A *TNR* Frameshift Variant in Weimaraner Dogs with an Exercise-Induced Paroxysmal Movement Disorder

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ABSTRACT: Background: Some paroxysmal movement disorders remain without an identified genetic cause.
Objectives: The aim was to identify the causal genetic variant for a paroxysmal dystonia–ataxia syndrome in Weimaraner dogs.
Methods: Clinical and diagnostic investigations were performed. Whole genome sequencing of one affected dog was used to identify private homozygous variants against 921 control genomes.
Results: Four Weimaraners were presented for episodes of abnormal gait. Results of examinations and diagnostic investigations were unremarkable. Whole genome sequencing revealed a private frameshift variant in the *TNR* (tenascin-R) gene in an affected dog, XM_038542431.1:c.831dupC, which is predicted to truncate more than 75% of the open read frame. Genotypes in a cohort of 4 affected and 70 unaffected Weimaraners showed perfect association with the disease phenotype.
Conclusions: We report the association of a *TNR* variant with a paroxysmal dystonia–ataxia syndrome in Weimaraners. It might be relevant to include sequencing of this gene in diagnosing humans with unexplained paroxysmal movement disorders. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: canine; neurogenetics; extracellular brain matrix; dystonia; episodic ataxia; precision medicine

Paroxysmal movement disorders are a rare group of diseases characterized by episodic involuntary movements that can include dystonia, dyskinesia, chorea, and ataxia.[1-4] They are divided into paroxysmal dyskinesias (characterized by transient episodes of hyperkinetic movements) and episodic ataxias (characterized by attacks of cerebellar ataxia) depending on the main movement.[1-4] Their cause can be primary (genetic) or secondary (acquired), and advances in next-generation sequencing have allowed the identification of genetic variants responsible for these disorders.[2,3] The large number of genes involved in the pathogenesis of paroxysmal movement disorders reflects a high complexity of molecular causes involved, including synaptic vesicle fusion, postsynaptic intracellular signaling, brain energy metabolism, neurotransmitter synthesis, ion channels, and solute carriers.[1,2] Despite advances in understanding the genetics of these disorders, there is still a number that remains without an identified cause, suggesting that other unidentified genes and disease mechanisms exist.[2-4]

The identification and clinical characterization of spontaneously occurring hereditary paroxysmal movement disorders in different dog breeds provide an opportunity to identify new genes and disease mechanisms involved in these rare diseases.[5-7] The unique population structure of purebred dogs, in which each breed arises from a limited number of founders, and canine reproduction with relatively large litter sizes provide advantages for genetic studies in dogs compared to humans.[8] In recent years, variants in four genes have

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Relevant conflicts of interest/financial disclosures: H.L. has served as a consultant for Kinship co., a company providing canine gene tests.

Funding agency: None.

Received: 7 December 2022; **Revised:** 30 January 2023; **Accepted:** 10 March 2023

Published online 6 April 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29391

been identified in dogs with paroxysmal movement disorders: juvenile paroxysmal dyskinesia in Markiesje dogs caused by a frameshift variant in *SOD1* (OMIA 002322-9615),[9] a paroxysmal dyskinesia in soft-coated Wheaten terriers with a missense variant in *PIGN* (OMIA 002084-9615),[10,11] and a paroxysmal hypertonicity syndrome in cavalier King Charles spaniels caused by a microdeletion in *BCAN*, encoding the brain-specific extracellular matrix protein brevican (OMIA 001592-9615).[12,13] Furthermore, a *PCK2* missense variant was identified in Shetland sheepdogs with paroxysmal exercise-induced dyskinesia (OMIA 001543-9615). However, the causality of this *PCK2* variant was not proven, and the paroxysmal movement disorder phenotype in these dogs might have been due to other reasons.[14]

Here, we describe a group of Weimaraner dogs with an autosomal recessive paroxysmal dystonia–ataxia syndrome associated with a novel homozygous variant in the tenascin-R (*TNR*) gene. TNR is a member of the tenascin family of extracellular matrix glycoproteins, which are expressed in the nervous system.

