine responsiveness and clinical outcome. The association of a homozygous 33insC mutation with severe infantile ESRD, resulting in early deaths in 2 out of 3 cases, warrants a choice for prenatal diagnostics in affected families.

6. AGXT mutational analysis in PH1 patients: the Italian job

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Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder caused by a deficiency of alanine-glyoxylate aminotransferase (AGT), which is encoded by a single copy gene (AGXT). Molecular diagnosis was used in conjunction with clinical, biochemical and enzymological data in order to evaluate genotype–phenotype correlation.

More than 50 patients (and in many cases their family members) were analysed in 10 years activity of the Italian laboratory for molecular diagnosis of PH1, formerly located in Trieste and since 2004 in Torino. During this time, several techniques have been developed for DNA analysis, from heteroduplex analysis, to DHPLC, Taqman assay and sequencing.

More than 20 different mutations were characterized, some of them never described before. For the new mutations, several approaches were used in order to define their pathogenicity. The disease in Italian patients was high heterogeneous from clinical and genetic point of view. Mutant alleles have been recognised in 80–90% of chromosomes, depending of techniques used. Mutations in exons 1, 2, 4 and 10 are more frequent in Italian patients. Normalized AGT activity seems to be lower in the severe form than in the adult form. The T444C mutation was more frequent in the severe form, while the opposite was observed for G630A. G630A mutation homozygotes had a higher AGT residual activity.

The presence of allelic heterogeneity of AGXT could be responsible, to some extent, for the phenotypic heterogeneity in PH1.

7. Alternative metabolic pathways

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The metabolic functions of the enzymes glycolate oxidase, keto-hydroxyglutarate aldolase, lactate dehydrogenase, alanine:glyoxylate aminotransferase and glyoxylate reductase in oxalate and glyoxylate metabolism have been firmly established. In addition, there is support for a role for D-amino acid oxidase oxidation of glycine to glyoxylate and the splitting of xylulose-1-phosphate to glycolaldehyde, a presumed precursor of glycolate, by aldolase. The roles of other metabolic pathways that lead to glyoxylate or oxalate synthesis are uncertain. Other potential substrates that have been proposed as potential precursors of oxalate include ethanolamine, serine, phenylalanine, tyrosine, tryptophan, fructose and glucose. The evidence supporting metabolism through these metabolic pathways will be evaluated as well as a discussion of technical difficulties in determining low levels of oxalate production free from artefactual oxalate synthesis. The availability of new instruments that will facilitate an examination of these presumptive pathways will also be discussed.

8. *AGXT* and *GRHPR*: the problems with gene nomenclature! (guest lecture)

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The role of the HUGO Human gene nomenclature committee (HGNC) is to provide a full name and a short form abbreviation, known as a symbol, for every human gene, and to ensure that these standard names are used in all publications and databases. We aim to provide a unique, meaningful, memorable and stable symbol for every human gene, and as far as possible to make sure that the orthologous gene in mice and other mammals has the same symbol. When possible the name attempts to indicate the normal function of the gene product. Almost everyone agrees in principle with this worthy goal, but its achievement is not always easy in practice. Sometimes a name which would be quite acceptable to workers within a field is already approved for another gene. This in fact what happened to the gene coding for alanine-glyoxylate aminotransferase, since the most obvious abbreviation, AGT, was already approved and well used for Angiotensinogen. Graciously but reluctantly the workers on hyperoxaluria accepted *AGXT*.

The situation with glyoxylate reductase/hydroxypyruvate reductase illustrates another common problem; two demonstrated activities of the same protein, with some disagreement about which is most important. The compromise symbol *GRHPR* was negotiated in 1999. Currently there are 24,419 approved symbols for human genes. Visit our website at <http://www.gene.ucl.ac.uk/nomenclature/>. Some of the new challenges will be discussed and comments will be welcome. Controversy is never far away!

9. Diagnostic algorithms: pitfalls and evolution

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Diagnosis of the primary hyperoxalurias (PH) has been facilitated by more specific, less invasive, and highly accurate diagnostic methods developed over time. It has also become more difficult due to appreciation of a broader range of disease expression, few laboratories world-wide with necessary expertise, variations in values and units of measurement with different assay methods, limited normal values data in infants and young children, the plurality of the hyperoxalurias, and the expense of newer diagnostic methods.

The diagnosis begins with informed clinical suspicion of PH in an individual patient (high sensitivity), followed by a logical sequence of testing designed to identify early those with other forms of urolithiasis or hyperoxaluria. Specific diagnostic methods are reserved for those with high likelihood of having PH. Efficiency, accuracy, and definitive diagnosis at reasonable cost are desired outcomes.

The range of clinical settings in which PH should be considered continues to expand. Confirmation of hyperoxaluria can be difficult, for example in infants with stones or nephrocalcinosis. More comprehensive normal ranges than previously available and new strategies can now be employed. Identification of causative factors in patients at risk for enteric hyperoxaluria yet with clinical findings suggesting PH can be challenging. In all patients, the

roles of glycolate and glycerate testing, liver biopsy, and molecular genetics are evolving.

As clinical behavior of the primary hyperoxalurias is better understood, information obtained during diagnosis increasingly has treatment and prognostic implications. A careful diagnostic strategy is important in achieving optimum care and outcome for each patient.

10. Evidence-based approach for the diagnosis of PH1

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Definitive diagnosis of Primary Hyperoxaluria type 1 has traditionally relied upon measurement of alanine:glyoxylate aminotransferase (AGT) activity in a liver biopsy. Increasingly a molecular genetics approach is being utilised for the diagnosis of this autosomal recessive disease.

Effective utilisation of a diagnostic test requires knowledge of diagnostic sensitivity and performance criteria e.g. accuracy, precision. To establish such an evidence base for a molecular screening test, we have evaluated the performance of DNA sequencing in exons 1, 4 and 7 of the *AGXT* gene, the site of three commonly occurring mutations.

DNA from 300 biopsy proven PH1 patients was amplified by PCR and selective sequencing of exons 1, 4 and 7 carried out. Mutations were identified in 70% of disease alleles and a molecular diagnosis (i.e. 2 defective alleles found) was possible in 50% of patients. While lower in sensitivity than either whole gene sequencing or liver biopsy analysis, the mutation screen nevertheless provides an excellent, non-invasive first line test.

Whole gene sequencing can substantially increase the sensitivity of DNA testing, but the likelihood of detecting novel and/or unproven sequence changes will be increased. In these cases or where no sequence changes are found, liver biopsy analysis will still be required for definitive diagnosis.

Issues arising from molecular analysis, including confirmation of pathogenicity, de novo mutations and the role of family studies will be discussed.

11. Glyoxylate reductase activity in blood cells and the diagnosis of primary hyperoxaluria type 2

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Background: Primary hyperoxaluria type 2 (PH2) is due to a deficiency of glyoxylate reductase/hydroxypyruvate reductase (GRHPR) activity. A definitive diagnosis of PH2 is currently made by the analysis of GR activity in a liver biopsy. GRHPR is expressed in most tissues, suggesting that utilizing more readily available cells could be used to determine GRHPR deficiency. In this study, we have identified GRHPR in red blood cells (RBC) and evaluated the potential of determining GR and D-glycerate dehydrogenase (DGDH) activity in blood mononuclear cells (BMC) as a diagnostic indicator of PH2.

Methods: Blood samples were obtained from normal subjects, and from primary hyperoxaluria patients at the Mayo Clinic. BMC and RBC were isolated and GR and DGDH activities measured in cell lysates.

Results: An assay of 20 normal individuals indicated that BMC contained a DGDH and GR activity of 0.97 ± 0.20 (range 0.62–1.45), and 10.6 ± 3.3 (range 8.3–16.6) nmoles/min/mg protein, respectively. The intra-assay coefficient of variation for DGDH and GR activity was 8.2 and 11.5%, respectively. BMC lysates from normal adult subjects and patients with PH1 showed similar GR and DGDH activities. This was confirmed by the presence of immunoreactive GRHPR protein by western blot analysis. In contrast, PH2 BMC lysates did not exhibit DGDH or GR activity, and showed no immunoreactive GRHPR. Red blood cells contained a DGDH activity of 1.6 ± 0.57 nmoles/min/mg haemoglobin ($n = 6$), and western blot analysis confirmed the presence of immunoreactive GRHPR protein.

Conclusion: These results suggest that the assay of DGDH or GR activity in BMC could be used as a minimally invasive diagnostic test for PH2. The presence of GRHPR in red blood cells suggests whole blood could also be used to diagnose PH2.

12. Current treatment algorithms

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Treatment options in patients with primary hyperoxaluria (PH) are scarce. Treatment and hence disease outcome dramatically relies on patients compliance. Hence, optimization of treatment is based on the patient's individual needs. (1) Treatment in stable renal function: Primary measure is adequate daily fluid intake. All other therapeutic regimen will be less effective, if fluid intake is insufficient. A fluid intake of >3 l per 1.73 m^2 BSA per day should be achieved, even if this is only possible via gastric tubing in infants and children. Special dietary recommendations are not particularly important, other than the avoidance of extremely oxalate rich nutrients. Pharmacological doses of pyridoxine may significantly reduce hyperoxaluria in ~30–50% of patients with PH type I. Certain mutations of *AGXT* appear associated with pyridoxine sensitivity and can be useful in guiding therapy. All patients with type I PH should receive B6 in increasing dosages at diagnosis to find proof of usefulness. If useless, medication may be stopped. Crystallization inhibitors like alkaline citrate, orthophosphate or magnesium are (equally) beneficial in increasing the urinary solubility. (2) Treatment in ESRF: in type I PH pyridoxine may help reducing the endogenous oxalate production. All renal replacement therapies, even a combination of hemo- and peritoneal dialysis, are insufficient in oxalate removal. Hence, early combined liver-kidney transplantation is the method of choice in most patients. (3) Future options: chaperone treatment, application of oxalate degrading bacteria or enzymes and hepatocyte transplantation are currently under investigation.

13. An update from the European PH1 liver/kidney transplant registry: short and long term outcomes

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Between 1984 and 2007 33 European Centres reported more than 140 liver transplants (usually in combination with a renal trans-

plant) in over 130 patients with an underlying diagnosis of primary hyperoxaluria (PH1). Mean age of onset of first symptoms was 5.6 ± 7.8 years with a mean delay to diagnosis of 3.5 ± 5.9 years. Diagnosis was based on biochemical findings initially backed up increasingly by AGT assays and latterly by genetic analysis identifying known mutations. The mean age at transplantation was 16.5 ± 11.4 years with a mean duration of dialysis of 3.2 ± 3.2 years. There was a family history in 42% of cases. Following transplantation 1, 2, 5 and 10 year patient survival rates were 85, 82, 79 and 68% and graft survival rates were 80, 73, 71 and 59% at the same time intervals. Patients who had been on dialysis for less than 2 years at the time of transplant were more likely to be assessed as being in good general condition at the time of transplantation. These patients had better survival than those who had been on dialysis for longer time periods and came to transplantation with evidence of marked systemic oxalosis. Combined liver/kidney transplantation gives excellent results in patients with PH1 although the results are poor when transplantation is delayed until advanced systemic oxalosis has developed.

The registry will continue to collect and analyse data from patients undergoing liver/kidney grafting for PH1. Current data are still being received and will be available for the presentation.

