Primary Hyperoxaluria Type 1: Update and Additional Mutation Analysis of the AGXT Gene

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ABSTRACT: Primary hyperoxaluria type 1 (PH1) is an autosomal recessive, inherited disorder of glyoxylate metabolism arising from a deficiency of the alanine:glyoxylate aminotransferase (AGT) enzyme, encoded by the AGXT gene. The disease is manifested by excessive endogenous oxalate production, which leads to impaired renal function and associated morbidity. At least 146 mutations have now been described, 50 of which are newly reported here. The mutations, which occur along the length of the AGXT gene, are predominantly single-nucleotide substitutions (75%), 73 are missense, 19 nonsense, and 18 splice mutations; but 36 major and minor deletions and insertions are also included. There is little association of mutation with ethnicity, the most obvious exception being the p.Ile244Thr mutation, which appears to have North African/Spanish origins. A common, polymorphic variant encoding leucine at codon 11, the so-called minor allele, has significantly lower catalytic activity in vitro, and has a higher frequency in PH1 compared to the rest of the population. This polymorphism influences enzyme targeting in the presence of the most common Gly170Arg mutation and potentiates the effect of several other pathological sequence variants. This review discusses the spectrum of AGXT mutations and polymorphisms, their clinical significance, and their diagnostic relevance.


KEY WORDS: AGXT; primary hyperoxaluria type 1; mutation; PH1

Introduction

Primary hyperoxaluria type 1 (PH1; MIM# 259900) is an autosomal recessive disorder of glyoxylate metabolism leading to overproduction of endogenous oxalate, which manifests as renal stones and/or nephrocalcinosis [Danpure, 2001]. Approximately 50% patients presenting in childhood will have end-stage renal failure by the age of 15 years [Latta and Broedehl, 1990], a situation which leads to systemic oxalosis with oxalate precipitation in the eye, heart, and bones, with significant morbidity and mortality. The incidence of PH1 is in the range of 1 in 120,000 live births [Cochat et al., 1995] with prevalence ranging from 1.05 × 10^-6 to 2.9 × 10^-6 in France, Switzerland, and the Netherlands [Cochat et al., 1995; Kopp and Leumann, 1995; Van Woerden et al., 2003], respectively. Excessive oxalate excretion is an indicator of this disease, although the test is not specific for PH1 and may actually be misleadingly reduced in renal failure. Thus more sophisticated tests, including genetic analysis [Rumsby et al., 2004; Williams and Rumsby, 2007] and/or enzymology [Allsop et al., 1987; Rumsby et al., 1997], are required to make a diagnosis.

The disease is caused by mutations in the AGXT gene (MIM# 604285) which encodes the hepatic, peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT; EC 2.6.1.44). The genomic sequence is comprised of 11 exons, spanning approximately 10 kb, and maps to chromosome 2q37.3 [Purdue et al., 1991b]. The entire sequence can be found within the contig NT_005416 (www.ncbi.nlm.nih.gov).

The full-length cDNA, which is ~1.7 kb on northern blot [Nishiyama et al., 1990], has an open reading frame of 1,176 bp. The 5’ UTR varies from 21 bp [Nishiyama et al., 1990] to 122 bp [Takada et al., 1990], with a 3’UTR of 287 bp [Nishiyama et al., 1990]. Primer extension analysis confirmed that the main transcription start site was located near to –45 bp, with other minor start sites up to –115 [Sato et al., 2002]. Deletion analysis of the 5’ flanking region indicated that the region up to –65 was important for expression of AGXT in a human liver cell line and that the region from –440 to –700 is potentially an important region for enhancer elements, with a number of putative functional binding sites identified [Sato et al., 2002].
The mature protein is an 86-kDa homodimer [Nishiyama et al., 1990; Noguchi and Takada, 1978], with each monomer binding one molecule of pyridoxal phosphate (PLP) [Ishikawa et al., 1996]. The terminal amino acids, KKL, of the human protein form an atypical peroxisomal targeting sequence that is necessary, but not sufficient, for peroxisomal uptake via the PTS1 targeting sequence receptor, Pex5p [Motley et al., 1995]. Another putative ancillary targeting sequence (PTS1A) has been found in the C terminal domain, between Val 324 and Ile 345 [Huber et al., 2005]. Both the PTS1A and the PTS1 lie on the surface of the AGT dimer in close proximity and their spatial arrangement may well be potentially important for interaction with human Pex5p or an additional intermediary [Huber et al., 2005; Knott et al., 2000]. A third region, from amino acids 59–66, has also been attributed a role in peroxisomal targeting [Ikeda et al., 2008]; although, given the location of this region along the dimer interface, it is difficult to see how this is possible.

