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NHLRC1 repeat expansion in two Beagles with Lafora disease

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Abstract

Lafora disease is a fatal genetic disorder with neurotoxic deposits of malformed insoluble glycogen. In humans it is caused by genetic variants in the *EPM2A* or *NHLRC1* genes. There is a known sequence variant in Miniature Wirehaired Dachshunds but not in different dog breeds, including in Beagle dogs in which the disease is relatively commonly reported. This case report describes the causative defect in two affected Beagles, namely the same massive expansion as in Miniature Wirehaired Dachshunds of a 12-nucleotide repeat sequence that is unique to the canine *NHLRC1* gene. This is the first sequence variant described in Beagles with Lafora disease, and so far the only Lafora disease genetic variant in dogs.

Keywords

Inherited metabolic brain disease; Myoclonic epilepsy; Reflex epilepsy; Polyglucosan storage disease; Canine

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INTRODUCTION

Lafora disease (LD) is a fatal autosomal recessive disorder caused by accumulation of Lafora bodies (LBs) in the brain. Human LD begins in adolescence and results from loss-of-function sequence variants in the *EPM2A* or *NHLRC1* (*EPM2B*) genes (Minassian 2002, Schoeman *et al.* 2002). The earliest reports of LD in dogs came in 1970, 1973 and 1976 in the latter two on Beagles. The disease has also been described in Miniature Wirehaired Dachshunds (MWHs), Basset Hounds and Miniature and Standard Poodles (Holland *et al.* 1970, Tomchick 1973, Cusick *et al.* 1976, Jian *et al.* 1990, Webb *et al.* 2009). A causative defect was identified in MWHs and a Basset ten years ago, namely massive expansion of a 12 bp repeat in the *NHLRC1* gene (Lohi *et al.* 2005). To date, no other sequence variants have been reported, despite the fact that Beagles and other breeds with LD continue to be seen regularly in clinical practice, and reported in the literature. The aim of this report is to describe the clinical presentation of LD and its causative defect in two affected Beagles.

CASE HISTORIES

Case 1

An 8-year-old 20.7-kg neutered male Beagle was referred for neurological examination with a two-month history of daily progressive myoclonic episodes. On examination, mild myoclonic movements of the head and intermittently of the whole body were detected. These were observed at rest and whilst ambulating. They could be elicited by gesturing near the patient's head and by audiogenic stimulation. The episodes were characterized by rapid symmetrical muscle contractions mainly of the neck and forelimbs without impairment of consciousness. Myoclonic jerks occurred separately and were of low intensity. Complete blood cell count, serum biochemistry, urinalysis, magnetic resonance imaging (MRI) and cerebrospinal fluid analysis were unremarkable.

Case 2

A 7.5-year-old 16.5-kg spayed female Beagle presented for a second opinion 12 months after onset of myoclonic jerks or startles and generalized seizures. The startles were first noticed at 6.5 years of age, becoming progressively worse. Subtle changes in mentation were noted some 3-4 months later. Generalized seizure activity was first observed 6 months after onset. The startles occurred spontaneously during sleep (hypnic jerks). In wakefulness they could be triggered by visual stimuli or changes in light (the patient moving into or through sunlight or shadows), waving one's hand in front of her face or by loud noises. They were characterized by sudden rapid backwards jerking of the head and neck. Gentle and slow petting would not elicit signs. The jerks varied from very mild facial myoclonic contractions to severe, whereby facial muscles, neck, trunk and limbs were affected, often resulting in the patient being "flung" backwards into a sitting position by extension of forelimbs and collapse of hindlimbs support (video). No apparent loss of consciousness or change in mentation was noted during these episodes. Recovery was rapid (seconds) and complete. Physical and neurological examinations were unremarkable and MRI, routine bloodwork, and serum thyroid, lead and mercury concentrations were normal.

LD was suspected due to previous descriptions in the breed and neurological signs. Genetic testing for the previously described *NHLRC1* repeat expansion was performed by Southern blotting: 10 µg of DNA was digested with DraIII and EcoRI overnight, separated on 1% agarose, nicked in the gel with HCl, denatured with NaOH, neutralized with Tris and transferred onto a Hybond-N membrane. Following pre-hybridization, the membrane was hybridized with a ³²P-labelled DNA fragment specific to *NHLRC1* (GeneBank/EMBL/DDJB # AY560905.1, assembly CanFam3.1, probe coordinates chr35:16,920,972-16,921,534). After several rounds of washes, the membrane was exposed onto X-ray film. Both dogs had the previously reported LD expansion sequence variant in homozygous state (Fig 1).

Follow-up

In case 1, owners were advised to keep the dog quiet and avoid stressful situations, without any specific medical treatment. A proprietary diet high in antioxidants^a was prescribed and owners reported significant improvement in the patient's clinical condition, with only sporadic myoclonus (twice a month). One year after diagnosis, the dog is alive and well-controlled without increased myoclonia.

In case 2, treatment with phenobarbital controlled seizures. Myoclonic startles were unaffected, although serum phenobarbital level was low (13.8 mg/L) at presentation for our opinion. Levetiracetam 250 mg three times daily was added. Initially, this resulted in noticeable decrease in startles, but this was short-lived. The patient was changed to a low-carbohydrate diet.^b Eighteen months after first clinical signs; she continues to experience myoclonic startles and infrequent generalized seizures.

