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Authors

Jankelunas, Leanne
Murthy, Vishal D
Chen, Annie V
et al.

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CASE REPORT

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Novel COL6A3 frameshift variant in American Staffordshire Terrier dogs with Ullrich-like congenital muscular dystrophy

Leanne Jankelunas[1] | Vishal D. Murthy[1] | Annie V. Chen[1] | Katie M. Minor[2] | Steven G. Friedenberg[2] | Jonah N. Cullen[2] | Ling T. Guo[3] | James R. Mickelson[4] | G. Diane Shelton[3]

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, Washington, USA

[2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

[3]Department of Pathology, School of Medicine, University of California San Diego, La Jolla, California, USA

[4]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

Correspondence
Vishal D. Murthy, Department of Veterinary Clinical Sciences, Bustad Hall, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA.
Email: vishal.murthy@wsu.edu

Abstract

Two (male and female) 10-month-old American Staffordshire Terrier littermates presented for progressive weakness, joint contracture, and distal limb joint hyperlaxity beginning around 6 months of age. Neurological examination, serum creatine kinase activity, infectious disease titers, cerebrospinal fluid analysis, and electrodiagnostic testing were performed. Muscle biopsies were collected for histopathology and immunofluorescence staining for localization of dystrophy associated proteins. Whole-genome sequencing (WGS) was performed on 1 affected dog. Variants were compared to a database of 671 unaffected dogs of multiple breeds. Histopathology confirmed a dystrophic phenotype and immunofluorescence staining of muscle cryosections revealed an absence of staining for collagen-6. WGS identified a homozygous 1 bp deletion in the *COL6A3* gene, unique to the first affected dog. Sanger sequencing confirmed the homozygous presence of the frameshift variant in both affected dogs. This report describes the clinical features and most likely genetic basis of an Ullrich-like recessively inherited form of congenital muscular dystrophy in American Staffordshire Terriers.

KEYWORDS
collagen, joint laxity, myopathy, sequencing

1 | INTRODUCTION

Ullrich congenital muscular dystrophy (CMD) is a human disease characterized by muscle weakness, proximal joint contractures, and hyperlaxity of the distal joints.[1] In humans, this disease shows a mostly autosomal recessive and rarely autosomal dominant inheritance pattern and is associated with variants in the *COL6A1*, *COL6A2*, or *COL6A3* genes with severity of clinical signs being associated with the specific genetic variant.[1] At this time, there are limited treatment options for this disease.[1]

In veterinary medicine, a limited number of CMDs are documented. However, to date, CMDs due to autosomal recessive mutations are documented in *COL6A1* in Landseer Newfoundland dogs (OMIA:001967-9615), in *LAMA2* in Staffordshire bull terriers (OMIA:002459-9615), and in *COL6A3* and *LARGE1* in Labrador retrievers (OMIA:002274-9615; OMIA:002460-9615).[2-5] Dogs with variants in *COL6A* present with clinical signs similar to those in people, including severe weakness, prolonged sleep, laxity of distal joints, angular contractures of proximal joints, and decreased range of

Abbreviations: bp, base pair; CMDs, congenital muscular dystrophies; SNP, single nucleotide polymorphism.

FIGURE 1 Abnormal posture displayed by 2 juvenile Staffordshire terrier dogs with Ullrich-like congenital muscular dystrophy. Note the kyphosis, partial contracture of the carpi, and hyperextension of the tarsi, metatarsophalangeal, and metacarpophalangeal joints.

motion of multiple joints.[3] The collagen-6 protein consists of 3 major α-chains (a1, a2, and a3), encoded by collagen-6 genes, that assemble intracellularly to form triple helical monomers.[3] These combine into dimers and tetramers which, extracellularly, go on to create the higher order extracellular fibrous collagen-6 protein structure that augments the structural stability of connective tissues and muscles.[3] Defects at any point in the formation of mature collagen-6 protein severely impairs sarcolemmal integrity resulting in myofiber degeneration and the ability of tissues to regenerate, resulting in the slowly progressive clinical signs in these patients.[3]

To date, there are no documented muscular dystrophies in American Staffordshire Terriers. In this case report, we describe a unique collagen-6 deficiency in 2 related American Staffordshire Terrier dogs of different sexes, along with the associated clinical, pathological, diagnostic, and genetic features identified with this unique condition. Knowledge of this disease and testing for the variant can help inform breeding practices. Additionally, because of the similarities to human Ullrich CMD, this disease in dogs is considered a type of "Ullrich-like" CMD and might prove valuable as a translational model for the development of treatments for the human disease equivalent.

