Bankwest *Landscope* Conservation VisaCard

Gilbert's Potoroo Recovery –

Measuring predisposition to oxalosis in wild potoroos

Final Report

Tony Friend

Science Division, Albany Research
120 Albany Hwy, Albany 6330

June 2005

Introduction

Gilbert's potoroo is Australia's most endangered mammal, surviving only in a population of less than 40 animals at Two Peoples Bay Nature Reserve near Albany. The risk of extinction of this population through wildfire is very high and in order to increase the chance of survival of the species, another population must be established as soon as possible.

Soon after the rediscovery of Gilbert's potoroos in 1994, a captive breeding colony was established as insurance against a catastrophic fire and in the hope of breeding animals for reintroduction to other sites. Since the establishment of the captive colony, however, five animals have died from kidney failure due to the widespread formation of calcium oxalate crystals in the kidney tubules. While this disorder, termed oxalosis or hyperoxaluria, can be due to a high intake of oxalate in food, analysis of the captive diet and the pen vegetation have shown that oxalate levels are low in both and thus have ruled out dietary intake as the primary cause.

Further investigations showed that captive Gilbert's potoroos with elevated urinary concentrations of glycolate and oxalate have a high chance of developing oxalosis, which generally results in death. In humans, both genetic and dietary influences are attributed to the disease. Two types of inherited disorders of oxalate metabolism have been identified, with the more common form (PH1) causing dysfunction of the enzyme alanine glyoxylate aminotransferase (AGT). AGT is involved in intermediary steps in amino acid and oxalate metabolism. All abnormalities of AGT resulting in hyperoxaluria are inherited, with an autosomal recessive pattern of inheritance. Other research has since shown, however, that affected Gilbert's potoroos show normal activity levels of AGT in the liver, so the disorder cannot be explained by this mechanism (Forshaw *et al*., in prep). Still, the familial relationships between the affected animals indicate that there is an inherited origin to the disease.

During previous investigations we screened all captive animals by measuring blood oxalate and glycolate concentrations in urine, but only three wild animals were sampled. In this project, we proposed to screen all wild animals that could be captured, in order to discover the extent of expression of the disease in the entire population of Gilbert's potoroos. By correlation with known relationships within the wild population (mother-young association), it might also possible to test the hypothesis that the disease is inherited. If the genetic basis of the disease is confirmed, knowledge of the predisposition of certain individuals to the disease would be useful in selecting individuals for use in captive breeding and translocation.

Methods

Gilbert's potoroos were trapped at Two Peoples Bay during our regular four-monthly monitoring sessions in March and July 2004, using permanently placed Sheffield cage traps baited with peanut butter, rolled oats and synthetic pistachio nut flavour. The captured animals were carried in black calico bags to the vehicle, parked on a firebreak as close as

possible to the trap site. Each potoroo was anaesthetised using a portable isoflurane anaesthetic machine. Urine was collected from the captured potoroos by applying manual pressure to the bladder. Under anaesthesia, even females with pouch young could be sampled with little risk to the young.

Sampling was carried out during the March and July 2004 trapping sessions, rather than just during the March session as originally intended. Additional samples were added in subsequent trapping sessions.

Determination of urinary levels of glycolate and oxalate was performed without charge in the Biochemistry Department, Princess Margaret Hospital for Children, Subiaco, under the direction of Dr Lawrence Greed.

Results

Urine samples were collected from a total of 13 wild potoroos under this project, with repeat samples from two animals. Oxalate and glycolate levels in wild potoroo urine are shown in Table 1, expressed as values relative to creatinine concentration, in order to standardise levels in urine of varying concentration. Values measured earlier in captive and wild potoroos are also shown, for comparison.

Only one wild potoroo (F66) showed elevated levels of glycolate and oxalate, similar to levels found in captive potoroos that died from hypoxaluria.

Discussion

This study has found a low occurrence of elevated oxalate and glycolate levels in the urine of wild potoroos. All captive animals that have been tested and have subsequently died from this disorder (F10, F19 and F32) have shown high urinary levels of these compounds. The five captive animals that died of hypoxaluria were F10, F17, F19, F32 and M36. F19, F32 and M36, as well as M28, were the young of F10 and M3. M11 was also F10's young, but was conceived in the wild and was not sired by male M3. M28 has low oxalate and glycolate levels and has survived to 9 years of age without developing hypoxaluria. F17 was captured from the wild and is of unknown pedigree (see Figure 1).

This pattern of kidney disease development amongst the progeny of M3 and F10 supports the hypothesis that the disorder has an inherited origin. If the inheritance is autosomal recessive as in PH1, it would be expected that F10 is homozygous and M3 is heterozygous for the allele responsible.