**Polymorphisms and Mutations in AGXT**

The nomenclature used in this work follows the HGVS guidelines (www.hgvs.org) and as such may differ from that presented in the original literature. The reference sequences used are NM_000030.2 (cDNA) and NT_005416 (genomic). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

While it is more typical to discuss mutations prior to the less important polymorphisms, AGXT has one common allelic variant, the so-called minor allele, which has potential functional and pathological significance. For this reason, the polymorphisms in the AGXT gene will be described first to set the context.

**Polymorphisms**

All the polymorphic variants currently described in AGXT are presented in Supp. Table S1.

A number of polymorphic variants are found in linkage disequilibrium and form the minor allele haplotype (AGT-Mi). The primary, invariant, change in this allele is the presence of the c.32C>T change, which encodes leucine at codon 11 instead of the proline found in the major allele (AGT-Ma) [Purdue et al., 1990]. Additional sequence changes on the minor allele are the presence of a 74-bp imperfect duplication in intron 1 containing the two further substitutions c.166–100A>C and c.166–96T>G [Purdue et al., 1991a], a variable number of tandem repeats (VNTR) in intron 4 [Danpure et al., 1994b], and several other single nucleotide changes—c.165+16A>G (Williams and Rumsby; Waterham; this work*), c.264C>T [Danpure et al., 1994a], c.358+13C>T [Von Schna Toben et al., unpublished observation]). The VNTR in intron 4 is composed of a 29–32-bp repeat unit and was originally identified as a Taq1 RFLP [Rumsby et al., 1992]. The largest allele has approximately 38 repeats, the smallest 12 [Danpure et al., 1994a,b]. AGT-Mi is, to date, always associated with the largest size repeat unit.

Other missense changes that appear to be neutral in their effect have been found in AGXT. In some cases these have been found in patients with a known pathological mutation. One of these, p.Thr9Asn, was originally described as a mutation but in fact has normal activity in vitro [Williams and Rumsby, 2007]; moreover, it was found in trans of the pathogenic mutation c.943–1G>T in the healthy mother of an affected child (Amoroso; this work). p.Ile279Thr, p.Ala280Val, and p.Val326Ile also have normal activity when expressed in vitro on AGT-Mi [Coulter-Mackie et al., 2003].

**Mutations**

A total of 146 mutations, scattered across the gene, have now been described, with all exons represented. The majority (75%) are point mutations, which include 73 missense, 19 nonsense, and a further 18 single-nucleotide changes affecting splice-site consensus sequences. The remaining 25% are major or minor deletions and insertions. For reasons of space, only novel variants are shown in Table 1. All known mutations (novel and previously reported) are given in Supp. Tables S2, S3, and S4. The supporting tables also give previously unpublished expression data for a large number of variants.

