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14. ABSTRACT
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**RPPR Final Report**  
as of 17-Aug-2018

Agency Code:

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**Agreement Number: W911NF-13-1-0036**

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**Report Date:** 10-Sep-2015

Date Received: 14-Aug-2018

**Final Report** for Period Beginning 11-Jan-2013 and Ending 10-Jun-2015

**Title:** Genetic Risk Factors For Lumbosacral Stenosis in Labrador Retriever Working Dogs (*Canis familiaris*)

**Begin Performance Period:** 11-Jan-2013

**End Performance Period:** 10-Jun-2015

**Report Term:** 0-Other

Submitted By: Cindy Jarvis

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**Distribution Statement:** 1-Approved for public release; distribution is unlimited.

**STEM Degrees:**

**STEM Participants:**

**Major Goals:** Lumbosacral stenosis (LS) is a structural narrowing of the spinal canal in the lower back region. This disease is an important cause of debilitation and early retirement in military working dogs. Early diagnosis and treatment are essential for maximizing active duty service for the dogs and mission readiness for the teams that depend on them. Labrador retrievers are commonly used as military working dogs and are also predisposed to LS. Current standard phenotyping methods for canine LS include clinical examination, radiography, computed tomography (CT), and magnetic resonance imaging (MRI). However, clinical signs of LS are often delayed in military working dogs because they are bred and trained for stoicism and high working drive. By the time clinical signs are detected, the disease is often too far advanced for effective treatment. Standard diagnostic imaging methods are based on qualitative assessments and therefore limit the use of robust statistical analyses. For research purposes, a noninvasive method for quantitative phenotyping of LS would therefore be beneficial. Computed tomography (CT) is an established method for qualitatively determining presence of LS, however at the time of this study, published evidence for a consensus on any such quantitative CT phenotypic traits was lacking in humans or dogs. The causes of LS in dogs are incompletely understood, however previous researchers have proposed that genetics may play a role. The predominant genetic approach for research in canine LS has been pedigree analysis (especially in the German shepherds); but no genetic association studies have been reported in any breed. The long-term goals of our research are to determine whether there may be genetic risk factors for LS in Labrador retriever military working dogs, and, if so, to develop screening tests that could be used to assist decision-making at the time of procurement.

**Accomplishments:** We developed a novel method for quantifying the phenotype of lumbosacral stenosis in Labrador retrievers using computed tomography (CT). We also identified the TTR (transthyretin) gene as a possible candidate gene using exome sequencing of affected and unaffected Labrador retriever military working dogs.

## RPPR Final Report as of 17-Aug-2018

The first study for the funded project was a retrospective, methods comparison design.<sup>1</sup> We used archived CT data from 3 veterinary hospitals and a sample of 25 Labrador retrievers to develop a novel quantitative CT phenotyping trait for LS. This trait was termed "fat area ratio or FAR" and was calculated as the ratio of the vertebral canal epidural fat area in a transverse CT slice relative to the vertebral body area in the same transverse slice. This measurement was found to have good agreement with the standard qualitative assessment of LS made by a board-certified veterinary radiologist. We also reported FAR values measured for each of the L5-S3 vertebral levels in dogs classified as LS positive or LS negative by the radiologist. We also described qualitative and quantitative characteristics of "funnel-shaped" lumbar vertebral canals in some of the Labrador retrievers.

The second study for the funded project was a prospective, exploratory design.<sup>2</sup> We analyzed the exome of 8 young Labrador retrievers (4 positive for LS and 4 negative for LS), selected from a pool of 40 Labrador retrievers at the Military Working Dog Training Center at Lackland Joint Base, San Antonio. All procedures were approved by and conducted in accordance with

requirements of the Institutional Animal Care and Use Committees at West Virginia University and the DOD Military Working Dog Veterinary Service. Phenotyping was performed for all 40 dogs using quantitative (FAR measurements) and qualitative (veterinary radiologist assessment) methods. Results of these assessments were used to select the 8 dogs best representing the extremes of the phenotype- LS positive and LS negative. Genetic analyses were performed using blood samples that had been collected at the time of CT scanning and stored on FTA cards. The DNA was extracted from blood samples and exomes were sequenced to identify and annotate exonic variants. Findings indicated that the TTR (transthyretin) gene could be a possible candidate gene for LS in Labrador retrievers (based on previous studies that have reported an association between human lumbar spinal stenosis and transthyretin protein amyloidosis). Six other annotated (SCN1B, RGS9BP, ASXL3, LRR16B, PTPRO, ZBBX) and 3 predicted genes (EEF1A1, DNAJ1A, ZFX) genes were identified with exonic variants in this study. These could also be candidate genes for LS in dogs, but they had no known published association with human lumbar spinal stenosis. Future studies are needed to further evaluate these candidate genes in a larger sample of affected and unaffected dogs.