2 | CASE REPORT

2.1 | History and clinical signs

Two 10-month-old American Staffordshire Terrier littermates, a female weighing 20 kg and a male weighing 22 kg, presented to the Washington State University Veterinary Teaching Hospital Neurology/Neurosurgery Service with a history of progressive gait abnormalities and joint contracture that began at approximately 6 months of age in the female and 7 months of age in the male. The owners reported that before the onset of these signs, no abnormalities had been detected in either dog; none of the remaining 5 littermates, dam or sire displayed clinical signs. Both dogs were on appropriate diets, up to date on vaccinations and deworming, and other animals in the home were healthy.

On presentation, physical examination on both dogs revealed diffuse muscle atrophy and multifocal joint contracture with reduced range of motion and marked thickening of the elbow and stifle joints, along with distal limb joint hyperlaxity (Figure 1). On neurological examination, both dogs revealed severe generalized weakness, such that they had difficulty rising and ambulating. Both dogs displayed ambulatory tetraparesis with a stiff, choppy, short-strided gait in all limbs without obvious ataxia. Both dogs required short periods of rest after advancing a few steps. Mentation and cranial nerves were normal. Additionally, weak withdrawal reflexes were seen in all limbs. The remainder of the spinal reflexes and postural reaction testing were normal. Mild spinal hyperesthesia was appreciated on direct spinal palpation of the male but not the female. Deficits were localized to the peripheral nervous system or primary musculoskeletal disease.

Both dogs had a mild leukocytosis characterized by neutrophilia, mild elevation in serum alanine amino transferase activity, mild hyponatremia, and mildly low serum creatinine concentrations. Creatine kinase (CK) activity was abnormal in both dogs (Female 2882 U/L, Male 10,776 U/L; ref: 0-314 U/L). Titers for *Neospora caninum*, *Toxoplasma gondii*, *Ehrlichia canis*, *Borrelia burgdorferi*, *Rickettsia rickettsii*, and *Babesia canis* were negative in both dogs. Radiographs of the right carpus, elbow, stifle, and tarsus were performed on the female and revealed polyarthropathy characterized by joint effusion. Radiographs of the lumbar and sacral vertebral column did not detect any abnormalities in the male.

Approximately 2 weeks after initial presentation, the male presented for reassessment and additional diagnostics. At this time, low head carriage and moderate lumbar and thoracic kyphosis was appreciated. The remainder of signs were relatively unchanged; however, no spinal hyperesthesia was detected on direct palpation. CBC at that time had normalized and serum CK activity was markedly improved (571 U/L; ref 0-314 U/L).

Cerebrospinal fluid analysis and cytology did not reveal any abnormalities. Synovial fluid cytology of the right tarsus revealed a moderate number of nucleated cells consisting of 98% mononuclear cells and 2% segmented nondegenerate neutrophils, consistent with a mild degenerative arthropathy. Abnormalities were not detected on

