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Cover Image: *Portrait of a Young Man with a Book* by Agnolo Bronzino.

CONTENTS

Letter from the Founding Facilitator for Volume 4, Issue 1

Richard J. Barohn, MD

This is the first issue of the fourth volume of the RRNMF Neuromuscular Journal. In this issue we again have two challenging "What's on your Mind?" pieces by Drs. Freeman and Frey. They continue to keep us thinking about what is imperfect in our health care system and how we can improve it. The "New Stuff" section has a number of interesting research articles. Dr. Li and the Duke group conducted a survey of myasthenia gravis patients to determine what effect the COVID-19 pandemic had on them. Dr. Katyal and colleagues focus on myasthenia gravis as well but from another perspective. They collected data on comorbid events in the first year of immunotherapy treatment. Dr. Bhai and my colleagues prepared a manuscript describing a prospective study on the effects of testosterone and transcutaneous muscle stimulation on strength and muscle mass in myotonic dystrophy. I think this article may win a record for the longest gestation time between a study being done and publication. I can briefly tell you the story about this. In 1986 when I was a fellow at the Ohio State University, Seth Kolkin, the outgoing fellow, and I as the incoming fellow, completed this study with John Kissel and Jerry Mendell. We did present it at the American Academy of Neurology and the abstract was published, but for reasons I still cannot recall we did not write the manuscript up. I have been carrying my file of this study around for decades. Recently, in my move from Kansas to Missouri, I uncovered the file. I worked with Salman, John Kissel and Seth Kolkin to create this manuscript which I still believe has relevant findings. So, a 27-year gestation! In the "Clinic Stuff" category, Adam Reynolds and Seattle team report a novel DOK-7 mutation causing a limb-girdle phenotype of congenital myasthenic syndrome. Drs. Jajwa Al-Bustani and Zulfqar Hussain report a case of a steroid responsive acute inflammatory demyelinating polyneuropathy induced by an immune check point inhibitor. In the third "Clinic Stuff" article, Nahee Park and colleagues at the Medical College of Wisconsin report a 21-month-old child with spinal muscular atrophy with a BICD2 mutation rather than the typical SMN1 chromosome 5 mutation. In the "Meeting Stuff" section I am pleased to report on the annual KCMD (Kansas City Musculoskeletal Disorders) Symposium held in December 2022. The agenda for the meeting and the abstracts are included. The KCMD group consists of investigators from the University of Kansas Medical Center, the University of Missouri-Kansas City, Kansas City University, and the University of Missouri- Columbia.

The artwork on the cover is from the Metropolitan Museum of Art and is a portrait of a man by one of my favorite Italian renaissance artists, Agnolo Bronzino.

Rick

FDA should protect the American people, and Pharma should pay!

Joshua Freeman, MD

This article originally appeared in Dr. Freeman's blog, Medicine and Social Justice.
https://medicinesocialjustice.blogspot.com/

The Food and Drug Administration (FDA) regulates drugs, and, I guess, food, although I don't know much about what they do in that area. It also does not approve certain drugs because they are not classified as drugs, but rather "nutritional supplements" or in new jargon "nutriceuticals". This is odd, because such a classification does not make them either safe or effective. If they ARE effective, do the good that is claimed for them, then of course they could have other effects, which could be bad. If they are biologically active, they could be harmful. The only way they can be presumed safe is if they have no effect. Oh, well.

The FDA has been prominent for several things in recent years, most commonly regarding the approval (or not) of drugs to treat COVID, and often for demonstrating that proposed treatments, even those endorsed by high-level elected government officials, were not effective. It also made news (and this blog, FDA approves Alzheimer's drug against the recommendation of its scientific panel. Be very concerned, June 21, 2021) by its approval of the Alzheimer's drug, Aduhelm, against the recommendation of its committee of scientific experts (eventually Medicare, the largest payer, refused to routinely pay for it, although it will in some situations).

A recent article in the *New York Times,* F.D.A.'s Drug Industry Fees Fuel Concerns Over Influence, discusses the controversy over "user fees" that the agency charges drug and medical device makers to help fund its work. Well, "help" may be incorrect, because such fees account for 75% of its budget. This requires annual negotiation between the agency and the trade organizations for the industry, and those negotiations often lead to concessions to the manufacturers. At the least, it creates a situation in which it appears that the manufacturers, rather than the public, are the agency's clients. The *Times* notes that 'The user fee program traces its roots to 1992, when AIDS activists pressed the F.D.A. to hasten drug approvals. About a decade later, drugs moved through the pipeline more quickly, averaging about 10 months from roughly 19 months,' seen at the time as a big victory for AIDS patients. Of course, it is important to note that speedier approval is only a good thing if the drugs being approved work for their intended purpose; speedier (or any) approval is *not* a good thing if the drugs do not work, no matter how much people with AIDS or any other disease, or their advocates, or physicians or scientists or drug manufacturers, wish they did.

If the pharmaceutical and medical device industry are going to pay for the costs of running the FDA, they should be charged enough to ensure that adequate staff and time are available for thorough review of drugs. The agency would otherwise be funded by general tax revenue, and it seems entirely just that the industry that makes money from those approvals (an INCREDIBLE amount of money; the drug industry is regularly by far the most profitable in the US) should pay for them rather than the rest of us. What is wrong is for those payers to have any influence on how the agency operates, what it does, or certainly what drugs are or are not approved. It is an insane idea to think that they should have influence because "they are paying for it" as if it were a business deal, and yet this seems to be the perspective of some influential politicians, such as Sen. Richard Burr (R-NC). *'Mr. Burr, a business-focused conservative, complained that the program burdens companies with negotiating with the agency over the fees, which he predicted would rise even higher.'* They should rise as high as they need to in order to fund the agency and the industry should have zero input into their policy decisions (as, indeed, the tobacco industry apparently does not over the 1200 FDA employees in its tobacco division, although the division is entirely funded by user fees).

This issue with the FDA is one (important) example of how, when industries are unsuccessful in "persuading" the government (though large cash donations) to entirely privatize a public function, they seek control of it anyway. In some cases this is a win-win for the industry and the government: the industry not only gets effective control of policy but very large influxes of money from the government to their business, and also gets to deny complete responsibility since it is a "government program". (See, for example, Medicare Advantage and the DCE/REACH program, "Private Equity": Profiteers in nursing homes, Medicare Advantage, DCEs, and all of healthcare, September 16, 2022.) Of course, there is a lose-lose part of the equation that involves the other two parties: the sick people who need treatments that are both effective and affordable, and the rest of us who are funding these donations to corporate coffers. Guess which group, winners or losers, has more people? Guess which gives more money to politicians?

It is tempting, when the nation's people want something done right (like protecting them from unsafe and ineffective drugs) but do not want to pay more taxes to make it work,

to enact things like "user fees". This is certainly fairer; it is why, for example, semi-trailers pay higher highway taxes than cars -- because they travel so many more miles and are so much heavier they cause far more damage to the roads. (You used to see bumper stickers on them that announced how much, until, presumably, they realized, that the other folks driving on the highway had little sympathy and probably cheered and felt it wasn't enough!) Thus charging the pharmaceutical companies who make so much money on drugs to pay for the FDA makes sense and is the way it should be, as long as they have no influence on the process. But that lack of influence is what irks Mr. Burr, and the drug makers who fund him.

Obviously, Burr is wrong, and so is the current process. Of the two sets of interests – the health of the American people and the profits of Big Pharma, the first should be the sole responsibility of the FDA, and the money to fund it should come from the second. Pharma will still make an exorbitant amount, no matter how much they and Sen. Burr cry that they do not have enough clout in the process to make even more, and they will continue to spend far more on marketing than on research and development.

And this should be the process for all government agencies. Fund them to protect the people from the profits of the companies that benefit.

Medicare Advantage — Whose Advantage Is It?

Donald Frey, MD

This article originally appeared in Dr. Frey's blog, A Family Doctor Looks at the World.
https://afamilydoctorlooksattheworld.com/

". . .my experience was that it was fine unless you get sick, in which case they severely limit your options, including getting a second opinion. I quit as soon as I could. Do not get this plan unless you know you'll never need any kind of serious medical care."—Eva, a former Medicare Advantage patient, expressing her frustration with the program.

At long last, election season is over. The shouting, screeching, wild claims and outright B.S. of non-stop political commercials are gone—at least for a short while.

But if you're somehow missing all of that, I have great news. You can still turn on your TV and hear a litany of monotonous, mind-numbing exaggerations. You can still go to your mailbox and find it stuffed full of slick marketing materials.

Of course I'm talking about Medicare Advantage. Just call our toll-free number.

As a physician and an Old Guy myself (I mean *really old*—I'm nearly 71, for God's sake!) I have a real concern about the future of Medicare. It's been around since 1965. Congress passed it so older Americans wouldn't have to choose between forgoing health care and getting crippled physically, or receiving health care and getting crippled financially.

Like most legislation, it was far from perfect. But it's still been a godsend for millions of older Americans. There's a whole chapter about Medicare in my book, if you're interested. For now, let's just look at a portion of Medicare. The part you constantly see on T.V.

The part that's threatening to bankrupt the entire Medicare program.

From the outset, private insurance companies have made money off Medicare. Private carriers have served as "intermediaries." That is, they got paid to process the claims submitted to Medicare.

They made plenty of money doing this. It just wasn't as much as they wanted.

So the insurance industry had to find another way to get at all of those Medicare bucks. In 1997—after intense lobbying—the industry convinced Congress to pass a plan that allowed older Americans to enroll in private programs, rather than Traditional Medicare. Instead of paying for an enrollee's medical expenses directly, Medicare would instead turn over a fixed sum of money to a private insurer to "manage" the patient's care. They called it Medicare Advantage.