**Missense Mutations**

Approximately 57% of the missense mutations have been expressed. With few exceptions, these mutant proteins have negligible activity and immunoreactivity and are essentially null alleles. The polymorphic background significantly influences activity in vitro, with lower activity often occurring in the presence of AGT-Mi. For example, p.Gly170Arg, p.Arg211Ser, and p.Pro233Cys, and p.Arg233His all have significant levels of catalytic activity and positive immunoreactivity when expressed on AGT-Ma, but negligible activity and immunoreactivity on AGT-Mi [Williams and Rumsby, 2007]. All these mutations have been found on the minor allele in patients and the effect in vivo if expressed on AGT-Ma could be partially functional. However, p.Ile244Thr, which also has significant activity when expressed on AGT-Ma [Lumb and Danpure, 2000], has been shown to still cause disease when on AGT-Ma.
Table 1. Novel Sequence Variants in the AGXT Gene

<table>
<thead>
<tr>
<th>Location</th>
<th>Sequence variant</th>
<th>Codon/effect</th>
<th>Major or minor haplotype</th>
<th>In vitro activity (% normal control) or how proven</th>
<th>Author attributions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>c.3G&gt;T</td>
<td>p.Met1Ile</td>
<td>Major</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>c.32C&gt;G</td>
<td>p.Pro11Arg</td>
<td>Major</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>c.107G&gt;A</td>
<td>p.Arg36His</td>
<td>Major</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>c.125G&gt;A</td>
<td>p.Gly42Glu</td>
<td>Major</td>
<td>8.5% on major; 5.4% on minor</td>
<td>Williams and Rumsby</td>
</tr>
<tr>
<td>Exon 1</td>
<td>c.167T&gt;A</td>
<td>p.Ile56Asn</td>
<td>Major/Minor</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>c.187G&gt;C</td>
<td>p.Gly63Arg</td>
<td>Major</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>c.205C&gt;T</td>
<td>p.Gln69X</td>
<td>Major/Minor</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>c.242C&gt;T</td>
<td>p.Ser81Ile</td>
<td>Major</td>
<td>Absent in 142 controls; conserved in 17/22 orthologous sequences</td>
<td></td>
</tr>
<tr>
<td>Exon 5</td>
<td>c.583A&gt;C</td>
<td>p.Met195Leu</td>
<td>Minor</td>
<td>0%</td>
<td>Salido</td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.614C&gt;T</td>
<td>p.Ser205Leu</td>
<td>Major</td>
<td>&lt;3% on major and minor</td>
<td>Acquaviva and Chevalier</td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.616C&gt;A</td>
<td>p.Ser205X</td>
<td>Major</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.661T&gt;C</td>
<td>p.Ser211Pro</td>
<td>Major</td>
<td>&lt;3% on major and minor</td>
<td>Williams and Rumsby</td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.806T&gt;C</td>
<td>p.Leu269Pro</td>
<td>Major</td>
<td>Unknown</td>
<td>Monoico</td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.823G&gt;C</td>
<td>p.Glu274Asp</td>
<td>Minor</td>
<td>Fully-conserved residue</td>
<td>Williams and Rumsby</td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.823A&gt;C</td>
<td>p.Ser275Arg</td>
<td>Major</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.854A&gt;G</td>
<td>p.Gln282Arg</td>
<td>Major</td>
<td>Absent in 50 controls</td>
<td>Monoico</td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.864G&gt;C</td>
<td>p.Gln282Asp</td>
<td>Major</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>c.851T&gt;C</td>
<td>p.Leu284Pro</td>
<td>Minor</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>c.853G&gt;T</td>
<td>p.Glu285X</td>
<td>Major</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>c.891T&gt;G</td>
<td>p.Tyr297X</td>
<td>Major</td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1079G&gt;A</td>
<td>p.Arg368Gln</td>
<td>Minor</td>
<td>&lt;1% on major and minor</td>
<td>Williams and Rumsby</td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1102G&gt;A</td>
<td>p.Ala368Thr</td>
<td>Minor</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>c.1151T&gt;C</td>
<td>p.Leu384Pro</td>
<td>Major</td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Intron 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.358+2T&gt;G</td>
<td>Splice donor</td>
<td></td>
<td>Amoroso</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.680+1G&gt;C</td>
<td>Splice donor</td>
<td></td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>c.680+1G&gt;A</td>
<td>Splice donor</td>
<td></td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.846+1G&gt;A</td>
<td>Splice donor</td>
<td></td>
<td>Williams and Rumsby</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>c.943–1G&gt;T</td>
<td>Splice acceptor</td>
<td></td>
<td>Monoico; Amoroso</td>
<td></td>
</tr>
<tr>
<td>Exon 10</td>
<td>c.1071+1G&gt;A</td>
<td>Splice donor</td>
<td></td>
<td>Acquaviva and Chevalier</td>
<td></td>
</tr>
</tbody>
</table>

*Reference sequence used is NM_000030.2. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.