14. The surgical management of renal tract calculi

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There are several modalities of treatment for stones within the renal tract, e.g. ESWL, PCNL, open pyelolithotomy and more recently laparoscopic procedures. The selection of mode of treatment depends on several factors—stone composition, stone bulk or mass, location, presence or absence of infection and/or obstruction.

Extra-corporeal shock wave lithotripsy (ESWL) is the method of choice for stones <2.0 cm in size. It is relatively safe, with the least amount of permanent damage to the renal parenchyma. A piezo-electric device produces the shock waves, which are focused on the calculus. The shattered fragments are passed in the urine over the following 4–6 weeks.

Percutaneous nephrolithotomy (PCNL) is technically challenging and involves introduction of instruments into the kidney to fragment and extract the calculus, but is always associated with permanent scars, both on the kidney and on the skin.

Open pyelolithotomy and nephrolithotomy are associated with significant renal parenchymal damage, although there is effective clearance of calculi and residual fragments can be treated with ESWL.

Recent advances include minimal access (laparoscopic) adaptations of open operations—laparoscopic pyelo- and nephrolithotomies.

15. Primary hyperoxaluria: diagnosis and management in developing countries

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Primary hyperoxaluria (PH) is a rare life threatening recessive metabolic disease which is therefore more frequent in populations

with a high rate of consanguinity, a frequent condition in developing countries for both religious and cultural background. Type 1 is likely considered to be the predominant form worldwide.

The diagnosis of index case uses to be delayed and investigation of presymptomatic siblings is often limited. Prenatal diagnosis is not universally accepted and premarital counselling should be preferred to pregnancy termination. In the presence of urolithiasis \pm nephrocalcinosis and impaired GFR, PH is very likely and can be confirmed by crystalluria, urine oxalate measurement, kidney or bone biopsy and fundus examination. DNA analysis may not have been developed on-site but it can be easily shipped; specific mutations have been reported in several ethnic groups. DNA analysis confirms the diagnosis of PH and may contribute to therapeutic strategy.

Improving early diagnosis of PH must lead to early aggressive conservative treatment when it is available, therefore slowing the progression of the disease. Due to both financial and logistic difficulties, combined liver-kidney transplantation cannot be proposed to PH1 patients, so that ethical limits must be discussed. Indeed therapeutic withdrawal may be considered in patients with severe infantile PH1 and aggressive dialysis strategy (daily hemodialysis \pm peritoneal dialysis) may be applied to older patients waiting for organ transplantation. Kidney transplantation is questionable.

Due to its relative frequency and because of life threatening issues, specific medical education programmes in countries with a high prevalence of PH1 are warranted.

16. Transgenic AGTKO mouse as a model system

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Primary hyperoxaluria type I (PH I) is an autosomal recessive disease caused by mutations in the alanine-glyoxylate-aminotransferase (*AGXT*) gene. The functional implications of the main *AGXT* mutations responsible for PH I have been analyzed in detail. In the last few years, we have learned that the most common missense mutations in the *AGXT* gene result in important protein conformational changes but the gene product is still produced and relatively stable. The structure of the AGT protein has been determined, making it possible to understand better the consequences of PH I mutations. This knowledge might also help design and test molecules that could have an impact on the protein conformation.

A genetically modified mouse, AGTKO, with a deletion in the homologous gene, *Agxt*, has been crossed with transgenic mice expressing human *AGXT* under the control of the transthyretin promoter/enhancer. By using *AGXT* cDNAs with the most common PH I mutation (G170R), in the minor haplotype, a “humanized” version of the AGTKO mouse has been produced. This new mouse model reproduces key aspects of the G170R mutation, such as mitochondrial mistargeting of the gene product. This animal model can be used for in vivo testing of new therapeutic approaches. It will be an important system to test the safety and efficacy of small molecules that affect AGT protein folding as a therapeutic tool to correct the most common PH1 mutation.

A similar approach has been carried out for another mutation of the minor haplotype: 1244T.

17. Yeast-based screen for AGT chemical chaperones

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We present two cell-based approaches that use the model eukaryote *S. cerevisiae* to identify pharmacological chaperones that rescue misfolding of disease variants of human AGT. The first approach relies on complementation of yeast lacking endogenous AGT (AGX1) with wild-type or disease variants of human AGT. The second approach involves coupling folding of AGT to an essential metabolic reporter protein, dihydrofolate reductase. In this approach, growth of yeast cells is dependent on activity and folding of the DHFR-misfolded protein fusion. In both assays, stabilization is observed as a change in temperature-sensitive growth of the yeast. Growth temperatures are optimized, such that factors that modulate growth, such as compounds that rescue folding, can be clearly observed. We show that wild-type and disease-associated AGT proteins show clear differences in growth in each system. We are currently optimizing each assay for high-throughput chemical screens. Hits from each screen will be tested for direct effects on stability of AGT using a mass-spectrometry based approach, termed SUPREX, that allows examination of direct ligand binding events. This method uses hydrogen-deuterium exchange and MALDI mass-spectrometry to report ligand-induced stability changes. We show preliminary results from SUPREX analysis of bacterially expressed wild-type/major allele AGT and minor allele/1244T AGT with known ligand pyridoxal phosphate (PLP) and inhibitor AOA.

18. The potential of stably-transformed CHO cells to screen for AGT and GRHPR chemical chaperones

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In order to investigate the metabolic complexity of cellular oxalate synthesis and how it might be manipulated by endogenous and exogenous factors, we have constructed a model system based on singly, doubly and triply stably transformed Chinese hamster ovary (CHO) cells¹. CHO cells do not normally express alanine:glyoxylate aminotransferase (AGT), glyoxylate/hydroxypyruvate reductase (GRHPR), or glycolate oxidase (GO), three of the most important enzymic determinants of oxalate synthesis. This allows the function of normal and mutant human AGT, GRHPR and GO to be studied singly and in combination without background interference from endogenous enzyme.

Enzyme function, in the presence or absence of potential pharmacological modifiers, can be assessed either by the measurement of oxalate, glyoxylate, glycine and glycolate synthesis or, perhaps more efficiently, by measuring cell survival in the presence of glycolate. Glycolate is normally fairly innocuous. However, in the presence of GO, glycolate is oxidised to glyoxylate, which is cytotoxic. Therefore, in the triple transformant, for example, any agent that increases GO activity or decreases AGT/GRHPR activity will increase indirect glycolate toxicity, whereas anything that does the opposite will decrease it. This provides a simple cellular system in which to test the effectiveness of chemical chaperones or inhibitors as potential therapeutics for PH1, PH2 or any other disorder of oxalate synthesis.

1. Behnam et al., *Biochem J* 394, 409–416, 2006

19. p53: structure, activity, rescue (guest lecture)

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Systematic mutagenesis has been one of the most powerful tools for elucidating the principles of protein folding and stability. Mutation is the principle cause of cancer, and the technology for studying simple folding can be transferred to the study of proteins involved in cancer. Some 50% of human cancers have mutations that inactivate the tumor suppressor p53. Virtually all of the oncogenic mutations reside in the core domain that binds specifically to DNA. We have found that a significant proportion of the mutations inactivate the protein by lowering its melting temperature to body temperature or below. This has raised the possibility of designing small molecules that rescue those oncogenic mutants simply by binding to the native state of the protein and hence raising its melting temperature by the principal of mass action. To understand further the structure of the protein and hence the rational design of drugs, we have solved the structure of oncogenic mutants at high resolution. We have engineered a more stable variant, which is biologically active and have solved the crystal structures of oncogenic mutants in this framework. Some cancer mutations cause surface cavities that are drug targets.

20. PH1 in the context of peroxisomal diseases

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Peroxisomal disorders (PDs) are usually subdivided into two groups including: (1) the peroxisome biogenesis disorders (PBD), with Zellweger syndrome as prototype, and (2) the single peroxisomal enzyme deficiencies with X-linked adrenoleukodystrophy as prototype. The main functions of peroxisomes in human beings include: (1) fatty acid beta-oxidation; (2) etherphospholipid biosynthesis; (3) fatty acid alpha-oxidation, and (4) glyoxylate detoxification. Hyperoxaluria type 1 (PH1) belongs to the group of single peroxisomal enzyme deficiencies and is the only peroxisomal disorder in which glyoxylate metabolism is perturbed. However, glyoxylate metabolism is also impaired in some of the peroxisome biogenesis disorders, notably Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD), which represent a spectrum of disease severity, with ZS being the most severe and IRD being the least severe disorder. Common to all three PBDs are liver disease, variable neurodevelopmental delay, retinopathy, and perceptive deafness. As a consequence of the defect in peroxisome biogenesis, virtually all the peroxisomal enzymes are deficient in cells from PBD patients. Notable exceptions are catalase and alanine glyoxylate aminotransferase (AGT), which apparently can mature properly in peroxisome deficient cells. Nevertheless, loss of the compartmental organization in peroxisome deficient cells with AGT distributed over the entire cytosol, may lead to the disturbed degradation of glyoxylate, at least partially. This is possibly due to the fact that under normal conditions glyoxylate is predominantly formed in peroxisomes and rapidly detoxified by AGT, whereas in peroxisome deficient cells AGT has to compete with other enzymes like lactate dehydrogenase, thus causing a less efficient conversion of glyoxylate into glycine.

21. Hepatocyte transplantation for the treatment of primary hyperoxaluria 1 in a mouse model

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Aims: To develop hepatocyte transplantation-based treatment strategies for primary hyperoxaluria type I (PH1), using an *Agxt-1* gene-deleted mouse model.

Principle and procedure: Since hepatocytes overproduce oxalate in PH1, for hepatocyte transplantation to be therapeutically effective, a large proportion of the mutant hepatocytes must be replaced by *Agxt*-competent hepatocytes. Such repopulation of the host liver requires preferential proliferation of the engrafted cells over host hepatocytes. Here we describe a preparative regimen combining hepatic X-irradiation (HIR) and expression of hepatic growth factor (HGF), which provides a competitive advantage to transplanted non-irradiated wildtype hepatocytes. Recipient *Agxt*^{-/-} mice were injected with 10¹⁰ IU of an adenovector expressing human HGF (Ad-HGF) and, 6 h later, underwent HIR (50 Gy), shielding other organs. After this, 10⁶ hepatocytes from *Agxt* competent C57Bl6-ROSA26-LacZ transgenic mice were transplanted by intrasplenic injection. Urinary oxalate levels and microscopic crystalluria were examined before and at intervals after the procedure.

Results: Histochemical staining of the recipient livers showed that 1 week after the procedure, ~0.5% of the hepatocyte mass consisted of donor hepatocytes. Proportion of the donor cells increased progressively to ~10, ~20 and >90% in 4, 8 and 24 weeks, respectively. In control groups receiving HIR or Ad-AHGF only, the repopulation did not exceed 2%. Following repopulation, urinary oxalate excretion was reduced significantly from pre-transplantation level of 1.51 ± 0.53 to 0.19 ± 0.006 mM (*P* < 0.01) (normal, 0.1–0.2 mM) and oxalate crystals became undetectable in urine sediments.

Conclusion: Preparative HIR and HGF expression resulted in therapeutically sufficient repopulation of the mouse liver with transplanted wildtype hepatocytes.