From the outset, any rational person could have seen this was going to be an expensive boondoggle, but we're not talking about rational people here. We're talking about Congress. Traditional Medicare had run an overhead (even with the claims processing being outsourced) of around 2-3%. Private insurers exceeded 10%. Even by third grade arithmetic standards, the numbers didn't add up.

And they still don't. Today, Traditional Medicare runs a 2% overhead. Advantage plans combined overhead and profit checks in at over 12%. That difference represents taxpayer dollars that don't pay for health care. Instead, they're eaten up by TV ads, marketing, and corporate bottom lines.

But money buys influence, and the insurance industry has plenty of both. And since its passage, Advantage plans have been marketed non-stop. They've become a gold mine for private insurers, but a multi-billion dollar drain on the Medicare Trust Fund.

But how can Advantage plans offer all of those "extras" like gym memberships, etc. and still be so profitable? Through the twin processes of "upcoding" and "care management" (which really means denying referrals and refusing to pay for treatment). Both are endemic in the Advantage world.

Upcoding works like this. The money the Medicare Trust Fund pays an insurer is based on the diagnoses listed for an individual patient. The more diagnoses, the greater the payment, *whether the patient actually receives any care for those diagnoses or not.* Through aggressive data mining, seniors are suddenly assigned diagnoses they've never heard of, never been treated for, and likely never will. But it adds big bucks to the insurer.

How widespread is this? According to the Office of the Inspector General, 4 of the 5 largest Advantage insurers are guilty of overbilling. Three have been charged with outright fraud.

Multiple whistleblower complaints have uncovered a scale of fraud that's unprecedented. In addition to the quote at the beginning of this post, further evidence reveals seniors have been lied to about what the plan covers, whether their doctor is included, and what treatments are available. That's right—I said outright lies.

But they'll be so convincing when you call that toll free number.

Estimates of how much all of this costs Medicare run upwards to $25 billion per year—money that would otherwise actually pay for care in Traditional Medicare.

But upcoding is only part of the story. Because Advantage plans are basically managed care products (unlike Traditional Medicare), patients are only allowed to receive care through a specific insurance-designated network—and pay through the nose if they go out of network. Claiming you didn't know the providers were out of network won't help. You'll still pay.

Think staying "in network" sounds simple? Think again. Some hospitals might be in network, but most of the doctors aren't. Sometimes the laboratory testing will be in network, but not the radiologists reading the X-Rays. For those expenses, you'll have to cough up the money yourself. And you probably won't find out until you get the bill.

And even if you stay within the network, testing, treatments, referrals, and even some admissions must first be approved by the insurer, resulting in long delays in care and often outright denials. A recent audit found that 18% of those denials were for treatments that Medicare was supposed to cover. And in each instance, the care was ordered by the patient's physician. It was the Advantage insurer who denied it.

One of the added financial drains from Advantage insurers is the fact that each year older Americans can sign up for a different plan. That's where the TV adds, mailings, and repeated badgering phone calls come in. It's high stakes marketing that gets thrown at Seniors year in and year out. And it's extraordinarily expensive.

"Ditch your traditional Medicare for our Advantage plan!" "No, ditch their Advantage plan for *our* Advantage plan!" "But ours gives you these benefits!" "But we give you *these* benefits?"

Often these products are sold on a commission basis, where the incentive for sales reps to shade the truth to older Americans, or simply outright lie, is enormous. And every phone call, every advertisement, every come-on, is paid from one source. Your tax dollars. And not a penny of it goes to pay for health care.

But don't some of those Advantage programs say they'll also pay for dental care? And vision? And home meals? And rides to the doctor? And trips to Mars on Elon Musk's spaceship?

Some do, some don't. And nobody pays for all of it (listen closely when that commercial says "they told me I *might* qualify for. . .)"

But shouldn't I want dental coverage? Of course. What Medicare Advantage plans do is take some of the thousands of extra dollars they receive from the Trust Fund and buy a policy that is available to everyone for $10-25 a month. Then they pocket the rest.

Forget the fact that I'm a doctor. I'm also a patient. And as a patient, I really don't give a damn about the bells and whistles in an insurance plan. I'm interested in something else.

Can I see any doctor I want, or just someone in my network (who may not be in the network tomorrow)? Can I get the tests my doctor orders, or do I have to wait until the insurance company approves them? Can I get admitted for treatment, or have to wait for the company's OK? How much will I ultimately be stuck paying in copays and deductibles after I've paid the premium—regardless of how low the premium seems at first (remember, there's no free lunch)?

According to an investigation by the Kaiser Family Foundation, insurers are now reaping twice the profit from Advantage plans as from their non-Medicare products.

This was never the intention of the Medicare program. And if it continues, Medicare's future is in serious jeopardy. Through clever (and expensive) marketing, nearly half of all Medicare recipients have signed up for Advantage plans. They're wildly popular.

That doesn't change the fact that these plans are bleeding the Trust Fund dry.

And to be honest, I'm also concerned about something other than just my own health care. I want Medicare to be there for my children and grandchildren.

According to news sources, some in Congress are demanding cuts in Medicare and an increase in eligibility age, claiming both are necessary to sustain the program.

Fine. But I hope those Senators and Representatives also realize that there are far greater savings in the $25 billion currently being lost through Advantage overpayments. If Congress has the courage to act, these dollars could quickly be recouped by moving the program back to the far more efficient Traditional Medicare, where overbilling would cease and care placed back in the hands of health care providers.

That would go a long way toward stabilizing the Medicare program. It could even pay for those dental benefits for *all* Medicare recipients.

If that were to happen, it would be a *true* advantage for all Americans.

Knowledge and perceptions of the COVID-19 pandemic among patients with myasthenia gravis: follow up survey

Yingkai Li, Ph.D;[1] Yohei Harada M.D;[1] Marjan Cobbaert, M.P.H;[2] Vern C. Juel, M.D.;[1] Lisa D. Hobson-Webb, M.D.;[1] Janice M. Massey, M.D.;[1] Karissa L. Gable, M.D.;[1] Shruti M. Raja, M.D.;[1] Natalia L. Gonzalez, M.D.;[1] Jeffrey T. Guptill, M.D. [1]

[1]Neuromuscular Division, Department of Neurology, Duke University Medical Center, Durham, NC 27710, USA

[2]Duke Clinical Research Institute, Durham, NC 27710, USA

ABSTRACT

Introduction: We previously conducted a survey study in April 2020 at the beginning of the SARS-CoV-2 (COVID-19) pandemic to understand how it affected patients with myasthenia gravis (MG). Since then, significant advances have occurred in the following areas: knowledge about the SARS-CoV-2 virus, infection risk mitigation, patient management, risks for MG patients, and a global vaccination program. We conducted a follow-up survey in February 2021 to assess how these advances impacted the care and perception of MG patients.

Methods: We conducted a prospective online survey study of MG patients at a large academic practice in the Duke Health System.

Results: Seventy-eight patients participated in the survey, including 55 from the previous survey and 23 newly identified patients. The top reported change in the interaction with healthcare providers was an increase in telemedicine visits (74%). Telemedicine visits' median satisfaction score (0-100 scale) was 74. Ninety-six percent of survey participants expressed concern about the pandemic, and nearly half showed anxiety based on the Generalized Anxiety Disorder-7 score. The top 3 concerns related to COVID-19 were getting hospitalized (62%), exacerbation (62%), and death (53%).

Discussion: Although the follow-up survey results were similar to the previous study, most patients switched from in-person clinic visits to telemedicine. The overwhelmingly large portion of patients continue to have concern and anxiety about the pandemic, but the patients with severe symptoms have higher anxiety scores.

Conclusion: This follow-up survey demonstrated the adjustment of MG patients to new methods of communication, the significant psychological impact of COVID-19 on them, and their good healthcare literacy.

Introduction

As of September 2021, more than 4.5 million people worldwide have died due to COVID-19.[1] Information during the pandemic has evolved more rapidly than any other health crisis in human history. There has been a need for real-world data regarding how the pandemic has affected the care of MG patients. In April 2020, we surveyed to understand how patients with MG were experiencing the COVID-19 pandemic, where they got their information, and how it affected their medical care. The measures they took to protect themselves.[3] To understand how changes in our understanding of COVID-19 have changed the care of MG patients and their perception of COVID-19, we conducted a follow-up survey in February 2021.

Methods

This is a prospective observational cohort study. Patients in the Duke Health System (Durham, North Carolina) were surveyed using REDCap. Details of our methods, eligibility criteria and statistical analysis were the same as described previously.[3]

Results

Survey timing and MG participant characteristics

The initial study approached 1,413 patients, and 75 patients completed the survey.3 Among the original 75 participants, 55 (71%) completed the follow-up survey. We also sent the survey to 937 patients who did not respond to our first survey invitation on February 10, 2021, and 23 of them consented and completed the survey, resulting in 78 total participants in the follow-up survey. Participant demographics in the initial and follow-up surveys were similar (Table 1).