\( \text{Minor allele defined as p.Pro11Leu.} \)

\( \text{Denotes authors contributing novel data.} \)

[Williams and Rumsby, 2007], implying that in vitro activity is a poor indicator of effect in vivo. It is possible that AGT-Mi negatively influences protein stability as mutant AGT proteins associated with AGT-Mi tended to aggregate into the insoluble pellet [Lumb and Danpure, 2000] and to have no immunoreactivity [Williams and Rumsby, 2007]. Furthermore, the mutations p.Gly41Arg, p.Phe152Ile and p.Arg233Cys on a major allele background were resistant to proteasomal digestion, but were degraded on a minor allele background [Coulter-Mackie and Lian, 2006, 2008].

Eight mutations have been found in exon 6, which contains the PLP cofactor binding site consensus sequence (amino acids 201–221). A Schiff base forms between Lys209 and PLP, with Ser158 and Asp183 playing a role in orientation of the cofactor in the pocket at the dimer interface [Zhang et al., 2003]. An artificial construct in which Lys209 was mutated to arginine predictably had no catalytic activity [Lumb and Danpure, 2000]. Other residues involved in cofactor binding are Gly 82, His83, Tyr260, and Thr263, the latter two contributed by the other monomer [Zhang et al., 2003]. Mutations have been described at codons 201, 209, 260, and 263, and some of these mutations have been found to affect the stability of the protein [Williams and Rumsby, 2007].
82 and 83, and the former is discussed in more detail later in the text.

The effect of some mutations that do have significant activity and/or immunoreactivity is discussed in more detail below.

**p.Gly41Arg and p.Gly41Val**

The p.Gly41Arg mutation is found mainly, but not exclusively, on AGT-Mi and tends to form core-like aggregates of AGT within the peroxisomal matrix [Danpure et al., 1993]. In vitro expression studies have shown that the activity of both the Gly41Arg and Gly41Val mutant proteins can be restored by denaturation and refolding [Coulter-Mackie et al., 2005b]. The Gly41 residue is situated in the center of the dimerization interface, in contact with its equivalent residue in the other monomer [Zhang et al., 2003]. Interestingly, inhibition of dimerization only occurs with p.Gly41Arg and not p.Gly41Val [Coulter-Mackie and Lian, 2006]. The large side-chain of the arginine moiety is thought to disrupt the dimerization interface, thus inhibiting dimer formation. This hypothesis is supported by the observation that the AGT-41Arg site is specifically cleaved by trypsin, suggesting Arg41 is exposed rather than protected in a dimer interface [Coulter-Mackie and Lian, 2008]. Normal AGT dimer with glycine at codon 41 is resistant to trypsin under the same conditions.

**p.Gly82Glu**

This mutation was first described in a patient with no AGT catalytic activity but positive for immunoreactivity, and the protein was correctly localized in the peroxisomes [Purdue et al., 1992]. The mutation occurs on the background of the AGT-Ma allele. The –NH group on the glycine residue provides one of the H bonds for the phosphate on pyridoxal phosphate (PLP). Based on modeling alone it was hypothesized that the glutamate side-chain might occupy the space required by the PLP [Danpure, 2006]. Subsequent biochemical and spectroscopic studies have, however, shown that the protein is still able to bind PLP and pyridoxamine monophosphate (PMP), the intermediary form in the transamination reaction. The binding affinity is, however, substantially reduced, to <50,000-fold with PMP and ~700-fold for PLP. The end result is a decrease in $K_{cat}$ while maintaining $K_m$ [Cellini et al., 2007].

