First Award

I. Title and Abstract (1pp)

Title: Biomechanical and Genetic Risk Factors for Osteochondrosis in Standardbred Pacers and Trotters

Rationale: Osteochondrosis (OC) is a common manifestation of developmental orthopedic disease in young horses that is influenced by both genetic and environmental risk factors. Some OC lesions may heal spontaneously, but those present after 8 months of age are typically permanent and nearly always require surgical intervention. While OC can affect individuals of any breed, Standardbreds have a particularly high prevalence of tarsal lesions (10-26%). Differences in tarsal OC lesion prevalence and distribution between Standardbred pacers and trotters have been reported, but it is unknown if these are due to differences in genetic risk, biomechanical forces related to gait, or a combination of the two.

Hypothesis/Objectives: We hypothesize that Standardbred pacers and trotters share genetic risk factors for OC, but that biomechanical differences in their natural gait patterns influence which early lesions heal and which become permanent. In **Objective 1** we will *prospectively follow the development of tarsal OC lesions in a cohort of Standardbred pacer and trotter foals* to determine if gait preference has an impact on lesion healing/persistence. In **Objective 2** we will *determine if previously validated genetic risk factors* (**Preliminary Data**) *are shared between pacers and trotters and determine if these risk alleles are associated with development and/or persistence of OC lesions* in our experimental cohort.

Study Design: 86 sire-matched foals (n = 43 pacer; n = 43 trotter) will be enrolled in this study between birth and 60 days of age. **Objective 1:** Four standard radiographic views of both tarsi will be taken using a portable digital x-ray unit at 60 day intervals until the foal is sold or reaches 12 months of age. Radiographs will be assembled serially for each foal and evaluated blindly for the presence/absence, as well as spontaneous healing or persistence, of OC lesions. Foals will also be observed in their normal paddock/pasture turnout and their activity will be recorded (amount of time spent moving and at which gait). Differences in presence, location, and progression of OC lesions, based on gait preference (pace vs trot) and activity will be determined. **Objective 2:** DNA from all foals will be submitted for single nucleotide polymorphism (SNP) genotyping on a custom Sequenom genotyping assay (**Preliminary Data**). Mixed model association analysis will be performed to identify risk alleles significantly associated with 1) development of any OC lesion during the course of the study; 2) development of a permanent OC lesion.

Preliminary Data: A genome-wide association study (GWAS) in 182 Standardbreds revealed regions associated with tarsal OC on equine (ECA) chromosome 14. Follow-up genotyping of 240 putative risk alleles using a Sequenom assay in the GWAS population and an independent population of Standardbreds (n = 139) resulted in validation of the two risk loci on ECA14 as well as risk loci on ECA10 and 21.

Expected Results: We expect that ≥70% of the foals will have evidence of OC at one or more time points, with ~20% developing permanent lesions. Further, the amount of time spent pacing/trotting will correlate with the location of permanent lesions (i.e. distal intermediate ridge of the tibia in pacers vs. medial malleolus in trotters). Finally, we expect that one or more putative risk alleles within the validated risk loci on ECA14, 10, or 21 will be associated with disease in our cohort, but that there will not be differences between pacers and trotters.

Budget and Timeline: 2 years, total budget [redacted], most of which is devoted to radiographic exams [redacted] and genotyping [redacted]. **Months 1-21**: sample collection and radiographs (Year 1 n = 60 foals; Year 2 n = 26 foals). **Months 17-19**: genotyping. **Months 21-24**: final data analysis and compilation of results.

Potential Impact for Animal Health: Prospective evaluation of differences in OC lesion formation between pacer and trotter foals will provide insight into the role that biomechanical forces related to gait may play in the development of disease. Further, this approach will allow us to determine if there are differences in genetic risk factors not just between pacers and trotters, but between horses who spontaneously heal their OC lesions and those who go on to develop permanent lesions. Defining the roles of these putative risk factors will both improve our understanding of the pathophysiology of OC and facilitate early interventions for at-risk horses to help reduce risk of clinical disease.

II. Letters
Candidate Letter of Intent

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Department of Veterinary Clinical Medicine
College of Veterinary Medicine
1008 West Hazelwood Drive
Urbana, IL 61802

25 June 2015

To the Selection Committee:

As a veterinarian board-certified in large animal surgery, I have an ongoing interest in musculoskeletal disease in horses as this is a major reason for presentation of patients for clinical evaluation, with complaints ranging from poor performance to impaired quality of life. As a researcher, I am especially interested in the role that genetics plays in developmental and degenerative joint disease. This encompasses predisposing genetic factors, changes in gene expression during the course of disease, and genetic therapies for treatment. I believe that clinician-scientists play a vital role in bridging the gap between benchtop research and clinical applications, and I hope to fulfill such a role as I advance in my career. I recently accepted a tenure-track Assistant Professor position in academic veterinary medicine and am working to establish an independent research program while maintaining involvement in clinical practice, the didactic and clinical teaching of veterinary students, and the training of interns and residents. The work outlined in this First Award proposal is an outgrowth of the projects I completed during my PhD training, and is the natural next step to support my growth into an independent researcher.

My previous research training has been diverse, ranging from cognitive psychology to molecular genetics, from very basic to purely clinical. I deliberately sought out these different experiences so that I would have a clear idea of where I wanted to focus my own research career. I discovered that while I enjoyed both basic and clinical research, I was most interested in being able to bridge the gap between them as a clinician-scientist. My PhD mentors supported my efforts to combine my clinical interest in orthopedics with my basic research interest in genetics, which resulted in a thesis project primarily focused on the genetic risk factors for ostoechondrosis (OC; a developmental orthopedic disease), and secondarily focused on genetic determinants of gait and performance in horses. Specific approaches used in the course of this work included single nucleotide polymorphism (SNP) genotyping, genome-wide association (GWA) analysis, traditional Sanger sequencing of candidate genes, and whole-genome sequencing analysis. I wrote 14 grant proposals related to this research, three of which were funded. To date, however, I have not received funding as a principal investigator for more than $20,000 for any single, extramural award. I have presented the results of my research at several national and international meetings since 2011 (e.g. Plant and Animal Genome XVIII Conference, 2011; Dorothy Russell Havemeyer Foundation 10th International Equine Genome Mapping Workshop, 2013; Plant and Animal Genome XXII Conference, 2014), and have abstracts accepted at two upcoming meetings (Dorothy Russell Havemeyer Foundation 11th International Equine Genome Mapping Workshop July 2015; American College of Veterinary Surgeons Surgical Summit October 2015). Additionally, my PhD research has generated four peer-reviewed publications to date (*Osteoarthritis Cart* 21:1638-1647, 2013; *J Hered* 105:163-172, 2014; *Anim Genet* 45:153, 2014; *Equine Vet J* 47:438-444, 2015), with a fifth manuscript submitted for review (*BMC Genomics*).

One of these publications described the effect of hock OC on race performance in a cohort of Standardbred pacers and trotters (*Equine Vet J* 47:438-44, 2015). An unexpected novel finding of this report was that there were gait-related differences for both OC lesion distribution and the overall risk for developing disease in our study population. These differences could have been due to biomechanical factors, genetic factors, or a combination of the two, but my existing data set did not allow further exploration of these possibilities. In collaboration with my new colleagues at the University of Illinois, including Dr. Kevin Kline (co-investigator on this award), I am now ideally positioned to follow up on this finding in this First Award by prospectively evaluating foals radiographically for the development and progression (healing/permanence) of OC lesions and correlating these findings with observed activity and natural gait preferences. Additionally, I will be able to evaluate specific risk alleles for OC in this group of foals based on recent work done in collaboration with my mentor, Dr. Molly McCue, in which we validated several genetic risk loci for hock OC by demonstrating association with disease in two independent populations of Standardbreds (work supported by a Morris Animal Foundation Pilot Award; manuscript submitted for review). Not only will I be able to compare these risk alleles between pacers and trotters, but also between foals that spontaneously heal their OC lesions and those that go on to require treatment for permanent lesions. This type of comparison has never been reported, and has the potential to open up an entirely new line of inquiry for me to pursue moving forward.

The work outlined in this First Award represents the next step towards a major goal of my planned independent research program, namely development and testing of a risk model for development of OC in individual horses based upon genetic variants of putative functional effect and environmental risk factors. The independent collaborations I have established at the University of Illinois will open up many future research opportunities by giving me access to a large local cohort of mares and foals. At the same time, the First Award supports my efforts to meet my training and career development goals under the ongoing guidance of my mentor, Dr. McCue, who is both an internationally recognized expert in my chosen field of equine genetics/genomics and a singularly successful researcher who has established a highly productive lab in her relatively short career.

In my eyes, my roles as a clinician and as a researcher are inherently intertwined, with each enhancing the other. My clinical training is the lens through which I frame my scientific inquiries, providing crucial context and perspective. As equine genetics research advances with the aid of next-generation technologies, allowing us to ask and answer more complex questions, the results will continue to be translated into tangible benefits for our patients. I certainly plan to be a part of this process as my career develops, and this First Award will help me take the next step towards my goal of becoming an independent clinician-scientist.

Sincerely,

Annette M. McCoy, DVM, MS, PhD
Diplomate, American College of Veterinary Surgeons
Assistant Professor, Equine Surgery
Department of Veterinary Clinical Medicine
College of Veterinary Medicine
University of Illinois

Mentor Letter

UNIVERSITY OF MINNESOTA

Twin Cities Campus

Veterinary Population Medicine
College of Veterinary Medicine

225 Veterinary Medical Center
1365 Gortner Avenue
St. Paul, MN 55108
Office: 612-625-7755

To the Review Committee:
June 20, 2015

It is my pleasure to write a letter indicating my strongest support for Dr. Annette McCoy's first award application. I have had the pleasure of working closely with Dr. McCoy since she began her PhD in my laboratory in 2010, and I have continued to collaborate with Dr. McCoy since she began her faculty position at the beginning of 2015. The objectives outlined in this first award are the follow-up to the project Annette worked on in my laboratory as a PhD student. As part of her thesis work, Annette phenotyped a population of Standardbred horses for osteochondrosis (OC), characterizing the impact of OC and early surgical intervention on racing performance and identifying genetic risk loci using genome-wide association (GWAS) analysis. During Annette's study on the impact of early surgical intervention on racing performance, she identified differences in tarsal OC prevalence and site predilection in Standardbred pacers and trotters, a finding that had not been previously reported. Further, Annette's GWAS study identified two chromosomal loci associated with tarsal OC, and Annette successfully obtained funding from Morris Animal Foundation (MAF) in 2014 to validate these findings in a second independent population of European Standardbred trotters. Annette has now successfully completed this MAF funded work and for the first time, validated a genetic locus identified for OC in a second independent horse population. The hypothesis in Annette's First Award comes directly from her findings in both her performance and genetic studies, and the work outlined in this proposal are the natural next steps to these findings.

The goals of the First Award are to support new investigators that are proposing the highest quality science. I can state that without a doubt that Dr. McCoy's proposal represents both. The proposed research in this proposal builds on the genetics/genomics and computational skills Annette acquired during her PhD, however, this work represents Annette's own intellectual pursuit and is feasible due to the new collaborations she has established since her arrival at the University of Illinois. The project plan is Annette's own design and represents an opportunity for Annette to establish her own independent research program. The objectives in this proposal fit perfectly into her long-term goal of developing a risk model of OC that incorporates both genetic and environmental (including biomechanical) risk factors. Further, the genetic risk factors identified will likely be informative across breeds and predilection sites, thus the impact of this work will extend beyond predicting risk for tarsal OC in the Standardbred. Finally, the prospective nature of the study will allow for an estimation of the number of OC lesions in foals that spontaneously heal and has the potential to identify the genetic factors that are responsible for spontaneous healing.

Funding of this proposal will also have an impact on the career of a truly outstanding scientist. I cannot overstate Annette's potential as an independent research scientist; she is highly intelligent, extremely organized, dedicated to her career, and an incredibly hard worker. During her PhD, Annette not only studied OC, but also capitalized on the population of Standardbred horses she used for her thesis to map two additional traits by genome wide association (GWAS), one for racing performance and another for gait. She has also worked with a collaborator to gather samples from > 100 Thoroughbred horses to eventually study the genetic basis of a juvenile osteoarthritis, and laid the groundwork for the functional genomics and proteomics evaluation of an experimental model of post-traumatic osteoarthritis in the horse. In addition to generating research data and research samples, during her tenure in my laboratory, Annette co-authored 14 grant proposals (3 funded), published 5 manuscripts and authored a book chapter. Annette has also mentored undergraduate students and veterinary summer scholars, and in 2012 the college awarded her the Vaughn Larson Award in recognition of her leadership. Annette has taken an active role in her professional

development outside of research, participating in the University's Preparing Future Faculty Program and in teaching in the didactic veterinary curriculum. Needless to say she has been highly productive, setting the bar for her fellow graduate students, and for those who will follow in her footsteps.