Information sources regarding COVID-19

The top five sources of COVID-19 information were other federal government sources (the U.S. Center for Disease Control (CDC), Food and Drug Administration (FDA), Dr. Anthony Fauci (director of the National Institute of Allergy and Infectious Diseases), and the U.S. Surgeon General), local healthcare providers, state governments, television news, and websites (Figure 1), respectively. The top three most-trusted sources were federal government sources (73%), local healthcare providers (64%), and state government (52%). The most remarkable change was a 19% increase in participants who rated state government as

one of the most trusted sources of info. The top three least-trusted sources were unchanged from the initial survey:

- Facebook/Twitter/other social media (59%)
- Presidential news conferences and addresses (52%)
- Word of mouth from friends and family (40%) (Supplemental table 1)

Interaction with the healthcare system

Compared to the initial survey, participants reported that the use of telemedicine visits increased (44% vs. 74%), fewer patients had appointments postponed or canceled (72% vs. 64%), and messaging through the electronic health record was essentially stable (43% vs. 49%). The median satisfaction score (0-100 scale) for telemedicine visits increased from 67 to 74 (IQR: 61, 92.5). We further analyzed the telemedicine satisfaction score among different follow-up visits and found that the scores increased with time (Figure 3A).

Concern and anxiety level among survey participants

Ninety-four percent of survey participants reported being either very concerned (62%) or somewhat concerned (32%) about COVID-19. The top concerns among participants were getting hospitalized (62%), MG exacerbation (62%), and dying from COVID-19 (53%) (Figure 2). Nearly half (49%) of survey participants had Generalized Anxiety Disorder (GAD)-7 scores suggestive of anxiety (33% mild anxiety, 11% moderate anxiety, and 5% severe anxiety) (Supplemental figure 1A). Patients with moderate symptoms had significantly higher GAD-7 scores than patients with no or mild symptoms (Supplemental figure 1B). When comparing the GAD-7 score changes among different follow-up visits, we found that the score decreased with the visits (Figure 3D). In addition, the ADL and Qol-15r scores dropped with time (Figure 3B, C).

Discussion and Conclusion

This follow-up survey showed interesting changes between April 2020 and February 2021. There was an increasing trend for the use of telemedicine and an improvement in patient satisfaction scores for telemedicine visits. The implementation of telemedicine was investigated even before the pandemic. Multiple studies showed favorable outcomes for patient care by allowing better communication and reducing the burdens of travel, especially for those with chronic neurological diseases.[4, 5] However, it had not been put into routine practice, likely due to the lack of incentive to change practice and concerns about the legality of telemedicine.[6] Because of the need for social distancing and stay-home orders placed in many regions in the countries, telemedicine has been widely implemented as an efficient way to reduce the transmission of the virus in the United States. Its feasibility and effectiveness for treating various neurological diseases were also reported during the COVID-19 pandemic.[7] While the first survey showed that 44% of the first participants reported telemedicine visits, 74% of the second survey participants used it. Similarly, Mayo Clinic reported an increase in telemedicine use by 2,000% by June 15, 2020, compared to before the COVID-19 pandemic.[8] There was also increased satisfaction among patients with telemedicine; the median satisfaction score was increased from 67 (IQR 50-79) to 74 (IQR 61-92.5), and the average of the score was increased with different follow-up visits in our survey, presumably on account of rising familiarity of providers and patients with the use of video communication modalities over time.

On the other hand, there were essentially no interim changes in participants' concern for COVID-19 and anxiety levels. Sixty-two percent of participants were very concerned, and 32% were somewhat worried about COVID-19 in present survey. This is similar to our first survey, where 69% of patients were very concerned.[3] Regarding their GAD-7 scores, 49% of survey participants showed anxiety, a 10% increase from the initial survey. Within different follow-up visits, the GAD-7 scores decreased gradually, but the ADL and Qol-15r scores increased with time. Also, we continued to see the correlation between the severity of symptoms and anxiety that we reported in the first survey. The top 3 issues they were concerned about were unchanged between the two surveys. Compared to April 2020, when we first conducted the survey, our community has learned more about how COVID-19 can affect the disease course of MG and its risks for MG patients from published works of literature and direct clinical experiences.[9-17] Our participants' concerns are scientifically reasonable from a medical perspective. It is likely because our study participants obtain information from trustable resources such as the federal or state government, health care providers, and the Myasthenia Gravis Foundation of America. Multiple studies consistently showed that the risk of contracting COVID-19 for MG patients appears to be no higher than that of other general populations.[11, 14] Businaro et al. interviewed 162 patients with MG and identified COVID-19 infection in 3 patients confirmed by PCR testing and eight without test.[11] The prevalence did not differ from the general population in the Pavia district.[11]

On the other hand, COVID-19 can trigger the exacerbation of MG as seen in other infection.[2, 15, 16] In a large cohort study of 93 patients with MG who developed COVID-19 symptoms, Jakubíková et al. reported 15% of patients developed MG exacerbation.[14] In their study, 38%

had severe pneumonia, and 11% died due to COVID-19 infection. They also reported that MG patients treated with rituximab had a high risk of death due to COVID-19. At the same time, other immune therapies, including azathioprine, mycophenolate mofetil, and ciclosporin, did not appear to affect the course of COVID-19. They also reported unsatisfied condition of MG with lower forced vital capacity, previous long-term CS treatment, especially in higher doses, older age, and the presence of cancer as risk factors for severe COVID-19 symptoms. To provide further real-world evidence related to COVID-19 and MG, an international physician-reported registry, COVID-19 Associated Risk and Effects in MG (CARE-MG), was launched.[18] The preliminary data from the registry revealed that among a total of 91 patients with MG, MG is worsening or crisis requiring rescue therapy, including intravenous immunoglobulin, plasma exchange, or steroid, was observed in 36 (40%) of patients. Although they reported a more significant proportion of patients developing worsening MG symptoms than other studies, these data should be interpreted with caution given the potential selection bias toward poor outcomes reporting.[14, 18] Further studies will enable us to understand the relationship between COVID-19 and MG.

The limitation of the study is unchanged from the initial survey. It includes the meager participation from minorities more significantly impacted by the pandemic, and our clinic population has very few uninsured patients.

In conclusion, our follow-up survey suggests that many MG patients have well-adjusted to remote communication with providers in the pandemic era. Also, from the evaluation scales the MG patients took, they seem to have been well-educated about the risks and consequences of contracting COVID-19 over time, even though we didn't include the direct questions in our survey. Although these results are promising, the current survey probably could not obtain information from those with limited access to broadband internet, computers, or insufficient technology literacy. To improve the care of MG patients in the entire community, further studies should be conducted to investigate the impact of COVID-19 among patients with poor virtual healthcare access.

Corresponding Author
Jeffrey T. Guptill, MD
DUMC Box 3403
Duke University Medical Center
Durham, NC 27710, USA
Office: 919.684.5422
Jeffrey.guptill@duke.edu

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References

1. WHO Coronavirus (COVID-19) Dashboard [online]. Available at: https://covid19.who.int/.

2. Gummi RR, Kukulka NA, Deroche CB, Govindarajan R. Factors associated with acute exacerbations of myasthenia gravis. Muscle Nerve 2019;60:693-699.

3. Li Y, Emmett CD, Cobbaert M, et al. Knowledge and perceptions of the COVID-19 pandemic among patients with myasthenia gravis. Muscle Nerve 2021;63:357-364.

4. Geronimo A, Wright C, Morris A, Walsh S, Snyder B, Simmons Z. Incorporation of telehealth into a multidisciplinary ALS Clinic: feasibility and acceptability. Amyotrophic lateral sclerosis & frontotemporal degeneration 2017;18:555-561.

5. Backhaus A, Agha Z, Maglione ML, et al. Videoconferencing psychotherapy: a systematic review. Psychological services 2012;9:111-131.

6. Patterson V. Neurological telemedicine in the COVID-19 era. Nature reviews Neurology 2021;17:73-74.

7. Rosellini I, Vianello M, Ghazaryan A, et al. Virtual visits for chronic neurologic disorders during COVID-19 pandemic. Neurol Sci 2021;42:2607-2610.

8. Demaerschalk BM, Blegen RN, Ommen SR. Scalability of Telemedicine Services in a Large Integrated Multispecialty Health Care System During COVID-19. Telemedicine journal and e-health : the official journal of the American Telemedicine Association 2021;27:96-98.

9. Anand P, Slama MCC, Kaku M, et al. COVID-19 in patients with myasthenia gravis. Muscle Nerve 2020;62:254-258.

10. Bhagavan SM, Ramaswamy SB, Govindarajan R. A case report of COVID-19 in refractory myasthenia: Outcome with remdesivir and dexamethasone. Medicine (Baltimore) 2021;100:e25701.

11. Businaro P, Vaghi G, Marchioni E, et al. COVID-19 in patients with myasthenia gravis: Epidemiology and disease course. Muscle Nerve 2021;64:206-211.

12. Camelo-Filho AE, Silva AMS, Estephan EP, et al. Myasthenia Gravis and COVID-19: Clinical Characteristics and Outcomes. Front Neurol 2020;11:1053.

13. Delly F, Syed MJ, Lisak RP, Zutshi D. Myasthenic crisis in COVID-19. J Neurol Sci 2020;414:116888.

14. Jakubíková M, Týblová M, Tesař A, et al. Predictive factors for a severe course of COVID-19 infection in

myasthenia gravis patients with an overall impact on myasthenic outcome status and survival. Eur J Neurol 2021.

15. Saied Z, Rachdi A, Thamlaoui S, et al. Myasthenia gravis and COVID-19: A case series and comparison with literature. Acta Neurol Scand 2021;144:334-340.

16. Salik I, Rodhouse HB, Barst S. Myasthenic crisis in the setting of Coronavirus Disease 2019 (COVID-19). J Clin Anesth 2020;67:110001.

17. Rzepiński Ł, Zawadka-Kunikowska M. COVID-19 pandemic year in a sample of Polish myasthenia gravis patients: an observational study. Neurologia i neurochirurgia polska 2021.

18. Muppidi S, Guptill JT, Jacob S, et al. COVID-19-associated risks and effects in myasthenia gravis (CARE-MG). Lancet Neurol 2020;19:970-971.