**p.Gly170Arg**

The most common AGXT mutation, c.508G>A (Gly170Arg), is associated with significant catalytic activity and immunoreactivity in liver biopsies [Danpure and Rumsby, 2004] to the extent that enzyme activity within the reference range has been found in some subjects [Rumsby, 2005]. The mutation occurs in ~24 to 37% of patients [Amoroso et al., 2001; Monico et al., 2007; Rumsby et al., 2004; Tarn et al., 1997], is always associated with AGT-Mi, and in vitro expression studies show that Arg170 expressed on this allele has approximately 50% of the activity of AGT-Mi alone [Coulter-Mackie et al., 2005b; Williams and Rumsby, 2007]. p.Gly170Arg is associated with a mistargeting phenotype in which AGT is found in the mitochondria, where it is unable to carry out glyoxylate detoxification [Purdue et al., 1990]. The mutation appears to inhibit, or slow, AGT dimerization. In its monomeric state, the N-terminal sequence of AGT-Mi can interact with the mitochondrial import receptor, Tom20, and the enzyme is taken up into the mitochondria [Lumb et al., 1999]. For this unusual phenotype to be manifest, it is essential that the Arg170 occurs on the background of the minor polymorphic allele (Leu11).

**Splice-Site Mutations**

Eighteen mutations have been found at the intron–exon boundary, 11 in splice-donor sites and seven in splice-acceptor sites (Table 1; Supp. Table S3). In the majority of cases, interference in splicing is assumed based on deviation from the consensus sequence. However, analysis of liver cDNA was possible in two cases, which showed that the c.776+1G > A mutation led to the inclusion of an additional 24 nucleotides into the mRNA (p.Met259fs), while the c.424–2A > G mutation caused a loss of 12 nucleotides at the beginning of exon 4 (p.Gly142_Gln145del) [Williams and Rumsby, 2007]. An additional point mutation, c.423G>T (p.Glu141Asp), may act through missplicing rather than amino acid substitution as it occurs in the last nucleotide of exon 3. In vitro expression studies of the p.Glu141Asp mutant AGT yielded a protein with 50% normal activity when expressed on the major allele and 100% on the minor allele (Supp. Table S2) with positive immunoreactivity on either background. The mutation was found on the major allele in vivo.

**Deletions and Insertions**

Thirty-one small deletions and insertions and duplications have been described (Table 1; Supp. Table S4). In most cases they arise in a region of the gene where repetition occurs; e.g., the run of eight cytosine residues in exon 1 is particularly prone to deletion/insertion mutations with one of these, c.33dupC (formerly known as c.33_34insC), with reported frequency varying from 11 to 15% of mutant alleles [Amoroso et al., 2001; Monico et al., 2007; Rumsby et al., 2004; Van Woerden et al., 2004]. An 8-bp deletion in exon 4, c.447_454delGCGTGCGT [Williams and Rumsby, 2007], has been documented predominantly, but not exclusively, in patients of Asian origin.

Major deletions have been documented in at least five cases in which all or part of the AGXT gene has been deleted [Coulter-Mackie et al., 2001, 2005a; Nogueira et al., 2000, Monico et al., 2007] (Waterham, this work). These mutations may be more common than suspected as, in the absence of Southern blotting, hemizygosity will not be detected unless gene copy number is assessed by other means; e.g. multiplex ligation probe amplification (MLPA) [Monico et al., 2007], or family studies are carried out. We recently identified a deletion mutation affecting exons 5 to 8 by loss of heterozygosity of linkage markers on a biopsy-proven patient following family studies (Williams and Rumsby, personal communication).

**De Novo Mutations**

To date, only one de novo mutation has been described, an insertion, c.33dupC, occurring in the run of cytosine nucleotides in exon 1 [Williams et al., 2006].

