L-2 hydroxyglutaric aciduria in a South African Staffordshire Bullterrier

Abstract: L-2 hydroxyglutaric aciduria is an autosomal recessive error of metabolism that manifests as an encephalopathy with seizures, tremors, ataxia and / or dementia as the most common presenting complaints. Some affected dogs show only subtle behaviour changes. Amongst canines the condition is best described in Staffordshire Bullterriers and although this is the first reported case in South Africa, we are aware of at least 3 other affected dogs in this country. Affected dogs have normal haematology, serum biochemistry and routine urine analysis. This paper discusses the advantages and limitations of the three main diagnostic modalities – magnetic resonance imaging, urine gas chromatography–mass spectrometry and genetic testing. It aims to increase awareness of the condition, assist diagnosis in encephalopathic dogs and improve detection of carriers in breeding stock.

Introduction: L-2 hydroxyglutaric aciduria (L2-HGAU) was first reported in humans in 1980 (Duran et al., 1980) and to date case reports on well over 100 patients have been published (Kranendijk et al., 2012, Steenweg et al., 2010). In 2003, Abramson and others reported on 6 affected UK Staffordshire Bullterriers (SBT) (Abramson et al., 2003). Since then, L2-HGAU has also been documented in three Yorkshire Terriers (YRT) (Sanchez-Masian et al., 2012, Farias et al., 2012) and one West Highland White Terrier (Garosi et al., 2005). The causative mutations in YRT differ from those in SBT (Penderis et al., 2007, Sanchez-Masian et al., 2012, Farias et al., 2012). Where all tested SBT have had the same mutation in the L2 hydroxyglutarate dehydrogenase gene (*L2-HGDH*) (Penderis et al.,

2007), over 80 different mutations of *L2-HGDH* have been demonstrated in affected people (Steenweg et al., 2010, Kranendijk et al., 2012).

Case history: A five year old 18.3 kg female neutered SBT was examined because of two episodes of bizarre behaviour or dementia. The first episode occurred 9 months prior to presentation, lasted for a day and resolved without treatment: the dog appeared disorientated and aggressive, was pacing and restless, panting and hyperactive. She did not respond to commands where she is normally biddable.

The second episode began 5 days prior to presentation and lasted for a week. Initially, she became aggressive toward the neighbour's dog (with whom she usually plays enthusiastically) and attacked the garbage bins. Signs gradually worsened and two days later she had stopped responding to commands, spent all night pacing, and appeared disorientated with impaired spacial perception and balance. She urinated indoors and showed no awareness of the fact that this had happened. On the third symptomatic day, she was treated with alprazolam (1.75 mg bid)(Adco-Alzem, Adcock Ingram Pharmaceuticals, Bryanston, South Africa) which caused transient sedation after which the above behaviour was resumed. The next day Depomycin® (dose not recorded) (procaine benzylpenicillin with dihydrostreptomycin, Intervet-Schering Plough, Isando, South Africa), alprazolam, dexamethazone (dose not recorded) (Dexa 0.2 Phenix, Virbac, Halfway House, South Africa) and phenobarbitone (30 mg bid) (Lethyl, Aspen Pharmacare, Gallo Manor, South Africa) had been administered. Her owners reported that she had appeared calmer and had slept for the first time in

2 days following these treatments. She was referred the following day, on day 5 of this episode.

On thinking back, her owners had observed mild ataxia for 11 months prior to presentation: she appeared a bit wobbly for a few seconds when she woke up, occasionally banged into walls on cornering and had difficulty climbing steps at times. Signs would wax and wane, and were too subtle to prompt consultation with a veterinarian.

On clinical examination the dog appeared disorientated – staring into space, becoming fixated on and staring at objects (e.g. a lead), pacing and circling, walking into a wall. A complete clinical and neurological examination revealed no further abnormalities.

Haematology, serum biochemistry, electrolytes and resting cortisol had been sent to a commercial laboratory by the referring veterinarian. Results were within normal limits. Urine analysis, resting ammonia and a bile acid stimulation test revealed only hyposthenuria (SG 1.005).

