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RRNMF NEUROMUSCULAR JOURNAL
Letter from the Founding Facilitator

The purpose of creating RRNMF Neuromuscular Journal was to build a venue for our colleagues in the field of neuromuscular medicine to have a hassle free and easy way to get published in a journal devoted to neuromuscular disorders. I wanted to have a journal that costs no money to run and that did not charge authors a fee for publishing their papers and no subscription fee.

We also wanted it to be completely on-line and open access. In addition, I wanted the authors to own the contents of what they wrote. I did not want the journal to have any copyright or ownership of what was published. I wanted healthcare researchers/authors to be in control of the journal and not a publisher or a society.

Of course, I did not want this to be a predatory open access journal that charged excessive fees to publish and preyed on susceptible young investigators who were under pressure to publish at any cost. And I wanted it to be a fun journal that we all could enjoy and not have to worry that the editor or editorial board or reviewers would insist that you make minor and unnecessary changes to your manuscript. I wanted to avoid "gotcha" reviewer and editor comments. We are doing peer review, but it will really be looking at the overall big picture of what your manuscript is about and what you are trying to say and not nit-picking the manuscript apart which happens in so many ways in many journals in the review and editing process.

The new technology to do journal publishing digitally with tools we have in the University of Kansas Libraries Digital Publishing Services unit has allowed us to do this at this point in time.

This is a part of KU Libraries (not the KU Press which is a different unit that publishes hard copy books). KU Libraries provides a Journal Management System that allows online journal publishing. The Library has purchased software from a company called OJS. I became aware of the capability at KU only a couple of years ago through meeting Marianne Reed who is the Digital Initiatives Manager. I had been exploring fee for service open access publishing vendors but then I found out this is available at KU for free! All it takes is a KU faculty member who wants to start a journal and who is willing to put together a team of colleagues who would help. The Library provides a project manager to assist the Faculty with online publishing. The Faculty and his/her colleagues (which do not have to be from KU) are the "editors and editorial staff". KU Libraries has over 40 digital journals. The only other medical one is the Kansas Journal of Medicine which we are patterning much of our organization after. Go to journals.ku.edu to see the entire list and from this website you can click to go on any of the journals, including RRNMF Neuromuscular Journal. Also, you can go directly to our journal by using journals.ku.edu/RRNMF.

Being able to publish in this way is truly part of the computer revolution in communication that began in the middle of the last century but is now having even a greater impact every day. The computer revolution has changed everything we do as a species and how we communicate. I am old enough to have started my academic career prior to the computer revolution when we sent typed manuscripts (yes on a real typewriter) and mailed them to a journal (with postage stamps). The editor would return it with red pencil marks that he did himself for you to make revisions. I recall the first time I bought a portable electronic typewriter as a resident (1983-1986) in which I could program a template for the history and physical and I used this because my handwriting was so bad. These early "computers" evolved quickly to desk and laptops. We all moved on to typing our manuscripts on desk and laptops in the 1980s, but we still mailed them into the journal office and they still came back with red pencil marks to correct. I have all my old submitted manuscripts from Drs Robert Daroff and Lewis Rowland (from the journal *Neurology*) with their pencil corrections which I cherish. In the appendix I am attaching my first article that was accepted into *Neurology* that was edited by Dr. Daroff (lightly!) and our snail mail correspondence. This was our Myoshi Myopathy clinical paper. Dr. Miller insisted we not call the disorder by someone's name so the title was shortened to Autosomal Recessive Distal Dystrophy in the galley state (1). Finally, journals moved to online submissions and the process went full electronic in the late 1990s. So, this computer revolution in publishing did not take long to evolve.

Now the process has become less expensive and streamlined and software programs are available so nonprofit institutions like the University of Kansas can purchase them to make it available to their faculty who want to start their own academic journals.

I see this as part of the evolution of our human species that has a continuing drive and need to communicate ideas. What this journal is about is facilitating IDEAS to be seen by others in an evolving communication format that is part of this new revolutionary era of communication via online technology. Our human ancestors began walking about 2 million years ago. Mankind/homo sapiens seem to have evolved probably 200,000 to 300,000 years ago. These early humans lived in hunter-gatherer societies. All communication was in small tribes, around the fire and in caves. Food was free by being part of the clan. Tribes moved around a lot and there were no permanent settlements. But even these early humans wanted to communicate ideas and we have evidence of this in paleolithic cave drawings. The earliest cave drawings in Europe date from 17,000 to 30,000 years ago in France and Spain. Picasso apparently said after seeing the amazing animal cave paintings in the Lascaux Cave in France, "They've invented everything". Recently, even earlier cave paintings were discovered in Borneo, Indonesia from 44,000 years ago. (2) The Indonesian drawings

showed wild pigs and buffalo and also part human /part animal figures that had snouts and tails. These animal-human figures are thought to indicate that the early humans who created them could conceive of things that did not exist in the natural world. Cave drawings are thought to represent the beginning of creative thinking. This is the dawn of the IDEA Revolution. It has also been called the "Cognitive Revolution". (3)

The Agricultural Revolution occurred about 10,000 years ago. Humans moved from hunter-gatherer nomadic clans to cities and they had to grow food in farms to support the growing masses of people. The populations exploded. The food had to be locked up. People had to pay for the food. Religion develops to give people an explanation of "what the heck is happening" and allowed people to believe that if their life is difficult now they should not worry as it will be better after they die in the afterlife, or that a supernatural being has got their back. For a wonderful easy-to-read discussion about this march through various revolutions and the impact of the agricultural and other revolutions see *Sapiens: A Brief History of Humankind* by Yuval Noah Harari (nonfiction) and the series of novels by Daniel Quinn which tells this story in a unique way. (3,4,5,6)

A number of civilizations emerged around the planet. The earliest cities and civilizations were formed in what is now Iraq in Mesopotamia and written language called cuneiform was probably invented somewhere around 3200 BC in Sumer. The development of writing I believe was the First Communication Revolution. In Babylon the Code of Hammurabi was written in 1754 BC, one of the earliest deciphered writings of significant length. Other civilizations emerged in Egypt, Indus River Valley, Asia, Persia and the Minoans in Crete with their own language and written alphabets. The Minoans developed a language that was then widely disseminated by the Phoenicians and became the forerunner of Greek. The Mayans in Mesoamerica developed their own writing system independently perhaps as early as 500 BC. Eventually, western civilization evolved in classical Greece and Rome. Paper was developed in China between 100 BC and 100 CE, a major advance in communication, which I consider the Second Communication Revolution. Paper had immense consequences to the book world. Books could now be carried by hand and no longer had to be transported in multiple individuals / sections on parchment.

Following the fall of the Roman Empire things went dark in the west for a thousand years. The Third Communication Revolution occurred with the development of the movable type and printing press by Johannes Gutenberg around 1440. Immediately ideas could be easily communicated in books and other paper products. The rate limiting step was being able to read. The printing press facilitated the blossoming of the Renaissance in art and science (which had begun a century earlier) with the re-emergence of classic Greek and Roman works and the development of new ideas that could be easily printed and disseminated in books.

The Enlightenment Revolution in the 1700s promoted individual freedom to lead our lives and furthered ideas in rational science and in social behavior. The Industrial Revolution began in the early 1800s with the development of machines that made things easier to do. It also allowed people to move even more often and faster to the cities. It also locked more of the food up as well. From the mid-1800s to the mid-1900s there was another revolution in science-the Postindustrial Scientific Revolution. Machines that had been developed now allowed scientists to make new discoveries in every sphere of science culminating in the understanding of the cell and the universe, along with amazing breakthroughs in medical knowledge and treatments. Surgery was possible, new drugs were developed that actually worked and DNA was discovered unlocking the secrets of life. The years between revolutions became shorter and shorter. We moved quickly from the postindustrial scientific revolution to the current Computer Revolution of the last half of the 20th century, the Fourth Communication Revolution, which has led to RRNMF Neuromuscular Journal.

This new journal is an outgrowth of our website RRNMF. I used to communicate with my close colleagues by snail-mail. At one point there were about six of us in a BBC - Barohn Book Club and I would actually mail books that I thought would be of interest to my friends. Then came emails. For years in the 1990s I resisted the use of email and had my secretary in Dallas print my emails and I would write my response in long hand and she would type it out and respond to the email for me. I just did not want to waste my time getting on a computer everyday to do emails. But when I moved to Kansas City and the University of Kansas in 2001, for the first three months I did not have a secretary. So, I was thrown into the deep water and had to begin doing emails myself.

My life has never been the same and I am not sure it is better for emails. But as a result of me learning how to do my own emails, in the early 2000s we began a chain email group that was the original BBC plus others, and we kept adding more neuromuscular doctors. The initial BBC and email chain crew as I recall was Jon Katz, Dave Saperstein, Carlayne Jackson, Tony Amato, John Kissel. We would email each other about cases that were challenging with difficult diagnosis and treatment issues. Todd Levine joined shortly after along with Tahseen Mozaffar and Aziz Shaibani. We called this growing group Rick's Real Neuromuscular Friends. In 2013 the opportunity came up to start a website with the help of the company Nufactor, Inc. who supplied us a webmaster. We created the RRNMF website so we could bring more neuromuscular doctors and health care professionals into the conversation. This has expanded so that now there are more than 2,000 health care providers and researchers interested in neuromuscular disease that are part of this unique communication vehicle. The website RRNMF.com is the portal to enter this electronic conversation. The RRNMF website also has other features like Sur-

veys, Grand Rounds presentations, Journal Club, but the highlight are the many cases discussed in the Forum. But the website did not have a true journal feature. Therefore, this new RRNMF Neuromuscular Journal is the next extension of electronic communication efforts in this space. We hope many of the cases that are posted on the RRNMF website get converted to case reports or case series for the journal. And we would like to see many of the ideas discussed on the RRNMF website get developed into New Discoveries/New Stuff (new original articles), Looking Back and Looking Forward at Stuff (review articles), Proposed Stuff (grants that have been submitted), What's on Your Mind? (Commentaries and Other Stuff), Meeting Stuff and Visual Stuff.

I am calling the journal leadership Facilitators rather than Editors to set a positive tone. The Facilitators will provide peer review for your submitted manuscripts. We do not plan to be overly critical about every punctuation or formatting style you choose or be argumentative about the point you are trying to make. We want to facilitate the process so you can get your manuscript published and so other neuromuscular researchers and health care providers can be aware of what's on your mind. We of course will offer constructive facilitation.

If you want to become an Associate Facilitator, send me an email or give me a call and we can discuss. We want to be inclusive, but I want to add Facilitators who get the spirit of what we are trying to do in order to promote publishing neuromuscular ideas.

Impact factor is not a concern of ours in creating this new journal. Marianne Reed assures me that articles published in the KU digital press series are easily searchable on Google Scholar which will index our papers within a week or two of publication. The current KU digital journals get thousands of hits annually on Google Scholar. Web of Science is another index and at least one KU Digital Journal is now indexed on Web of Science. We may eventually apply to get indexed by Web of Science. We know there is a lot of interest by authors to have their paper indexed in PubMed. We will get there. The Kansas Journal of Medicine is indexed in PubMed. There is a threshold of papers a journal has to have published before we can apply PubMed to begin getting indexed. It appears that once we have published 26 papers and have been in operation for a year, we can do that.

But even if we are not in PubMed at this time, I do not see that as a major issue. Our goal is to simply disseminate ideas in the field of neuromuscular disorders in an open access, free publishing format. If we are successful (and I am sure we will be) then issues like PubMed and perhaps even impact factor will take care of themselves. In the meantime, you will have to decide which of your ideas you want to publish in this new journal versus the other neuromuscular journals we all currently use to publish our work (Neurology; Muscle and Nerve; Journal of Clinical Neuromuscular Disease, JAMA Neurology, etc.). I trust that you will all have a pretty good idea which of these journals you want to shoot for as you contemplate your publication journey. We know it is not likely you will send a manuscript that you want to be published in Annals of Neurology or New England Journal of Medicine to RRNMF Neuromuscular Journal. But I am confident we will get our share.

So, enjoy this inaugural issue of RRNMF Neuromuscular Journal and please submit manuscripts. We plan to publish this approximately every other month at this time. Maybe at some point it will be monthly if we get a lot of submissions. The interesting thing about electronic journals is that you can publish an issue whenever you want and either often or as infrequent. But we will of course try to be consistent on publishing issues.

I want to thank Marianne Reed who for over a year has walked me through this new process of communicating ideas. I also want to thank Amanda Sebok my Executive Assistant who has helped me a great deal to get this journal launched. And also, thanks to the initial Facilitator team that bounced all these ideas around for over a year: Todd Levine, Jon Katz, Mazen Dimachkie, Mamatha Pasnoor and Laura Herbelin.

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APPENDIX

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Edited

Miyoshi Myopathy

(Autosomal Recessive Distal Dystrophy)

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ABSTRACT

We describe five new cases of Miyoshi myopathy and emphasize the distinctive clinical and laboratory features of this distal muscular dystrophy. Symptoms began at age 15-25, the gastrocnemius muscles were selectively involved, and creating kinase was elevated more than 10 times normal. The EMG showed abundant brief motor units with numerous fibrillations. Dystrophic features without vacuoles were best seen in the biceps femoris muscle. Asymptomatic creatine kinase elevation was ~~observed~~ present years prior to the development of weakness. The disorder appears to be inherited in an autosomal recessive pattern. Miyoshi myopathy can be distinguished from other distal muscular dystrophies. We propose a new classification for the distal muscular dystrophies.

Introduction

Patients with primarily distal weakness and histologic evidence for muscular dystrophy are rare and may be difficult to classify (1). Welander described the first large series of distal myopathy (2) and, since then, there have been a number of reports describing other forms (3-12). Miyoshi and others have described an autosomal recessive myopathy with early adult onset, early involvement of gastrocnemius muscles, and markedly elevated creatine kinase (13-16). These Japanese reports remained unnoticed in the Western medical literature until recently (17-21). We describe five new cases of Miyoshi's myopathy and propose a classification system for the distal myopathies.

Case Reports (Table 1)

Case 1

A 27 year old man complained of a two year history of slowly progressive leg weakness. He had difficulty running and could not stand on his toes. He could rise from a chair without difficulty. He had no pain, sensory symptoms, cramps, or fasciculation. The patient had previously been on active duty in the U. S. Air Force for six years and had no difficulty with running or walking (including the annual 1.5 mile run).

The family history was negative, although we were not able to examine his parents or a 20 year old sister.

On physical examination, the abnormalities were restricted to the legs. The gastrocnemius muscles were atrophic but without fasciculation. Strength in the lower extremities (MRC scale 0-5) (22) revealed 5 knee extension, ankle

dorsiflexion, eversion, and inversion; 4+ hip flexion, abduction, and extension; 4 knee flexion; 4- ankle plantar flexion. Sensory testing and strength in other muscles was normal. Muscle stretch reflexes were normal except for absent ankle jerks. He could not walk on his toes, but could heel walk normally. He could rise from a chair and from squatting without difficulty.

Laboratory evaluation: creatine kinase (CK) was 10,050 IU/L (normal up to 195 IU/L); erythrocyte sedimentation rate (Westergren) was normal (2mm/hr). Motor and sensory nerve conduction studies were normal. Despite the absent ankle jerks and atrophic gastrocnemius muscles, an H reflex with a normal latency (27.5 msec) was easily obtained over the soleus muscle. Concentric needle electromyographic (EMG) examination in the lower extremities revealed grade 2+ fibrillation potentials [0 to 4 grading system(23)] in the medial gastrocnemius, biceps femoris; grade 1+ in the vastus lateralis and tensor fascia lata; and no fibrillations in the anterior tibialis or lumbosacral paraspinous muscles. Numerous polyphasic motor units were present in weak muscles, with units ranging from 4 to 15 msec in duration and 100uv to 2 mv in amplitude. Motor unit recruitment was full at minimal effort in weak (grade 4) lower extremity muscles except for the gastrocnemius. In this muscle many areas were devoid of voluntary motor units. In the upper extremity the deltoid and triceps muscles were normal; the biceps muscle had 1+ fibrillations, occasional polyphasic units, and normal recruitment, with the distal arm and hand muscles being normal. A CT scan and myelogram of the lumbosacral spine were normal.

A vastus lateralis muscle biopsy revealed a slight increase in variability of fiber size, ranging from 15 to 60 microns in diameter (fig 1). Rare atrophic angular fibers were seen. Three necrotic fibers ware present; one was engulfed

by mononuclear inflammatory cells. No central nuclei were present and there was no increase in connective tissue. Because the changes observed were minimal, a lateral gastrocnemius muscle biopsy was performed. The muscle was pale and resisted excision. On light microscopy, the muscle was replaced almost entirely by connective tissue with only a few islands of small (4 to 25 microns) muscle fibers (fig 2). A third muscle biopsy, from the biceps femoris, revealed changes intermediate from the previous two specimens (fig 3-4). There was marked variation in fiber size (4 to 120 microns). Atrophic fibers were of both round and angular configurations and some stained darkly with the NADH stain. In several areas, the angular fibers were seen in small collections. Approximately thirty necrotic fibers, often undergoing phagocytosis by mononuclear inflammatory cells were present. Central nuclei occurred in 10% of muscle fibers, and fiber splitting was present. Connective tissue was increased. No vacuoles were present. With the ATPase stains, a normal distribution of fiber types were seen, without type grouping. While a few of the features noted above suggested slight neuropathic changes, the overall biopsy pattern was most consistent with a muscular dystrophy.

Prednisone was instituted at 100 mg daily for two weeks, then 100 mg. every other day. After three months there was no improvement and the CK remained between 6,000 and 10,000. Over the last year off Prednisone, there has been moderate progression.

Case 2

A 20 year old female gave a one year history of difficulty standing on her toes, walking up stairs, and rising from a deep knee bend. Her symptoms were

particularly apparent while, when horseback riding, she was unable to plantar flex her ankles to get her toes down into the stirrups. The symptoms evolved gradually over many months. There was no pain or sensory loss. The patient had previously been active physically and had been a high school cheerleader.

The family history was negative. Three brothers and both parents were living and well.

There was no facial or upper extremity weakness or atrophy. The gastrocnemius muscles were atrophic with normal bulk of the extensor digitorum brevis, peroneal and anterior tibial muscles. Muscle strength testing revealed grade 5 hip flexors and abductors; grade 4+ knee extension and knee flexion, grade 4+ ankle dorsiflexion and grade 4 ankle plantar flexion. Muscle stretch reflexes in the arms were normal, the knee jerks were depressed and the ankle jerks were absent. She could not walk on her heels or toes and there was a Gower's sign.

Serum CK was 9,440 IU/L (normal less than 89 IU/L). Nerve conduction studies were normal. EMG of the medial gastrocnemius and anterior tibialis revealed 1+ fibrillation potentials. Other details were unavailable.

A muscle biopsy of the lateral gastrocnemius showed marked fibrosis and remaining muscle fibers were small and round, with some necrotic fibers. Biopsy of the vastus lateralis revealed only minimal changes, with an occasional degenerating or necrotic fiber.

Over the next two years no objective change in strength or CK was noted although the patient complained of progressive weakness.

Case 3

A 24 year old man complained of a seven year history of weakness in his calves. Initially there was aching and cramping in the calves and he subsequently developed great difficulty walking on his toes, and to a much lessor extent on his heels. Over the next five years he noted a gradual worsening of the distal lower extremity strength and progressive wasting of his calves. More recently he had difficulty walking up stairs and getting out of low seats. Six months before our evaluation he began noticing symptoms of weakness and aching in his arms, particularly of the triceps and extensor muscles of the forearm.

The past medical history was notable for juvenile diabetes mellitus for which he had required insulin for nine years, with good serum glucose control ranging from 130 to 150 mg/dl. A glycosylated hemoglobin was 7.9% (normal less than 8%). The family history was negative for muscle weakness or wasting.

On examination, there was marked atrophy of the legs with prominent extensor digitorum brevis muscles. No atrophy was noted in the upper extremities. Strength was normal in the arms except for grade 4 elbow and finger extensors. In the legs, hip flexion and knee extension were slightly weak but hip abduction and knee flexion were normal. Distally, there was a gradation of weakness, with the ankle plantar flexors being the weakest (4-), ankle dorsiflexors were 4, and ankle everters were 4+. Tendon reflexes were normal except for absent ankle jerks. The sensory exam showed only minimal vibration loss at the toes.

The CK ranged from 4,000 to 7,050 IU/L over several years. Nerve conduction studies were normal. The EMG revealed fibrillation potentials in the

medial gastrocnemius (3+), anterior tibialis (1+), and peroneus longus (1+), and none in the vastus lateralis, lumbar paraspinous muscles, triceps or deltoid. Motor units in the gastrocnemius were polyphasic, brief, and low amplitude, but in the anterior tibialis and peroneus longus there were both long and short duration polyphasic units. Motor units were normal in the vastus lateralis, triceps and deltoid. The recruitment pattern was full in weak muscles.

Biopsy of the medial gastrocnemius revealed that much of the muscle specimen had been replaced by connective tissue. Of the remaining fibers, there was a marked variability in size, ranging from 4 to 80 microns in diameter. Some of the atrophic fibers formed small clusters. Occasional necrotic fibers but no inflammatory cells were seen. Fibers with internal nuclei and split fibers were present. Subsequently, a biceps femoris biopsy was performed. As in Case 1, while there was some increase in the endomysial connective tissue, it was not as severe as in the gastrocnemius biopsy. Muscle fiber size varied from 4 to 140 microns. There were over 50 necrotic fibers singly and in groups, many undergoing phagocytosis by macrophages and lymphocytes. Many of the smaller fibers occurred in groups of up to 15 fibers. These small fibers frequently stained intensely with NADH oxidative stain. Fiber splitting was present. No type grouping was seen with the ATPass stains. No vacuoles were present.

The patient was given azathioprine (2.5 mg/kg) for three months and Prednisone, 60 mg./day, was added for a further three months with no benefit. Weakness has progressed during the last year of follow-up, but the patient remains ambulatory without assistance.

Case 4

A 24 year old ~~female~~ woman complained of weakness of eight years duration. At the age of 17 she noted "tired" legs and difficulty rising from a squat. The CK ranged from 3,000 to 8,000 IU/L (normal less than 188 IU/L). A muscle biopsy of the vastus lateralis showed the possibility of polymyositis and led to treatment with Prednisone (60 mg./day), and then methotrexate (25 mg./week intravenously) over the next three years without benefit.

The patient was one of four siblings. A 21 year old brother had a similar pattern of weakness and has been previously described (21). His CK was found to be elevated (4,000 to 7,000 IU/L) for eight years prior to the first development of gastrocnemius weakness at age 23. A quadriceps muscle biopsy showed mild myopathic changes but a gastrocnemius biopsy revealed marked replacement by dense connective tissue. Two other brothers and both parents are alive and well.

At age 24, she had atrophy and wasting of the gastrocnemius muscles. Strength testing in the arms was normal except for shoulder abductors (4+), wrist flexors (4+) and interossei (4-). Hip and knee flexors were mildly weak (4+). Ankle dorsiflexion was normal; ankle inversion and eversion were grade 5 on the right and 4 on the left, and plantar flexion was grade 2 bilaterally. Extensor hallucis longus was grade 5 and flexor hallucis longus was grade 3. Tendon reflexes were normal except for absent ankle jerks.

CK was 2,258 IU/L (normal less than 195 IU/L). Nerve conduction studies were normal. EMG revealed 2+ fibrillation potentials in the left rectus femoris, with 1+ fibrillation in the vastus medialis, medial gastrocnemius and anterior tibialis. In all of these muscles, motor unit potentials were

polyphasic with reduced amplitude and duration. The recruitment patterns were full even in very weak muscles.

Vastus lateralis muscle biopsy revealed fiber size variability and scattered necrotic fibers. Occasionally the necrotic fibers were surrounded and engulfed by mononuclear inflammatory cells. Fibers with central nuclei were present. No clusters of atrophic fibers were seen and there were no vacuoles. Endomysial connective tissue was not increased.

The patient is still ambulatory without assistance but there has been significant progression over the last three years of follow-up.

Case 5

A 15 year old ~~female~~ girl was found to have an asymptomatic elevation of CK to 3,650 IU/L during routine blood tests prior to tonsillectomy. The family history was unremarkable, with three siblings and both parents free of neuromuscular symptoms or signs. Her neurologic examination was normal. Laboratory investigations included normal thyroid function and nerve conduction studies. EMG revealed no fibrillations and motor units ranged from 3 to 8 msec. in duration without polyphasia and with normal recruitment in distal and proximal upper and lower extremity muscles. A vastus lateralis muscle biopsy showed muscle fiber size variability from 20 to 80 microns with a rare scattered necrotic fibers and without inflammatory cells. Internal nuclei were seen in 10% of fibers. Endomysial connective tissue was not increased.

The patient remained normal for the next three years but her CK ranged from 5000 to 10,000 IU/L. At age 17 she first noted pain in her calf muscles after exercise. At age 19 she complained of poor exercise tolerance. A repeat vastus lateralis biopsy revealed muscle fiber size variation ranging from 5 to 125

micrometers with occasional (30%) internal nuclei. There were fifty necrotic fibers, a few undergoing phagocytosis. Atrophic fibers were both angular and rounded and were randomly distributed. Connective tissue was moderately increased. The NADH reaction showed ten dark angulated fibers but otherwise fibers had a normal intramyofibrillar network. Phosphorylase and myoadenylate deaminase were present.

She continued to complain of aching in her calves after exercise and at age 18 her calves first appeared thin and she had difficulty hopping and walking on her toes. Her ankle reflexes became diminished. By age 20 there was marked gastrocnemius wasting, inability to toe walk, and absent ankle jerks. She had difficulty climbing stairs and was unable to wear high-heeled shoes. Her CK remained 50 to 100 times normal.

Discussion

The five patients described above show the characteristic clinical and laboratory features of the autosomal recessive distal muscular dystrophy first reported by Miyoshi (13-17) and later by others (Table 2) (18-21). The salient clinical features of this disorder are: 1) Onset between age 15 and 25; 2) early and predominant involvement of the gastrocnemius muscles with relative sparing of the anterior tibialis; 3) elevation of serum creatine kinase, 10 to 150 times normal; 4) biopsy evidence of a chronic active myopathy without vacuoles; and 5) probable autosomal recessive inheritance, although sporadic cases are common. The course is steadily progressive. All five of our patients worsened during follow-up although they all remained ambulatory. There was no response to immunosuppressive therapy with corticosteroids or antimetabolites in the three patients treated.

These features characterize our five cases and those of Miyoshi (17 cases) (17), Nonaka, et al. (4 cases) (20), and three recent cases described by Galassi, et al. (21) (Table 2). Kuhn, et al. (18) reported two brothers with similar clinical features but the only muscle biopsy reported was of the vastus lateralis, which showed only slight changes. Biopsies of this muscle in our patients were also slightly abnormal.

Several points regarding the muscle biopsy deserve mention. The slight myopathic changes noted in biopsy of the vastus lateralis generally consist of rare scattered necrotic muscle fibers, occasionally being engulfed by inflammatory cells, with slight muscle size variability. However, most of the muscle fibers in the vastus lateralis biopsies are normal and the connective tissue is only minimally increased. Biopsy of the gastrocnemius muscle on the other hand shows severe fibrosis often with only a few remaining small, round fibers, or so called "end-stage muscle." Biopsies from the biceps femoris muscle showed the most representative changes. There was marked fiber size variability, with both hypertrophy and atrophy. Many muscle fibers were in various stages of necrosis and phagocytosis. In one case, these changes were interpreted as inflammatory myopathy (case 4 above). However, there were no inflammatory cells in the perimysial connective tissue, around blood vessels or in the endomysial connective tissue except when associated with necrotic fibers. Connective tissue proliferation was scant in the vastus lateralis, intermediate in the biceps femoris, and severe in the gastrocnemius. Small angular fibers formed clusters, often staining darkly with the NADH stain, reminiscent of a neuropathic process; however, the other histologic features, along with the characteristic changes in the vastus lateralis and gastrocnemius muscles, as

well as the characteristic clinical and electrodiagnostic findings, clearly indicate this is a primary muscle disease.

There is only one report of an autopsied case (17). In this patient the gastrocnemius was nearly totally replaced by fat; the anterior tibialis and biceps femoris had moderate dystrophic changes, and other proximal limb muscles were only minimally affected. Anterior horn cells and peripheral nerves were normal. Because of the wide range of biopsy changes in different muscles, we recommend biopsy of the biceps femoris.

The EMG findings are characteristic of a chronic myopathy. Modest numbers of fibrillation potentials were present in all patients, except case 5 who was studied before she was symptomatic. In general, motor unit potentials were brief in duration, low amplitude and polyphasic. In some patients (cases 1 and 3), a second population of polyphasic motor units was observed with longer durations, as has been reported in other dystrophies (24) and in chronic inflammatory myopathies (25). The recruitment pattern was full in weak muscles and thus characteristic of a primary muscle disease. An exception to this was occasionally seen in the very weak gastrocnemius muscles which, when severely replaced by connective tissue, showed a reduced recruitment pattern with the few remaining units firing very rapidly.

In Case 5, marked CK elevation preceded clinical evidence of gastrocnemius weakness by several years. This presymptomatic "hyper-CK-emia" was also described by Galassi, et al. (21) in two of their three cases. The first symptoms were aching in the calves with exercise (cases 3 and 5), or a tired, weak feeling in the legs (cases 1, 2, and 4). The earliest signs were the inability to toe walk and depression or loss of ankle tendon reflexes.

Gastrocnemius atrophy developed later.

Several of the clinical and laboratory features of this form of distal myopathy resemble those of inclusion body myositis (IBM) (26-30). In IBM, distal weakness is usually prominent, and the EMG and muscle biopsy have features resembling a neuropathic process (29). However, there are important differences that distinguish IBM and Miyoshi myopathy. In IBM, the distal muscle weakness almost always involves finger flexors and the quadriceps; the gastrocnemius muscle is not preferentially involved. The serum CK is only slightly increased in IBM. Finally, the muscle biopsy in IBM contains characteristic rimmed vacuoles and 15-18 nm filaments either within the nuclei or at the edge of the vacuoles on electron microscopy.

In 1986, Markesbery and Griggs (1) proposed a classification scheme for hereditary distal myopathies: (1) late adult onset autosomal dominant distal myopathy of Welander with onset in the hands; (2) late adult onset autosomal dominant distal myopathy with onset in the legs; (3) early adult onset autosomal recessive or sporadic distal myopathy with onset in the hands or legs. With the characterization of Miyoshi myopathy, this latter category must now be further subdivided (Table 3) as discussed below.

The most familiar late onset form was described by Welander (2). It is an autosomal dominant distal myopathy with onset in the 5th decade, and weakness beginning in the hands. Distal leg involvement occurs in the anterior compartment. The CK is either normal or mildly increased. The histologic changes noted by Welander, and later by Edstrom (29), ranged from mild myopathic changes to severe fibrosis, and vacuoles were observed in some cases.

The other form of late adult onset autosomal dominant distal myopathy

begins in the legs and has been reported in non-Scandinavian patients (3,4). The anterior compartment muscles are most affected and the CK is normal or mildly elevated. Muscle biopsy reveals a vacuolar myopathy.

It is now clear that there are two distinct types of early adult onset distal myopathies with autosomal recessive inheritance. One usually begins in the anterior compartment (5-12), although rarely it begins in the hands. The CK tends to be moderately elevated (<10 times normal) and the muscle biopsy reveals a severe vacuolar myopathy; the prognosis is generally good with a relatively benign course in most patients (12).

By contrast, the condition described herein as well as by Miyoshi and others can now be classified as a separate early adult onset distal muscular dystrophy (13-21) (Table 3). As described above, these patients present with gastrocnemius weakness and atrophy, markedly elevated CK (10 to 150 times normal), a dystrophic biopsy without vacuoles and a more rapidly progressive course with greater disability.

The observations on treatment with corticosteroids and azathioprine or methotrexate in our patients are uncontrolled. Nonetheless, the objective evidence of worsening in our cases makes it unlikely that patients with Miyoshi myopathy will respond to immunosuppressive treatment, despite the recent results described in Duchenne's dystrophy (32).

Thus, the muscular dystrophies with predominantly distal involvement include at least four separate disorders, each with distinctive clinical and laboratory features, and each probably reflecting different gene lesions.

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Table 1: SUMMARY OF CLINICAL DATA

CASE	SEX	AGE AT ONSET	EARLY GASTROC WEAKNESS	SERUM CK(IU/L)	BIOPSY	EMG
1	M	25	+	10,050	G - end stage/fibrosis VL - minimal myopathy BF - intermediate; dystophy	MUP - poly, brief REC - full FIBS - +
2	F	19	+	9,440	G - end stage/fibrosis VL - minimal myopathy	MUP - poly REC - full FIBS - +
3	M	17	+	7,050	G - end stage/fibrosis BF - severe dystrophy	MUP - poly, brief REC - full FIBS - +
4	F	16	+	8,000	VL - minimal myopathy	MUP - poly, brief REC - full FIBS - +
5	F	15	+*	11,040	VL - minimal myopathy	Normal**

* - At age 22, 7 years after high CK noted

** - When clinically asymptomatic at age 15

G = gastrocnemius
VL = vastus lateralis
BF = biceps Femoris

MUP - motor unit potential
poly - polyphasic
REC - recruitment
FIBS - fibrillations

Table 2: DISTAL MYOPATHY OF MIYOSHI: Literature Summary

AUTHOR	# PATIENTS	SEX	AGE AT ONSET	CREATINE KINASE	GASTROCNEMIUS WEAKNESS
1. Miyoshi et al. (1967/1986)	17	8 M 9 F	12-30	20 to 100 X Normal	17/17
2. Kuhn, et al. (1981)	2	2 M	17;20	25 X Normal	2/2
3. Alderson, et al. (1985)	1	1 M	19	12 X Normal	1/1
4. Nonaker, et al. (1985)	4	2 M 2 F	21;29 17;28	40-100 X Normal	4/4
5. Galasi, et al. (1987)	3	3 M	15;18;19	62-153 X Normal	3/3
6. Barohn, et al. (1991)	5	2 M 3 F	17;25 15;16;19	30-150 X Normal	5/5

TABLE 3

CLASSIFICATION OF DISTAL MYOPATHIES

Late adult onset (Welander Myopathy) - Type I (refs 2,31)

Autosomal dominant

Onset in hands

Later leg involvement in anterior compartment

CK normal or slightly increased

Biopsy-variable; vacuolar myopathy in some cases

Late adult onset - Type II (refs 3,4)

Autosomal dominant

Onset in legs - anterior compartment

CK normal or slightly increased

Biopsy - vacuolar myopathy

Early adult onset - Type I (refs 5-12)

Autosomal recessive or sporadic

Onset legs - anterior compartment

CK - increased, usually <10 X normal

Biopsy - vacuolar myopathy

Early adult onset (Miyoshi Myopathy) - Type II (refs 13-21)

Autosomal recessive or sporadic

Onset legs - posterior compartment

CK - increased 10 to 150 X normal

Biopsy - dystrophy without vacuoles

gastrocnemius often "end-stage"

LEGENDS

Figure 1. Case 1. Vastus lateralis. Muscle fiber size variability with one necrotic fiber. Hematoxylin and eosin, X 376.

