Molecular Etiology of Primary Hyperoxaluria Type 1: New Directions for Treatment

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Key Words
Primary hyperoxaluria type 1 · Alanine:glyoxylate aminotransferase · AGXT · Liver transplantation · Enzyme replacement therapy · Pyridoxine · Gene therapy · Chemical chaperones · Kidney stones · Calcium oxalate

Molecular Etiology of PH1

Primary hyperoxaluria type 1 (PH1, MIM 259900) is a rare autosomal-recessive disorder caused by a deficiency of the liver-specific enzyme alanine:glyoxylate aminotransferase (AGT). AGT deficiency results in increased synthesis and excretion of the metabolic end-product oxalate and deposition of insoluble calcium oxalate in the kidney and urinary tract. Classic treatments for PH1 have tended to address the more distal aspects of the disease process (i.e. the symptoms rather than the causes). However, advances in the understanding of the molecular etiology of PH1 over the past decade have shifted attention towards the more proximal aspects of the disease process (i.e. the causes rather than the symptoms). The determination of the crystal structure of AGT has enabled the effects of some of the most important missense mutations in the AGXT gene to be rationalised in terms of AGT folding, dimerization and stability. This has opened up new possibilities for the design pharmacological agents that might counteract the destabilizing effects of these mutations and which might be of use for the treatment of a potentially life-threatening and difficult-to-treat disease.

Abstract
Primary hyperoxaluria type 1 (PH1) is a rare autosomal-recessive disorder caused by a deficiency of the liver-specific enzyme alanine:glyoxylate aminotransferase (AGT). AGT deficiency results in increased synthesis and excretion of the metabolic end-product oxalate and deposition of insoluble calcium oxalate in the kidney and urinary tract. Classic treatments for PH1 have tended to address the more distal aspects of the disease process (i.e. the symptoms rather than the causes). However, advances in the understanding of the molecular etiology of PH1 over the past decade have shifted attention towards the more proximal aspects of the disease process (i.e. the causes rather than the symptoms). The determination of the crystal structure of AGT has enabled the effects of some of the most important missense mutations in the AGXT gene to be rationalised in terms of AGT folding, dimerization and stability. This has opened up new possibilities for the design pharmacological agents that might counteract the destabilizing effects of these mutations and which might be of use for the treatment of a potentially life-threatening and difficult-to-treat disease.

Enzyme Heterogeneity
A number of different enzyme phenotypes have been identified in PH1. Some patients have a complete, or nearly complete, absence of AGT immunoreactivity and catalytic activity (CRM-/ENZ-), while others have nor-
mal, or near normal, levels of AGT immunoreactivity but almost no catalytic activity (CRM+/ENZ-). Yet other patients, totalling about one third of the total, have significant levels of both AGT immunoreactivity and catalytic activity (CRM+/ENZ+) [4].

Each of these crude categorizations of enzyme phenotype can be related to specific functional abnormalities of AGT at the molecular and cell biological levels. For example, in CRM--/ENZ-- patients, either no AGT protein is synthesised or if it is then it is very rapidly degraded. In CRM+/ENZ-- patients, AGT fails to bind its cofactor pyridoxal phosphate [5]. In CRM+/ENZ+ patients, disease is due to a remarkable trafficking defect in which AGT is mistargeted from its normal location in the peroxisomes to the mitochondria [6]. Intermediate enzyme phenotypes also exist. For example, in some CRM+/ ENZ-- patients, AGT is aggregated into peroxisomal cores [7].

**AGT Structure**

AGT is a homodimeric protein, each subunit possessing 392 amino acids and molecular mass of about 43 kDa [8]. The X-ray crystal structure of normal AGT has been solved to a resolution of 2.5 Å (PDB 1H0C) [9]. AGT forms an intimate dimer each subunit of which is comprised of three structural domains. The first 20 or so residues make up an N-terminal extension that wraps over the surface of the other subunit. The next 260 or so residues form the ‘large domain’ that contains most of the active site and the dimerization interface. The final C-terminal ‘small domain’ consists of about 110 residues and contains, among other things, the principal and ancillary peroxisomal targeting information [10, 11]. Each subunit binds one pyridoxal phosphate which forms a Schiff base with Lys209. The large surface area of the dimerization interface explains the high stability of the AGT dimer. Although the interaction of the N-terminal extension of one subunit with the surface of the other subunit is unlikely to make any major contribution to stability to the dimer once formed, it is believed to play a significant role in the dimerization process per se. Dimerization is very important, not only because evidence suggests that monomeric AGT has vastly reduced catalytic activity [5], but also because it is unstable leading to aggregation and rapid degradation [7]. Knowledge of the crystal structure of AGT not only provides insights into the processes of AGT folding, dimerization and catalytic activity, but also helps us understand the effects of mutations and polymorphisms on these processes (see below).