Materials and Methods

Animals

Four Weimaraner dogs with a paroxysmal dystonia–ataxia syndrome were included in this study. They were from three litters of different and reportedly healthy parents, with 2 cases being littermates. Residual EDTA (ethylenediaminetetraacetic acid) blood samples were retained from all cases for genetic investigation. Samples from dams, sires, or other littermates could not be obtained. Ethical approval was granted by the ethics committee of the School of Veterinary Medicine of the University of Glasgow.

Clinical Investigations

All cases were examined and investigated by veterinary neurologists. Investigations included blood samples for hematology and biochemistry, fructosamine, lactate, pyruvate, acetylcholine receptor antibodies, enzymatic testing for storage diseases, magnetic resonance imaging (MRI) of the brain and spinal cord, cerebrospinal fluid analysis, electomyography, motor nerve conduction velocities, urine organic acids, muscle and nerve biopsies, and serologies for *Toxoplasma* and *Neospora*.

Sequencing and Genotyping

EDTA blood samples from all 4 cases were collected, and genomic DNA was isolated. The genome of 1 case was sequenced at 21.5× coverage on an Illumina Novaseq 6000 instrument (Illumina, Zurich, Switzerland). Mapping and variant calling were performed with respect to the UU_Cfam_GSD_1.0 reference genome assembly as previously described.[15] The gathered sequence data were compared to 921 genomes of control dogs of different breeds and filtered for homozygous private protein-changing variants (Tables S1 and S2). Identified variants were genotyped in all 4 cases via Sanger sequencing. The candidate variant was additionally genotyped in 70 control Weimaraner dogs.

Results

Clinical Description and Investigations

Four Weimaraner dogs (3 males and 1 female) from three different litters were presented for episodes of abnormal gait characterized by increased muscle contractions (dystonia), ataxia, and hypermetria, leading to occasional collapse. Kyphosis and low head carriage were also consistent features (Video S1; Table 1). Parents and some of the littermates were reported to be clinically normal in all cases. The age of onset was 3 to 7 months. Increased emotional arousal or exercise was reported to trigger the abnormal episodes, which could occur multiple times daily for 5 to 15 minutes. Two dogs displayed intermittent anisocoria associated with the episodes.

Resting physical and neurological examinations were unremarkable in all cases, although the reported abnormalities were elicited by short periods of exercise in 3 dogs. Results of diagnostic investigations, including hematology, biochemistry, urine organic acids, lactate and pyruvate levels, enzymatic testing for storage diseases, acetylcholine receptor antibodies, muscle and nerve biopsies, MRI (brain and spinal cord), cerebrospinal fluid analysis, and electrophysiology, were mainly unremarkable. Treatment with fluoxetine (1 mg/kg once a day) in 2 dogs resulted in a dramatic reduction in episode severity and frequency as soon as it started. Interestingly, when fluoxetine was stopped after a few months in one of the cases, the episodes reoccurred, and frequency increased.

Genetic Analysis

Comparing the sequence data of the affected dog to 921 control dogs revealed 1030 homozygous private variants. Only four of those variants were called with moderate or high impact using SnpEff software[16] and thus predicted to be protein changing. Genotyping of the affected dogs showed that only one of those variants was homozygous in all 4 cases (Table S3). The remaining variant was a single-nucleotide duplication in *TNR*, chr7:23,940,980dupC (UUCfam_GSD_1.0) (Fig. 1), which is a known candidate gene for "neurodevelopmental disorder, nonprogressive, with spasticity and transient opisthotonos" in humans (OMIM 619653). The canine variant XM_038542431.1:

TABLE 1 *Signalment, investigations, episode characteristics, and response to treatment*