Figure 2. Case 1. Lateral gastrocnemius. A. Marked muscle fiber loss with small atrophic fibers and replacement of muscle by dense connective tissue and fat. Hematoxylin and eosin, X 53. B. Higher magnification of an adjacent area shows muscle fiber size variability and central nuclei and dense connective tissue. Hematoxylin and eosin, X 376.

Figure 3. Case 1. Biceps femoris. Intermediate changes compared to Figures 1 & 2, with variable muscle fiber size, hypertrophic and small round fibers. Hematoxylin and eosin, X 376.

Figure 4. Case 3. Distal tapering with posterior compartment (gastrocnemius) atrophy.

Sciatic Neuropathy with Preserved Sensory Nerve Action Potentials, A Case Series

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ABSTRACT

Background. Sciatic neuropathy is differentiated from lumbosacral radiculopathy based on the finding of abnormal sensory nerve action potentials (SNAPs). Cases of sciatic neuropathy with intact SNAPS have not been well described.

Methods. A retrospective analysis of 12 patients with sciatic neuropathy in a single institution.

Results. We describe 12 patients in whom a sciatic neuropathy was diagnosed based on a combination of history, physical exam, radiological and electrodiagnostic (EDX) findings. Lower extremity SNAPs were found to be within normal range in all patients, although SNAP amplitude asymmetry between both sides was observed in 3. Included patients were young (mean age of 40.3 years) and mostly female (9 patients).

Conclusions. Sciatic neuropathy may occur with a relative sparing of sensory fibers. Recognition of this group of patients should help to avoid making a misdiagnosis of lumbosacral radiculopathy.

Keywords: *sciatic nerve, sciatic neuropathy, radiculopathy, nerve conduction study, sensory nerve action potential, electromyography.*

Introduction

The sensorimotor function of the sciatic nerve includes innervation of muscles and skin in the regions of the posterior thigh, the lower leg, and the foot. Due to sciatic nerve's large size, either the peroneal (fibular) or the tibial branch could be preferentially affected while leaving the other branch relatively spared.[1-4] However, sciatic neuropathy affecting predominantly motor or sensory branches has not been well characterized. In the current study, we describe 12 patients with sciatic neuropathy showing preserved sensory responses on nerve conduction studies (NCS).

Methods

The study was approved by our institutional review board. The electronic medical record at our institution was searched for patients who presented to the neuromuscular center with lower extremity sensorimotor symptoms and received electrodiagnostic (EDX) testing between 2004 and 2018. Patients with clinical and electrophysiological diagnosis of sciatic neuropathies, but showing normal sural and superficial sensory nerve action potentials (SNAPs) defined by laboratory standards were included.

Data collection included patient age, sex, past medical history, affected side, mechanism of injury, onset time, sensory deficit, muscle strength examination, time until EDX study, EDX and radiological findings, and follow-up information.

Results

Clinical information

Twelve patients were identified (Table 1). The average age was 40.3 years old (range 16-59), and 9 were females. Left sciatic neuropathy was present in 5 patients and right in 6. In addition, patient 12 had a significant right sciatic neuropathy, and a coexisting mild left sciatic neuropathy. Only data related to the right sciatic neuropathy from patient 12 were included for further analysis. Limited data on 4 patients were included in one prior publication.[4]

Sensory complaints of paresthesia, numbness and pain were present in all patients. On exam, reduction of pinprick and/or touch sensation was documented in 10 patients, reduction of vibration sensation in 2, and normal sensory exam in 1. Lower extremity muscle weakness was encountered in 11. In all patients, initial symptoms occurred at the distal lower extremity, and none presented with lower back or radicular pain. Magnetic resonance imaging (MRI) of the lumbar spine was performed in 8 patients. None showed contributory findings with the exception of 1 patient (patient 3), in whom a moderate left L5 nerve root compression was observed, though her clinical presentation and EDX findings were consistent with a left sciatic neuropathy.

Electrophysiological findings

On sensory NCS, bilateral superficial peroneal SNAPs were obtained in all patients, and bilateral sural SNAPs in 10 (Table 2). All SNAP responses were present, with their amplitudes and latencies falling within the normal ranges based on our laboratory standards. In 7 patients (patients 1 to 7), no significant asymmetry was observed on bilateral superficial peroneal and sural SNAPs. Amplitude asymme-

Table 1. Clinical Information of 12 patients with sciatic neuropathy

Patient No.	Age	Sex	Affected Side	Etiology	Time to deficit	MRI of pelvis or thigh
1	35	F	left	perioperative, trans-vaginal hysterectomy	immediate	normal
2	48	F	left	popliteal nerve block	unknown	normal
3	59	M	left	vasculitis: Churg-Strauss syndrome	10 days	not done
4	48	F	right	chronic exertional activity (yoga)	immediate	normal nerve, abnormal surrounding soft tissue
5	20	F	right	perioperative, colectomy	immediate	normal nerve, abnormal surrounding soft tissue
6	41	F	left	perioperative, hip surgery	unknown	nerve difficult to visualize, abnormal surrounding soft tissue
7	41	F	left	perioperative, breast reduction	immediate	not done
8	49	F	right	perioperative, foot surgery	unknown	not done
9	52	F	right	awaken from sleep	unknown	enlarged nerve with enhancement
10	45	F	right	fall	immediate	normal nerve, abnormal surrounding soft tissue
11	16	M	left	perioperative, hip surgery	immediate	normal nerve, abnormal surrounding soft tissue
12	30	M	right>left	prolonged sitting	immediate	not done

Abbreviations: F, female; M, male

try (defined as being less than 50% of that on the unaffected sided) on the superficial peroneal SNAP was observed in patients 8 and 10, and on the sural in patient 9. On motor NCSs, abnormally reduced peroneal or tibial compound muscle action potentials (CMAPs) were observed in 10 patients. Amplitude asymmetry on the peroneal or tibial CMAPs was observed in patients 8 and 9, respectively (Table 2). No distal latency prolongation, conduction slowing, conduction block or temporal dispersion was observed on motor NCS. Frequencies of abnormal electromyography (EMG) findings (fibrillations, positive wave discharges, long duration motor unit potentials, or reduced recruitment) on needle EMG of individual lower extremity muscles are listed in Table 3. EMG exam of the gluteus medius (N=12), gluteus maximus (N=11) and lumbosacral paraspinal muscles (N=9) were normal.

Etiology analysis

In 6 patients (patients 1, 5, 6, 7, 8, and 11), sciatic neuropathy occurred perioperatively. In patient 4, MRI revealed severe gluteus minimus tendonitis, greater trochanter bursitis, and edema in the long head of the biceps femoris muscle producing compression of the sciatic nerve. These were felt to be related to exertional activity associated with intense Yoga practice of long duration. In patient 9, sciatic neuropathy developed upon awakening from sleep, and resolved gradually within the next 8 months. A similar right sciatic neuropathy occurred 6 years prior, also resulting in a complete recovery within 6 months. It was felt the sciatic neuropathy in patient 9 was secondary to a compressive etiology due to liability to external pressure. In patients 10 and 12, sciatic nerve compression occurred following falling accident and prolonged sitting, respectively. In patients 2 and 3, sciatic neuropathy was related to a popliteal nerve block procedure and a partially treated eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), respectively.

Radiological findings and follow-up

Eight patients underwent MRI of the pelvis and/or the thigh for the evaluation of sciatic nerve (Table 1). MRIs were normal in 2, showed abnormal sciatic nerve in 1, and revealed surrounding soft tissue abnormality without clear abnormalities in the sciatic nerve itself in the remaining 5 patients.

Three patients (25%) were lost to follow up. Among the remaining 9 patients with a follow-up period ranging from 7 days to 31 months, 4 patients (33%) had no or suboptimal

Table 2. Sensory and motor nerve conduction studies

Patient No.	Timing of EDX study (days)	Side	Sural SNAP amplitude/latency (uV/ms)	Superficial peroneal amplitude/latency (uV/ms)	Peroneal-EDB amplitude/latency (mV/ms)	Peroneal-TA amplitude/latency (mV/ms)	Tibial-AH amplitude/latency (mV/ms)
1	30	**L**	18.3/3.4	23.1/3.0	1.3/5.2	6.3/3.1	16.5/3.4
		R	22.5/4.0	28.4/2.8	5.1/4.5	NA	NA
2	520	**L**	5.2/4.3	5.2/2.6	3.8/4.0	4.6/3.2	2.0/4.3
		R	9.3/3.2	10.0/2.8	4.4/4.8	4.5/3.5	8.9/3.8
3	210	**L**	11.5/3.6	8.5/3.1	2.4/4.0	NA	11.4/3.7
		R	11.2/3.2	6.5/3.1	NA	NA	NA
4	1100	L	20.3/3.1	17.0/2.8	NA	NA	9.6/3.6
		R	16.2/3.0	14.5/2.8	4.2/3.5	7.5/3.0	4.6/4.4
5	40	L	30.6/3.4	26.8/2.5	7.4/4.8	6.3/1.4	19.8/2.4
		R	22.6/4.0	21.9/2.9	5.6/3.0	7.4/1.6	14.0/4.8
6	21	**L**	12.7/4.0	9.4/4.0	4.2/5.5	2.7/3.1	10.4/5.3
		R	12.8/3.8	9.8/3.4	6.8/5.1	6.0/3.0	10.1/5.0
7	28	L	13.0/3.3	10.8/3.0	5.9/2.9	4.2/2.1	8.1/3.1
		R	14.8/3.0	9.2/2.9	0.9/3.4	5.5/2.9	8.2/3.5
8	65	L	15.3/3.7	12.1/2.8	10.6/3.5	6.7/5.5	NA
		R	11.3/3.8	5.9/3.3	4.6/3.1	5.9/5.3	9.2/4.1
9	30	L	14.7/3.7	18.4/2.4	3.8/3.6	7.4/2.6	10.8/4.4
		R	5.9/4.6	11.2/3.6	1.8/4.7	6.8/3.8	4.7/5.0
10	32	L	7.8/4.0	15.3/2.2	3.8/3.8	6.0/3.9	13.5/3.1
		R	6.7/3.6	6.9/2.9	NR	4.3/3.8	2.0/6.3
11	90	**L**	9.2/3.6	7.7/2.8	0.6/4.6	5.7/3.6	NR
		R	NA	10.0/3.0	2.6/3.8	5.0/3.0	11.6/3.3
12	43	L	NA	15.2/2.2	1.3/4.3	5.0/2.9	NA
		R	25.7/3.3	16.7/2.6	1.9/3.9	5.0/2.7	17.8/3.4

Bolded indicates involved side of sciatic neuropathy. Underlined numbers indicate abnormal results based on lab reference ranges.

Abbreviations: EDX, electrodiagnostic; L, left; R, right; SNAP, sensory nerve action potential; mV, millivolt; ms, millisecond; S, superficial; EDB, extensor digitorium brevis; TA, tibialis anterior; AH, abductor hallucis; NA: not assessed. NR: no response.

recovery, and 5 patients (42%) had a complete or near-complete recovery of sensorimotor deficits. No surgical decompression of the sciatic nerve was performed in any patients.

Discussion

The differential diagnoses of sciatic neuropathy include common fibular neuropathy, lumbosacral (L5 or S1) radiculopathy, and lumbosacral plexopathy. EDX testing is frequently needed to achieve a definite diagnosis. Among these entities, lumbosacral radiculopathy is the most common, being associated with normal sensory NCSs.[1] In our patients, a diagnosis of lumbosacral radiculopathy was ruled out based on the following: (1) an initial presentation of unilateral distal lower extremity sensory and motor deficits rather than lower back or radicular pain; (2) etiologies or circumstances rendering the sciatic nerve to compression or inflammation; (3) normal needle examination findings of the gluteus medius, gluteus maximus and/or lumbosacral paraspinal muscles; and (4) lack of significant findings on MRI of the lumbar spine in the majority of patients. A diagnosis of lumbosacral plexopathy was additionally ruled out with a lack of gluteus medius and gluteus maximus involvement on EMG.

Table 3. Needle electromyography findings

Patient No.	Side	EDB	TA	PL	AH	BFLH	TP/FDL	BFSH	ST	MG
1	L	abnl	abnl	abnl	NA	NA	nl	abnl	nl	abnl
2	L	abnl	abnl	NA	abnl	NA	abnl	nl	nl	nl
3	L	abnl	abnl	NA	abnl	NA	abnl	NA	nl	nl
4	R	abnl	abnl	abnl	NA	abnl	NA	NA	abnl	abnl
5	R	abnl	abnl	NA	abnl	abnl	abnl	abnl	abnl	abnl
6	L	abnl	abnl	abnl	nl	abnl	nl	abnl	abnl	nl
7	R	abnl	abnl	abnl	nl	abnl	abnl	abnl	abnl	nl
8	R	abnl	nl	abnl	abnl	nl	abnl	nl	nl	nl
9	R	abnl	abnl	l	abnl	NA	nl	abnl	abnl	abnl
10	R	abnl	abnl	abnl	abnl	NA	abnl	abnl	abnl	abnl
11	L	abnl	abnl	NA	abnl	NA	abnl	nl	abnl	nl
12	R	abnl	abnl	NA	abnl	NA	abnl	nl	nl	abnl
% of abnl		100	92	86	80	80	73	60	58	50

Abbreviations: EDB, extensor digitorium brevis; TA, tibialis anterior; PL, peroneus longus; AH, abductor hallucis; BFLH, biceps femoris long head; TP/FDL, tibialis posterior/flexor digitorum longus; BFSH, biceps femoris short head; ST, semitendinosus; MG, medial gastrocnemius; L, left; abnl, abnormal; NA, not assessed; nl, normal; R, right.

Previously, Yuen et al[2] described a series of 100 patients with sciatic neuropathy. In 9% of patients, normal unilateral sural and superficial peroneal sensory SNAPs were recorded. However, interpretation was limited due to a lack of sensory NCS of the contralateral lower extremity. In our study, bilateral superficial peroneal sensory NCSs were performed in all 12 patients and bilateral sural sensory NCSs in 10. All obtained SNAPs fell within the normal range according to our laboratory standard. Therefore our study confirmed the presence of a group of sciatic neuropathies that may relatively spare sensory fibers. Amplitude asymmetry on sural or superficial peroneal SNAPs was observed in 3 patients, with lower amplitudes being seen on the affected side but still falling within normal range. Thus it is important to perform sensory NCSs on both sides in the evaluation of unilateral sciatic neuropathy. It is worthwhile pointing out that all patients in this study had sensory complaints and/or abnormal sensory examination findings despite preserved SNAPs.

Our observations seem to indicate that sensory and motor fibers in the sciatic nerve can be differentially affected, either due to anatomical separation within the sciatic nerve or intrinsic quality differences between sensory and motor fibers. The interpretation based on anatomical separation seems to be supported by several previously published studies demonstrating the somatotopic fascicular organization of human sciatic nerves.[5-6] Results of human sciatic nerve dissection and fascicle mapping by Gustafson et al[6] revealed that fibers of the superficial peroneal, deep peroneal and sural cutaneous remains fascicular and independent within the sciatic nerve. Therefore it seems plausible that the selective involvement of the motor fibers seen in our patients be explained on the basis of fascicular anatomy.

This group of patients with sciatic neuropathy was mostly young, and had a higher female to male ratio when compared to previous studies.[2,4] Yuen et al[2] previously reported that most of their patients with intact SNAPs revealed mild axonal loss changes with normal or near normal CMAP amplitudes. In our study, a reduction of CMAP amplitude was observed in 10 patients, and sensory sparing were seen in patients with both mild (e.g., patient 5) and severe (e.g. patient 11) sciatic neuropathies.

We are uncertain about the evolutional changes on EDX studies due to a lack of follow-up EDX data. SNAPs

likely evolve with the disease course and may improve with the removal of triggering factors or worsen as the disease progresses. Three patients in our study had a disease course of longer than 6 months. This seems to suggest the relative sparing of sensory fibers in sciatic neuropathy may persist for an extended duration in some patients.

In conclusion, our study demonstrates that the presence of intact sural and superficial peroneal SNAPs does not rule out a diagnosis of sciatic neuropathy. A diagnosis of sciatic neuropathy should be based on a combined analysis of clinical circumstance, symptoms and signs, EDX data and radiological findings. Sciatic neuropathy with preserved SNAPs is preferentially seen in young females. The mechanism for the differential involvement sensory and motor fibers in the sciatic nerve merits further study.

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Safety of Needle Electromyography in Critically Ill Patients

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ABSTRACT

Introduction. To evaluate the safety of needle electromyography (EMG) in critically ill intensive care unit (ICU) patients who are on anticoagulants and have comorbidities that increase the risk of bleeding and infections.

Methods. We conducted a retrospective chart review of critically ill patients who underwent needle EMG studies. The most common complications following needle EMG were reviewed and classified based upon common terminology criteria for adverse events (CTAC) criteria. Descriptive statistics were reported using the frequencies and percentages for categorical variables. Mean and interquartile range is used for continuous variables. All analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS Statistic Version 21, IMB Inc., Chicago, IL.

Results. Twenty-nine patients were included. 17 (58.6%) were males with a mean age of 60.8 +/- 16.7 years. The mean PT, PTT, and INR were 15.2 sec, 36.5 seconds, and 1.13, respectively. Fourteen (48.2%) patients in this cohort were treated with low molecular weight heparin (LMWH), and an additional 8 (27.5%) patients were administered subcutaneous (SC) heparin for deep vein thrombosis prophylaxis. Therapeutic heparin was being used in 3 (10.3%) patients and sequential compression devices (SCDs) in 4 (13.7%) patients. A total of 228 muscles were tested. Among them, 38 (16.6%) were deep muscles. There were no major bleeding complications at the time of the procedure and for the next seven days in any of the patients, including those with multiple medical comorbidities. All our patients met the grade 1 scale in the severity of adverse events criteria proposed by CTCAE.

Conclusion. Needle EMG is safe in critically ill ICU patients on anticoagulants and multiple comorbidities including those that increase the risk of bleeding and infection.

Keywords: *Needle EMG, ICU patients, Electro-Diagnostic Study, Safety of EMG, EMG in Critically Ill.*

Introduction

Needle electromyography (EMG) is routinely performed in patients with suspected myopathy and peripheral nervous system disorders. The needle EMG portion of the electro diagnostic testing can be a painful and uncomfortable procedure but is generally well tolerated and poses very little risk to patients (1). The rare complications associated with the needle EMG include local bleeding, hematoma, infection, pneumothorax, and nerve injury (2). There are no absolute contraindications to the needle EMG procedure according to the guidelines proposed by the American Association of neuromuscular and electro diagnostic medicine (AANEM). The diagnostic yield of these studies, in many cases, outweighs the risks associated with the procedure in making a definite diagnosis (3). The safety of needle EMG has been studied extensively in a healthy population, patients on antiplatelet medications, and anticoagulant medications such as warfarin and non-vitamin K oral anticoagulants (4). Performing a detailed neurological examination in critically ill patients can be difficult, and electro diagnostic studies provide valuable information in these patients. Patients who are critically ill and in the intensive care unit (ICU) have multiple comorbidities and are sometimes admitted in the ICU for an underlying neuromuscular disorder.

However, the safety of needle EMG in critically ill ICU patients remains unknown. The objective of our study is to determine the safety of needle EMG procedures in critically ill ICU patients.

Methods

Basic demographic and the coexisting ICU comorbid conditions, coagulation studies such as prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR) within 24 hours before the needle EMG procedure were reviewed. Thrombocytopenia defined as a platelet count of less than 150,000 (5). Detailed histories, along with the indications for the usage, type of anticoagulation, and the concurrent usage of antiplatelet, were reviewed.

Needle EMG was performed as per the standard protocol and included both superficial and deep muscle groups (we classified muscles as deep if the muscle needed to be reached by traversing through another muscle or located next to a bony prominence) (6).

All the patients in our study underwent nerve conduction studies which include the motor (median, ulnar, tibial and fibular nerves), sensory nerve conduction studies (median, ulnar, superficial fibular and sural nerves), repetitive nerve stimulus, phrenic nerve conduction studies, and needle electromyography as per the standard guidelines proposed by the American Association of neuromuscular & electro diagnostic Medicine (AANEM) (6).

A retrospective chart review of commonly reported complications such as infection, bleeding, pneumothorax, compartment syndrome, and necrotizing fasciitis reviewed for seven days following the procedure. The infections during and after the needle EMG in critically ill patients were classified based on the definition of infection in patients with sepsis proposed by the international sepsis forum consensus conference (7). The hemorrhagic complications were classified based on the bleeding academic research consortium definition for bleeding (8). All our patients had a chest x-ray on the following day, the results were reviewed for pneumothorax, and electronic medical records (EMR) were reviewed for the occurrence of compartment syndrome. Finally, the severity of the adverse events was classified based on the common terminology criteria for adverse events (CTCAE) (9).

Descriptive statistics were reported using the frequencies and percentages for categorical variables. The mean and interquartile range is used for continuous variables. All analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS Statistic Version 21, IMB Inc., Chicago, IL.

Results

Twenty-nine patients were included in this study. Reason for the exclusion of other patients is described in the figure 1.

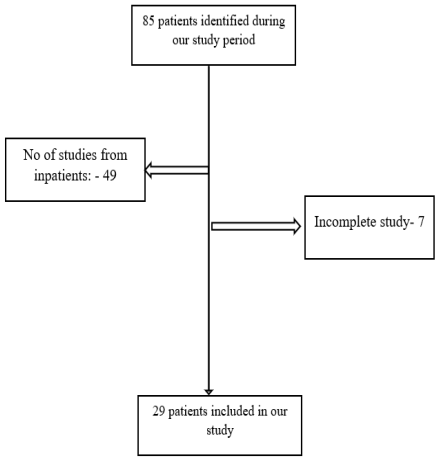

Figure 1: Reason for exclusion

Among them, 17 (58.6%) were males, and 12(41.4%) were females with a mean age of 60.8 +/- 16.7 years (mean +/- SD). The mean prothrombin time (PT) and partial thromboplastin time (PTT) were 15.2 (normal 13.8- 15.8 seconds) and 36.5 seconds (normal 27-37seconds), respectively. The mean International Normalized Ratio (INR) was 1.13 (normal INR- 0.9-1.1) of 23 patients who were on anti-coagulation, excluding the four patients with subcutaneous compression devices. Thrombocytopenia was seen in 7 (23%) patients. Fourteen (48.2%) patients in this cohort were treated with low molecular weight heparin (LMWH), and an additional 8(7.5%) patients were administered subcutaneous (SC) heparin for deep vein thrombosis prophylaxis. Therapeutic Intravenous (IV) heparin was being used in 3 (10.3%) patients and sequential compression devices (SCDs) used in 4 (13.7%) patients. Five (17.2%) patients were on aspirin, and the rest of them were not on any antiplatelet therapy. Twenty (69%) patients had at least one comorbid condition such as cardiovascular disease, malignancy, renal failure, or diabetes mellitus. The most common indication for needle EMG study was a generalized weakness in 21 (72.4%), difficulty to wean off the ventilator in 4 (13.7%), bilateral lower extremity weakness in 2 (6.8%), hemi-facial weakness in 1(3.4%), and neck weakness in 1 (3.4%) patient. Out of 29 patients, 26 underwent upper and lower extremity nerve conduction studies, and three patients exclusively underwent repetitive nerve stimulus along with needle EMG. Among 26 patients, four of them who had difficulty in weaning from the ventilator underwent phrenic nerve conduction studies, MRI of the C-spine, and ultrasound of the diaphragm. At the time of needle EMG study, 8 (33.3%) patients met the definition for the infection proposed by the international sepsis forum consensus conference. A total of 228 muscles were tested. Among them, 38 (16.6%) were deep muscle groups. The muscle groups are summarized in table 1.

None of the patients required any immediate intervention to prevent bleeding, and none had visible clinical hematoma following the procedure, including those patients who had thrombocytopenia. Also, none of the patients required a radiological evaluation for hematoma during the next seven days of chart review. Twenty-one (72.5%) patients had no signs of infection, including superficial and deep tissue infections, and this remained unchanged at seven-day follow-up. None of the patients had a pneumothorax (on follow up imaging: chest x-ray) or compartment syndrome. All our patients met the grade 1 scale in the severity of adverse events criteria proposed by CTCAE. Diagnosis of the un-

Table 1: Muscle groups tested in critically ill patients who underwent needle EMG.

Muscles checked	Frequency of muscles
Deltoid	25
First Dorsal interossei	25
Biceps	22
Triceps	13
Iliopsoas	8
Trapezius	5
Cervical paraspinal	2
Thoracic paraspinal	2
Lumbar paraspinal	1
Vastus medialis	25
Mentalis	1
Tensor fascia lata	10
Frontalis	1
Orbicularis oculi	1
Gastrocnemius	24
Pronator teres	17
Tibialis posterior	1
Extensor digitorum communis	9
Tibialis anterior	27
Rectus femoris	1
Extensor indicis pollicis	3
Abductor pollicis brevis	5
Total	228

derlying neuromuscular condition was made in 93% of the patients that underwent needle EMG study, and the details summarized in table 2.

Discussion

In this study, we assessed the safety of needle EMG in patients who are critically ill and found the procedure to be relatively safe with only mild complications based on CTCAE criteria. No patients had secondary complications such as infection, hematoma, pneumothorax, compartment syndrome for seven days following the electro diagnostic studies.

Superficial and deep tissue infections are rarely reported in patients who underwent EMG studies, and the risk is estimated to be less than 1/10,000 (2). Burris et al. reported

Table 2: Summary of diagnosis from electrodiagnostic study

Diagnosis	No. of patient
Critical illness neuropathy/ Myopathy	7(24.1%)
Guillain-Barré Syndrome and its variants	7(24.1%)
Generalized Sensory motor Polyneuropathy	4(13.7%)
Motor Neuron disease	4(13.7%)
Myasthenia gravis	2 (6.9%)
Normal	1(3.4%)
Inflammatory Myopathy	1(3.4%)
Vasculitis Neuropathy	1(3.4%)
Inconclusive	2(6.9)

the first case of cellulitis after the needle EMG, and another case series described six patients who had skin and soft tissue infection (10, 11). In both studies, EMG was done using a reusable needle. We believe the reported cases of infection may have been due to improper sterilization techniques rather than the standard electro diagnostic procedure itself. The previous practice of performing needle EMG by using a reusable needle has been replaced by using disposable fine concentric needle electrodes. We are unaware of superficial infection after the introduction of disposable needles in clinical practice. In our study, 21 out of 29 who are critically ill with multiple comorbidities underwent needle EMG, and none of them had superficial skin infections or usage of antibiotics on follow up. These findings suggest that needle EMG testing might be safe even in critically ill patients with multiple comorbidities. 8 of the 29 patients were taking antibiotics during needle EMG studies and continued to be on them during follow up. Of the five patients in our study with lymphedema that underwent EMG procedure, none of the patients had an infection or required antibiotics on the follow up.

The AANEM recommends exercising caution while performing needle EMG in patients with platelets counts less than 50,000 IU, INR >1.5-2.0, or PT >1.5-2.0 seconds. (AAEM guidelines, 1999) (3). In our study, seven patients had thrombocytopenia, and their platelet counts ranged between 95,000-1, 40,000 number/ vol. Units, and none of them had immediate bleeding complications. Clinically significant bleeding complications are very rare following needle EMG procedure, and isolated case reports have been

reported in patients on therapeutic anticoagulation (12, 13, 14). The overall incidence of subclinical hematoma identified by ultrasound and other studies involving both superficial and deep muscle groups after a needle EMG procedure ranges between 0.62 and 1.45% (4, 15). Both studies included patients on therapeutic warfarin, antiplatelet agents, and healthy controls. In our study, three patients were on therapeutic anticoagulation at the time of the study. Very limited data is available on the safety of electro diagnostic studies in patients on prophylactic subcutaneous heparin or low molecular weight heparin. Gertken et al. did a retrospective chart review of 370 patients who underwent electro diagnostic studies and magnetic resonance imaging (MRI) of the spine within one week following the procedure. Four of those patients were on prophylactic anticoagulation (2 patients were on heparin while one patient each was on LMWH and dalteparin), and none had a symptomatic or asymptomatic hematoma on follow up imaging (16). In our study, 21 (72.5%) patients were on prophylactic anticoagulation and did not require immediate intervention or evaluation for hematoma following the procedure though, none of them had follow-up imaging for the assessment of hematoma. We believe patients may or may not have had asymptomatic bleeding following needle EMG, and it is consistent with the grade 1 scale in the severity of adverse events criteria proposed by CTCAE.

Compartment syndrome is a very rare complication after the standard needle EMG study. In our literature review, two isolated case reports with compartment syndrome following electro diagnostic studies were identified. In both cases, symptoms started immediately and slowly progressed in the next few hours before it became clinically evident. Accidental damage to the blood vessels might be the potential cause in these patients (17, 18). None of our patients had compartment syndrome even in the presence of multiple comorbidities and lymphedema.

Pneumothorax is a rare and potentially lethal complication after the needle EMG procedure. The high-risk muscle groups include serratus anterior, supraspinatus, rhomboids, diaphragm, trapezius, and cervical paraspinal muscles. Kassardjian et al. retrospectively reviewed 64,490 patients that were diagnosed with pneumothorax over 18 years. Among them, only seven patients had pneumothorax primarily due to electro diagnostic studies. In the same study, 22 patients also had a pneumothorax, which is temporally associated with EMG studies but was believed to be due to other causes such as lung biopsy or thoracoc entesis. In their series, the risk for the development of pneumothorax is higher in the serratus anterior (0.445%) and diaphragm (0.149%) followed by trapezius (0.117%) with the lowest risk from cervical (0.004%) and thoracic (0.003%) paraspinal muscles (19). All of the patients that had symptoms due to the pneumothorax the diagnosis were confirmed within 24 hours following electro diagnostic study. In our study population, we sampled only the trapezius and paraspinal muscles, and none had symptomatic pneumothorax in the follow-up imaging using a chest x-ray.

The following are the limitations of this study. The procedure technique varies between different electromyographers, making it difficult to generalize the conclusions from our findings. It is possible that the patients might have had asymptomatic bleeding secondary to needle EMG during follow-up. Few patients were already on antibiotics for various reasons before the needle EMG, it was difficult to assess for infection rate secondary to the procedure. Finally, it is noteworthy to mention that the authors were not blinded to the patient's condition, i.e., the comorbid conditions and the anticoagulation status. Another limitation is that the diaphragm and serratus anterior was not examined in any patient in this series.

Conclusion

Needle EMG of commonly tested superficial and deep muscles is safe in critically ill ICU patients who are on anticoagulants and have multiple medical co-morbidities including those that increase the risk of infection and bleeding.

Summary

Electro diagnostic studies like nerve conduction studies and needle EMG is gold-standard test in the evaluation of peripheral nerve disorders. American Association of Neuromuscular and Electro diagnostic Medicine (AANEM) recommends that there is no absolute contraindication to perform needle EMG. However, they recommend being cautious in patients on medically induced coagulopathy and thrombocytopenia. Patients admitted to ICU have multiple comorbidities and prone to have complications from the medical procedures and diagnostic evaluations. However, the safety of the needle EMG in critically ill is unknown. Our stud evaluated the safety of needle EMG in patients with multiple comorbidities and evaluated for various complications like bleeding, infection, pneumothorax, and compartment syndrome. No major complications are found based on our study. Also, all our patients met the grade 1 scale in the severity of adverse events criteria proposed by CTCAE.

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Botulinum Toxin for the Treatment of Lower Limb Cramp Pain in Patients with Amyotrophic Lateral Sclerosis

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ABSTRACT

Background. Muscle cramps and pain associated with them can be seen in patients with amyotrophic lateral sclerosis (ALS) and are known to reduce the quality of life. Pharmacological treatment may not benefit all patients in treating these cramps. We assess the efficacy of Onabotulinum toxin A (BTX-A) in the treatment of lower limb cramps in patients with ALS.

Methods. This retrospective chart review included a total of ten patients with ALS who suffered from pain due to lower limb cramps and were managed with BTX-A. Data including patient demographics, visual analog pain scale at different intervals during follow up, ALS functional rating scale and site of onset of ALS symptoms were documented. The pain score at baseline (before administration), at 3 months follow up and at 6 months follow up were compared using Wilcoxon test to assess BTX-A's efficacy.

Results. A significant improvement in average pain score due to cramps from baseline to the 6-month interval with a change of 3.1±0.7 ($p<0.05$,95%CI) was seen on the pain scale. No adverse events were noted during administration or post injections.

Conclusion. Local BTX-A administration is an efficacious and safe procedure for improving pain associated with cramps in patients with ALS.

Introduction

Muscle cramps are involuntary contractions of an individual muscle or muscle group which can range from mild to severe, and is known to reduce a patient's quality of life by negatively affecting sleep and causing pain lasting for days. [1,2] Various physiological states are commonly associated with cramps, including pregnancy and fatigue. [3-7]. There are two main theories describing the origin of a cramp - the unusual arousal of terminal branches of motor axons, and hyperactive motor neurons in the spine. [3,7-14] These two processes can occur simultaneously thereby leading to muscle hyperactivity presenting as a cramp.[9]

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by degeneration of anterior horn cells. Cramps are one of the most commonly reported symptoms with up to 90% of the patients diagnosed with ALS having them during the course of the disease.[15,16,17] A study reported that about 56% patients with cramp require treatment for the same.[18] They have also been reported to be the presenting symptom and may precede weakness and wasting by several months.[19,20] Cramps in ALS patients are caused by excitation of glutamate, triggering the random release of motor nerves, which eventually progress to muscle fibers.[4]

Currently, treatment for ALS is centered around symptom management.[21-23] Typical treatments for cramps and the pain associated with it include antiepileptic drugs, quinine, and magnesium supplements, which are known to have unpleasant effects and are not efficacious.[24-27] The use of vitamin E, gabapentin, and quinidine have been investigated for the relief of cramps in ALS patients, but none were successful in alleviating cramps.[28-31] Mexiletine, a sodium channel blocker has been demonstrated to reduce cramp and cramp induced pain without affecting the progression of the disease.[32] Another effective management modality which has proved to be efficacious is the use of onabotulinum toxin A (BTX-A) which works by preventing acetylcholine release at the neuromuscular junction thereby relaxing the muscle and ceasing the pain caused due to cramps.[33,34,35]

The purpose of our study is to assess the efficacy of botulinum toxin in reducing lower limb cramp pain in ALS patients.

Materials and Methods

Our study is a retrospective chart review of patients that attended a University based hospital approved by the Institutional review board (IRB) and the IRB waived the need to collect informed consent for this study. The study population included patients with ALS aged more than 18 years who were undergoing care our hospital for the same.

These patients had undergone BTX-A administration for managing pain due to cramps by the same physician. Only patients with lower limb cramps and at least 6 months follow up during the study period were included. All patients in this study had tried and failed two or more medications for cramps (either reached maximum dose with no

benefit or had side effects resulting in discontinuation or dose limitation). The standardized injection sites for BTX-A included bilateral gastrocnemius (50 units each), quadriceps (50 units each) and intrinsic muscles of the foot (50 units each). They received injections every three months to coincide with their regular clinic visit. A total of 10 patients fulfilled the criteria and were made a part of the study.

Information including age, gender, race, site of onset of ALS, ALS functional rating scale score and visual analog pain score was collected for these 10 patients. The visual analog pain score was recorded at baseline before the administration of botulinum toxin and was followed up at 3-month and 6-month intervals from the first injection.