Table 1. Characteristics of MG-COVID19 Survey Participants (N=78)			
	First batch follow-up	Second batch	Total
	N(%) or median (IQR)	N(%) or median (IQR)	N(%) or median (IQR)
N	55	23	78
Female sex (%)	26 (45%)	11 (47.8%)	37 (47.4%)
Age (y)	66(59-73.3)	65(55-71)	66(57.5-73)
Race			
Black or African American	1 (1%)	1 (4%)	2 (3%)
American Indian or Alaska Native	0 (0%)	0 (0%)	0 (0%)
Asian	0 (0%)	0 (0%)	0 (0%)
Native Hawaiian or Other Pacific Islander	0 (0%)	0 (0%)	0 (0%)
White	54 (99%)	22 (96%)	76 (97%)
Other	0 (0%)	0 (0%)	0 (0%)
Prefer not to respond	0 (0%)	0 (0%)	0 (0%)
Ethnicity (%)			
Hispanic or Latino	1 (1%)	1 (4%)	2 (3%)
Not Hispanic or Latino	54(98%)	22 (96%)	76 (97%)
Not reported	0(0%)	0(0%)	0(0%)
Antibody Status (%)			
AChR-Ab	17(31%)	5(22%)	22(28%)
MuSK-Ab	2(4%)	0(0%)	0(0%)
Other Ab	3(5%)	1(4%)	4(5%)
Seronegative	10(18%)	5(22%)	15(19%)
Did not know	23(42%)	12(52%)	35(45%)
Self-reported MG symptoms			
No symptom	14(25%)	10(43%)	24(31%)
Mild	27(49%)	7(30%)	34(44%)
Mod	11(20%)	0(0%)	0(0%)
Severe	3(5%)	6(26%)	9(12%)
Self-reported overall disease severity (0-100)	25(11-53)	19(9-53)	25(10.5-53)
MG-ADL (IQR)	4 (2-6.25)	6 (2-8)	4 (2-7.5)
MG-QOL15r (IQR)	5 (2-11.25)	8 (2.5-12.5)	6 (2-11.75)
MG treatments			
Pyridostigmine	32 (58%)	14 (61%)	46 (59%)
Corticosteroids	18(33%)	5 (22%)	23 (29%)
Other oral Immunosuppressives	28(51%)	12(53%)	40(51%)
Eculizumab	1(1%)	0(0%)	1(1%)
Rituximab	0(0%)	0(0%)	0(0%)
No treatment	3(5%)	4(17%)	7(9%)
Education			
High school diploma or equivalency (GED)	6(11%)	2(9%)	8(10%)
Associate degree (junior college) or vocational degree/license	9(16%)	0(0%)	9(12%)
Bachelor's degree	20(36%)	12(52%)	32(41%)
Master's degree	16(29%)	5(22%)	21(27%)
Professional (MD, JD, DDS)	3(5%)	4(17%)	7(9%)
None of the above	1(2%)	0(0%)	1(1%)
Total household income			
<$25,000	3(5%)	4(2%)	7(9%)
$25,000-<$50,000	6(11%)	2(9%)	8(10%)
$50,000-<$75,000	13(24%)	2(9%)	15(19%)
$75,000-<$100,000	8(15%)	3(13%)	11(14%)
$100,000-<$150,000	5(9%)	7(30%)	12(15%)
≥$150,000	13(24%)	6(26%)	19(24%)
Prefer not to respond	7(13%)	2(9%)	9(12%)

Abbreviation: Y-year, Ab-Antibody, MG-ADL –-myasthenia gravis activity daily life, MG-QOL15r –myasthenia gravis quality of life.

Figure 1. COVID-19 related concerns among survey participants.

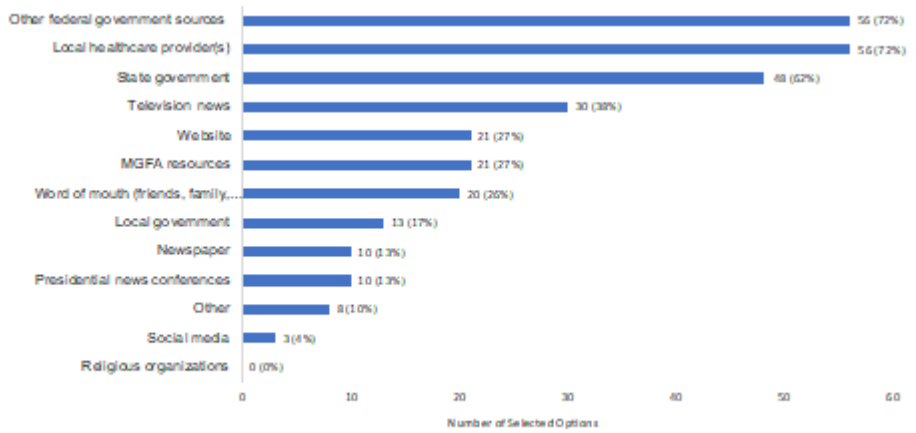


Figure 2. Top COVID-19 information sources used by responders

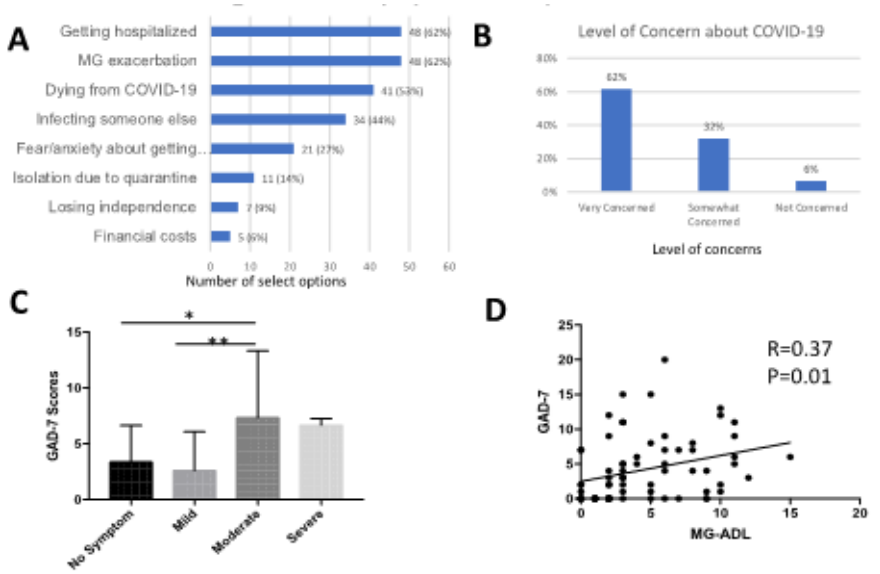
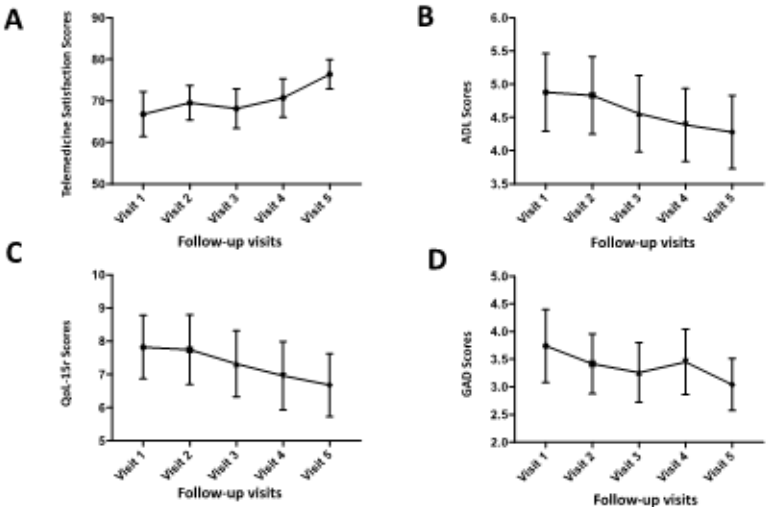
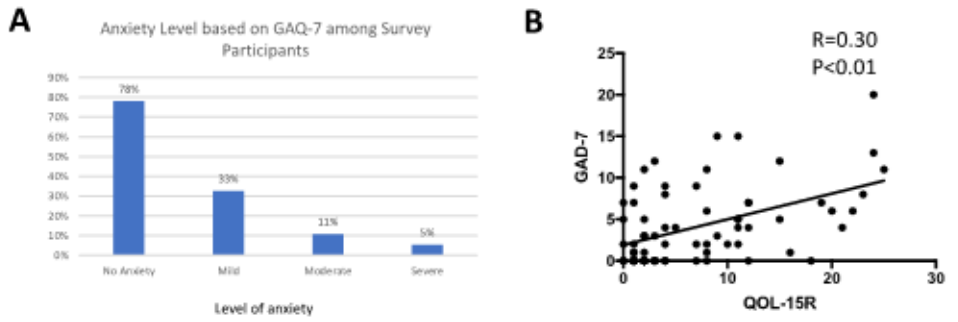


Figure 3. Scores change with follow-up visits.



A, Telemedicine satisfaction score changes with follow-up visits.
B, ADL score changes with follow-up visits.
C, QOL-15r score changes with follow-up visits.
D. GAD-7 score changes with follow-up visits.
Visit 1, the time of the first visit around April-2020; Visit 2, the time of the first visit around May-2020; Visit 3, the time of the first visit around June-2020; Visit 4, the time of the first visit around July-2020; Visit 5, the time of the first visit around October-2020.

Supplemental figure1. GAD-7 scores and QOL-15r scores.



