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SHORT COMMUNICATION

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DYRK1B haploinsufficiency in a Holstein cattle with epilepsy

Abstract

In this study, epilepsy with focal seizures progressing to generalized seizures was diagnosed in a 6-month-old Holstein heifer. The seizures were characterized by a brief pre-ictal phase with depression and vocalization. During the ictal phase eyelid spasms, tongue contractions, nodding and abundant salivation were observed, rapidly followed by a convulsive phase with bilateral tonic, clonic or tonic–clonic activity and loss of consciousness. Finally, during the postictal phase the heifer was obtunded and disorientated, unable to perceive obstacles and hypermetric, and pressed its head against objects. In the inter-seizure phase, the heifer was clinically normal. Neuropathology revealed axonal degeneration in the brainstem and diffuse astrocytic hypertrophic gliosis. Whole genome sequencing of the affected heifer identified a private heterozygous splice-site variant in *DYRK1B* (NM_001081515.1: c.-101-1G>A), most likely resulting in haploinsufficiency owing to loss-of-function. This represents a report of a *DYRK1B*-associated disease in cattle and adds *DYRK1B* to the candidate genes for epilepsy.

Epilepsy is a complex group of neurological disorders characterized by spontaneous recurrent seizures. According to the Veterinary Epilepsy Task Force, epilepsy can be classified into idiopathic (genetic) epilepsy and structural epilepsy (Berendt et al., 2015). Furthermore, the seizure types can be classified according to their semiology as focal epileptic seizures, generalized seizures or focal epileptic seizures evolving into generalized epileptic seizures. The latter are characterized by a seizure that begins with regional motor, automatic and/or behavioral signs, rapidly followed by a convulsive phase with bilateral tonic, clonic or tonic–clonic movements and loss of consciousness (De Risio et al., 2015).

Epilepsy has previously been described as a challenging multifactorial disorder with a complex genetic background in several domestic animal species (Charalambous et al., 2023), including cattle (OMIA:000344-9913), dogs (OMIA:000344-9615), cats (OMIA:000344-9685), chickens (OMIA:000344-9031), horses (OMIA:000344-9796) and rabbits (OMIA:000344-9986). In dogs, various genetic variants associated with epilepsy have been reported including a *PITRM1*-related recessively inherited form of epilepsy with mitochondrial dysfunction and neurodegeneration (Hytönen et al., 2021), myoclonus epilepsy in *NHLRC1*-related Lafora disease (Denholm et al., 2014), benign familial juvenile *LGI2*-related epilepsy (Seppälä et al., 2011) and *DIRAS1*-related generalized myoclonic epilepsy with photosensitivity (Wielaender et al., 2017). In addition, a recessive variant in *LOC430486* causing epilepsy has been described in chickens (Douaud et al., 2011).

In this report, a Holstein heifer was studied that was referred to the Clinic for Ruminants of the University of Bologna at 6 months of age, weighing 125 kg, with a history of multiple seizures since birth. The parents were reported to be healthy.

On admission, the heifer showed a poor general condition, reduced skeletal development and a dull coat. No significant alterations were noticed on neurological examination. A complete blood count, serum biochemistry, venous blood gas, urinalysis, cerebrospinal fluid analysis and serology for Schmallenberg virus, *Neospora caninum*, blue-tongue virus and *Toxoplasma gondii* were performed. No abnormalities were found in the complete blood count and venous blood gas. The serum biochemistry showed a moderate increase in creatinine kinase (325 U/L; reference interval 88–292). No biochemical abnormalities were found in the cerebrospinal fluid. All serological tests were negative.