Results

Out of the 10 patients seven were males. Table 1 summarizes the patient demographics of our study. Our study showed the average ALS functional rating scale score amongst these patients to be 36.5±5.01. The average pain score due to cramps at baseline (before the administration of BTX-A), at 3 months follow up and at 6 months follow up were 8.8±0.7 (8-10), 6.8±1.5 (4-9) and 5.7±1.4 (4-8) respectively.

The change in average pain score from baseline to 3 months follow up was 2±0.8 (p=0.05,95%CI) with correlation coefficient of 0.782. However, a significant improvement in the score from baseline to the 6-month interval with a change of 3.1±0.7 (p<0.05,95%CI) was noted. Figure 1 demonstrates the change in pain scores from before administration to 3 month and 6-month intervals. No adverse either during the procedure or during interval between injections were reported.

Table 1: Patient demographics

Characteristics of the patients	Detail
Age (years)	64.1± 5.48
2.Gender (M/F)	7/3
Ethnicity	
• Caucasians	9
• African American	1
Site of onset of ALS	
• Lower limb	8
• Upper limb	2

Statistics

The analysis of the data included summarizing patient demographics and pain scores in form of descriptive statistical variables including mean, standard deviation and ranges. Comparison of the pain scale at different intervals was done using Wilcoxon signed rank test and a correlation between them was done. All statistical analyses were done using SPSS v22 software (IBM, Armonk, NY).

Discussion

ALS is characterized by progressive weakness in absence of pain and sensory loss. Although not a cardinal symptom in ALS, pain is bound to occur to patients at some point during their illness. Cramps are a leading cause of pain in patients with ALS and have been reported to affect 92% of ALS patients in the USA. Occurring on an average of 5.3 cramps per day, they do not correlate with the disease severity or duration.[16] They have been reported to occur most commonly in the calf and thigh followed by hand and foot. Cramps were reported to trend down from the first year of the disease to the second and third year although its prevalence appears to be stable from the first to the third years of ALS and increase during months of illness prior to diagnosis.[16] This was one of the rationales why we chose the specific sites for injecting BTX-A in these patients.

Our study assessed the efficacy of BTX-A which has been reported to effectively manage cramps in other conditions including benign fasciculation syndrome and diabetic neuropathy. [33,36,37] The property of relaxing the muscle by preventing acetylcholine release at the neuromuscular junction has been utilized in ALS patients to manage spasticity, sialorrhea and dysphagia. [38,39,40] Table 2 summarizes the different studies that used medications to manage pain as a result of cramps in patients with ALS.

A relatively recent trial by De Carvalho in 2010 aimed to assess the efficacy of Memantine when compared to placebo in 63 participants of the study reported the failure of Memantine as a cramp relieving agent.[47]

A study in 2016 involving 60 patients from 10 centers randomized 1:1:1 to placebo, mexiletine 300mg/d or mexiletine 900mg/d and followed for 12 weeks. Mexiletine 300 mg/d was found to be safe and well tolerated whereas Mexiletine 900mg/d was associated to more discontinuations. Large dose dependent reductions in muscle cramps frequency and intensity were reported in patients who were on Mexiletine. A dose dependent reduction in pain intensity was also noted when compared to placebo (300

Table 2: Summary of studies conducted with different medications that were targeted to provide relief to ALS patients with cramps.

Authors	Reference	Year of study	Medication used	Outcome
Norris et al	41	1979	Baclofen	Diminution of cramps in placebo arm and Baclofen arm
Blin et al	42	1989	L-threonine	No improvement in cramps
Blin et al	43	1992	L-threonine	No improvement in cramps
Gil et al	44	1992	L-threonine alone, L-threonine + L-isoleucine and L-valine combination	No change in cramps
Desnuelle et al	45	2001	Riluzole, Riluzole + Vit E	No significant difference in cramps between treatment and placebo group
Miller et al	30	2001	Gabapentin	No difference between treatment and placebo group.
Meininger et al	46		Xaliproden	No significant change in cramp characteristics.
Weber et al	48	2010	THC	No significant change in cramp characteristics.
De Carvalho et al	47		Memantine	No significant difference between two groups.
Weiss et al	32	2016	Mexiletine - 300mg/d and 900mg/d	Dose dependent reduction in pain intensity
Oskarsson et al	49	2018	Mexiletine	13 out of 18 patients had significant reduction in cramps due to administration of drug.
Our study		2019	Onabotulinum toxin A	Significant reduction in average pain score from before administration to 6-month interval

mg Mexelitine: 37% placebo, p=0.058; 900mg mexiletine: 16% of placebo, p=0.025%). There was a decrease in ALS FRS-R that was observed and was not different from that seen in the placebo group.[32] A multi-center, double blinded, placebo control cross over trial of Mexiletine involving reported the reduction of cramp frequency in 18 of 20 patients out of which 13 reductions were attributed to the treatment ($p<0.05$). One of the patients discontinued the study due to dizziness while the other initiated an open label mexiletine therapy.[49] As compared to Mexiletine, the adverse effect profile of BTX-A is less severe which is reflected in our study. None of our patients complained of any adverse effects and all patients continued using this option. The improvement in average pain score from before administration to follow up at 3 months was insignificant while that from before administration to at 6 months follow up was significant implying that approximately 6 months were needed to get significant improvement in pain due to cramps. This implied that BTX-A may be a good option to manage pain due to cramps in a long-term setting.

Our study however has notable limitations including a small sample size and the use of a subjective pain scale to

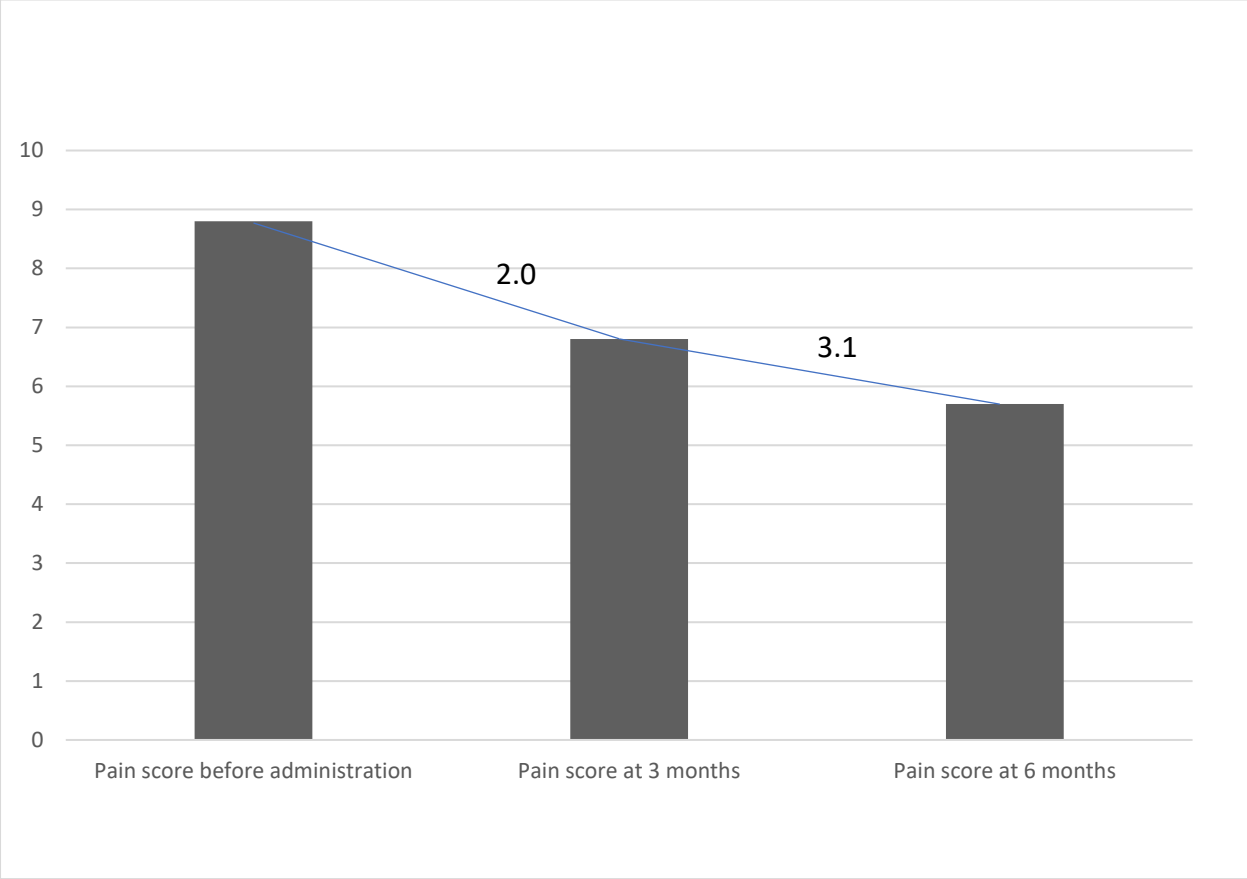

Figure 1: Graph showing the change in average pain score at different intervals of time during the patient's follow up. The number on top of the graph denotes the decrease in average pain score from the score noted before administration of botulinum toxin to that time interval.

assess the change in pain from last visit. We also lacked objective cramp measures. Larger studies including randomized clinical trials should be conducted to have a definitive conclusion about the efficacy and tolerability of using botulinum toxin in the management of pain due to cramps in patients with ALS.

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Neuromuscular Disease Update: What's of Note from 2019-2020?

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Keywords: *neuromuscular disorders, update, spinal muscular atrophy, myasthenia gravis, diabetic neuropathy, inclusion body myositis, Pompe disease.*

As I have constructed these in recent years, this review runs "in reverse" from skeletal muscle retrograde to the motor neuron. All studies were published in 2019 or 2020, and within one year of preparation of this bulleted syllabus. I focus mainly on management issues but there is a bit of pathogenesis mixed in. I hope the review provides a framework for some of the advances in our field in the last year.

Muscle Disease: Late-onset Pompe and enzyme replacement therapy

- Prior well-designed randomized, controlled studies have established the efficacy of enzyme replacement therapy (ERT) with recombinant human α-glucosidase in infantile and late-onset forms of Pompe disease, an autosomal recessive, previously progressive and even fatal myopathy.
- In adults, ERT has demonstrated benefits in ambulatory function and muscle strength, stabilization of pulmonary function and increased survival. However, follow-up studies through 3 years have suggested that these benefits wane with secondary decline over time.
- Late-onset patients in the initial LOTS trial have now been followed for 10 years or longer, providing better understanding of long-term benefits from ERT.
- The prospective, multicenter cohort study (class IV evidence) of 30 patients who were initially enrolled in the only randomized placebo-controlled trial or the extension study of ERT in late-onset Pompe disease found that most patients benefit long-term, but that a minority will experience decline after 3 to 5 years.[1]
- Overall, 93% of patients had initial benefit. At last follow-up (median 9.8 yrs), 52% continued to have a better than baseline 6 minute walk test or upright FVC than their baseline. There were no clear predictors for an initial or secondary response.

Muscle Disease: Pathogenesis of inclusion body myositis (IBM)

- Histologic features of myonuclei and mitochondria along with aggregation of myofibrillar cytoplasmic proteins have fed the degenerative hypothesis for IBM. Yet, an autoimmune pathogenesis remains actively argued.
- In a GWAS/big-data study of 411 muscle biopsies, including 44 of IBM, 77 with other inflammatory myopathies, 188 with other forms of myopathy (hereditary) and 106 normal muscle specimens, a signature pattern was seen for IBM, distinguishing it from others.[2]
- Highly differentiated CD8+ T-cell effector memory and killer cell lectin-like receptor G1 (KLRG1)+ cells identified in IBM. KLRG1 found in T-cells invading IBM myofibers, and these cells were also increased in IBM patient blood.
- These highly differentiated cells were poorly proliferative and are known to be resistant to immunosuppressive therapy.
- Targeting these cytotoxic T-cells has therapeutic potential in this treatment-refractory muscle disease.

Neuromuscular Disease: Social functioning and fatigue

- Chronic fatigue is the main factor in reduced social participation per 60% of surveyed neuromuscular patients.
- Aerobic exercise and/or cognitive behavioral therapy have reduced fatigue levels in a variety of neuromuscular disorders including FSH and myotonic dystrophy, mitochondrial myopathy.
- The Energetic intervention study[2] enrolled 53 patients with a variety of neuromuscular disorders in a 1:1, rater-blinded controlled trial. Intervention subjects underwent aerobic training for a total of three 30 minute sessions for 4 months in addition to energy conservation management strategy training (eight 90-minute sessions) and ten relapse prevention sessions at home.[3]
- Subjects who underwent the Energetic intervention scored significantly better on the Canadian Outcome Performance Measure (COPM) vs. controls. They also showed significant improvement

on the 6 minute walk test, depression scales and activity measures.
- Fatigue and anxiety scales did not show a significant difference between the two populations
- Overall 72% of intervention subjects had a ≥2 point improvement on the COPM vs. only 25% of control subjects, yielding a NNT of 2.3.

Myasthenia gravis: Preoperative IVIg in generalized patients undergoing procedures

- Plasma exchange and intravenous immunoglobulin both used to prepare MG patients for surgery. No prospective data on need for such preoperative therapies in well-controlled generalized MG patients.
- A double-blind, placebo-controlled randomized trial of IVIg 0.4 gm/kg x5 days vs. saline was performed in 45 generalized MG patients, 43 of whom were AChRAb+. The primary outcome was myasthenic crisis with secondary outcomes including length of stay and QMG scores. Most of the procedures were thymectomy or GI-related.[4]
- There was only one crisis episode in a 63 year-old woman with thymoma who developed bilateral pleural effusions and a left phrenic nerve palsy post-op. There were no significant differences in QMG scores or time in hospital.
- A pre-op QMG score of <8 and FVC>70% predict that MG patients will tolerate surgery without the need for preparatory IVIg.[4]

Myasthenia gravis: FcRn antagonists as new therapeutic approach

- These agents are being widely studied in immune-mediated disorders. They block the neonatal Fc receptor that normally ensures IgG homeostasis by rescuing IgG from degradation in lysosomes. FcRn antagonists lower all IgG subtypes quickly, peaking at approximately a 70% reduction, similar to plasma exchange.
- The first full report of a randomized, placebo-controlled trial of an FcRn antagonist studied efgartigimod, an IgG1 mutated Fc portion. A total of 24 patients were randomized 1:1 to efgartigimod 10 mg/kg weekly infusions for 4 weeks vs. placebo. Patients were followed through week 11.[5]
- The primary outcome was safety, and no SAEs were reported. AEs were predominantly mild and were balanced in frequency compared to placebo.
- Secondary outcomes assessed several MG outcome scales. Clinical benefit could be seen at one week that persisted on some measures through week 11. Significant separation vs. placebo was seen on the QMG at one, MG-ADL at two and MG-QOL15r at three time points during the study.[5]
- Phase 2 to 3 studies of this mechanism of action are underway for MG and other immune-mediated disorders.

Painful diabetic peripheral neuropathy: costs and complications

- A longitudinal analysis of a large commercial and Medicare claims database was used to compare costs of painful diabetic polyneuropathy (DMPN) vs. DMPN without pain and DM without neuropathy.[6] Patients were followed a median of 856 days.
- Of 360,559 total patients diagnosed with these conditions between 2010-2015, only 5% had non-painful DMPN vs. 23% with painful DMPN. So the cost differentials between painful DMPN and DM are likely underestimated.
- Baseline outpatient medication costs for painful DMPN patients were 1.67-2.13x as high than the other two patient groups.
- Painful DMPN patients were 2x as likely to receive opiates. Being on opiates increased the costs of care at 1 year by $7,000.
- Lower limb infections, amputations and falls were all significantly more likely in the painful DMPN group.[6]

Spinal muscular atrophy: real-world experience with nusinersen and combination therapy

- Between 2016 and 2019, FDA approved both nusinersen, an antisense oligonucleotide that increases full-length SMN production by altering SMN2 pre-mRNA splicing, and SMN1 gene replacement via AAV9. Combination therapy has not been formally tested.
- An Italian multicenter study assessed 12-month outcomes in 85 SMA type 1 patients ranging in age from 2 months to almost 16 years of age.[7] Sixty-one of the children had 2 SMN2 copies, 18 had 3 copies, 2 had one copy. The remainder were unknown.
- Significant group improvements in the CHOP-INTEND were seen except those starting nusinersen

after age 5 years and on the HINE-2 except those starting nusinersen after age 2 years.

- Improvement on functional scales and on parent/patient surveys were mainly in the motor domain. Less improvement noted related to gastrostomy, non-invasive ventilation and tracheostomy. Of note, an additional 7 children required gastrostomy and 2 required tracheostomy during the 12-month period.[7]
- Two infant boys presenting with hypotonia at 2 months commenced on intrathecal nusinersen loading doses at 5 to 5.5 months of age followed by the single AAV9 intravenous infusion at 9 months.[8]
- Both boys are gaining motor milestones with improved CHOP-INTEND scores, reduced BiPAP needs, enhanced functional skills per parents
- No adverse events or laboratory abnormalities were observed including platelets, LFTs.[8]
- Combination therapy is largely uncharted, with insurance coverage, treatment order implications. The potential for long-term side effects is unclear.

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Peripheral Nerve Hyperexcitability Following Titanium Marker Placement

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Keywords: *peripheral nerve hyperexcitability*, *titanium*.

INTRODUCTION

Peripheral nerve hyperexcitability (PNH) is characterized by spontaneous and continuous muscle activity, caused by hyperactive motor nerve terminal or neuromuscular junction. PNH can be associated with a variety of peripheral nerve disorders that can be inherited, autoimmune, metabolic or toxic.[1] Here we describe a patient of PNH following titanium marker placement during a breast biopsy.

CASE PRESENTATION

A 48 year-old Caucasian female with no prior medical history was noted to have microcalcifications in her left breast on mammogram. Biopsy revealed fibrocystic disease with no evidence of malignancy. During biopsy, a titanium marker was placed in the left breast for repeat imaging. One week later she developed stiffness in her heels while walking, followed by sensations of tingling in bilateral hands, bilateral feet and chest tightness. Within the following month, she noticed widespread muscle tightness that was prominent at the initiation of limb movement and became less noticeable with repeated motion. Frequent muscle spasms also appeared in her lower extremities. Her exam revealed increased tone in bilateral lower extremities, mild weakness in distal arms and legs, diffuse hyporeflexia, and prominent fasiculations in eyelids, tongue, and all extremities. Her lab work was significant for an elevated creatine kinase level of 885 U/L (reference range: 30-220 U/L) and a low vitamin B12 level of 193 pg/mL (reference range: 221-700 pg/mL). Acetylcholine receptor and muscle specific receptor tyrosine kinase antibodies were negative. Electromyogram (EMG) showed persistent continuous motor unit activity during attempted relaxation, doublets and triplets, myokymic discharges and cramp potentials in essentially all examined muscles, indicative of PNH. Motor unit potential configurations were difficult to analyze due to overriding spontaneous activity but appeared unremarkable. An extensive work up including inflammatory markers, heavy metal screening, serum paraneoplastic antibody panel, including voltage gated potassium channel antibodies, glutamic decarboxylase antibodies, computed tomography of chest; abdomen and pelvis was unrevealing. Cerebrospinal fluid analysis was normal. She was treated with carbamazepine 200 mg twice daily which led to significant symptomatic relief within 1 week. Patient also took B12 supplementation with correction of B12 levels subsequently.

The titanium marker was removed at 3 months, which led to no clinical improvement. The dose of carbamazepine was steadily increased to 200 mg four times a day and was associated with improvement in fasciculations, stiffness and gait. At 10 months, repeat EMG demonstrated a partial improvement in the amount of spontaneous discharges. She was treated with 5 cycles of plasmapheresis which led to short-lived improvement of stiffness, fasciculations and gait. Prednisone at a daily dosage of 40 mg was initiated, resulting in improvement of stiffness and weakness. Subsequently prednisone dose was gradually tapered. At 66 months following the initial onset, she remained minimally symptomatic on a combination regime of prednisone 10 mg per day and carbamazepine 200 mg four times a day.

DISCUSSION

Peripheral nerve hyperexcitability syndrome commonly results from ion channel dysfunction from either decreased potassium conduction or persistent sodium channel activity, or enhanced neuromuscular junction transmission from acetylcholinesterase inhibition. In our patient, an extensive work up for known causes of PNH was unrevealing. While the possibility of coincidence cannot be totally excluded, the close temporal relationship between titanium marker placement and onset of PNH may indicate an association. There have been reports of PNH occurring secondary to heavy metal exposure such as gold, platinum, mercury, lithium, and manganese.[2-6] The mechanism of PNH in these cases is typically due to toxicity resulting in ion channel dysfunction or acetylcholinesterase inhibition.[2-6]

Titanium is used extensively in surgical and radiological procedures due to its sustainability and biocompatible properties. It has been demonstrated that titanium may

alter the kinetics of voltage-gated potassium channel currents resulting in changes in neuron excitability.[7] However, a direct toxicity of ion channel may not be the underlying mechanism of PNH in our patient, as removal of the titanium marker did not lead to significant improvement. Recent studies also suggested that metals such as titanium, nickel, mercury and gold could trigger systemic autoimmune or autoinflammatory syndromes in humans.[8-9] The observed clinical improvement with prednisone and plasmapheresis treatment in our patient is supportive of an immune mediated etiology. Given the widespread use of titanium in surgical and radiologic procedures, clinicians should consider inquiring about titanium or other metal exposure in PNH syndromes.

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Anterior Spinal Extradural Cyst Mimicking Hirayama Disease and Amyotrophic Lateral Sclerosis

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Keywords: *anterior extradural spinal cyst, Hirayama disease, amyotrophic lateral sclerosis, myelopathy.*

Introduction

Hirayama disease (HD) is a focal motor neuron disorder that occurs in teenagers or young adults secondary to dynamic compression of the cervical spinal cord. Classically it manifests as insidious and progressive muscle wasting and weakness of the upper extremity, thus is often considered in the differential diagnosis of amyotrophic lateral sclerosis (ALS).[1] Spinal extradural cyst accounts for approximately 1% of spinal cord space-occupying lesions.[2] Here we describe a patient presenting with slowly progressive upper extremity weakness that was initially suggestive of HD or ALS, but was later found to have an anterior extradural cerebrospinal fluid collection secondary to spinal dural defect. We would like to alert readers that anterior spinal extradural cyst should be in the differential diagnosis of slowly progressive cervical motor neuron dysfunction. A brief description of this patient was presented previously.[3]

Case Presentation

A 47-year-old right-handed male with a past medical history of hypertension presented with progressive painless asymmetrical weakness and atrophy of bilateral upper extremities. Six years prior to presentation, he experienced sudden onset of severe mid-back pain while walking downstairs which forced him to sit down immediately. Back pain persisted for the next 24 hours; along with severe headache and gait imbalance. An evaluation by a emergency medicine physician was non-revealing. Within the next year, he began to notice a gradual worsening weakness in the right upper extremity with difficulty extending and griping his fingers. Intermittent muscle twitching was observed in his right hand, right upper arm and left forearm. Over the next several years, atrophy of the right pectoralis, right hand and forearm muscles became evident. There was no paresthesia or pain, bowel or bladder dysfunction, or involvement of bilateral lower extremities.

Neurologic examination at year six following symptomatic onset demonstrated asymmetrical (right more than left) atrophy of the forearm flexors, extensors, abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles. Fasciculations were observed in the bilateral triceps, right forearm flexors and bilateral FDI muscles. Muscle strength testing was graded as follows (right/left, Medical Research Council grade): deltoid 5/5; biceps 5/5; triceps 3/4; wrist flexors 3/5; wrist extensors 4/5; FDI 3/4; abductor digiti minimi 2/4; flexor pollicis longus 4/5 and APB 4/4. Muscle strength examination in the lower extremity was normal. Bilateral triceps tendon reflexes were hypoactive while bilateral quadriceps and right Achilles tendon reflexes were hyperactive. Remainder of his neurologic examination was normal.

Electrophysiological findings

Sensory nerve conduction studies of the right upper extremity and the right median compound muscle action potential (CMAP) were normal. The right ulnar CMAP amplitude was reduced. Needle examination revealed fibrillation potentials and long-duration polyphasic motor unit potentials in the following muscles of the right upper extremity: FDI, APB, extensor indicis, pronator teres, triceps, and lower cervical paraspinals. Nerve conduction studies and needle electromyography of the right lower extremity were normal. Electrodiagnostic findings were supportive of a focal motor neuron disorder or cervical polyradiculopathy affecting the C7, C8 and T1 segments or roots. It was felt that the patient may suffer from HD or a slow variant of ALS.

Additional investigation

Cervical spine MRI with and without contrast showed minimal lower cervical cord atrophy and moderate disc herniation at the C5/6 and C6/7 levels without significant cord compression. Flexion and extension MRI showed no anterior shifting of the posterior dura or posterior venous engorgement that would be suggestive of Hirayama disease. Axial T2-weighted image showed the presence of bilateral small hyperintense lesions consistent with a "snake-eyes appearance" in the lower cervical cord (Figure 1). MRI

myelogram revealed the presence of an anterior extradural fluid collection that extended from C5 to L2 (Figure 1). CT myelogram revealed pooling of contrast in the cervical neural foremen, indicating cerebrospinal fluid leakage. Further review of the MRI showed the presence of anterior spinal cord herniation through an anterior dural defect at T2-T3 level (Figure 1).

Clinical course

Patient declined dura repair surgery. He underwent an upper thoracic epidural blood patch treatment. Follow-up exam at one year showed no further progression of his weakness and muscle atrophy. Patient noted he could run quicker, and his stamina also improved. Patient declined follow-up testing. Telephone follow-up for the next 4 year indicated a stable course without further worsening.

Discussion

The occurrence of spinal cysts is associated with surgery, trauma, neural tube defect, and arachnoiditis.[4] Anterior extradural spinal cysts mimicking HD or ALS has been described previously.[5-6] Rahmlow et al described a 34-year old man with a 5-year history of progressive forearm weakness and atrophy. MRI showed a longitudinally extensive anterior spinal extradural cyst extending from C2 to L1. CT myelogram revealed a slow cerebrospinal fluid leak. Patient's strength improved after receiving surgical cyst drainage and fenestration.[5] Schmalbach et al. described 3 male patients who presented to ALS clinic with weakness and fasciculations in the upper extremities. Clinical and electrophysiological studies in these patients was in accordance with a diagnosis of possible ALS by the revised El Escorial criteria.[7] In all 3 patients, repeat cervical spine MRI several years into the course showed the presence of anterior spinal cysts. Cyst resection in 1 patient led to clinical improvement.[6] Authors stressed the importance of repeating cervical spine MRI later in the course for the purpose of identifying cervical extradural cyst that may have been missed with initial imaging.[6] Like our patient, all 5 patients presented with an asymmetrical upper extremity weakness and atrophy with a symptomatic duration of 5 years or longer.[5-6]

The etiology of cyst formation in our patient could be related to a minor trauma. Anterior spinal cysts may cause motor neuron dysfunction by compressing anterior spinal arteries leading to compromised microcirculation and eventual anterior horn cell death or compressing ventral roots.[6] MRI of the cervical spine in our patient showed hyperintense lesions with a snake-eyes appearance in the anterior cervical spinal cord. This appearance was previously described in spinal cord ischemia due to thrombosis or dissection as well as in which HD, further supports a mechanism of chronic ischemia as the cause of anterior horn dysfunction.[8-10]

A surgical repair was offered to our patient but he declined. Instead, a less invasive approach of epidural blood

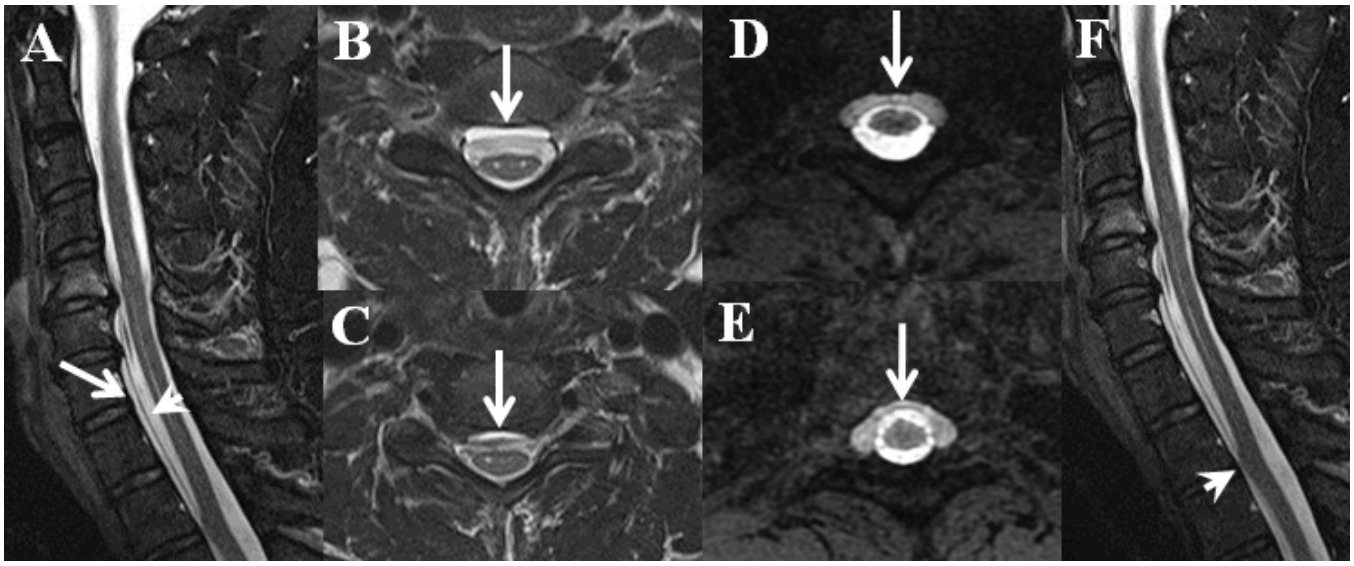

Figure 1: MRI findings. Sagittal cervical spine MRI (A) shows the presence of anterior dura (arrowhead) and a longitudinal anterior extradural spinal cyst (arrow). The extradural cyst is better visualized on the axial T2 weighted MRI sequence (arrows in B and C) and axial MRI myelogram (arrows in D and E). Hyperintense lesions in the anterior cervical cord show "snake-eyes appearance" (B and C). Arrowhead in F indicates the location of spinal cord herniation through an anterior dural defect.

patch was accepted by the patient to repair the dural defect. We were unclear whether such a treatment fixed the dural defect due to a lack of follow-up MRI. However, disease stabilization and improvement occurred in our patient following treatment, suggesting a possible efficacy.

Conclusion

Anterior extradural spinal cyst should be in the differential diagnosis of slowly progressive upper extremity weakness and atrophy of long duration, and its identification via appropriate imaging may lead to possible curative treatment.

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Contractures in Myopathies

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ABSTRACT

The two videos show physical examination of two patients with contractures. These questions will be answered in the next issue of RRNMF Neuromuscular Journal, along with further discussion as to how to approach a case of muscle contracture and myopathy, as well as teaching points.

Keywords: *Contractures, Neuromuscular, Myopathy.*

Questions:

Q1/ A 34-year-old man who walked on his toes as a child and had Achilles tendon surgery. As he grew older, he developed weakness of the triceps and knee flexors and extensors. He had two healthy brothers and no family history of muscle disease. Examination findings are shown in the vide number 1. CPK was: 477 IU/L, Electromyography (EMG) showed mixed long and short duration MUAPs in the tested proximal muscles. (see video 1)

Cardiac involvement is typically a feature of the following myopathy:

A. Oculopharyngeal muscular dystrophy (OPMD)
B. Emery-Dreifuss muscular dystrophy (EDMD)
C. Facioscapulohumeral muscular dystrophy (FSHD)
D. Collagen VI myopathies

Q2/ A 32-year-old woman who walked on tiptoes at age 5 years for which she had an elongation of the Achilles tendon bilaterally. She had one healthy sister and no family history of muscle disease. She developed a fixed mild proximal legs weakness since childhood. CPK level was slightly elevated and EMG was myopathic. Physical findings are shown in video 2.

Contractures are common in the following myopathies:

A. Limb Girdle muscular dystrophy type 2 B (LGMD2B)
B. Myotonic dystrophy
C. Bethlem myopathy
D. FSHD

ALS Patients Demand

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Explanation Regarding the Proposed Grant: Patients and health care providers were discussing the possibility of doing a multiple drug "cocktail" trial for amyotrophic lateral sclerosis (ALS). When this U01 PRA/RFA grant opportunity was released in 2015 we thought it might be a good vehicle to attempt to get this funded. The goal was to leverage multiple sites funded by NIH Clinical and Translational Science Awards (CTSA sites) and also include other sites (total 25 sites). After a great deal of discussion, we decided on a three-arm study and the drug cocktail was designed to potentially attack the pathophysiologic processes of neuroinflammation, motor neuron hyperexcitability and glutamate excitotoxicity. In all three study arms patients were to receive standard of care which included access to riluzole, PEG, BIPAP and then they would be randomly assigned to one of three arms:

1) tamoxifen (20mg: 2 times/day) and ranolazine (500 mg: 2 times a day
2) tamoxifen (20mg: 2 times/day) and mexiletine (200 mg: three times/day
3) tamoxifen (20 mg: 2 times a day) and memantine (20 mg: 2 times/day)

Subjects were to be randomly assigned using a Bayesian adaptive design process that we used successfully in the PCORI funded comparative effectiveness drug study for neuropathic pain.

We called the project ALS PATIENTs DEMAND which stood for the ALS Patient-Driven Electronic-based Multidrug Adaptive Network Design clinical trial

Because the grant was to NCATS and the goal was to introduce novel trial designs that could be extrapolated to other diseases, we also had an aim to utilize a number of new initiatives to streamline regulatory oversight, ensure meaningful patient -engagement, enhance recruitment and decrease the burden of outcome collection.

We divided the sites into three regions and proposed to use IRB reliance models at U California -Irvine on the west coast (Dr. Tahseen Mozaffar as Irvine sites PI and leader of the west coast group), at Univ. Kansas as the lead in the Greater Plains Collaborative PCORnet network, (Dr. Jeffrey Statland as site PI) and in the NIH Create consortium based in Miami (Dr. Michael Benatar site PI and leader of the CReATe group).

We proposed to use the then recently created EPIC downloadable ALS clinic templates to collect the data.

We proposed two-way video web-based interactions with patients so they would not have to come in for as many visits.

We believed the study could create a model for multicenter research studies seeking to more efficiently maximize network-level collaborations to study any rare disease.

This was an ambitious proposal that did get scored (41) but this was not in a fundable range.

At the time these U01 grants allowed for direct costs of nearly 1 million dollars a year for 5 years.

We had to apply via an XO2 preproposal mechanism to apply for the large study. The XO2 was submitted in the summer of 2015 and was accepted in the fall of 2015. We submitted the full proposal in early 2016.

We have attached PDFs of the Specific Aims page, the Research plan, and the Critiques.

The reviewers were very critical of our attempt to use three IRBs to control the study and in retrospect this was a valid criticism. They stated this could potentially jeopardize the safety of the study. This is why they concluded the protection of human subjects "was unacceptable" along with some toxicology concerns. Even with the three central IRB approaches we still had intended for Univ Kansas to be the primary CCC/DCC, but this did not come across in the proposal. They thought we were saying the three CTSA hubs (Kansas, Miami, Irvine) each were responsible for all the DCC/CCC activities of the sites using their IRBs. That was not our intention.