**Uniparental Isodisomy**

The occurrence of the c.33dupC mutation has also been described as a result of maternal isodisomy [Chevalier-Porst et al., 2005]. The patient had partial uniparental isodisomy (UPD) for the telomeric end of chromosome 2q (2q37-ter), ~4.7 Mb. It was deduced that UPD occurred as a postzygotic event, as microsatellite analysis showed that the rest of 2q and 2p were derived from both parents. The possibility of a deletion of the paternal AGXT gene was disproved by assessment of gene copy number.
Evidence of Founder Effects

Most mutations are found within a wide range of ethnic groups; however, two—c.731T>C (p.Ile244Thr) and c.454T>A (p.Phe152Ile)—may have founder effects. The former is found in individuals of Spanish North African origin, occurs with a high frequency in the Canary Islands, and is thought to originate in the island of La Gomera [Lorenzo et al., 2006; Santana et al., 2003]. c.454T>A has been found at a relatively high frequency (19%) in patients from the Netherlands and was homozygous in four patients [Van Woerden et al., 2004]. The prevalence of this mutation has been found to be much lower in other patient cohorts (e.g., 6.8% in the Canadian patient cohort) [Rumsby et al., 2004], suggesting a founder effect in the Dutch population.

Recombinant and Complex Alleles

In several PH1 cases, more than one missense change has occurred on a single allele. In some cases one of the changes is a known mutation with documented pathogenicity so it does not cause problems for diagnosis. For example, p.Val336Asp was found in cis with p.Gly170Arg (p.[Val336Asp;Gly170Arg]) [Van Woerden et al., 2004]. As p.Gly170Arg is a known mutation, the change at codon 336 was assumed to be of lesser or no importance. However, expression studies have now confirmed the pathogenicity of the p.Val336Asp mutation [Coulter-Mackie and Lian, 2008]. Other combinations of sequence changes present a more complex diagnostic conundrum. For example, three missense mutations were identified in a symptomatic patient. These changes were c.[65A>G;557C>T;590G>T], which encode p.[Asn22Ser;Ala186Val;Arg197Leu] and were identified as being on the same allele by family studies. In the absence of expression studies it is difficult to know whether these changes are significant or whether one of the amino acid changes is more influential than another. In another patient with biopsy-proven PH1, both p.[Cys173Trp;His174Asn] were found in a homozygous state (Williams and Rumsby, this work). From the crystal structure of AGT, it is likely that the p.Cys173Trp change is the more important change, as it disrupts an alpha helix. Furthermore, another missense mutation affecting this residue, p.Cys173Tyr, is associated with severely reduced catalytic activity and negative immunoreactivity [Williams and Rumsby, 2007]. Two related patients with the neonatal form of PH1 were both found to be homozygous for p.[Arg289His;Leu298Pro] [Rinat et al., 1999]. Leu298 is a fully-conserved amino acid while Arg289 is not, and the substitution of proline at codon 298 will lead to disruption of an alpha helix. It remains to be seen whether both of these missense changes are in fact pathogenic.

Biological Significance

Crystal Structure

The availability of the crystal structure [Zhang et al., 2001, 2003] has allowed the prediction of the effects of certain mutations on enzyme activity. Each protein subunit of the homodimer is composed of a large N-terminal domain (residues 22–282) and a smaller C-terminal domain (283–292) containing the peroxisomal targeting sequence [Zhang et al., 2003] and other ancillary targeting information required for import [Huber et al., 2005]. The 21 amino acids at the N terminus wrap around the outside of the other monomer, stabilizing the dimeric protein (Fig. 1). There is also evidence that this region facilitates the dimerization process itself, as reflected by the observation that AGT fails to dimerize in vitro when the first 37 amino acids are absent [Lumb et al., 1999]. AGT-Mi with a leucine at position 11 is thought to allow more movement of the N-terminal amino acids, resulting in formation of an alpha-helix, which is able to act as a mitochondrial targeting sequence [Lumb et al., 1999].