During the first 4 days after hospitalisation, the heifer showed no changes in the physical and neurological examinations compared with the initial examination. Five days after admission, an episode of seizure was observed, which started with a brief pre-ictal phase characterized by depression and vocalization (Figure 1a). During the ictal phase, the heifer showed eyelid spasms, tongue contractions, nodding and sialorrhea (Figure 1b), rapidly followed by a convulsive phase with bilateral tonic, clonic or tonic–clonic activity, and loss of consciousness (Figure 1c). The ictus lasted 5 min, followed by a postictal phase of 15 min, which was characterized by obtundation and disorientation, inability to perceive obstacles,

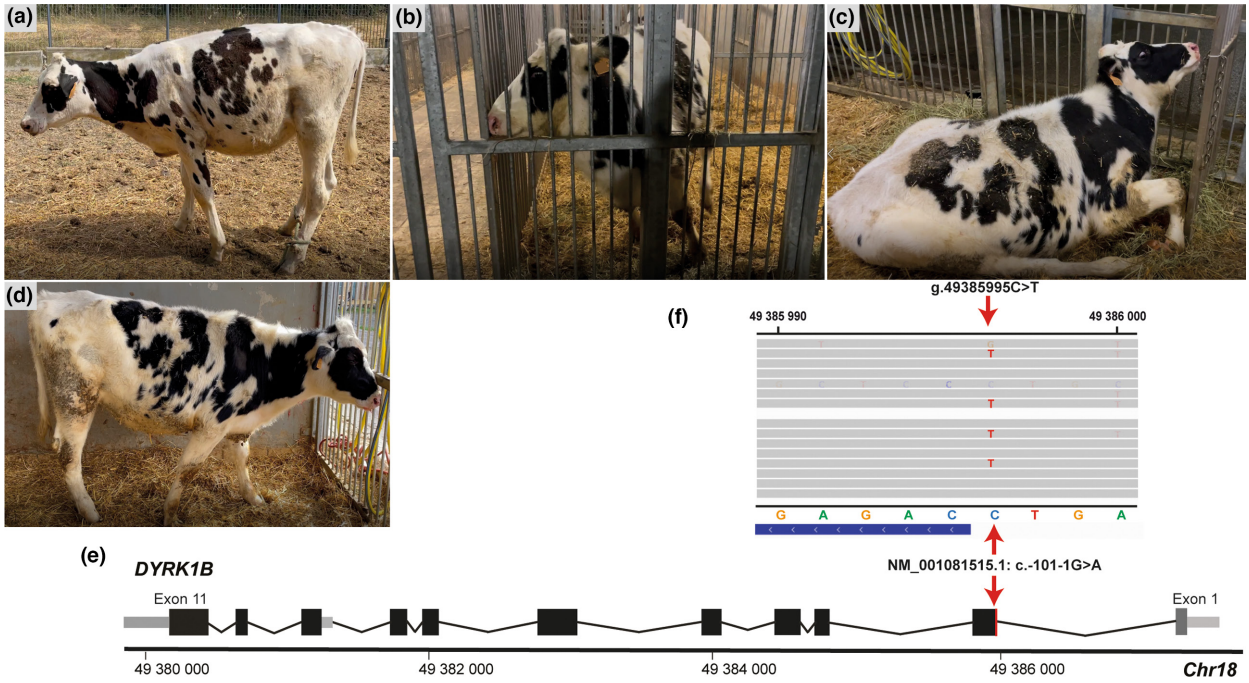

FIGURE 1 *DYRK1B*-associated idiopathic epilepsy with focal seizures progressing to generalized seizures in a Holstein heifer. (a) Pre-ictal phase characterized by depression. (b) Beginning of ictal phase started with eyelid spasm, tongue contraction, nodding and sialorrhea. (c) Ictal phase characterized by a convulsive stage with bilateral tonic, clonic or tonic–clonic activity and loss of consciousness. (d) Postictal phase characterized by obtundation and disorientation, inability to perceive obstacles and head pressing. (e) Structure of *DYRK1B* showing the exon 2 variant located on chromosome 18. (f) IGV screenshot showing the chr18:49385995C>T splice-site variant in the affected heifer.

hypermetria and head pressing (Figure 1d). The heifer then recovered a normal posture and mental status.