Regarding the cocktail approach, some of the reviewers thought this was not novel as it had been used in cancer and HIV studies. We felt these reviewers did not appreciate the difficulties in doing this for ALS.

Only one reviewer addressed the drugs in the cocktail. They stated tamoxifen was not well justified and that each drug had side effect profiles and that the side effects of each drug "could be viewed as exacerbating the ALS disease process"!

They really liked the Bayesian adaptive design.

In talking to leaders at NCATS after we received the critiques, they encouraged us not to do a cocktail study.

When we resubmitted the proposal, we engaged the new NIH funded Trial Innovation Network resources. Johns Hopkins is one of the TINS and we applied for a consultation on how to improve our proposal and we were accepted into the TIN program. We worked for nearly a year to improve the application and resubmitted with more simplified trial design comparing mexiletine and ranolazine and also randomizing sites to enrolling subjects as traditional urban research centers (TURCs) or mobile innovation research centers (MIRCs) to also test the hypothesis that we can just as easily do research remotely. This application did not do much better with a score of 40. We continued to work with the TIN and now we believe we have further improved the trial which was resubmitted in March 2020.

1. Specific Aims

New translational science tools and approaches for more rapidly advancing health research to the common goal of improved cures and treatments are especially needed for studies of rare diseases. The goals of this application are 1) to create a model for leveraging existing national research initiatives and new translational science tools to build the infrastructure to run multi-site studies in rare diseases; and 2) for proof of concept, to use this CTSA-based national research infrastructure to test the hypothesis that drug combination therapy in amyotrophic lateral sclerosis (ALS) will be more effective than standard of care alone. ALS is a rare progressive neurodegenerative disease which is uniformly fatal. Traditional approaches to developing therapies have failed in ALS, yielding only a single FDA approved therapy with a modest benefit on survival. Thus, there is a pressing need for new therapeutic approaches in ALS. One such approach used to treat cancer and HIV has been drug combinations targeting different pathological pathways. We convened an ALS patient and caregiver focus group who expressed overwhelming interest in using a drug 'cocktail' approach to ALS therapy. Patients also frequently state they feel left out or abandoned once their disease progresses beyond the earliest stages, the most common focus of most ALS clinical trials. Therefore, we designed a patient-driven clinical trial to assess which of three drug cocktails targeting different pathological pathways are the most effective in slowing disease progression in ALS: the **ALS Patient**-**D**riven **E**lectronic-based **M**ultidrug **A**daptive **N**etwork **D**esign clinical trial (**ALS PATIENTs DEMAND**). For **ALS PATIENTs DEMAND** we will leverage existing major initiatives to simplify the regulatory process, to connect electronic health records (EHRs) of large academic ALS centers, and to roll out common data elements through the EHR and via CTSA funded REDCap databases to build a large national ALS clinical trial network—providing the bandwidth to study large numbers of ALS patients, and importantly, to broaden study inclusion criteria to include ALS patients often excluded from traditional clinical trials.

Aim 1: To leverage existing research initiatives and introduce new innovations to streamline regulatory oversight, ensure meaningful patient-engagement, enhance recruitment, decrease the burden of outcome collection, and hasten results dissemination using 3 CTSA Coordinating Centers and 25 sites (19 CTSAs). Specifically:

a. We will compare the regulatory efficiencies across 3 different networks: two IRB reliance models (Greater Plains Collaborative: a PCORNet CDRN; and the University of California Regulatory System); and one central IRB (the ALS Rare Disease Clinical Research Network). We will compare the time to regulatory approval, time from regulatory approval to first patient enrollment, and rates of accrual.

b. We will create a patient engagement plan which incorporates the patient voice into all aspects of the clinical trial: protocol development, recruitment, retention, study conduct, and dissemination of results.

c. We will use EHR-defined computable phenotypes for patient screening, and compare this approach to traditional recruitment strategies in the clinic or via patient advocacy groups.

d. We will leverage the availability of EPIC downloadable ALS clinic templates for the primary set of outcomes to compare outcomes collected by this EHR-i2b2 interface with those collected by using REDCap database links within the local clinic work-flow. We also will explore whether this approach reduces the burden on patients, caregivers, and health care providers.

e. We will implement a patient visit and adverse event monitoring system via a two way web-based video system already in production to reduce the burden of participation and ensure retention.

Aim 2: To determine which of three drug regimens added to standard of care has the greatest efficacy for slowing ALS disease progression. For this aim we will conduct a prospective 12 month three-arm Bayesian response adaptive randomization clinical trial. Drug combinations will repurpose FDA approved drugs for other indications which act on potential ALS pathological pathways (neuroinflammation, motor neuron hyperexcitability, and glutamate excitotoxicity). Informative priors and stopping criteria will be derived from the PROACT data base of 8500 patients. The diverse ALS population in our national ALS network (over 4700 patients) and the informative priors derived from PROACT will allow us to broaden our inclusion criteria to include patients often excluded from current clinical trials.

Our proposed collaboration among CTSA coordinating centers to create a model for assembling study-specific infrastructure for rare diseases will <u>not only serve as a blueprint for future clinical trials in ALS, but also will inform any multi-center clinical trials seeking to more efficiently maximize network-level collaboration to study any disease.</u>

2. Research Strategy

A. Statement of the Problem and its Significance to Translational Science

The goal of this application is to create a model whereby we will leverage existing national research initiatives and translational science tools to create the infrastructure needed to run multi-site studies in rare diseases. We intend to show that deploying innovative translational science approaches can accelerate testing of putative therapeutics for rare diseases. By definition a rare disease in the US is one where < 200,000 people are affected; however, taken together there are approximately 7000 rare diseases(1). This represents a significant burden to the US health care system. Barriers to developing new therapies for rare diseases include: 1) the need to use multiple sites to recruit sufficient numbers of patients to obtain statistical rigor; 2) difficulties with regulatory oversight for large multicenter studies causing delays in start-up and increasing study costs; 3) difficulties with assembling efficient networks for data collection, while minimizing patient burden; 4) ensuring patient and caregiver voices are heard throughout the therapeutic development process; 5) using technology to overcome barriers in distance or medical infirmity to allow all eligible patients to participate in the research enterprise. The national CTSA system gives us an unprecedented opportunity to use our existing infrastructure to build on the models for IRB reliance, use the national REDCap database infrastructure, use CTSA based patient engagement initiatives, leverage new health care technology development, and formalize existing multi-institution relationships to address a large unmet medical need. We propose to leverage the broad CTSA-based national research infrastructure and the regulatory structure of 3 established clinical research networks to conduct a clinical trial in amyotrophic lateral sclerosis (ALS).

ALS is a progressive uniformly fatal neurodegenerative disease. The median age of onset is between 50 and 60 years, where individuals are at the peak of earning, which places a tremendous financial and emotional burden on patients, family members, and their communities(2). ALS is characterized by spasticity and hyperreflexia from the loss of upper motor neurons in corticospinal tracts and from muscle weakness, fasciculations, and atrophy due to lower motor neuron degeneration and death in the anterior horns of the spinal cord(3). Disease progression leads to limb paralysis, loss of speech, swallowing, and respiratory functions, and ultimately death. While ALS is regarded as a rare disease, affecting only ~21,000 people in the US at any point in time, the incidence (at 2 per 100,000) matches that of common neurological diseases such as multiple sclerosis(4-6). Moreover the lifetime risk of dying from ALS is about 1 in 400(7). ALS therefor appears rare only because we have no effective therapies and because the disease is fatal.

Traditional approaches to therapy development have so far failed in ALS, yielding only one FDA approved drug, Riluzole, which prolongs life by 2-3 months(8, 9). The current standard of care for ALS patients is primarily supportive with the goal of maximizing quality of life(10). The 2009 American Academy of Neurology recommendations for the management of ALS patients state that in addition to Riluzole, enteral nutrition via percutaneous endoscopic gastrostomy (PEG) should be considered to stabilize body weight in patients with impaired oral intake, and noninvasive ventilation (NIV) should be offered in order to prolong survival and slow the impact of declining forced vital capacity (FVC). PEG or NIV can extend life by approximately 6 months if the treatments are adhered to and applied early(10, 11).

Since traditional approaches to therapy development have failed, we urgently need to apply innovative translational science approaches to ignite a paradigm shift in the way we approach therapy development for this fatal neurodegenerative disease(12). Thus, as a first test of our proposed approach, we will establish the ALS Patient-**D**riven **E**lectronic-based **M**ultidrug **A**daptive **N**etwork **D**esign (**ALS PATIENTs DEMAND**) infrastructure needed specifically for studying ALS treatment options and determining which of three drug combination regimens has the greatest effect on slowing ALS disease progression.

The significance of this project is: 1) it will present a model for leveraging existing national infrastructure and translational science innovations for clinical trials in rare diseases; and 2) it will answer the question of whether drug combinations work better than standard of care in ALS, which would have an impact on patient care.

B. Rationale

Establishing an innovative CTSA-based national research infrastructure will allow us to contribute to advancing translational science by critically assessing innovations such as patient-engagement, streamlining regulatory oversight, and using other new initiatives (e.g., IRB reliance agreements) and translational science tools (e.g.,

EHR-i2b2 interface capabilities for downloadable clinic templates). Our project also is innovative in being the first to rigorously test the efficacy of drug combination regimens for ALS.

Accumulating evidence points to multiple pathological processes being active in ALS – this raises concerns that the disease cannot be halted or slowed by simply targeting one of these mechanisms. Important lessons for approaching ALS can be learned from other diseases: in HIV they found that only by targeting multiple steps in the pathological cascade could they affect a significant health impact on controlling HIV; and in cancer the use of combination therapies which target multiple pathological pathways has essentially become standard of care for many malignancies (3, 13, 14). The exact underlying cause of ALS motor neuron degeneration may remain uncertain; however, convincing evidence, supports the role of a number of pathological pathways—including glutamate excitotoxicity, neuroinflammation, and motor nerve hyperexcitability (15-17). Each of these pathways has FDA approved drugs for other indications than ALS currently available on the market. Many of these drugs have shown promise in ALS studies *in vitro*, in animal models, or in small often underpowered clinical trials(18). This is frustrating to both patients and clinical researchers, as many currently available and potentially effective drugs are not being tried or are being discarded in ALS due to lack of money or initiative. We have convened two patient and caregiver focus groups who have expressed overwhelming interest in using a drug 'cocktail' approach to ALS therapy. By creating drug combination regimens which target multiple pathological pathways we may be able to slow progression in a more profound and lasting fashion than any one drug alone.

The ability to obtain statistical rigor to test drug combination regimens in ALS requires large number of patients and multiple sites participating across the country. ALS patients are seen in either an Amyotrophic Lateral Sclerosis Association (ALSA) or Muscular Dystrophy Association (MDA) sponsored clinics, usually at tertiary care centers. While the vast majority of our ALS patients are seen in ALS specialty clinics, most of which are affiliated with CTSA academic centers, our ability to pool resources and recruit large populations of ALS patients for studies has been limited. This is not due to lack of interest from patients. The existing CTSA national infrastructure, combined with three large existing network initiatives, provide an unprecedented opportunity to consolidate resources and build on these existing initiatives to advance translational science.

With respect to the specific proposed trial, if any of the tested drug combinations prove effective in ALS; this will have a dramatic and immediate impact on patients, their family members, and communities. All of the proposed drug combinations repurpose FDA approved medications currently used for other indications *which should all be available in generic preparations by completion of this trial*. Thus, most ALS patients could obtain immediate access to and benefit from these proposed treatments.

Equally important, if the study design innovations proposed here prove feasible this may not only transform the way we approach clinical trials in ALS and rare diseases, but also will inform any multi-center clinical trials seeking to more efficiently maximize network-level collaboration to develop new therapeutics for any disease.

C. Strategy and Methodology

Aim 1a. *We will compare the regulatory efficiencies across 3 different networks: two IRB reliance models and one central IRB.*

Collaboration: In order to maximize efficiency and streamline design of the **ALS PATIENTs DEMAND** infrastructure we will leverage three existing research networks (Figure 1):

- We will designate three CTSA Coordinating Centers (CTSA CC), each of which plays a key role in an established research network.
- The CTSA CCs will share the workload of reviewing the 25 sites, thus gaining efficiencies in the start-up process.
- There are 5 sites not affiliated with a CTSA CC. These unaffiliated sites will be added to a CTSA CC.
- All sites are ALS specialty centers with long histories of working together in smaller existing research consortia: i.e. the Western ALS Study Group, the Northeast ALS Consortium, the ALS Research Group, or in prior investigator-initiated ALS studies.

The three research networks included in this study are: **1) the Patient Centered Outcomes Research Network (PCORnet) Greater Plains Consortium (GPC) (19), 2) The University of California Biomedical Research Acceleration, Integration, and Development (UC BRAID)(20), and 3) The Clinical Research in**

ALS and Related Disorders for Therapeutic Development (CReATe), a Rare Diseases Clinical Research Consortium that forms part of the NIH's Rare Diseases Clinical research Network. Within the GPC there are ALS specialty clinics that already have demonstrated the ability to combine resources to perform ALS research by conducting a survey of ALS patients using a single IRB of record. UC BRAID also has mature reliance architecture in place which will be used for this study. CReATe has an ongoing natural history study to better understand the phenotypic variability in ALS (that will not compete with the current proposed study), and a central IRB model in place. Part of CReATe is the ALS patient registry (**CReATe Connect)**, with hundreds of ALS patients registered, who have agreed to be contacted for future studies.

Our organizational structure will include one CTSA CC site representing each of the above networks, and 25 ALS specialty centers spread across the country (20 CTSAs, Figure 1). These CTSA CCs will be: 1) the University of Kansas Medical Center (KUMC) which serves as the data coordinating site for the GPC and the overall lead site for this study; 2) The University of California Davis (UC-Davis), which will coordinate with the UC BRAID system; and 3) The University of Miami, which serves as the main coordinating site for CReATe.

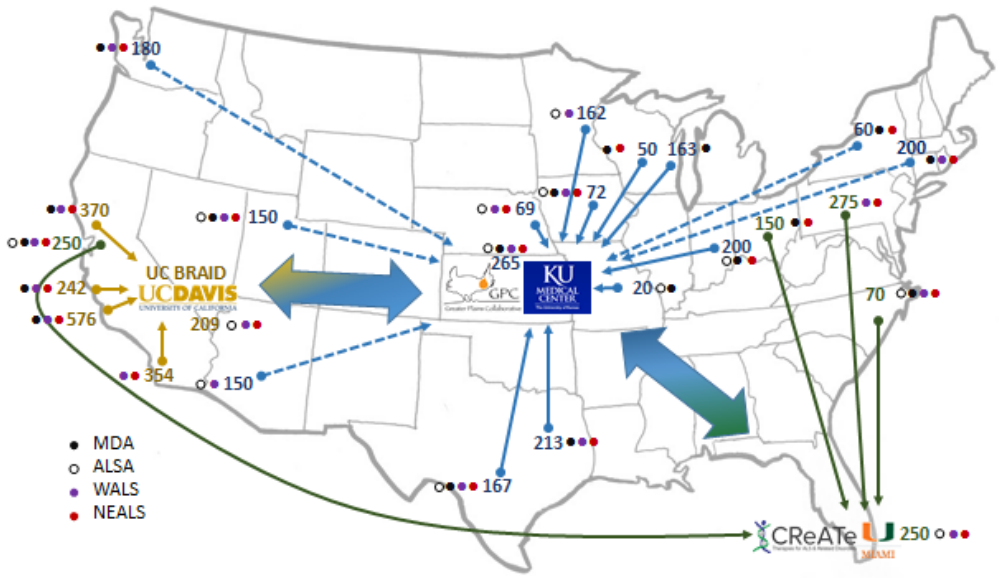


Figure 1. The three CTSA CCs and their existing research networks include 25 academic centers. The numbers represent estimated ALS clinic populations. Yellow=UC BRAID, Blue=GPC, Green = CReATe, *MDA = Muscular Dystrophy Association Clinic, ALSA = Amyotrophic Lateral Sclerosis Association Clinic, WALS = the Western ALS Study Group, NEALS = the Northeast ALS Consortium. Dotted lines represent sites not currently in GPC, UC BRAID, or CReATe.*

The organization structure will stem from the 3 CTSA CCs. We will compare the existing IRB regulatory models of these networks on key study timelines.

The GPC, UC BRAID and CReATe will be responsible for providing regulatory oversight of institutions in their existing networks. The workflow will include: Each of the CTSA CC ALS physicians will interact the with an **ALS PATIENTs DEMAND** steering committee (see below) to produce the final study protocol; and then the 3 CTSA CC regulatory members will create a common informed consent form. The KUMC IRB will coordinate with the other two CTSA CCs to facilitate communication and administration of regulatory tasks necessary for a successful start-up. The KUMC IRB is well situated to lead this cooperative model, and:

- Has experience in serving as the coordinating center for 3 PCORI funded trials,
- Has a dedicated reliance coordinator,
- Will lead development of the universal consent form to be used at all sites and customized only in limited areas relevant to local information (this important step will speed the approval of consent and other patient-facing materials by the 3 IRBs),
- Will centralize version control for all materials submitted to the three reviewing IRBs, and
- Will disseminate any updates to protocol or patient materials, safety monitoring updates (adverse events, DSMB reports, etc.)

A study steering committee was established for the design phase of this study and included: each of the CTSA CC PIs and ALS doctors, a patient representative, the study informatics officer, statistician, a PhD pharmacist, and representatives from patient advocacy groups. In addition the steering committee sought specific input

from patients who had participated in prior focus groups who agreed to be contacted for this study.

***Innovation*:** Our innovation is creating a nation-wide **ALS PATIENTs DEMAND** infrastructure not only to perform a large multi-center ALS clinical trial that uses established research networks with IRB reliance agreements in place, but also to test comparative efficiencies of different IRB reliance agreements and to serve as a model for assembling similar infrastructure to run multicenter clinical trials for any rare disease.

***Translation*:** Comparatively testing existing IRB reliance models and will inform translation by determining which is the most efficient on key factors that often delay or slow clinical trials. *Statistical considerations:* We will investigate the relative efficiency of each network by comparing their sites using outcomes: number of days to IRB approval and number of days from regulatory approval until the first patient is enrolled. Our first key metric will be **IRB Duration** (as defined in the proposed CTSA Common Metrics): "the time in days between the date that the application for IRB review is received by the IRB office and the date of final approval granted by the IRB with no IRB-related contingencies remaining." Our second metric is time from IRB approval to first enrollment—will track efficient study start-up processes once IRB approval has been granted. Since these are time-to-event outcomes we will use Bayesian Weibull models, including a censoring indicator for sites that may not have achieved an event within the study time, to assess this metric. We will follow rates of accrual across the different networks, and determine demographic features which could affect accrual rates. We will compare regulatory efficiency to KUMC's (GPC), and UC BRAID past values on these metrics. Identifying mechanisms to improve these metrics address an intermediate barrier to getting trial results into practice for ultimate patient health benefit.

This model of assembling research networks using existing infrastructure in a study-specific fashion can directly benefit patients with rare diseases, by allowing the assembly of the large multi-institute trials which will be necessary to bring scientific rigor to testing new therapeutics, but also pragmatic questions of patient care.

***Partnership*:** Dr. Richard Barohn is the overall study PI and KUMC CTSA PI and is responsible for coordinating all members of the study team. He will work with his collaborating CTSA PIs Drs. Lars Berglund (UC Davis) and Ralph Sacco (Miami) to ensure smooth operation of the informatics and regulatory conduct of the study. The lead ALS doctors, Michael Benatar (Miami), Bjorn Oskarsson (UC Davis), and Jeffrey Statland (KUMC) will coordinate within their respective CTSA CC to ensure smooth recruitment and retention of ALS patients across all 25 sites. Dr. Barohn, an experienced ALS multicenter trial investigator, is the PI on two FDA-OPD R01 ALS studies (for rasagiline and memantine). He has led the Western ALS (WALS) Consortium, participates in the Northeast ALS (NEALS) Consortium, and been on the executive committee of the ALS Research Group (ALSRG). He was instrumental in developing the ALS Common Data Element Forms (ALSCDE). Dr. Sacco, CTSA PI at the University of Miami, is Chairman of Neurology and was the PI of the Northern Manhattan Study which described the greater incidence of stroke in the Hispanic population. Dr. Berglund has been the PI of the UC Davis CTSA since 2006 and is the Senior Associate Dean for Research at the UC Davis School of Medicine. He has extensive experience with both basic and clinical research, serving as PI for several NIH R01 grants. Michael Benatar, MD, PhD is a Professor of Neurology at the University of Miami, Chief of the Neuromuscular Division, Executive Director of the Kessenich Family ALS Center, and holds the Walter Bradley Chair in ALS Research. He is the PI for CReATe (U54), an FDA funded trial of arimoclomol in SOD+ familial ALS (R01), the ongoing MDA and ALSA funded Pre-symptomatic familial ALS (Pre-fALS) study, and the University of Miami NeuroNEXT hub (U10). Björn Oskarsson, MD is an Associate Professor at the University of California – Davis and directs their Multidisciplinary ALS clinic. He has been in numerous clinical treatment trials and epidemiological studies in ALS. Jeffrey Statland, MD is an Assistant Professor of Neurology at KUMC, helps run both MDA and ALSA clinics, is a current CReATe ALS research fellow, and co-investigator on the GPC ALS projects.

Barriers: 1) *IRB Reliance, and IRB coordination between CTSA CC sites* – by building on existing consortia that use IRB reliance agreements and selecting sites with a history of working together, we expect to accelerate the timeline for study approval. The three CTSA CCs are committed to working together to solve issues in a timely fashion that may come up in start-up (see Letters of Support).

***Defining Success*:** We have already developed 3 research networks involving 20 CTSA sites. We are going to demonstrate cooperation among the 3 networks, with regard to recruitment and trial innovation. We will determine if any network has superior efficiency and why. We will then disseminate our experience to the entire CTSA community via the CTSA leadership channels.

Aim 1b. *We will create a patient engagement plan which incorporates the patient voice into all aspects of the clinical trial.*

Collaboration**:** Increasingly, collaboration with patients has become critical for translational research. True **patient engagement** reflects commitment to transparency, the practice of open, bidirectional communication, and an appreciation for the lived experience that only patients can provide as members of the research team. Investigators at the 3 lead CTSA sites have decades of community-engaged and community-based participatory research experience in frontier, rural, minority, disease-specific, and underserved communities in diverse geographic settings (FL, CA, KS) and are skilled at meshing investigators' concerns with those of participating patients, communities and stakeholders. Our investigators have a history of maintaining ongoing personal relationships with ALS patients and other stakeholders relevant to this application. These trusted relationships have and will continue to provide the project with established connections to further engage patients in and with this study. The community/patient engagement faculty from the 20 CTSA sites will serve as models to assist the non-CTSA participating sites that may not have established engagement programs.

Our comprehensive framework for patient engagement is the "ALS engagement ecosystem" (Figure 2), developed and refined with patient input. Each engagement element informs the other, and each makes a unique contribution. The model is a visual tool that ensures investigators understand the complex and unique contributions the non-academic members make to the team. It also is a useful reference point for addressing organizational and representational issues for decisions at all levels and for the design of the study.

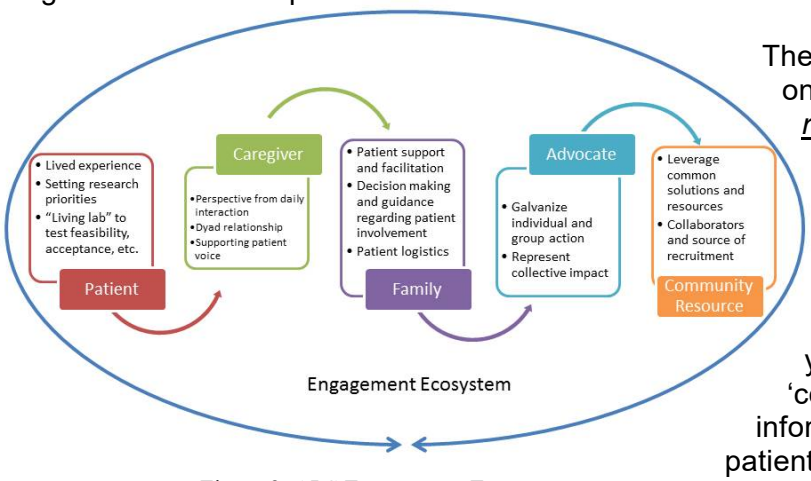


Figure 2. ALS Engagement Ecosystem

The approach for this project stemmed from ongoing dialogue with patients and families – *many of whom knew they would not likely benefit from participation given their advancing ALS.* Using facilitated discussion groups, these individuals asked the investigators to operationalize the "cocktail" design they discussed. Our ALS physicians discussed this issue with their patients and conversations yielded overwhelming support for a drug 'cocktail' approach to therapy. Using this information, we then convened a patient and patient/caregiver dyad focus group by phone (to reduce transportation and cost burden to participants). Four key findings from this focus group helped shape the approach for this study:

1. First, patients shared enthusiastic support for a multidrug study and agreed that better understanding the specific treatments proposed in **ALS PATIENTs DEMAND** would benefit patients.
2. Second, the patients and caregivers were passionate about ensuring that the study would be available to as many patients as possible. They discussed this topic at length. They understood that opening inclusion criteria would require more patients, and they suggested using functional criteria for inclusion, rather than disease duration.
3. Third, the participants said they preferred to use their personal computers or tablets to input their own patient-reported outcomes. Patients, as well as caregivers, were unanimous in sharing how stressful and energy-draining additional study visits are for both of them, so they want to provide as much information from home as possible.
4. Finally, the group overwhelming wants to stay involved throughout the study and to continue to advise and help in any way possible—especially in dissemination strategies that will assist the ALS community to learn about the study's findings.

They called on the investigators to be bold, to "think big," and to do all they could to remove barriers in order for more patients to become involved in research that could lead to better treatments. We look forward to continuing to work with our patient and caregiver partners.

Innovation**:** In addition to patient and caregiver input already obtained for the study design we will keep patients and caregivers involved throughout the running of the clinical trial.

- We will designate patient engagement leaders at each of the CTSA CCs

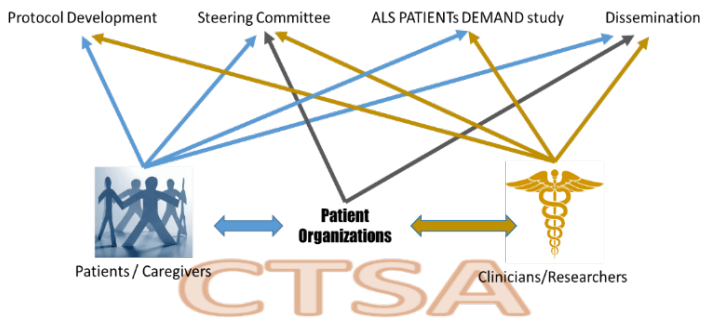
- We will create patient engagement circles and identify a pool of patients and their caregivers who can be called upon on an as-needed basis for focus groups to address specific study concerns regarding conduct, recruitment, and retention
- We will designate patient/caregiver representatives to formally participate in the **ALS PATIENTs DEMAND** study Steering Committee
- We will use both patient-representatives and our patient engagement circles to help disseminate study findings, as well as leverage existing patient outreach technologies from the MDA and ALSA clinics, and **CReATe Connect** ALS patient registry

Each of the 3 CTSA CCs will have engagement facilitator to ensure efficient and effective engagement activities at the network level. A lesson learned from our early work is that designated patient engagement leaders who can reach out and communicate with the entire engagement ecosystem need to be in place at the earliest possible point of the project. This ensures a consistent liaison function between the various engagement stakeholders and ensures maintaining fidelity so that no aspect of the study moves forward without the input or review from relevant stakeholders. For most participating sites, these trained site engagement facilitators will be the staff of their CTSA award community engagement programs. In addition to engaging patients as collaborators and full members of research teams, our patient engagement activities will address effective mechanisms for encouraging patients to become participants in research.

Translation: Comprehensive engagement, necessary for translation, must include active stakeholder involvement in project oversight, monitoring and leadership functions, not just for recruitment. We include stakeholders on all project committees and at all stages of the research, from early concept development through dissemination of findings. We will hold monthly team meetings (using GoToMeeting, or phone conferencing) to discuss study status. Patient representatives, site investigators, and data and safety monitoring board members will be on these calls. The specific composition of these groups will be established to ensure perspectives and input from patients, caregivers, MDA and ALSA clinics, and the CTSA CC engagement officers. We are sensitive to not burdening patient/caregiver/family stakeholders and overtaxing individuals willing to participate in this work. For example, participation at in-person team meetings can be exhausting, so use of online meeting resources like GoToMeeting.com and telephone conference calls that can be done from home always will be offered. Likewise, meeting times will be variable to be as convenient as possible for everyone.

We also will adopt a novel online platform developed at the Mid America Chapter of the ALSA. This innovation introduces a strategy to encourage camaraderie and increase knowledge penetration about the study. Co-developed with a private health IT firm, (HeartToHeart Network, LLC), the **ALS Care Portal** provides a way to communicate effectively and on an ongoing basis with ALS patients and facilitating the translation of our findings.

Upon completion of this study, we will establish a study communication committee composed of volunteers from our patient and caregiver collaborators and from registry and advocacy partners (MDA, ALSA, Prize4Life, and the **CReATe Connect** Registry). Results will be communicated through the broad network of ALS specialty centers from all 3 networks (GPC, UC BRAID, and CReATe). *The ability to mobilize patient and caregivers and engage them at each stage of the **ALS PATIENTs DEMAND** study, and to partner them with investigators and patient advocates will create an environment where all major stakeholders are directly involved, thus maximizing the impact of potential study findings, and accelerating their implementation into ALS clinical practice.*



Ultimately the success of **ALS PATIENTs DEMAND** is a collaborative effort (Figure 3). By combining three large existing networks, using ALS specialty centers, many which operate ASLA and MDA clinics, and bringing the patient voice into trial planning and conduct, we will create a flexible, highly leveraged ALS trial infrastructure, responsive to individual site level concerns and individual patient concerns, and will use this network to test combination therapies to halt disease progression in ALS.

Figure 3. Effective research into rare diseases is a collaborative effort – from the patient, to patient organizations, to ALS physicians. **ALS PATIENTs DEMAND** is built on CTSA infrastructure.

***Barriers*:** *Maintaining patient engagement throughout the complete study process* is one major barrier for this aim. We have a track record of maintaining patient engagement through our PCORI sponsored projects. We already have a strong commitment from patients and caregivers to serve on our Steering Committee. As any study is a fluid process, and success requires adapting to unforeseen circumstances, we have identified CTSA CC engagement leaders who will adapt our engagement efforts throughout study conduct.

***Defining Success*:** We will develop a broad engagement plan, demonstrate which engagement initiatives are the most successful, and disseminate the lessons across the CTSA consortium, and ALS research networks. We will define success for this aim as 1) established patient engagement circles and use of topic-specific focus groups to help with conduct, recruitment, and retention; 2) engaged patient representatives; and 3) a dynamic communication committee for study result dissemination.

Aims 1c-e. *We will use EHR-defined computable phenotypes for patient screening, and compare this approach to traditional recruitment strategies in the clinic or via patient advocacy groups. We will leverage the availability of EPIC downloadable ALS clinic templates for the primary set of outcomes to compare outcomes collected by this EHR-i2b2 interface with those collected by using REDCap database links within the local clinic work-flow. We will implement a patient visit and adverse event monitoring system via a two way web-based video system already in production to reduce the burden of participation and ensure retention.*

***Collaboration*: ALS PATIENTs DEMAND** includes 25 sites with varying capabilities for EHR-i2b2 (Informatics for Integrating Biology and the Bedside)(21) interface and different EHR systems (e.g. Epic/Cerner). Despite the diversity many features unite the study sites—including membership in broad research networks (GPC, UC BRAID, and CReATe), and most importantly, the underlying CTSA infrastructure resources (e.g., REDCap) at 20 of the proposed sites. We will build on the informatics infrastructure set up by the CTSA CCs to implement broad data 'packages' that will be within the technical operating capabilities at each site. Russ Waitman, PhD, PI of the GPC and head of informatics for the KUMC site, will take an overall coordinating role for this project. KUMC Informatics has integrated and augmented two widely used CTSA technologies (REDCap(22) and i2b2(21)) to CReATe HERON (Healthcare Enterprise Repository for Ontological Narration)—an i2b2-based data repository of EHR data from the KU Hospital and clinics integrated with biospecimen, a research participant registry, and national data—and have used REDCap as a low cost method for data capture and secure data delivery from HERON.(23) KUMC Informatics also has extensive experience through its leadership of the GPC, a PCORnet Clinical Data Research Network (CDRN) of 12 sites associated with 8 CTSAs and geographically dispersed over 1300 miles.(19) We have invested a major effort to develop and publish open source rich Extract/Transform/Load (ETL) software methods to facilitate data and methods sharing and are solidly positioned to tackle and contribute to new informatics advances in support of translational science. Dr. Waitman will work collaboratively with his counterparts at UC-Davis (Nick Anderson) and the University of Miami (Nick Tsinoremas). The ability to leverage existing PCORnet initiatives like an EHR-i2B2 interface and computable EHR phenotypes (GPC and at UC BRAID sites), and the ability to build on the common CTSA REDCap infrastructure, will maximize the roll out of existing technologies. *While logistically challenging the data innovations proposed here are feasible within the time frame of the grant.*

***Innovation*:** For the **ALS PATIENTs DEMAND** we will implement the following innovations: 1) we will use EHR computable phenotypes to assist with patient recruitment; 2) we will collect outcomes during the clinic work flow using an EHR-i2b2 interface, or customized REDCap link built into standard work-flow; and 3) we will use two way video to follow AEs or perform study visits for patients not able to travel into clinic.

Recruitment: At sites which have the capability (GPC, UC BRAID), we will use EHR computable phenotypes defined by diagnosis, standard diagnosis codes (e.g. ICD10), number of visits, and status as living. We will compare this recruitment approach to a standard approach of recruiting patients through clinics, use of advocacy groups (MDA and ALSA), and use of the **CReATe Connect** ALS registry.

- KUMC will define study cohort computable phenotype and study recruitment strategy using i2b2 queries developed for the PCORnet ALS cohort characterization. Recruitment will use a.) Manual

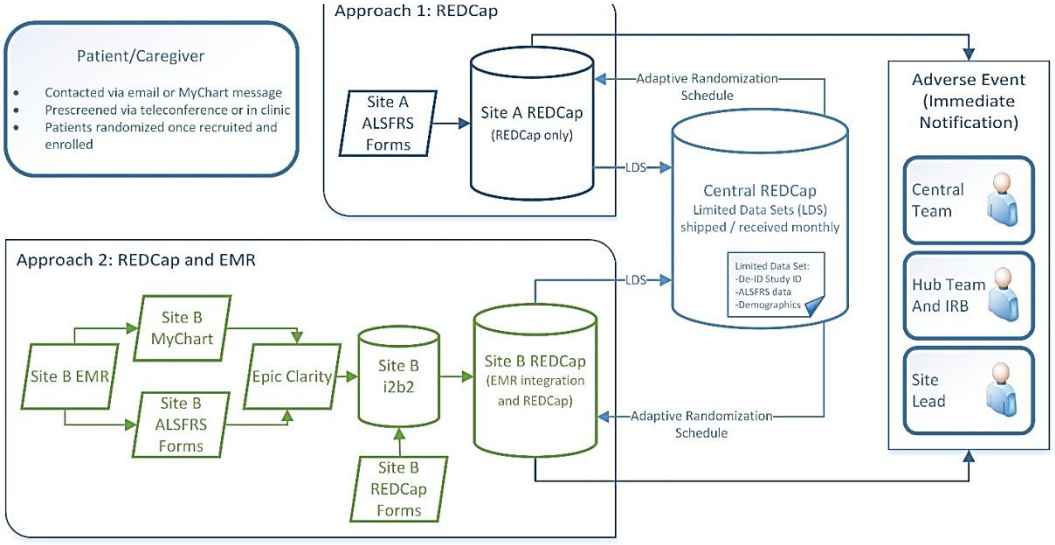
screening model (where coordinators enter prescreening info in REDcap), b.) Direct email to REDCap workflow for sites for prescreening of patients, and, (c.) MyChart to REDCap for the advanced sites.