**Effects of Mutations and Polymorphisms on the Properties of AGT**

AGT is encoded by the *AGXT* gene which is located on chromosome 2q37.3 and is comprised of 11 exons spreading over 10 kB [12]. Two main polymorphic variants have been identified in European and North American populations. The ‘minor’ allele differs from the ‘major’ allele by the presence of 32C→T and 1020A→G nucleotide substitutions, which lead to Pro11Leu and Ile340Met amino acid replacements, respectively [13]. In addition, the minor *AGXT* allele contains a 74-bp duplication in intron 1 [14]. Unlike most polymorphisms in most genes, the Pro1Leu replacement does have significant affects on the properties of AGT. For example, it decreases its specific catalytic activity by two thirds, slows down the rate of AGT dimerization, especially at elevated temperatures [5], and it redirects a small proportion (about 5%) away from the peroxisomes towards the mitochondria [13]. None of these effects appears to be too detrimental, as the presence of the polymorphism does not lead to any obvious adverse clinical phenotype. However, it does play an important role in PH1, because its presence appears to sensitise AGT to the adverse effects of a number of missense mutations, including the two most common ones, which are predicted to have no phenotype in the absence of the polymorphism [5].

Over 50 different mutations have been identified so far in the *AGXT* gene, ranging from missense and nonsense point mutations, deletions, insertions, and splice site variants [15]. Many of these mutations segregate with a specific polymorphic allele, and those that segregate with the minor *AGXT* allele have their effects significantly affected by the presence of the Pro11Leu polymorphism [5]. The effects on enzyme function of only a few of these mutations have been characterized in any detail. The best studied are described below and summarized in table 1.

**Gly170Arg**. The most common mutation found so far is a 508G→A missense point substitution which leads to a Gly170Arg amino acid replacement [13]. This has an estimated allelic frequency of about 30% in unclassified European and North American PH1 patients. This mutation has little or no effect on the properties of AGT when present on its own. However, when located on the minor allele (i.e. with the Pro11Leu polymorphism), the two interact synergistically to delay AGT dimerization [5, 16] and redirect 90% of the enzyme away from its normal location in the peroxisomes to the mitochondria [6, 13, 17]. Although still catalytically active, mistargeted AGT is metabolically inefficient. The Pro11 is located in the
middle of the N-terminal extension [9]. Its replacement by Leu generates a functionally weak mitochondrial targeting sequence (MTS), the efficiency of which is enhanced by the Gly170Arg replacement [18]. This is because the two together slow down the rate of AGT folding and dimerization [5, 16]. This unparalleled explanation for a human genetic disease highlights one of the main differences between the peroxisomal and mitochondrial protein import pathways. Whereas peroxisomes can import fully folded cofactor-bound functionally active oligomeric proteins [19, 20], mitochondria can only import unfolded, or loosely folded, monomers [21, 22].

The locations of Pro11 and Gly170 in the crystal structure of AGT suggest a possible mechanism by which they might functionally interact. The Pro11Leu replacement of AGT allows the N-terminal extension to adopt a conformation typical of a mitochondrial targeting sequence (MTS) [13, 17, 18]. However, it cannot work efficiently because AGT still dimerizes rapidly (albeit somewhat slower than when the polymorphism is absent), so that the N-terminal extension (now a cryptic MTS) still remains partially tethered to the surface of the opposing subunit. The additional presence of the Gly170Arg replacement delays dimerization further, so that the N-terminal extension cannot interact with the surface of the opposing subunit and is then free to interact with the mitochondrial import receptor TOM20 [23, 24]. Because mitochondrial import seems to exert a hierarchical dominance over peroxisomal import [25], AGT is redirected away from the peroxisomes towards the mitochondria.