A, GAD-7 scores among all participants.
B, GAD-7 scores and disease severity
C, Correlation between GAD-7 scores and MG-QOL-15r.
D. Correlation between GAD-7 scores and MG-ADL.
GAD-7 scores were grouped as No anxiety: 0-4; Mild anxiety: 5-9; Moderate anxiety 10-14; Severe anxiety: 15-21.
*, p value < 0.05.**, p value < 0.01.

Supplement table 1. Most trusted and least trusted sources of COVID-19 information according to survey participants*

Sources Rank	Most Trusted	Least Trusted
1	Other federal government sources (73%)	Facebook, Twitter, other social media (59%)
2	Your local healthcare provider(s) (64%)	Presidential news conferences and addresses (52%)
3	State government (governor, state health department) (52%)	Word of mouth (friends, family, etc.) (40%)
4	MGFA resources (27%)	Television news (29%)

*Total does not equal 100% patients selected 3 options.

Emergent Comorbid Events in First Year of Immunomodulatory Treatment in Adults with Generalized Myasthenia Gravis treated in a Neurology Clinic: A Retrospective Review

Nakul Katyal, MD,*,[1] Brian Blankenship, MD[1],Naureen Narula, MD,[†] Eric Grisham, BAS,* and Raghav Govindarajan, MD*

*University of Missouri School of Medicine, Columbia, MO; [†]Staten Island University Hospital, Staten Island, NY
[1] Contributed equally to this work.

ABSTRACT

Background: Current treatments for myasthenia gravis, including immunomodulatory therapies, are associated with significant comorbid events.

Method: Retrospective chart review of all adults diagnosed with generalized myasthenia gravis in our clinic over 5 years to evaluate potential associations between treatment regimens and emergent comorbid events according to system organ class. Comorbid events were categorized by affected system organ class as endocrine, neuropsychiatric, musculoskeletal, gastrointestinal, pulmonary, cardiovascular, urologic, infectious, and hematologic. MG treatment regimens at the latest available date during the 1-year follow-up were categorized by corticosteroid use and further stratified by medication class and combination therapy.

Result: A total of 68 patients were included in the analysis (corticosteroid group, n = 43; non-corticosteroid group, n = 25). We found no significant differences in the frequencies of comorbid events between patients whose regimens included corticosteroids and patients with corticosteroid-free regimens.

Conclusion: Patients who received corticosteroid treatments did not experience higher comorbid events than those receiving non-corticosteroid treatments.

Keywords: Corticosteroids; immunoglobulins; immunomodulation; myasthenia gravis; pyridostigmine

Introduction

Myasthenia gravis (MG) is an autoimmune disorder characterized by chronic fatigable skeletal muscle weakness caused by dysfunction at the neuromuscular junction that affects 14 to 20 per 100,000 individuals in the United States.[1,2] While traditional treatments for MG, including acetylcholinesterase inhibitors, immunosuppressants, and immunomodulatory drugs, can be effective and enable most patients to achieve a normal life expectancy, these treatments are associated with multiple side effects and comorbid events that can significantly impact patient quality of life, compounding the social and quality-of-life burdens imparted by the disease itself.[3-8] The risk of these events depends on medication choice, drug dosage, treatment duration, and patient characteristics.[9] The impact of MG treatments on the emergence of comorbid events within the first year of diagnosis is poorly understood. In this study, we conducted a retrospective chart review of patients with generalized MG diagnosed and treated for at least 1 year at a single neurology clinic to examine potential associations between treatment regimens and emergent comorbid events.

Materials and Methods

Participants

We retrospectively examined electronic medical records (EMRs) for all adult patients (≥18 years of age) diagnosed with MG from 2011 to 2015 with ≥1 year of follow-up at a single neurology clinic (University Hospital Neurology and Sleep Disorders Clinic at University of Missouri, Columbia, MO, USA). Institutional review board approval was obtained prior to study. IRB #2010001HS. Patients were included if their EMR reflected acetylcholine receptor (AChR) antibody detection, decremental response on low-frequency repetitive nerve stimulation, or abnormal jitter on single-fiber electromyography.[10] The population was limited to those with a Myasthenia Gravis Foundation of America class II to IV clinical classification. Patients with muscle-specific tyrosine kinase antibodies were not included. Patient and disease characteristics, preexisting conditions, medication history, treatment regimens, and comorbid events recorded after diagnosis were extracted from EMRs (Table 1).

Treatments

Our clinic used oral corticosteroid therapy for patients with AChR-positive and seronegative (ie, AChR- and muscle-specific tyrosine kinase-negative) MG. The initial dose (5 mg/day) was gradually increased as needed (low dose, 10–20 mg/day; high dose, 40–50 mg/day). Corticosteroid-sparing agents (mycophenolate 1000 mg twice daily or azathioprine 200 mg/day) were typically added unless there was a contraindication or the patient refused. Pyridostigmine (60 mg 3 times daily)

was used as symptomatic therapy for all patients unless they chose to discontinue because of poor tolerability or efficacy. Intravenous immunoglobulin (IVIg) was added to maintenance treatment (1000 mg/kg every 4 weeks) when the corticosteroid dose could not be increased to an effective level or used as a rescue treatment (1 mg/kg as needed) from worsening symptoms. IVIg could be used alone if the patient refused or was nonadherent to corticosteroid treatment.

Data Analysis

For this analysis, MG treatment regimens at the latest available date during the 1-year follow-up were categorized by corticosteroid use and further stratified by medication class and combination therapy. Patients included in the corticosteroid group were categorized into the following MG treatment cohorts: (1) high-dose corticosteroids only; (2) pyridostigmine and high-dose corticosteroids; (3) pyridostigmine, high-dose corticosteroids, and corticosteroid-sparing agents; or (4) IVIg, low-dose corticosteroids, and corticosteroid-sparing agents (**Table 1**). Patients included in the non-corticosteroid group were categorized into the following MG treatment cohorts: (1) pyridostigmine only; (2) pyridostigmine, corticosteroid-sparing agents, and IVIg as needed; (3) maintenance IVIg only; or (4) maintenance IVIg and pyridostigmine.

Comorbid events that occurred within 1 year of MG diagnosis were categorized by affected system organ class: endocrine, neuropsychiatric, musculoskeletal, gastrointestinal, pulmonary, cardiovascular, urologic, infectious, and hematologic. Once categorized, an independent, board-certified neurologist reviewed all events to ensure proper classification. The comorbid events were recorded in a systematic and uniform fashion in every clinic visit. Using the comorbid event categories, we compared event incidences between the corticosteroid and non-corticosteroid groups and the MG treatment cohorts.

Continuous and categorical data were summarized with descriptive statistics including means with standard deviations and frequencies. Categorical analyses were performed using GraphPad Prism software (version 7.0; La Jolla, CA, USA) and are presented as odds ratios (ORs) and 95% confidence intervals (CIs); differences were considered statistically significant at $P < 0.05$.

Results

Patient Characteristics

A total of 68 patients were included in the analysis (corticosteroid group, n = 43; non-corticosteroid group, n = 25). The demographic and disease characteristics of the groups were generally well balanced, although preexisting medical conditions and medication history were more common among patients in the non-corticosteroid group (**Table 1**). Most patients across both groups had preexisting conditions and were taking non-MG medications at baseline.

Emergent Comorbid Events

The patient EMRs contained 47 comorbid events (corticosteroid group, n = 27; non-corticosteroid group, n = 20) (**Table 2**). Two patients in each group experienced >1 event, and no patients reported >2 events. A smaller proportion of patients in the corticosteroid group experienced ≥1 event versus the non-corticosteroid group (25 of 43 [58.1%] vs 18 of 25 [72.0%], respectively); however, there was no statistically significant difference between groups in the overall incidence of comorbid events. Most comorbid events occurred in the neuropsychiatric, infections, endocrine, and musculoskeletal system organ class categories. The majority of endocrine emergent comorbid conditions in the corticosteroid group were likely to be steroid-related. Similar to the overall results, there was no statistically significant difference in the incidence of comorbid events within each system organ class between both groups.

There were no statistically significant differences between corticosteroid treatment cohorts for the other system organ classes. We found no statistically significant differences when comparing the frequencies of system organ class events in the non-corticosteroid treatment cohorts.

Discussion

The tolerability challenges of corticosteroids for the treatment of MG are well established, particularly over the long term, and are a motivation for combining corticosteroids with other immunomodulatory and corticosteroid-sparing agents.[3,11] Previous studies suggest that the development of corticosteroid-related comorbid events positively correlates with the duration of corticosteroid treatment and may emerge as late as 3 years after treatment initiation.[12,13] In our study, patients who received corticosteroid treatments did not experience higher comorbid events than those receiving non-corticosteroid treatments. Given the need for many patients with MG to maintain long-term corticosteroid use and the limited 1-year follow-up of our analysis, additional follow-up time in our population may have yielded a higher frequency of corticosteroid-related comorbid events.

We observed higher proportions of patients in the non-corticosteroid group that reported preexisting conditions and medication histories in comparison to the corticosteroid group, which may have influenced the frequency of emergent comorbid events in this group.