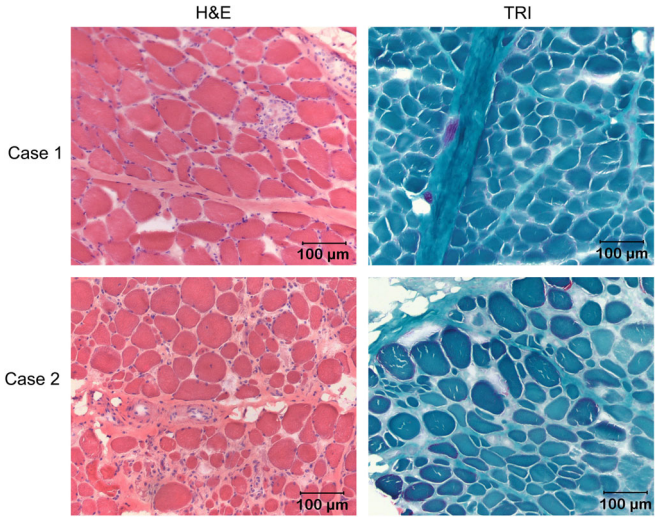

FIGURE 2 Cryosections from the vastus lateralis muscles of Cases 1 and 2 showed similar pathological changes including excessive variability in myofiber size with scattered atrophic fibers having a round shape, fibers containing internal nuclei, sporadic necrotic fibers undergoing phagocytosis, and mild endomysial fibrosis. H&E and trichrome stains. Bar in lower right corner of all images = 100 µm.

cytology of the remaining joints. Electromyography revealed mild generalized fibrillation potentials and positive sharp waves within some of the evaluated musculature (gastrocnemius, cranial tibial, semitendinosus, vastus lateralis, and biceps femoris) of the right pelvic limb. Abnormalities were focally present in these muscle bellies and seen primarily at superficial levels but not deeper levels. Abnormalities were not detected on testing motor nerve conduction velocity and compound muscle action potential amplitudes of the peroneal nerve. Appropriate waveforms were elicited with normal velocity (60 m/s). While abnormal, these findings did not support a specific myopathy or neuropathy.

Biopsies of the triceps, vastus lateralis, and cranial tibial muscles, and the common peroneal nerve were submitted under refrigeration to the Neuromuscular Disease Laboratory, Veterinary Medical Teaching Hospital, University of California, Davis. Samples were frozen in isopentane pre-cooled in liquid nitrogen and stored at −80°C until further processed by a standard panel of histochemical stains and reactions. After preliminary review, frozen muscle blocks were sent to the Comparative Neuromuscular Laboratory, University of California San Diego. Histopathology revealed an excessive variability in myofiber size in all muscles, with fiber diameters ranging from 13 µm to 101 µm (Figure 2). Scattered atrophic fibers had a round shape and were of both fiber types. Sporadic necrotic fibers, histochemical type 2C regenerating fibers, and mild endomysial fibrosis were also identified. No specific abnormalities were identified in the frozen sections of the common peroneal nerve. Based on these changes, a congenital myopathy with a dystrophic phenotype was suspected.

Indirect immunofluorescence staining of quadriceps muscle cryosections (8 µm) from the affected male and an archived control normal dog was performed as described.[6] Sections were incubated with monoclonal or polyclonal antibodies against collagen-6 (gift of Eva Engvall, 3G7, direct apply),[7] laminin α2 (gift of Eva Engvall, 1B4, direct apply)[8] and the rod domain of dystrophin (1:20; NCL-DYS1; Case 1; Figure 3). Staining for collagen-6 localization was not detectable in cryosections from the affected dog but normal in control archived muscle, while staining for laminin α2 and the rod domain of dystrophin were similar in the affected dog and control. Stainings for other sarcolemmal and extracellular matrix proteins, including those for the carboxy terminus of dystrophin, caveolin3, dysferlin, emerin, α, β, and γ sarcoglycans, β dystroglycan, utrophin, and spectrin were similar to control (not shown).

Histopathology of the cranial tibial and vastus lateralis muscles of the affected female revealed changes similar to the male, including active muscle degeneration, scattered Type 2C regenerating fibers, and mild endomysial fibrosis. Similar to the male, indirect immunofluorescence staining revealed undetectable collagen-6 (Case 2; Figure 3). These findings in both affected dogs were consistent with collagen-6 deficient, Ullrich-like CMD. Since a form of CMD associated with deficiency of collagen-6 was confirmed, whole-genome sequencing (WGS) was performed to identify a likely causal variant.