As Dr. McCoy's primary mentor I will continue to be involved in her research training and on-going project development. As Dr. McCoy has been establishing her own laboratory and ongoing projects at the University of Illinois, we have continued to be in contact 2-3 times per month for Skype meetings. Annette has also contributed to the on-going training of students in my laboratory by being involved in our weekly data analysis meetings (via Skype) and has often volunteered to help with feedback for upcoming research presentations, both via reviewing slides and by providing input during presentation "practice sessions". Moving forward (at least until Annette's own lab becomes busy enough that we can no longer impose on her time), Annette will continue to be involved in some of the on-going activities in my laboratory. I view this as a win-win situation; these interactions provide Annette the opportunity to help mentor graduate students and also give my students an opportunity to get additional feedback and/or a different perspective. In addition to nearly weekly discussions and her on-going involvement with my laboratory group, Annette and I have identified opportunities to develop clinical research projects, hone her skills in grant and manuscript writing, network with investigators both inside and outside the University of Minnesota and the University of Illinois, and to further develop her teaching skills. As stated above, the research project outlined in this proposal stems from the work on OC that she initiated during her PhD, and also from other collaborations she has independently developed. To support these and other on-going projects, I have worked with Dr. McCoy to identify potential funding sources and continue to mentor her in the grant writing process. Additionally, I will work with Dr. McCoy to ensure that she publishes a minimum of 2-3 (or more) high quality manuscripts each year to ensure that she stay on track in her career development. However, in large part Dr. McCoy's independence will be facilitated by her taking sole ownership of the OC and OA projects she has initiated in my laboratory. Both OC and OA are disease processes that are in alignment with Dr. McCoy's clinical training; whereas my clinical training and interests are in internal medicine with the main projects in the my lab being focused on muscle, metabolic, and neurologic diseases/biology.

My previous mentoring experience includes being a graduate faculty member in 3 graduate programs at the University of Minnesota. I serve as a faculty mentor for both T32 and T35 awards, and I am the co-director of our post-doctoral T32 in Comparative Medicine and Pathology. I have served/am serving as the primary mentor for 6 PhD students and 3 MS students, and 3 post-doctoral trainees, and as the primary clinical mentor for 5 internal medicine residents. Two of my PhD students have completed their degrees and a third will defend in August 2015. One of three MS students has completed her masters and gone on to a PhD degree at the University of Minnesota. Two of three post-docs have completed their training. Of the PhD and post-doctoral students who have completed their training, three have become faculty members (2 tenure track) and the forth went into industry. I am also currently the co-advisor for 2 PhD students and have served on an additional 10 graduate committees (5 PhD and 5 MS). I currently informally mentor another tenure track faculty member at the University of Minnesota, including serving on a K01 award mentoring committee, mentoring on the balance between research and clinical work and serving as a co-investigator on a canine genomics grants.

In summary, Annette is a very bright, talented, and motivated individual. Her academic career and productivity to date has been stellar; she is most deserving of a First Award and will take full advantage of the opportunity. I have had the pleasure of watching her grow as a scientist during her PhD and would be honored to continue to mentor her. I appreciate your consideration of her application.
Sincerely,

Molly McCue DVM, MS, PhD, Diplomate, American College of Veterinary Internal Medicine
Associate Professor, University of Minnesota College of Veterinary Medicine

Letter of Support from Department Head

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Department of Veterinary Clinical Medicine

College of Veterinary Medicine
1008 West Hazelwood Drive
Urbana, IL 61802

June 23, 2015

To: Morris Animal Foundation

I am writing this letter to indicate my support for Dr. Annette McCoy's proposal, "Biomechanical and Genetic Risk Factors for Osteochondrosis in Standardbred Pacers and Trotters." We were pleased to have Dr. McCoy join our department in January, 2015 as an Assistant Professor in our Equine Medicine and Surgery section.

The Department of Veterinary Clinical Medicine is strongly committed to Dr. McCoy's research work, and have provided her a furnished office and an assigned research laboratory. She will receive ample startup funding to be used for her research projects. In addition she will have access to shared equipment and space in the department and college. Dr. McCoy's clinical and teaching activities will be restricted to 25% to allow her to focus on her research activity. Her research appointment will be increased if the level of external funding supporting scholarship is obtained.

The Department and College are committed to the development, advancement and retention of its faculty members. As Department Head, I am personally committed to these goals and will attempt to provide all necessary resources for Dr. McCoy to be successful.

Sincerely,

Karen L. Campbell, DVM, MS
Diplomate, ACVIM & ACVD
Professor and Department Head

III. Resubmission Summary: N/A

IV. Name, Institution, and E-mail Address of PI and all Co-Investigators (1pp)

Annette M. McCoy, DVM, MS, PhD, DACVS; University of Illinois; [redacted]

Molly E. McCue, DVM, MS, PhD, DACVIM; University of Minnesota; [redacted]

Kevin H. Kline, PhD; University of Illinois [redacted]

V. Study Proposal (5pp limit)

Specific, Testable Hypothesis and Objectives

Our long-term goal is to develop a predictive model that will allow for estimation of an individual horse's risk for the development of osteochondrosis (OC). A crucial component of this is improved understanding of the interplay between the genetic and environmental factors that underlie disease risk. We have recently reported differences in tarsal OC lesion prevalence and distribution between Standardbred pacers and trotters (see **Literature Review**), however it is unknown if these are due to differences in genetic risk, biomechanical forces related to gait, or a combination of the two. We have identified genetic risk loci for tarsal OC that are shared between Standardbred pacers and trotters (see **Preliminary Data**), therefore, *we hypothesize that Standardbred pacers and trotters share genetic risk factors for OC, but that biomechanical differences in their natural gait patterns influence which early lesions heal and which become permanent*. To address this hypothesis, in this proposal we will:

Objective 1: Prospectively follow the development of tarsal OC lesions in a cohort of Standardbred pacer and trotter foals to determine if gait preference has an impact on lesion healing/persistence.

Objective 2: Determine if previously validated genetic risk factors are shared between pacers and trotters, and determine if these risk alleles are associated with development and/or persistence of OC lesions in our experimental cohort.

Justification, Significance, and Literature Review

Osteochondrosis (OC) is a commonly diagnosed developmental orthopedic disease in the horse, as well as other domestic animal species (particularly the dog and pig), which is characterized by abnormal cartilage within a joint that occurs secondary to focal failure of endochondral ossification (the process by which a cartilage template becomes bone in the limbs of a growing animal). It is a complex disease, with interactions of genetics and environment (e.g. diet, exercise) determining expression and severity of lesions. Manifestations of disease can vary from mild to severe, and there is evidence that many lesions heal spontaneously (van Weeren and Barneveld, 1999; Dik et al., 1999). However, "permanent" OC lesions (present after 8 months of age [Dik et al., 1999]) nearly always require surgical intervention to prevent ongoing joint damage. If left untreated, OC can potentially lead to severe degenerative joint disease and can be career- or even life-threatening. Prevention of OC is an as-yet unattained goal of the equine industry, and thus this disease represents an animal health and welfare issue requiring ongoing research efforts.

OC is recognized across many breeds, with reported overall prevalence ranging from 6.25% in feral horses in the Western United States (Valentino et al, 1999) to greater than 40-50% in European Warmblood and Coldblood breeds (Lepeule et al, 2009; Wittwer et al, 2006). In addition to differences in overall prevalence, it is recognized that lesions are more common at one predilection site (i.e. fetlock, tarsus, or stifle) than another in different breeds. In Warmbloods, for example, average reported prevalence of OC lesions in the fetlock is 22.3%, while average prevalence in the tarsus and stifle are reported to be 11.5% and 7.0%, respectively (e.g. Stock et al., 2005; van Grevenhof et al., 2009). By comparison, in Standardbreds, OC of the tarsus is most common, with an average reported prevalence of 14.7% as compared to 3.3% and 6.3% in the fetlock and stifle, respectively (e.g. Grondahl and Dolvik, 1993; Lykkjen et al., 2012; Ricard et al., 2013). These differences in predilection sites and prevalence between breeds may be due to population differences in environmental and/or genetic risk factors, but this is not yet completely understood.

It has been postulated that OC could be caused either by abnormal forces on normal cartilage or by normal forces on abnormal cartilage (Pool, 1986). The evidence for the role of exercise in the development of OC is mixed, with one large study showing that the amount of exercise during the first 5 months of life affected the distribution but not the total number of lesions (van Weeren and Barneveld, 1999), and another reporting that reduced or irregular activity in the first weeks of life increased severity of lesions (Lepeule et al., 2013). However, to date there have been no studies examining the role that differences in natural gait preferences may play on the development of OC. Recently, we reported differences in tarsal OC lesion prevalence and distribution between Standardbred pacers and trotters (McCoy et al., 2015). Trotters were significantly

more likely to be affected with OC than were pacers. Further, among OC-affected individuals, the odds of a trotter having a medial malleolus (MM) lesion were 5 times higher than a pacer, while the odds of a pacer having a lesion at the distal intermediate ridge of the tibia (DIRT) were 3.7 times higher than a trotter. Since the natural pacing gait is demonstrated in young pacing-bred foals before any training occurs (United States Trotting Association, 2013), it is possible that gait-specific biomechanical forces may have an effect on lesion development and/or distribution. At least three differences between the biomechanics of the trot and the pace have been reported that may have biological significance (Drevemo et al., 1980; Wilson et al., 1988a; Wilson et al., 1988b; Robilliard et al., 2007). In objective 1, prospective radiographic evaluation of foals that preferentially pace and those that preferentially trot will be used to identify differences in the development and persistence of OC lesions between these two groups. This will determine if the gait-based differences are related to overall OC incidence, differences in the site(s) of primary lesion development and/or differences in primary lesion healing.

Pacing and trotting lines are carefully maintained and are as genetically distinct as any two separate breeds (Cothran et al., 1987). Thus, a second possibility is that genetic risk factors for OC may vary between pacers and trotters, and that differences in genetic risk factors are responsible for differences in prevalence and lesion localization. We recently performed a genome-wide association study (GWAS) in a cohort of Standardbreds with tarsal OC that was specifically selected to minimize the effect of environmental confounders on disease association (see **Preliminary Data**). Two distinct loci on equine (ECA) chromosome 14 were found to be associated with OC in this population. Putative risk alleles from within our GWAS regions of interest, as well as from chromosomal regions previously reported to be associated with equine OC, were evaluated in both our GWAS population and an independent population of Standardbreds (see **Preliminary Data**). This work resulted in validation of the two risk loci on ECA14, as well as risk loci on ECA 10 and 21. These data support our hypothesis that genetic risk factors for OC are in fact shared between pacers and trotters, although it is possible that modifying population-specific risk factors also play a role in disease manifestation.

Prospective evaluation of differences in OC lesion formation between pacer and trotter foals will provide insight into the role that biomechanical forces related to gait may play in the development of disease. Further, this approach will allow us to determine if there are differences in modifying genetic risk factors not just between pacers and trotters, but between horses who spontaneously heal their OC lesions and those who go on to develop permanent lesions. Defining the roles of these putative risk factors will both improve our understanding of the pathophysiology of OC and is an important step in developing a predictive model for OC that can facilitate management changes and early disease intervention in at-risk individuals.

Preliminary Data

GWAS in discovery population: The discovery cohort was comprised of 182 similarly bred Standardbred yearlings born on a single breeding farm in the eastern United States between 2007 and 2012 and raised under similar management conditions (70 affected with tarsal OC, 112 unaffected). Horses were genotyped on either the Illumina Equine SNP50 or SNP70 beadchips. Markers were imputed to a set of 74,595 single nucleotide polymorphism (SNP) markers for analysis (McCoy and McCue, 2014). Mixed model association analysis with gender and gait covariates was performed utilizing GEMMA software (Zhou and Stephens, 2012), which incorporates a relationship matrix to account for population structure. Five SNPs located within two distinct regions on ECA14 were most highly associated with OC, and were nearly genome-wide significant after a Bonferroni correction was applied based on the number of effective independent markers ($p < 5.1 \times 10^{-5}$; corrected genome-wide significance level 1.86×10^{-6} [Li et al., 2012]). Repeat GWAS after imputation to nearly 250,000 markers identified the same regions of association on ECA14, defined by 30 SNPs at $p < 4.76 \times 10^{-5}$.