TABLE 1. Patient Demographics, Disease Characteristics, and Treatments

	Corticosteroid Group (n = 43)	Non-corticosteroid Group (n = 25)
Age at diagnosis, mean (SD), y	60.5 (16.8)	63.7 (15.7)
Male	28 (65.1)	11 (44.0)
Race		
White	42 (97.7)	23 (92.0)
Black	1 (2.3)	2 (8.0)
Antibody status		
AChR-positive	37 (86.0)	21 (84.0)
AChR- and MuSK-negative	6 (14.0)	4 (16.0)
Thymectomy status		
Yes	8 (18.6)	6 (24.0)
No	35 (81.4)	19 (76.0)
MGFA class		
Class II	20 (46.5)	13 (52.0)
Class III	13 (30.2)	7 (28.0)
Class IV	10 (23.3)	5 (20.0)
Preexisting conditions		
Pulmonary	22 (51.2)	15 (60.0)
Cardiovascular	31 (72.1)	25 (100.0)
Gastrointestinal	12 (27.9)	13 (52.0)
Endocrine	17 (39.5)	15 (60.0)
Hematologic	4 (9.3)	3 (12.0)
Medication history		
Antihypertensives	26 (60.5)	22 (88.0)
Antiplatelets	12 (27.9)	10 (40.0)
Inhalational bronchodilators	18 (41.9)	13 (52.0)
Lipid-lowering medications	15 (34.9)	10 (40.0)
Proton pump inhibitors	18 (41.9)	14 (56.0)
Diabetes medications	16 (37.2)	19 (76.0)
MG treatment regimen		
Pyridostigmine only	—	10 (40.0)
Pyridostigmine + corticosteroid	34 (79.1)	—
Pyridostigmine + CSA	—	11 (44.0)
Pyridostigmine + corticosteroid + CSA	4 (9.3)	—
Corticosteroid only	3 (7.0)	—
IVIg only	—	2 (8.0)
IVIg + pyridostigmine	—	2 (8.0)
IVIg + pyridostigmine + corticosteroid	2 (4.7)	—

Data are shown as n (%) unless specified otherwise.
AChR, acetylcholine receptor; CSA, corticosteroid-sparing agent; IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle-specific kinase; SD, standard deviation.

TABLE 2. Emergent Comorbid Events Stratified by System Organ Class

System/Complication, n (%)	Corticosteroid Group (n = 43)	Non-corticosteroid Group (n = 25)	Odds Ratio (95% CI)	P
Endocrine				
Glucose intolerance	3 (7.0)	—	7.28 (0.5182–186.9000)	0.128
Diabetes mellitus	1 (2.3)	—		
Pancreatitis	1 (2.3)	—		
Neuropsychiatric				
Peripheral neuropathy	1 (2.3)	5 (20.0)	0.41 (0.2100–1.2356)	0.132
Anxiety	2 (4.7)	—		
Cramp fasciculation	1 (2.3)	—		
Depression	1 (2.3)	—		
Headache	—	1 (4.0)		
Multiple sclerosis	1 (2.3)	—		
Sciatica	1 (2.3)	—		
Sleep apnea	—	1 (4.0)		
Stroke	—	1 (4.0)		
Musculoskeletal				
Osteoporosis	3 (7.0)	—	3.16 (0.3600–23.4960)	0.317
Bicep tendon tear	1 (2.3)	—		
Osteopenia	1 (2.3)	—		
Rotator cuff tear	—	1 (4.0)		
Gastrointestinal			0.57 (0.0342–9.5568)	0.690
Gastritis	—	1 (4.0)		
Peptic ulcer	1 (2.3)	—		
Cardiovascular			3.07 (0.1417–66.5951)	0.470
Hypertension	2 (4.7)	—		
Urologic			0.10 (0.0050–2.3400)	0.150
Acute kidney injury	—	1 (4.0)		
Renal stone	—	1 (4.0)		
Hematologic			0.18 (0.0342–9.5568)	0.690
Pancytopenia	—	1 (4.0)		
Pulmonary			0.10 (0.0050–2.3400)	0.150
Bronchitis	—	1 (4.0)		
COPD	—	1 (4.0)		
Infections				
Pneumonia	2 (4.7)	1 (4.0)	0.78 (0.2890–2.2950)	0.697
Urinary tract infection	—	3 (12.0)		
Chronic sinusitis	1 (2.3)	—		
Clostridioides difficile	—	1 (4.0)		
Disseminated varicella sepsis	1 (2.3)	—		
Epididymitis	1 (2.3)	—		
Necrotizing fascitis	1 (2.3)	—		
Otitis media	1 (2.3)	—		
Total*	27	20	0.42 (0.1324–1.3441)	0.144

*Patients may have experienced >1 emergent comorbid event.
CI, confidence interval; COPD, chronic obstructive pulmonary disorder.

The existence of comorbidities has contributed to poorer outcomes in patients with MG and is considered a risk factor for infections in most autoimmune disorders.[14,15] In our study, the decision to start a patient on a specific medication regimen was made after reviewing their preexisting comorbidity profile and choosing the best available medication option. Preexisting comorbidities and patient age can influence the identified medication related comorbid events [14,15]

Although generally well tolerated, treatment with pyridostigmine has been associated with muscarinic and nicotinic side effects,[3,6,11] and azathioprine can cause flu-like symptoms,[3] which may have compounded the corticosteroid side effects. The use of high-dose corticosteroids in combination with corticosteroid-sparing agents shortly after diagnosis to achieve early symptom control may have potentiated corticosteroid-related endocrine and neuropsychiatric effects.[9]

The prevalence of comorbid events associated with immunomodulatory treatment underscores the importance of oversight by an experienced neurological center.[9] In one study, outcomes for patients with MG were rated as significantly improved when care was provided by neuromuscular specialists versus other physician types despite clinical severity at onset.[16]

There are some limitations to our study. The findings of this retrospective study of EMRs at a single institution may not be reflective of outcomes in other care settings. Moreover, the analysis was limited to the first year after MG diagnosis, which may have excluded comorbid events that emerged after long-term treatment. Lastly, the small sample size and lack of control group limit the robustness of our findings.

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Corresponding Author

Nakul Katyal, MD, University of Missouri School of Medicine, 1020 Hospital Drive, Columbia, MO 65212; Tel.: 573-882-1515; Fax: 573-884-0070 (e-mail: katyaln@health.missouri.edu).

Disclosures

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References

1. Gilhus NE. Myasthenia gravis. *N Engl J Med*. 2016;375:2570-2581.

2. Howard Jr JF. Clinical overview of MG. Myasthenia Gravis Foundation of America; 2015. Available at: https://myasthenia.org/For-Professionals/Clinical-Overview-of-MG. Accessed June 29, 2020.

3. Farmakidis C, Pasnoor M, Dimachkie MM, et al. Treatment of myasthenia gravis. *Neurol Clin*. 2018;36:311-337.

4. Cutter G, Xin H, Aban I, et al. Cross-sectional analysis of the Myasthenia Gravis Patient Registry: disability and treatment. *Muscle Nerve*. 2019;60:707-715.

5. Nagane Y, Murai H, Imai T, et al. Social disadvantages associated with myasthenia gravis and its treatment: a multicentre cross-sectional study. *BMJ Open*. 2017;7:e013278.

6. Skeie GO, Apostolski S, Evoli A, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. *Eur J Neurol*. 2010;17:893-902.

7. Bacci ED, Coyne KS, Poon JL, et al. Understanding side effects of therapy for myasthenia gravis and their impact on daily life. *BMC Neurol*. 2019;19:335.

8. Juel VC, Massey JM. Myasthenia gravis. *Orphanet J Rare Dis*. 2007;2:44.

9. Sanders DB, Evoli A. Immunosuppressive therapies in myasthenia gravis. *Autoimmunity*. 2010;43:428-435.

10. Howard Jr JF. Electrodiagnosis of disorders of neuromuscular transmission. *Phys Med Rehabil Clin N Am*. 2013;24:169-192.

11. Gilhus NE, Tzartos S, Evoli A, et al. Myasthenia gravis. *Nat Rev Dis Primers*. 2019;5:30.

12. Palace J, Newsom-Davis J, Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. *Neurology*. 1998;50:1778-1783.

13. Rozsa C, Lovas G, Fornadi L, et al. Safety of long-term combined immunosuppressive treatment in myasthenia gravis--analysis of adverse effects of 163 patients. *Eur J Neurol*. 2006;13:947-952.

14. Gilhus NE, Nacu A, Andersen JB, et al. Myasthenia gravis and risks for comorbidity. *Eur J Neurol*. 2015;22:17-23.

15. Gilhus NE, Romi F, Hong Y, et al. Myasthenia gravis and infectious disease. *J Neurol*. 2018;265:1251-1258.

16. Dunand M, Botez SA, Borruat FX, et al. Unsatisfactory outcomes in myasthenia gravis: influence by care providers. *J Neurol*. 2010;257:338-343.

The Effects of Testosterone and Transcutaneous Muscle Stimulation on Strength and Muscle Mass in Myotonic Dystrophy

Salman Bhai MD[1,2*], Alexis Lizarraga MD[3*], Morgan McCreary PhD[2], Seth Kolkin MD[3], John Kissel MD, Richard J. Barohn MD[4]

*Denotes co-first authors

[1]Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX

[2]Neuromuscular Center, Institute for Exercise and Environmental Medicine,Texas Health Presbyterian, Dallas, TX

[3]Department of Neurology, University of Rochester, Rochester, NY

[4]Department of Neurology, University of Missouri, Kansas City, MO

ABSTRACT

In myotonic dystrophy type 1 (DM1) quadriceps weakness often results in severe functional limitations and genu recurvatum. To improve quadriceps strength the effects of isometric tetanic contractions using transcutaneous muscle stimulation (TMS) and testosterone enanthate (TE) were assessed. Ten DM1 subjects underwent unilateral TMS 6 hours per day for 14 days. The stimulated leg was randomly assigned and sham stimulation was done on the opposite leg by transcutaneous nerve stimulation. Muscle mass was estimated by cross-sectional area computed tomography and strength was measured by Cybex ergometry. Following the initial TMS period, 8 of 10 subjects were given a 12-week course of TE (3 mg/kg/wk) followed by 14 days of TMS. Neither TMS nor TE improved strength. Following 12 weeks of TE, there was an average increase in muscle mass of at least 8.7 +/- 1.6 cm^2. These findings are consistent with the TE—increased muscle mass in DM1 as measured by creatinine clearance and total body potassium. The dissociation of mass and strength following TE and the failure of exercise to improve strength may have significance in characterizing the muscle defect in DM1.