22. Ins and outs of enteric oxalate transport

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Not only does the mammalian intestine absorb oxalate by energy-dependent transcellular pathways, it also exhibits the ability to support net, transcellular oxalate secretion in a segment-specific manner. In general, small intestinal segments and proximal colon spontaneously secrete oxalate, while distal colonic segments from healthy animals actively absorb oxalate. Functionally, net oxalate secretion across the intestine can serve as an extra-renal avenue for oxalate excretion and ways to exploit this, especially in patients with primary hyperoxaluria, can theoretically reduce the burden of oxalate excretion via the kidneys and minimize the risk of hyperoxaluria, oxalosis, and kidney failure. Compelling evidence has emerged from a

number of human and animal studies suggesting that *Oxalobacter* sp. can play an important role in reducing intestinal oxalate absorption. The results of all of these studies are consistent with the notion that *Oxalobacter* can degrade intraluminal, dietary-derived oxalate and reduce the amount of oxalate available for absorption. However, a more provocative question that we have begun to address is whether *Oxalobacter* can derive oxalate from systemic sources possibly by initiating or enhancing intestinal oxalate secretion. We recently showed that *Oxalobacter formigenes* can modulate intestinal oxalate transport in rats by inducing colonic oxalate secretion and a positive consequence of this bacterium-enterocyte interaction is a significant reduction in urinary oxalate excretion due to the induced enteric oxalate shunt. Clearly, an understanding of the mechanistic basis for bacterial cell modulation of intestinal oxalate handling is fundamental to future efforts in identifying which strains of bacteria and/or bacterial products will be effective in the treatment of hyperoxaluria and calcium oxalate stone disease.

23. Oxabact™: a potential treatment for primary hyperoxaluria

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GI tract can act both as a site for oxalate absorption and its excretion through enteric elimination. Pharmacological approaches to maximize enteric elimination of oxalate can be used to lower oxalate excretion via kidneys to prevent hyperoxaluria, oxalosis and renal failure. Oxabact™ therapy utilizes *O. formigenes* mediated degradation of oxalate in the intestine to reduce urinary oxalate levels. Its oral administration to the GI tract increases the degradation of oxalate and generates a suitable trans-epithelial gradient to promote the removal of endogenously produced plasma oxalate by enteric elimination. Among a variety of oxalate degrading enzymes, *O. formigenes* has been selected for its high efficiency to degrade oxalate in the GI environment. There is also a very low risk with its administration since this bacterium is a part of normal gut flora in humans, it is non pathogenic and there have been no reports of any systemic infections with this bacterium.

Hatch et al. 2006 have demonstrated that *O. formigenes* administration can induce colonic secretion/excretion of endogenously produced oxalate in a rat model thereby reducing urinary oxalate excretion. The safety and efficacy of *O. formigenes* treatment has been investigated in two open label studies in PH patients (Hoppe et al. 2006). The decline in urine and plasma oxalate observed during treatment provides clinical verification of enteric elimination of oxalate. The benefit of this treatment was observed in patients with normal renal function, after end stage renal failure and after transplantation. Oxabact™ treatment for 4 weeks produced >60% reduction in mean urinary oxalate excretion compared to baseline levels in PH patients with their native functional kidneys. This was accompanied with significant decrease in plasma oxalate levels. The treatment was well tolerated and no safety concerns were raised during this 4-week study.

The long term efficacy of Oxabact™ to reduce urinary oxalate levels in PH patients is now being assessed in a multicentric randomized double blind placebo controlled trial.

24. Efficacy of an oral crystalline enzyme in a mouse genetic model for primary hyperoxaluria

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Hyperoxaluria is a major risk factor for recurrent urolithiasis and progressive nephrocalcinosis. Primary hyperoxaluria (PH-1), caused by a deficiency of hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT-1), leads to overproduction of oxalate, progressive nephrocalcinosis, kidney function loss, oxalemia, and systemic oxalosis. Therapies for patients with PH-1 are limited.

The search for an effective therapy for oxalate removal has been hindered by both the lack of an oxalate-degrading enzyme that is stable and active throughout the stomach and intestine, and the absence of PH-1 animal models. We hypothesized that oral therapy with a highly specific oxalate-degrading enzyme, formulated as cross-linked enzyme crystals (ALTU-237), could significantly reduce hyperoxaluria and decrease the severity of renal injury, provided that the enzyme was both active and stable in the degradative conditions of the gut. We evaluated the efficacy of ALTU-237 therapy on the reduction of hyperoxaluria, prevention of nephrocalcinosis, preservation of kidney function, and survival in EG-challenged AGT1KO mice that mimic human PH-1. We orally administered daily doses of 5, 25, and 80 mg ALTU-237, mixed with food, for 32 days.

Oral therapy with ALTU-237 resulted in a significant reduction (30–50%) in urinary oxalate excretion when compared to controls. Hyperoxaluria was maximally suppressed in mice fed with the highest (80 mg) dose, where a constant urinary oxalate reduction of 40–50% resulted in the total prevention of nephrocalcinosis, renal failure, and death. The efficacy of ALTU-237 presents a realistic option for treatment of hyperoxaluria.

25. Understanding oxalosis

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Oxalate occurs normally in the body and is found in food such as spinach, rhubarb, chocolate and tea. Variable amounts of oxalate are absorbed by the gut into the blood system. Oxalate also occurs in the bloodstream as part of the normal working of cells known as cell metabolism. This oxalate is normally passed out in urine. Certain individuals have inherited genes that result in abnormal or defective breakdown of oxalate in the body. The two main enzymes that are responsible for oxalate breakdown (AGT and GR enzymes) are found predominantly in the liver. Patients who suffer from deficiencies of these enzymes have a disease called oxalosis or primary hyperoxaluria. As a result of these enzyme deficiencies oxalate can build up in the bloodstream and form a hard insoluble substance called calcium oxalate. This calcium oxalate can settle in kidney tissue causing calcification of the kidneys as well as in the urinary pipe system causing kidney stones.

There is a great variability in people who suffer from oxalosis, some individuals are unaware that they have an illness at all,

others pass kidney stones whereas some are more severely affected and will develop kidney failure in early life.

26. Usefulness of liver biopsy and genetics in diagnosis of primary hyperoxaluria

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A clinician who suspects a diagnosis of primary hyperoxaluria will ask the laboratory to perform a number of tests of increasing difficulty. These tests are not readily available and are usually only done in centres specialising in this work. Here at UCLH we receive samples from as far afield as Brazil and Australia and as close to home as the Institute of Child Health. Because these tests are rare they tend to be done in batches, and therefore the results can take some time to become available (e.g. from 2 days to 3 months depending on the test).

The first test is measurement of oxalate in a urine sample. This test is usually done more than once to confirm that it is high. A 24 h urine collection is best but this can be a problem in children so a random sample is usually taken from a child. This test will simply confirm that a raised oxalate is present. It does not tell us why it is high.

The next test may be a liver biopsy. With a small amount of liver material (about the size of an orange pip) we can measure the enzymes which cause PH1 and PH2 to see if one of them is absent. If it is, then the diagnosis of PH is made.

DNA testing is increasing in popularity because it is done on a simple blood sample. With this test we can look for the change in the DNA (=mutation) which causes the disease. If we find a change we can also look for it in other family members. DNA-based tests can also be used for prenatal diagnosis.

27. What is in store for the future? The importance of basic research

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Basic laboratory research underpins almost all advances in the clinical management of human disease. This can be seen very clearly in the case of primary hyperoxaluria, particularly PH1, where new discoveries have been followed rapidly by improvements in patient care. For example, the discovery of liver AGT deficiency as the basic enzyme defect in PH1 in 1986 led immediately to the development of a definitive diagnostic test based on the enzyme assay of a percutaneous needle liver biopsy, as well as the first ever prenatal diagnosis. It also led to the introduction of liver transplantation as a form of enzyme replacement therapy that can result in a metabolic cure. The discovery of the mutant gene *AGXT* in 1990 enabled the identification of nearly 100 mutations over the next 15 or so years, which in turn has allowed significant improvements in postnatal and, particularly, prenatal diagnosis. The latter can now be carried out using standard amniocentesis and DNA analysis in the first trimester (i.e. within the first 14 weeks of gestation).

New laboratory findings are likely to continue benefiting patient care in the future. For example, the recent determination of the three-dimensional structure of AGT (in 2003) has provided the foundation for further advances, particularly in the development of

new drugs that might be able to counteract the untoward effects of mutations in the *AGXT* gene and lead to a new treatment for PH1.

As can be seen with PH1, although basic laboratory research might appear to be rather remote from the patient's immediate needs, it provides the foundation for material advances in patient care.

28. Treatment options

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Treatment options in patients with primary hyperoxaluria (PH) are scarce. Disease outcome relies on early diagnosis and treatment instalment, but also on patient compliance. Optimization of treatment is based on the patient's individual needs. (1) Treatment in stable renal function: adequate daily fluid intake is most important. All other therapeutic regimen will be less effective, if fluid intake is insufficient. A fluid intake of > 3 l per day should at least be achieved, even if this is only possible via gastric tubing in infants and children. Special dietary recommendations are not particularly important, other than the avoidance of extremely oxalate rich nutrients. Pharmacological doses of vitamin B6 may significantly reduce hyperoxaluria in ~30–50% of PH type I patients and especially in those with certain mutations of the disease specific gene. All patients with PH I should receive B6 in increasing dosages at diagnosis to find proof of usefulness. If useless, medication may be stopped. Crystallization inhibitors like alkaline citrate, orthophosphate or magnesium are (equally) beneficial in increasing the urinary solubility. (2) Treatment in renal failure: in type I PH vitamin B6 may help reducing the oxalate production by the liver. All dialysis methods, even a combination of hemo- and peritoneal dialysis, are insufficient in oxalate removal. Hence, early combined liver-kidney transplantation is the method of choice in most patients. (3) Future options: treatment with newly synthesized small proteins, with oxalate degrading bacteria or enzymes and liver cell instead of whole liver transplantation are currently under investigation.

29. The surgical management of kidney stones

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There are several different methods to treat stones within the urinary system (kidneys, ureters and bladder), e.g. ESWL, PCNL, open operations and more recently Laparoscopic procedures. The selection of type of treatment depends on several factors—stone composition, stone bulk or mass, location, presence or absence of infection and/or obstruction.

Extra-corporeal shock wave lithotripsy (ESWL) is the method of choice for stones less than 2.0 cm in size. It is relatively safe, with the least amount of long-term damage to kidney tissue. A machine produces the shock waves, which are then focused on the stone. The shattered fragments are passed in the urine over the a prolonged period, sometimes for 4–6 weeks afterwards

Percutaneous nephrolithotomy (PCNL) is technically challenging and involves introduction of instruments into the kidney to fragment and to break and remove the stone. This method is always associated with permanent scars, both on the kidney and on the skin.

Open operations are associated with significant kidney damage, however there is effective clearance of stones and residual fragments can be treated with ESWL.

Recent advances include laparoscopic (keyhole) adaptations of open operations—these have the advantage of smaller scars, but are technically demanding.

The treatment of stones, although challenging, is effective in clearing the kidney of stones, while preserving maximum function.