Validation of risk loci: Approximately 1.5 million alleles, discovered via whole-genome sequencing of 18 horses from the discovery population (9 affected with OC, 9 unaffected) were evaluated from a total of 32 regions that were either identified as regions of interest in our GWAS or were chromosomal regions

previously reported to be associated with hock OC. 240 putative risk alleles from loci on 10 chromosomes were prioritized for follow-up according to predicted functional effect and segregation with OC status in the sequenced horses. These alleles were genotyped in both the GWAS cohort (above) and an independent validation cohort consisting of 139 Norwegian Standardbreds (60 affected with tarsal OC, 79 unaffected; described in Lykkjen et al., 2010) using a custom high-throughput Sequenom assay. Mixed model association analysis was performed in GEMMA with sex and gait included as covariates. Variants from within the two GWAS loci on ECA14 were most highly associated with disease status in both populations (p=0.0004-0.022 in the GWAS cohort; p=0.014-0.049 in the validation cohort). Variants with $p < 0.05$ from chromosomal regions on ECA10 and 21 were also shared between the two populations. These additional regions were identified from the GWAS, although they were less significantly associated with OC than the regions on ECA14. The association of putative risk alleles from within the same regions with disease status in two independent populations of Standardbreds suggest that these are true risk loci in this breed. Work is ongoing to evaluate specific risk alleles within these loci.

Experimental Methods and Design

Objective 1: Prospectively follow the development of tarsal OC lesions in a cohort of Standardbred pacer and trotter foals to determine if gait preference has an impact on lesion healing/persistence.

Rationale and working hypothesis: We recently reported differences in OC prevalence and lesion distribution between Standardbred pacers and trotters (McCoy et al., 2015), but the retrospective nature of this study did not allow further investigation of this finding. Prospective evaluation of the development and persistence of OC lesions, combined with field observation of foals to assess their activity and preferred gait will provide insight into the role of gait-related biomechanical forces in the development of disease. We hypothesize that pacer and trotter foals develop similar early OC lesions, but that biomechanical differences in their natural gait patterns influence which lesions heal and which become permanent.

Study cohort: 86 sire-matched Standardbred foals (n = 43 pacer; n = 43 trotter) will be enrolled in this study before 60 days of age (see below for power calculation). Foals will be raised under similar management conditions on one of three breeding farms in the central United States, located within 75 miles of each other. Approximately 80 foals are raised each year among the participating farms, and most of the foals are sold at breed-recognized yearling sales each fall. We expect to enroll the majority of foals needed during the first foaling season (n = 60) and the remainder during the second foaling season (n = 26).

Power calculation for sample size: The overall prevalence of tarsal OC in our cohort, based on historical expectations from the participating breeding farms and reported breed prevalence, is expected to be approximately 20%. Lesions of the distal intermediate ridge of the tibia (DIRT) and medial malleolus (MM) are the most commonly reported across all studies of tarsal OC. We previously reported that DIRT lesions were present in 70% of affected individuals (expected overall prevalence 14%) with a relative risk (RR) of 3.7 for pacers compared to trotters, and that MM lesions were present in 36% of affected individuals (expected overall prevalence 7.2%) with a RR of 5.01 for trotters compared to pacers. Based on expected prevalence and RR, power calculations to determine sample size was performed using the formula:

$$N = 4 / \text{prevalence} * (\sqrt{(RR)} - 1)^2$$

This results in a required sample size 34 horses per group for DIRT lesions, and 37 horses per group for MM lesions. We selected the larger of these, and added 15% to account for potential loss to follow-up, resulting in a final sample size of 43 pacers and 43 trotters.

Serial radiographs: Four standard radiographic views – lateral, cranio-caudal, dorsolateral-palmaromedial oblique, and dorsomedial-palmarolateral oblique – of both tarsi will be taken using a portable digital x-ray unit beginning within one week of turning 60 days of age, and then at 60 day intervals until the foal is sold or reaches 12 months of age (i.e. 60 ± 7, 120 ± 7, 180 ± 7, 240 ± 7, 300 ± 7, 360 ± 7 days). Foals will be excluded from data analysis if they are lost to follow-up before 8 months of age. Routine physical exams will be performed on each foal on each day that radiographs are collected. Radiographs will be taken using

routine physical restraint (halter/leadrope) whenever possible; for foals that are intractable to physical restraint alone, a single dose of sedation (xylazine 0.2mg/kg IV) will be administered. Best practices for radiographic safety (ALARA principles) will be followed at all times. Based on pilot work we have recently performed in foals ranging from 2 weeks to 3 months of age, with our projected personnel and equipment resources, we expect to be able to radiograph 10-20 foals in a day.

Field observation: Foals will be video monitored in their normal paddock/pasture turnout at least 1 day (8-12 hours) per week. The video will subsequently be evaluated for the following activities: lying down, standing/nursing, walking, trotting and/or pacing, and moving at a faster gait (canter and/or gallop). A standardized activity log will be maintained for each foal to record the number of minutes (rounded to 30 second intervals) spent on each activity for a minimum of two consecutive hours each week.

Data analysis: Radiographs will be randomized and blindly evaluated for the presence or absence of OC lesions. They will then be assembled serially for each foal to determine where lesions developed and whether they healed spontaneously or became permanent. An OC lesion will be considered permanent if it is seen on radiographs of a foal ≥8 months of age. Differences in presence (yes/no), location (DIRT, MM, lateral trochlear ridge of the talus [LTR], medial trochlear ridge of the talus [MTR]) and progression (healed/permanent) of OC lesions between pacers and trotters will be determined using Chi-squared analysis. Survival analysis (logrank statistic) will be used to determine if there is a difference in lesion development and healing between pacers and trotters over time.

Overall activity will be assessed as percent time spent moving (combined time at the walk, trot/pace and canter/gallop divided by total observation time) during all observation periods prior to each set of radiographs (i.e. each 60 day period will be considered separately). Pacing will be evaluated in all foals as a categorical variable (observed/not observed). For those foals observed to pace, it will further be evaluated as a percentage of their total activity. Logistic regression models will be constructed to test the relationship between overall activity (in each 60 day period) and pacing with categorical outcome variables: 1) presence/absence of any OC lesion; 2) presence/absence of specific OC lesions (DIRT, MM, LTR, MTR); and 3) whether an observed OC lesion healed or became permanent. Sex and sire will also be included as predictor variables. For example:

$$OC_{R3} = \mu + activity_{T1} + activity_{T2} + pace + sex + sire + \varepsilon$$

where the outcome is the presence of an OC lesion (yes/no) on the third set of radiographs (R3), pace is a categorical predictor, and activity levels during each of the two 60-day periods prior to the third set of radiographs are considered separately (T1, T2).

Expected Outcomes, Potential Pitfalls, and Alternative Approaches: Based on previously published studies (e.g. Dik et al., 1999), we expect ≥70% of the foals will have evidence of tarsal OC at one or more time points, with ~20% developing permanent lesions. Further, differences in preferred gait will affect the location of permanent lesions (i.e. DIRT in pacers, MM in trotters). The biggest potential pitfall for this objective is that we may not have as many foals affected with OC as we expect, resulting in insufficient power to detect differences between groups. Should this appear to be the case based on preliminary analysis of findings from year 1, we will plan to enroll additional foals during the second year of the study. There are sufficient numbers of foals raised on the three participating farms that this could be easily accomplished without having to recruit additional participants. Should this become necessary, internal funds will be committed to accomplish the additional radiographs.

Objective 2: Determine if previously validated genetic risk factors are shared between pacers and trotters, and determine if these risk alleles are associated with development and/or persistence of OC lesions in our experimental cohort.

Rationale and working hypothesis: The presence of OC across domestic horse populations, including a feral horse population (Valentino et al., 1999), as well as shared major predilection sites and lesion morphology suggests a unified underlying pathophysiology and shared genetic risk across breeds. Our recent validation

of shared risk loci for tarsal OC in two independent populations of Standardbreds (see **Preliminary Data**) supports this idea. Thus, we hypothesize that Standardbred pacers and trotters share genetic risk factors for OC, and further, that alleles from within validated risk loci on ECA14, 10, and 21 will be associated with disease in our study cohort.

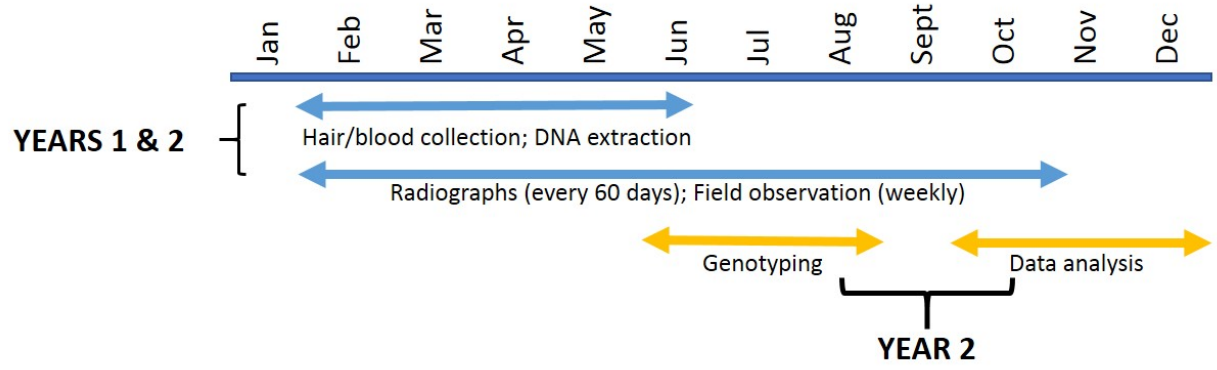
Study cohort and sample collection: The study cohort is described under **Objective 1**. Hair roots and/or whole blood will be collected from all foals at the time of study enrollment (<60 days of age). DNA will be isolated from collected samples using the Gentra® Puregene® Blood Kit (Qiagen, Valencia, CA) per manufacturer recommendations. Quantity and purity of extracted DNA will be assessed using spectrophotometric readings at 260 and 280nm (NanoDrop 2000, Thermo Scientific, Wilmington, DE).

Genotyping: Genotyping will be completed by Neogen GeneSeek (Lincoln, NE). The Sequenom assay has been previously designed and all SNPs validated in our GWAS cohort (see **Preliminary Data**). This assay includes 338 SNPs multiplexed into wells of 48; 98 of these SNPs are ancestry informative markers (AIMs) included to help control for population structure, while 240 are SNPs selected from chromosomal regions of interest associated with OC. Evaluation of additional risk alleles (not included in the Sequenom assay) within validated risk loci on ECA14, 10, and 21 is ongoing. We will genotype these risk alleles in our study cohort via restriction fragment length polymorphism (RFLP) or Sanger sequencing, as appropriate.

Data analysis: Genotyping data will be analyzed using mixed model association analysis as implemented in the GEMMA software (Zhou and Stephens, 2012). Outcomes for which models will be created are 1) development of any OC lesion during the course of the study; and 2) development of a permanent OC lesion. Association testing will be performed using the options to create a centered relatedness matrix (-gk2) and perform all three possible frequentist tests: Wald, likelihood ratio, and score (-fa4). The relatedness matrix will be constructed using the AIMs. Gender and gait will be included in the model as covariates. SNPs will be pruned prior to analysis using the default GEMMA parameters of minor allele frequency (MAF) < 1% and missingness < 95%.

Expected Outcomes, Potential Pitfalls, and Alternative Approaches: Our study cohort includes individuals of the same breed and with OC lesions in the same location (tarsus) as our GWAS and validation cohorts (see **Preliminary Data**). Further, our GWAS cohort was comprised of both pacers and trotters. Thus, we expect that putative risk alleles found within validated loci on ECA14, 10, and 21 will be associated with disease in our entire study cohort and that there will not be difference between pacers and trotters. However, it is possible that there are true differences in modifying alleles conferring disease risk between these two groups. We are addressing this potential limitation by using a Sequenom assay that contains SNPs from multiple chromosomal regions of interest associated with OC in other populations of horses. If SNPs from other loci are associated with disease in our study cohort, this will need to be investigated further and would be the subject of future studies.