Keywords: myotonic dystrophy; testosterone; transcutaneous muscle stimulation; muscle mass

Introduction

Muscle atrophy in myotonic dystrophy (DM1) is characterized by depressed muscle protein synthesis in the absence of accelerated degradation (1). Since exercise and testosterone can enhance muscle protein synthesis, we examined their effects on quadriceps muscle mass in DM1 (2,3). This was achieved using testosterone enanthate (TE) and, as a form of stimulated exercise, transcutaneous muscle stimulation (TMS), both alone and in concert (4–6). Our objective was to focus on the thigh muscles since quadricep muscle weakness and atrophy often result in severe functional limitation in patients with DM1.

For DM1, past studies have had mixed results, with benefits primarily in the arena of symptomatic treatment for myotonia rather than weakness. Mexiletine is well-tolerated by DM1 patients and improves myotonia but does not improve muscle strength or 6-minute walk distance (7–11). In a smaller study of 12 patients, imipramine showed improvements in grip and percussion myotonia (12). Creatine monohydrate tested in DM1 patients did not show significant benefits in muscle strength, though myalgias improved in a minority of myotonic dystrophy type 2 patients studied (13–15). A small study of five patients with DM1 reported subjective and objective (vigorimeter grip strength, electromyogram myotonic discharges) improvements with selenium and vitamin E supplementation, though a larger double-blind placebo-controlled trial did not show benefit (16–18). Thus, there is a clear gap in therapeutics, though several interventions are in the pipeline.

TMS has been studied in DM1 patients in various forms with suggestion that strength and functional status can improve, though other studies suggest no benefit to strength and functional outcome (19–24). One study tested functional electrical stimulation cycle training in four DM1 patients and showed improved muscle strength and endurance (25).

Hormone therapy, including growth hormone, dehydroepiandrosterone sulfate, testosterone, and insulin-like growth factor 1, that can improve muscle protein anabolism have been of high interest, though results have not been promising in regards to improvement of muscle strength and functional status (26–33). Testosterone has been shown to improve muscle mass but not strength in DM1 (34). Hormone therapy has not been tested in conjunction with TMS in this patient population.

Given the possible synergistic effects of TMS and TE, we tested the impact of these modalities on improving muscle mass and strength.

Methods

The study was conducted at the Ohio State University (OSU) and was approved by the OSU institutional review board.

Patient Selection

Ten male DM1 patients volunteered and gave informed consent to participate in this protocol. The mean age of patients was 31 years (range 25 to 35 years). All patients had myotonia of hand grip and percussion myotonia of the thenar muscles, that was confirmed by needle electromyography. In addition, all patients had a family history for DM1 and exhibited extremity weakness. Genetic testing was not performed. In particular, all patients had knee extensor (quadriceps) muscle weakness: no better than Muscle Research Council (MRC) grade 4.

Evaluation of Muscle Mass

Computed tomography (CT) planimetry was used to measure the cross-sectional area of the thigh muscles. The level scanned was standardized to be one third the distance from the gluteal furrow to the level of minimum circumference just above the knee. The level was marked and maintained throughout the study. Measurements were made at the viewing console using a joystick device to guide a cursor around the area of interest. The contours of the quadriceps and the femur were outlined. Quadriceps muscle size was the area inside a curve separating the subcutaneous tissue from the muscle, minus the area of the femur.

Evaluation of Strength

Electromechanical isokinetic ergometry (Cybex dynamometer, Tumex Inc., Bay Shore, New York) was used to measure knee extensor strength at velocities of 60 degrees per second and 180 degrees per second. The strength was the mean of nine measurements taken during three individual settings over a two-day period.

Study Design

Phase 1. During the first phase, 10 male DM1 volunteers were admitted to the Clinical Research Center (CRC) and underwent unilateral TMS of the quadriceps muscle group. A Medtronic Respond II 3128 Neuromuscular Stimulator was utilized. A surface electrode was placed over the mid belly of the muscle. Prior to stimulation, the subject had each leg splinted to maintain the knees in 10 degrees of flexion. The stimulation was increased to produce a maximal, non-painful muscle contraction for 5 seconds every minute. Two three-hour stimulation periods were performed each day for 14 days. At the end of each treatment period, the knee splints and electrode were removed and the subjects were allowed to resume normal activity. The stimulated leg was splinted and had been randomly assigned. The control leg received sham stimulation at identical stimulation points over the anterior thigh during the same period with transcutaneous nerve stimulation (TENS). TENS produced no muscle contraction.

Phase 2. During the second phase, eight subjects (2 dropped out due to pain) had weekly intramuscular (IM) injections performed, in one leg (quadriceps muscles that were previously subjected to TMS), of TE, 3 mg/kg/week, for 12 weeks as outpatients.

Phase 3. During the final phase, eight subjects received TE and returned for a second CRC admission. Just as in phase 1, the same randomly assigned leg received 14 days of TMS while the control leg had sham stimulation with TENS.

Statistical methods

All analyses were performed in R (version 4.5.0) (35). All plots were generated using ggplot2 (36). Linear mixed effects models were fit using lmerTest (37).

A linear mixed effects model was fit to the change from baseline for each of the three phases. Subject-specific and subject-phase-specific random effects were included to capture the within-subject correlation amongst measurements for each leg. Covariates included in the mixed effects model included treatment group (non-stimulated side served as reference), phase (post-stimulation served as reference), and the interaction of treatment group and phase. T-test was used to compare changes in strength across the different phases.

Results

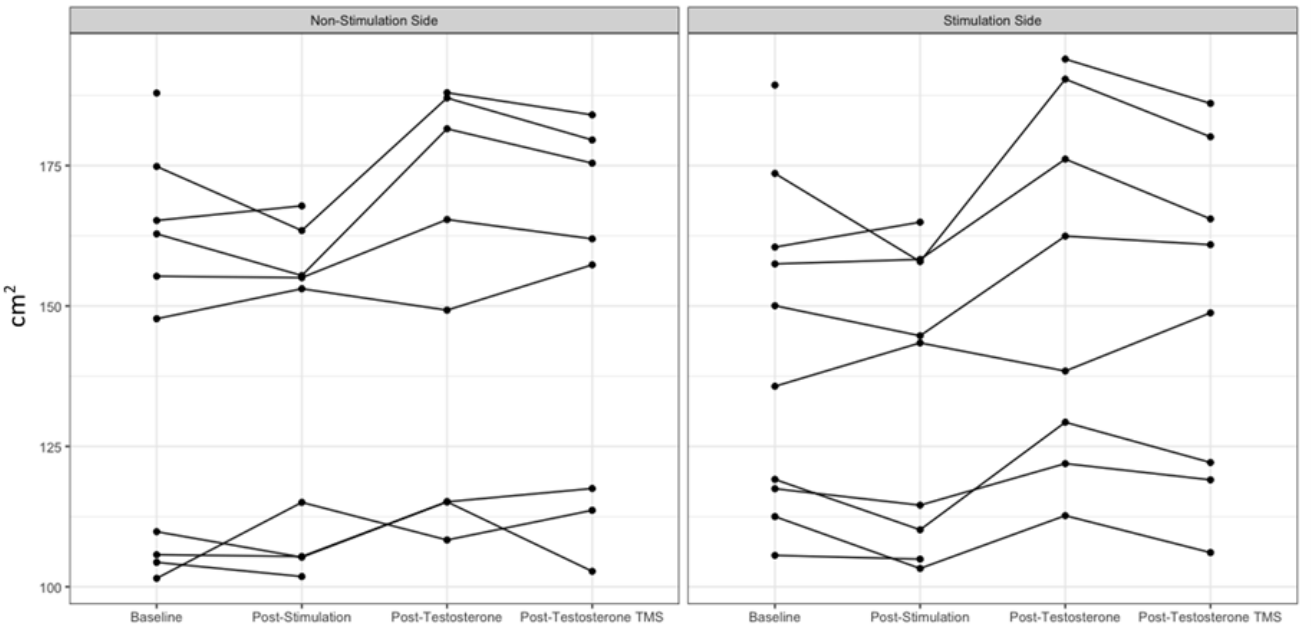
The data suggest that relative to baseline (table 1), there is increased muscle mass post-testosterone ($p = 0.008$) and post-testosterone with TMS ($p = 0.04$). However, relative to the non-stimulation side receiving TENS, there was no significant difference during any of the three phases in the stimulation side, relative to baseline. TMS did not produce any significant increase in muscle mass. Figure 1 shows the changes in muscle size across the study.

Relative to the previous phase (table 2), there was an increase in muscle mass post-testosterone ($p = 0.04$). However, relative to the non-stimulation side, there was no significant difference during any of the three phases, relative to the previous phase (Table 3). The combination of TE and TMS did not result in larger muscle mass compared to the TENS leg.