30. Transplant options in PH1

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PH1 causes renal failure by overproduction of oxalate, largely by the liver. This oxalate can only be removed from the body by being filtered through the kidneys where in combination with calcium it forms stones and results in cumulative damage to the kidney. This results in deteriorating renal function over a period of months to years and ends with the patient requiring dialysis. Initially before the key role of the liver in producing excess oxalate was appreciated patients were often offered kidney transplantation as a treatment for their renal failure but these usually failed quickly due to recurrence of oxalate stones. Some specialised centres were able to get better results by special high intake fluid treatments but the underlying error in the liver was not corrected and the management was difficult. Once the key nature of the enzyme problem in the liver was appreciated combined liver kidney transplantation was performed and we now have long term results showing that this is a good treatment: allowing both correction of the liver problem and a successful kidney transplant. Following combined liver and kidney transplantation 1, 2, 5 and 10 year patient survival rates were 85, 82, 79 and 68% and graft survival rates were 80, 73, 71 and 59% at the same time intervals. A smaller number of patients have been treated with a liver transplant alone before the patients own kidneys have been too badly damaged to continue working but the timing of such operations is difficult to judge.

The options will be discussed together with the longer term implications of having a transplant operation.

31. What is a patient registry, and how can it help me?

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The international registry for primary hyperoxaluria (PH) is a tool to obtain information regarding this rare disease, in order to collectively advance knowledge, and ultimately improve the quality of life of PH patients and their families.

Due to its rarity, most doctors will care for only a few PH patients. Symptoms and problems caused by the disease can differ from patient to patient. This makes it difficult to learn how oxalate affects kidneys and other body systems, and difficult to learn what treatments work best. If patients and doctors can share what they know about PH, everyone together will learn more about the disease.

The patient registry is a location where such information is brought together, analyzed, shared, and used to advance understanding of PH. The purpose of this Registry is to identify as many PH patients as possible from around the world, to determine how the disease behaves during the course of their lifetimes, and to

understand factors that affect its severity. The more patients participate, the more valuable the information. The resulting collection of information is a powerful tool that can be used to:

- Increase understanding of PH.
- Provide evidence to guide patient care.
- Form the basis for future research studies of new treatments. The registry goal of collecting medical information on a large number of patients will help all doctors and scientists to more fully understand the primary hyperoxalurias and to learn better ways of caring for patients with this disease.

32. Welcome to the OHF

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The oxalosis and hyperoxaluria foundation (OHF) is a voluntary, not-for-profit health organization established in 1989. The mission of the OHF is to seek the cause, improve the clinical treatment and discover the cure of hyperoxaluria and oxalate stone disease and enhance the quality of life of patients and their families. In the fight against these diseases, the OHF is a recognized leader through its support of research, professional education, patient programs, public education and governmental and legislative affairs.

The OHF has supported millions of dollars in research and continues to be a leader in funding some of the best science in the world investigating cures and treatments for hyperoxaluria and oxalate stone disease.

In addition to its commitment to research, the OHF believes that no one should have to face hyperoxaluria or oxalate stone disease alone. The OHF offers practical and ongoing support in the form of a Web Site, Patient Support Network, OHF-talk, a Resource Directory and comprehensive informational and educational materials and services.

Volunteers from all walks of life give generously of their time and talents to implement the OHF programs. They provide professional guidance and help raise vitally needed funds.

For more information, visit: <http://www.ohf.org>.

33. The German PH self support group

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The German PH self support group was founded in 2005. Since, more than 50 patients or family members joined us. Most of the members experienced a hard road until diagnosis of primary hyperoxaluria. Clearly, diagnosis would have been made earlier, if all medical personnel would have known about the existence of such a rare metabolic disorder. Therefore, we found that better education, information and other measures are definitively necessary to increase knowledge on primary hyperoxaluria to increase the chances of early diagnosis and appropriate instalment of treatment. Hence, we set out to start regular meetings primarily for patients and their family members, but will also organize specific education for medical personnel. In addition we produced a web site with all available information for patients with primary hyperoxaluria, not only available in German, but in most European languages and with information about disease specific experts in each country (see

<http://www.ph-selbsthilfe.org>). We were and are involved in European agencies either dealing with rare diseases per se (Eurordis, ACHSE) or with the evaluation of new treatment options (EMA). We, of course, have goals for the next years to come, which includes building up a European PH self support group in close partnership with the Oxalosis and Hyperoxaluria Foundation (OHF) from the USA. We recently agreed that we share board members with the OHF. Next to that, further educational programs, fund raising projects and support of research efforts are on our top priority list. We welcome international membership—come, join us!

34. Combined liver–kidney transplantation: the red cross children's hospital experience

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Objectives: To document our experience of combined liver–kidney transplantation in paediatric patients.

Methods: A retrospective folder review of 5 patients, who underwent combined liver–kidney transplantation between January 1997–June 2006.

Results:

Indication for transplantation: primary hyperoxaluria ($n = 4$), polycystic kidney disease with congenital hepatic fibrosis ($n = 1$).
Age at diagnosis: 2.8–12.2 year (mean 6.8 year)
Age at transplant: 6.5–13.9 years (mean 11.7 year)
Weight at time of transplant: 16.5–53 kg (mean 31.6 kg)
Primary hyperoxaluria:

- The diagnosis was confirmed by liver biopsy (1 patient) and stone analysis (3 patients)
 - All the patients with primary hyperoxaluria required dialysis for 5–36 months (mean 20 months) before transplant
 - Waiting time for transplantation varied from 4.7–36 months (mean 19.8 months)
 - GFR at time of transplant ranged from 6–10 ml/min/1.73 m² (mean 7.8).
 - Postoperative haemodialysis with hyperhydration (>3l/m²/day) and maximal diuresis was required in 2 hyperoxaluria patients using gastrostomies.
 - One patient developed recurrence of oxalate crystal deposits in her graft kidney due to poor compliance with fluid intake. Her current GFR is 32 ml/min/1.73 m² after 4 years.
 - The remaining 3 patients have stable creatinine < 110 µmol/l with calculated GFR of 60–104 ml/min/1.73 m² (mean 77).
 - Long-term liver function remained stable in all of these patients.
 - One patient died 6 days post transplant due to hepatic artery thrombosis and primary non-function of the renal graft.
- Outcome:** With follow-up between 2.3–9.7 years (mean 5.1 year) we currently report patient and graft survival of 80% at 5 years.

Conclusions: Combined liver–kidney transplants have been successful in our program with close attention to prevention of recurrence of stones and preservation of the graft.

Long waiting lists result in increased morbidity and mortality pre- and post-transplant, due to oxalate crystal deposition resulting in end-organ damage.

35. Perturbation of the amino-terminal sequence of human mutant alanine:glyoxylate aminotransferase (AGT^{LRM}) corrects the mistargeting of the protein and restores its physiological function

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Human alanine:glyoxylate aminotransferase (AGT) is localized predominantly in hepatocyte peroxisomes. Mutant AGT^{LRM} (a polymorphism/amino acid substitution combination) is the most common genetically abnormal form associated with primary hyperoxaluria-1 (PH1). AGT^{LRM} expressed in COS cells is catalytically active, but is mistargeted to mitochondria.

Aims: To determine: (a) if such mistargeting occurs in AGT^{LRM} expressed in vivo, (b) whether perturbation of the N-terminal mitochondrial localization signal affects the intracellular localization and function of AGT^{LRM}.

Methods: Adenovectors (10⁹ IU) expressing human wtAGT or AGT^{LRM}, with or without N-terminal FLAG-tag, were injected into *Agxt-1* gene deleted (*Agxt*^{-/-}) mice (model of PH1). One week later, urine was collected and hepatocytes were isolated. Intracellular location of the expressed forms of AGT was determined by confocal microscopy, using Mitotracker-Red and Pex19 immunofluorescence to visualize mitochondria and peroxisomes, respectively.

Results: In mice expressing wtAGT with or without FLAG, but not those expressing AGT^{LRM}, urinary oxalate, glyoxylate and glycolate excretion were reduced to normal and oxalate crystalluria was abolished. Surprisingly, expression of AGT^{LRM} with an N-terminal FLAG normalized urinary oxalate and glyoxylate excretion and abolished crystalluria. WtAGT with or without FLAG and FLAG-AGT^{LRM} were localized predominantly in peroxisomes, whereas AGT^{LRM} without FLAG was localized mainly in mitochondria.

Conclusions: The polymorphism–mutation combination in AGT^{LRM} may weaken the C-terminal peroxisomal localization signal, whereby the mutant enzyme is mistargeted to mitochondria via a N-terminal mitochondrial localization signal. Perturbation of the latter by an N-terminal FLAG may restore its peroxisomal localization and physiological function.

36. Human glycolate oxidase 1: a structural and biochemical examination of a possible target for hyperoxaluria treatment

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Human glycolate oxidase 1 (GO1) catalyzes the FMN-dependent oxidation of glycolate to glyoxylate. In a second reaction, GO1 is also able to further oxidize glyoxylate to oxalate. The calcium salt of oxalate is highly insoluble and readily precipitates from solution. Here, we report the crystal structures of GO1 complexed with sulfate, glyoxylate, and an inhibitor, DSTC. The protein shows the same α8/β8 fold of other α-hydroxy acid oxidases, most

notably spinach glycolate oxidase. A loop region disordered in crystal structures from other enzymes in this family is visible for the first time. The GO1/DSTC complex indicates that this loop may be involved in a disordered to ordered transition that occurs upon substrate binding. Differences in the active site residues between the three structures indicate that the conformational flexibility of Trp110 may allow GO1 to react with α -hydroxy acids of various chain lengths. Additionally, the kinetic parameters of GO1 for glycolate and glyoxylate have been determined. This analysis indicates that the oxidation of glycolate to glyoxylate is the primary reaction catalyzed by the enzyme while the oxidation of glyoxylate to oxalate is most likely not biologically relevant. However, the role of GO1 in glyoxylate production may prove important in disease etiology, especially in cases such as primary hyperoxaluria type 1.

37. Structural basis for the substrate specificity in human glyoxylate reductase/hydroxypyruvate reductase

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Human glyoxylate reductase/hydroxypyruvate reductase (GRHPR) is a D-2-hydroxy-acid dehydrogenase found predominantly within the liver that is essential for the removal of the metabolic by-product glyoxylate. Primary hyperoxaluria type 2 (PH2) diseases, which are characterised by increased urinary oxalate levels, formation of kidney stones and renal failure, correlate with mutations in the gene for GRHPR leading to diminished enzyme activity. To understand the molecular basis of GRHPR activity, we have determined the crystal structure of human GRHPR to 2.2 Å resolution. In this structure there are two homodimers of GRHPR in the crystallographic asymmetric unit: in each homodimer, one monomer is present as a ternary complex (enzyme + NADPH + reduced substrate) complex, and the other as a binary (enzyme + NADPH) form. Each GRHPR monomer contains two distinctive domains: a larger coenzyme-binding domain which forms most of the dimer interactions, and a catalytic domain containing the active site residues. This is the first crystal structure of a true ternary complex of an enzyme from this family and confirms earlier proposals of the mode of substrate binding, stereospecificity and likely catalytic mechanism for these enzymes. GRHPR utilises both glyoxylate and hydroxypyruvate

as substrates, but not pyruvate. The composition of the active site cleft, including the protrubence of a tryptophan residue (Trp141) from the neighbouring subunit, provides an explanation for the substrate selectivity of GRHPR. The crystal structure can also be used to explain the reduction in GRHPR activity associated with missense mutations of the gene for this enzyme that lead to PH2.

38. Measurement of enteric oxalate absorption (EnOxA) in hyperoxaluric calcium oxalate stone formers (CaOx SFs) using gas chromatography/mass spectrometry (GC/MS)

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Increasing evidence supports a role for the gastrointestinal tract in maintaining Ox homeostasis. Recent appreciation of *Oxalobacter formigenes* colonization, angiotensin-mediated Ox secretion, and function of the *SLC26A6* anion transporter in influencing Ox metabolism underscores a need for accurate measurement of EnOxA in hyperoxaluria.