Ile244Thr. The second most common missense mutation is a $731T \rightarrow C$ nucleotide substitution which encodes for an Ile244Thr amino acid replacement [26]. This has an overall allelic frequency of about 9% in PH1 patients. This mutation also works synergistically with the Pro11Leu polymorphism to give massively reduced AGT immunoreactivity and catalytic activity, presumably due to accelerated AGT degradation. In vitro studies have shown that the combined presence of the Pro11Leu and Ile244Thr replacements leads to AGT aggregation [27], although this has not been detected in patients. Analysis of the crystal structure of AGT gives little in the way of clues about how these replacements might achieve their effects on AGT. It is possible that they work similarly to the Pro11Leu+Gly170Arg combination as they would also be expected to interfere with the inter-subunit tethering of the N-terminal extension [9]. For reasons that are not understood, although Pro11Leu+Ile244Thr also inhibits AGT dimerization, they do not cause mistargeting to mitochondria.

Gly82Glu. All other missense mutations in the AGXT gene are much rarer than Gly170Arg and Ile244Thr, and have been found in only a limited number of families. The 245G→A substitution, which leads to a Gly82Glu

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Allelic frequency in PH1</th>
<th>Associated polymorphic allele</th>
<th>Effect on AGT properties (on appropriate polymorphic background)</th>
<th>Likely success of chemical chaperones</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH1-specific mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>508G→A</td>
<td>Gly170Arg</td>
<td>30%</td>
<td>minor</td>
<td>dimerization inhibited, 90–95% mistargeted from peroxisomes to mitochondria</td>
<td>good</td>
</tr>
<tr>
<td>33_34insC</td>
<td>–</td>
<td>13%</td>
<td>major</td>
<td>correct polypeptide not synthesized</td>
<td>zero</td>
</tr>
<tr>
<td>731T→C</td>
<td>Ile244Thr</td>
<td>9%</td>
<td>minor</td>
<td>aggregates, degradation accelerated</td>
<td>good</td>
</tr>
<tr>
<td>121G→A</td>
<td>Gly41Arg</td>
<td>1%</td>
<td>minor/major</td>
<td>dimerization inhibited, aggregates in peroxisomes, degradation accelerated</td>
<td>poor</td>
</tr>
<tr>
<td>245G→A</td>
<td>Gly82Glu</td>
<td>1%</td>
<td>major</td>
<td>cofactor binding blocked</td>
<td>zero</td>
</tr>
<tr>
<td>613T→C</td>
<td>Ser205Pro</td>
<td>9%</td>
<td>major</td>
<td>degradation accelerated</td>
<td>good</td>
</tr>
<tr>
<td>Minor allele polymorphisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32C→T</td>
<td>Pro11Leu</td>
<td>50%</td>
<td>[minor]</td>
<td>specific activity decreased by two thirds, dimerization slowed, 5% mistargeted from peroxisomes to mitochondria</td>
<td>good</td>
</tr>
<tr>
<td>1020A→G</td>
<td>Ile340Met</td>
<td>50%</td>
<td>[minor]</td>
<td>no effect</td>
<td>N/A</td>
</tr>
</tbody>
</table>

1 Gly170Arg and Ile244Thr have no discernable effect in the absence of the Pro11Leu polymorphism of the minor allele.
2 Distribution in hepatocytes from a normal individual homozygous for the minor allele.
3 Predicted likelihood of success of chemical chaperones in counteracting the aberrant enzyme phenotypes resulting from particular mutations or polymorphisms (N/A, not applicable, as Ile340Met has no detectable phenotype).
replacement, has only been found only on the major AGXT allele (i.e. without the Pro11Leu polymorphism) [28]. Patients homozygous for Gly82Glu are characterized by normal levels of correctly targeted AGT, but zero catalytic activity. Structural analysis shows that the reason for this is because, when glycine is replaced by glutamate, the side-chain of the latter blocks the pyridoxal phosphate binding site [5, 9]. Because cofactor binding to Lys209 is essential for the normal catalytic cycle, the resulting enzyme has no catalytic activity.

Gly41Arg. The 121G→A substitution, which leads to a Gly41Arg replacement, has been found on both the minor and major AGXT alleles [7, 29]. Some evidence suggests that disease might be more severe when present on the former rather than the latter. In the presence of Pro11Leu, this mutation results in significant diminution of immunoreactive AGT, presumably due to accelerated degradation. The small amount of immunoreactive AGT remaining is without catalytic activity and appears to be aggregated into intra-peroxisomal cores [7]. Some AGT is also present in the mitochondria, but whether this is greater than the amount of mitochondrial AGT found in normal individuals homozygous for the minor allele is unclear. Structural analysis shows that Gly41 is located right in the middle of the dimerization interface in very close proximity to its opposite number in the other subunit [9]. If glycine were to be replaced by arginine, then the large side chain of the latter would prevent the apposition of the dimerization interfaces, thereby preventing dimerization. The intrinsic instability of the monomer would lead to its aggregation and accelerated degradation.