- UC BRAID has the University of California Research eXchange (UC-ReX) Data Explorer which enables UC investigators to identify potential research study participants at the five UC medical centers. That system can be searched in a similar fashion to the HERON system in the GPC.

As proof of concept for this approach, the GPC used direct patient input to develop a revised version of the ALS Functional Rating Scale (ALSFRS-DEMAND) which patients complete from home. They then used the existing IRB reliance model to approve a protocol to conduct a survey of GPC ALS patients, using EHR computable phenotypes to identify patients, and combining survey response data with existing EHR demographic data as proof of concept for the approach. In that study we identified > 2000 ALS patients and greater than 50% of those who returned the survey stated they would be interested in participating in a clinical trial of combination therapy.

Outcome collection: We have designed two pathways for data collection. *Both approaches keep protected health information (PHI) local, and transmit de-identified data to the KUMC central REDCap architecture*. As shown in Figure 4 below, each site in **ALS PATIENTs DEMAND** will implement one of the two proposed data collection approaches. For Approach 1 (REDCap-only), data will be collected in a site level REDCap survey. For Approach 2 (EHR and REDCap), several sites will pilot this proposed advanced approach to data collection that leverages site level Epic-EHRs and REDCap.

***Figure 4**. Informatics Architecture for ALS Patient DEMAND*



For Approach 1, KUMC Medical Informatics (KUMC MI) will design and develop REDCap data dictionaries to be used to deploy the primary data collection instruments in REDCap installations at each site. Deployment at each site will be done via webinars with KUMC Informatics team members providing hands on support. KUMC MI team also will help the REDCap site teams deploy KUMC MI-developed REDCap plugins to use for notifying both site level and KUMC study team members of an occurrence of a Serious Adverse Event. A limited dataset version of the REDCap study data shared by the site with KUMC will inform the interim analyses for the Bayesian Adaptive Design. KUMC MI will coordinate with the site REDCap administrators to extract/upload the data and to deploy new randomization schedules derived from the interim analysis.

For Approach 2, sites will leverage EHR data collection and patient portal (MyChart) features in addition to the REDCap features of Approach 1. In this approach, patients will complete pre-screening via the site's Epic EHR patient portal, MyChart. Post-enrollment outcome measures, including ALSFRS, will be documented in the EHR along with REDCap. Initial work will involve implementing the EPIC forms and integrating them into test and development environments at each site. After validation the forms will be deployed in a production environment for use in the study. KUMC Medical Informatics also will develop additional ETL methods to integrate the measures collected at each site through their EHR and REDCap into a site-level i2b2 in order to enable sharing limited dataset versions of study data with the central study team for interim and final analysis. Data extraction will leverage the R Data Builder module developed by KUMC MI. The ETL code and R data builder will be tested at KUMC before distribution to all the sites. KUMC will host substantive, individual webinars with each site during installation of the shared code. In addition to these technical webinars, KUMC MI will provide training for study coordinators involved at sites implementing either approach.

Two-way web based video: KUMC also will leverage its experience deploying secure two way video communications through its https://telehousecalls.org application. Telehousecalls was developed for secure in-home patient/caregiver/provider communication with funding from the National Science Foundation and has been used to support both pediatric behavioral health and pediatric primary care consults for vulnerable populations through a grant from the REACH Healthcare Foundation.(24) For **ALS PATIENTs DEMAND**, Telehousecalls will be available for patients and caregivers as a platform for communication and assessments with providers. It will be modified to best support this trial with patients who may be affected by mobility issues. The ALSFRS-R can be collected via video interview and this two-way video can be used for adverse event (AE) reporting. The latter enables personal interaction and assures participants that their symptom is understood investigators that more serious AEs are not missed. This two-way video technology is adaptable to a variety of platforms (both PC and Mac, Android and iPhone) and enables patients who can no longer physically travel to the clinic for study visits to continue to participate in the study.

***Translation*:** The ability to assemble flexible clinical trial networks using existing CTSA, regional network, and NIH funded network infrastructure is transformative for patients with rare diseases like ALS. Our approach to such 'assembled' study-specific networks needs to be flexible, and **ALS PATIENTs DEMAND** will be a model for future studies. Key concepts such as: keeping PHI local; using EHR for both recruitment and data collection; using modern communication modalities to facilitate including patients who may not physically be able to travel to study centers; and creating work-throughs for assembling networks across multiple institutions all are addressed by **ALS PATIENTs DEMAND,** providing proof of concept that such a study can practically be assembled and conducted.

***Partnership*:** The key partnerships for Aim 1c-e will include the informatics officers at KUMC and the other two CTSA CCs, data personnel at each of the 25 participating sites, the local investigators, and the patients and caregivers participating in the study. The CTSA framework of sites with informatics people already familiar with REDCap makes the informatics portion of this study possible.

***Barriers*:** *Creating a universal REDCap data base with links to local EHRs* can be challenging, but we have already successfully used the proposed model in a current GPC ALS survey. For sites where we cannot provide live links via the EHR, patients will be provided web browser bookmarks for their tablet or computer. *Patient selection and recruitment via EHR* also can be challenging, but we also already have used computable phenotypes to identify ALS patients at the GPC and UC BRAID sites. That most ALS patients are seen in ALS specialty clinics allows us to recruit in clinic, from clinic rosters, or from regional ALSA or MDA patient lists as well.

***Defining Success*:** We will compare the frequency of recruitment via the EHR computable phenotype to traditional clinic / advocacy based recruitment. We will survey both patients and providers regarding data capture techniques, to determine if the current model reduces the overall study burden. We will disseminate the successful translational science approaches developed here to the CTSA consortium, and use this as a model for future ALS studies.

Aim 2. *To determine which of three drug regimens added to standard of care has the greatest efficacy for slowing ALS disease progression.*

***Collaboration*: ALS PATIENTs DEMAND** will be a three-arm, 12 month open label, response adaptive response randomized study involving 25 sites (and 20 CTSAs) associated with one of three cooperating regulatory networks (Figure 1). We will enroll 300 ALS participants. The sites included all have ALS specialty clinics, and include: 11 MDA certified ALS clinics; 5 ALSA certified clinics; and 9 clinics which run both MDA and ALSA certified clinics. Many of these sites have considerable experience participating in ALS clinical trials, and include: 16 sites who are members of the Western ALS Study Group (WALS) and the Northeast ALS Consortium (NEALS), 6 sites who are members of NEALS, and 2 sites who are members of WALS. Together WALS and NEALS have conducted over 21 interventional or observational ALS studies. Together the **ALS PATIENTs DEMAND** national ALS cohort is over 4700 patients. ALS patients seen in these clinics represent the full spectrum of disease, both genders, all races, and diverse socioeconomic status. Study visits will be designed to coincide with routine clinic visits, and will use the EHR patient portal, telephone calls, and video conferencing to collect information on patient functional status and adverse events between study visits. We will allow study personnel to report outcomes using the EPIC standardized forms via the EHR or via REDCap links during clinic visits.

***Innovation*:** The key innovations for Aim 2 are: 1) opening up of the entry criteria for the study, 2) the use of targeted combinations therapies, 3) the use of a Bayesian Adaptive trial design and the PROACT data set for informative priors; 4) entry of study data directly into EHR at the time of clinic visits, and by patients in the patient portal between visits; and 5) use of EPIC/EHR downloadable ALS clinic templates.

1) Patient characteristics: clinical trials of experimental interventions in ALS use narrow inclusion criteria which exclude most ALS patients by limiting studies to patients with symptom onset within 2 years, and FVC >75% predicted. This excludes more than half of patients who will have FVC < 75% predicted at diagnosis(25). In focus groups patients and their family members made it clear they find this approach makes them feel *left out* of studies of new or promising therapies. They suggested a more reasonable approach for inclusion would be by baseline functional status. Therefore, our inclusion criteria will be: a) A clinical diagnosis by a study investigator of ALS; and b) ALSFRS-R $\geqslant$ 20 (moderately affected); and our exclusion criteria will be: a) Any medication contra-indications for the particular drugs being studied; b) inability to provide informed consent; and c) current pregnancy or lactation.

2) Interventions: Considering the seriousness of the disease, the lack of robust efficacy of Riluzole (the only approved treatment for ALS), and limited options for further treatment, there remains a pressing unmet medical need for effective treatments for ALS. Three of the more promising pathological mechanisms with existing FDA registered drugs which could be repurposed for ALS are neuroinflammation, glutamate excitotoxicity, and nerve hyperexcitability. By creating drug combination therapies which target multiple pathological pathways we may be able to slow progression in a more profound and lasting fashion than any one drug alone.

Inflammation*:* Inflammatory monocytes and macrophages in the CNS have been shown to be involved both pathologically and in the rate of progression in ALS.(26-28) The importance of inflammation has been seen in both preclinical and animal model data.(27) The inflammation is associated not only with locally acting microglia, but also circulating inflammatory cells, which release cytokines believed to play a role in neurodegenerative processes, and to be harmful to motor neurons.(29, 30) Inflammation also is found in post mortem tissue from ALS patients.(29) The synthetic nonsteroidal drug tamoxifen is widely used in chemotherapy for breast cancer. A phase 2 randomized, dose ranging, selection trial of tamoxifen in ALS showed significant improvement in survival (P= 0.04) in those randomized to a 20 mg, 30 mg, or 40 mg daily tamoxifen treatment cohorts combined together.(31) For each dosage, survival was better at the higher dosage.(32, 33) Tamoxifen also may be neuroprotective – as metabolites have antioxidant actions since they are strong intramembranous scavengers of peroxyl radicals.

Nerve Hyperexcitability: Recent studies suggest that neuronal hyperexcitability may play a pathogenic role in ALS. Whole cell recordings from both embryonic and early postnatal SOD1G93A spinal motor neurons demonstrate increased persistent sodium current.(34, 35) Increased repetitive firing of cortical motor neurons following injection of current using current clamp conditions in SOD1G93A mice relative to age-matched controls has been shown to correlate with cortical hyperexcitability in the mutant mice.(36) Cell culture models for ALS have shown direct toxic effects of motor nerve hyperexcitability.(37, 38) Mexiletine and ranolazine are both FDA approved agents which act to reduce motor neuron hyperexcitability.(39-42). Ranolazine has been shown to block persistent sodium currents believed to play a key role in axonal neuro-degeneration and to block brain sodium channel excitability, suggesting both central and peripheral actions on axonal excitability. Two small human trials of mexiletine in ALS showed promising early results.(41, 43) Both studies were small, and in the only controlled study they did not see any change in the ALS Functional Rating Scale; however a slowing of the decline in respiratory function was seen in the lower-dose mexiletine group, and researchers did find a dose-dependent reduction in muscle cramps with mexiletine compared to placebo(44).

Glutamate excitotoxicity: Increased activation of N-methyl-D-aspartate (NMDA)-type glutamate receptors accounts, at least in part, for excitotoxic neuronal damage—potentially contributing to a wide range of acute and chronic neurologic disorders(45). Memantine is a non-competitive NMDA receptor antagonist that may reduce the effects of glutamate mediated excitotoxicity(46). Inhibition of excessive NMDA receptor activity by memantine, via a mechanism of noncompetitive open-channel blockade, can ameliorate excessive production of NO, protein misfolding, and neurodegeneration(47). Memantine has been shown to prolong survival in a mutant SOD1 transgenic mouse model of ALS. The data demonstrated that mutant SOD1 transgenic mice

survived longer when treated with memantine than placebo controls (p=0.03)(48). A small open label study of memantine suggested a possible slowing in the rate of progression (p=0.10), which persisted in 8 patients taking memantine for > 2 years compared to historical controls(49). A second small randomized controlled study showed memantine to be safe but did not demonstrate slowing of progression(50). This study, however, was powered to detect a large (50%) reduction in rates of progression, so was likely underpowered for a meaningful clinical effect.

Each arm of the study will consist of standard of care (SOC) as detailed in the AAN Guidelines (access to Riluzole, PEG for nutrition, and BIPAP as indicated) plus one of three drug combinations:(10)
1) tamoxifen (20 mg: 2 times/day) and ranolazine (500 mg: 2 times/day);
2) tamoxifen (20 mg: 2 times/day) and mexiletine (200 mg: 3 times/day); and
3) tamoxifen (20 mg: 2 times/day) and memantine (20 mg: 2 times/day).

The drug interactions for the proposed study arms were reviewed by a consulting pharmacist PhD.

3) Bayesian Adaptive Design: We can vastly improve the efficiency of our study by using an adaptive Bayesian study design, informative priors and interval analyses to adapt randomization during the study to favor drug combinations which interval analyses suggest are beneficial. Informative priors can be drawn from The Pooled Resource Open-Access ALS Clinical Trials data base (PRO-ACT), the largest database ever created of clinical data on ALS patients. PRO-ACT contains over 8500 fully de-identified clinical patient records, and more than 8 million longitudinally collected data points. Patients will be randomized to one of three treatment arms (groups) with a maximum number of patient's nmax= 300 (see Protocol Synopsis for specific power calculations and modeling). The primary endpoint used to drive adaptive randomization and stopping criteria is: the average disease progression (monthly measures of ALSFRS-R) from enrollment to 52 weeks. A longitudinal model using early estimates from 26 weeks will allow early adaptive randomization to promote a smaller, faster, but more powerful trial. Interim analysis will occur after 100 patients have 52 week data and every 8 weeks thereafter. These data will inform an updated adaptive randomization schedule. We will "stop for success" if the probability a treatment is best is > .965. For interim analyses, all data are used on all enrolled patients with at least 26 weeks of data. For the final analysis: (1) a treatment is best if pr(it is best) > .95 or (2) a treatment is loser if pr(it is best) < .01. It was deemed the most likely effect size will be a disease progression of 1 point/month for usual care, but only 0.75 point/month for the best drug combination (an effect size greater than current SOC)(8) . For example, in the "One Best" case, the study design has 94% power to find the best treatment with an estimated 238 subjects, trial duration of 135 weeks, and 47% of the subjects in the winning group. Type I error rate is 5%. A pre-specified subgroup analysis, suggested by our patient group, will use a Bayesian ANOVA to estimate the interaction of gender and site of onset with drug.

***Translation*:** By leveraging existing CTSA and PCORnet programs we can accelerate the **ALS PATIENTs DEMAND** milestones (Table 1). We already have used the EHR to computer phenotype ALS patients, and can use this to accelerate recruitment. The two-way video system is already in production. We have used REDCap surveys embedded in an EHR link, or through web interface, in our PCORnet ALS patient survey.

Table 1. ALS PATIENTs DEMAND 5 year timeline

		Study Year				
Aim	**Milestone**	**1**	**2**	**3**	**4**	**5**
Aim 1	**IRB**					
	Patient Engagement					
	Screening EHR-defined phenotypes					
	Two way video / AE					
	EHR-i2b2 data package / REDCap					
	PRO Outcomes via Web/Tablet					
Aim 2	**Recruitment**					
	Clinical Trial					
	Analysis / Dissemination					

The ALS clinical and research community have agreed on standard clinical measurement tools to assess outcomes, and the **ALS PATIENTs DEMAND** study will use these tools. Standard functional and symptom scales include: the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R), forced vital capacity, and the ALS Global Impression of Change scale.(51) These scales have

largely been adopted by the **ALS PATIENTs DEMAND** clinics at this time, and because of the simplicity of these forms, the ease of creating REDCap surveys for the forms, and a commitment from Electronic Privacy Information Center (EPIC) to make them available to clinics using EPIC EHRs, it is feasible to collect them in **ALS PATIENTs DEMAND**.

Primary outcome: Functional status is the primary outcome. The ALS Functional Rating Scale (ALSFRS) was designed to assess the ability of ALS patients to perform activities of daily living and to detect functional changes during clinical trials(52). Precedent for using this scale in clinical trials stems from the only positive treatment trial of riluzole in ALS, which showed slower decline in treated patients relative to placebo(53, 54). The ALSFRS-R, a revised version of the ALSFRS, is a quickly administered, by research personnel or study staff, (five minutes) ordinal rating scale that assesses capability and independence in 12 functional activities. These include six bulbar-respiratory functions, three upper extremity functions (writing, cutting food, and dressing), and three gross motor functions (walking, climbing, and turning in bed). Each activity is recorded to the closest approximation from a list of five choices, scored 0-4, with the total score ranging from 48 (normal function) to 0 (no function). The ALSFRS-R has been used extensively in previous clinical trials and validity has been established by correlating ALSFRS-R scores with quantitative strength testing and changes in strength over time (55-63).

For this project we further revised the ALSFRS-R and created the ALSFRS-DEMAND. We modified the ALSFRS-R so that it would be patient user friendly and so patients could fill it out themselves. The ALSFRS- R was sent to approximately 20 ALS patients throughout the GPC region and their caregivers. They were asked if the scale was difficult to understand; if there were items they felt should be dropped from the ALSFRS-R; or if new items should be included. We held two focus group conference calls where the patients discussed and commented on the scale. Patient focus group recommendations included a need for clarification in meanings of some of the words in the ALSFRS-R, but overall the impression was that this instrument reflected the types of functional limitations they experienced on a daily basis. However, there also were several items patients believed should be added to the ALSFRS-R: a question about pain; a question about emotional liability; and a general non-denominational question about faith. These were added and our new ALSFRS-DEMAND survey is designed to be completed by patients between clinic visits and can be completed via the EHR patient portal or an individualized email link to a REDCap database.

Currently Riluzole is the only approved medication for patients with ALS, which extends life by only 2-3 months. If any of the drug combination proposed here proves effective in ALS, this will have a dramatic and immediate impact on patients, their family members, and communities. All of the proposed drug combinations are readily accessible registered FDA medications used for other indications, and should be available in generic formulations by the end of the study. It would be expected most ALS patients interested could obtain access to the proposed treatments, and so benefit. In addition if the study design innovations proposed here prove feasible this also may transform the way we approach therapies in ALS by:

***Partnership*: ALS PATIENTs DEMAND** is fundamentally a partnership between the patient and caregivers who will have an active role in study design, conduct, and dissemination of results; the engagement officers; the patient advocacy groups who will have role in recruitment and dissemination; the GPC, UC BRAID, and CReATe who will provide regulatory oversight; and the 25 participating ALS specialty clinics across the country.

***Barriers*:** *Rolling out a Bayesian adaptive design across multiple CTSAs across the country* will be challenging. However, we have used this approach to randomization in a current PCORI sponsored study evaluating pain medications in patients with small fiber neuropathies. The current model of using the EHR-i2b2 interface *or* REDCap links embedded in the clinic workflow allows a backup mechanism built into the study design to ensure we will be able to perform interim analysis and adjust randomization. *Difficulty with recruitment* is another challenge. We believe our combined networks, which cover approximately 4700 ALS patients, and the broadening of our inclusion criteria, will lessen this challenge for our goal of recruiting 300 participants. However, if we do encounter difficulty we will add additional sites and assign them to their closest regional CTSA CC.

Defining Success: The ultimate success of this study would be to identify a treatment arm which slows down ALS progression. In addition we will consider ALS PATIENTs DEMAND successful if we: 1) meet our

enrollment and study completion timeline; 2) if at least half of the sites can enter data directly into the EHR at the time of clinic visits (the remainder using REDCap links), and this can be successfully abstracted; 3) if patients can use the patient portal to enter data between study visits; and 4) use of two way video for adverse event monitoring.

Ultimately developing the infrastructure proposed for **ALS PATIENTs DEMAND**, demonstrating the feasibility of conducting a large national multi-site study, and disseminating the innovations in efficiency back across the CTSA consortium will provide a viable model for repurposing drugs for use in ALS, and for testing new therapeutics for rare diseases, and potentially any disease.

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1U01TR001815-01 BAROHN, RICHARD

RESUME AND SUMMARY OF DISCUSSION: This is a new application for the Collaborative Innovation Award, Clinical and Translational Science Award (CTSA) Program (U01) from the University of Kansas Medical Center entitled “ALS Patients Demand.”

The purpose of this application is to create a model for building infrastructure to run multisite-studies in rare diseases by leveraging existing resources, and the applicants plan to use the CTSA-based national research infrastructure to test the hypothesis that drug combination therapy in amyotrophic lateral sclerosis (ALS) will be more effective than standard of care alone. Strengths of the application include the urgent need for drug combination therapy studies to target ALS as single drug therapies failed to stop the disease progression; the well-experienced Principal Investigator (PI); the plans to integrate existing coordinating centers from Clinical and Translational Science Institutes (CTSIs) and rare disease networks; the plans to use Bayesian adaptive designs in the proposed three arm clinical trial of three drug combinations in ALS patients; and the excellent resources at the partnering institutions. The plans to use electronic health records (EHRs) in addition to RedCap to identify subjects and transmit data and video conferencing for study follow-up visits are additional strengths. The proposed patient engagement plan to involve stakeholders from early stages of study design and the plans for patient-driven clinical trial to assess ALS combination therapies are innovative. Weaknesses include the lack of clear information on how interim metrics will be obtained from three institutional review boards and the three CTSA hubs to improve the regulatory submissions or recruitment efforts and the inadequate information on the expected side effects of the proposed combination drug therapy. Although there are plans to conduct trial visits of patients by video conferencing, there are no alternate plans described to visit them if they cannot participate by video. The lack of systematic pharmacovigilance during the clinical trial and of a real time, study-wide, centralized live database are major weaknesses that will have an impact on the ability to implement adaptive randomization and interim analyses, and also increase the risk for human subjects. This resulted in a rating of unacceptable for protection of human subjects.

Overall, the application received an Impact/Priority Score of 41; the committee recommended the budget as requested.

DESCRIPTION (provided by applicant): New translational science tools and approaches for more rapidly advancing health research to the common goal of improved cures and treatments are especially needed for studies of rare diseases. Amyotrophic lateral sclerosis (ALS) is a rare progressive neurodegenerative disorder caused by loss of motor neurons in the brain and spinal cord which is invariably fatal. Traditional approaches to developing therapies have failed in ALS, yielding only a single FDA approved therapy with a modest benefit on survival. Thus, there is a pressing need for new therapeutic approaches in ALS. Accumulating evidence points to multiple pathological processes being active in ALS – this raises concerns that the disease cannot be halted or slowed by simply targeting one of these mechanisms. One approach used to treat cancer and HIV has been to use drug combinations targeting different pathological pathways. The goals of this application are 1) to create a model for leveraging existing national research initiatives and new translational science tools to build the infrastructure to run multi-site studies in rare diseases; and 2) as proof of concept, to use this CTSA-based national research infrastructure to test the hypothesis that drug combination therapy will slow disease progression in ALS. Several institution level innovations will make such a study feasible. We will leverage IRB reliance agreements across 3 large networks (comprised of 25 sites and 20

CTSAs) to increase the efficiency of regulatory oversight. We will build on principles of patient engagement we utilized in focus groups to involve patients and caregivers in the design, conduct and dissemination of results for our study. We will maximize the use of the electronic health records (EHR) to identify eligible participants using automated systems based on diagnosis codes and clinic visits. We will build a large REDCap data infrastructure based on the common underlying CTSA infrastructure, and compare data collection using REDCap to data capture using EPIC downloadable ALS clinic templates and the EHR-i2b2 interface. We will implement a two way web-based video system for adverse event reporting, and to enable ALS patients no longer physically able to come to clinic to remain in the trial. All of the institutional level innovations will complement innovations at the level of the clinical trial. We will utilize a Bayesian response adaptive design to test which of 3 drug combinations is most effective in slowing disease progression in ALS. If any of the drug combinations proposed here prove to be effective in ALS, this will have an immediate impact on patients, the family members, and communities. All of the proposed drug combinations are readily accessible medications currently prescribed for other indications which could be repurposed for ALS, and all should be available in generic preparations by completion of this trial. Our proposed collaboration among CTSA Coordinating Centers and model for assembling study-specific infrastructure will not only serve as a blueprint for future clinical trials in ALS and other rare diseases, but also will inform all multi-center clinical trials seeking to more efficiently maximize network-level collaboration to study any disease.

PUBLIC HEALTH RELEVANCE (provided by applicant): Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting the voluntary motor system which is invariably fatal. Patient focus groups expressed overwhelming interest in using a drug `cocktail' approach to ALS therapy, with drugs targeting different pathological pathways. We will create a national network of CTSA sites and implement novel innovations in patient engagement, regulatory oversight, patient recruitment, and outcome collection to conduct a patient-driven clinical trial to assess which of 3 drug cocktails are the most effective in slowing disease progression in ALS: the ALS Patient-Driven Electronic- based Multidrug Adaptive Network Design clinical trial (ALS PATIENTs DEMAND).

CRITIQUE

Critique 1

Significance: 1

Investigator(s): 1

Innovation: 5

Approach: 4

Environment: 1

Overall Impact

Therapeutic development in rare disorders is severely limited by the number of available subjects and their possibility to participate in trials. In fast aggressive diseases like ALS, the time it takes to initiate trials directly and negatively impacts the number of available subjects. Despite the availability of national networks and CTSAs, there is still a need for better coordination of efforts to increase recruitment and accelerate trial initiation. New technology offers the possibility of remote patient participation in trials, minimalizing the site visits, very burdensome in this disease. A separate problem

that has been plaguing ALS and other rare disorders, is the failed attempts to halt or stop the disease progression by targeting one abnormal pathway at the time (one trial-one target), in diseases where multiple pathways combine to cause a rapid, irreversible pathology. This ambitious application sets up to both, create a supra structure to efficiently run large, multicenter clinical trials in ALS (Aim 1) and to test this structure while investigating the first combination therapy approach (Aim 2) to ALS. Both aims, if successful, can have a great impact in the field of ALS therapeutics, as well as set up the bases to similar approaches for other rare disorders. If successful, this project has the potential to highly impact the field. The impact of this application is diminished by the lack of tight pharmacovigilance. Though the drugs proposed for combination are FDA approved, with the subsequent large amount of available safety information, their systematic long term combination is unique to this study and has not undergone rigorous toxicology evaluation. Though adverse events (AEs) reporting is clearly delineated, there is a lack of systematic and centralized laboratory and AEs pharmacovigilance, which could identify safety signals before they become an issue. Since these combination trials are most likely to be conducted by academic networks such as this one, the investigators need to establish industry-standard safety and data monitoring.

Significance

Strengths

- This application addresses a critical barrier to therapeutic studies in ALS: time to study initiation and patient recruitment and multi-target therapeutic approach. Despite large advances in ALS pathophysiology and genetics, ALS clinical trials, one after another, have been disappointing, failing to translate encouraging pre-clinical (and small clinical) study results. The reasons might lay on the study design, but also on the fact that in very rapid and aggressive diseases like ALS a single target approach is likely to provide very small benefit, and thus result in negative trials. Combination therapy, however, introduces another challenge, requiring much larger number of subjects to adequately power the studies. This application addresses these issues by providing a plan to create a supra-structure including three CTSA hubs and 25 centers across North America, including all the well-established ALS networks, to increase efficiencies in regulatory approval and patient recruitment.
- The application also includes utilizing clinical EHR in addition to RedCap to identify subjects, collect and transmit data. They are making a tremendous effort to include patient's input in all aspects of the trial, which is the mandate in rare disorders. It is also bringing telemedicine into the trial design, which will facilitate patient's participation and follow up. Even if partially successful, this application will provide valuable information on how to conduct these large multi-center trials in an academic setting, and what kind of efficiencies (or deficiencies) might result from such efforts. It has the potential to change how the field is moving forward and as such it is highly significant.

Weaknesses

- Lack of systematic pharmacovigilance during the clinical trial and of a study-wide centralized live database increases the risk for human subjects and adds unnecessary risk to the overall conduct of the study.

Investigator(s)

Strengths

- Excellent, experienced investigators, all leaders in the field of ALS therapeutic research.
- Investigators have history of collaborating and participating in multi-center ALS trials.

- Three CTSA hubs are involved in the project, with the central hub being at University of Kansas Medical Center.

Weaknesses

- None.

Innovation

Strengths

- Though none of the proposed methodologies or concepts are novel, these are novel in the field of ALS.
- Video conferencing for study follow up visits and patient portal and involvement in trial design and data dissemination are clear strengths.

Weaknesses

- Large supra-structures to conduct clinical trials and combination trials have been established for decades in the field of oncology and HIV, and are thus not novel.

Approach

Strengths

- Aim 1 will provide metrics on different institutional review boards (IRBs) reliance and central IRB approval methods, which could result in more efficient, unified regulatory submissions in future trials.
- Three different coordinating centers will be used to accelerate regulatory approvals and launching trial at the 25 centers by dividing efforts, increasing trial initiation efficiencies.
- Patient engagement and input is sought from the conceptualization of the clinical trial to the data monitoring and dissemination.
- Clinical databases (Electronic Health Records (EHR)) will be used to identify patients for trials. The investigator shows feasibility, successfully identifying more than 2000 ALS patients through EHR phenotyping, fifty percent of which returned a survey positively backing up combination therapy trials.
- It could be efficient to integrate the clinical EHR and Epic databases and RedCap for data collection and for patient recruitment, though this also could be a very time-consuming effort and very error prone.
- Combination therapies are needed for ALS. The drugs chosen for this trial have a clear rationale and target important pathological pathways, and proof of activity has been shown in animal models or small trials. The investigators have thought about the pharmacodynamics interactions that could affect data results and consideration to these are included in the data analysis plan.
- Primary outcome for the trial is ALS progression as measured by one well-established, validated, clinical relevant functional scale. This makes the study simple, cheaper, and relevant. Though the lack of biomarkers could result in a type 2 error, the bar is set high for disease modification and that is reasonable.
- A Bayesian adaptive design will help to delineate wining combinations and potentially decrease the needed number of subjects.
- The PIs have addressed barriers and proposed alternative plans.
- There are clear plans to disseminate results and to measure success of the projects.

Weaknesses

- Although Aim 1 is strong on its conceptualization, it becomes weaker on the outcome front. It is not clear how the metrics to be obtained from the three types of IRBs and the three CTSA hubs' recruitment efforts will be translated into improving the mid-way regulatory submissions or recruitment efforts. Since the study is large and will last at least five years, it would have been stronger to have interim data analysis of Aim 1 metrics, and use this information to correct or improve ongoing regulatory submissions and recruitment efforts based on what is being learned from the first years.
- The main weakness of this application is having three different CTSA hubs acting as separate coordinating centers, including the collection and management of interim data. A lack of centralized live database hinders the clinical trial pharmacovigilance. They propose a risk-based review, randomly performed by each CTSA hub. Though AEs are clearly being collected and transferred monthly to a central database, laboratory analysis are said to be reviewed only by PIs, and data not entered and transferred on electronic case report forms. Monitoring of AEs will be done at each one of three CTSA hubs and then every four months reviewed by a Data and Safety Monitoring Board (DSMB). That means that there is no continuous, centralized pharmacovigilance, where one person or team will be looking at trends in labs and AEs study wide. This could result in safety signals being missed until a significant AE is reported.
- The DSMB will review data every four months, and it is not clear that this includes labs (not mentioned). Though these are FDA approved drugs, they all have a significant side effect profiles and their combination has not been systematically studied in humans or toxicological studies.
- The lack of a central data system also introduces several potential complications, which will make the study implementation and conduct inefficient and error prone. There are no centralized data quality check tools and no data monitoring plan. Since they will be using a Bayesian adaptive design, they would rely on clean, real time data, arriving to some central location, and it is not clear how they will smoothly achieve this with once a month limited data transfers and no ongoing data management and cleaning.
- They have plans to conduct trial visits by video if patient cannot come to the site, but no systematic efforts to have a home nurse visit the patient to collect vitals and blood work. The latter should be part of any missed visit to assure patient is safe.
- Inclusion criteria are broad, which is good, but there is no statistical language on how the heterogeneous population will be taken into account in the data analysis.

Environment

Strengths

- The central coordinating centers, and all participating institutions, are ALS centers of excellence and well established research networks.
- There are letters of support from all three IRBs.
- The project builds on the existing strengths and resources of the CTSA program, and at the individual investigators Institutions and CTSA hubs.

Weaknesses

- None.

Protections for Human Subjects

Unacceptable. Though the drugs proposed for combination are FDA approved, with the subsequent large amount of available safety information, their systematic long term combination is unique to this study and has not undergone rigorous toxicology evaluation. Though AEs reporting is clearly delineated, there is a lack of systematic and centralized laboratory and AEs pharmacovigilance, which could identify safety signals before they become an issue. This is an unacceptable risk to humans.

Inclusion of Women, Minorities and Children

Acceptable. Study will recruit both sexes and adults. Children are not included, as ALS is very rare in children.

Vertebrate Animals

Not Applicable.

Biohazards

Not Applicable.

Select Agent Research

Not Applicable.

Resource Sharing Plans

Acceptable. The investigators propose clear ways of sharing and disseminating study results.

Budget and Period of Support

The budget for a centralized, systematic pharmacovigilance monitoring and reporting is not included. The safety monitoring budget is at 0.12 calendar months per year. This is a very low effort for pharmacovigilance in a 300 patient trial. The data management relies on one person with an effort of 4.8 calendar months per year. It appears low for the type of decentralized database and potential issues that could be found from different sources.

Critique 2

Significance: 2

Investigator(s): 1

Innovation: 1

Approach: 4

Environment: 3

Overall Impact

ALS is a devastating progressive disease, where few therapeutic options exist. Translation from pre-clinical efficacy studies in mouse models to efficacious approaches in human patients has largely failed, leaving patients few options for treatment. This application plans to establish a trans-CTSA clinical trial

network for ALS clinical studies, leveraging key domain expertise at participating institutions. A key goal is to begin combination therapy clinical trials; an approach that patients are requesting. The application is highly innovative in many ways. There is extensive patient engagement in trial content, focus and design, and an ongoing elaborate means of soliciting patient feedback. This is extended to encouraging contact between patients through established infrastructures. The application takes the important strides made in different coordinating centers, both within Clinical and Translational Science Institutes (CTSIs), and outside (e.g. Clinical Research in ALS and Related Disorders for Therapeutic Development (CReATe)), and works very hard at integrating these. The combination drug trial, requested by patients, is also highly innovative, particularly the use of the Bayesian adaptive design, the ALS Patient-Driven Electronic-based Multidrug Adaptive Network Design (ALS PATIENTs DEMAND) clinical trial patient recruitment and phenotyping tools, and broad inclusion criteria. The weaknesses centered on feasibility. The applicants have chosen to tackle a large number of problems such as integration of networks, multiple coordinating centers, broadening of inclusion criteria with new outcome measures, three drug combinations (where there is scant evidence that each shows efficacy in ALS individually), and large-scale patient involvement simultaneously. Any one of these is important, with significant innovation if successful. But with such a large (n=300 patients) and complicated study, there is a relatively high risk that the data once (and if) obtained may be difficult to interpret. There are also significant side effect profiles for each drug individually, and concerns about combined side effects (over and above drug metabolic interaction).