Anaesthesia was induced with propofol at 3.33 mg/kg iv (Propofol 1%, Fresenius, Fresenius Kabi, Halfway House, South Africa) and was maintained with a constant rate infusion of propofol 0.2 mg/kg/min in saline (5 ml/kg/min) (Sabax Sodium Chloride 0.9%, Adcock Ingram, Johannesburg, South Africa). The following MRI sequences were acquired using an 8 channel head array coil in a GE 1.5T Signa Excite HD MRI scanner: transverse T2, transverse T2 FLAIR, sagittal T1, sagittal T2 and coronal T2 FLAIR and axial T1. Further sagittal and transverse T1 sequences were acquired after intravenous administration of 1.8

mmol gadalinium (Dotarem, Gd-DOTA 27.93% m/v, 0.5 mmol/ml, Guerbet, Villepinte, France). A subtle bilateral symmetrical T2 hyperintensity was evident involving the regions of the basal ganglia, central and posterior tectum. No contrast enhancement was evident and no other abnormalities were reported. MRI changes were consistent with a metabolic or toxic encephalopathy.

Random urine samples were collected from the patient (free flow) and from a control dog (cystocentesis) and were submitted to the UCT/NHLS laboratory (Red Cross Children's Hospital, Cape Town) for organic acid analysis by gas chromatography – mass spectrometry (GC-MS) as previously described (van der Watt et al., 2010). The organic acid spectrum from the patient revealed a significant lactic aciduria (lactic acidosis) and a large 2-hydroxyglutaric acid component (Figure 1); this latter organic acid was quantitated, using an internal standard, at 360 mmol/mol creatinine. 2-Hydroxyglutaric acid was not detected in the control canine urine (Figure 1). This organic acid profile was highly suggestive of L2-HGAU as previously reported in SBTs (Abramson et al 2003). Urine or plasma amino acid levels were not quantified.

EDTA blood was submitted to Inqaba Biotec (Hatfield, Pretoria, South Africa) to determine if the causative mutation was present. The DNA was extracted and followed with a polymerase chain reaction (PCR) to amplify the gene of interest. The *L2-HGDH* gene is located on canine chromosome 8. The causative mutation is a known to be in exon 10 in SBT and consists of two single-nucleotide substitutions with one T nucleotide separating them (c[1297TRC; 1299cRt]. This leads to the substitution of two adjacent amino acids: proline is replaced with leucine at position 433 and tyrosine with histidine at position 434 (Penderis et al., 2007). Penderis *et al.* (2007) described a second mutation in exon 10, but as this

mutation does not lead to an amino acid substitution it not tested. An ABI PRISM™3130 Genetic Analyzer was used to determine the nucleotide sequences in the amplicons. The dog was shown to be homozygous for the dicodon mutation in *L2-HGDH* (figure 2).

The patient has been maintained on low doses of phenobarbitone (30 mg in the morning and 45 mg in the evening; serum phenobarbitone 49.8 μmol/l – therapeutic range 43-172). Her owners consider her back to normal and she has not displayed any further severe episodes during the year following diagnosis. Supplementation with riboflavin 100 mg daily started immediately after diagnosis.

Discussion: This is the first report of L2-HGAU in SBT in South Africa. Samples from 200 South African SBT were submitted to Inqaba Biotec for determination of the dogs' *L2-HGDH* status between 2009 and 2012. Most samples were submitted by breeders. The laboratory detected 46 heterozygotes (23%) and 4 (2%) affected dogs (pers. comm., H van der Zwan, 4 December 2012). These dogs originated from different breeding lines and lived in kennels dispersed throughout South Africa. This confirms that the mutation is present in the South African SBT population and dogs showing clinical signs of L2-HGAU are likely to present to vets periodically.

A study of 130 normal UK SBT and 131 normal Finnish SBT detected *L2-HGDH* heterozygotes in 11% of both populations (Short et al., 2010). The same authors searched for the SBT *L2-HGDH* mutations in over 1000 epileptic dogs of other breeds and found no carrier dogs (Short et al., 2010). Thus the mutations

described by Penderis et al are either specific to the SBT breed or occur at a very low frequency in epileptic dogs of other breeds (Penderis et al., 2007). The higher proportion of carriers and affected dogs in the population tested by Inqaba Biotec is expected because theirs was not a random sampling of a population.