Ser205Pro. Ser205Pro is a rare mutation identified in a Japanese PH1 patient [30]. It is found on the major AGXT allele. This mutation results in accelerated degradation so that patients in which it is found are CRM-/ENZ- [31]. Structural analysis suggests that the replacement of serine by proline at this site would necessitate a large conformational change in the backbone of one of the β-strands of AGT that would completely disrupt the main chain hydrogen bonding of the central β-sheet [9]. This would make AGT highly unstable and lead to its rapid degradation.

33_34C. The second most common mutation is the 33_34CinsC insertion, which has an overall allelic frequency of about 13% in PH1 patients [15]. Patients homozygous for this mutation have no AGT immunoreactivity or catalytic activity. This is unsurprising and requires no knowledge of the structure of AGT to understand. This insertion causes a frame shift, so that the coding for AGT is lost and the protein cannot be made.

Table 2. AGT-specific therapeutic procedures in PH1

<table>
<thead>
<tr>
<th>Therapeutic procedure</th>
<th>Mechanism of action</th>
<th>Mutational applicability</th>
<th>Timescale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme replacement therapy (liver transplantation)</td>
<td>replaces defective enzyme</td>
<td>all mutations</td>
<td>current</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>increases conversion of apo-enzyme to holo-enzyme, stabilizes holo-enzyme (?)</td>
<td>Gly170Arg only (?)</td>
<td>current</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>replaces defective gene stabilises defective protein</td>
<td>all mutations</td>
<td>future (?)</td>
</tr>
<tr>
<td>Chemical chaperones</td>
<td></td>
<td>many (but not all) missense mutations</td>
<td>future (?)</td>
</tr>
</tbody>
</table>

New Directions for Treatment

PH1 has been known about, and treated with varying degrees of success, for eighty years. Absence of any understanding of the molecular etiology of the disease meant that many treatments were introduced on a trial-and-error basis and dealt mainly with the later stages of the disease process. Little progress was made until 1986 when the basic cause of the disease (i.e. AGT deficiency) was discovered. Since then there have been enormous changes to all aspects of the clinical management of PH1. Some of the most important advances have been concerned with treatment (table 2).

Classic Treatments

The classic treatments for PH1 (i.e. those that we in use before the mid-1980s) involved decreasing the solubility product of CaOx in the urine, decreasing the chances of crystallization, crystal growth and crystal adherence to the tubule or collecting duct wall, and reacting to the effects of kidney failure (i.e. dialysis and kidney trans-
plantation). Most of these approaches to treatment are still in use today, to one extent or another. Although potentially lifesaving, at least in the short term, these treatments at best slow down the rate of disease progression, rather than cure it. This is because they do not tackle the basic defect (i.e. hepatic AGT deficiency). Kidney transplantation, especially, is problematic because even though the new kidney may very well provide an immediate solution to the problem of kidney failure, it is highly likely to succumb to the ravages of CaOx deposition, as did the original organ.

**Pyridoxine Therapy**

One classic treatment that, in retrospect, probably did address the basic cause of the disease was pyridoxine treatment. For reasons that are not at all clear, pyridoxine therapy was introduced as a treatment for PH1 long before AGT deficiency was discovered. Whatever the reason, it has been well-recognised for many years that pharmacological doses of pyridoxine can be very effective at arresting disease progression in 10–30% of patients (reviewed in [2]). Pyridoxine (vitamin B₆) is metabolised to pyridoxal phosphate (PLP) in the body, and PLP is the essential cofactor of AGT, as it is for all aminotransferases. Despite this knowledge, it is not at all clear how pyridoxine actually works in these responsive PH1 patients.

It was noticed some time ago that patients were more likely to be responsive to pyridoxine treatment if they had significant levels of residual AGT (i.e. they were CRM+/ENZ+) [2, 32]. As these patients, in general, have the Gly170Arg mutation and mitochondrial AGT, it followed that patients with mistargeted AGT were the ones responsive to pyridoxine. Formal evidence in support of this has come recently from a retrospective analysis of PH1 patients at the Mayo Clinic in Rochester, Minn., USA [33]. This study showed that pyridoxine responsiveness was clearly related to the presence of the Gly170Arg mutation. In general, patients homozygous for Gly170Arg were more responsive than those expressing only one Gly170Arg allele.