Case	Signalment	Investigations	Episode characteristics	Response to treatment
Case 1	Female neutered Age at onset: 7 mo Consanguinity: unknown	Hematology, biochemistry, EMG, MNCV, CSF, urine organic acids, muscle and nerve biopsies, lactate and pyruvate, *Tox*., *Neo*., AChR	Duration: 5–10 minutes Frequency: multiple times a day Trigger: exercise and EA Site of onset: pelvic limbs Body distribution: generalized Ataxia: yes Dystonia: yes (pelvic limbs) Hypermetria: yes Collapse: occasionally Other signs: kyphosis, neck down	Diazepam: mild response Phenytoin: mild response Baclofen: moderate response
Case 2	Male entire Age at onset: 3 mo Consanguinity: unknown	Hematology, biochemistry, fructosamine, EMG, MNCV, CSF, MRI (brain and spinal cord)	Duration: 5–10 minutes Frequency: multiple times a day Trigger: exercise and EA Site of onset: pelvic limbs Body distribution: generalized Ataxia: yes Dystonia: yes (pelvic limbs) Hypermetria: yes Collapse: occasionally Other signs: kyphosis, neck down	Diazepam: mild response Baclofen: moderate response
Case 3	Male entire Age at onset: 6 mo Brother of case 4 Consanguinity: yes	Hematology, biochemistry, EMG, MNCV, CSF, enzymatic testing for storage diseases, *Neo*., MRI (brain)	Duration: 10–15 minutes Frequency: multiple times a day Trigger: exercise and EA Site of onset: pelvic limbs Body distribution: generalized Ataxia: yes Dystonia: yes Hypermetria: yes Collapse: occasionally Other signs: kyphosis, neck down, and anisocoria that persist for up to 12 hours after the episodes	Fluoxetine: excellent response
Case 4	Male entire Age at onset: 6 mo Brother of case 3 Consanguinity: yes	Hematology, biochemistry, EMG, MNCV, urine organic acids, CSF, *Tox*., *Neo*., MRI (brain and spinal cord)	Duration: 5–15 minutes Frequency: multiple times a day Trigger: exercise and EA Site of onset: pelvic limbs Body distribution: generalized Ataxia: yes Dystonia: yes Hypermetria: yes Collapse: no Other signs: kyphosis, neck down, and anisocoria that persist for up to 12 hours after the episodes	Fluoxetine: excellent response

Abbreviations: EMG: electromyography; MNCV: motor nerve conduction velocity; CSF: cerebrospinal fluid; *Tox*.: *Toxoplasma gondii* serology; *Neo*.: *Neospora caninum* serology; AChR: acetylcholine receptor antibodies; EA: emotional arousal; MRI: magnetic resonance imaging.

c.831dupC is predicted to result in a frameshift and truncation of about 77% of the wild-type open reading frame of the encoded TNR protein, XP_038398359.1: p.(Asn278Glnfs*38). Genotyping of 70 control Weimaraner dogs showed the expected correlation for an autosomal recessive mode of inheritance (Table 2).

Discussion

We describe 4 young Weimaraner dogs with a paroxysmal dystonia–ataxia syndrome. We identified a frameshift variant in the *TNR* gene (XM_038542431.1: c.831dupC) and demonstrated that the genotypes at this

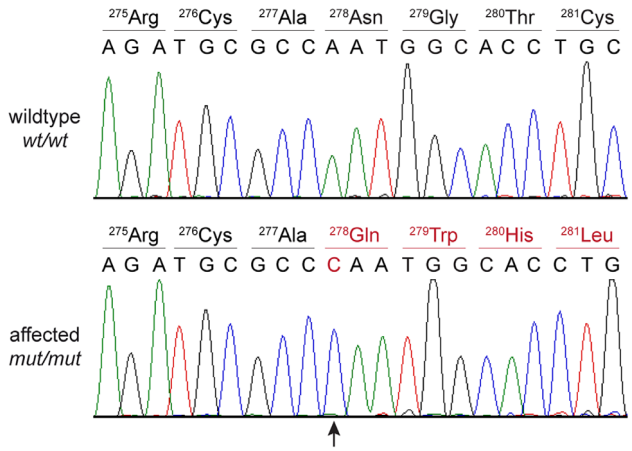

FIG. 1. Details of the *TNR*:c.831dupC variant. Sanger sequencing electropherograms of a wild-type control and an affected dog are shown. The duplicated C in the affected dog is indicated with an arrow. The shifted reading frame with the altered amino acid codons is shown in red. [Color figure can be viewed at wileyonlinelibrary.com]

variant were consistent with the disease phenotype assuming a recessive inheritance pattern.