L2-HGAU has been proposed as a deficiency of "metabolic repair". L2-hydroxyglutaric acid (L2-HGA) has no known metabolic function. It is produced because mitochondrial L-maleate dehydrogenase is not specific for its primary substrate, oxaloacetate, and reduces α-ketoglutarate as well. Normally, metabolic repair ensues when L2-HGA is converted back to α-ketoglutarate by L2-hydroxyglutaric acid dehydrogenase (L2-HGDH) (Rzem et al., 2004) – see Figure 3. When a mutation in the *L2-HGDH* gene reduces or abolishes the enzyme's function L2-HGA accumulates in the brain, CSF, plasma and urine (Rzem et al., 2004, Steenweg et al., 2010). L2-HGA has been shown to cause oxidative stress. It inhibits mitochondrial creatinine kinase in the cerebellum and may also inhibit glutamate dependent pathways as it is a structural analog of this common neurotransmitter substance (Rzem et al., 2004).

Affected humans usually show clinical signs from an early age, but as signs may be subtle and / or non-specific, diagnosis may be delayed (Weimar et al., 2012, Patay et al., 2012, Abramson et al., 2003). Generally, clinical signs are slowly progressive. Clinical signs shown by both species include ataxia, dementia (manifesting variably in dogs with head pressing, getting trapped in corners, hyperactivity, aggression, lethargy or attention seeking) and seizures (Farias et al., 2012). Dogs show behaviour changes and / or loss of learnt behaviour while behavioural problems are reported in 32% and psychomotor delay in 93% of humans. In dogs, disorientation, hypermetria and tremors are reported while

82% of humans show specifically cerebellar ataxia with intention tremors (Steenweg et al., 2010). 38% of people also show extrapyramidal signs ie involuntary movements, inability to initiate movement, tremor and muscle spasm. Dog owners have reported muscle stiffness and fatigue with exercise while 40% of human patients show hypotonia progressing to spasticity (Abramson et al., 2003). Some dogs show periodic increases in clinical signs that last for minutes to days. This is not typical of humans. In contrast, macrocephaly and a possible increase in risk of brain tumours are features of the condition in man but these signs have not been reported in dogs (Aghili et al., 2009, Kranendijk et al., 2012). Many dogs will show only one or 2 of the above signs (Abramson et al., 2003). In both species the extent of the neurological compromise does not appear to be related directly to the severity of the aciduria (Abramson et al., 2003). In humans, it has not been possible to associate different mutations with different disease manifestations (Steenweg et al., 2010).

Both species show T2 hyperintensity in peripheral subcortical white matter, as well as grey matter of the thalamus, basal ganglia, globus pallidus, caudate and dentate nuclei on MRI (Steenweg et al., 2010, Penderis et al., 2007). Diffuse oedema is sometimes reported, manifesting as a subtle T1 hypointensity without contrast enhancement (Abramson et al., 2003). In addition, humans may show cerebellar atrophy and macrocephaly (Kranendijk et al., 2012). Because the extensive grey matter changes in dogs are usually symmetrical, they may be overlooked by inexperienced viewers and referral to a veterinary diagnostic imaging specialist is advised.

A full post mortem study of an affected SBT found no macroscopic changes. Histopathological changes were restricted to the brain: There were marked

spongiform changes in the astrocytes of the cerebral cortex, thalamus, cerebellum and brainstem. Grey matter was most severely affected (Scurrell et al., 2008). In humans, spongiform changes are most severe in the subcortical white matter (Penderis et al., 2007). Thus histopath changes correspond to those found on MRI. It is not clear why the distribution of the most severe lesions differs between humans and dogs (Abramson et al., 2003).

Routine haematology, serum biochemistry and in house urine analysis are typically normal (Scurrell et al., 2008) and the hyposthenuria in this patient was probably a side effect of phenobarbitone treatment.