Native AGT contains two catalytic sites, each being contributed by one of the monomers. Gly82 is positioned in the center of the catalytic site, from where it is hydrogen bonded to the PLP cofactor. The rare p.Gly82Glu mutation results in intact, stable AGT protein with positive immunoreactivity, but no catalytic activity as it is unable to bind PLP. Another mutation c.1079A (p.Arg360Gln) has now been found in several, unrelated PH1 patients with positive immunoreactivity, but undetectable catalytic activity upon liver biopsy analysis. Recombinant mutant AGT protein containing this mutation also has no catalytic, but positive immunoreactivity (Williams and Rumsby, this work). This enzymatic phenotype is not surprising given that Arg360 was identified in the crystal structure to be the critical residue required for binding the alanine substrate [Zhang et al., 2003].

Animal Model

A mouse model of PH1 has been made by targeted mutagenesis of the homologous gene, Agxt, in embryonic stem cells [Salido et al., 2006]. These mice are hyperoxaluric, the males having a higher oxalate excretion than females. Approximately one-half of the males form oxalate bladder stones although this was dependent on the genetic background; those with a pure C57BL/6 background did not show urolithiasis. Injection of adenovirus vector containing wild-type human AGXT into Agxt−/− mice led to demonstrable enzyme catalytic activity and immunoreactive AGT protein in the liver biopsies after 7 days and oxalate excretion fell after 14 days. By 30 days urinary oxalate increased again, suggesting partial loss of the transgene.

Genotype–Phenotype Correlation

Attempts have been made to establish a genotype–phenotype relationship for PH1 based on age of onset of disease and responsiveness to pyridoxine therapy. The age of onset for homozygotes of the three common mutations, c.33dupC, c.508G>A, and c.731T>C, are shown in Figure 2. While individuals homozygous for the null mutation, c.33dupC, tend to present earlier than c.508G>A, there is considerable overlap and, rather surprisingly, one patient with c.33dupC did not present until over 40 years of age [Rumsby et al., 2004]. Another family with a history of early onset disease in five siblings, all of whom died before 3 years of age, was found to have another sibling diagnosed on genetic testing at age 20 years but who was still asymptomatic [Frischberg et al., 2005]. The late presentation of some patients may actually indicate that the prevalence of this disease is underestimated [Van Woerden et al., 2007].

Genotype–phenotype correlations are hard to demonstrate, and even patients with the same genotype can have entirely different courses of disease. For example, more than 90% of PH1 cases in the Canary Islands are due to p.Ile244Thr, with almost all patients homozygous. Even in this genetically isolated cohort there is no uniform distinct phenotype apparent [Lorenzo et al., 2006].

One area where genotype–phenotype correlation has been found is with respect to pyridoxine responsiveness. The AGT...
enzyme requires pyridoxine as cofactor and one form of treatment is to supplement the diet with pharmacological amounts of pyridoxine [Gibbs and Watts, 1970]. This approach has reduced oxalate excretion in a number of cases and there is data to suggest that those with the p.Gly170Arg mutation are particularly suited for this approach. Homozygotes for this mutation who had normal renal function at the time of diagnosis appeared to be the most likely to respond [Monico et al., 2005b; Van Woerden et al., 2004]. Whether this reflects the fact that this mutation produces a functional protein and the pyridoxine is simply improving folding of mature protein and therefore enhancing peroxisomal uptake is not clear. However, other patients have been documented as pyridoxine responsive with essentially null alleles [Amoroso et al., 2001], suggesting that responsiveness may also be through other, possibly glyoxylate-utilizing, enzymes.

Diagnostic Strategies

In the vast majority of cases mutations completely ablate enzyme activity, introducing frameshifts, nonsense mutations, or splice defects. While there are a lot of “private” mutations, there are also commonly occurring changes that can be used as the basis for clinical diagnosis. The c.33dupC, c.508G > A and c.731T > C mutations were found to account for ~34.5% of mutations in a group of 287 patients with PH1 and test sensitivity was 62.3% [Rumsby et al., 2004].