TNR is a member of the tenascin family of extracellular matrix glycoproteins.[17] It is involved in neurite outgrowth and neural cell adhesion, proliferation and migration, axonal guidance, myelination, and synaptic plasticity.[17,18] It is exclusively expressed in the nervous system, mainly by oligodendrocytes, but also by some neurons in the central nervous system and by Schwann cells in the peripheral nervous system. Its expression increases perinatally, and it represents a major component of perineuronal nets in adults, which are a specialized kind of matrix that ensheathes subtypes of neurons that regulate synaptic plasticity.[17,18] The role of TNR in human pathology is just starting to be elucidated. Variants in *TNR* have recently been reported to cause a nonprogressive neurodevelopmental disorder with spasticity and transient opisthotonos (OMIM 619653).[18-20] Although all human patients shared some common traits affecting motor function, the severity of the phenotype varied.[18-20] Brain MRI of patients showed variable degrees of delayed myelination and abnormalities in the structure of the corpus callosum, something that was not observed in the dogs from this study.[18] In addition, *TNR* has been described as a candidate risk gene for familial Parkinson's disease.[21,22] Our findings in dogs suggest that it might be relevant to include sequencing of this gene in the diagnosis of humans with unexplained paroxysmal movement disorders.

TABLE 2 *Association of the genotypes at the* TNR*:c.831dupC variant with paroxysmal movement disorder in 74 Weimaraner dogs*

Phenotype	*wt/wt*	*wt/mut*	*mut/mut*
Paroxysmal movement disorder (n = 4)	–	–	4
Control dogs (n = 70)	68	2	–

The extracellular matrix plays an important role in neuronal glial interactions, and so far, three genetic causes of inherited dystonia have interactions or contribute to extracellular matrix homeostasis.[23] Interestingly, this is the second gene encoding a brain extracellular matrix protein, causing a paroxysmal movement disorder in dogs, suggesting a role of extracellular matrix in central nervous system motor function. Previously, a paroxysmal hypertonicity syndrome in cavalier King Charles spaniels was found to be associated with a variant in *BCAN*, encoding brevican (OMIA 001592-9615).[12,13] The clinical phenotype had similarities with the Weimaraner dogs from our study, and episodes were also triggered by increased emotional arousal. Something unusual in 2 Weimaraner dogs from this study was the presence of intermittent anisocoria associated with the movement disorder episodes. To our knowledge, this has not been reported before in other paroxysmal movement disorders. The episodes observed in the dogs from this study had some similarities with episodic ataxias in humans, as there was hypermetria and truncal ataxia, but they lacked other inter-ictal signs, such as myokymia or nystagmus. Another important feature of the episodes observed in these dogs was dystonia, with increased muscle tone and kyphosis. Therefore, we decided to classify these episodes in the group of dystonia–ataxia syndromes, which encompass many human genetic disorders.[24] It has been suggested that molecular pathways of ataxia and dystonia are closely related, and the cerebellum seems to play an important role in the control of both.[25]

TNR is an important constituent of the perineuronal nets, and in knockout mice, their distribution, composition, and function are altered.[26,27] *Tnr*$^{-/-}$ knockout mice display altered levels of excitatory and inhibitory synapses, with an enhancement of excitatory synaptic transmission in some parts of the brain, such as the hippocampus.[28,29] The clinical signs observed in the dogs from this study probably emerge secondary to alterations in the synaptic balance between inhibitory and excitatory neurons at the perineuronal nets. Interestingly, the dogs in the present study responded to fluoxetine treatment. Fluoxetine is a selective serotonin reuptake inhibitor that has been previously used successfully in another movement disorder of dogs affecting Scottish terriers in which altered serotonergic function is suspected.[30-32] Previous studies have shown that fluoxetine promotes structural changes in inhibitory neurons in the cerebral cortex of adult mice, probably through alteration of the extracellular matrix surrounding them, which could also explain the good and sustained response observed in these dogs,

although if this was the main mechanism of action we would have expected to see a slower onset of effect.[33,34]