Significance

Strengths

- ALS is a relatively common neurological disorder with no effective treatment. Efforts to develop therapeutic approaches are highly significant.
- Efforts to leverage and integrate multiple existing networks are highly significant.

Investigator(s)

Strengths

- The proposed investigative team is outstanding. The PI brings extensive clinical trial and clinical experience in ALS to the collaborative network. The participating CREATE network and CTSA hubs all have extensive resident experience to carry out the proposed roles.

Innovation

Strengths

- The model of integrating different existing coordinating centers both from the CTSIs and rare disease networks under the umbrella of broader CTSI infrastructure is innovative. There are clear strengths to the participating institutions and great strides that have been made in data collection and access, and trans-center data queries.
- The patient engagement aim is innovative. There is increasing recognition that stake holders should be involved from early stages of study design, and the EU seems to be ahead of US in this. The proposed (relative elaborate) effort to include stake holders is impressive, and innovative.
- The patient-driven desire to undertake a clinical trial of combination therapies is innovative.
- The use of Bayesian adaptive designs in the proposed three arm clinical trial of three drug combinations in approximately 300 ALS patients is innovative.

- By focusing on repurposed drugs, there is no need for the complication of an IND. A letter from FDA confirmed this.

Approach

Strengths

- The proposed ALS DEMAND network describes an interesting structure, where there are three sub-hubs managing a total of 25 recruitment sites. The major hub is the Kansas CTSI (parent institution of the ALS DEMAND network) managing 14 sites, a University of Miami site managing an existing network of four ALS recruitment sites (CREATE network), and the UC system UC BRAID network servicing four recruitment sites via UC Davis. The rationale provided is that this will share the workload, thus gaining efficiencies. In fact Aim 1 will test this rationale by providing metrics during the trial (Aim 2) startup phase, comparing each site in terms of IRB approvals, contracts, initial enrollment, and subject accrual metrics.
- Aim 1b describes a patient engagement plan. This is very well described and impressive in scope and depth. This is a major strength.
- The application builds on existing network strengths, including the CREATE ALS network.
- Patients' phenotypes will be collected using a computed method via an integrated EHR between the 25 sites. The applicants acknowledge that there is significant heterogeneity between sites regarding i2b2, EHR systems, and computing infrastructure. Integrating the EHRs to the point of computable phenotypes sounds intimidating. However, the applicants provide a good example of receiving data on 2,000 ALS subjects via the GPC on ALSFRS-DEMAND at home survey. It could be argued that this is a small step towards integrated computed phenotyping via EHRs, but a step in the right direction nonetheless, and a strength.
- The applicants propose two distinct data acquisition methods (RedCap alone; EHR/RedCap/Epic). While this is inclusive of heterogeneous sites, it also complicates the conduct of an already complex trial.

Weaknesses

- Typically clinical trials have a single coordinating center. The structure of three coordinating centers is unusual. While it is stated that this will share workload and gain efficiencies, this rationale is not entirely transparent.
- The applicants wish to promote combination therapy trials. These are often problematic, as the individual drugs may not have shown efficacy individually, optimization of doses is made much more challenging when studying multiple drugs simultaneously, and developing effective clinical trial designs to accurately monitor both safety and efficacy can be difficult. The investigators cite both HIV and cancer as success stories. While they are indeed success stories, one could argue that key biomarkers were critical to the successful testing of combination therapies in these (viral load in HIV; molecular targets in cancer). ALS seems to lack such key biomarkers, and thus the translation of success in HIV and cancer may not be easily accomplished in ALS.
- There are many well-established ALS clinical trial networks. The CREATE network is integrated into the proposed CTSI large network via University of Miami. However, the applicants should provide a clear contrast to these pre-existing resources, providing a justification for how the proposed CTSI network is value added. Indeed, there is considerable overlap in the proposed U01 project and these pre-existing ALS-focused networks.
- Including stake holders in the consideration of clinical trial designs, while innovative, is also risky. As the applicants describe, the patients would like combination therapies across a very broad range of disease severity. Of course, this same inclusiveness can make a trial very difficult to carry out, with the risk of disparate outcome measures with variable relevance to

specific disease subgroups. As the combination drug design (sans biomarkers) is already quite challenged, adding the broad inclusion criteria may lead to a trial stands a risk of not providing much interpretable data, and thus could become unethical in its broad inclusion. Similarly, the effort to promote patient communication during the trial with the ALS Portal is innovative, it stands the risk of introducing additional bias as well, further complicating interpretation of outcome data.

- The bulk of the proposed study is to carry out a three arm clinical trial of three drug combinations in ALS. The rationale for choosing these drugs, as well as the doses, is not well described. It is acknowledged that this is a short grant application, and there is not adequate room for a thorough justification. The choice of tamoxifien as an anti-inflammatory agent is not well justified. Moreover, each of the drugs individually has side effect profiles, and this is not discussed adequately. Tamoxifen is reported to cause reduced cognition. Mexiletine has been reported to show nausea in 40%, coordination problems in 10%, and tremor in 13% of patients. Memantine was halted in trials of multiple sclerosis due to problems with neurological impairment. Ranolazine shows dizziness in 10% and constipation in 10% of patients. All these side effects could be viewed as exacerbating the ALS disease process.

Environment

Strengths

- There are multiple proposed participants, and thus multiple environments. Overall, the combined environment is outstanding.

Weaknesses

- There are acknowledged challenges with integrating the multiple networks.

Protections for Human Subjects

Acceptable.

Inclusion of Women, Minorities and Children

Acceptable.

Vertebrate Animals

Not Applicable.

Biohazards

Acceptable.

Resource Sharing Plans

Acceptable.

Critique 3

Significance: 2

Investigator(s): 2

Innovation: 3

Approach: 4

Environment: 2

Overall Impact

The investigators propose to utilize CTSA-based national infrastructure to conduct a clinical trial to evaluate drug combination therapy to slow disease progression in ALS. They will take advantage to IRB agreements between sites to improve efficiency in regulatory oversight and hence expedite study start-up and mid-stream approval of protocol revisions. They will utilize existing data capture resources at the sites to recruit and collect data from patients. They also propose to use Bayesian adaptive design to treatment randomization and early stopping. The investigators will also include input from the community members (patients and caregivers) in their design, conduct and dissemination of study results. While the investigators propose a bold initiative to conduct a large trial in a rare disease with no real treatment option, there are limitations in their approach that will impact adaptation of their methodology to other rare diseases. Key limitations include informatics support and lack of an existing network with IRB agreements to expedite study start-up.

Significance

Strengths

- The investigators aim to test drug combinations to slow the progression of ALS using vast CTSA infrastructure. If successful, it will be a major breakthrough in providing treatment options to ALS patients.
- The investigators hope to provide an example of how to utilize CTSA infrastructure to conduct a multi-site trial.

Weaknesses

- The methods proposed to implement the trials may not be readily transferable to other trials or disease areas.

Investigator(s)

Strengths

- The applicants have put together a strong team of experienced researchers that are likely to succeed in their efforts. The role of each CTSA hub is well defined.

Innovation

Strengths

- The investigators propose to utilize several existing approaches to successfully conduct an important trial in ALS patients. These concepts that have been tested and evaluated in other settings include: use of central IRB or use of existing IRB agreements in a network of sites; incorporating patient and community input in design, conduct and dissemination of study results; use of EHR to screen potential patients; use of two-way web based video to reach and capture key data from patients with mobility issues; and use of adaptive design for randomization and early stopping for efficacy or futility.

Weaknesses

- Some of these concepts may not be easily transferable to other rare disease populations.

Approach

Strengths

- The overall study design is reasonable with a good likelihood of achieving the objective of testing the hypothesis that the proposed drug combinations will slow the progression of ALS.

Weaknesses

- The key weakness, a function of independent databases at each CTSA hub, is the data collection and management system. The lack of a central data system makes the study implementation very in-efficient, resource intensive and potentially error prone. There is no real time central database with monthly downloads of data from individual sites. There are no centralized data quality check tools. Safety reports cannot be real time either. Every update of CRFs will be time consuming and cannot be pushed from a central system. These will have an impact on the ability to implement adaptive randomization and interim analyses. This is not an ideal model to emulate for other studies.
- It is not clear why the KUMC IRB is developing informed consent forms and disseminating updates to study protocols. This is a task typically held by lead study PI and their staff.
- The use of time from IRB approval to first enrollment to evaluate IRB performance does not make sense. The application suggests that they expect some sites will not enroll any participant hence the need to censor time to first enrollment.
- While the idea of expanding inclusion criteria makes sense, it would be good to account for patient's baseline status in primary analysis or use some sort of stratified randomization approach. The impact of patient's functional status at baseline on primary endpoint is not addressed.
- While the proposed Bayesian adaptive design seems reasonable, the investigators should have presented why this method is preferable over other adaptive designs.
- The study timeline should allow for at least six months of data analyses after last patient last visit. The current plan can have patients in follow-up in Year 5 of the grant.
- The timing of interim analyses (100 in some places and 90 in other) and statistical descriptions are confusing. It is not clear whether the primary outcome change from baseline or functional rating scale at 12 month follow-up.
- It is not clear if all interim data from a patient will be used to predict 12 month score or just the six month value.
- Risk based monitoring is not at all described.
- It is not clear if the drug will be labeled for the study and also if patients are expected to pay for the drug costs.

Environment

Strengths

- The environment is adequately suited for successful completion of the trial.

Protections for Human Subjects

Acceptable.

Inclusion of Women, Minorities and Children

Acceptable. Children are not expected to be in this study as the median age of disease diagnosis is around 60 years.

Vertebrate Animals

Not Applicable.

Biohazards

Not Applicable.

Select Agent Research

Not Applicable.

Resource Sharing Plans

Acceptable.

Budget and Period of Support

Adequate.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): UNACCEPTABLE

Though the drugs proposed for combination are FDA approved, with the subsequent large amount of available safety information, their systematic long term combination is unique to this study and has not undergone rigorous toxicology evaluation. Though AEs reporting is clearly delineated, there is a lack of systematic and centralized laboratory and AEs pharmacovigilance, which could identify safety signals before they become an issue. This is an unacceptable risk to humans.

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 U01 TR001815-01; PI Name: Barohn, Richard J.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

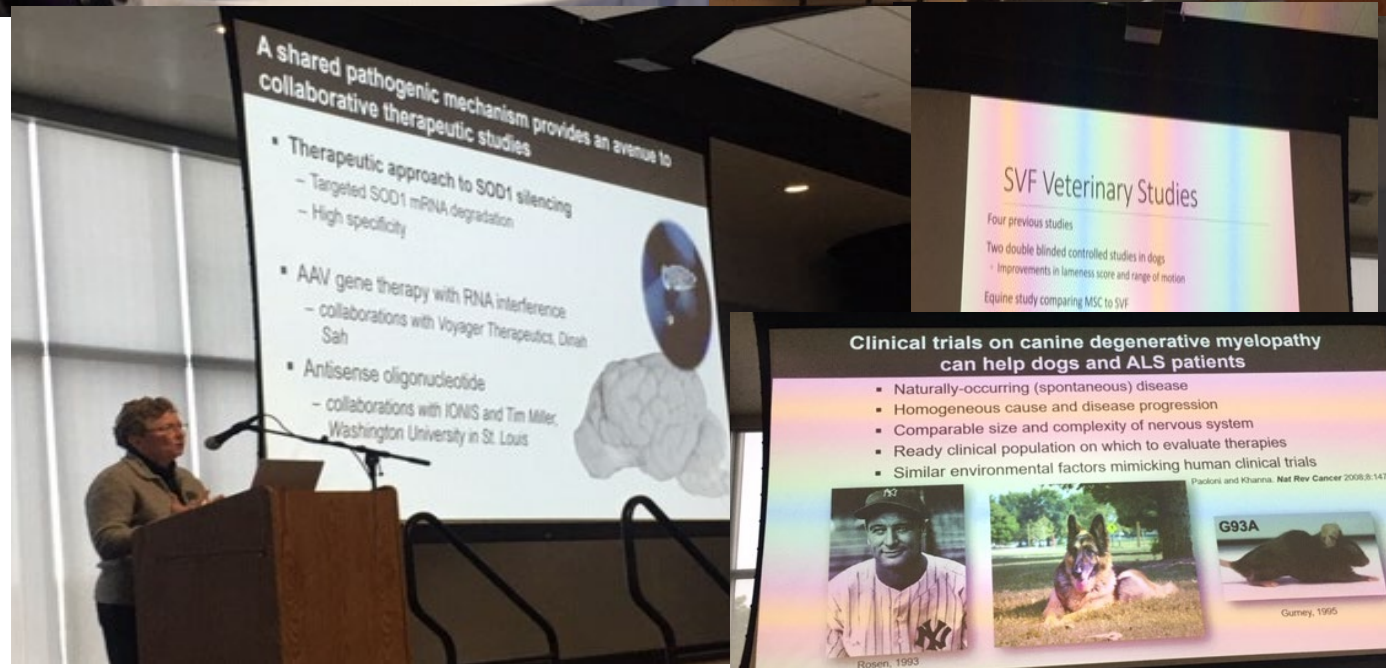
4th Annual Symposium on Musculoskeletal and Neuromuscular Disorders

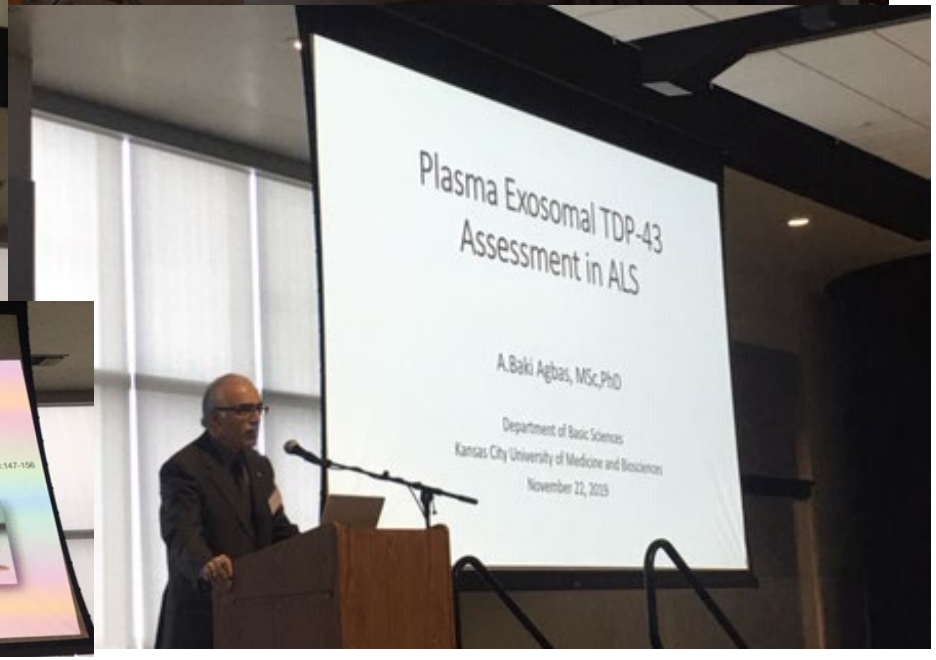
University of Missouri-Kansas City November 22, 2019

Below are some of the abstracts and the agenda for the 4th Annual Symposium on Musculoskeletal and Neuromuscular Disorders which was held on the University of Missouri -Kansas City campus on November 22, 2019. Also, some pictures I took on my cell phone. This is a consortium of four universities: University of Kansas Medical Center; University of Missouri - Kansas City; University of Missouri - Columbia; and Kansas City University (the regions Osteopathic medical school. The Vice Chancellors of Research of the respective universities formed the consortium in 2015 to promote faculty at each institution who are interested in musculoskeletal disorders to work together. Musculoskeletal disorders for the purposes of KCMD included disorders or biology of bone, joints, cartilage, muscle, peripheral nerve, neuromuscular junction and anterior horn cell and other motor neuron related disorders. So, it is a broad group of conditions and investigators. We have an annual meeting in the fall where investigators from all the universities meet and mingle and present their research. In January, we put out an RFA for grants that would be done by faculty on at least two campuses on a research project. We fund two or three a year with funding between $ 30,000 and $50,000 per project. We then have the teams present their findings at subsequent KCMD meetings.

Richard J. Barohn, MD

Photos from
4th Annual Symposium on Musculoskeletal and Neuromuscular Diseases
University of Missouri Kansas City





Kansas City Musculoskeletal Diseases Consortium
4th Annual Symposium on Musculoskeletal and Neuromuscular Diseases
November 22, 2019
Pierson Auditorium, Atterbury Student Success Center
University of Missouri Kansas City
5000 Holmes, Kansas City, MO 64110

10:00 am - 10:15 am	**Welcome and Opening Remarks: Richard J. Barohn, MD,** Vice Chancellor for Research, The University of Kansas Medical Center**, Executive Director, KCMD Consortium**
10:15 am – 10:30 am	Pamela Tran, KUMC, and Erin Bumann, UMKC, *"Role of genetic interaction between ciliary paralogs, Thm2 and Thm1, in postnatal skeletogenesis"* – **2019 Pilot Grant Award Winner Presentation**
10:30 am – 10:45 am	Charlotte Phillips, MU, and Sarah Dallas, UMKC, *"Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta"* – **2019 Pilot Grant Award Winner Presentation**
10:45 am – 11:00 am	David Upchurch, KSU, *"Administration of Adipose-derived Stromal Vascular Fraction and Platelet Rich Plasma in Dogs with Coxofemoral Osteoarthritis"*
11:00 am – 11:15 am	**Group Speaker Q&A – Rick Barohn**
11:15 am – 11:30 am	Joan Coates, MU, *"Canine Degenerative Myelopathy in Translation to ALS"*
11:30 am – 11:45 am	A. Baki Agbas, KCU, *"Plasma Extracellular Vesicles TDP-43 assessment in ALS"*
11:45 am – 12:00 pm	Mark Johnson, UMKC, *"Estrogen Action in Bone and Muscle Crosstalk"*
12:00 pm – 12:15 pm	**Group Q&A – Ed O'Connor**
12:15 pm – 1:45 pm	**Lunch and Posters**
1:45 pm – 2:00 pm	Douglas Haase, KUMC, *"Association between cartilage NFAT1 levels and severity of radiographic osteoarthritis in human hips"*
2:00 pm – 2:15 pm	Nicole Nichols, MU, and Teresa Lever, MU," Upregulating *neuroplasticity in surviving motor neurons to preserve upper airway function in a novel motor neuron disease model"*
2:15 pm – 2:30 pm	Patty Kluding, KUMC, *"Sedentary behavior and diabetic neuropathy"*
2:30 pm – 2:45 pm	**Group Q&A – Chris Liu**

2:45 pm – 3:00pm	**BREAK and Posters**
3:00 pm – 3:15 pm	Timothy Cox, UMKC, *"Understanding clinical variability in craniofacial birth defects: opportunities to reduce the burden of reconstructive surgeries"*
3:15pm – 3:30 pm	Steven Segal, MU, DDW Cornelison, MU, and Bret Ulery, MU, *"Interactions among tissue components during skeletal muscle regeneration"*
3:30 pm – 3:45 pm	Michael Wacker, UMKC, *"Bone-Heart Crosstalk"*
3:45 pm – 4:00 pm	Leslie Lyons, MU, and Gary Johnson, MU, *"Precision/Genomic Medicine in Companion Animals – Rapid Identification of Musculoskeletal and Neuromuscular Disease Models"*
4:00 pm – 4:15 pm	Duaa Jabari, KUMC, "Studying *phenylbutyrate in inclusion body myositis – Rational and study design"*
4:15 pm – 4:30 pm	**Closing Remarks: Chris Liu, PhD,** Vice Chancellor for Research Office of Research and Economic Development, UMKC, **KCMD Executive Committee Member, KCMD Consortium**

The Executive Committee of the Kansas City Musculoskeletal Diseases Consortium:

- Richard J. Barohn, M.D. Vice Chancellor for Research, The University of Kansas Medical Center and KCMD Executive Director
- Yusheng (Chris) Liu, PhD, Vice Chancellor for Research Office of Research and Economic Development, UMKC
- Mark A. McIntosh, Ph.D. Vice President for Research and Economic Development, UM System, and Vice Chancellor for Research, Graduate Studies and Economic Development, University of Missouri
- Edward R. O'Connor, Ph.D., MBA, FACHE, Provost and Executive Vice President for Academic, Research, and Student Affairs, Chief Academic Officer, Kansas City University

Symposium Speakers:

Kansas City University (KCU)

A. Baki Agbas, Professor of Biochemistry, Department of Basic Sciences

Kansas State University (KSU)

David Upchurch, Assistant Professor, Small Animal Soft Tissue Surgery

University of Kansas Medical Center (KUMC)

Douglas Haase, Resident, Department of Orthopedic Surgery

Duaa Jabari, Assistant Professor, Department of Neurology

Patty Kluding, Professor and Chair, Physical Therapy and Rehabilitation Science

Pamela Tran, Associate Professor, Department of Anatomy and Cell Biology/Kidney Institute

University of Missouri (MU)

Joan Coates, Professor, Neurology and Neurosurgery; Director, Comparative Neurology Program, Department of Veterinary Medicine and Surgery

D Cornelison, Professor, Biological Sciences, Professor, Department of Molecular Microbiology and Immunology

Gary Johnson, Associate Professor, Veterinary Pathobiology, Veterinary Pathobiology

Teresa Lever, Associate Professor, Department of Otolaryngology

Leslie Lyons, Gilbreath-McLorn Endowed Professor, Comparative Medicine; Director, Feline Genetics and Comparative Medicine Laboratory, Department of Veterinary Pathobiology

Nichole Nichols, Assistant Professor, Department of Biomedical Sciences; Investigator, Dalton Cardiovascular Research Center

Charlotte Phillips, Professor, Department of Biochemistry and Child Health

Steven Segal, Curators Distinguished Professor; Margaret Proctor Mulligan Professor in Medical Research, Department of Medical Pharmacology and Physiology

Bret Ulery, Assistant Professor, Department of Biomedical, Biological, and Chemical Engineering

University of Missouri-Kansas City (UMKC)

Erin Bumann, Assistant Professor, Department of Oral and Craniofacial Sciences

Timothy Cox, Endowed Chair in Musculoskeletal Tissues, Department of Oral and Craniofacial Sciences, School of Dentistry

Sarah Dallas, Lee M. and William Lefkowitz Endowed Professor, Department of Oral and Craniofacial Sciences

Mark Johnson, Professor and Chair, Department of Oral and Craniofacial Sciences, School of Dentistry

Michael Wacker, Associate Dean of Academic Affairs, Associate Professor and Vice Chair Biomedical Sciences Department

Poster Presentations:

University of Kansas (KU)

Jonathan Brumberg, Multisession brain-computer interface performance using a switch-scanning augmentative and alternative communication device by individuals with ALS

University of Kansas Medical Center (KUMC)

Yomna Badawi, Degeneration of ALS mouse neuromuscular junctions analyzed using super resolution microscopy and ameliorated using human mesenchymal stem cells

Ryan Funk, Utilization of the Collagen-Induced Arthritis Mouse Model to Evaluate Molecular Biomarkers of Methotrexate Efficacy in Autoimmune Arthritis

Aaron LacKamp, Ileus and dysautonomia can contribute to significant morbidity in Guillain-Barre syndrome independent of peripheral motor involvement

Takashi Matsuda, Applying Human Umbilical Cord Derived Mesenchymal Stem Cells for the Treatment of Amyotrophic Lateral Sclerosis

Mehrdad Maz, The Prevalence and Patterns of Celiac Disease Associated Arthropathy and Coexistence of Celiac Disease with Rheumatic Disorders in a Single Tertiary Medical Center

Mehrdad Maz, Efficacy of Mycophenolate Mofetil in the Treatment of Rheumatoid Arthritis Associated Interstitial Lung Disease

Mehrdad Maz, Pattern of Arthropathy in Patients with Cystic Fibrosis

Peter Rowe, ASARM reverses hyperphosphatemia, prevents calciphylaxis-like lesions, corrects renal, bone brain and cardiovascular calcification in a rat model of chronic kidney disease

Peter Rowe, Nephrogenic Systemic Fibrosis is induced in high phosphate diet CKD rats exposed to Gd3+ Binding Contrast Agents (GBCA): Role of ASARM peptides

Jinxi Wang, Treatment of posttraumatic arthrofibrosis using high intensity ultrasound and laser in a novel rabbit model of knee contracture

Mingcai Zhang, Mast cell deficiency amplifies inflammatory response in a mouse model of Kawasaki's disease

University of Missouri (MU)

Lauren Borkowski, Accessory inspiratory muscle (e.g., pectoralis minor) activity is increased in a rodent model of respiratory motor neuron loss

Joan Coates, Positron Emission Tomography Spinal Imaging in a Canine Model of ALS

Rebecca Dirkes, Voluntary Wheel Running Partially Compensates for the Effects of Global Estrogen Receptor-α Knockout on Cortical Bone in Young Male Mice

Brian Flesner, Multi-Modal Pain Assessment of Client-owned Dogs with Primary Bone Tumors

Ashley Kloepper, Developing Swallow-Evoked Potentials (SwEPs) to Identify Pathological Neural Generator Sources for Targeted Dysphagia Treatment

Kerry McDonald, Preload-induced ventricular dysfunction in isolated hearts from Duchenne Muscular Dystrophy ($DMD^{mdx\text{-}4CV}$) mice

Charlotte Phillips, Skeletal muscle mitochondrial dysfunction and whole body metabolic alterations in a mouse model of osteogenesis imperfecta

David Schulz, Neurostimulation of Bladder Efferents After Spinal Cord Injury to Preserve Autonomic Ganglion Function

University of Missouri-Kansas City (UMKC)

Thiagarajan Ganesh, Biomechanical Role and Strength of the Anterolateral Ligament in the Rotational Control of the Knee

Loretta Laughrey, Multi-scale finite element analysis of: 3D multiplexed images to examine bone mechanotransduction and heterogeneous activation of β-catenin signaling in osteocytes.

Hammad Mumtaz, Jaw morphogenesis: a budding role of neural crest in mineral density, microarchitecture, and calcification of bone

Joel Robinett, Regulation of Myofilament Force and Loaded Shortening by Skeletal Myosin Binding Protein-C

Kun Wang, Overlapping properties and functions of matrix vesicles and exosomes/extracellular vesicles in bone cells

PRESENTATION ABSTRACTS

Kansas City University

Title: **Plasma Exsomal TDP-43 assesment in ALS**

Author: *A. Baki Agbas, MSc, Ph.D., Yajaira Marin Esqueda, Edina Kosa*
Department of Basic Sciences, Kansas City University of Medicine and Biosciences, Kansas City, MO

Abstract: Blood-based biomarkers are in high demand for monitoring the prognosis and early diagnosis of the neurodegenerative diseases. Our purpose is to study the extracellular vesicles and their protein content that may be assign as a surrogate biomarker for ALS. Extracellular vesicles (EV) are excreted from cells into surrounding media and can be found in many, if not all, body fluids. Due to their small size (10-1000 nanometer), EVs can penetrate through blood brain barrier that makes them great interest in the search for biomarkers. We are working on optimizing the isolation of extracellular vesicles and their sub-groups (i.e., microvesicles and exosomes) from serum/plasma samples obtained from human ALS patients and healthy subjects. In initial phase of this project. We have isolated the exosomes from human plasma by using a commercial kit procedure based on the capture of water molecules, which otherwise form the hydrate envelope of particles in suspension. This method is easy to use and does not require any specialized equipment. Our laboratory has developed an interest to validate TDP-43 and their derivatives as a potential blood-based surrogate biomarker for ALS. We have demonstrated that isolated exosomes from human plasma contain TDP-43 and its chemical derivative, phospho-TDP-43. The results from these assays will provide a valuable information about the TDP-43 protein profile. The outcome of this pilot study will establish an optimized working assay protocol to isolate, verify, and analyze the profile of misfolded/aggregated proteins in extracellular vesicles that can be assign for a potential surrogate biomarker for ALS.

Kansas State University

Title: **Administration of Adipose-derived Stromal Vascular Fraction and Platelet Rich Plasma in Dogs with Coxofemoral Osteoarthritis**

Author: *David Upchurch, DVM*

Abstract: **Objective:** To evaluate the safety and effect of a single simultaneous intra-articular and intravenous injection of autologous adipose-derived stromal vascular fraction (SVF) and platelet rich plasma (PRP) on coxofemoral osteoarthritis (OA) in dogs.
Methods: This was a randomized, double-blind, placebo-controlled prospective trial of intra-articular and intravenous SVF and PRP for coxofemoral OA. Dogs with coxofemoral OA causing lameness or discomfort were evaluated by orthopedic exam, visual lameness score, Canine Brief Pain Inventory (CBPI), goniometry, visual analogue scale (VAS), and pressure-sensitive walkway (PSW) at week 0 (baseline), and 4, 8, 12 and 24 weeks after injection. Joint radiographs were scored at 0 and 24 weeks.

Results: Twenty-two client-owned dogs with OA of the coxofemoral joints were enrolled (12 placebo-control, 10 SVF-treated). CBPI pain severity scores were lower in the treatment group at 24 weeks compared to the placebo group ($p=0.042$). The VAS score for the treatment group was significantly greater at 0 weeks than at 4, 8, or 24 weeks ($p<0.05$). When dogs with low quartile baseline PVF were compared, the treatment group had statistically higher PVF at all post-injection time points when compared to the placebo group. After SVF injection, fewer dogs in the treated group were lame compared to the control group.
Clinical Significance: This study is the first to utilize objective data from PSW as an outcome measure for dogs treated with SVF and PRP for coxofemoral OA. No adverse events were noted. Improvements were noted in some measured parameters in the treated dogs.

University of Kansas Medical Center

Title: **Decreases in articular cartilage NFAT1 levels associated with increased radiographic osteoarthritis severity in human hips**

Author: *Douglas R. Haase[1], MD, Resident in Orthopedic Surgery, University of Kansas Medical Center, Kevin Hodge[1], MD, Resident in Orthopedic Surgery, University of Kansas Medical Center, Perwaiz Nawabi[2], BS, Kansas City University of Medicine and Biosciences, Jinxi Wang[1], MD, Ph.D., Professor of Orthopedic Surgery, University of Kansas Medical Center*

Abstract: **Purpose:** Nuclear factor of activated T cells (NFAT1) has been found to be integral to joint homeostasis. NFAT1 knockout mice develop premature osteoarthritis in young adults. The purpose of this study was to evaluate the association of human cartilage NFAT1 levels with radiographic osteoarthritic changes observed in hip joints.

Methods: 135 articular cartilage samples were collected from patients undergoing hip arthroplasty and their NFAT1 mRNA levels were measured by quantitative real-time RT-PCR . Three independent reviewers examined the preoperative hip radiographs in a blinded manner. Radiographic grading of osteoarthritis severity included the Kellgren and Lawrence (KL) classification and the Osteoarthritis Research Society International (OARSI) atlas. Patients were divided into five cohorts based on their NFAT1 levels compared to control patients with healthy cartilage.

Results: The cohort with moderately decreased NFAT1 had an average increase in KL score of 0.44 (p=0.008), while the cohort with severely decreased NFAT1 had an average increase of 0.66 (p=0.0001) compared to the control patient group. Additionally, the cohort with moderately decreased NFAT1 had an average increase in superior joint space narrowing of 0.77 (p=0.006), while the severely decreased cohort had an average increase of 1.23 (p=0.00002). Significant associations between decreased NFAT1 levels and elevated superior-sided osteophyte scores were also observed.

Conclusions: This study found that moderate and severe decreases in human articular cartilage NFAT1 levels are associated with significantly increased radiographic osteoarthritic changes. While this study has several limitations, it provides initial evidence regarding the association of decreased NFAT1 levels and osteoarthritis severity in humans.

Title: **Studying Phenylbutyrate in Inclusion Body Myositis- Rational and Study Design**

Author: *Duaa Jabari, M.D., Richard J. Barohn, M.D., Department of Neurology*

Abstract: Inclusion body myositis (IBM) is the most common acquired muscle disorder after age 50. It is a progressive debilitating disease with no available treatment. The muscle biopsy in IBM shows both inflammatory and degenerative features but the lack of response to immunomodulatory treatments suggest that IBM is a degenerative disorder with secondary inflammatory changes. This is supported by abnormal accumulation of amyloid-beta protein precursor, and its proteolytic fragment amyloid-beta along with other misfolded proteins. Many similar neurodegenerative disorders, termed "protein-misfolding disorders" are characterized by the accumulation of intracellular or extracellular protein aggregates. A highly conserved class of proteins called molecular chaperones has evolved to prevent such midfolding or repair or clear misfolded protein. Phenylbutyrate, an orally active chemical chaperone approved by the US Food and Drug Administration for treatment of urea cycle disorders, mimics the function of intracellular molecular chaperones in preventing protein aggregation and oligomerization. Phenylbutyrate showed positive effect on the muscle cell model of IBM by improving lysosomal activity, ameliorating consequences of impaired autophagy and decreasing vacuolization. This provides rational to study this medication in patients with IBM. We plan to conduct an open label study to evaluate the safety and tolerability of phenylbutyrate in IBM and to look for any early signal of effectiveness. With the preliminary data obtained from this study, we will proceed with larger randomized, placebo-controlled safety and efficacy study. If phenylbutyrate is found to be effective in treating IBM, it will be the only available treatment for this disease.

Title: **Sedentary behavior and diabetic neuropathy**

Author: *Patricia Kluding, Ph.D., Physical Therapy & Rehabilitation Science*

Abstract: **Background:** Sedentary behavior is associated with multiple metabolic risk factors in people with type 2 diabetes (T2D); when sitting for a prolonged time, postural muscles are inactive, directly leading to impaired postprandial glucose and insulin action. People with diabetic peripheral neuropathy (DPN) may be more sedentary due to pain, sensory loss and fall risk. Exercise intervention studies have demonstrated improved neuropathy symptoms and function; however, it is unknown whether sedentary behavior interventions have a similar effect.

Methods: First, a cross sectional study of sedentary adults with T2D (n=59, mean age 64±7) assessed the relationship of sedentary behavior with HbA1C. Participants wore an activity monitor for 7 days to determine average daily sitting time. Second, a pilot study was completed in a subgroup (n=9) to assess a sedentary behavior intervention with actigraphy feedback, vibrotactile prompts, and personalized activity counseling.

Results: Participants were sedentary for 68% of waking hours or 11.08 ± 2.31 hours/day. Multiple linear regression analysis showed that lower HbA1C (β= 0.40; 95% CI: 14.43, 58.13) was associated with increased sedentary time, independent of time spent in moderate-vigorous physical activity. The pilot study showed a significant decrease in total sitting time and decreased HBA1C following the intervention.

Conclusion: A sedentary behavior intervention may address many barriers to traditional exercise programs for this population, as it induces minimal physiological stress or pain and is potentially more sustainable. Further research is necessary to study this type of intervention in a large randomized trial.