Timeline



VI. Animal Involvement Justification

Morris Animal Foundation

Animal Involvement Justification

(From the proposal guidelines, single-spaced, no page limit)

Morris Animal Foundation (MAF) is dedicated to funding scientifically sound, relevant and humane studies that specifically address the health and well-being of animals. All studies receiving funding must follow MAF's Health Study Policy for Animals Involved in Research (adopted October 18, 2008), which was written to ensure that each and every animal involved in a MAF funded health study receives excellent, compassionate care throughout the study. MAF shall not fund health studies which require euthanasia as an endpoint or the induction of disease or injury, unless the nature of the disease or condition to be studied is of such significance for improving animal health that such means are justified, and that meaningful information can be obtained in no other way. Furthermore, MAF will not fund any study that induces or allows pain or distress unless such pain or distress can be controlled by appropriate anesthetic, analgesic, tranquilizing drugs, or nursing care. Click here for the full Health Study Policy.

A. If this study does not involve live animals please indicate here by N/A: _____

B. Does this study involve biological samples, tissues, etc.? __**yes**_____

If yes, describe in detail what samples will be used and where & how they will be (or were) acquired. Note: Morris Animal Foundation reserves the right to request a copy of the Institutional Animal Care and Use Committee (IACUC) application/approval and other relevant applications/approvals (e.g., wildlife permit) covering the original collection of samples, including archived samples. MAF reserves the right to request IACUC (or equivalent) review and approval for any Foundation study ***regardless of the Institution's requirements***. This would include the use of archived samples as well as clinical trials.

- **Hair root samples and/or whole blood will be collected from each individual included in the study for the purpose of DNA extraction. Samples will be acquired from the animals located on their home farm. Routine restraint (i.e. halter and lead rope) should be sufficient to allow collection of these samples; sedation will not be required.**
- **Foals will be enrolled in this study between birth and 60 days of age. Standard radiographic views (lateral, cranio-caudal, dorsolateral-plantarolateral oblique, and dorsomedial-plantarolateral oblique) of both tarsi (hocks) will be taken using a portable digital x-ray unit starting at 60 days of age (±7 days) and then at 60 day intervals until the foal is sold at a yearling sale or reaches 12 months of age. Radiographs will be taken using routine physical restraint whenever possible (i.e. halter and leadrope); if a foal is intractable to physical restraint alone, a single dose of sedation (xylazine 0.2mg/kg IV) will be administered. In the case of unweaned foals, the mare will be routinely restrained (halter/leadrope) within sight of the foal while radiographs are taken. Best practices for radiographic safety (ALARA principles) will be followed at all times, including the use of personal protection equipment (lead aprons, thyroid shields, and gloves). Total time for each radiographic exam (based on pilot work in foals 2 weeks-3 months of age) is expected to be 15-20 minutes.**
- **Foals will also be video monitored in their normal paddock/pasture turnout at least one day a week. The video will be reviewed for at least 2 consecutive hours of observation per foal,**

and activity will be recorded (amount of time spent moving, and at which gait). Observation may also be done in person.

C. If this study involves live animals, succinctly address the following: (please restate the questions and directives).

1. What species will be studied? **Equine (horse)**
2. State the status of your IACUC application/approval. All recipients of MAF funding will be required to submit the entire IACUC protocol and document. A copy of the IACUC approval should not be included with the application, but it is required before funding can be awarded.

- **The protocol for hair root/blood collection for the purpose of DNA isolation has been reviewed and approved by the University of Illinois IACUC (protocol #15031 2/20/2015).**
- **The protocol for serial radiographs and field observation has been reviewed and approved by the University of Illinois IACUC (protocol #15086 5/18/2015)**

3. List the USDA category for pain and distress (B, C, D, E): __**C**____

   Note: Any study beyond category C will require review by MAF's Animal Welfare Advisory Board (AWAB). In general MAF does not fund studies beyond category C (category D studies will only be considered if they conform with MAF's Health Study Policy, category E studies will not be considered).

4. Does this proposal involve client-owned animals? __**yes**_____

   If yes, the protocol for client-owned animals must be approved by the appropriate peer review committee before the project is funded. *If this proposal involves client-owned animals, an informed client consent form must be submitted with this proposal. For a suggested list of items to be considered in an informed client consent form, click here.*

- **A copy of the client consent forms is included for review. These forms have been approved by the University of Illinois Veterinary Teaching Hospital and have also been reviewed as part of the IACUC approval process.**

5. Explain how animals will be acquired (e.g., client-owned, USDA licensed breeder, institutional "herds" or "colonies") and verify that the animals are suitable for the study (e.g., have no physiologic, physical or pharmacologic issues that would interfere with results)

- **Individuals included in the study will be University- or client-owned foals that are housed at their home farm. There is no physiologic, physical, or pharmacological issue that would be anticipated to interfere with the study results. If a foal sustains an unrelated illness or injury during the course of the study, they will be excluded from the final analysis.**

6. How many animals will be used? _**86**_____

   a. Summarize numerical justification

- **The overall prevalence of hock osteochondrosis (OC) in Standardbreds is expected to be approximately 20%, based on numerous radiographic survey s in the literature. Lesions of the distal intermediate ridge of the tibia (DIRT) and medial malleolus (MM) are the most commonly reported across all studies of OC in the hock.**
- **Previous work we have done looking at OC in Standardbred pacers and trotters (McCoy et al. *Equine Vet J* 47:438-444, 2015 DOI:10.1111/evj.12297) suggests the following expectations for prevalence and relative risk (RR) of specific lesions:**
  - **DIRT – prevalence ~14% (70% of affected individuals), RR 3.7 for pacers compared to trotters**

    - **MM – prevalence ~7.2% (36% of affected individuals), RR 5.01 for trotters compared to pacers**
- **Power calculations based on the expected prevalence of lesions was completed using the formula: N = 4 / prevalence * (sqrt(RR) – 1)^2)**
    - **RR 3.7, prevalence 14%: N = 34 in each group**
    - **RR 5, prevalence 7.2%: N = 37 in each group**
- **Based on these calculations, and accounting for 15% loss to follow-up, we plan to enroll 43 pacers and 43 trotters in the study, for a total of 86 horses**

7. Does this study induce disease, injury, pain or distress in animals? Note: any study requiring the induction of disease, injury, pain, or distress will have an additional evaluation by MAF's AWAB.

- **No, this study does not induce disease, injury, pain (beyond momentary discomfort from a hair root collection or venipuncture), or distress in animals. All of these foals are regularly handled as part of their normal routine.**

If yes,

a. Defend the necessity of experimental design

b. Explain how pain and/or distress will be controlled

c. Justify that no alternative, including clinical studies, can be used to accomplish study objectives and the disease/condition to be studied is of such significance for improving the health of the species.

8. Explain the environment and housing conditions (quality of life) in which the animals will live (address species-appropriate exercise, enrichment, socialization, veterinary care, etc.)

- **The horses in this study will be housed at their home farms. Management of these horses will proceed per standard protocol for these locations and will not be affected by participation in the study.**

9. What will happen to the animals upon completion of the study?

If adoption, explain the adoption process. Provide assurance that whenever possible and when in the animal's best interest, investigators shall make companion animals available for adoption at the end of the study or return the animals to the owner/responsible agency in an environment that promotes animal welfare and excellent quality of life.

- **After completion of the study, the horses will return to their regular management protocol. Their disposition will not be affected by participation in the study.**

10. If euthanasia, provide the following additional information (note: any study requiring euthanasia as an endpoint will have an additional evaluation by a MAF's AWAB.

- **Euthanasia is not an endpoint for this study.**

i. Total number that will be euthanized and justification for numbers

ii. Method of euthanasia

iii. Justification that no alternatives can be used to accomplish study goal(s) and that the disease/condition to be studied is of such significance for improving the health of the species that a terminal endpoint is deemed necessary.

iv. Reason for euthanasia in lay language (this wording may be shared with staff, donors and media)

v. Provide objective criteria for determining when euthanasia is appropriate or necessary (note: Morris Animal Foundation wants assurance that an animal will not be allowed to suffer and that monitoring for pain and suffering is adequate)

Note: Morris Animal Foundation does not consider the use of CO2 alone to be an appropriate method of euthanasia

Please note:

1. If an animal is used in an invasive study, MAF may require that a guarantee is provided, through principal investigator and institutional signatures that the animal will not participate in any future invasive study or procedure
2. MAF does not allow inclusion of ancillary data in MAF funded research that includes animal use protocols not in agreement with our Health Study Policy, even if it is obtained using other funding sources.
3. Morris Animal Foundation considers euthanasia acceptable when an animal develops unanticipated illness or injury that results in pain and suffering that cannot be alleviated with standard veterinary interventions.

VI. Animal Involvement Justification: Informed Client Consent Forms

OWNER CONSENT FORM	**Owner or Agent Name** _____ **Horse Name or Tattoo** _____ **(Check here ☐ if additional animals are listed on page 2)**

Biomechanical and Genetic Risk Factors for Osteochondrosis in Standardbred Pacers and Trotters
Development of Osteochondrosis in Standardbred Pacers and Trotters

Clinical Investigators: Annette McCoy, DVM, MS, PhD, DACVS. Veterinary Teaching Hospital, University of Illinois, Urbana, IL

Purpose of Study: Prospectively evaluate differences between pacer and trotter foals in the development of osteochondrosis lesions in the hock.

Eligibility: Standardbred foals (pacer- and trotter-bred) less than 60 days of age are eligible for enrollment in the study. Study participation will continue until the foals are sold at yearling sales and/or reach 12 months of age.

Procedures: Standard radiographic views (lateral, cranio-caudal, dorsolateral-plantaromedial oblique, and dorsomedial-plantarolateral oblique) of both tarsi (hocks) will be taken using a portable digital x-ray unit at 30-60 day intervals from the time of enrollment until the foal is sold at a yearling sale or reaches 12 months of age. If needed, a single dose of intravenous sedation will be administered to obtain radiographs. Foals will also be observed in their normal pasture/paddock turnout at least weekly, for a minimum of 2 hours, and their activity will be recorded (amount of time spent moving, and at which gait). Observation may be done in person or by video monitoring.

Possible Benefits / Owner Incentives: Radiographs will be made available to the owners at no charge, if desired. While this study does not provide any additional immediate financial benefits to you, in the future, information obtained will help us to better understand osteochondrosis, a disease that has enormous economic impact in the equine industry.

Associated and Unforeseen Risks: This study involves routine restraint for serial radiographs +/- intravenous sedation. None of these procedures should pose a significant risk to any participant. Rarely, horses may have an adverse reaction to sedative drugs. All reasonable efforts will be made to minimize known or potential risks associated with all procedures, and therefore there will not be compensation in case of problems arising from participation in this study. There will be no cost to you for participation in this study.

Confidentiality: Neither you nor your horse(s) will be mentioned by name in any report arising from this study. Results pertaining specifically to your horse(s) may be released to you or your designated representative upon request after completion of the study; otherwise all records will remain entirely confidential

Compensation and Financial Obligations: There will be no compensation or financial obligation associated with participation in this study.

Questions about this project may be directed to: Dr. Annette McCoy, Department of Veterinary Clinical Medicine, University of Illinois, at [redacted]

Acknowledgements:

Please note: *Before being accepted into any clinical trial, all animals must be evaluated and officially enrolled by the study principal investigator. All owners must sign an official study consent form before their pet will be accepted into a clinical trial. The cost of pre-evaluations may be the responsibility of the owner. Please be sure to contact the study investigator if you have any questions or concerns.*

I understand that my horse's participation in this study is entirely voluntary. Refusal to participate or to continue participation carries no medical penalty, and I am free to withdraw my horse from this study at any time without medical penalty or prejudice. I understand that my voluntary removal will constitute disqualification from further participation in this study.

I also understand that my horse may be required to withdraw from the study for violation of eligibility requirements, or noncompliance with restrictions and/or procedures during the study. This also constitutes disqualification. I may also be required to withdraw from the study to protect my horse's health (such as with the occurrence of significant injury, adverse reactions, or illness whether or not a consequence of the study), or if the study is terminated early.