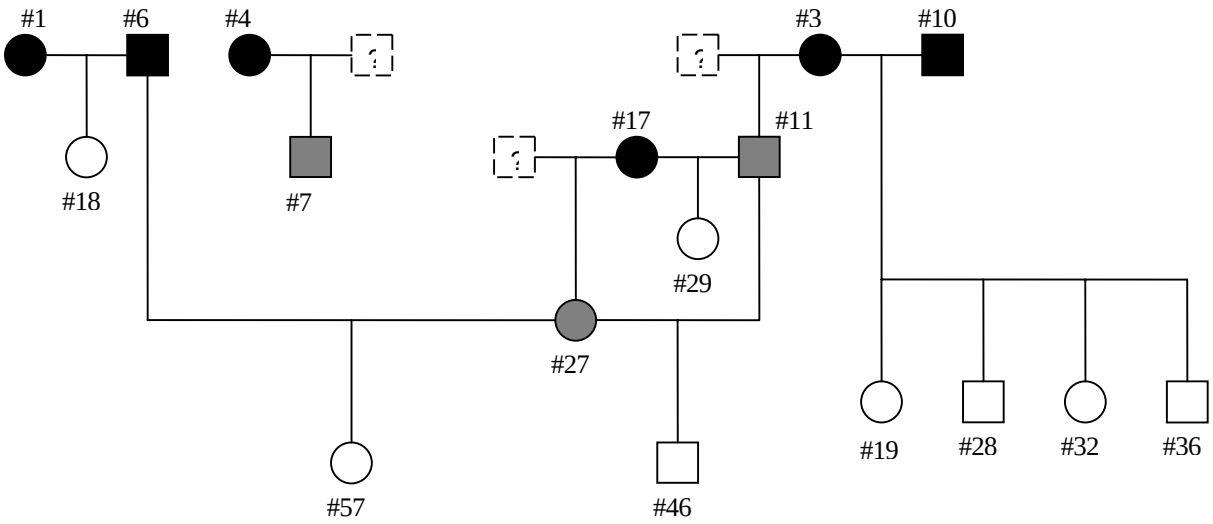

Figure 1: Status and known relationships of individuals in the captive colony of Gilbert's Potoroo. Key: Circles = Female; Squares = Male; Dotted square with ? = unknown father of wild born young; Black = wild caught animal (unknown parentage); Grey = wild born animal (one parent known); White = captive born animal (both parents known); # = unique number allocated to each (captive or wild) animal.

The low occurrence of elevated oxalate and glycolate levels in wild animals (only one instance in a total of 16 wild animals now screened) does not allow any testing of the inheritance hypothesis. This finding is fascinating as the only affected animal, female 66, has not been found with young in all the time since her first capture in 2002, until her removal in 2005 from Mount Gardner into captivity in the breeding facility at Two Peoples Bay. No other female potoroo in the trapping record has shown such a high degree of infertility. As F10 and M3 produced young regularly in captivity, however, there can be no absolute link between high oxalate/glycolate and lack of reproduction.

Management of the Gilbert's potoroo population should take these findings into account, although the low occurrence of affected animals necessitates the continued testing of the wild population. Repeated testing of wild and captive animals will show whether the levels change markedly within an individual's life.

The removal of F66 from the wild due to her lack of breeding is a clearly beneficial strategy as it allows a breeding female to take over her space. Also, however, if the inheritance hypothesis is accurate, she should also be removed because she carries the means to pass on this disease, should she eventually breed. The removal of F66 to captivity was carried out in February 2005.

Costs

Costs		**$**
Vehicle running	30 trips @ $60	1800
Materials (isoflurane, oxygen)	2 bottles isoflurane @ $150 2 cylinders medical oxygen @$ 31	362
Veterinary charges (3 trips)	8 hrs @ $80 420 km driven @ $0.50	850
Total		$3012

Acknowledgments

I would like to thank Dr David Forshaw for valuable collaboration during this project. Dr Kay McIntosh attended three field trips and provided training in isoflurane anaesthesia. Stephanie Hill and Sara Hands gave excellent technical assistance during the field survey and anaesthetic procedures. Dr Lawrence Greed of Princess Margaret Hospital for Children kindly provided the analysis of the urine samples without charge. Field assistance was provided by volunteers belonging to the Gilbert's Potoroo Action Group (Inc.) and Murdoch University Honours students Jeremy Lee and Jill Meinema.

Reference

Forshaw, D., Horwitz, A. M., Ellard, K., Friend, J.A., Greed, L., Metz, M. and Courtenay, J. (in prep.). Renal oxalosis and hyperglycolic aciduria in Gilbert's Potoroos - a possible inherited metabolic disease. *Journal of Wildlife Diseases.*

Potoroo	Creatinine mmol/L	Oxalate µmol/ mmol creatinine	Glycolate µmol/ mmol creatinine
Captive			
F1	*4.5*	*81*	*36*
M3	*6.29*	*100*	*34*
M3	*12.0*	*99*	*24*
M6	*8.0*	*92*	*63*
M7	*4.81*	*129*	*30*
M7	*14.9*	*86*	*21*
M11	*5.1*	*93*	*54*
F19[a]	*2.57*	*394*	*1180*
F19[a]	*2.39*	*117*	*5757*
F19[a] *(necropsy)*	*1.87*	*186*	*14550*
F32[a]	*5.2*	*300*	*2321*
F10[a]	*5.6*	*441*	*6488*
F18	*4.4*	*87*	*33*
F27	*3.6*	*74*	*38*
M28	*2.9*	*46*	*31*
Wild			
M24	*13.6*	*78*	*31*
M37	*27.1*	*173*	*63*
M45	*13.0*	*243*	*71*
M44	8.94	213	144
M47	4.47	109	32
F50	5.1	222	48
F63	4.2	121	48
F66 [b]	5.7	500	1704
M68	5.6	124	52
F69	4.7	134	75
M72	5.4	113	36
F78	4.1	113	63
F78	3.1	127	65
F92	3.2	159	67
M83	6.4	78	103
M83	5.7	117	57
F89	4.1	129	120
M94	4.1	178	119

Table 1. Urinary levels of creatinine, oxalate and glycolate in wild and captive Gilbert's potoroos. Italicized entries refer to results of previous investigations.

[a] Animals that died with renal oxalosis.

[b] Wild animal with markedly elevated urinary oxalate and glycolate.

Normal urine reference interval (n=24):

Oxalate <225 µmol/mmol creat

Glycolate <125 µmol/mmol creat