**Training Opportunities:** Nothing to Report

**Results Dissemination:** One dissertation and two peer-reviewed publications.

**Honors and Awards:** Nothing to Report

**Protocol Activity Status:**

**Technology Transfer:** Nothing to Report

### **PARTICIPANTS:**

**Participant Type:** PD/PI

**Participant:** Jianbo Yao

**Person Months Worked:** 1.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

**Funding Support:**

**Participant Type:** Co PD/PI

**Participant:** Jeryl Jones

**Person Months Worked:** 1.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

**Funding Support:**

**RPPR Final Report**  
as of 17-Aug-2018

Other Collaborators:

**Participant Type:** Other Professional

**Participant:** Meenakshi Mukherjee

**Person Months Worked:** 3.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

**Funding Support:**

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12. DISTRIBUTION/AVAILABILITY STATEMENT Contents of the report can be made publicly available.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The project has been completed and results have been published in 1 dissertation and 2 peer-reviewed publications. We developed a novel method for quantifying the phenotype of lumbar stenosis in Labrador retrievers using computed tomography (CT). We also identified the TTR (transthyretin) gene as a possible candidate gene using exome sequencing of affected and unaffected Labrador retriever military working dogs (see Final report document).					
15. SUBJECT TERMS military working dog, Labrador retriever, lumbar stenosis, candidate genes					
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**Protocol Title** Genetic risk factors for lumbosacral stenosis in working Labrador Retriever dogs

## **FINAL REPORT**

### **Statement of the problem studied**

Lumbosacral stenosis (LS) is a structural narrowing of the spinal canal in the lower back region. This disease is an important cause of debilitation and early retirement in military working dogs. Early diagnosis and treatment are essential for maximizing active duty service for the dogs and mission readiness for the teams that depend on them. Labrador retrievers are commonly used as military working dogs and are also predisposed to LS. Current standard phenotyping methods for canine LS include clinical examination, radiography, computed tomography (CT), and magnetic resonance imaging (MRI). However, clinical signs of LS are often delayed in military working dogs because they are bred and trained for stoicism and high working drive. By the time clinical signs are detected, the disease is often too far advanced for effective treatment. Standard diagnostic imaging methods are based on qualitative assessments and therefore limit the use of robust statistical analyses. For research purposes, a noninvasive method for quantitative phenotyping of LS would therefore be beneficial. Computed tomography (CT) is an established method for qualitatively determining presence of LS, however at the time of this study, published evidence for a consensus on any such quantitative CT phenotypic traits was lacking in humans or dogs. The causes of LS in dogs are incompletely understood, however previous researchers have proposed that genetics may play a role. The predominant genetic approach for research in canine LS has been pedigree analysis (especially in the German shepherds); but no genetic association studies have been reported in any breed. The long-term goals of our research are to determine whether there may be genetic risk factors for LS in Labrador retriever military working dogs, and, if so, to develop screening tests that could be used to assist decision-making at the time of procurement.

### **Summary of the most important results**

The first study for the funded project was a retrospective, methods comparison design.<sup>1</sup> We used archived CT data from 3 veterinary hospitals and a sample of 25 Labrador retrievers to develop a novel quantitative CT phenotyping trait for LS. This trait was termed “fat area ratio or FAR” and was calculated as the ratio of the vertebral canal epidural fat area in a transverse CT slice relative to the vertebral body area in the same transverse slice. This measurement was found to have good agreement with the standard qualitative assessment of LS made by a board-certified veterinary radiologist. We also reported FAR values measured for each of the L5-S3 vertebral levels in dogs classified as LS positive or LS negative by the radiologist. We also described qualitative and quantitative characteristics of “funnel-shaped” lumbar vertebral canals in some of the Labrador retrievers.

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## **Bibliography**

1. Mukherjee M, Jones JC, Holásková I, Raylman R, Meade J. Phenotyping of lumbosacral stenosis in Labrador retrievers using computed tomography. *Vet Radiol Ultrasound*. 2017;58:565–580. <https://doi.org/10.1111/vru.12520>
2. Mukherjee M, Jones J, Yao J. "Lumbosacral Stenosis in Labrador Retriever Military Working Dogs - an Exomic Exploratory Study" (CGAE-D-17-00007R2). *Canine Genet Epidemiol*. 2017;4; 12. <https://doi.org/10.1186/s40575-017-0052-6>