I have not withheld information regarding my horse's medical history. I acknowledge that I have read and understand this consent form and all my questions have been answered to my satisfaction. I have been given a copy of this consent form if I have requested a copy. I am aware that this research has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois (UI).

As a volunteer, I give my informed consent to the Board of Trustees of the UI and the Veterinary Teaching Hospital (VTH) to enroll my horse in this study, according to the explanations and conditions presented in this document. I agree to hold harmless the Board of Trustees of the UI, the VTH, and its officers, employees, agents and assigns from any and all liability, claims and actions that may arise from participation in this study.

IACUC Approval #: 15086

_____	_____	____ / ____ / 20_____
Owner's Printed Name (or authorized agent)	Signature of Owner (or authorized agent)	Date
_____	_____	____ / ____ / 20_____
Witness' Printed Name	Signature of Witness	Date
Laura Garrett		
VTH Director/Chief of Staff	Signature of Director/Chief of Staff	Date: May 20, 2015

My additional horses that I approve to be included in the above study/studies	
Horse Name or Tattoo	**Horse Name or Tattoo**

Owner's Printed Name (or authorized agent)	Signature of Owner (or authorized agent)	____ / ____ / 20____ Date

(Original to medical records)

OWNER CONSENT FORM	**Owner or Agent Name** _____ **Horse Name or Tattoo** _____ **(Check here ☐ if additional animals are listed on page 2)**

Biomechanical and Genetic Risk Factors for Osteochondrosis in Standardbred Pacers and Trotters
Genetic Factors Underlying Disease and Performance Traits in the Horse

Clinical Investigators: Annette McCoy, DVM, MS, PhD, DACVS. Veterinary Teaching Hospital, University of Illinois, Urbana, IL

Purpose of Study: Identify genes and alleles in the horse that underlie 1) genetic risk for the development of osteochondrosis, and 2) the ability to perform alternative gaits.

Eligibility: Known phenotype for OC (affected or unaffected) based on radiographs and/or surgical records, and/or known phenotype for alternative gait (gaited or non-gaited individual) based on history or official race records.

Procedures: A single 10ml blood sample and hair root sample will be collected for the purpose of DNA extraction. The DNA samples will be examined using known markers from the horse genome to identify differences between horses.

Possible Benefits / Owner Incentives: While this study does not provide any immediate financial benefits to you, in the future, information obtained will help us to better understand diseases and performance traits that have enormous economic impact in the equine industry.

Associated and Unforeseen Risks: This study involves routine restraint for a single blood draw from the jugular vein and collection of hair root samples. None of these procedures should pose a significant risk to any participant. Rarely, horses may develop a clot or infection at the site of blood collection that in isolated cases may lead to long-term health problems. All reasonable efforts will be made to minimize known or potential risks associated with all procedures, and therefore there will not be compensation in case of problems arising from participation in this study. If heat or swelling is noted over the jugular vein, please contact your local veterinarian for immediate care. There will be no cost to you for participation in this study.

Confidentiality: Neither you nor your horse(s) will be mentioned by name in any report arising from this study. Results pertaining specifically to your horse(s) may be released to you or your designated representative upon request after completion of the study; otherwise all records will remain entirely confidential

Compensation and Financial Obligations: There will be no compensation or financial obligation associated with participation in this study.

Questions about this project may be directed to: Dr. Annette McCoy, Department of Veterinary Clinical Medicine, University of Illinois, at [redacted].

Acknowledgements:

Please note: *Before being accepted into any clinical trial, all animals must be evaluated and officially enrolled by the study principal investigator. All owners must sign an official study consent form before their pet will be accepted into a clinical trial. The cost of pre-evaluations may be the responsibility of the owner. Please be sure to contact the study investigator if you have any questions or concerns.*

I understand that my horse's participation in this study is entirely voluntary. Refusal to participate or to continue participation carries no medical penalty, and I am free to withdraw my horse from this study at any time without medical penalty or prejudice. I understand that my voluntary removal will constitute disqualification from further participation in this study.

I also understand that my horse may be required to withdraw from the study for violation of eligibility requirements, or noncompliance with restrictions and/or procedures during the study. This also constitutes disqualification. I may also be required to withdraw from the study to protect my horse's health (such as with the occurrence of significant injury, adverse reactions, or illness whether or not a consequence of the study), or if the study is terminated early.

I have not withheld information regarding my horse's medical history. I acknowledge that I have read and understand this consent form and all my questions have been answered to my satisfaction. I have been given a copy of this consent form if I have requested a copy. I am aware that this research has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois (UI).

As a volunteer, I give my informed consent to the Board of Trustees of the UI and the Veterinary Teaching Hospital (VTH) to enroll my horse in this study, according to the explanations and conditions presented in this document. I agree to hold harmless the Board of Trustees of the UI, the VTH, and its officers, employees, agents and assigns from any and all liability, claims and actions that may arise from participation in this study.

I approve the participation of my horse(s) in the following studies:

□ Investigation of genetic risk factors underlying **OC** (willing to share with us if your horse ever had OC/OCD)

□ Investigation of genetic risk factors affecting **gait**

IACUC Approval #: 15031

_____	_____	____ / ____ / 20_____
Owner's Printed Name (or authorized agent)	Signature of Owner (or authorized agent)	Date
_____	_____	____ / ____ / 20_____
Witness' Printed Name	Signature of Witness	Date
Brendan McKiernan		Feb. 24, 2015
VTH Director/Chief of Staff	Signature of Director/Chief of Staff	Date

My additional horses that I approve to be included in the above study/studies

Horse Name or Tattoo	Horse Name or Tattoo

Owner's Printed Name (or authorized agent)	Signature of Owner (or authorized agent)	____ / ____ / 20____ Date

(Original to medical records)

VII. Recombinant DNA/Biohazards: N/A

VIII. Facilities and Equipment

Foals will be recruited for this study from the University of Illinois (UIUC) Horse Farm and from 2 nearby private breeding farms that routinely collaborate with Dr. Kline (Co-investigator). Approximately 80 foals are raised each year among the participating farms, and most of the foals are sold at breed-recognized yearling sales each fall. Management is similar among the facilities, including exercise regimens: foals are kept with their dams in a large box stall or small run for the first ~2 weeks of life, and then are turned out in a large paddock or pasture with the other foals/mares. After weaning, the foals are turned out together. At each facility, personnel experienced in handling foals and mares will be available to assist with restraint during exams. Large box stalls will be available for all exams. Radiographs will be taken with a MinXray HF8020 portable high frequency veterinary x-ray unit with a wireless plate and laptop running Metron software for digital image capture. Radiographs will be uploaded as DICOM images to the UIUC College of Veterinary Medicine PACS system for long-term secure storage. Video observation will be performed using a GoPro HERO3+ camera with waterproof housing and mounting hardware allowing it to be attached to a fence. Digital video will be uploaded to Dr. McCoy's (PI) lab server space for storage and analysis.

All necessary lab work will be performed in Dr. McCoy's laboratory. Dr. McCoy has approximately 400 sq ft of laboratory space. Laboratory equipment available in the McCoy lab includes: 2 water baths, a dry bath, 8 pipetters, 2 multi-channel pipetters, 1 each repeater pipette and serological pipette, 2 BioRad horizontal gel electrophoresis with 26 well combs and power supplies, 1 BioRad mini-horizontal gel electrophoresis unit, 1 Bio-Rad T100 96 well plate format thermal cycler, a refrigerated microcentrifuge, Thermo Scientific Sorvall ST16R 15,200 rpm refrigerated table-top centrifuge, Nanodrop 2000 spectrophotometer, fume hood with UV light, Spectrolinker XL-1500 UV crosslinker, one 15 cu. ft -20 C freezer, and one 20 cu. ft. -80 C freezer. Equipment in the neighboring colleague's lab that will be available includes additional pipetters, thermal cyclers, and water baths, as well as a fluorescent plate reader and gel imaging system, biopulverizers, and a Spex mill. Shared larger equipment available within the college includes a Millipore water purification system and 4 ABI Real-Time PCR systems.

Available computing resources in the McCoy lab include 2 windows computers, both Dell dual quad (8) processor workstations, with a dual installed Linux virtual machine, and maintain (Linux and/or Windows) versions of PLINK, R (SNP Matrix, Pedigreemm, HClust, etc), PHASE, fastPHASE, Sequencher, and others. The computers are also be fully equipped with word processing, spreadsheet, and database software. Additionally, Dr. McCoy has access to the UIUC High-Performance Biological Computing center (HPCBio). This includes access to HPCBio's computational infrastructure (including two Campus Clusters with 512 computing nodes each, and the Blue Waters supercomputer with 1.5 petabytes of memory and 25 petabytes of disk storage) and a broad suite of genomics software. Large data sets are stored both locally and remotely at HPCBio. Remote access to these resources is available.

Sequenom assay genotyping will be performed by Neogen GeneSeek (Lincoln, NE). Sanger sequencing will be performed by the the University of Illinois Roy J Carver Biotechnology Center's High-Throughput Sequencing and Genotyping Unit. The facility maintains ABI 3730xl DNA Analyzers for fragment analysis and Sanger sequencing and offers custom oligonucleotide preparation.

IX. Cited References

Cothran EG, MacCluer JW, Weitkamp LR, Bailey E (1987), Genetic differentiation associated with gait within American standardbred horses. *Anim Genet.* 18:285-296

Dik KJ, Enzerink E, van Weeren PR (1999), Radiographic development of osteochondral abnormalities, in the hock and stifle of Dutch Warmblood foals, from age 1 to 11 months. *Equine Vet J Suppl* 31:9-16.

Drevemo S, Dalin G, Fredricson I, Hjertén G (1980), Equine locomotion: 1. The analysis of linear and temporal stride characteristics of trotting Standardbreds. *Equine Vet J* 12:60-65.

Grondahl AM, Dolvik NI (1993), Heritability estimations of osteochondrosis in the tibiotarsal joint and of bony fragments in the palmar/plantar portion of the metacarpo- and metatarsophalangeal joints of horses. *J.Am.Vet.Med.Assoc.* 203:101-104.

Lepeule J, Bareille N, Robert C, Ezanno P, Valette JP, Jacquet S, Blanchard G, Denoix JM, Seegers H (2009), Association of growth, feeding practices and exercise conditions with the prevalence of Developmental Orthopaedic Disease in limbs of French foals at weaning. *Prev.Vet.Med.* 89:167-177.

Lepeule J, Bareille N, Robert C, Valette JP, Jacquet S, Blanchard G, Denoix JM, Seegers H (2013), Association of growth, feeding practices and exercise conditions with the severity of the osteoarticular status of limbs in French foals. *Vet.J.* 197:65-71**.**

Li MX, Yeung JM, Cherny SS, Sham PC (2012), Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Hum Genet.* 131:747-756.

Lykkjen S, Roed KH, Dolvik NI (2012), Osteochondrosis and osteochondral fragments in Standardbred trotters: prevalence and relationships. *Equine Vet.J.* 44:332-338.

McCoy AM, McCue ME (2014), Validation of imputation between equine genotyping arrays. *Anim Genet* 45:153.

McCoy AM, Ralston SL, McCue ME (2015), Short- and long-term performance of Standardbred pacers and trotters after early surgical intervention for tarsal osteochondrosis. *Equine Vet J* 47:438-444.

Pool RR. Pathogenesis of developmental lesions. In: McIlwraith CW ed. *Proceedings Panel on Developmental Orthopedic Disease.* 1986;22-25.

Ricard A, Perrocheau M, Courouce-Malblanc A, Valette JP, Tourtoulou G, Dufosset JM, Robert C, Chaffaux S, Denoix JM, Guerin G (2013), Genetic parameters of juvenile osteochondral conditions (JOCC) in French Trotters. *Vet.J.* 197:77-82.

Robilliard JJ, Pfau T, Wilson AM (2007), Gait characterisation and classification in horses. *J Exp Biol* 210:187-197.

Stock KF, Hamann H, Distl O (2005), Estimation of genetic parameters for the prevalence of osseous fragments in limb joints of Hanoverian Warmblood horses. *J.Anim Breed.Genet.* 122:271-280.