What is clear is that pyridoxine exerts its effects on oxalate synthesis due to its effects on AGT. What is uncertain, however, is how it does it. The answer may not be known until a liver biopsy from a PH1 patient is analysed for AGT catalytic activity, immunoreactivity, and subcellular distribution, before and after pyridoxine treatment. As AGT mistargeting is thought to be due, at least in part, to a decrease in protein stability (see above), one possibility is that high concentrations of PLP stabilise AGT and correct its targeting. Although many cofactors are known to stabilise the proteins with which they interact, there is no evidence of this happening with PLP and AGT. In fact the only evidence so far suggests that neither PLP nor pyridoxine can correct AGT targeting in an in vitro tissue culture system [34]. A significant proportion of the enzyme in normal liver might exist in the inactive apo form [35]. Large concentrations of PLP would be expected to shift the equilibrium away from the apo form towards the catalytically active holo form. The resulting increase in the specific activity of AGT could explain the beneficial effects of pyridoxine therapy. Presumably it would be the 5–10% of the AGT remaining in the peroxisomes which would be the most important in this respect, but increasing the activity in the mitochondria might also be beneficial. Another possibility is that increased intracellular levels of PLP might have a direct effect on the total amount of AGT protein in hepatocytes, either by increasing its rate of synthesis or decreasing its rate of degradation [35].

**Enzyme Replacement Therapy by Liver Transplantation**

Identification of the defective enzyme in a metabolic disease often opens up the possibility of enzyme replacement therapy (ERT). This usually involves the purification of large amounts of enzyme, either isolated from a natural source or following production of recombinant protein, and then injection of the purified enzyme at regular intervals. This is best exemplified by lysosomal storage disorders, such as Gaucher disease (for a review see Beutler and Grabowski [36]). For these diseases, the enzyme is taken up by endocytosis. The endosomes fuse with lysosomes, so that the enzyme enters into the compartment where it is required to work (i.e. the lysosomes). Unfortunately, such an option is not available for peroxisomal diseases, such as PH1. Inside hepatocytes, AGT is located in the peroxisomes, an organelle that does not interact with endosomes or have any other interaction with the extracellular space. So that AGT taken into the cell will not be able to reach the necessary compartment (i.e. the peroxisomes), but instead would probably also make its way to the lysosomes where it would be degraded.

In humans, the vast majority of the body’s AGT is located in liver parenchymal cells (i.e. hepatocytes) [37–39], opening up the possibility that liver transplantation could be used as a form of ERT. Such a procedure has a number of benefits. In particular, liver transplantation is expected to reintroduce most of the body’s requirements for AGT, already in the correct cell and intracellular com-
Gene Therapy

As with most genetic diseases, characterization of the dysfunctional gene allows the possibility of gene therapy. Although the relative benefits of different gene therapy strategies in PH1 have been discussed [48], none have yet to be put into practice. One of the potential problems highlighted very early on was the particular need to be able to transduce a large percentage of the patient’s hepatocytes with the normal AGT gene, rather than maximising the total amount of AGT expressed in the organ as a whole. This is because, unlike many other hereditary metabolic diseases, PH1 is a disease of overproduction (of oxalate) not of failure to degrade (as is the case in other metabolic disease such as the lysosomal storage disorders). Any hepatocytes not expressing AGT will continue to synthesise oxalate excessively, irrespective of what its neighbours are doing. AGT overproduction in one hepatocyte cannot compensate for underproduction in its neighbour. Thus a large percentage of hepatocytes need to be transduced with the normal AGT gene. Although no PH1 patients have received gene therapy to date, it remains a viable proposition for the future once better (i.e. more efficient) vectors have been designed.

Chemical Chaperones

It is increasingly being realised that many, possibly most, missense mutations in human genetic disease cause their effects by decreasing protein stability. This can disturb protein folding and oligomerization, leading to a multitude of downstream effects, such as aggregation, accelerated degradation and mistargeting [49]. Several studies have shown that if the destabilizing effects of mutations can be overcome, then the resulting protein requires its functional activity. One of the best examples of this is the ΔF508 mutation in the CFTR gene that is the prime cause of cystic fibrosis. This mutation destabilises the protein so that it aggregates in the endoplasmic reticulum from which at least some is re-exported back out into the cytosol for degradation by the proteosomes. Very little reaches its normal destination in the plasma membrane. But that which does appears to work fairly normally. Treatments which non-specifically stabilise proteins, such as lowering temperature or addition of osmolytes, such as glycerol, enable the CFTR protein to fold properly. This prevents aggregation in the ER, so that the protein can make its way to the plasma membrane unhindered where it is functionally active [50–52].