DISCUSSION

LD is a glycogen metabolism disorder manifesting as progressive myoclonic epilepsy (Minassian 2002). In humans, besides LD, inherited progressive epilepsies include neuronal ceroid lipofuscinosis (NCL), sialidosis, Unverricht-Lundborg disease and myoclonic epilepsy with ragged red fibres (Shahwan *et al.* 2005). From these, only NCL and LD have so far been identified in dogs (Awano *et al.* 2006). The associated myoclonic jerks frequently involve head and forelimbs (Gredal *et al.* 2003, Sainsbury 2011). In our cases, photosensitive whole-body myoclonus, sleep myoclonus and generalized seizures were also present.

Unlike the human and e.g. the feline gene, the canine *NHLRC1* gene sequence has the particularity of containing a three-copy repeat of a 12-nucleotide sequence. All canine cases to date (Lohi *et al.* 2005), including the Beagles in the present study, are caused by expansions of this repeat. Prior to identification of the LD genes, detection of LBs in biopsies of peripheral organs (skin, muscle, liver) were used for diagnosis. However, presence and extent of LBs in these locations is highly variable and the rate of false-negativity in our canine and human experience, and in the literature, is high (Gredal *et al.* 2003, Minassian 2002). We did not biopsy the current cases because the diagnosis was

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evident from the combination of clinical signs and presence of the previously identified common *NHLRC1* expansion sequence variants.

EPM2A encodes the only known glycogen phosphatase, laforin (Tagliabracci *et al.* 2008), and *NHLRC1* the malin ubiquitin E3 ligase, which regulates laforin (Tiberia *et al.* 2012). Loss of function of either results in formation and then precipitation, aggregation and accumulation of malstructured and insoluble glycogen (LBs), which lead to the epilepsy. The precise path from dysregulation of glycogen phosphate to abnormal glycogen architecture is still not understood.

Next to MWHs, LD in dogs has most frequently been described in Beagles. We now show that at least in some Beagles the LD mutation is the same as in other breeds. Occurrence of the same mutation in unrelated breeds suggests a recurring event. This is likely explained by the uniquely canine dodecamer repeat sequence in *NHLRC1*. In human genetics, a similar situation occurs not in LD, the genes of which do not contain the canine-specific repeat, but in another of the progressive myoclonic epilepsies, Unverricht-Lundborg disease. In that disease, humans (but not dogs) have a three-copy dodecamer repeat in the *EPM1* gene, and Unverricht-Lundborg disease in almost all patients is due to expansion of this repeat (Lehesjoki & Koskiniemi 1999).

LD therapy is currently limited to management of seizures and myoclonus (Shahwan *et al.* 2005). In dogs, seizures may be controlled with phenobarbital and potassium bromide. Myoclonus may respond temporarily to levetiracetam which was used in our second case, a response that is well-described in humans (Rusbridge *et al.* 2005). Early data suggests that *EPM2A* and *NHLRC1* safeguard neurons against accumulating carbohydrates and work has focused on therapy with low-carbohydrate and ketogenic diets. Feeding exclusively of a proprietary antioxidant-rich diet^a reduced myoclonus in MWHs with LD (Rusbridge *et al.* 2005, Cardinali *et al.* 2006). This same diet^a was also reported to improve aging-related cognitive deficits (Landsberg 2005). In our first case, a high-antioxidant diet^a did improve myoclonus. On the other hand, a low-carbohydrate diet^b, used in our second case did not. The low-carbohydrate diet we and others used is merely low in carbohydrate and not ketogenic. It is unlikely that a properly ketogenic diet can be used as therapy in dogs because of the highly efficient peripheral utilization of ketones in this species (de Bruijne & van den Brom 1986). Thus, antioxidant-rich diets might be more effective for canine LD than low-carbohydrate ones, although these tentative conclusions await further work in larger groups of animals.

While there will all but certainly be other *EPM2A* and *NHLRC1* sequence variants in future cases of canine LD, the results to date and the nature of the dodecamer repeat unique to dogs suggest that dogs have, in relative terms, a degree of predisposition to LD. Given the severity of the disease, specific genetic diagnosis is crucial to prevent potential spread in purebred dogs. In breeds known to have the repeat expansion, genetic testing can be used to identify carriers and affected individuals. In general, genetic counsellors recommend continuing to use carriers in a breeding program but only with dogs clear of the genetic variant so that no clinically affected dogs are produced. This helps preserve desirable, often necessary, genetic

diversity within the breed (Proschowsky *et al.* 2003). Dogs homozygous for a mutant allele should not be bred except under extreme circumstances.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

LD	Lafora disease
LBs	Lafora bodies
MWHD	Miniature wirehaired dachshund
MRI	Magnetic resonance imaging
NCL	Neuronal ceroid lipofuscinosis

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Fig 1.

NHLRC1 Southern blot. Affected Beagle dogs (lanes 1 and 4) have homozygous expanded alleles. The dodecamer expansion in these dogs is approximately the same size as in affected Miniature Wirehaired Dachshunds (MWHs) in which the mutation was originally described. In the latter the repeat expansion was measured to vary between 19 to 26 copies (versus two or three copies in the wild-type canine sequence).