Valentino LW, Lillich JD, Gaughan EM, Biller DR, Raub RH (1999), Radiographic prevalence of osteochondrosis in yearling feral horses. *Vet.Comp Orthop.Traumatol.* 12:151-155.

van Grevenhof EM, Schurink A, Ducro BJ, van Weeren PR, Van Tartwijk JM, Bijma P, van Arendonk JA (2009), Genetic variables of various manifestations of osteochondrosis and their correlations between and within joints in Dutch warmblood horses. *J.Anim Sci.* 87:1906-1912.

van Weeren PR, Barneveld A (1999), The effect of exercise on the distribution and manifestation of osteochondrotic lesions in the Warmblood foal. *Equine Vet J Suppl* 31:16-25.

Wilson BD, Neal RJ, Howard A, Groenendyk S (1988a), The gait of pacers 1: kinematics of the racing stride. *Equine Vet J* 20:341-46.

Wilson BD, Neal RJ, Howard A, Groenendyk S (1988b), The gait of pacers 2: factors influencing pacing speed. *Equine Vet J* 20:347-351.

Wittwer C, Hamann H, Rosenberger E, Distl O (2006), Prevalence of osteochondrosis in the limb joints of South German Coldblood horses. *J.Vet.Med.A Physiol Pathol.Clin.Med.* 53:531-539.

United States Trotting Association. Standardbred Breed Information, Available at: http://fanguide.ustrotting.com/standardbred-breed.cfm. Accessed Nov 18, 2013.

Zhou X, Stephens M (2012), Genome-wide efficient mixed-model analysis for association studies. *Nat.Genet.* 44:821-824.

X. Budget

PROPOSAL BUDGET

Note: First Award – complete year 1, year 2 and total only. **Pilot Study** – complete year 1 only. **Fellowship Training** – complete salary, fringe benefits, indirect costs and total for year 1 and year 2 only.

Category	Year 1	Year 2	Year 3	Total
Personnel: 1. Principal investigator (Annette McCoy)* 2. Co-investigator #1 (Kevin Kline) 3. Co-investigator #2 (Molly McCue) 4. Technician (Sulalita Chaki) Salary (10% effort * $31,751) Fringe benefits (39.14%) 5. Student Assistant (TBD) Salary (200 hours @ $10/hr) Fringe benefits (0.15%) Total Salaries & Wages	[redacted]	[redacted]	[redacted]	[redacted]
Supplies & Expenses: 1. Radiographs ($100/foal/session) 2. Sequenom genotyping ($40/sample) 3. Consumable supplies 4. Sequencing and RFLP genotyping 5. Statistical consulting 6. Conference travel Provide justification in the designated section. Total Supplies & Expenses:	[redacted]	[redacted]	[redacted]	[redacted]
Animal Use & Care: Animal Purchase: N/A Animal Per diem: N/A Total Animal Care: N/A	[redacted]	[redacted]	[redacted]	[redacted]
Subtotal of All Categories:	[redacted]	[redacted]	[redacted]	[redacted]
Maximum of 8% - Indirect Costs:**	[redacted]	[redacted]	[redacted]	[redacted]
Grand Total Requested from MAF:	[redacted]	[redacted]	[redacted]	[redacted]

* Salary requests for principal investigators must be clearly defined and justified in the following budget justification section. You may request salary for technicians, residents, graduate students, and postdoctoral fellows, based on their percentage of time involved in the project

** Indirect costs may be claimed only if you are charged for indirect costs by your institution for work carried out in this proposal. **You must make this calculation yourself.** If your institution charges less than 8%, claim only that amount and indicate the percentage.

XI. Itemized Budget Justification

Personnel

Dr. Annette McCoy (PI): Dr. McCoy is a veterinarian board-certified in Large Animal Surgery with research experience in equine genetics and genomics and clinical expertise in musculoskeletal disease in the horse. Dr. McCoy generated the preliminary data for this proposal. She will coordinate and oversee the radiographs and field observation for Aim 1 of this proposal. She will also be primarily responsible for radiograph interpretation and will verify scoring of activity levels from video observations. For Aim 2, Dr. McCoy will oversee sample preparation and will perform all data analysis. Dr. McCoy will be responsible for primary drafts of manuscripts for publication.

Dr. Molly McCue (Mentor/Co-I): Dr. McCue is a veterinarian board-certified in internal medicine with extensive research experience in equine genetics and genomics, particularly in the area of statistical genetics. Dr. McCue is the Mentor for this First Award and will provide guidance and subject expertise during data analysis and interpretation and will collaborate on production of manuscripts for publication. Dr. McCue and Dr. McCoy will have weekly meetings via Skype to discuss the progress of the project.

Dr. Kevin Kline (Co-I): Dr. Kline is a professor in the Animal Science department with a shared research and extension appointment, specializing in horse production and management. He runs the University of Illinois horse farm and has extensive connections in the Standardbred community. Dr. Kline will oversee recruitment of foals into the study and will collaborate on production of manuscripts for publication.

Sulalita Chaki (Technician): Ms. Chaki is a technician in Dr. McCoy's laboratory experienced in numerous molecular biology techniques. She will perform DNA isolation, PCR, and genotyping by sequencing and RFLP. We are requesting 10% salary support (plus fringe) for Ms. Chaki.

Student Assistant: We are requesting salary support for 200 hours (plus fringe) for a student assistant for this project. The student will have primary responsibility for field monitoring (setting up cameras for video observation, reviewing and logging videos) and will assist with taking radiographs as needed.

Supplies & Expenses

Radiographs: Radiographs will be taken at 60, 120, 180, 240, 300, and 360 days (+/- 7 days) of age. Images will be obtained with a digital x-ray unit and stored on the hospital server. The cost of 1 full set of hock films (including use of equipment and personnel, plus data storage) is [redacted] we anticipate 6 sets of films per foal. 86 foals x [redacted]. Because films will be taken over 2 foaling seasons, this breaks down to [redacted] in Year 1 (n = 60 foals) and [redacted] in Year 2 (n = 26 foals).

Sequenom genotyping: Primers and multiplex design for the Sequenom assay were completed during the preliminary work for this proposal. Genotyping will cost [redacted] per horse. [redacted]

Consumable supplies: We are requesting [redacted] in each year of the award for consumable supplies that will be used during sample preparation as well as genotyping by sequencing and RFLP. This includes the cost of disposable plastic ware, PCR primers, agarose gels, polymerases, restriction enzymes, etc.

Sequencing and RFLP genotyping: Additional risk alleles (not included in the Sequenom assay) will be genotyped in our study cohort via restriction fragment length polymorphism (RFLP) or Sanger sequencing. The cost for sequencing is [redacted]/sample. We are requesting [redacted] in the second year of the study which will allow us to genotype approximately 15 variants per horse in our entire cohort.

Statistical Consulting: We will work with experts at ATLAS Statistics (University of Illinois College of Liberal Arts and Sciences) during our final data analysis. We are budgeting for 15 hours of consultation time in the second year of the study. [redacted]

Conference Travel: We are requesting [redacted] in the final year of the study to fund PI travel to two conferences – one focused on clinical medicine/orthopedics, and one on genetics. This is based on projected expenses related to conference registration, airfare, and accommodations.

XIII. Prior MAF Support during last three years for McCoy (PI)

MAF ID # D15EQ-813
Validation of Putative Genetic Risk Alleles for Osteochondrosis in Standardbreds
Funding period: January 2015- Dec 2015
Total Amount: [redacted]

Brief summary of the objectives: Osteochondrosis (OC) is a widely recognized manifestation of developmental orthopedic disease that affects weanling and yearling horses across breeds. Manifestations of OC can vary from mild to severe, but nearly always require surgical intervention to prevent ongoing joint damage. While OC is known to be influenced by environmental risk factors, heritability estimates suggest that as much as 50% of disease risk is due to genetics. It is likely that genetic risk results from a combination of alleles in several different genes. We have identified a number of putative genetic risk alleles for OC in a cohort of Standardbred yearlings, and in this proposal we seek to validate these findings in an independent population. Identification and validation of specific risk alleles is crucial to developing a genetic risk model for OC that could be applied to individual horses. *We hypothesize that one or more alleles underlying OC risk are present within the genomic regions identified in our discovery population*. The **objective** of this proposal is to confirm that specific putative risk alleles also associate with disease in an independent population of Standardbreds and can thus be considered to be true risk alleles. Deep resequencing of the regions surrounding validated alleles will be completed to confirm that the associated allele is the true functional allele. Horses included in this study are Norwegian Standardbreds (n = 162; 80 affected with hock OC, 82 unaffected). DNA will be submitted for genotyping on a previously designed Sequenom genotyping assay. Mixed model analysis will be performed to identify alleles significantly associated with OC in the validation cohort. Those alleles which are associated with OC in both the discovery and validation populations will be considered to be true risk alleles. Random forest analysis will be used to determine the relative contribution of individual variants to OC and to illuminate potential interactions between variants. Deep Sanger resequencing will be performed around each of the alleles confirmed to be associated with OC in the validation cohort to make sure that functional alleles are not missed.

Summary of results to date: A GWA analysis was performed in 182 Standardbred horses from a single breeding farm in the United States (70 affected with tarsal OC, 112 unaffected). The GWA was performed using GEMMA (Genome-wide Efficient Mixed Model Analysis) software, incorporating a relatedness matrix constructed from a LD-pruned marker set. Two distinct loci on ECA 14 were most highly associated with OC status in this cohort ($p=1.8x10^{-4}$ to $8x10^{-6}$). Eight additional regions of interest were identified on 7 other chromosomes, including ECA10 ($p=3.9x10^{-4}$ to $8.3x10^{-5}$) and 21 ($p=4.3x10^{-4}$ to $1.5x10^{-4}$). Variant discovery was subsequently performed via whole-genome sequencing in 18 horses (9 OC cases and 9 controls). 215,712 variants were identified within the 10 regions of interest identified in the GWA, and 1,271,635 variants were identified from 22 previously reported regions of association for tarsal OC. These 1,487,347 variants were prioritized based on predicted functional effect and segregation with OC status. 240 variants from regions on 10 chromosomes were selected for follow-up genotyping using a Sequenom genotyping assay. The 240 variants, along with 98 ancestry informative markers (AIMs), were genotyped in both the 182 horses in the GWA cohort as well as an independent validation cohort of 139 Norwegian Standardbreds (60 affected with tarsal OC, 79 unaffected).

Within the GWA cohort, variants from ECA10 (n=2, p=0.0076-0.015), 14 (region 1, n=3, p=0.019-0.049; region 2, n=4, p=0.0008-0.018), and 21 (n=5, p=0.008-0.043) were most highly associated with OC status after GEMMA mixed model analysis. Within the validation cohort, the variant most highly associated with OC status (p=0.0014), as well as two additional highly associated SNPs (p=0.0089-0.0058), were from "region 1" on ECA14, the top GWA region of association in the discovery population. Three additional genotyped variants highly associated with OC status in the Norwegian horses were from chromosomal regions of interest previously reported in a GWA in this same population (ECA1 and ECA3).

We have successfully demonstrated shared risk loci for OC in two independent populations of Standardbreds. This is the first successful validation of findings from a genome-wide association study for OC in horses. The genotyping results from this study are included in a manuscript that will be submitted before the end of July 2015.

List of Publications: McCoy, AM, Beeson, SK, Lykkjen, S, Ralston, SL, **McCue, ME**. Identification and Validation of Risk Loci for Osteochondrosis in Standardbreds. *BMC Genomics*
List of Presentations: None to date
List of patents resulting from MAF awards: none at this time.