2.2 | Whole-genome sequencing, homozygosity mapping, and Sanger sequencing

Genomic DNA was extracted from archived frozen muscle of both affected dogs (Qiagen DNeasy Blood and Tissue kit) according to the manufacturer's protocol. Genomic DNA from the male dog was submitted to GeneWiz (South Plainfield, New Jersey) for WGS. A polymerase chain reaction-free library was prepared and sequenced in 1 lane of an Illumina HiSeq 4000 sequencer. Reads were mapped against the dog reference genome assembly (CanFam4) as described[9,10] and are available in NCBI's Short Read Archive at https://www.ncbi.nlm.nih.gov/sra/PRJNA950365. Variants from the proband were compared to a private WGS database of 671 other dogs, including 1 other American Staffordshire Terrier, revealing 271,345 variants unique to the proband. Among these variants was a homozygous 1 base pair (bp) deletion frameshift variant in candidate gene *COL6A3* (chr25:48287602CG>C, NP_001096685.1:p.Pro2133ArgfsTer109; Figure 4). This variant alters and truncates ~33% of the protein, including Von Willebrand factor type A, a Fibronectin type-III, and BPTI/Kunitz Inhibitor domains.

Analysis of chromosome 25 single nucleotide polymorphism genotypes derived from the WGS of the sequenced case revealed a large region of homozygosity surrounding the *COL6A3* g.48287602CG>C variant that extends from positions 41,878,709 to 51,674,494.

Sanger sequencing utilizing primers 5′-ACACTCAGCTCGTGCCC TAT-3′ and 5′-TCAGGATCTGTGGGTTTTCC-3′ resulted in a 494 bp amplicon and confirmed homozygosity for the 1 bp deletion in both affected littermates.

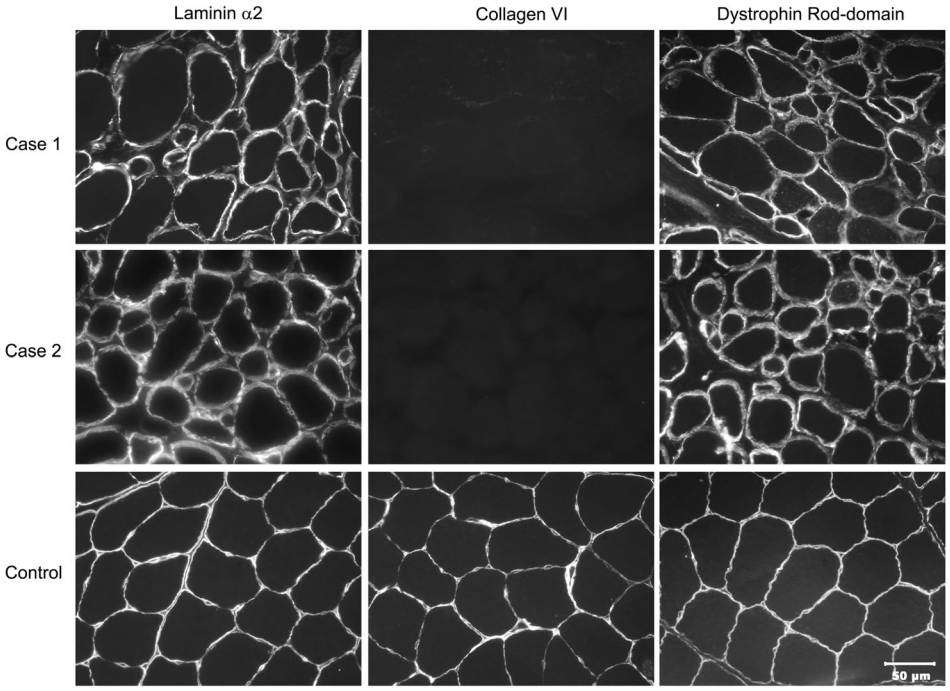

FIGURE 3 Immunofluorescence staining of the vastus lateralis muscle from Cases 1 and 2, and an archived control muscle, stained with antibodies against laminin α2, collagen-6 and the rod domain of dystrophin. Staining for collagen-6 was absent in Cases 1 and 2 and showed a normal staining pattern in the archived control muscle. Staining for laminin α2 and the rod domain of dystrophin was similar to control in both cases. Bar in lower right image = 50 µm for all images.