The animal was hospitalised for 18 months, during which the seizure frequency was inconstant, ranging from one to four seizures per week and not more than one seizure per day. The epileptic seizures always began as focal seizures that developed into generalized seizures similar to the episode described above (Video S1), with a duration of ictus of 2–10 min and a post-ictal phase ranging from 15 to 20 min. The inter-ictal neurological examination was normal. The heifer developed several traumatic lesions in its mouth and limbs secondary to the seizures. The animal showed a deterioration in its general condition owing to the extension of these traumatic lesions and was euthanized for ethical reasons at 24 months of age and necropsied. Gross pathology revealed no significant abnormalities. Histological examination of eyes, liver, spleen kidney, heart and skeletal muscle did not reveal any lesion. In the brain, single randomly scattered small to large axonal spheroids of homogeneous to granular appearance were observed in the brainstem (Figure S1), but without a clear topographical pattern. Additionally, throughout the brain, many astrocytes showed a pale to vesicular nucleus (hypertrophy; Figure S1). The Bergmann glia in the cerebellar cortex appeared proliferated and thickened. Purkinje cell axons and dendrites were prominent, and the molecular layer showed increased cellular density. High numbers of small-sized Purkinje cells were located in the internal granule cell layer of the cerebellum (Figure S1). Immunohistochemistry for glial fibrillary acidic protein revealed plump astrocytes in the cerebellar white matter. The histological findings were consistent with axonal degeneration and astrocytic gliosis, and the ectopic position of Purkinje cells in the internal granule cell layer. The lesions were mild and not specific for any known degenerative or developmental disease in the heifer.

Given the clinical history and phenotype, the heifer was suspected to have epilepsy with focal seizures progressing to generalized seizures. In addition, the neuropathological phenotype was compatible with a genetic degenerative or metabolic disorder.

Therefore, we hypothesized a genetic etiology for this disease and performed a genetic analysis. DNA was extracted from EDTA-blood of the affected heifer using standard methods. Whole-genome sequencing (WGS) was performed as previously described (Jacinto et al., 2022). Reads were mapped to the ARS-UCD1.2 assembly (Rosen et al., 2020), including 1204 Holstein. The Integrative Genomics Viewer (IGV) (Robinson et al., 2017) version 2.0 software was used for visual inspection of genomic regions containing candidate variants. Assuming a dominant heterozygous variant as the cause of this disease, the WGS data were filtered for heterozygous coding variants that were present in the heifer and were absent in all remaining cattle, identifying three variants with a predicted high or moderate impact effect on three different

TABLE 1 Results of whole-genome sequencing variant filtering of the Holstein heifer affected by idiopathic epilepsy.

Filtering step	Homozygous variants	Heterozygous variants
All variants in the affected heifer	4 328 702	2 960 897
Private variants in the affected heifer using 943 cattle genome controls	656	592
Protein-changing in the affected heifer using 943 cattle genome controls	4	5
Remaining protein-changing private variants using a global control cohort of 4540 cattle genomes and subsequent IGV inspection	0	3

genes (Table 1), all of which were confirmed all as true variants by IGV inspection (Table S1). In addition to a missense variant in *ARMH4* and an in-frame deletion in *SCARF1*, neither of which is predicted to be deleterious, a single variant affects an interesting functional candidate gene for the disease under study (Figure 1e). This heterozygous variant at chr18:49385995C>T represents a splice-site variant at the beginning of exon 2 of the *DYRK1B* (NM_001081515.1: c.-101-1G>A; Figure 1e,f). It is predicted to disrupt proper splicing, and therefore most likely represents a loss-of-function (LOF) variant.

We speculate that the identified variant in *DYRK1B* arose from a *de novo* mutation event in the germline of the parents or during early embryonic development of the affected heifer, as both parents were reported to be healthy. However, to prove that the identified variant in *DYRK1B* indeed occurred *de novo*, genotyping of the parents would be needed but unfortunately no biological material was available.