XIII. Prior MAF support during last three years for McCue (Mentor)

MAF ID # D15EQ-029
Role of endocrine disrupting chemicals in equine metabolic syndrome.
Funding period: January 2015- Dec 2015
Total Amount: [redacted]

Brief summary of the objectives: Our prior work has demonstrated that equine metabolic syndrome (EMS) and its metabolic components, including hyperinsulinemia, adiposity and key biochemical and hormonal measures, are influenced by both environmental and individual animal factors. We have collected 11 morphometric, biochemical and hormonal phenotypes along with epidemiologic and environmental data from 610 horses/ponies from 166 farms. Multi-level regression modeling demonstrated that 23-49% of the variability in phenotype was due to shared environment (farm). Individual factors (ie. age, breed, sex, laminitis status, obesity) accounted for 3-16%, and environmental factors (ie diet, exercise, season) accounted for 4-18% of the phenotypic variation in the data. However, a large portion of the phenotypic variability in this cohort was not explained by our 16 predictors. Thus, a significant amount of metabolic variation in EMS remains unexplained, which we believe is due in large part to as yet unmeasured environmental risk factors and undetermined specific genetic risk alleles. Epidemiologic studies have linked environmental exposure to synthetic and naturally occurring chemicals that disturb endogenous endocrine signaling pathways ('endocrine disrupting chemicals' [EDCs]) with underlying components and long term consequences of metabolic syndrome (MetS) in humans; and our preliminary data links potential EDC exposure to laminitis and abnormal insulin responses in horses. Specifically, we have examined proximity of our cohort of horses to US EPA 'Superfund sites' containing EDCs as a possible contributor to environmental risk. Horses from farms < 30 miles of a Superfund site were significantly more likely to have a history of laminitis (p=0.002) and have higher insulin responses post oral sugar challenge (OST INS, p=0.00005) when compared to horses on farms > 30 miles from a Superfund site, suggesting that exposure to POP/EDCs may play a role in the phenotypic variation seen in our cohort. Concurrently, our genetic studies on EMS have identified 3 non-synonymous SNPs in the trans-activation domain of the equine aryl hydrocarbon receptor (*AHR*) gene. The aryl hydrocarbon receptor (Ahr) is a ligand-dependent transcription factor that mediates a wide range of cellular effects resulting from exposure to synthetic and naturally occurring chemicals, including EDCs. One of the *AHR* SNPs that we identified (*Val556Met*) was associated with OST INS values (p=0.002) and two haplotypes across 7 SNPs in the *AHR* trans-activation domain were strongly associated with OST INS (p=0.0007 and 0.0009; overall p=0.0002). Taken together, these data suggest that further investigation of the relationship between these *AHR* variants and metabolic traits, and a potential interaction between *AHR* genotype and environmental EDCs, is needed.

We hypothesized that EDCs are an important environmental factor for the development of EMS and that genotypic variation in the AHR may mediate the effects of EDCs in horses. Our **objectives** in this proposal are to: **1) Determine the role of endocrine disrupting chemicals in metabolic variation in horses, and 2) to identify interactions between EDCs and *AHR* genotype.** We propose to quantify EDCs in banked serum samples from 139 Welsh Ponies from 14 farms and 161 Morgan horses from 18 farms (total n=300 from 32 farms) using cell-based reporter bioassays that measure dioxin and organochlorine compound levels based on their ability to interact with the Ahr and estrogen receptors (ER), respectively. Toxic equivalency factors (TEFs) will be calculated from the Ahr assay and β-estradiol equivalents will be calculated from the ER assay. TEFs and β-estradiol equivalents will be correlated to EMS phenotypic responses using multi-level, multivariate, multiple regression modeling. The roles of *AHR* genotype and genotype-by-environment (ie EDC level) interaction, will be determined by inclusion of *AHR* genotype in the statistical models.

Progress to date: Frozen heparinized serum samples from all 300 horses were to BD Systems in the Netherlands in the second week of March. BDS has generated data on the first 50 samples for both the DR-CALUX and the ERα-CALUX. DR-CALUX results are as anticipated. ERα-CALUX assays are lower than

expected, which BDS believes is due to the fact that estrogens in horse serum are mainly present as sulfate conjugates. BDS is currently evaluating de-conjugation procedures before moving forward with additional ERα-CALUX assays.

List of Publications: We anticipate a minimum of one publication for each objective upon completion.

List of Presentations: None to date

List of patents resulting from MAF awards: none at this time.

MAF ID# D15EQ-031
Gene Loci for Recurrent Exertional Rhabdomyolysis in Thoroughbreds and Standardbreds
Funding period: January 2015- Dec 2015
Total Amount: [redacted]

Brief summary of the objectives: Approximately 5 - 10% of Thoroughbred (TB) and Standardbred (STB) racehorses suffer from recurrent exertional rhabdomyolysis (RER), in which they exhibit sporadic bouts of painful cramping and muscle cell damage following mild to moderate exercise. This condition also affects TB and TB crossbreds used for performance events such as three day eventing, hunter jumper, and barrel racing competitions; the same or a closely related form of exertional rhabdomyolysis is present in many other breeds including the Quarter Horse. Losses due to RER come from the cost of veterinary care, lost training time, and less frequent participation in competition. Previous research is consistent with an underlying genetic basis to RER susceptibility, with gender, temperament, diet, age, and activity being contributing factors. We hypothesize that genes of moderate to major effect underlie RER susceptibility in both TB and STB horses, and the goals of this proposal are to identify these gene loci and their underlying functional mutations associated with RER risk in both breeds. This will be achieved through three objectives: **1)** an enhanced genome-wide association analysis (GWAS) with SNP markers and high-density haplotype analysis to precisely locate RER susceptibility loci; **2)** identifying the sequence variants in these regions through whole genome sequencing of multiple cases and controls; and, **3)** genotyping high-priority variants, based on their allele frequencies in the sequenced horses and their predicted SNP effects, in the entire case and control population. We will first impute our existing GWAS data to 670K genome-wide SNPs to increase the genetic mapping power, and confirm the chromosomal regions and underlying haplotypes associated with RER risk in each breed. We will then use whole genome sequencing (10 cases and 10 controls in each breed) to identify the sequence variants in the RER-associated regions; these variants will be prioritized based on their predicted effects on gene or encoded protein function and estimated allele frequencies in cases vs controls. The highest-priority variants will then be genotyped on the entire cohort to identify the most likely RER functional mutations.

Summary of results to date: Using genotype data from an equine haplotype map resource being developed in our laboratory, we have now imputed the whole genome SNP data to greater than 1,450,000 SNPs in the Thoroughbreds and 540,000 in the Standardbreds with >90% imputation accuracy and minor allele frequency of greater than 5%. Using these data we have now repeated the GWAS analysis in each population independently and across both breeds. This has led us to concentrate our GWAS to 2 loci (ECA16 and ECA30) in the Thoroughbreds and 1 locus (ECA14) in the Standardbreds, with one locus (ECA11) shared by both breeds. We have also selected and submitted the 10 Standardbreds (5 RER and 5 controls) for whole genome sequencing based on haplotypes at the ECA14 and ECA11 loci, and selected the 10 Thoroughbreds to be sequenced based on haplotypes at the ECA11, ECA16, and ECA30 loci.

List of Publications: We anticipate a minimum of one publication for each objective upon completion.

List of Presentations: We will also be reporting on the results of this project at the Havemeyer Equine Genome Mapping Workshop in Hannover Germany in July 2015.

List of patents resulting from MAF awards: none at this time.

XIII. Prior MAF support during last three years for Kline (Co-investigator)

None

XIV. Biographical Data

NAME	POSITION TITLE
Annette Marie McCoy	Assistant Professor, Dep't of Veterinary Clinical Medicine University of Illinois College of Veterinary Medicine 1008 W Hazelwood Dr., Urbana, IL 61802

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Michigan State University, East Lansing, MI	B.S.	05/2002	Animal Science (GPA 3.98/4.0)
Michigan State University, East Lansing, MI	D.V.M.	05/2006	Veterinary Medicine (GPA 3.93/4.0)
University of Minnesota, St. Paul, MN	Internship	06/2006-07/2007	Large Animal Medicine and Surgery
Colorado State University, Ft. Collins, CO	M.S.	05/2010	Clinical Sciences (GPA 4.0/4.0)
Colorado State University, Ft. Collins, CO	Residency	07/2007-07/2010	Equine Surgery and Lameness
University of Minnesota, St. Paul, MN	Ph.D.	09/2010-5/2014	Comparative and Molecular Biosciences (GPA 4.0/4.0)
University of Minnesota, St. Paul, MN	Post-doctoral	5/2014-10/2014	Computational Genetics

A. Role: Principal Investigator

B. Positions and Honors

Positions

Professorial Assistant, Michigan State University 1999-2001
Research Assistant, Michigan State University 2001-2002
Veterinary Research Scholar, Kansas State University 5/2004-8/2004
Internship, Large Animal Medicine and Surgery, University of Minnesota 2006-2007
Residency, Equine Surgery and Lameness, Colorado State University 2007-2010
Postdoctoral Fellow/PhD Candidate, University of Minnesota 2010-2014
Postdoctoral Associate, University of Minnesota 5/2014-10/2014
Assistant Professor, University of Illinois 2015-present

Honors

Board Certification: Large Animal Surgery (American College of Veterinary Surgeons) 2011
Stephen J. O'Brian Award (American Genetic Association) 2015
Doctoral Dissertation Fellowship (Univ of Minnesota) 2013-2014
NIH T32 Training Grant in Comparative Medicine and Pathology 2010-2013
Council of Graduate Students Travel Award (Univ of Minnesota) 2013
Vaughn Larson Scholarship Award (Univ of Minnesota) 2012
AAEP Foundation Past Presidents' Research Fellow 2011
ACVS Resident's Forum 2010, 2nd place, Large Animal Clinical Research Presentation
Mitzy H. Yount Memorial Scholarship (Colorado State Univ) 2009-2010
Doctor of Veterinary Medicine, *With Highest Honors*
J.P. Hutton-W.F. Riley Equine Award (Michigan State Univ) 2006
Phi Zeta Award (Michigan State Univ) 2006

CVM Alumni Council Student Leadership Award (Michigan State Univ) 2006
Theriogenology Award (Michigan State Univ) 2006
Phi Zeta Research Day Award for Best Oral Presentation by a Veterinary Student (Michigan State Univ) 2004
Bachelor of Science in Animal Science, *With Highest Honors*
Richard Lee Featherstone Society Prize (Michigan State Univ) 2002
American Society of Animal Science Scholarship Award (Michigan State Univ) 2002
Alumni Distinguished Scholarship (Michigan State Univ) 1999-2003

Professional Organizations
American College of Veterinary Surgeons
American Veterinary Medical Association
American Association of Equine Practitioners
The Society of Phi Zeta, Zeta Chapter
Orthopaedic Research Society
Veterinary Orthopedic Society

C. Peer-reviewed Publications

1. **McCoy AM**. 2015. Animal models of osteoarthritis: comparisons and key considerations. *Vet Path* 2015 June 10 [Epub ahead of print] DOI:10.1177/0300985815588611.

1. **McCoy AM**, Ralston SL, McCue ME. 2015. Short- and long-term performance of Standardbred pacers and trotters after early surgical intervention for tarsal osteochondrosis. *Equine Vet J* 47:438-444 DOI: 10.1111/evj.12297.

2. **McCoy AM**, McCue ME. 2014. Validation of imputation between equine genotyping arrays. *Anim Genet* 45:153 DOI: 10.1111/age.12093.

3. **McCoy AM**, Schaefer R, Petersen JL, Morrell PL, Slamka MA, Mickelson JR, Valberg SJ, McCue ME. 2014. Evidence of positive selection for a glycogen synthase (*GYS1*) mutation in domestic horse populations. *J Hered* 105:163-172. DOI: 10.1093/jhered/est075.

- selected for the 2015 Stephen J. O'Brian Award from the American Genetic Association

4. **McCoy AM**, Toth F, Dolvik NI, Ekman S, Ellemann J, Olstad K, Ytrehus B, Carlson CS. 2013. Articular osteochondrosis: a comparison of naturally-occurring human and animal disease. *Osteoarthritis Cart* 21:1638-1647. DOI: 10.1016/j.joca.2013.08.011. PMCID:3815567.

5. **McCoy, AM**, LR Goodrich. 2012. Use of a radiofrequency probe for tenoscopic-guided annular ligament desmotomy. *Equine Vet J* 44:412-415. DOI: 10.1111/j.2042-3306.2011.00454.x.

6. **McCoy AM**, ES Hackett, AE Wagner, KR Mama, DA Hendrickson. 2011. Pulmonary gas exchange and plasma lactate in horses with gastrointestinal disease undergoing emergency exploratory laparotomy: a comparison with an elective surgery patient population. *Vet Surg* 40:601-609. doi: 10.1111/j.1532-950X.2011.00840.x [Epub 2011 May 3].

7. **McCoy AM**, ES Hackett, RJ Callan, BE Powers. 2009. Alimentary-associated carcinomas in Vietnamese pot-bellied pigs: 5 cases (2000-2008). *J Am Vet Med Assoc* 235:1336-1341.

8. Beilock S, BI Bertenthal, **AM McCoy**, TH Carr. 2004. Haste does not always make waste: expertise, direction of attention, and speed versus accuracy in performing sensorimotor skills. *Psychonomic Bulletin and Review* 11:373-379.