Routine GC-MS detects the presence of 2-hydroxyglutaric acid in the urine but does not determine chirality. L2-HGAU must be differentiated from D-2 hydroxyglutaric aciduria, isolated cases of which have been reported in dogs (Abramson et al., 2003). In humans, two types of congenital D-2 hydroxyglutaric aciduria as well as D,L-2 hydroxyglutaric aciduria have been described (Kranendijk et al., 2012). To complicate matters in man, D-2 hydroxyglutaric aciduria has been associated with other errors of metabolism eg a skeletal dysplasia resulting in metaphyseal chondromatosis and multiple Acyl-CoA dehydrogenase deficiency and has been observed as an acquired mutation in patients with glioma and acute myeloid leukaemia (Kranendijk et al., 2012). There is no reason why these errors of metabolism could not occur in dogs, thus in theory all of these need to be considered as a differential diagnosis for a dog with high 2-hydroxyglutaric acid levels in the urine. In humans these conditions may often be distinguished by their clinical signs and MRI changes but confirmation of chirality and genetic testing are necessary for a complete work-up (Kranendijk et al., 2012) .

When performing GC-MS, elevated lysine in the CSF, plasma and urine have been noted in some humans and dogs with L2-HGAU, while some had elevated urine cysteine and / or arginine (Abramson et al., 2003) and / or lactate (Scurrell et al., 2008). As high urine lysine decreases absorption of cysteine and arginine in the proximal convoluted tubules, increased loss of these four amino acids may be interrelated (Abramson et al., 2003).

As many different mutations of *L2-HGDH* can result in a non-functional enzyme and clinical signs of L2-HGAU, dogs with mutations that have not yet been described or included in the test may be missed if one relies solely on the genetic test. Thus genetic screening and GC-MS have complimentary roles to play in the diagnosis.

L2-HGAU is incompletely understood and there is no specific treatment for dogs or humans at present (Penderis et al., 2007). Humans with better characterized organic acidurias are treated with dietary protein restriction and appropriate amino acid, mineral and vitamin supplementation (Penderis et al., 2007, Kolker et al., 2012, van der Watt et al., 2010). The efficacy of such supplementation of precursors is likely to vary depending on the nature of the underlying mutation as well as whether any normal L2-HGDH is present in the particular patient. Supplementation with 100 mg riboflavin once daily improved neurological function and markedly decreased L2-HGA secretion in the urine of a teenaged boy with L2-HGAU (Yilmaz, 2009). Riboflavin (Vitamin B2) is bound to an ADP molecule to form flavin adenine dinucleotide (FAD). FAD is a co-factor for L2-HGDH (Yilmaz, 2009). A diet supplemented with FAD and levocarnitine chloride improved clinical signs in another human (Samuraki et al., 2008). A

lysine restricted diet was offered to one dog with concurrently elevated plasma lysine levels but the patient refused to eat it (Farias et al., 2012). In dogs with seizures, phenobarbitone at 3 mg/kg appears effective at controlling clinical signs (Abramson et al., 2003, Sanchez-Masian et al., 2012).

Conclusion: To our knowledge, this is the first report of L2-HGAU in a South African SBT. The aim here is to raise awareness of this condition in the SBT breed in South Africa for two reasons: The first is so that colleagues can encourage breeders to screen breeding stock prior to mating. At least 46 carrier dogs have already been identified in South Africa and 11% of two overseas convenience samples of clinically normal SBT were carriers. Heterozygotes are asymptomatic but unwitting mating of two carriers is likely to result in clinically affected pups. Some affected dogs have clinical signs that are vague and may even be considered a cute quirk or 'just a Staffie thing' by some owners. Yet even mildly affected individuals should not be bred because all offspring will at least be carriers and subsequent homozygous offspring could be a great deal more compromised. The second reason is so that colleagues consider genetic screening on pet SBT showing consistent neurological signs, which potentially avoids having to do more invasive diagnostic testing. There may be further mutations causing L2-HGAU in dogs, just as there are over 80 described in man. For this reason, urine GM-CS should be determined in dogs showing consistent clinical signs or MRI changes but whose genetic test suggests they are unaffected.

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