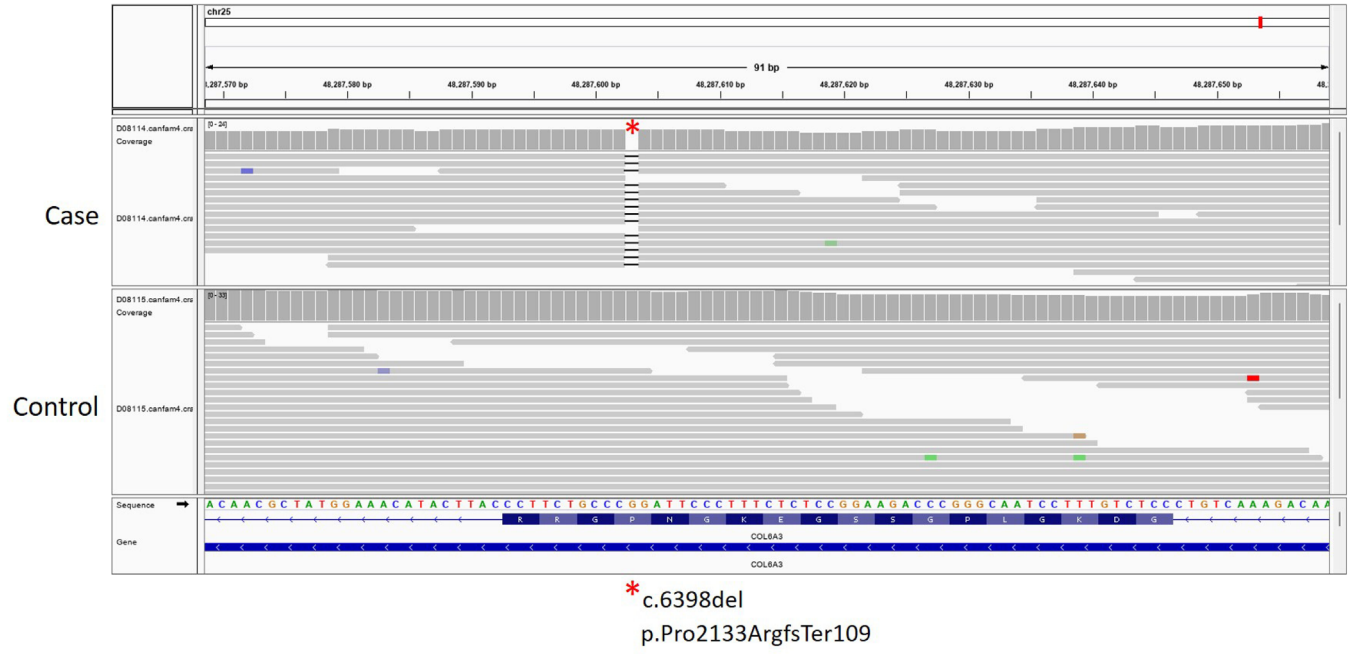

FIGURE 4 *COL6A3* 1 bp deletion (chr25:48287602CG>C, c.6398del p.Pro2133ArgfsTer109) detected with whole-genome sequencing. A 91 bp region flanking exon 21 is shown in the case (upper) and a control (lower) dog aligned to CanFam4. The (*) in the case dog indicates the location of the deleted nucleotide.

2.3 | Treatment

After diagnostic testing, the dogs were maintained on nonsteroidal anti-inflammatory medications and custom orthotic braces were applied to the thoracic limbs. However, the dogs failed to adapt to the orthotics, which were discontinued, and the dogs were allowed to dictate their own activity level. Concurrently, formal rehabilitation therapy under the supervision of a certified canine rehabilitation practitioner was initiated. This primarily involved weekly underwater treadmill sessions (3-8 minutes with single break; speed gradually increasing from 0.1 to 0.6 miles per hour) and dry needling as well as infrequent cold laser therapy, passive range of motion and massage on an as-needed basis. These were well tolerated by both dogs. This combination of rehabilitation, nonsteroidal anti-inflammatory

medications and time resulted in substantial improvement in ability to rise and ambulate without assistance. At the time of writing, both affected dogs are alive (at approximately 20 months of age) and have maintained the ability to rise and ambulate unassisted, with an owner-reported good quality of life.