Using GC/MS, we assessed EnOxA in hyperoxaluric (urine Ox excretion (Uox) > 0.46 mmol/1.73 m²/24 h) CaOx SFs. Following an oral Ox load (100 mg/BSA ¹²C-Ox and 20 mg/BSA ¹³C₂-labelled Ox), Uox was measured in 2 time periods (T1 = 0–6 h, T2 = 6–24 h). ¹³C₂-Ox and ¹²C₂-Ox were measured by GC/MS using the trimethylsilyl derivative. Total Ox was measured using Ox oxidase. % EnOxA was calculated.

In most patients and controls, > 75% of the Ox load was absorbed in T1, while in 2 CaOx SFs (pts 3 AND 4), EnOxA was more evenly distributed over 24 h. This variability may suggest proximal vs. distal sources of EnOxA, with implications for treatment.

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PT	Type	Uox (mmol/BSA/ 24 h)	Plasma Oxalate (umol/L)	Serum Creatinine at Last Follow-up	Oxalic Acid (mg)	Oxalic-1,2 ¹³ C ₂ acid (mg)	% EnOxA	
							T1	T2
1	Idiopathic	0.38–0.75	NA	1.0	100	20	3.5	0.3
2	Idiopathic	0.68	8.2	0.8	100	20	2.4	0.6
3	nonPH1PH2	0.24–1.48	3.4	1.9	104	20.8	2.3	3.2
4	Enteric	0.57–1.04	3.6	1.3	100	20	3.1	6.0
5	Enteric	1.21	2.0	0.9	100	20	14.3	1.7
6	Enteric	0.42–1.32	2.9–5.4	0.7	67.9	13.9	24	ND
7	nonPH1PH2	0.97–2.19	1.0–3.1	0.9	57.8	11.6	8.1	ND
8	Enteric	0.70–1.00	1.5	1.5	107.5	21.5	8.6	ND
Mean Control (n = 7)							4.5	0.8

39. Luminex-flexmap tag/anti-tag mlpa (multiplex ligation dependent probe amplification) technology for detection of gene copy alterations in *AGXT*, the gene mutated in primary hyperoxaluria type 1 (PH1)

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Complex gene rearrangements are estimated to account for 2–5% of disease causing mutations in monogenic disease. Systematic assessment of gene copy alterations in *AGXT* has not been performed previously. To ascertain whether large gene deletions or

insertions might account for disease-causing alleles in PH1, we developed a quantitative gene dosage assay using Luminex-FlexMAP MLPA technology. Of 48 unrelated liver-biopsy proven PHI probands whose *AGXT* was fully sequenced, we screened 21 purely homozygous for *AGXT* mutations, showing loss of heterozygosity across known *AGXT* SNPs or in whom whole gene sequencing failed to reveal mutations. Since only 2 large partial *AGXT* deletions (5' UTR_IVS5, 5'UTR_IVS7) and a single case of maternal isodisomy of 2q37.3 have been reported for PHI, we initially limited probe design to exons 1, 4 and 11. Following detection of a new Ex 11_3'UTR del, we designed 6 additional probes (exons 9 and 10, 1 kb, 2 kb, 80 kb, 0.25 MB, and 1 MB from the 3' UTR) to delineate its extent. The new Ex 11_3'UTR del, detected in a proband for whom a second mutation had not been identified, was found to extend between 2 and 80 kb from the 3' UTR (Fig. 1).

We conclude that MLPA technology is suitable for detection of *AGXT* gene copy alterations, complementing *AGXT* sequencing for improved sensitivity of *AGXT* mutation detection. Its application appears especially useful to screen for hemizyosity in purely homozygous patients for rare mutations, or in those for whom whole *AGXT* sequencing is uninformative.

40. Determinants of renal outcome in the primary hyperoxalurias

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The primary hyperoxalurias (PH) demonstrate marked clinical variability, including the time to kidney failure. Data in the International PH Registry was used to identify parameters that predict renal outcome.

The Cox proportional hazards model was used to evaluate predictors of time from Dx to ESRD: PH type, urinary oxalate (U_{Ox}), age at first symptoms (Sx), age at diagnosis (Dx), urinary citrate (U_{cit}), urinary calcium (U_{ca}), baseline estimated glomerular filtration rate ($eGFR$).

Among 141 pts in the Registry, ESRD developed in 27 before PH Dx. They were excluded from analysis. For the remaining 114 pts, at Dx age was 11.9 ± 13.1 years, $eGFR$ 97.3 ± 38.6 ml/min/1.73 m², U_{Ox} 2.07 ± 0.98 mmol/1.73 m²/24 h. Median (25th,75th) f/u was 17.8 (9.4, 36.2) years, with 25 pts developing ESRD. The percentage free of ESRD was 88, 70, and 45% at 10, 20, and 30 years after Dx, respectively. $eGFR$ and U_{ox} at diagnosis were univariate predictors of ESRD. U_{ox} remained significant ($p = 0.038$) adjusted for $eGFR$. For pts with $U_{ox} < \text{median}$ 20 year renal survival following Dx was 100% compared to 65% in those with $U_{ox} > \text{median}$ ($p < 0.001$).

ESRD hazard ratios are listed below:

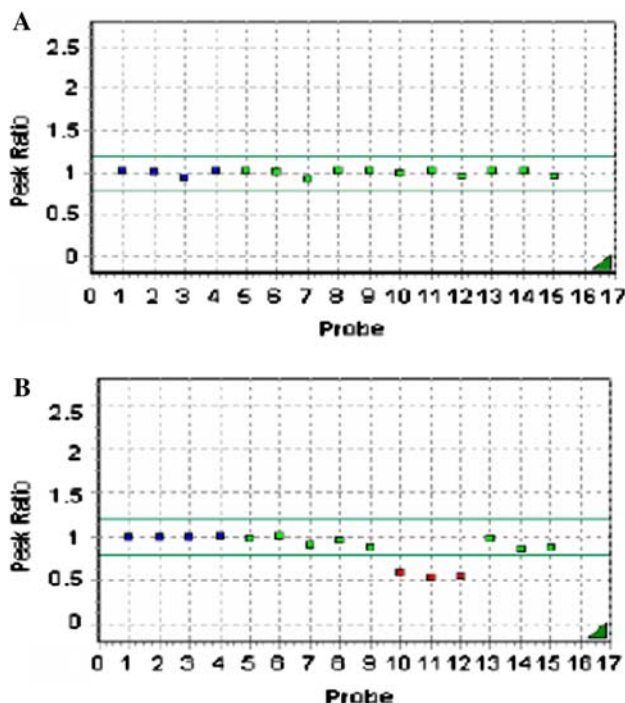


Fig. 1 MLPA analysis. The 5 control (BCAR3, CTNNB, HIRA, TNFRSF7, DMD) and 10 experimental probes (*AGXT* exons 1, 4, 9, 10, 11, 3' 1 kb, 3' 2 kb, 3' 80 kb, 0.25 and 1 MB) are listed sequentially. Panel A = female control, Panel B, PH1 proband with exon 11 *AGXT* deletion extending between 2 and 80 kb from the 3'UTR. For the control probes, BCAR3, breast cancer anti-estrogen resistance 3, chromosome 1; CTNNB1, catenin (cadherin-associated protein), beta 1, chromosome 3; TNFRSF7, tumor necrosis factor receptor superfamily 7, chromosome 12; HIRA, HIR histone cell regulation defective homolog A, chromosome 22; DMD, dystrophin, exon 11, gene dosage control, X-chromosome

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	GFR ml/min/BSA	Uca mg/24 hr	Ucit mg/24 hr	Uox mmol/BSA	PHI (vs other)
N	101	65	54	82	108
ESRD	0.64*	0.98*	1.00*	2.2	> 99 (na)
Hazard Ratio (95% CI)	(0.54,0.76) *10 unit incr.	(0.88,1.09)	(0.98,1.03)	(1.2,3.9)	
p-value	< 0.001	0.74	0.76	0.007	< 0.001

Therefore, PHI type, lower eGFR and higher U_{Ox} at Dx impart a worse renal prognosis. Strategies to reduce U_{Ox} may be beneficial.

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41. Correlates of kidney calcification in primary hyperoxaluria

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Stone formation is a nearly universal feature of primary hyperoxaluria (PH), yet the numbers of stones vary markedly between patients. Renal damage from stones, nephrocalcinosis and/or urologic procedures could contribute to chronic kidney disease.

Clinical information was collated from presentation and follow-up visits in 141 patients enrolled in the International PH Registry including number of stones by imaging; stones passed; urological procedures; urine chemistries; serum creatinine; and measured or estimated GFR. Correlations were assessed by Spearman correlation coefficient.

Average patient age at last f/u was 24.3 years (25th, 75th = 9.6, 36.1), and kidney imaging was documented in 96%. Overall, 72% passed a stone; mean number passed/year = 0.38. One or more procedures were required by 67% of patients ever, with a

mean number of 0.22 procedures/yr. Nephrocalcinosis occurred in 45% of all patients; it was rarely newly-detected after age 30. Urine oxalate was a weak risk factor for stone events, while increased urinary citrate and volume appeared protective (Table). Stone burden or nephrocalcinosis did not correlate with loss of GFR over time.

Therefore, stone events are a hallmark of PH averaging about 1 every 3 years. Urinary oxalate, citrate, and volume all may influence stone formation. However, stone burden does not correlate with renal outcome, suggesting that other effects of PH may mediate loss of kidney function over the course of the disease.

42. Generation of tetracycline-inducible stably-transfected cell lines that express altered levels of the candidate crystal binding molecules annexin ii and hyaluronan

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Calcium oxalate (CaOx) crystal adhesion to tubular cells appears important in the evolution of renal stones associated with marked hyperoxaluria. Recent *in vivo* and *in vitro* observations implicate both annexin II (AxII) and hyaluronan (HA) as crystal binding molecules (CBMs). To further define its role, rat inner medullary collecting duct (cIMCD) cells were transiently transfected using 2 separate Morpholino anti-sense oligonucleotides (MO) designed against Rat AxII (NM_007585). After 5 transfection cycles, AxII expression was decreased by 20% (MO-1) and 40% (MO-2), correlating to a 23% ($P < 0.01$) and 43% ($P < 0.001$) decrease in CaOx crystal binding compared to control. Since transient

Table for abstract 41

	Measure of Stone Burden				Correlation Recent Labs w/ Stone Burden: r					
	Mean	Med	25th/75th	Min/Max	UOx/BSA	Pl Ox	U Ca	U Cit	U Vol/BSA	GFR
Avg. stones per imaging study	3.3	2.0	0/4	0/20	-0.18	0.28*	0.06	-0.37*	-0.28*	-0.10
Average stone events/year (passed + procedures)	0.59	0.25	0.06/0.65	0/6.8	0.23*	0.27*	-0.03	-0.16	0.09	-0.10

BSA body surface area; Ca calcium; GFR glomerular filtration rate; Max maximum; Med median; Min minimum; Ox oxalate; Pl plasma; U urine; Vol volume

* $P < 0.05$ for test of true $r = 0$

transfection was inefficient and produced variable AxII suppression, stable tetracycline-inducible cell lines that express siRNA against the mouse AxII (NM_007585) and Hyaluronan synthase 2 (HAS-2; NM_008216) genes were next generated. Five siRNA sequences against each gene were cloned into pRNATin-H1.2/Neo (AxII) and pRNATin-H1.2/Hygro (HAS-2). Mouse collecting duct (M1) cells were transfected using Nucleofector II system, and stably transfected cells were selected using specific antibiotics. Resulting cell lines are stable in culture, and upon tetracycline-induction of siRNA demonstrate ~50% and 65% decrease in AxII and HA expression, as assessed by fluorescent microscopy. Cell proliferation rates were reduced in the presence of tetracycline in both transfected cell lines, but not controls. In experiments that carefully control for the variable growth rates, CaOx crystal binding to the transfected cells appears reduced. Therefore, these stable, inducible cell lines are an excellent *in vitro* model to assess the relative importance of candidate CBMs.