In humans, no reports of the use of fluoxetine for treating paroxysmal movement disorders could be found, but movement disorders have been reported as side effects of its use.[35,36]

To our knowledge, the affected dogs represent the first domestic animals described with a *TNR*-related disease. Our results enable genetic testing, which can be used to avoid the unintentional breeding of further affected dogs. In addition, the studied dogs might serve as a spontaneous large animal model to further understand the role of *TNR*, the extracellular matrix, and perineuronal nets in movement disorders. ■

Acknowledgments: We thank Sini Karjalainen for technical assistance. We thank the Next Generation Sequencing Platform of the University of Bern for performing the high-throughput sequencing experiments and the Interfaculty Bioinformatics Unit of the University of Bern for providing high-performance computing infrastructure. We acknowledge the Dog10K genomes project and all researchers who deposited dog whole genome sequencing data into public databases. We thank the dog owners who donated samples and participated in the study.

Data Availability Statement

Data is available

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Cross-Ethnic Variant Screening and Burden Analysis of *PTPA* in Parkinson's Disease

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ABSTRACT: Background: Recently, homozygous variants in *PTPA* were identified as the disease cause for two pedigrees with early-onset parkinsonism and intellectual disability. Although the initial link between *PTPA* and parkinsonism has been established, further replication was still necessary.

Objectives: To evaluate the genetic role of *PTPA* in Parkinson's disease (PD).

Methods: We analyzed rare variants of *PTPA* in cohorts of Asian and European ancestries ($N_{case} = 2743$, $N_{control} = 8177$) with whole-exome sequencing, and further explored the functional effect of the target variant.

Results: One patient with early-onset PD from a consanguineous family carried the homozygous variant p.Met329Val, while her parents and elder sister with heterozygous p.Met329Val were healthy. This patient developed minor cognitive decline within 1 year, with a Montreal Cognitive Assessment (MoCA) score dropping from 28 to 25. Functional exploration with overexpression studies suggested that this variant was associated with decreased protein phosphatase 2A (PTPA) protein level by affecting protein stability, but not mRNA expression.

Conclusions: These results have broadened the mutation spectrum of *PTPA*, and paved the way for further research into the role of *PTPA* in PD. © 2023 International Parkinson and Movement Disorder Society.

Key Words: Parkinson's disease; *PTPA*; rare variant; protein level

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by heterogeneous motor and non-motor manifestations.[1] The disease etiology is complex, and mounting evidence has demonstrated the important role of genetic factors in PD.[2,3] Along with the wide application of next-generation sequencing and burgeoning research into the genetic background of PD, over 90 common risk variants have been identified.[4] However, a number of pedigrees still have no identifiable genetic causes, suggesting that more risk genes require exploration. Rare variants, which might make a major contribution to the missing heritability, could help improve understanding of the disease pathogenesis.[2]

Recently, two homozygous variants (p.Met298Arg, p.Ala171Asp) in *PTPA* (*protein phosphatase 2A*) were identified as the disease cause in two pedigrees of African descent.[5] The patients had early-onset parkinsonism and intellectual disability. The study further demonstrated that *PTPA* ortholog knock-down in *Drosophila* neurons induced a significant impairment of locomotion in the climbing test. Although the initial links between *PTPA* and parkinsonism have been established, further replication from additional cohorts is still necessary, especially in populations of different ancestries.

In this context, we analyzed rare variants of *PTPA* in PD cohorts of Asian and European ancestries, respectively. We identified a novel homozygous rare variant p.Met329Val in a patient with early-onset PD. Further

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Financial disclosure and conflict of interest: Nothing to report.

Funding: This research was supported by funding from the National Key Research and Development Program of China (Grant No. 2021YFC2501200), the Sichuan Science and Technology Program (Grant No. 2022ZDZX0023 and 2021YJ0415), and the National Natural Science Foundation of China (Grant No. 81901294 and 81871000).

Received: 16 October 2022; **Revised:** 25 February 2023; **Accepted:** 28 March 2023

Published online 12 April 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29411