NAME	POSITION TITLE
Molly Elizabeth McCue	Associate Professor; Veterinary Population Med Department College of Veterinary Medicine, University of Minnesota 225 VMC, 1365 Gortner Ave, St. Paul, MN 55108

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Kansas State University, Manhattan Kansas	BS	1998	Animal Science
Kansas State University, Manhattan Kansas	DVM	2000	Veterinary Medicine
University of Georgia, Athens Georgia	Internship	2001	Large Animal Medicine and Surgery
Kansas State University, Manhattan Kansas	MS	2004	Clinical Sciences (Epidemiology)
Kansas State University, Manhattan Kansas	Residency	2004	Equine Internal Medicine
University of Minnesota, St. Paul Minnesota	PhD	2007	Comparative and Molecular Biosciences
University of Minnesota, St. Paul Minnesota	Post-doctoral	2008	Genetic Epidemiology

A. Role: Mentor/Co-investigator

B. Positions and Honors

2008-2013 Assistant Professor, University of Minnesota
2009-present Faculty, Paul and Sheila Wellstone Muscular Dystrophy Center, Uof Minnesota
2013-present Faculty, Microbial and Plant Genomics Institute, University of Minnesota
2013-present Associate Professor, University of Minnesota
2014-present Informatics Institute Transdisciplinary Faculty Fellow, University of Minnesota
Board Certification*:* Diplomate American College of Veterinary Internal Medicine (Large Animal)
2014 Inventor Recognition Award University of Minnesota
2013-present NSRP8 Equine Genome Co-coordinator
2011 NRSP8 Equine Genome Workshop Chair
2010 NRSP8 Equine Genome Workshop co-Chair
2008 Inventor Recognition Award University of Minnesota
2007-2008 Morris Animal Foundation Fellow
2007-2008 University of Minnesota Doctoral Dissertation Fellow
2007-2008 University of Minnesota Women's Leadership Institute
2007 Best Graduate student research Award University of Minnesota
2004-2007 NIH Comparative Medicine and Pathology Post-Doctoral Fellowship
Doctor of Veterinary Medicine *Summa Cum Laude*
Bachelor of Science, Animal Science and Veterinary Medicine *Magna Cum Laude*

C. Selected Peer-Reviewed Papers (last 2 years, out of 48 total)

** indicates manuscript by a graduate or ^ post-doctoral student in Dr. McCue's laboratory*

1. ^Petersen JL, Mickelson JR, Valberg SJ, **McCue ME.** Genome-wide SNP data shows little differentiation between the Appaloosa and other American stock horse breeds. ***Animal Genetics*** *Article first published online: 22 MAY 2015 DOI: 10.1111/age.12301.*
2. **McCue ME**, Geor RJ, Shultz NE. Equine Metabolic Syndrome: a complex disease influenced by genetics and the environment. ***Journal of Equine Veterinary Science*** 35 (5) (2015): 367-375.
3. ^Petersen JL, Valberg SJ, Mickelson JR, **McCue ME**. Haplotype diversity in the equine myostatin gene with focus on variants associated with race distance propensity and muscle fiber type proportions ***Animal Genetics*** *published online: 26 August 2014 DOI: 10.1111/age.12205.* PMID:25160752.

4. *McCoy AM, Ralston SL, **McCue ME**. Short- and long-term racing performance of Standardbred pacers and trotters after early surgical intervention for tarsal osteochondrosis. ***Equine Veterinary Journal*** 47:438-444, 2015 *DOI: 10.1111/evj.12297.* PMID:24819047.
5. Schubert M, Ermini L, Der Sarkissian C, Jónsson H, Ginolhac A, Schaefer R, Martin MD, Fernández R, Kircher M, **McCue ME**, Willerslev E, Orlando L. Characterization of ancient and modern genomes by SNP detection and phylogenomic and metagenomic analysis using PALEOMIX. ***Nature Protocols*** 9:1056-1082, 2014. PMID:24722405.
6. *McCoy AM, Schaefer R, Petersen JL, Slamka MA, Morrell PL, Mickelson JR, Valberg SJ, **McCue ME**. Evidence of Positive Selection for the *GYS1* mutation in Equine Polysaccharide Storage Myopathy. ***Journal of Heredity*** 105:163-172, 2014. PMCID: PMC3920812.
7. Martinson K, Coleman B, Rendahl AK, **McCue, ME**. Estimation of body weight and development of a body weight score for adult equines using morphometric measurements. ***Journal of Animal Science*** 92:2230-2238, 2014. PMID:24663191.
8. ^Fritz KL, Hendrickson JA, Kaese HJ, Rendahl AK, Dynes KM, Lucio MA, Cuomo FM, Brinkmeyer-Langford CL, Skow LC, Valberg SJ, Mickelson JR, Rutherford MS, **McCue ME**. Genetic risk factors for insidious Equine Recurrent Uveitis in Appaloosa horses. ***Animal Genetics*** 45:392-399, 2014. PMID:24467435.
9. ^Petersen JL, Cleary K, Mickelson JR, **McCue ME**. The American Quarter Horse: Population Structure and relationship to the Thoroughbred. ***Journal of Heredity*** 105:148-162, 2014. PMCID: PMC3920813.
10. *McCoy AM, **McCue ME**. Validation of imputation between equine genotyping arrays. ***Animal Genetics*** 45:153, 2014. PMCID: PMC4000747.
11. *Teixeira, R, Rendahl AK, Anderson, S, Sigler, D, Buchanan, B, Coleman, B, Mickelson, JR, **McCue, ME**. Coat color genotypes and risk and severity of melanoma in gray Quarter Horses. ***Journal of Veterinary Internal Medicine*** 27:1201-8, 2013. PMID:23875712.
12. Lykkjen, S, Dolvik, NI, **McCue, ME**, Rendahl, AK, Mickelson, JR, Roed, KH. Equine developmental orthopaedic diseases – a genome-wide association study of first phalanx plantar osteochondral fragments in Standardbred trotters. ***Animal Genetics***. 44:766-9, 2013. PMID:23742657.
13. ^Petersen JL, Mickelson JR, Cothran EG, Rendahl AK, Andersson LS, Axelsson J, Bailey E, Bannasch D, Binns MM, Borges AS, Brama P, de Câmara Machado A, Distl O, Felicetti M, Fox-Clipsham L, Graves KT , Guérin G, Haase B, Hasegawa T, Hemmann K, Hill EW, Leeb T, Lindgren G, Lohi H, Lopes MS, McGivney BA, Mikko S, Orr N, Penedo MCT, Piercy RJ, Raekallio M, Rieder S, Røed KH, Silvestrelli M, Swinburne J, Tozaki T, Vaudin M, Wade CM, **McCue ME**. Genetic diversity in the modern horse illustrated from genome-wide SNP data. ***PLoS One*** *2013: 8(1): e54997 DOI:10.1371/journal.pone.0054997.*
14. ^Petersen JL, Mickelson JR, Rendahl AK, Valberg SJ, Andersson LS, Axelsson J, Bailey E, Bannasch D, Binns MM, Borges AS, Brama P, de Câmara Machado A, Capomaccio S, Cappelli K, Cothran EG, Distl O, Fox-Clipsham L, Graves KT , Guérin G, Haase B, Hasegawa T, Hemmann K, Hill EW, Leeb T, Lindgren G, Lohi H, Lopes MS, McGivney BA, Mikko S, Orr N, Penedo MCT, Piercy RJ, Raekallio M, Rieder S, Røed KH, Swinburne J, Tozaki T, Vaudin M, Wade CM, **McCue ME**. Genome-wide analysis reveals selection for important traits in domestic horse breeds. ***PLoS Genetics*** *2013: 9(1): e1003211, DOI:10.1371/journal.pgen.1003211.*

NAME	POSITION TITLE
Kevin Halden Kline	Professor of Animal Sciences and Extension Specialist, Horses College of ACES, University of Illinois 388 Animal Sciences Lab, 1207 W Gregory Dr., Urbana, IL 61801

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Illinois, Urbana, IL	BS	05/1978	Animal Sciences
University of Illinois, Urbana, IL	MS	05/1980	Animal Sciences
University of Illinois, Urbana, IL	PhD	05/1987	Animal Sciences

A. Role: Co-investigator

B. Positions and Honors

8/1981 – 1/1988 Extension Specialist – Academic Professional; University of Illinois, Urbana, IL
1/1988 – 6/1994 Assistant Professor of Animal Sciences; University of Illinois, Urbana, IL
6/1994 – 6/2001 Associate Professor of Animal Sciences and Extension Specialist, Horse; University of Illinois, Urbana, IL
6/2001 – present Professor of Animal Sciences and Extension Specialist, Horse; University of Illinois, Urbana, IL

Honors

2013 College of ACES Spitler Teaching Award (University of Illinois)
2008 College of ACES Senior Faculty Award for Excellence in Extension (University of Illinois)

Other Positions

Director, Horsemen's Council of Illinois (HCI)
Chair, Illinois Equine Foundation
Consultant, State Racing Commission

C. Selected Peer-reviewed Publications

1. Schliewert E, Lascola K, O'Brien R, Clark-Price S, Wilkins P, Foreman J, Mitchell M, **Kline K**. Comparison of radiographic and computed tomography images of the lung in healthy neonatal foals. *Am J Vet Res* 2015; 76:42–52.
2. Heffron B, Benoit M, Bishop J, Costello S, Hurt L, Simpson L, Taddei L, **Kline K**, Negrusz A., Equine total carbon dioxide testing in Illinois in 2012. J Anal. Toxicol. 2014 Oct; 38(8):536-40.
3. Warren S, Gonzalez P, Kline K. Feed form affects growth and stomach ulcers in yearling horses. *Journal of Agricultural Science and Technology A* (2014) 449-453.
4. Flores, R.S., C.R. Byron and **K.H. Kline**. 2011. Effect of Feed Processing Method on Average Daily Gain and Gastric Ulcer Development in Weanling Horses. *Equine Vet J*. 31: 124-128.
5. Butudom, P, J. H. Foreman, **K. H. Kline** and E. L. Whittem. 2010. Validation and comparison of two methods of measuring lactate in equine plasma. *Equine Vet. J*. 42 (suppl.38): 155-160.
6. Bonoma, T.A., A.C. Brogren, **K.H. Kline** and K.M. Doyle. 2008. Effects of feeding distillers dried grains with solubles on growth and feed efficiency of weanling horses. *J. Equine Vet. Sci*. 28: 725-727.

7. Markey, A.D. and **K.H. Kline**. 2006. Effects of Dietary Fat and Yeast Culture Supplementation on Total Tract Digestibility by Horses. *The Professional Animal Scientist*. 22: 261-266.
8. **Kline, K.H.,** D. Fitzpatrick, L.Taddei, and A. Sukta. 2006. Effect of Dose of Furosemide on Plasma TCO2 Changes in Standardbred Horses. *J. Equine Vet. Sci*. 26: 317 – 321.
9. Andrew, JE, **K.H. Kline** and J.L. Smith. 2006. Effects of Feed Form on Growth and Blood Glucose in Weanling Horses. *J. Equine Vet. Sci*. 26: 349-355.
10. **Kline, K.H**., L.P. Frey, J.H. Foreman and J.T. Lyman. 2005. Effects of intravenous sodium bicarbonate and sodium acetate on equine acid-base status. *J. Equine Vet. Sci*. 25: 349 – 354.
11. **Kline, K.H.** Milkshakes for the Horse. 2004. *J. Equine Vet. Sci*. 24: 374 – 378.
12. Bush, J, Freeman D, **Kline K**, Merchen N, Fahey G. 2001. Dietary fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility by horses. *J. Anim. Sci*. 79: 232 – 239
13. Frey, L. P., **K. H. Kline**, J. H. Foreman and J. T. Lyman. 2001. Using calcium carbonate as an osmolar control treatment for acid-base studies in horses. *J. Anim. Sci*. 79: 1858 – 1862.
14. Vischer, C. M., J. H. Foreman, P. D. Costable, G. J. Benson, **K. H. Kline**, D. E. Freeman, K. L. Campbell and T. C. Grubb. 1999. Hemodynamic effects of thyroidectomy in sedentary horses. *Am. J. Vet. Res*. 60: 14-21.
15. Frey, L. P. and **K. H. Kline**. 1995. Effects of pre-race exercise, frusemide, sex and ambient temperature on blood sodium, bicarbonate and pH values in Standardbred horses. *Equine Vet. J*. 27, 170-174.

XV. Letters of Support: N/A