Whole-gene sequencing is a readily available option for the AGXT gene as it is relatively small. However, the high frequency of some mutations, e.g. 33_34insC, c.508G > A and c.731T > C, make a two-step process more cost effective [Williams and Rumsby, 2007]. The first step, focusing on exons 1, 4, and 7, has been shown to find at least one mutation in 70% of cases of PH1 and to make a diagnosis (i.e. two mutations found) in 50%. These figures are obtained from a patient cohort in whom PH1 was confirmed by enzyme analysis; one would expect somewhat lesser success with a clinically symptomatic population. Audit of referred liver biopsies shows that at least 30% patients will not have PH1 (Williams and Rumsby, unpublished results). We found that while 50% of patients have one or more mutation on screening exons 1, 4, and 7, extending this to whole-gene sequence allows a diagnosis of PH1 to be made in 70%. Do the 30% without PH1 reflect poor clinical evaluation or are there mutations in areas not covered by gene sequencing, e.g., promoter, enhancer, or intronic regions, or simply missed, such as major deletions? A review of previous publications suggests that the latter is unlikely as whole-gene sequencing failed to identify a mutation in only 1% of alleles from biopsy-proven PH1 patients [Amoroso et al., 2001; Milosevic et al., 2002; Monico et al., 2007; Van Woerden et al., 2004]. Thus,
if genetic testing were to become first line, liver biopsy will still be required in a small number to confirm/exclude PH1. As mentioned previously, family studies may indicate a major deletion occurring in one allele, which would not be picked up by analysis of the proband alone. Mutations affecting the promoter have not yet been described, but could potentially lead to a misclassification of non-PH1 if testing relies on DNA sequencing alone.

Confirmation of mutations in parental DNA is essential to confirm that the mutations are on separate alleles. There are several cases in which two or more (nonpolymorphic?) missense changes have occurred on the same allele [Rinat et al., 1999; Van Woerden et al., 2004] (Williams and Rumsby, this work). The significance of these changes is not yet known, although in one case, the changes did not track with disease in the family and therefore PH1 was excluded.

Significance of Novel Sequence Changes

When novel sequence changes are revealed by DNA sequencing, it is not always easy to predict pathogenicity. Absence in 100 normal controls or sequence conservation data is helpful but not entirely foolproof. For example, AGT-Pro11 is highly conserved across all species and yet variants have been described in which this residue is changed to leucine (c.32C>T), histidine (c.32C>A), or arginine (c.32C>G). The leucine and histidine variants both have similar activities in vitro, ~50% that of the wild type. Homozygosity for Leu11 (the “minor allele” discussed earlier) is not associated with any pathological consequences on its own and therefore it is likely that AGT-His11, with similar activity, has no detrimental effect on AGT. In contrast, AGT-Arg11 has no in vitro activity (Williams and Rumsby, this work) and therefore must have pathogenic consequences.

Another variant of unknown significance is p.Ile279Thr, which has been found in patients with known mutations [Coulter-Mackie et al., 2005a; Monico et al., 2007]. This missense change, when associated with AGT-Ma, has normal activity [Coulter-Mackie et al., 2005a]. However, it has also been found on the minor allele of PH1 patients in the absence of other significant changes in the AGXT gene (Acquaviva and Chevalier, Waterham; this work) and so its status as mutation or nonpathological sequence variant, particularly when expressed on AGT-Ma, is still unclear.

Future Prospects

Mutations in the AGXT gene have been found in more than 99% of patients with PH1. This success rate is likely to influence diagnostic procedures, making limited genetic analysis the first line in any testing procedure, followed by liver enzyme analysis or whole-genome sequencing for more complex cases. As yet, no mutations have been described in the promoter region of AGXT and it is possible that this type of mutation may account for the 1% of disease alleles not yet found. An exciting future prospect is the potential of chemical chaperones to rescue some of the missense mutations by counteracting their destabilizing effects [Lumb et al., 2003; Coulter-Mackie and Lian, 2008].

References