In this respect, PH1 is like other genetic diseases. Many of the missense mutations in the AGXT gene are predicted to decrease the stability of AGT and, therefore, stabilising agents should correct the aberrant enzyme phenotypes. Formal proof in principle has been obtained for the Pro11Leu+Gly170Arg and Pro11Leu+Ile244Thr polymorphism-mutation combinations, which lead to peroxisome-to-mitochondrion mistargeting, and aggregation/accelerated degradation, respectively. In the case of the former, various treatments known to stabilise proteins non-specifically, such as decreasing the temperature or the addition of glycerol, can normalise AGT targeting in vitro [34]. In the case of the latter, glycerol, betaine, and other non-specific chemical chaperones, have been shown to prevent aggregation, also in vitro [27].

It is likely that such treatments would also do the same for at least some other, but certainly not all, missense mutations (table 1). For example, although they would probably also work for AGT containing the Ser205Pro mutation, they are unlikely to work for missense mutations that cause their effects for reasons unrelated to protein stability, such as Gly82Glu (see above). Another possible example of where protein stabilising agents would not be expected to work is in the case of the Gly41Arg mutation. This mutation leads to AGT destabilization, but it does so by preventing AGT dimerization allowing the protein monomer to aggregate in the peroxisomes and be prematurely degraded. The basic problem (i.e. the presence of the large arginine side-chain preventing apposition of the dimerization interfaces) would be unlikely to be affected by the presence of chemical stabilization agents. Also it is clear that nonsense mutations, insertions, deletions and splice-site mutations would not be open to this approach, because the coding for the correct polypeptide does not exist. This rules out, for example, the second most common mutation 33_34insC.
Although the common Pro11Leu polymorphism is a component of 'normal' AGT, albeit that encoded by the polymorphic minor AGXT allele, it might be a better target for chemical chaperones that any of the PH1-specific mutations. There is no doubt that it has a more marked phenotype than does either of the most common mutations it segregates and functionally interacts with. Thus, whereas Gly170Arg and Ile244Thr appear to cause AGT no adverse effects when present on their own, Pro11Leu on its own decreases specific catalytic activity, slows down the rate of dimerization, especially at high temperatures, and generates an N-terminal mitochondrial targeting sequence that directs about 5% of the polypeptide to the mitochondria. In addition, Pro11Leu appears to sensitise AGT to the untoward effects of several other PH1-specific mutations, including Gly170Arg and Ile244Thr (see above). Directing the design of chemical chaperones against the effects of the Pro11Leu polymorphism has the advantage that it would catch all the mutations that segregate and functionally interact with it.

There is another consideration that might make the use of Pro11Leu as the principal target for chemical chaperones even more important. It has been speculated previously that Pro11Leu might have a role to play in determining an individual's susceptibility to idiopathic calcium oxalate kidney stone disease [53]. However, the suggested relationship is not simple, as it is diet dependent. It has been suggested that Pro11Leu is more likely to be beneficial (i.e. decrease the chances of CaOx kidney stones) in individuals whose diets are high in meat, and is more likely to be disadvantageous (i.e. increase the chances of CaOx kidney stones) in those whose diets are low in meat (e.g. vegetarians). Compatible with this suggestion is the recent finding that the frequency of the Pro11Leu polymorphism is much higher in populations in which the ancestral diet is extremely meat-rich than it is in those in which the ancestral diet is more mixed or more vegetarian [53]. The use of anti-Pro11Leu chemical chaperones in the general populations could be a two-edged sword. It could decrease the susceptibility to CaOx stones in individuals carrying the polymorphism who were vegetarian, but increase it in those that ate a lot of meat. The likely success, or otherwise, of chemical chaperones in being able to counteract the effects of the specific mutations and polymorphisms discussed above is summarized in table 1.

Whatever the best target for chemical chaperones is, it is clear that extensive work will be required to find small molecules that bind to and stabilise AGT with a much greater affinity than the agents used so far. In the long run, it will be worth it because not only will it lead to enormous improvements in the treatment of a potentially life-threatening disease that is otherwise difficult to treat, but also because such an agent might have wider benefits as a prophylactic against CaOx stone disease in general.

References