Title: **Role of Genetic Ineraction between Cilliary Paralogs, Thm2 and Thm1, in Postnatal Skeletogenesis**

Author: *Pamela Tran, Ph.D., University of Kansas Medical Center, Erin BUmann, DDS, Ph.D., University of Missouri-Kansas City. 2019 Pilot Grant Award Winner Presentation*

Abstract: Skeletal dysplasias affect 1:5,000 births and range in severity from perinatal lethality to craniofacial dysmorphogenesis and short stature. These disorders are often heritable, and identification of the causative mutations has been instrumental in improving diagnosis, understanding mode of inheritance, and identifying therapeutic targets. The translational impact of finding causative loci underscores the need to continue to identify and study novel genes involved in skeletal development. Primary cilia are signaling organelles that receive mechanical and chemical cues, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. One such ciliopathy is Jeune Syndrome, in which mutations in *THM1*, a cilia gene and Hedgehog (Hh) modulator, have been identified. We have generated a knock-out mouse of *Thm2,* which is a paralog of *Thm1* and a gene that is largely uncharacterized. In contrast to *Thm1*-null mouse late embryos which show shortened long bones, *Thm2*-null mice are healthy and reach adulthood, suggesting that *Thm2* alone is dispensable for development. Since paralogs can have redundant roles, we have generated *Thm2-/-; Thm1+/-* (triple allele) mutant mice. These triple allele mutant mice appear normal at birth, but are markedly smaller than their littermates by postnatal day (P)14, and some do not survive to weaning. We hypothesize that by regulating mechanosensing and Hh signaling, genetic interaction between *Thm2* and *Thm1* is critical for postnatal skeletal development. To examine our hypothesis, we propose to examine *Thm2-/-; Thm1+/-* skeletal and ciliary phenotype and to examine role of *Thm2-/-; Thm1+/-* in Hh signaling in skeletal phenotype. Investigations will include quantifying skeletal defects using microCT imaging, examining functional role of genetic downregulation of Hh signaling in triple allele mutant mice, and examining mechano- and chemotransductory capabilities of triple allele mutant primary cilia in chondrocytes. This project will provide new insights into genetic interactions underlying skeletal development, and will establish *Thm2* as a novel locus important for cilia-mediated mechanosensation and signaling.

University of Missouri

Title: **Canine Degenerative Myelopathy in Translation to ALS**

Author: *Joan R. Coates, DVM, MS, Diplomate ACVIM (Neurology), University of Missouri, Columbia MO*

Abstract: Canine degenerative myelopathy (DM) is an adult-onset, progressive neurodegenerative disease that shares important similarities to amyotrophic lateral sclerosis (ALS) including clinical presentation and progression, pathological features, and superoxide dismutase 1 gene (*SOD1*) mutations. The E40K SOD1 mutation is widespread among companion dogs and leads to enzymatically actively SOD1 aggregate accumulations within cells, the putative mechanism of disease in DM and in both familial and sporadic forms of human ALS. This shared pathologic mechanism provides an avenue to direct therapeutic approaches toward SOD1 silencing. The similarities between the canine and human nervous systems, the homogeneity in onset and clinical progression of disease, and the ability to longitudinally analyze and collect samples from affected dogs during treatment make companion dogs with DM a uniquely valuable large animal disease model for therapeutic development and optimization. Moreover, companion dogs with DM represent a clinical population confounded by complexities in diagnosis, comorbidity and environmental and genetic diversity similar to those encountered in a human clinical trial setting. While experimental models are vital for understanding biologic mechanisms and early screening of novel therapies, development of a clinically relevant, time efficient *predictive* model system is paramount to therapeutic advancements. Thus, incorporation of veterinary clinical trials into the ALS treatment development paradigm will enhance translational efficiency by identifying and optimizing those therapies most likely to generate clinical benefit. A multi-institutional network of veterinary clinician-scientists focused on DM can be refined and formalized to establish a veterinary platform trial for DM to serve as a "translational accelerator" for ALS and DM therapies.

Title: **Interactions among tissue components during skeletal muscle regeneration**

Author: *Steven S. Segal[1], DDW Cornelison[2], Bret D. Ulery[3], Medical Pharmacology and Physiology[1], Biological Sciences[2], Biomedical, Biological, and Chemical Engineering[3], University of Missouri, Columbia MO*

Abstract: Skeletal muscle is the largest tissue in the body and is necessary for motility, respiration, and energy generation. Contractile myofibers make up the bulk of muscle tissue, but are surrounded by blood vessels which supply oxygen and nutrients, motor nerves which control contraction, satellite cells which regenerate damaged myofibers, and fibroblasts/interstitial cells which interact biophysically and biochemically with the other resident cell types. In the context of both development and regeneration after injury, the constituent cell types tend to be studied as isolated systems, rather than as interconnected components of the same tissue. Our collaboration is designed to identify key interactions among resident tissue components that regulate coordinated regeneration, with the goal of identifying signaling pathways that can be targeted for biochemical and/or bioengineered interventions to improve muscle function and repair. Our laboratories have been developing physiological (Segal, intravital imaging), molecular (Cornelison, stem cell biology), and engineered (Ulery, biomaterials) approaches to investigate how muscle fibers and microvessels integrate their responses to injury and in regeneration. Current work is focused on understanding how loss of one tissue component (e.g., muscle fibers or blood vessels) affects regeneration of the others and on developing 3D coculture systems and cellularized scaffolds to recapitulate multicomponent interactions. Our preliminary data suggest that inhibiting angiogenesis disrupts myofiber regeneration after acute injury, while loss of regenerated myofibers results in respecification of microvessels from the morphology associated with myofibers to that seen in adipose. Inhibition of either angiogenesis or myogenesis negatively impacts reinnervation.

Title: **Precision/Genomic Medicine in Companion Animals- Rapid Identification of Musculoskeletal and Neuromuscular Disease Models**

Author: *Leslie Lyons, Ph.D., Joan Coates, DVM, Gary Johnson, DVM, PhD, College of Veterinary Medicine, University of Missouri*

Abstract: Precision/Genomic Medicine can be implemented in dogs and cats, which not only leads to improvements in their health care, but also expediates the identification of new biomedical models. Less than one drug is approved for each billion dollars of research and development. Large animal models are clearly an asset to drug development and gene therapies by providing a different biological system to evaluate physiological effects. Importantly, these disease models permit studies of therapy intervention using similar procedures as those in human patients. Demonstrating dosing and delivery paradigms and safety of therapy in the large animal disease models will provide key supportive data and improve the probability of clinical trial success. However, the model must exist before it can be used, including detailed genetic and clinical phenotyping. As part of the MU systems strategic initiative, Precision/Genomic Medicine, as a diagnostic opportunity, is being offered to veterinary patients that have clinical presentations likely to be caused by heritable DNA variants. Our research has had dedication and successes in the discovery of many musculoskeletal and neuromuscular diseases in cats and dogs.

Researchers and veterinary clinicians at the University of Missouri (MU), College of Veterinary Medicine are world class in the genetic definition of canine and feline neuromuscular diseases. In cats, investigators have genetically defined several musculoskeletal diseases as well as several neuromuscular syndromes including, hypokalemia, myotonia congenita, a generalized myopathy, multiple systems degeneration, Niemann-Pick Type C, an inherited neurological syndrome with forebrain commissural malformations, ventriculomegaly and interhemispheric cysts. Canine degenerative myelopathy is now a disease model in companion dogs for amyotrophic lateral sclerosis. At least ten different forms of neuronal ceroid lipofuscinosis have been identified and one has impacted the translation of a therapy into children. MU has the bioinformatic skills, computational resources, clinical medicine, diagnostics, and investigative team to rapidly define new large animal biomedical models for musculoskeletal and neuromuscular disease in pets.

Title: **Upregulating neuroplasticity in surviving motor neurons to preserve upper airway function in a novel motor neuron disease model**

Author: *Teresa Lever, Ph.D., Department of Otolaryngology, Nicole Nichols, Ph.D., Department of Biomedical Sciences, University of Missouri*

Abstract: Motor neuron diseases (MNDs; *e.g.,* amyotrophic lateral sclerosis/ALS and primary lateral sclerosis/PLS) result in life-threatening alterations in upper airway function (*i.e*., swallowing and breathing), primarily due to degeneration of the hypoglossal (XII) axis (*i.e.,* upper motor neurons/UMNs and lower motor neurons/LMNs controlling the tongue). Despite its critical importance, upper airway function has seldom been studied in MNDs; thus, effective treatments remain to be discovered. The fundamental goals of this study are to understand how XII axis degeneration impairs function and coordination of swallowing and breathing in a novel model of MND, and to utilize this model for translational treatment discovery. Specifically, intralingual injection of cholera toxin B conjugated to saporin (CTB-SAP) in rats mimics numerous aspects of dysphagia in MNDs including: 1) targeted death of XII LMNs and corresponding degenerative changes throughout the XII axis (XII nerve, UMNs, and tongue); 2) decreased XII motor output; 3) reduced tongue strength and motility; and 4) impaired swallowing. Here, we will use a multidisciplinary approach to test the central hypothesis that upper airway function/coordination can be preserved in the face of XII LMN degeneration by harnessing the therapeutic potential of tongue exercise to upregulate neuroplasticity *via* neurotrophic factor expression in spared XII axis motor neurons. Our pilot data suggest that tongue exercise in CTB-SAP rats preserves tongue strength and motility as well as swallowing and breathing patterns/coordination. This work may identify noninvasive, cost-effective strategies to preserve upper airway function and improve the quality and duration of life for patients with MNDs.

Title: **Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta**

Author: *Charlotte Phillips, Ph.D., Department of Biochemistry and Child Health, University of Missouri, Sarah Dallas, Ph.D., Department of Oral and Craniofacial Sciences, University of Missouri-Kansas City*

Abstract: Osteogenesis imperfecta (OI) is a heritable disorder of skeletal fragility, ranging in severity from mild with few fractures to perinatal lethal with marked bone deformity and fragility. There is no cure; treatment is limited to surgical intervention and anti-resorptive drugs, both with limited success. Bone is mechanosensitive, responding and adapting to its mechanical environment to strengthen the skeleton, and contracting muscles are one of the largest physiological loads that bone experiences. Recent studies demonstrated that 80% of OI patients exhibit muscle weakness. To investigate the pathogenesis of OI muscle weakness, we examined the mitochondria, generators of the cellular energy needed for contraction, in the osteogenesis imperfecta mouse model (*oim)* with muscle weakness. We found *oim* skeletal muscle has severely compromised mitochondrial function. This opens up potential therapeutic targets to enhance muscle and bone weakness, but also raises the question as to whether bone cell mitochondrial function is similarly compromised. The goal of this pilot project is to test whether mitochondrial dysfunction is a major contributor to pathogenic muscle weakness and skeletal fragility in OI through investigation of three aims: 1) to determine if the compromised respiration rates in isolated *oim* skeletal muscle mitochondria are associated with altered Ca^{+2} homeostasis and production reactive oxygen species, 2) determine whether cultured primary *oim* calvarial cells have compromised mitochondrial bioenergetics and metabolism, and 3) to determine how the dynamic, morphological and functional properties of mitochondria are altered in *oim* bone and muscle cells using live cell and intravital imaging approaches. This innovative hierarchical approach (from isolated mitochondria through to live cell/intravital imaging) will competitively position this unique interdisciplinary team for future external grant submissions to NIH /NIAMS.

University of Missouri – Kansas City

Title: **Role of Genetic Interaction between Cilliary Paralogs, Thm2 and Thm1, in Postnatal Skeletogenesis**

Author: *Erin Bumann, DDS, Ph.D., University of Missouri-Kansas City, Pamela Tran, Ph.D., University of Kansas Medical Center.*

2019 Pilot Grant Award Winner Presentation

Abstract: Skeletal dysplasias affect 1:5,000 births and range in severity from perinatal lethality to craniofacial dysmorphogenesis and short stature. These disorders are often heritable, and identification of the causative mutations has been instrumental in improving diagnosis, understanding mode of inheritance, and identifying therapeutic targets. The translational impact of finding causative loci underscores the need to continue to identify and study novel genes involved in skeletal development. Primary cilia are signaling organelles that receive mechanical and chemical cues, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. One such ciliopathy is Jeune Syndrome, in which mutations in *THM1*, a cilia gene and Hedgehog (Hh) modulator, have been identified. We have generated a knock-out mouse of *Thm2,* which is a paralog of *Thm1* and a gene that is largely uncharacterized. In contrast to *Thm1*-null mouse late embryos which show shortened long bones, *Thm2*-null mice are healthy and reach adulthood, suggesting that *Thm2* alone is dispensable for development. Since paralogs can have redundant roles, we have generated *Thm2-/-; Thm1+/-* (triple allele) mutant mice. These triple allele mutant mice appear normal at birth, but are markedly smaller than their littermates by postnatal day (P)14, and some do not survive to weaning. We hypothesize that by regulating mechanosensing and Hh signaling, genetic interaction between *Thm2* and *Thm1* is critical for postnatal skeletal development. To examine our hypothesis, we propose to examine *Thm2-/-; Thm1+/-* skeletal and ciliary phenotype and to examine role of *Thm2-/-; Thm1+/-* in Hh signaling in skeletal phenotype. Investigations will include quantifying skeletal defects using microCT imaging, examining functional role of genetic downregulation of Hh signaling in triple allele mutant mice, and examining mechano- and chemotransductory capabilities of triple allele mutant primary cilia in chondrocytes. This project will provide new insights into genetic interactions underlying skeletal development, and will establish *Thm2* as a novel locus important for cilia-mediated mechanosensation and signaling.

Title: **Understanding clinical variability in craniofacial birth defects: opportunities to reduce the burden of reconstructive surgeries.**

Author: *Timothy C. Cox, Department of Oral & Craniofacial Sciences, School of Dentistry, & Department of Pediatrics, School of Medicine, University of Missouri-Kansas City, Missouri.*

Abstract: Non-syndromic cleft lip/palate (NS-CL/P) affects ~1 in 700 live births. It is widely considered to be a complex trait, encompassing a spectrum of clinical presentations. Despite the identification of many population-level genetic variants contributing to an individual's risk of clefting, it remains impossible to predict whether a child will be born with NS-CL/P or how severe the presentation will be. As severity is the major determinant of surgical burden, there is a pressing need to understand additional risk factors.

Manipulation of the maternal diet is an attractive avenue to modify an embryos genetically-determined risk. We have initially focused on Vitamin A as it is solely acquired from our diet yet essential for early facial development. Furthermore, dietary vitamin A deficiency is among the most common nutritional deficiencies worldwide. We are exploiting a unique mouse model that facilitates control of dietary vitamin A availability to address whether embryos carrying defined genetic risk factors for CL/P are hypersensitive to normal fluctuations in maternal dietary vitamin A bioavailability.

Preliminary data will be presented on the impact of transient reductions in maternal vitamin A availability on cleft-labile embryos. Ongoing efforts are aimed at investigating different genetic sensitivities, the impact of timing and duration of the deficiency, and whether low level supplementation can reduce cleft severity and hence minimize the surgical burden for patients. We hope these studies will open up the prospect of simple interventional treatments for genetically at-risk individuals much like that of folate supplementation for reducing the risk of neural tube defects.

Funding: Endowment in Dental and Mineralized Tissue Research.

Title: **Compromised Mitochondrial Function in the Pathogenesis of Osteogenesis Imperfecta**

Author: *Charlotte Phillips, Ph.D., Department of Biochemistry and Child Health, University of Missouri, Sarah Dallas, Ph.D., Department of Oral and Craniofacial Sciences, University of Missouri-Kansas City*

Abstract: Osteogenesis imperfecta (OI) is a heritable disorder of skeletal fragility, ranging in severity from mild with few fractures to perinatal lethal with marked bone deformity and fragility. There is no cure; treatment is limited to surgical intervention and anti-resorptive drugs, both with limited success. Bone is mechanosensitive, responding and adapting to its mechanical environment to strengthen the skeleton, and contracting muscles are one of the largest physiological loads that bone experiences. Recent studies demonstrated that 80% of OI patients exhibit muscle weakness. To investigate the pathogenesis of OI muscle weakness, we examined the mitochondria, generators of the cellular energy needed for contraction, in the osteogenesis imperfecta mouse model (*oim)* with muscle weakness. We found *oim* skeletal muscle has severely compromised mitochondrial function. This opens up potential therapeutic targets to enhance muscle and bone weakness, but also raises the question as to whether bone cell mitochondrial function is similarly compromised. The goal of this pilot project is to test whether mitochondrial dysfunction is a major contributor to pathogenic muscle weakness and skeletal fragility in OI through investigation of three aims: 1) to determine if the compromised respiration rates in isolated *oim* skeletal muscle mitochondria are associated with altered Ca^{+2} homeostasis and production reactive oxygen species, 2) determine whether cultured primary *oim* calvarial cells have compromised mitochondrial bioenergetics and metabolism, and 3) to determine how the dynamic, morphological and functional properties of mitochondria are altered in *oim* bone and muscle cells using live cell and intravital imaging approaches. This innovative hierarchical approach (from isolated mitochondria through to live cell/intravital imaging) will competitively position this unique interdisciplinary team for future external grant submissions to NIH /NIAMS.

Title: **Estrogen Action in Bone and Muscle Crosstalk**

Author: *Mark L. Johnson, Nuria Lara-Castillo, Erica Jackson, Mark Dallas, Julian Vallejo, Jennifer Rosser, Ellie Ray, Derrick Nelson, Ganesh Thiagarajan and Michael Wacker*

Abstract: Estrogens play important roles in bone metabolism and muscle mass and function throughout the lifespan in both females and males. Evidence has accumulated that beyond the mechanical coupling of the bone and muscle, these two tissue communicate with each other biochemically. We hypothesize that loss of estrogen mediated signaling with aging impairs crosstalk signaling between bone and muscle. Estrogen signals through both genomic (via estrogen receptors; ERα and ERβ) and non-genomic mechanisms. We are using several animal models that have targeted deletion of ERα and ERβ in bone (oy-cKO-ER) or skeletal muscle (sm-cKO-ER) to dissect the individual roles of these estrogen receptors in each tissue across aging and in bone-muscle crosstalk. Ovariectomy of TOPGAL mice (β-catenin reporter mice) attenuates load-induced activation of β-catenin signaling in osteocytes, which is a prerequisite for bone formation in response to mechanical loading. Osteocyte targeted deletion of ERβ (oy-cKO-ERβ) resulted in decreased trabecular BV/TV and trabecular number with increased trabecular separation in male mice compared to control littermates, but not females. It also increased the modulus of elasticity in male oy-cKO-ERβ mouse femurs, but decreased this property in female oy-cKO-ERβ mice. The elastic stiffness of male oy-cKO-ERβ femurs increased with no effect in females. Interestingly, male oy-cKO-ERβ soleus muscles recovered to a lesser extent following fatigue *ex vivo*, but female oy-cKO-ERβ mice muscles showed no changes. Preliminary data from the sm-cKO-ERβ mice demonstrated a left shifted load:strain relationship, implying a change in the biomechanical properties of those tibiae. Using an *in vitro* model (TOPflash-MLO-Y4 osteocyte like cells) to further dissect the role of estrogen and estrogen receptors in bone and muscle crosstalk, we have demonstrated that conditioned media from C2C12 myotubes, but not myoblasts, produces a factor that synergizes with Wnt signaling. Purification and identification of this factor is currently ongoing. Additionally, estrogen receptor inhibition resulted in attenuation of Wnt activation of the pathway *in vitro*. Collectively these studies have demonstrated a sex and estrogen receptor isoform specific role for estrogen action in bone and muscle. Furthermore, these studies demonstrated that estrogen receptor deletion in bone or muscle consequently alters the properties of the other tissue, implying crosstalk communication and its control is regulated differently in males and females.

Title: **Bone-Heart Crosstalk**

Author: *Michael Wacker, Julian Vallejo, Mark Gray, Derek Wang, Nuria Lara-Castillo, Mark Dallas, Mark Johnson*

Abstract: Bone has previously been thought of to be a static organ, however, its role as a significant endocrine organ is now fully established and appreciated. Our bone and muscle collaborative has identified several factors released by bone (PGE2 and Wnt3a) that can regulate skeletal muscle. Another factor released by bone, fibroblast growth factor 23 (FGF23), can regulate phosphate via interactions with the kidney and parathyroid. My laboratory has previously demonstrated that FGF23 can also directly increase calcium entry in cardiomyocytes and increase cardiac contractility. We have now shown that pathological levels of FGF23 experienced during end stage chronic kidney disease can induce arrhythmias in isolated heart preparations and prolong the QT interval on EKG measurements ($n=10$; $P<0.01$). These data along with clinical studies demonstrating an association between osteoporosis and heart health has led us to further hypothesize that there may be factors released by bone that modify cardiovascular function to match bone strain. We have conducted initial studies in which isolated, paced, Langendorff-perfused mouse hearts were treated with conditioned media from MLOY4 cultured cells (osteocyte-like cell line) that underwent fluid flow sheer stress. Applications of various percentages of media (0.075%-7.5%) induced on average greater than 30 premature contractions/min in paced*, ex vivo* mouse hearts ($n=5$; $P<0.05$), which may indicate there is a factor increasing the excitability of cardiac myocytes. The exact mechanism for this response remains to be determined. Alteration of cardiac function by bone response to mechanical loading is a new area of research focus.

POSTER ABSTRACTS

University of Kansas

Title: **Multisession brain-computer interface performance using a switch-scanning augmentative and alternative communication device by individuals with ALS**

Author: *Jonathan Brumberg & Kevin Pitt*

Abstract: In this study we examined the performance of a motor-based brain-computer interface (BCI) access method to a switch-scanning augmentative and alternative communication device by four individuals with amyotrophic lateral sclerosis and three control participants without any neurological disease or injury. Participants with ALS completed 12 sessions either in a lab setting (N=2) or at home (N=2) while control participants completed 3 sessions in a lab setting. In addition to BCI tests, participants with ALS also completed a comprehensive BCI screening and assessment tool for identifying sensory, cognitive and motor (imagery) skills likely important for successful BCI performance, as well as ratings on fatigue, satisfaction, frustration, and motivation before and after each session. The results of this study found that two of four participants with ALS were able to increase their performance over time. Performance and frustration levels influenced satisfaction, with performance being largely tracked by symptom severity. Participants without ALS also varied in their performance, though all were able to control the device at levels above those with ALS. This study demonstrates that BCI-based control of augmentative and alternative communication devices is possible, and future training programs for individuals who may use BCI access methods may be enhanced by collecting user feedback regarding fatigue, frustration, and satisfaction.

University of Kansas Medical Center

Title: **Degeneration of ALS mouse neuromuscular junctions analyzed using super-resolution microscopy and ameliorated using human mesenchymal stem cells**

Author: *Yomna Badawi[1], Sudheer Tungtur[1], Tomohiro Tanaka[1], Rupal Soder[2], Richard Barohn[3], Kazuhiro Shigemoto[4], and Hiroshi Nishimune[1]*

[1]Department of Anatomy and Cell Biology, [2]Midwest Stem Cell Therapy Center, [3]Department of Neurology, University of Kansas Medical Center, Kansas City, KS, 66160, USA [4]Geriatric Medicine, Tokyo Metropolitan Institute of Gerontology, Itabashi, Japan

Abstract: Presynaptic active zones play an essential role as synaptic vesicle release sites for synaptic transmission. In this study, stimulated emission depletion (STED) super resolution microscopy analysis supports our molecular mechanism suggesting that laminin β2 anchors presynaptic voltage-gated calcium channels (VGCC) in front of postsynaptic junctional folds. PQ-type VGCC can then function as a scaffolding protein for active zone–specific proteins. Based on the knowledge obtained from wild-type NMJs, we evaluated the active zone proteins in NMJs of amyotrophic lateral sclerosis (ALS) mouse models. ALS is a neurodegenerative disorder in which NMJ denervation occurs before the death of motor neuron cell bodies in the spinal cord, suggesting a "dying-back" neuropathy. The mechanisms underlying NMJ denervation in ALS remain unknown. The pathogenesis of ALS may involve changes in protein levels, which are important for the maintenance of NMJ active zones and regulation of neurotransmission. For this purpose, we analyzed active zone proteins in NMJs of $SOD1^{G93A}$ mice, at an early, pre-symptomatic stage (P85) and a symptomatic stage (P140). Interestingly, we found that the quantity of laminin β2 and active zone proteins Bassoon, Piccolo, and PQ-type VGCC decreased in innervated NMJs of ALS mice compared to age-matched wild-type mice.

As a therapeutic source of laminin β2, we evaluated stem cells as vectors to express and secrete proteins that promote NMJ maintenance and are neuroprotective. This could prove to be an efficient and a long-term delivery system for laminin β2 to reduce NMJ denervation and increase the quality of life of ALS patients.

Title: **Utilization of the collage-induced arthritis mouse model to evaluate molecular biomarkers of methotrexate efficacy**

Author: *Ryan S. Funk, Pharm.D., Ph.D.*

Abstract: Methotrexate (MTX) is an anti-folate therapeutic and is the cornerstone of disease-modifying therapy in the treatment of autoimmune arthritis, including rheumatoid arthritis and juvenile idiopathic arthritis. Despite its established efficacy, a major barrier to its effective use is a highly variable and unpredictable response profile with approximately 1 in 3 patients failing to respond to initial therapy. Therefore, a critical need exists to identify clinical biomarkers of MTX efficacy to guide clinicians in the early optimization of drug therapy. This study utilizes the collagen-induced arthritis (CIA) mouse model as a model of autoimmune arthritis to evaluate molecular biomarkers of MTX response. Most notably, we demonstrate the dose-dependent efficacy of MTX in the CIA mouse model and find that mice fail to appreciably metabolize MTX into its biologically active intracellular form (i.e. polyglutamate metabolites). Further, we demonstrate that MTX therapy is associated with the depletion of intracellular folates and that this anti-folate effect is directly associated with various measures of drug efficacy. Together, this work demonstrates the dose-dependent efficacy of MTX in the CIA mouse model and directly links its efficacy with its pharmacological activity as an anti-folate.

Title: **lleus and dysautonomia can contribute to significant morbidity in Guillain-Barre syndrome independent of peripheral motor involvement.**

Author: *Aaron LacKamp, M.D.*

Abstract: Guillain Barre Syndrome (GBS) is a humorally-mediated inflammatory polyradiculoneuropathy. The rate of respiratory failure can be predicted by the rapidity of onset of symptoms. At KU, endotracheal intubation has occurred in 9 out of 129 cases based upon a search of de-identified records using the HERON database.

Findings: Respiratory failure may occur in GBS as a result of diaphragmatic weakness and can be anticipated based upon bedside spirometry. We present a case of precipitous respiratory failure in spite of normal spirometry due to gut failure. The patient had pharyngeal weakness, failed a swallow evaluation, ileus, colonic gaseous distention, and did not have a bowel movement for 7 days prior to his hypoxic respiratory failure. There was cephalad displacement of the diaphragm and the patient had developed shunting in the left lung due to atelectasis in the left lower lobe. The patient required prolonged mechanical ventilation until resolution of the ileus and colonic distention.

In this case dysautonomia and branchiomotor weakness were disproportionate to the degree of peripheral motor weakness. There was no appreciable respiratory muscle weakness prior to precipitous hypoxic respiratory failure. Dysautonomia, ileus, and hyponatremia may suggest hypothalamic involvement of GBS. The identification of non-motor symptoms is associated with increasing severity of motor weakness, however in this patient non-motor symptoms were uncoupled in terms of severity from peripheral motor involvement.

Conclusion: Possible hypothalamic effects of Guillain-Barre Syndrome: ileus, and dysautonomia may independently contribute to significant morbidity and may be future areas of investigation.

Title: **Applying Human Umbilical Cord Derived Mesenchymal Stem Cells for the Treatment of Amyotrophic Lateral Sclerosis**

Author: *Takashi Matsuda[1], Yomna Badawi[1], Kleiton Silva[4], Rupal Soder[2], Richard Barohn[3], Tadashi Yoshida[4], and Hiroshi Nishimune[1]*

[1]Department of Anatomy and Cell Biology, [2]Midwest Stem Cell Therapy Center, [3]Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA, [4]Department of Medicine and Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, MO, USA

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by a gradual loss of motor neurons. Studies indicate that neuromuscular junction (NMJ) denervation occurs in the early stages of the disease while neuronal cell bodies in the spinal cord remain intact. We found that the synapse organizer laminin β2 decreases in NMJs of ALS model SOD1^{G93A} mice, and transgenic expression of laminin β2 in skeletal muscles of SOD1 ^{G93A} mice ameliorates NMJ denervation. The objectives of this study are (1) to determine whether human umbilical cord-Wharton's jelly derived mesenchymal stem cells (hMSCs) from multiple donors secrete laminin β2 and neurotrophic factors at similar levels, and (2) to evaluate hMSCs derived from different donors in vivo in SOD1^{G93A} mice for NMJ maintenance and life-span extension. We discovered that hMSCs derived from multiple donors can increase the secretion of laminin β2 and neurotrophic factors by stimulation in media supplemented with growth factors. Furthermore, we confirmed that unilateral injection of hMSCs increased NMJ innervation rates and average myofiber cross sectional area in MSC-injected muscles compared to non-injected contralateral muscles. Then, the hMSCs obtained from two donors were transplanted separately into SOD1 ^{G93A} mice by combined intrathecal and bilateral intramuscular injections. Importantly, injection of hMSCs improved neuromuscular function of injected SOD1 ^{G93A} mice, and prolonged the lifespan of SOD1 ^{G93A} mice compared to the vehicle injected SOD1 ^{G93A} mice. Thus, injection of hMSCs obtained from donors could be an efficient treatment method to improve the quality of life of ALS patients.

Title: **Identification of the Prevalence and Patterns of Celiac Disease Associated Arthropathy and Coexistence of Celiac Disease with Rheumatic and/or Inflammatory Disorders in a Single Tertiary Medical Center**

Author: *Anita Moudgal[1], Pooja Bhadbhade[2], Ammar Haikal[2], Mehrdad Maz[2], [1]The University of Kansas School of Medicine, [2]Division of Allergy, Clinical Immunology and Rheumatology, Department of Medicine; The University of Kansas Medical Center*

Abstract: **Background:** Celiac disease (CD) is a gluten-sensitive enteropathy that develops in genetically predisposed individuals. Arthropathy has been reported as an extra-intestinal manifestation of CD. The pathogenesis of arthropathy is unclear; however, the immunologically mediated mucosal injury may lead to absorption of immune complexes or gut-derived antigens which may provoke antibody mediated autoimmune diseases including rheumatic diseases and arthropathy. We report a retrospective chart review of 35 confirmed cases of CD associated arthropathy and coexisting rheumatic or inflammatory disorders.

Methods: Heron database was used to search for patients seen by Gastroenterology and/or Rheumatology between January 2008 to January 2018 with suspected CD based on ICD-9 code for CD. These charts were retrospectively reviewed to determine who had confirmed CD, joint pain and pattern of involvement, dietary compliance, and other inflammatory or rheumatic disorders. The diagnosis of CD was based on serology specific for tissue transglutaminase (tTG) antibody, endoscopic and histological results, compliance with gluten free diet, and clinical judgement.

Results: A total of 95 patients with suspected CD were identified. 35/95 had confirmed CD. Of the 35 patients, 29 were female and 34 self-identified as Caucasian. The average age of patients was 49 years. Of the 35 patients, 22 patients did not have a co-existing rheumatic or inflammatory disorder. This cohort of 22 patients endorsed symptoms in the following distribution: 82% (18/22) had peripheral only, 0% (0/22) had axial only, and 18% (4/22) had peripheral and axial involvement. Of the 22 patients, 54% (14/22) endorsed arthropathy in small joints, 91% (20/22) in medium joints, and 82% (18/22) in large joints. Improvement in arthralgia after transitioning to a gluten-free diet was reported in 4/22 patients. A total of 13/35 with confirmed CD diagnosis had a rheumatic and/or inflammatory disorder. Rheumatoid Arthritis had the highest prevalence at 11% (4/35) followed by IBD (3), UCTD (2), Sjorgen's Syndrome (1), Scleroderma (1), Symmetric Inflammatory Polyarthritis (1), and Psoriasis (1). Fibromyalgia, a non-inflammatory condition, was reported in 6% (2/35) of patients. 3 patients were not seen by rheumatology.-

Conclusion: CD associated arthropathy and coexistence of CD with rheumatic and/or inflammatory disorders are under-recognized. Our data demonstrates that 63% of patients had CD associated arthropathy who appear to be a unique subset separate from those with coexisting CD and rheumatic/inflammatory disorders. Identifying the association and pattern of arthropathy in CD will aid in management of patients who either present to a gastroenterologist with extra-intestinal manifestations or to a rheumatologist with gastrointestinal manifestations. The awareness that CD can coexist in rheumatic diseases will aid rheumatologists in more effective recognition and mangement of patients with such presentations.

Title: **Efficacy of mycophenolate mofetil in the treatment of rheumatoid arthritis associated interstitial lung disease**

Author: *Amos, J.,[1] Kendall, J.[1], Moran, R.[1], Krause, M.[1], Schmidt, P.[1], Hall, C.[2], Hamblin, M.[2], Maz, M[1]. [1]Division of Allergy, Clinical Immunology and Rheumatology, [2] Division of Pulmonary and Critical Care, Department of Medicine; The University of Kansas Medical Center*

Abstract: **Background:** Interstitial lung disease (ILD) as an extra-articular manifestation of rheumatoid arthritis (RA) can lead to significant morbidity and mortality. There is limited data on the efficacy of mycophenolate mofetil (MMF) in the treatment of RA associated ILD.

Methods: This retrospective chart review identified patients with a clinical diagnosis of RA and interstitial lung disease at a single tertiary academic medical center who were treated with MMF for at least 3 months between 1/01/2005 and 12/31/2018. Patients were identified by diagnosis codes, and then reviewed to confirm clinical diagnoses. Data regarding concurrent therapies including glucocorticoids, and pulmonary function tests, infections and hospitalizations were also collected.

Results: 26 patients were identified; 17 female (65.4%) and 23 Caucasian (88.5%) with a mean age of 57.8 at the time of diagnosis of RA. Rheumatoid factor (RF) was positive in 20 (76.9%), anti-cyclic citrullinated peptide antibody (ACPA) in 16 (61.5%) and 10 (38.5%) were seropositive for both, while 3 (11.5%) patients were seronegative. The mean time to diagnosis of ILD after diagnosis of RA was 40.6 months with a range of -50 to 504 months. Seven (26.9) patients had an ILD diagnosis prior to RA.

Fourteen (53.8%) patients had usual interstitial pneumonia (UIP), 5 (19.2%) had nonspecific interstitial pneumonia (NSIP), 3 (11.5%) had organizing pneumonia (OP), and 4 (15.4%) had other forms such as mixed UIP and NSIP. The average duration of MMF therapy was 24.9 months with an average maximum daily dose of 2163.5mg. 11 (42.3%) patients were concurrently on rituximab (RTX), 1 (3.85%) on methotrexate, 1 (3.85%) on certolizumab, and 1 (3.85%) on sulfasalazine. 15 (57.7%) patients were on concurrent prednisone ≥10mg daily and 6 (23.1%) were on prednisone <10mg daily for at least one month.

Average FVC for all patients was 62.4% (SD 21.9) predicted at the time of initiation of MMF, 61.9% (SD 23.0) predicted at 6 months, and 64.8% (SD 25.9) predicted at 12 months. In aggregate, 13 (50%) patients on MMF had stable or improved FVC over the 12-month period, of whom 6/13 (46.1%) were on concurrent RTX. Among patients on combination MMF and RTX; 6/11 (54.5%) had stable or improved FVC over the 12-month period.