43. Patterns of elevated plasma organic acids in primary hyperoxaluria

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Background: The amounts of oxalate, glycolate and L-glycerate excreted in urine are important diagnostic indicators of primary hyperoxaluria. Their relative concentrations in plasma have not been fully characterized due to measurement difficulties.

Methods: Plasma and urine samples were obtained from the Mayo Clinic Hyperoxaluria Center for a cohort of patients with Type 1 PH ($n = 35$), type 2 PH ($n = 6$) and unclassified PH (not 1 or 2; $n = 8$). Patients continued prescribed medications during collections, including pyridoxine if indicated. Corresponding clinical data was obtained from the International PH Registry. Plasma and urine glycolate, and glycerate were assessed using ion chromatography coupled with mass detection. Oxalate was measured by conductivity detection under conditions that prevented ascorbate conversion to oxalate.

Results: Plasma glycolate levels were elevated in PH1 ($61.8 \pm 62.2 \mu\text{M}$) vs PH2 ($16.6 \pm 4.0 \mu\text{M}$) ($P < 0.001$) while plasma glycerate levels were elevated in PH2 ($149.5 \pm 15.9 \mu\text{M}$) vs PH1 ($8.1 \pm 6.8 \mu\text{M}$) ($P < 0.001$). In UPH, plasma glycolate ($9.0 \pm 4.8 \mu\text{M}$) and glycerate ($7.7 \pm 3.1 \mu\text{M}$) levels were both normal. Amongst PH1 patients, glycolate levels were highest in pyridoxine unresponsive patients ($158.3 \pm 71.6 \mu\text{M}$), intermedi-

ate in partially responsive patients ($47.7 \pm 37.4 \mu\text{M}$), and low in those responsive ($17.8 \pm 18.3 \mu\text{M}$) ($P < 0.01$ for comparisons between groups). Urinary glycolate and glycerate concentrations and excretion rates only roughly correlated with plasma levels. All patients with PH2 had markedly elevated plasma glycerate levels, whereas all pyridoxine non-responsive PH1 patients and about half of partially responsive PH1 patients had elevated plasma glycolate levels.

Conclusions: Plasma glycolate and L-glycerate levels appear to be useful diagnostic markers for PH1 and PH2, and plasma glycolate determinations show promise to assess pyridoxine responsiveness in PH1. Normal plasma glycerate and glycolate levels in UPH provides further evidence that these patients have a different phenotype.

44. Type of AGXT mutation (mistargeting, missense or truncating) correlates with phenotype in primary hyperoxaluria type 1 (PH1)

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Inference of genotype-phenotype correlations in PHI has been hindered by limited availability of AGXT genotyping in similarly treated & definitively ascertained patients with long-term follow-up (F/U). To evaluate the association of mutation type with phenotype, we utilized AGXT genotyping in patients from the International primary hyperoxaluria registry.

We classified 46 genotyped PH1 patients into four groups: (1) mistargeting homozygous (M/M) (2) missense homozygous (SS) (3) truncating homozygous (TT), (4) truncating/mistargeting (T/M) or missense (T/S) heterozygous. Associations between group and clinical factors (age at symptoms (Sx) and diagnosis (Dx), urinary oxalate (Uox) at Dx, % normal AGT activity, age at ESRD, and nephrocalcinosis) were evaluated. Analysis for age at ESRD was by non-parametric ANOVA, Chi-square, and life-table methods.

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Allele 1/ Allele 2 (Group)	<i>n</i>	Age at PH Sx ^a	Age at PH Dx ^a	U ox/BSA at Dx ^a	% Normal AGT Activity ^a	ESRD by age 30 (%)	Nephro-calcinosis (%)
M/M (1)	14	12 (11)	27 (19)	1.2 (0.7)	19 (7)	0	23.1
S/S (2)	10	14 (13)	23 (12)	1.5 (1.1)	17 (19)	17	50.0
T/T (3)	5	12 (15)	14 (19)	2.4 (0.9)	9 (3)	0	20.0
T/M of T/S (4)	17	6.4 (6.4)	7.4 (7.4)	3.2 (0.8)	14 (6)	37	50.0
Groups equal:	<i>P</i> =	0.31	0.002	0.0004	0.061	0.36	0.36
Grp 1,2 vs 3,4	<i>P</i> =	0.082	<0.0001	<0.0001	0.15	0.65	0.46

^a Mean (SD)

Median (25th, 75th %ile) age at last F/U was 25 (17, 42) years.

Heterozygosity for mutation type is associated with earlier age at symptoms & diagnosis, higher baseline Uox, and a trend toward earlier ESRD.

45. Comprehensive mutation screening in 55 type 1 primary hyperoxaluria (PH1) probands shows feasibility of a gene-based diagnosis

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Restriction enzyme-based screening for the 3 most common *AGXT* mutations (G170R, c.33_34insC, I244T) has yielded a molecular diagnosis sensitivity of 34.5% (2 mutations detected in only 99 of 287 liver-biopsy proven PH1 probands). To assess the diagnostic relevance of performing whole gene sequencing in PH1, and to expand further on the heterogeneity of *AGXT*, we sequenced the entire coding region of *AGXT* in 55 unrelated PH1 probands from the Mayo Clinic Hyperoxaluria Center.

G170R accounted for 37% of our PH1 alleles. c.33_34insC occurred with the next highest frequency (11%), followed by F152I and G156R (frequencies of 6.3 and 4.5%, respectively), both surpassing the frequency (2.7%) of I244T, the previously reported third most common pathogenic change.

We detected 28 new *AGXT* variants (21 mutations and 7 polymorphisms), with highest frequencies on exons 1, 4 and 7. If limited to these 3 exons, molecular analysis sensitivity was 77%, compared to 98% for whole gene sequencing.

To establish the pathogenicity of all previously described and newly discovered *AGXT* missense variants, we also developed a classification strategy based on the scheme developed by Grantham, performed a multi-sequence alignment and screened 50 control samples of predominantly European and North American descent.

These are the first data in support of comprehensive *AGXT* analysis for the diagnosis of PH1, obviating a liver biopsy in most well characterized patients. We also report evidence for the pathogenic basis of all *AGXT* missense variants, including evolutionary conservation data in a multi-sequence alignment and use of a normal control population.

46. ESRF and systemic oxalosis indistinguishable from primary hyperoxaluria type 1 (PH1) in short bowel syndrome

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ESRF and consequent systemic oxalosis are frequently encountered in PH I, but this scenario is rarely seen in patients with secondary hyperoxaluria (SH). Here we report a 39 year-old female patient with Crohn's disease diagnosed at age 9 years. Multiple bowel perforations necessitated repeated small intestine resections which lead to short bowel syndrome. From the age of 11 she developed recurrent calciumoxalate (CaOx) urolithiasis treated by stone extraction and ESWL. In 2002 advanced renal insufficiency and nephrocalcinosis was noted and the patient developed ESRF within a year. Kidney biopsy in 2002 and bone marrow biopsy carried out 16 months after initiating hemodialysis for treatment-resistant anemia, revealed both extensive CaOx deposition. After PH I was ruled out by liver biopsy, the patient continued, despite systemic oxalosis, only with regular hemodialysis for another 15 months. After 31 months on hemodialysis she received her first kidney graft. Mobilisation of body oxalate stores in the absence of appropriate medical management lead to early graft loss 3 months later. Her plasma Ox levels at this time were continuously >80 µmol/l and kidney graft biopsy showed excessive CaOx deposition. She recently received a second kidney graft, which shows stable kidney function under better patient handling. Current plasma oxalate is 22.5 µmol/l at a serum creatinine of 1.3 mg/dl, urinary oxalate ranges from 0.5–2.84 mmol/24 h. This case demonstrates that severe SH can produce systemic oxalosis clinically indistinguishable from PH I. There is no fundamental difference regarding the toxicity of endogenous versus exogenous oxalate.

47. Delayed diagnosis in primary hyperoxaluria and its consequences: quo vadis for children with recurrent kidney stones?

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A significant number of patients with either form of primary hyperoxaluria (PH) are only diagnosed late or even in ESRF. Stone disease appears to be a urological disease in view of most patients and physicians. However, "is stone removal always followed by adequate diagnostic evaluation", a question which is especially important in children with recurrent stone disease? We report on an exemplary case of a now 15 year old boy with recurrent kidney stones. First episodes of nephrolithiasis of the right kidney occurred at age 8 years. Until age 14 years multiple stone removal procedures (ESWL or pyelolithotomy) were necessary and a lower pole resection was performed. In addition, intravenous urographies, renal scintigraphies and other imagine studies were done. Since age 13 years stones also formed in the left kidney and were treated consecutively. Before removal of the right completely damaged kidney an adult nephrologist reported on a normal urinary calcium excretion and recommended a high daily fluid intake. Finally, at age 15 years, still suffering from recurrent kidney stones, he was evaluated by the Division of Experimental

Urology, University of Bonn and finally received his diagnosis of PH type II at our institution. This is not an isolated case, but rather expresses a “normal patient’s history”. How to prevent from such a scenario? Education, patient’s registries, self support groups and even publications of such case reports may lead way to earlier diagnostic evaluations. Children, however, should be seen in a paediatric nephrology setting even after the first stone event.

48. Renal function in children with primary hyperoxaluria type 1 on conservative treatment

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The renal function of 27 children with primary hyperoxaluria type 1 (PH1) was retrospectively studied once medical treatment had been started. The diagnosis was based either on alanine:glyoxylate aminotransferase (AGT) enzymatic deficiency or on the presence of specific mutations in the patient or his siblings. The conservative measures included high fluid intake (i.e. 2 to 4 l/m² per day), urine alcalinisation (potassium or sodium citrate/bicarbonate), and pyridoxine (10 to 20 mg/kg per day) in responsive subjects. The median follow-up was 7.1 [1.6–17.6] years. Median age at first symptom was 29 [0.5–94] months, 6 children were diagnosed on familial screening. Median age at start of treatment was 4.1 years [0.5 months–12.3 years], and 10.3 [2.8–21.2] years at the end of follow-up. Seventeen children were pyridoxine responsive. Initial renal impairment was present in 11 patients, 6 already had chronic renal failure. Median baseline GFR (estimated by the Schwartz formula) was 92 mL/min per 1.73 m² [29–180] and median final GFR in patients without ESRD (*N* = 23) was 110 mL/min per 1.73 m² [140–179] : 20 patients had stable renal function, 7 experienced a decrease in GFR by more than 20 mL/min per 1.73 m² (4 of whom presented with initial renal failure), and 4 patients progressed to ESRD. Fourteen patients suffered from stone passage during follow-up (mean of 2.1 [0–7] episodes per patient) and required either ESWL or open surgery in a mean number of 1.5 cases per patient [0–8].

