Brief Note

NHLRC1 dodecamer repeat expansion demonstrated by whole genome sequencing in a Chihuahua with Lafora disease

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Running title: ***NHLRC1 repeat expansion in a Chihuahua***

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Background: Lafora disease is an autosomal recessive disorder that causes myoclonic epilepsy[1,2,3]. The disease is characterized by the presence of polyglucosan inclusion bodies (Lafora bodies), predominantly in the central nervous system. More than 90% of human Lafora disease cases are caused by genetics variants in either *EPM2A*, encoding the laforin glucan phosphatase or *NHLRC1* encoding the NHL repeat containing E3 ubiquitin protein ligase 1, which has also been termed EPM2B or malin[1,2,4]. Lafora disease in animals has similar clinical signs as the human disease, including spontaneous and reflex myoclonus, jerks and generalized tonic clonic seizures. Lafora disease has been reported in the dog[2], cat[5], cow[6], and fennec fox[7]. In dogs, Lafora disease is one of the most commonly recognized structural-metabolic epilepsies and is inherited as an autosomal recessive condition. It is most frequent in Miniature Wirehaired Dachshunds, Basset Hounds and Beagles, and has also been reported in the Miniature and Standard Poodle, Pointer and Corgi[2,8,9]. A single disease causing variant has been found in dogs[2]. It consists of a massive expansion of a GC-rich dodecamer repeat sequence in the canine *NHLRC1* gene, leading to loss of function of the gene. The wild type allele of this repeat consists of 2 copies of a 12 bp motif in most mammalian species. In normal dogs and other canids 2-3 copies are present. The pathogenic alleles leading to Lafora disease in dogs were reported to contain 14-26 copies of this repeat[2]. Genetic testing and carrier detection is not routinely available as the extremely GC-rich dodecamer repeat expansion impedes PCR-based diagnostic approaches. Currently, a Southern blot based test is offered by the Hospital for Sick Children, Toronto, and represents an official DNA screening test recommended by the UK Kennel Club[9].

Case description: A 10-year old female spayed Chihuahua was presented at the Small Animal Hospital of the University of Bern. Lafora disease was suspected based on neurological examination, seizures semiology, MRI and CSF findings. An EDTA blood sample was collected for further genetic analysis.

Whole genome sequencing: An Illumina TruSeq PCR-free DNA library was prepared from genomic DNA of the affected dog. A total of 226'174'936 2 x 150 bp reads were obtained on a NovaSeq 6000 and mapped to the CanFam 3.1 reference genome yielding a 25.6x genome coverage as described[10]. The sequence data were deposited in the European Nucleotide Archive (ENA), project accession PRJEB16012, sample accession SAMEA4848714. The WGS data showed the presence of the previously described 12 bp repeat expansion on both *NHLRC1* alleles (Figure S1).

Comments: The previously described *NHLRC1* dodecamer repeat expansion was identified in a Chihuahua with Lafora disease demonstrating that this disease also exists in the Chihuahua breed. WGS based on a PCR-free DNA library is a suitable method to genotype this variant. During the preparation of this manuscript, another case of Lafora disease in a Chihuahua with genetically confirmed *NHLRC1* repeat expansion was presented at a conference[11].

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