There were 26 hospitalizations in 8 (30.8%) different patients; 17 (65.4%) for infections, 3 (11.5%) for respiratory failure, and the remainder were for cardiovascular events. There were 10 outpatient infections treated with antimicrobials. There were 9 (34.6%) deaths by the end of the study period.

Conclusion: In this cohort of 26 RA patients with ILD, treatment with MMF as mono or combination therapy with RTX was associated with stable FVC in 50% of patients at 12-month. About 55% of patients on combination MMF and RTX showed stability or improvement in FVC. Patients treated with MMF compared to combination MMF and RTX had similar FVC values at the end of the study period. Further studies are needed to better understand the efficacy and safety of MMF or combination therapy with RTX in RA associated ILD.

Title: **Pattern of Arthropathy in Patients with Cystic Fibrosis**

Author: *Daniel Pham; Mehrdad Maz, MD; Michael Crosser, MD; Megan Krause, MD*
Department of Internal Medicine, Division of Allergy, Clinical Immunology, & Rheumatology, The University of Kansas Medical Center

Abstract: **Background:** Arthropathy is a rare but debilitating manifestation of cystic fibrosis (CF) that has no formal definition. This study attempts to characterize the spectrum of joint pains in CF patients.

Methods: A retrospective chart review was conducted on 246 adult CF patients who were seen at a single tertiary care center between January 1, 2008 and December 31, 2017. Charts were individually reviewed for description of joint symptoms. Patients were excluded if they had an alternative explanation for joint symptoms. Association of joint symptoms with pulmonary exacerbation were abstracted and defined based on clinical diagnosis of the treating provider. Pattern of joint involvement, duration of symptoms (acute as defined by <6 weeks and chronic >6 weeks), and therapies utilized for joint pain were also abstracted.

Results: In the overall cohort of 246 adult CF patients, 43 (17%) had unexplained joint symptoms. In the overall cohort, 128 (52%) were female and 42 (97%) self-reported as Caucasian. In those with unexplained joint symptoms, 28 of 43 (65%) were female. Most commonly, the joint symptoms were not associated with pulmonary exacerbations (22, 51%). In 15 (35%) patients there was association between joint symptoms and pulmonary exacerbations while in 6 (14%) it was not specified. In 18 patients, the duration of symptoms were described and 14/18 had symptoms lasting less than 6 weeks. Knee (23), ankles (13), hips (10), and wrists (10) were the most affected joints. However, both small and large joints were affected. Both symmetric and asymmetric presentations were noted. No individuals had findings of sacroiliac joint involvement. There were no individuals with hypertrophic osteoarthopathy in this cohort. The most commonly used medications were NSAIDs. In the overall cohort of CF patients who had unexplained joint symptoms, therapies utilized were NSAIDs (25, 58%), acetaminophen (10, 23%), and prednisone (9, 21%). In terms of the use of DMARDs, hydroxychloroquine was the most frequently used (7, 16%) followed by sulfasalazine (1, 2.3%). No biologics were used in this cohort. There were no reports of fluoroquinolone associated tendinopathy or voriconazole induced periostitis.

Conclusion: This study characterizes the variety of joint symptoms in CF patients and expands on current knowledge. Females were most likely to experience joint symptoms and the knee was most commonly affected joint. Understanding the diverse spectrum of joint symptoms will result in greater recognition and improvement in quality of life for these patients.

Title: **ASARM reverses hyperphosphatemia, prevents calciphylaxis-like lesions, corrects renal, bone brain and cardiovascular calcification in a rat model of chronic kidney disease.**

Author: *Peter S. N. Rowe, Jason R. Stubbs, Shiqin Zhang, Timothy Fields, Alan S. Yu and Ellen T. McCarthy.*

Abstract: **Background:** Abnormalities in mineral metabolism, bone and vascular calcification occur in Chronic Kidney Disease (CKD-MBD). Cognitive function also declines as the disease progresses. Bone ASARM peptides are strong inhibitors of mineralization and induce hypophosphatemia by inhibiting phosphate uptake from the gut. We hypothesize treatment of CKD-MBD rats with ASARM peptides will reverse hyperphosphatemia, correct mineralization defects and improve mortality.

Methods: To test our hypothesis, we used a rat 5/6 Nephrectomy experimental model (NEPHREX) and sham operated rats (SHAM) as controls. Male rats (16 wk, 250 gm) were fed a high phosphate diet to worsen mineral metabolism defects (2% P, 2000 IU Vit D and 0.8% Ca; TEKLAD 170496). ASARM peptide was infused continuously for 4 weeks using subcutaneous implantation of Alzet osmotic pumps. Sera collections were taken at the beginning and end of the study.

Results: NEPHREX rats treated with ASARM-peptide showed major reductions in hyperphosphatemia, and improved renal, bone, brain and cardiovascular calcification compared to controls treated with vehicle. Also, the high phosphate diet NEPHREX rats developed sub-dermal medial blood vessel calcification and calciphylaxis like lesions. The subdermal blood vessel calcifications did not occur in 56-NEPHREX rats treated with ASARM-peptide.

Title: **Nephrogenic systemic fibrosis is induced in high phosphate diet CKD rates exposed to Gd3+ binding contrast agents (GBCA); Role of ASARM peptides.**

Author: *Peter S. N. Rowe, Aditi Gupta, Timothy Fields, Travis Hagedorn, and Ellen T. McCarthy.*

Abstract: **Background:** High contrast Magnetic Resonance Imaging (MRI) requires the use of Gadolinium Binding Contrast Agents (GBCAs). Subsets of chronic kidney disease (CKD) patients exposed to GBCAs develop Nephrogenic Systemic Fibrosis (NSF), a progressive disease that leads to acute morbidity and death. Our previous work showed circulating ASARM-peptides bind to GBCAs and induce release of toxic Gd . Bone-derived ASARM peptides induce hypophosphatemia and bone-mineralization abnormalities. We hypothesize increased levels of acidic ASARM-peptide exacerbates release of free Gd resulting in an NSF pathology with reduced ectopic mineralization defects.

Methods: To test our hypothesis, we used a rat 5/6 Nephrectomy CKD disease model (NEPHREX). Male rats (16 wk, 250 gm) were fed a high phosphate diet (2% P, 200IU Vit D and 0.8% Ca; TEKLAD 170496). ASARM peptide was infused continuously for 4 weeks using subcutaneous implantation of osmotic pumps. As controls, co-implanted osmotic pumps were used to co-infuse SPR4 peptide - a peptide that neutralizes ASARM. Sera collections were taken at the beginning and end of the study. Three consecutive, daily bolus injections of Gd -containing contrast agent (Omniscan , gadodiamide) were given 3 days after pump implantation through surgically implanted jugular-vascular-catheters.

Results: NEPHREX rats treated with Omniscan and ASARM developed severe skin pathology, behavioral abnormalities, and joint abnormalities that were consistent with NSF. Computed tomography (CT) showed renal, brain,heart dermal metastatic calcifications and bone defects in Omniscan treated Rats. ASARM peptide treatment corrected the Omniscan induced skin, bone and soft tissue mineral abnormalities and corrected the hyperphosphatemia.

Conclusion: Our study shows CKD rats fed a high phosphate diet and treated with Omniscan develop severe NSF like pathology. ASARM infusion prevents Omniscan induced subdermal calcification, corrects mineral defects and hyperphosphatemia. In conclusion, ASARM peptides induce release of free Gd from GBCAs but reduce mineralization pathology. These findings have clinical importance for GBCA use in inherited or acquired renal bone-mineral loss disorders with increased circulating ASARM-peptides.

Title: **Treatment of posttraumatic arthrofibrosis using high intensity ultrasound and laser in a novel rabbit model of knee contracture**

Author: *Authors: David Hazlewood, Yi Feng, Qinghua Lu, Xinmai Yang, Jinxi Wang*
Presenter: Jinxi Wang

Abstract: Post-traumatic joint contracture induced by scar tissues can leave patients in a permanent state of pain and disability, which is difficult to resolve by current treatments. This study examined the therapeutic effect of pulsed high-intensity laser (PHIL) and pulsed high-intensity focused ultrasound (PHIFU) for posttraumatic joint contracture due to arthrofibrosis, with short pulses for prevention of tissue damage. Rabbit knee contracture was induced by surgical capsular damage. Twenty-one rabbits were divided into four groups: untreated control (n=5), PHIL (n = 5), PHIFU (n = 5), and PHIL + PHIFU (n = 6). Maximum knee extension of the surgically modified rabbit knee was compared to that of the contralateral control knee over the course of 16 weeks. The results revealed that the rabbits in the untreated control group maintained a consistent level of joint contracture, while rabbits in each of the treatment groups had improved range of motion, eventually leading to a restoration of normal joint extension. Average recovery time was 7.6 ± 1.5 weeks for the PHIL treatment group, 9.8 ± 3.7 weeks for the PHIFU group, and 7.6 ± 2.2 weeks for the combined group. Histopathology demonstrated reduced density and accelerated resorption of scar tissues in the treated knee joints. This study provides evidence that both PHIL and PHIFU are effective in treating posttraumatic arthrofibrosis in rabbits, and warrant further investigations into the underlying mechanisms and optimal parameters of PHIL and PHIFU therapies in a larger number of animals.

Title: **Mast cell deficiency amplifies inflammatory response in a mouse model of Kawasaki's disease**

Author: *Jason M Springer, Mingcai Zhang, Ryan Funk, Ossama Tawfik, Naohito Ohno, Noriko N Miura, Mehrdad Maz, Kottarappat N Dileepan*
Division of Allergy, Clinical Immunology & Rheumatology, Department of Medicine, University of Kansas Medical Center, Kansas City, KS, USA

Abstract: **Background:** In Kawasaki's disease (KD) higher serum IL-6 in the first week of the disease has been shown to be a risk factor for the development of coronary artery aneurysms. In this study, we used the *Candida albicans* water-soluble fraction (CAWS) mouse vasculitis model for KD, to determine the role of mast cells on IL-6 homeostasis.
Methods: Wild-type male C57Bl/6 (WT) or mast cell-deficient (MC) mice were intraperitoneally injected with Phosphate-buffered saline (PBS) or CAWS (2 mg/mouse) daily for 5 days. mice were sacrificed at either 7 or 14 days after the last injection. Aortic root Inflammatory score was blindly accessed.
Results: Seven MC mice injected with CAWS died unexpectedly within 24 hours of the first CAWS injection. MC-CAWS mice had higher systemic IL-6 compared to WT-CAWS at 7 days and 14 days. By 14 days mice in MC-CAWS had significantly higher serum INFγ compared to WT-CAWS mice. TNFα was higher in MC-CAWS compared to WT-CAWS at both 7 days and 14 days. The average AIS was higher in the MC-CAWS group compared to WT-CAWS at both 7 days (1.5 vs 1.3) and 14 days (3.7 vs 2.4).
Conclusion: By 14 days, mast cell deficient mice exposed to CAWS developed higher systemic levels of both IL-6 and INFγ, two important cytokines in pathogenesis of aortitis and coronary arteritis. By histology, mast cell deficient mice have higher inflammatory scores. This supports the novel concept that mast cells play a protective role in inhibiting the initial systemic inflammatory response in Kawasaki's disease.

University of Missouri

Title: **Accessory inspiratory muscle (i.e., pectoralis minor) activity is increased in a rodent model of respiratory motor neuron loss**

Author: *Lauren F. Borkowski and Nicole L. Nichols, Department of Biomedical Sciences, University of Missouri, Columbia, MO*

Abstract: Patients with neuromuscular diseases experience loss of respiratory motor neurons (*i.e.,* phrenic and intercostal) resulting in ventilatory failure, and ultimately death. There are currently no significant treatments to prolong or correct for these breathing deficits. Genetic rodent models of motor neuron loss develop global symptoms (*e.g.,* dysphagia, limb paralysis, etc.), so we have developed an inducible model of only respiratory motor neuron death in order to study how motor neuron loss impacts respiration and to develop therapeutic interventions. Briefly, adult rats are intrapleurally injected with cholera toxin B conjugated to saporin (CTB-SAP) that is retrogradely transported to the phrenic and intercostal motor nuclei of the spinal cord, which results in selective elimination of phrenic and intercostal motor neurons. Despite deficits in maximal ventilatory capacity following CTB-SAP, eupneic ventilation is maintained. Our preliminary data suggest that one way eupnea may be maintained in CTB-SAP rats is *via* the recruitment of G-coupled protein receptor-dependent pathways to cause respiratory plasticity in the phrenic motor nucleus over the course of phrenic motor neuron death. However, our preliminary data also indicate that diaphragmatic amplitude is decreased at baseline in CTB-SAP rats *vs*. controls; thus, phrenic respiratory plasticity may only account for a portion of the maintenance of eupneic ventilation. We hypothesize that eupneic ventilation following respiratory motor neuron loss may also be maintained through the recruitment of accessory inspiratory muscles (*e.g*., the pectoralis minor muscles). The pectoralis minor muscles actively elevate the ribs upward and outward to move the chest wall following increased ventilatory demand. Pectoralis minor muscles are not normally utilized for eupnea, but have been shown to increase activity with disease or injury (*e.g.* ALS, spinal cord injury, and bilateral diaphragmatic paralysis). To begin to test this hypothesis, we are studying pectoralis minor output *via* electromyography in anesthetized, spontaneously breathing control and CTB-SAP rats. Our preliminary data suggest that indeed pectoralis minor activity is increased in CTB-SAP rats *vs*. controls, suggesting that the pectoralis minor may also be recruited to maintain eupneic ventilation. Future studies will evaluate the recruitment of other accessory inspiratory muscles (*e.g*., scalenes, sternocleidomastoid, etc.) over the course of respiratory motor neuron loss, and whether these muscles (including the pectoralis minor) are necessary for eupnea in CTB-SAP rats. This furthers our understanding of the potential contribution accessory inspiratory muscles, specifically the pectoralis minor, have on the maintenance of eupneic ventilation following respiratory motor neuron loss.

Title: **Positron emission tomography spinal imaging in a canine disease model of ALS**

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Abstract: Canine degenerative myelopathy (DM) is a progressive adult-onset neurodegenerative disease that has similarities to amyotrophic lateral sclerosis (ALS). A hallmark of CNS tissues within ALS patients, DM dogs and SOD1 mutant rodents is the loss of the excitatory amino acid transporter (EAAT2) protein on CNS astrocytes, which causes regional excitotoxic injury. Recently, a new positron emission tomography (PET) imaging fluorine-18 radiolabeled tracer, ^{18}F-FAA, which targets the CNS EAAT2 protein has been synthesized. The purpose was to detect EAAT2 changes in DM dogs relative to healthy controls (HC) by ^{18}F-FAA spinal PET imaging.

Companion owned dogs were recruited as HC or DM affected. The cohorts included HC [age range: 5-11 years, 1 male and 6 females] and DM affected [age range: 9-14 years, 4 males and 2 females]. Multiple tracer doses were afforded per production run, with high molar activity (83-730 TBq/mmol) and purity (>95%). The V_T estimates were derived with a two compartment (one tissue) kinetic. A significant statistical difference in ^{18}F-FAA tracer V_T was found for T5-T12 thoracic spine, where the DM cohort had a lower V_T value than the HC cohort (ANOVA p=0.0307). A significant V_T change in cervical spine (HC vs. DM) was not found. We conclude that the HC vs. DM cohort findings demonstrate that ^{18}F-FAA PET imaging is sensitive for the detection of thoracic EAAT2 protein target changes. Thus, longitudinal PET imaging in DM dogs is advancing in order to assess progressive EAAT2 changes in spine regions and brain.

Title: **Voluntary Wheel Running Partially Compensates for the Effects of Estrogen Receptor-α Knockout on Cortical Bone in Male Mice**

Author: *Rebecca K. Dirkes, Nathan C. Winn, Thomas J. Jurrissen, Dennis B. Lubahn, Victoria J. Vieira-Potter, Jaume Padilla, Pamela S. Hinton, University of Missouri- Columbia, MO*

Abstract: **Introduction:** Loss of estrogen activity negatively impacts bone mass and strength, whereas exercise is osteogenic. Here, we determined whether exercise could compensate for the negative effects of estrogen receptor-α (ERα) loss.

Methods: At 12 weeks of age, male ER-α knockout (KO) and wild-type (WT) mice were given a high-fat diet and randomized to exercise (EX) treatment or sedentary (SED) control, resulting in four groups: WT-SED, WT-EX, KO-SED, and KO-EX (n=8-10 per group). After 10 weeks animals were sacrificed, and tibiae collected. Cortical geometry and trabecular microarchitecture were assessed via micro-CT. Biomechanical properties were assessed via three-point bending. Two-way ANCOVA with body mass was used to test the effects of KO and EX on cortical geometry and biomechanical properties; two-way ANOVA was used to test the effects of KO and EX on other outcomes.

Results: EX decreased body mass and body fat percentage, regardless of genotype. KO had lower total bone area, marrow area, and cortical thickness of the tibia mid-diaphysis versus WT. There was a significant genotype-by-exercise interaction for cortical area; EX increased cortical area in the KO animals, such that KO-EX was not different from WT animals. EX increased cortical thickness regardless of genotype. There were no differences in biomechanical properties among groups. KO improved measures of trabecular microarchitecture compared to WT, with no effect of EX.

Conclusion: Loss of ERα negatively impacts cortical bone geometry in young, sedentary, male mice, but exercise started after skeletal maturity can partially compensate for the loss of ERα activity.

Title: **Multi-modal Pain Assessment of Client-owned Dogs with Primary Bone Tumors**

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Abstract: **Introduction:** Current evidence for pain/gait outcomes in canine osteosarcoma is described by relatively few studies. Subjective owner questionnaires, force plate analysis, and serum biomarkers have been utilized to assess response. We aimed to use multiple diagnostics to objectively evaluate pain relief after therapeutic intervention in dogs with primary bone cancer. We hypothesized that intervention would cause objective evidence of pain control.
Methods: Evaluations of 8 dogs with primary bone cancer included ^{18}F-FDG PET/CT scans, motion analysis, validated owner questionnaires (CBPI), and serum N-telopeptide (NTx) concentration. Dogs were staged and had ^{18}F-FDG PET prior to treatment, and day 0, 7, 14, and 28-day CBPI, serum banking for NTx, orthopedic exam, and gait analysis. Dogs treated with radiation underwent day 28 ^{18}F-FDG PET.
Results: Four dogs were amputated; four received neo-adjuvant zoledronate and hypofractionated radiation therapy. CBPI revealed significant improvements in pain severity and pain interference scores compared to baseline. Positive changes in peak vertical force (16.7%) and vertical impulse (29.1%) were noted at day 28. Dogs receiving zoledronate and RT had a significant (at least 30%) reduction in serum NTx from baseline compared to amputated dogs ($p=0.029$). Max_{SUV} and Intensity values from PET scans decreased while Tumor Uniformity significantly increased in irradiated tumors; Gross Tumor Volume did not change.
Conclusions: Owner questionnaires, gait analyses, serum NTx, and maximum SUV on ^{18}F-FDG PET/CT scans showed improved pain relief in dogs receiving zoledronate and radiation therapy. Larger, prospective studies are warranted to identify the best objective indicator of pain relief.

Title: **Developing Swallow-Evoked Potentials (SwEPs) to Identify Pathological Neural Generator Sources for Targeted Dysphagia Treatment**

Author: *Ashley Kloepper, Joseph Arnold, M2, Brian Kinealy, M4, Chandler Haxton, Nicole Nichols, PhD, Kazutaka Takahashi, PhD, (Ilker Ozden, PhD) Department of Biomedical, Biological and Chemical Engineering, (Teresa Lever, PhD) Department of Otolaryngology-Head and Neck Surgery*

Abstract: **Objectives:** Dysphagia is a poorly understood complication of many neurological diseases, often leading to fatal aspiration pneumonia. To address this clinical gap, we are adapting the fundamentals of auditory brainstem response testing to investigate dysphagia in rodents. Here, we share our progress toward developing a swallow evoked potential (SwEP) protocol in healthy mice and rats, in preparation for future studies with rodent models of neurogenic dysphagia.

Methods: Twenty C57BL6/SJL mice (4-8 months) and 20 Sprague Dawley rats (3-4 months) of either sex were used for SwEP protocol development. While lightly anesthetized and immobilized in ear bars, needle electrodes were inserted at multiple sites on the skull (subcutaneous) and ventral neck (intramuscular) for recording of EEG and swallow-related EMG activity, respectively. A chemical stimulus (2.7% citric acid) was applied to the oropharynx to evoke swallowing, confirmed by EMG bursts in synchrony with endoscopic pharyngeal constriction. SwEP responses were extracted from EEG signals time-locked to the onset of EMG swallowing activity during a 10-minute period post-stimulus application.

Results: Swallows were more frequent and consistent in rats versus mice. The averaged SwEP waveform consisted of 8 peaks within 10 ms for rats and 5 peaks within 6 ms for mice, immediately preceding the onset of EMG swallowing activity.

Conclusions: We developed a minimally invasive evoked potential protocol to investigate swallow-related neuropathology in rodents. Methodological optimization is underway, along with optogenetic experiments to unmask the corresponding neuroanatomical source(s) for each SwEP peak. Ultimately, we envision this work may accelerate the discovery of targeted dysphagia therapeutics.

Titile: **Preload-induced ventricular dysfunction in isolated hearts from Duchenne Muscular Dystrophy (DMD$^{mdx-4CV}$) mice.**

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Abstract: DMD is an inherited muscle wasting disease caused by absence of dystrophin. Clinically, DMD-associated muscle weakness presents early in life, progresses rapidly, and causes premature death. Subclinical signs of cardiac disease present early and usually progress to dilated cardiomyopathy in late stage DMD patients. Currently, it is unknown how dystrophin deficiency causes dystrophic cardiomyopathy and heart failure. Our group has undertaken a project to (i) examine underlying molecular mechanisms of dystrophic cardiomyopathy, (ii) identify novel diagnostic and prognostic biomarkers of disease progression, and (iii) identify novel gene therapies to ameliorate ventricular dysfunction. In this study we examined the tolerance of young male Dmd$^{mdx-4CV}$ dystrophic mice to sustained elevation in ventricular preload. Hearts were isolated, cannulated via both the aorta and left atrium, and perfused with physiological saline solution in Langendorff mode (i.e., no ventricular preload). In the absence of ventricular preload hearts of wildtype mice and Dmd$^{mdx-4CV}$ mice exhibited similar pressure development (60±6 mmHg wildtype versus 64±8 mmHg Dmd$^{mdx-4CV}$). However, following pre-load challenge (20 mmHg in working heart mode for 30 minutes), hearts from Dmd$^{mdx-4CV}$ mice exhibited impaired pressure development (43±9 mmHg) versus wildtype mice (76±7 mmHg, $P<0.05$). The Dmd$^{mdx-4CV}$ hearts also had visible signs of damage in response to higher preloads. Taken together, the studies support susceptibility to stretch-induced ventricular damage and dysfunction in male Dmd$^{mdx-4CV}$ hearts. Future work will address sub-cellular mechanisms of stretch-induced ventricular dysfunction by systematic assessment of regulated cell death pathways, altered Ca^{2+} handling, and varied myofilament structure/function in Dmd$^{mdx-4CV}$ mice.

Title: **Skeletal muscle mitochondrial dysfunction and whole-body metabolic alterations in a mouse model of osteogenesis imperfecta**

Author: *Victoria Gremminger[a] , Emily Harrelson[a], Laura C. Schulz[b], R. Scott Rector[c] , Charlotte L. Phillips[a]*

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Abstract: Osteogenesis imperfecta (OI) is a heritable connective tissue disorder with 85% of patients having type I collagen gene defects. OI occurs in approximately 1:15,000 livebirths and can be subdivided into four types based on clinical severity from mild with few fractures to perinatal lethal. Although bone fragility is the most common manifestation, intrinsic muscle weakness affects roughly 80% of OI patients. Homozygous osteogenesis imperfecta murine (*oim/oim*) mouse modeling moderately severe human OI type III have inherent muscle weakness and exhibit severe mitochondrial dysfunction. *Oim/oim* mitochondrial dysfunction was evidenced by significant reductions in gastrocnemius mitochondrial respiration rates, exhibiting only 35-48% of wildtype [Wt] mitochondrial respiration rates and citrate synthase activity. Mitochondria play essential roles in the metabolism and bioenergetics of the cell. To investigate parameters associated with metabolic health we measured glucose tolerance, energy expenditure, VO_2 consumption, VCO_2 production, and evaluation of the respiratory quotient (RQ) in Wt and *oim/oim* mice. RQ ratios (CO_2 expelled:O_2 consumed) can predict the primary fuel sources being utilized. Although glucose tolerance was not altered in male *oim/oim* mice, energy expenditure and VO_2 consumption were increased and RQ reduced relative to WT mice; suggesting a change in metabolic fuel preference. Male *oim/oim* mice also exhibited increased lean mass and reduced fat mass. While further evaluation of these parameters is still required, preliminary data suggests that *oim/oim* mice may exhibit a metabolic phenotype with potential changes in metabolic fuel utilization, which may be associated with the previously observed mitochondrial dysfunction and compromised skeletal muscle force.

Title: **Neurostimulation of Bladder Efferents After Spinal Cord Injury to Preserve Autonomic Ganglion Function"**

Author: *David J. Schulz, Division of Biological Sciences, University of Missouri-Columbia*

Abstract: It is now apparent that spinal cord injury (SCI) results in substantial changes in neurons and neural networks below the site of the injury as a result of loss of input, even though the cells of these networks are not directly injured. For example, in motor systems these changes in excitability below the injury manifest as spasm due to hyperexcitability in motor neuron populations. While these ideas have been investigated more in motor and sensory systems, there is a paucity of work in the autonomic nervous system in this regard, and virtually none of these concepts or approaches have been applied to efferent control of bladder and bowel function. Functional recovery or improvement will remain elusive if we do not understand these changes, as they are critical for the success of regenerated inputs, artificial interfaces, or pharmacological interventions. We use a rodent model of SCI to demonstrate how loss of descending inputs alters the gene expression, cellular properties, and activity of spinal motor and peripheral bladder innervating neurons below the site of injury. We have demonstrated that these injuries substantially reconfigure the gene expression profiles of these target tissues, as well as change the excitability and synaptic integration of peripheral neurons that directly innervate target organs such as the bladder. We are currently working towards designing and deploying implantable stimulation devices to determine whether acute stimulation shortly after injury can prevent or ameliorate these changes, with the hope that by preserving the underlying neural architecture in uninjured tissue below the site of injury, prognosis for functional recovery or efficacy of therapeutic intervention will be greatly enhanced.

University of Missouri- Kansas City

Title: **Biomechanical Role and Strength of the Anterolateral Ligament in the Rotational Control of the Knee**

Author: *Amy Whitaker[b], Matthew Daggett[b], Barth Wright[b], Kyle Barner[b], Catherine Mayer[b], Loretta Laughrey[a], Jayda Jones[a], Anthony Pitter[a] and Thiagarajan Ganesh[a]*
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Abstract: The anterolateral ligament (ALL) is a ligament located between the femur and tibia. The ligament's importance and role has recently been discovered. It has been hypothesized by researchers that the ALL controls the rotational stability of the tibia/femur. In Anterior Cruciate Ligament (ACL) construction surgery, the proposed connection between ALL and ACL is often disregarded. Understanding the biomechanics of the knee joint and the role that the anterolateral ligament plays in rotational stability is crucial to making good surgical decisions. To study the contribution of the ALL to the rotational control of the knee, we designed and fabricated a testing apparatus capable of applying torsional and axial loads simultaneously to the knee using the BOSE ® testing machine. Then a testing protocol was designed and developed to evaluate the contributions of the anterior and posterior bands of the ALL to torsional stability of unembalmed (fresh frozen) cadaveric knees when positioned at various degrees of flexion. The results of the biomechanical analysis of the ALL from the mechanical testing and strain analysis using the non-contact strain measurement technique called Digital Image Correlation (DIC) analysis of strains are presented in this poster.

Title: **Multi-scale finite element analysis of: 3D multiplexed images to examine bone mechanotransduction and heterogeneous activation of β-catenin signaling in osteocytes.**

Author: *Loretta E. Laughrey, Nuria N. Lara-Castillo, LeAnn M. Tiede-Lewis, Sarah L. Dallas, Mark L. Johnson, Thiagarajan Ganesh, University of Missouri - Kansas City: School of Computing and Engineering, School of Dentistry*

Abstract: Wnt/β-catenin signaling in osteocytes is known to be necessary for bone formation. We have observed that cyclic compression loading of the mouse forearm results in heterogeneous activation of Wnt/β-catenin signaling in osteocytes at the mid-shaft of the ulna. This is in contrast to results from previous bone finite element (FE) models, which predict a homogeneous osteocyte response.

To develop a more detailed understanding of mechanotransduction between bone loading and Wnt/β-catenin activation, we have developed realistic, predictive computer FE models that incorporate tissue imaging data to compute bone strains in the lacunar walls in response to macroscopic bone loading and to correlate those strains with β-catenin activation in osteocytes.

In preliminary studies, Micro-CT scans and confocal fluorescence images of murine bones were collected. The images were converted into finite element models using the Materialise Innovation Suite®, and strain analysis was done using the FEBio Software Suite. β-catenin activation was assessed using fluorescence intensity values from a LacZ reporter. Pearson correlation was used to identify relationships between strain and β-catenin activation for individual osteocytes within the same bone sample.

Using this experimental process with bone from mice in three age cohorts, we will look for patterns in bone strain that may suggest new ways to mechanically induce better bone stimulation for fracture and osteoporotic patients.

Title: **Jaw Morphogenesis: a budding role of cranial neural crest in bone mineral density and microarchitecture**

Author: *Hammad Mumtaz, Kathleen Nguyen, Brianne Schmiegelow, LeAnn Tiede-Lewis, Maria Gonzalez, and Erin Bumann*

Abstract: Mandibular bone reconstruction is still the only option for craniofacial jaw defects, trauma, and cancer. An in-depth study of jaw bone development is crucial to develop non-surgical options. We can learn a lot about jaw bone formation and morphogenesis by studying vertebrates. Our lab uses two commercially-available avian with differences in jaw size and shape, quail and duck. We have shown previously that osteoblast lineage, cells involved in bone deposition, derived from cranial neural crest control bone mineral density (BMD) during development. The objective of this study was to determine species-specific differences in BMD and microarchitecture. Fertilized eggs of quail and duck were incubated, mandibles collected at stages of early bone deposition (HH36) or remodeling (HH39), and analyzed by osteomeasure or microcomputed tomography. Duck had an average BMD of about 140mg calcium hydroxyapatite (CaHA)/cm^3, which was significantly higher than the quail 115mg CaHA/cm^3 at HH39 ($p< 0.0005$). Quail had significantly more BMD volume from 90-120mg CaHA/cm^3, while duck had significantly more BMD volume from 180-270mg CaHA/cm^3 at HH39 ($p<0.01$). From HH36 to HH39 significant differences were seen in bone surface to bone volume (BS/BV) and trabecular thickness in both quail and duck ($p<0.05$). Trabecular width was significantly higher in duck compared to quail at HH39 ($p< 0.01$). No significant differences were found in BS/BV, trabecular number, or width between species of the same stage. Species-specific differences were evaluated but further studies are needed to determine the precise mechanisms by which these properties are controlled.

Title: **Regulation of myofilament force and loaded shortening by skeletal myosin binding protein-c**

Author: *Joel Robinett, Department of Medical Pharmacology and Physiology, University of Missouri*

Abstract: Skeletal Myosin Binding Protein-C (sMyBP-C) is a 125-140 kDa protein located on each half-thick filament in a region known as the C-zone. In this study we investigated mechanisms by which sMyBP-C regulates myofilament function using rat permeabilized skeletal muscle fibers. Slow-twitch skeletal muscle fibers were mounted between a force transducer and motor and Ca^{2+} activated to produce a range of forces. Contractile properties were measured including stretch-induced transient force overshoot, force development rates, and loaded sarcomere shortening. In slow-twitch fibers, protein kinase A (PKA) treatment (*i*) augmented phosphorylation of sMyBP-C, (*ii*) doubled the magnitude of the relative transient force overshoot at low Ca^{2+} activation levels, and (*iii*) increased force development rates at all Ca^{2+} activation levels. We also investigated the role that sMyBP-C phosphorylation state plays in loaded sarcomere shortening. We tested the hypothesis that MyBP-C acts as a brake to filament sliding within the myofilament lattice by measuring sarcomere shortening as thin filaments traversed into the C-zone during lightly loaded slow-twitch fiber contractions. Before PKA, shortening velocity decelerated as sarcomeres traversed from ~3.10 to ~3.00 μm. After PKA, sarcomeres shortened a greater distance and exhibited less deceleration during similar force clamps. Following sMyBP-C dephosphorylation, sarcomere length traces displayed a brief recoil (i.e., "bump") that initiated at ~3.06 μm during loaded shortening. Our results suggest sMyBP-C and its phosphorylation state regulate sarcomere contraction by a combination of cross-bridge recruitment, modification of cross-bridge cycling kinetics, and alteration of drag forces that originate in the C-zone.

Title: **Overlapping functions of matrix vesicles and extracellular vesicles in bone**

Author: *Kun Wang, LeAnn M. Tiede-Lewis, Lora A. Shiflett, Donggao Zhao,Jennifer L. Rosser, Andrew Keightley, Lynda F. Bonewald, Sarah L. Dallas*, University of Missouri Kansas City, Kansas City, MO.

Abstract: A rEcent paradigm in cell-cell communication involves cell shedding of extracellular vesicles (EV) (exosomes and microvesicles), which deliver their protein, mRNA and miRNA cargo to target cells to alter their function. Using Dmp1-mGFP mice expressing a membrane-GFP in osteocytes, we have shown that osteocytes shed EV, which can signal to osteoblasts, be released into the circulation or be deposited in bone ECM. Matrix vesicles (MV) are another type of ECM-bound vesicle that initiate mineralization in calcified tissues. Because EV and MV have similar characteristics it was proposed that MV are a specialized type of anchored exosome. To examine this, we compared the properties and function of MV and EV from IDG-SW3 cells, a model of osteoblast/osteocyte transition.

In Dmp1-mGFP mice, GFP+ve vesicles were observed in the ECM of bone and dentin. Confocal and electron microscopy showed that osteocyte-enriched SW3 cells release similar vesicles and confirmed ECM-bound MV containing mineral crystals. EV were isolated from SW3 cell culture supernatants and MV were isolated from the cell layer. Both EV and MV were enriched in mineralization-related proteins, alkaline phosphatase, annexin A5 and PHOSPHO1, with comparable levels of mRNAs for osteocyte-expressed genes *Dmp1, E11, Rankl,* and *HIF1α*. Treatment of undifferentiated SW3 cells with EV or MV from mature SW3 cells increased Dmp1-GFP and induced comparable changes in osteocyte gene expression (upregulation of *Dmp1* and *RankL;* no change in *E11/gp38;* downregulation of *Phex, Col1a1* and *TNAP*). Both EV and MV induced equivalent mineralization in SW3 cells. These data show that EV and MV from SW3 cells overlap in composition and function, supporting the concept that MV are a specialized exosome that nucleates mineralization and functions in cell-cell signaling in bone.