Assuming a recessive homozygous variant as the cause of this disease, the WGS data were filtered for homozygous coding variants present in the heifer and absent in all controls, and no variants were identified.

The affected gene, *DYRK1B*, encodes the dual specificity tyrosine-phosphorylation-regulated kinase 1B protein, which has both serine/threonine and tyrosine kinase activities and plays a crucial role in rDNA double-strand break repair and rDNA copy number maintenance (Dong et al., 2021; Leder et al., 1999; Mercer & Friedman, 2006). DNA damage and repair is a continuous process necessary for the maintenance of genomic integrity. Double-strand breaks are the most toxic type of DNA damage and require appropriate repair by specialized players. Double-strand break repair is particularly important for post-mitotic, non-dividing, long-lived cells of the central nervous system (Scully et al., 2019; Thadathil et al., 2019). A decrease in these mechanisms leads to a disruption of the neuronal networks through the loss of neuronal populations. Therefore, they disrupt vital motoric functions (Marnef et al., 2017). In humans, neuronal double-strand break may contribute to the etiopathogenesis of neurological disorders such as amyotrophic lateral sclerosis, primary lateral sclerosis, spinal muscular atrophy, spinocerebellar ataxia, dementia-associated neurodegenerative diseases and cerebral hemorrhage (Provasek et al., 2022).

Both *DYRK1A* and *DYRK1B* appear to play important roles in neurogenesis (Abbassi et al., 2015; Kokkorakis & Gaitanou, 2020), but much more is known about *DYRK1A*. In humans, LOF variants in *DYRK1A* have been associated with autosomal dominant mental retardation 7 (OMIM #614104) characterized by epilepsy, autism and intellectual disabilities accompanied by microcephaly (Courcet et al., 2012; O'Roak et al., 2012). In mice, germline *de novo* variants in *Dyrk1b* have been associated with neurological abnormalities including reduced startle reflex, impaired gait, increased prepulse inhibition and abnormal auditory brainstem response (MGI:1330302). In humans, *DYRK1B* has been associated with abdominal obesity-metabolic syndrome 3 (OMIM #615812) when caused by dominant missense variants (Keramati et al., 2014; Mendoza-Caamal et al., 2021). More recently, a familiar case of abnormal cognition and metabolic syndrome was associated with a dominant splicing variant predicted to lead to LOF (Orenstein et al., 2022). The authors suggested that it is possible that LOF variants in *DYRK1B* contribute to the abnormal neurologic phenotype (Orenstein et al., 2022). In addition, recent large-scale data from human genome sequencing studies presented in the Genome Aggregation Database (gnomAD) (Karczewski et al., 2020) showed that the probability of a LOF intolerance score for *DYRK1B* was 0.97, meaning that this gene falls into the class of haploinsufficient LOF genes. We therefore speculate that the identified variant in *DYRK1B* in the diseased heifer leads to haploinsufficiency. Given the rarity of the variant, the prediction of haploinsufficiency and the known function of *DYRK1B*, the identified splice-site variant may be the cause of the observed disease, although it has not previously been associated with epilepsy in mammals, including humans.

In conclusion, our report describes the clinical, pathological and genetic findings in a Holstein heifer with idiopathic epilepsy with focal seizures progressing to generalized seizures. We propose here the first candidate causal *DYRK1B* variant associated with epilepsy. Our study highlights once again that the genetics of spontaneously occurring disorders in cattle is a valuable translational model system (Jacinto et al., 2021). Further

functional studies into DYRK1B's role in epilepsies are needed.

KEYWORDS

cattle, DNA double-strand, idiopathic epilepsy, precision medicine, seizure

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The WGS data are available under the study accession no. PRJEB18113 at the European Nucleotide Archive (www.ebi.ac.uk/ena; SAMEA111531462).

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