Mutation-Specific Correction of Aberrant AGT Phenotypes in Transgenic Knockout Mice

Primary hyperoxaluria type 1 (PH1), which is the more common of two well-characterized hereditary hyperoxalurias, is caused by mutations in the enzyme alanine:glyoxylate aminotransferase (AGT). If AGT does not work properly, then the body's synthesis and excretion of the metabolic end-product oxalate increases, and insoluble calcium oxalate crystallises out in the kidney and urinary tract. This dammages the kidneys, and they might eventually have to be replaced. Although a minority of PH1 patients can be treated successfully with large doses of vitamin B6, the long-term treatment options for most patients are very limited. Liver transplantation can be used as a form of enzyme replacement therapy in non-vitamin-B6-responsive patients. However, the short and long-term complications of this procedure make it far from ideal. New forms of treatment are urgently required.

In the present proposal, the main goal was to make mice which express normal and mutant human AGT rather than mouse AGT. These "humanised" mice will then be used to determine whether chemical chaperones work *in vivo* as they do *in vitro*. In addition, these mice will be used in an attempt to determine the precise mechanism of action of vitamin B6 in certain patients. These studies should not only shed light on the mechanism of action of chemical chaperones and vitamin B6, but also provide the foundation for far more extensive investigations that would screen for drugs that could be used in the treatment of patients with PH1.

We have succeeded in producing transgenic mice expressing the most common mutations responsible for PH1 (G170R and I244T in the minor haplotype) and these mutations have been introduced in Agt-deficient mice. We have shown that these mice reproduce important features of the human disease, and are a good model to test new therapies. We have also generated transgenic lines expressing the most common human wild type allele (AGT major haplotype). These animals have shown very good levels of AGT expression, and we could demonstrate that the human wild type allele complements the mouse Agxt gene, resulting in an expression of AGT protein with peroxisomal localization. These experiments demonstrate that the signals for subcellular localization of human AGT are conserved in the mouse model.