In conclusion, an early aggressive conservative management may preserve renal function of compliant PH1 children.

49. Molecular chaperones enhance protein solubility and enzymatic activity of mutant AGT expressed in *E.coli*

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Primary hyperoxaluria type I is a disorder of glyoxylate metabolism, caused by mutations in the alanine-glyoxylate-aminotransferase (*AGXT*) gene. More than 60 disease causing mutations in the *AGXT* gene have been reported so far, missense mutations being the predominant group.

Functional analysis of the two most common mutations present in the minor haplotype (which includes the common polymorphism Pro11Leu), G170R and I244T has shown that these mutations result in aberrant protein conformations leading to either subcellular mistargeting or protein aggregation. In vitro studies have also shown that molecular chaperones can influence the mechanism of disease in these mutations.

E.coli expression is a useful approach to assess the functional consequences of single amino acid substitutions. Most of the mutant *AGXT* alleles expressed so far in *E.coli* result in insoluble proteins that accumulate in inclusion bodies, while wild type AGT (major haplotype) is easily expressed as a soluble protein.

We have coexpressed mutant *AGXT* alleles together with plasmids with genes for the main prokaryotic molecular chaperones: GroES, GroEL, dnaK, dnaJ, grpE, and tig, and several combinations of them.

Coexpression of GroES-EL resulted in significant improvement on the production of soluble forms of protein encoded by the main mutant alleles described in the minor haplotype: G170R and I244T. Substantial enhancement of enzymatic activity was achieved in soluble lysates coexpressing GroE. There is a parallelism between the reported beneficial influence of chemical chaperones on the expression of mutant forms in cell culture and the results obtained in *E.coli*.

50. Cardiac manifestations in primary hyperoxaluria

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Primary hyperoxaluria (PH), results in oxalate overproduction and systemic oxalosis. Frequency of cardiac involvement is unknown. A retrospective review of 92 PH patients at the Mayo Clinic from 1948–2006 was completed. Seventy-six (83%) had PH type 1 with mean follow up of 11.9 years. Clinically indicated cardiac testing [ECG (*n* = 32), ECHO (*n* = 26)] was done in 38 (41%) of patients. Group A had abnormal cardiac findings while group B had normal findings.

Table for abstract 50

	Mean age (±SD)	Median plasma oxalate (μmol/l) (range)	Median GFR (ml/min/1.73 m ²) (range)	% ESRD
A (<i>n</i> = 27) 71%	41.6 (18.5)	14.8 (2.0, 86.3)	19.0 (6.8, 89.4)	74
B (<i>n</i> = 11) 29%	31.4 (10.9)	6.7 (2.4, 144.0)	49.9 (4.5, 98.0)	64
		<i>p</i> = 0.89	<i>p</i> = 0.44	<i>p</i> = 0.70

Table for abstract 50

Cardiac Abnormalities (<i>n</i> = 27)	<i>n</i>	%
Conduction Abnormalities (ECG)	5	18.5
LV Abnormalities	25	92.6
Systolic dysfunction	11	40.7
Diastolic dysfunction	22	81.5
Valve Abnormalities	2	7.4

Cardiac abnormalities are common in PH patients with ESRD. Young age and absence of other known cardiac disease in the majority suggests calcium oxalate deposition, although other causes can not be excluded. Regardless, PH patients with impaired renal function should be considered for cardiac surveillance. We hypothesize myocardial oxalate infiltration initially results in diastolic dysfunction. Progressive deposition may culminate in conduction disturbances. A prospective study using more sensitive tools of myocardial dysfunction (2D twist, strain rate, vector velocity imaging), cardiac CT or MRI may be useful in detection of cardiac manifestations of oxalosis.

Research Support: Grants from the National Institutes of Health (DK 73354, DK64865), the Oxalosis and Hyperoxaluria Foundation, and Mayo Foundation.

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51. Cardiac involvement in primary hyperoxaluria: evidence based systematic overview

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Background: In primary hyperoxaluria (PH), oxalate overproduction can result in renal and systemic oxalosis. Cardiac manifestations include arrhythmias, conduction defects or heart failure. The true prevalence of cardiac involvement is not known, as only scattered case reports are available.

Methods: A computerized MEDLINE (1/1966–12/2006), EMBASE (1/1988–12/2006), Web of Science (1/1993–12/2006) and Cochrane Database of Systematic Reviews 2006 identified 34 cases of cardiac involvement attributed to PH.

Results: The following table summarizes reported cardiac findings among 34 patients identified in the literature review:

Conclusions: There is currently no information on the prevalence of cardiac involvement in PH. Mortality appears to be high. Patients are young, the majority presenting with heart failure, heart block and ventricular hypertrophy. The most sensitive tools for assessing cardiac involvement are not established; chest X-ray, ECG and echocardiography are the current mainstay. Novel echocardiographic techniques (strain rate imaging, myocardial twist), cardiac Computed Tomography and Magnetic Resonance Imaging may be more sensitive tests that require further prospective study.

Table for abstract 51

	No. of patients (%) (<i>n</i> = 34)
AGE, range (mean \pm SD)	4–66 (29 \pm 16) years
Gender	
Male	15 (44)
Female	19 (56)
Clinical presentation	
Heart failure	14 (41)
Syncope	3 (9)
Pericardial friction rub	3 (9)
Arrhythmia	2 (6)
Chest pain	2 (6)
ECG	
Heart Block	12 (35)
Bundle Branch Block	4 (12)
Extrasystole	3 (9)
Bradycardia/tachycardia Syndrome	1 (3)
Ventricular tachycardia	1 (3)
Myocardial histopathology	
Oxalate Crystals	34 (100)
Ventricular Hypertrophy	12 (35)
Fibrinous Pericarditis	3 (9)
Outcome	
Death	23 (68)
Improvement of Symptoms and Histopathology	3 (9)
Stroke	1 (3)
Awaiting renal transplantation	1 (3)
Heart failure	1 (3)
Not reported	5 (15)

52. Vascular involvement in primary hyperoxalosis: an evidence-based systematic overview

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Background: Primary hyperoxaluria (PH) is characterized by excess serum calcium oxalate levels causing renal and extrarenal injury. Vascular manifestations include vasculitis, ischemic limbs, bowel and death. The prevalence of vascular involvement is not known with only scattered cases reports. A systematic overview of the literature was performed to characterise clinical presentation and outcomes of such patients.

Methods: A computerized search of Ovid Medline (Jan 1950 to 30 May 2007), Embase (January 1988 to 30 May 2007) and Cinahl (January 1982 to 30 May 2007). 23 cases of vascular involvement in PH were identified. Search terms used: Primary heproxaluria vasculitis; primary hyperoxalosis, vasculitis; PH, vascular disease; PH, vascular.

Conclusions: Little information exists on vascular involvement in PH. Morbidity and mortality is high (death and limb loss in 2/3). The majority are young females(74%). Vascular deposition of calcium oxalate crystals presents as skin vasculitis; limb/mesenteric

Table for abstract 52

Clinical findings among PH patients with vascular involvement.	
<i>N</i> = 23	No. of patients (%) (<i>n</i> = <i>X</i>)
Age, range (mean \pm SD)	10–54 (34 \pm 13) years
Gender	
Male	6 (26)
Female	17 (74)
Clinical presentation	<i>N</i> = 23 (%)
Limb ulcers	4 (17)
Gangrene	3 (13)
Abdominal pain	2 (9)
Stroke	2 (9)
Skin	
livedo reticularis	12 (52)
(1 livedo racemosa)	
Limb: Upper	9 (39)
Lower	21 (61)
Artery : Mesenteric	3 (13)
Carotids	1 (4)
Cerebral	1 (4)
Diagnosis	
Biopsy	14 (61)
CT angiography	6 (26)
Ultrasound	2 (9)
MR Angiography	1 (4)
Outcomes	
All deaths	8 (38)
Cardiac related	3 (37)
Abdo related (sepsis/gangrene)	5 (63)
Amputations:	6/21 (29%)
1limb	4
2 or more limbs	2

ischemia or gangrene. Stroke is uncommon. The lower limbs are affected more than upper limbs followed by mesenteric vessels. Vasculitic skin manifestations occur in 52% of cases. Amputation was necessary in 6 (29%) of cases. Death occurred in 8 (38%) of cases (63% abdominal gangrene/sepsis; 37% cardiac). The most sensitive tools for assessing vascular involvement are clinical exam and CT angiography. Prospective reporting via a National/ International Registry on vascular involvement is thus important to allow a better understanding of the frequency, complications and optimal management of vascular involvement in PH.

53. Primary hyperoxaluria in Italy

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Since 1990 the suspicion of primary hyperoxaluria in over 300 patients referred to our centre was confirmed by biochemical diagnostic protocol (Petrarulo et al. 1998) in about 50 patients. We considered in this study 47 PH1 and 2 PH2. Most were from Italy, 7 from other Mediterranean countries, age at presentation varies from 1 month to 49 years (median 6 y). AGT and GGT activities in liver biopsies were measured in 34. For 33 patients it was possible to define pyridoxine responsiveness by comparing plasma oxalate and glycolate before and after oral supplementation.

We standardized a PCR-DHPLC strategy and analyzed the 11 exons of the *AGXT* gene in 18 DNA samples (6 new and 12 previously known positive controls). Overall, in 89 out of 94 *AGXT* and 4/4 *GRHPR* alleles a mutation was detected by DNA sequencing. Twenty-three different variants were found, of which 9 unpublished: one in the promoter (c.-23G > A), to our knowledge the first ever described, 3 missense, 1 nonsense, 1 frameshift, 1 in frame deletion, and 2 splice site mutations. Pathogenicity of the missense changes was supported by the standard criteria of evolutionary conservation of the affected residue and absence in 80 healthy controls. In particular, Gly47Arg ("minor" haplotype) was predicted by molecular modelling to affect the dimerization energy, Ser81Leu ("major" haplotype) is adjacent to residue Gly82 in the PLP site. This study shows that DHPLC represents a feasible and sensitive diagnostic approach, and contributes to better characterize the mutational spectrum of PH in Italy.

54. Audit of sequencing service for the primary hyperoxalurias

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We have shown that molecular genetics can provide an alternative to enzymology for the diagnosis of primary hyperoxaluria¹. A service is offered from this laboratory to users worldwide and is part of the UK Genetic Testing Network (UKGTN). We performed an audit of the sequencing service for the primary hyperoxalurias and evaluated the quality of sequencing output, turnaround time and diagnostic outcome for mutation screen and whole gene sequencing.

Methods: The study covered a 2 year period from April 2005 to March 2007. Sequencing data was assessed for length of read, quality of output both internally and from EQA data (EMQN sequencing scheme). Turnaround time was assessed as date of receipt in the laboratory to date of reporting.

Results: The standards set for turnaround time was 15 days for mutation screen and 40 days for whole gene sequence, with 83% and 94% respectively falling within these limits.

The results of mutation screen were consistent with our previously published data in that a diagnosis (2 mutations) was made in 50% index cases (14/28). Whole gene sequencing for *AGXT* (*n* = 18) and *GRHPR* (*n* = 10), made a diagnosis in 11 cases (10 PH1, 1 PH2) of which 4 could have had the diagnosis made by mutation screen alone.

Conclusion: An effective, quality assured, diagnostic service is offered for the genetic analysis of primary hyperoxaluria type 1 and 2.