3 | DISCUSSION

This report details a 1 bp deletion frameshift variant in the *COL6A3* gene associated with CMD in American Staffordshire Terrier dogs and identifies a third likely disease-causing variant of this gene in dogs. The most common form of muscular dystrophy in dogs is X-linked and associated with variants in the *DMD* gene resulting in deficiency of the sarcolemmal membrane protein dystrophin (OMIA:001081-9615).[11] X-linked muscular dystrophy affects many breeds of dogs and is generally associated with a poor prognosis. Autosomal recessive or dominant forms of limb-girdle muscular dystrophy associated with variants in the sarcoglycan (*SCG*) genes are reported in Boston terrier (OMIA:002122-9615)[12] and miniature dachshund dogs (OMIA:002305-9615).[13] Most recently, variants in the collagen-6 (*COL6A*) genes are reported in Labrador retrievers (OMIA:002274-9615),[4] Landseer Newfoundland dogs (OMIA:001967-9615),[2] and in this report, an American Staffordshire Terrier.

The clinical presentation of collagen-6 deficiency, including Ullrich CMD, is similar in dogs and people, with the development of signs beginning as early as 6 months of age and slowly progressing.[4] The 2 affected American Staffordshire Terrier dogs in this study displayed joint flexion, kyphosis, and deformities from a young age similar to that of collagen-6 deficiency cases in humans, Labrador retrievers, and Landseer Newfoundland dogs.[1,2,4,14] Distal joint laxity and angular contractures of proximal joints seen in the dogs in this report are similar to those reported in Labrador retrievers with *COL6A3* mutations.[4] These changes are not reported in the Landseer Newfoundland dogs with *COL6A1* mutations, and are not reported in other congenital myopathies, suggesting these findings could be hallmark features of *COL6A3* Ullrich-like muscular dystrophy.[2,14,15] Further distinguishing the *COL6A1* variant from the *COL6A3* variant, Landseer Newfoundland dogs display severe weakness and prolonged sleeping- signs that are not reported for the dogs in the present report or the Labradors in the previous study.[2,4] The Landseer Newfoundland dogs were euthanized by 5-15 months of age, suggesting a more severe progression associated with that variant.[2,14] In contrast, at the time of writing, at approximately 20 months of age, both dogs in the present report are alive. This is more consistent with the Labrador retrievers, that are reported to be alive at 31-90 months of age.[4] In humans, there is a spectrum of disease, from early severe Ullrich CMD to the milder Bethlem myopathy.[1] The age of onset, distal joint laxity and joint contractures seen in the present report most closely matches the classical presentation of moderate progressive Ullrich CMD reported in humans.[1]

Both dogs were treated with underwater treadmill therapy and nonsteroidal anti-inflammatory medications and have maintained the ability to rise and ambulate without assistance. No signs of cardiac or respiratory disease, commonly associated with other progressive muscular dystrophies including X-linked dystrophin deficiency, were identified in either dog. This highlights the short-term prognosis and ability for reasonable quality of life with appropriate supportive care. The long-term prognosis remains guarded, as the disease is progressive and there are no specific treatments available for affected humans or dogs. While physical rehabilitation therapy is a part of supportive treatment in humans, in general, there are no clear recommendations or guidelines for rehabilitation therapy for muscular dystrophy in dogs. Most dystrophic dogs are euthanized as their mobility declines, and they are unable to support weight.[3]

Collagen-6 deficiency in humans and other breeds of dogs is typically an autosomal recessive condition. While the 2 dogs in this report were obtained from a breeder, further genetic and pedigree information could not be obtained for analysis. However, given that both affected dogs were homozygous for this *COL6A3* variant, it can be presumed that both parents were heterozygous. The affected dog was homozygous for an approximately 10 Mb region of chromosome 25, containing the *COL6A3* gene. This large, conserved region provides evidence for a very recent mutation, inbred cases, or both. As the prevalence of this variant within the greater population of American Staffordshire Terriers remains unknown, routine screening of breeding animals could help recognize the scale of this disease and help eliminate it from the breed.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Leanne Jankelunas https://orcid.org/0000-0001-9556-0921
Vishal D. Murthy https://orcid.org/0000-0002-2879-9593
Steven G. Friedenberg https://orcid.org/0000-0002-7510-2322
G. Diane Shelton https://orcid.org/0000-0002-3332-1359

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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