1. Rumsby et al., Kid Int 66:1–5 (2004);

Williams and Rumsby, Clin Chem 53: 1216–1221 (2007)

55. Single center experience with transplantation strategies in children with primary hyperoxaluria type 1 (PH1)

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Deficiency or mistargeting of alanine-glyoxylate amino-transferase (AGT) leads to hepatic overproduction of oxalate, which may lead to urolithiasis, nephrocalcinosis and ultimately end-stage renal disease. Cure of the metabolic defect is only possible with liver transplantation (LTX). Preemptive LTX was promoted to prevent chronic kidney disease, however the timing of this procedure is difficult in view of the heterogeneity of PH1 and effective conservative treatment. Combined liver/kidney transplantation (LKTx) is able to cure metabolic defect and replace renal function and is effective and indicated for the patient with ESRD. Sometimes a sequential approach (first liver, then kidney) has been recommended. We report on 10 patients with PH1 since 1995 that underwent transplantation procedures for PH1 at a median age of 4.1 (range 1.4–8.9) years in our center. In the first two patients on dialysis a sequential strategy was planned but both died in the early postoperative phase due to infectious complications. 4 patients underwent preemptive LTX. Three patients with sufficient residual renal function still have normal renal function after 9.8–10 years of follow-up. One patient with a GFR 27 ml/min/1.73 m² reached ESRD 5.5 years after PLTX and received a kidney graft later. 4 patients with ESRD received a combined LKTx. Three have excellent outcome with follow-up of 1.3–3.1 years, while one patient required dialysis again after 7 years due to mobilization of systemic oxalate pools. Growth was normal in patients after PLTX and LKTx. In summary and conclusion transplantation procedures are a challenge in PH1 but recent results are encouraging. Preemptive LTX remains an option despite the difficulties in timing the procedure. LKTx is indicated for patients in ESRD and is possible even for young children

56. Renal handling of potentially lithogenic dietary carbohydrates in a stone-free population: mechanistic clues for preventing calcium oxalate urolithiasis

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Studies have demonstrated that ingestion of glucose, xylitol and sorbitol can independently raise urinary calcium and oxalate but can also decrease urinary phosphate. In South Africa, urolithiasis in the black population is extremely rare while in the white population it occurs with the same frequency as in Europe. Investigations of this phenomenon have suggested that the handling of lithogenic dietary agents in the two population groups is different.

10 healthy male subjects from each population group participated in a double blind study in which they were required to ingest solutions of glucose, xylitol and sorbitol (20 g in 250 ml water) for seven days, while following a controlled diet. On day 7, blood samples were taken 30 min after ingestion of the sugar solutions

while urines were collected hourly over 3 h. These were analysed for glucose and routine biochemical parameters respectively. While the same changes in some parameters were observed in both groups, two changes were unique. Firstly, urinary phosphorus decreased significantly in blacks but not in whites after glucose ingestion. Secondly, urinary oxalate increased significantly in whites but did not change in blacks after sorbitol ingestion.

These results suggest that the mechanism involving reduction of urinary phosphate excretion is more active in blacks than in whites while that involving the transformation of sorbitol to oxalate is suppressed to a greater extent in the former group. It may therefore be prudent for researchers seeking strategies for preventing calcium oxalate and calcium phosphate urolithiasis, to investigate protocols which mediate these mechanisms.

57. Chromatographic determination of organic acids in selected foods relevant to calcium oxalate stone disease

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Hyperglycolaturia is sometimes seen without hyperoxaluria in calcium oxalate stone formers. Assessing whether this is of dietary origin is often hindered by the lack of reliable data on the levels of glycolate in common foods.

A Dionex High Performance Anion Exchange chromatography with auto-suppressed conductivity detection was used for the analysis of glycolate, oxalate, citrate and lactate content of some common foods. The acids were separated on a high capacity Ion Pac AS11-HC column (2 × 250 mm) with potassium hydroxide (generated electrolytically) as mobile phase. Resolution of oxalate, glycolate, glyoxylate, glycerate, citrate and lactate was achieved with an overall run time of 60 min. Performance and recoveries of the various organic acid anions were good. Apart from dilution (1/10) and filtration (0.45 µm), no other pre-treatment procedures were used.

High levels of glycolate were found in grapefruit juice, lager beer and white wine (>100 mmol/L) whilst moderate levels in lychee, guava and red grape juice (>30 mmol/L).

These should be avoided in patients who form calcium oxalate stone disease.

The method is simple, reliable and yields quantitative results.

58. Infantile oxalosis: management of primary hyperoxaluria type1 (PH1) in the first year of life

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Infantile oxalosis has been proposed to have a severe clinical course despite a lack of genotype phenotype correlation. Other factors, such as infection or dehydration may be responsible for early end-stage renal failure (ESRF). Management is difficult not only with regard to vascular access. We therefore present our experience with two infantile cases of PH1.

Patient 1 (born 12/2005) presented after respiratory infection at age 3 months in ESRF. Immediately, peritoneal dialysis (PD) was initiated. PH was suspected due to hyperechogenic kidneys and confirmed by kidney biopsy. Additional haemodialysis (HD) was started to optimise oxalate removal. However, he remained anuric. Plasma oxalate was high, and compound heterozygous mutations in *AGXT* were identified.

Patient 2 (born 6/2006) repeatedly vomited at age 2.5 months. Ultrasound revealed hyperechogenic kidneys. The diagnosis was made by raised urinary oxalate, glycolate, and plasma oxalate, and confirmed by reduced hepatic AGT-activity. Genetic studies showed a heterozygote G508A mutation. PD was started at age 3 months in good clinical conditions with reduced GFR but normal urine output. Additional HD succeeded only temporarily because of vascular access problems.

At present, both patients are at home in excellent clinical conditions despite more than 12 months combined HD and PD (pt. 1) and more than 7 months of PD (pt. 2). They are listed for liver and kidney transplantation.

We encourage early and intensive dialysis in PH1-patients presenting in infancy. Outcome of ESRF has improved with increasing experience of renal replacement therapy in infancy, yet only successful transplantation offers a long term perspective.

59. Primary cultures of renal proximal tubule cells derived from individuals with primary hyperoxaluria

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The primary hyperoxalurias (PH1 and PH2) are a group of inherited disorders caused by deficiencies of two enzymes, alanine:glyoxylate aminotransferase and glyoxylate reductase respectively. Mutations in either of these enzymes leads to endogenous oxalate overproduction primarily in the liver, but most pathological effects are exhibited in the kidney ultimately leading to end stage renal failure and systemic oxalosis. The variety of presenting features in patients with identical genotypes suggests that as yet unidentified genes influence susceptibility to stone development, and our goal is to identify such genes.

To provide a non-invasive means of investigating possible differences in gene expression in the renal tubules exposed to oxalate we have derived primary cultures of renal proximal tubule cells from the urine of individuals with PH. Urine samples from seventeen PH patients have been collected for the isolation of proximal tubule cells. The isolated cells displayed an epithelial morphology, which did not alter on sub-culture, and expressed the epithelial markers pan-cytokeratin and ZO-1. The proximal tubular nature of the isolated cells was confirmed by positive immunocytochemistry for γ -glutamyl transpeptidase and RT-PCR for the expression of aminopeptidase A. Furthermore, the cells were negative for the expression of the distal tubular and collecting duct markers, uromodulin and aquaporin 3 respectively, as assessed by RT-PCR. Mutation analysis confirmed that the cultured cells had the same genotype as the leucocytes of the patients and also expressed glyoxylate reduc-

tase at the mRNA level, illustrating their potential physiological value.

Our preliminary results show that primary cultures of human proximal tubule cells can be obtained from urine of patients with PH, providing the means with which to study the impact of these diseases on the kidney. By defining mechanisms influencing PH development, we may better understand why the disease progresses at different rates in patients with the same genetic abnormality, allowing specific strategies to be devised to slow down or prevent the clinical consequences of the disease.

60. The effect of oxalate exposure on the cell biology of human renal proximal tubule cells

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The primary hyperoxalurias (PH) are inherited diseases of endogenous oxalate overproduction, which exhibit most of their pathological effects in the kidney as a result of chronic exposure to elevated oxalate concentrations. At present, it is unclear how high levels of oxalate initiate damage to renal tubules and ultimately lead to chronic renal damage and kidney failure. Our hypothesis is that kidney damage is not simply explained by gross structural damage from oxalate crystals and stones but that the cell biology of renal tubular epithelia is significantly altered by exposure to high concentrations of free oxalate predicted to appear in the glomerular filtrate of PH patients.

We exposed HK-2 proximal tubule cells to pathophysiological concentrations of oxalate (50 μ M) and assessed changes in gene expression following oxalate exposure, by microarray analysis 1 hour and 24 h after treatment ($n = 3$ for each group). Over 100 genes showed statistically significant changes of greater than 1.5 fold in treated cells, at both time points. Strikingly, our microarray data indicated that several genes coding for proteins involved in the cytoskeleton (including the primary cilium) and cell polarity, such as pericentrin, inversin, dynein and kinesin were altered in response to oxalate exposure. In addition, the expression genes coding for α -catenin, collagen IV α 3, and collagen IV α 6, proteins that are involved in cell-cell and cell-matrix adhesion, were deregulated following oxalate exposure. We subsequently confirmed the presence of selected genes altered in the microarray in human proximal tubule cells and validated alterations in expression using quantitative real-time RT-PCR. We are currently examining the effects of oxalate on the cell biology of the HK-2 line, using immunohistochemistry to assess the integrity of the cytoskeleton, tight junction formation and focal adhesion complex assembly.

Our preliminary microarray data lead us to suggest that changes in expression of these genes may contribute to the initial steps in PH nephropathy. The wide genotype-phenotype variation seen in PH supports a hypothesis that there are modifying genes, which modulate the effect of oxalate in the kidney. We propose that some of the genes highlighted in our microarray studies are candidates for such modifiers and, in future, polymorphisms of these genes could be sought in PH patients and correlated with the severity of kidney disease.

61. Primary hyperoxaluria remains undiagnosed in patients with hyperoxaluria and recurrent urolithiasis

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Background: High incidence of end-stage renal disease at the time of diagnosis occurs in up to 60% of adult Primary Hyperoxaluria (PH) patients. Prevention of renal insufficiency is possible after timely diagnosis, especially in patients who respond to pyridoxine

treatment by decreasing urinary oxalate levels. Patients with hyperoxaluria may not have received adequate diagnostic follow-up and are therefore missed. Our aim was to investigate possible underdiagnosis of PH patients from results of urinary oxalate levels in a routine laboratory.

Methods: Urinary oxalate, glycolate and L-glycerate was measured, and, if necessary, a genetic analysis was performed in all patients with elevated urine oxalate levels that were ever found by screening at the department of clinical chemistry in our center.

Results: Elevated oxalate levels had been found in 32 out of 150 patients (males 91) in seven years. Twenty-five of these patients participated in this study. All had a history of urinary stones. A 24-h collection of urine revealed normo-glycolic hyperoxaluria in six patients: four with bowel disease, one without co-morbidity and one with a childhood onset of symptoms and hyperoxaluria. Genetic analysis revealed a homozygous Gly170Arg mutation on the minor allele in this patient.

Conclusion: Primary hyperoxaluria is more common in The Netherlands than previously found. The finding of hyperoxaluria should lead to further urine screening in order to detect PH1 immediately and initiate specialized clinical management without delay.