Table 1: Changes in muscle size relative to baseline

	Estimate (cm^2)	Std. Dev.	p-value	95% CI
Post Stimulation	-0.78	2.27	0.73	(-5.10, 3.49)
Post Testosterone	8.92	3.18	0.008	(2.79, 14.91)
Post Testosterone TMS	6.72	3.18	0.04	(0.58, 12.71)
Difference in Stimulated Side at Post Stimulation	-2.76	3.07	0.37	(-8.54, 3.02)
Difference in Stimulated Side at Post Testosterone	3.49	4.47	0.44	(-4.93, 11.92)
Difference in Stimulated Side at Post Testosterone TMS	1.12	4.47	0.80	(-7.31, 9.55)

Figure 1: Muscle size across the different phases of the study separated by non-stimulated and stimulated leg



Ten participants started the study and eight completed the study. The figure shows changes to muscle area (cm^2) across the study.

Table 2: Changes in muscle size relative to each phase

	Estimate (cm^2)	Std. Dev.	p-value	95% CI
Post Stimulation	-0.56	2.94	0.85	(-6.11, 5.00)
Post Testosterone	9.21	2.62	0.04	(1.12, 17.31)
Post Testosterone TMS	-1.65	4.29	0.70	(-9.74, 6.44)
Difference in Stimulated Side at Post Stimulation	-2.76	4.29	0.30	(-7.77, 2.24)
Difference in Stimulated Side at Post Testosterone	7.07	3.82	0.08	(-0.23, 14.36)
Difference in Stimulated Side at Post Testosterone TMS	0.39	3.82	0.92	(-6.91, 7.69)

Table 3: Changes in muscle size across phases between non-stimulated and stimulated leg

	Non-Stimulation Side	Stimulation Side
Median Baseline (IQR)	151.52 (57.91)	142.88 (41.87)
Median Post-Stimulation (IQR)	153.07 (50.03)	143.43 (47.76)
Median Post-Testosterone (IQR)	157.33 (67.80)	150.44 (52.26)
Median Post-Testosterone TMS (IQR)	159.65 (59.94)	154.85 (47.81)
Change from Baseline		
Post-Stimulation	-0.31 (7.15)	-2.93 (9.77)
Post-Testosterone	8.13 (6.25)	7.40 (9.46)
Post-Testosterone TMS	7.18 (7.42)	4.77 (8.36)
Change Relative to Previous Phase		
Post-Stimulation	-0.31 (7.15)	-2.93 (9.77)
Post-Testosterone	9.81 (14.57)	13.58 (11.47)
Post-Testosterone TMS	-3.93 (5.93)	-6.88 (5.93)

Table 4: Impact of TMS, TE, and TMS with TE on strength in the non-stimulated and stimulated sides

Phase	Angular velocity	TMS vs TENS	Mean (N/m)	Standard error
Phase 1 (TMS vs TENS)	60 degrees/sec	TMS	4.3	2.69
		TENS	5	3.79
	180 degrees/second	TMS	4.4	1.61
		TENS	3.5	1.71
Phase 2 (TE only)	60 degrees/sec	TMS	2.88	1.11
		TENS	1.75	1.95
	180 degrees/second	TMS	0.88	1.7
		TENS	0.88	1.26
Phase 3 (TE + TMS)	60 degrees/sec	TMS	5.13	1.3
		TENS	3.38	3.82
	180 degrees/second	TMS	2.63	1.93
		TENS	1.38	1.51

In phase 1, there was no significant difference in Cybex strength measurement at 60 degrees or 180 degrees per second between TMS and TENS (p= 0.5). In phase 2, TE treatment for three months showed no effect on muscle strength compared to baseline (p= 0.15). In phase 3, there was no increase in strength between TMS or TENS in combination with TE treatment (p= 0.15) (table 4).

Discussion

The results reported here are a direct demonstration of increased thigh muscle mass following three months of TE administration in DM1. This supports previous indirect evidence by Griggs et al. that TE can increase muscle mass as estimated by creatinine excretion and total body potassium as well as the evidence that TE increased muscle protein synthesis in DM1 (38). Testosterone, an anabolic steroid, has also been shown to increase muscle mass in trained athletes (39). Its mechanism of action in those athletes includes the induction of protein synthesis in skeletal muscle and an anticatabolic effect that counteracts the catabolic influence of endogenously stimulated glucocorticoids. Despite the increased muscle mass and congruence with other studies, we were unable to demonstrate that TE produced an increase in strength in association with increased muscle mass.

In distinction to the effects of TE in DM1, TMS failed to show the increase in thigh muscle size seen after nine days in paraplegic patients with upper-motor neuron lesions (40). Our randomized controlled trials showed no effect on strength after 14 days of TMS. Because of the duration of TMS and the limitations of ergonometric, a small effect on strength could have been missed.

Novel approaches to improving strength in DM1 are needed given the lack of disease-modifying pharmacologic

therapies. Nonpharmacologic approaches including TMS and strength training, or the combination of the two, have been tested not only in DM1, but also in a variety of other neuromuscular disorders (41–45). Neither strength training nor aerobic exercise appear to alter clinical outcomes measures in DM1, though there is conflicting data (46,47).

Other therapeutics are in development to achieve a disease modifying effect, such as antisense oligonucleotide targeted to the 3' untranslated region of the DMPK gene, which has shown promise in mouse models by improving strength (48). Adeno-associated virus type 6-mediated administration of miR30 RNAi hairpins to target the pathogenic *HSLAR* transgene in mice showed molecular and physiologic benefits (49). Various medications approved for other indications continue to be tested for use in DM1, including metformin and chloroquine. A small phase II study of metformin suggested a beneficial effect on mobility and gait abilities in myotonic patients (50). Chloroquine led to functional improvement in drosophila and mouse models of DM1 (51). In terms of improving symptoms, novel approaches are also being explored, such as with robotics and transcranial magnetic stimulation (52,53). With advances in robotics, exoskeleton assisted rehabilitation training was trialed in one patient with DM1 that showed improvement in strength and functional status (53).

Though this study did not show a benefit for strength of TE and TMS in isolation or in combination, research and drug development are actively being pursued. Several studies, including this, have shown that increasing muscle mass alone is not sufficient for improvement in strength, which may be related to defective function of diseased myofibers. TMS alone without an exercise program is not sufficient to lead to strength improvements. Future studies should not simply work to increase muscle mass but also increase strength.

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Corresponding Author
Salman Bhai, MD
5323 Harry Hines Blvd
Dallas, Texas 75390
Phone: 2143456521
Email: Salman.Bhai@UTSW.edu

References

1. Griggs RC, Jozefowicz R, Kingston W, Nair KS, Herr BE, Halliday D. Mechanism of muscle wasting in myotonic dystrophy. Ann Neurol. 1990 May;27(5):505–12.

2. Goldberg AL, Etlinger JD, Goldspink DF, Jablecki C. Mechanism of work-induced hypertrophy of skeletal muscle. Med Sci Sports. 1975;7(3):185–98.

3. Griggs RC, Halliday D, Kingston W, Moxley RT. Effect of testosterone on muscle protein synthesis in myotonic dystrophy. Ann Neurol. 1986 Nov;20(5):590–6.

4. Gould N, Donnermeyer D, Pope M, Ashikaga T. Transcutaneous muscle stimulation as a method to retard disuse atrophy. Clin Orthop. 1982 Apr;(164):215–20.

5. Williams JG, Street M. Sequential faradism in quadriceps rehabilitation. Physiotherapy. 1976 Aug;62(8):252–4.

6. Eriksson E, Häggmark T. Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery. A preliminary report. Am J Sports Med. 1979 Jun;7(3):169–71.

7. Fossati B, Sansone V, Panzeri MC, Zanolini A, De Ambroggi G, Cappato R, et al. Tolerability and safety of mexiletine in patients with myotonic dystrophy type 1 over time. J Neurol. 2009;256(SUPPL. 2):S128–9.

8. Sansone V, Panzeri M, Zanolini A, Fossati B, Meola G, Testa M, et al. Effects of mexiletine on cardiac parameters, muscle strength and myotonia in myotonic dystrophy type 1. Med Genet. 2009;21(3):435–6.

9. Logigian EL, Martens WB, Dilek N, Barbieri CA, Annis CL, Thornton CA, et al. Mexiletine is an effective antimyotonia treatment in myotonic dystrophy type 1. Neurology. 2010;74(18):1441–8.

10. Heatwole C, Luebbe E, Rosero S, Eichinger K, Martens W, Hilbert J, et al. Mexiletine for myotonic dystrophy type 1 (DM1): Results from a randomized, placebo controlled, clinical efficacy trial. Muscle Nerve. 2018;58(Supplement 1):S3.

11. Heatwole C, Luebbe E, Rosero S, Eichinger K, Martens W, Hilbert J, et al. Mexiletine in Myotonic Dystrophy Type 1: A Randomized, Double-Blind, Placebo-Controlled Trial. Neurology. 2021;96(2):e228–40.

12. Gascon GG, Staton RD, Patterson BD, Konewko PJ, Wilson H, Carlson KM, et al. A pilot controlled study of the use of imipramine to reduce myotonia. Am J Phys Med Rehabil. 1989;68(5):215–20.

13. Schneider-Gold C, Beck M, Wessig C, George A, Reiners K, Toyka KV, et al. Creatine monohydrate in DM2/PROMM: A double-blind placebo-controlled clinical study. Neurology. 2003;60(3):500–2.

14. Tarnopolsky M, Mahoney D, Thompson T, Naylor H, Doherty TJ. Creatine monohydrate supplementation does not increase muscle strength, lean body mass, or muscle phosphocreatine in patients with myotonic dystrophy type 1. Muscle Nerve. 2004;29(1):51–8.

15. Walter MC, Reilich P, Lochmuller H, Kohnen R, Schlotter B, Hautmann H, et al. Creatine monohydrate in myotonic dystrophy: a double-blind, placebo-controlled clinical study. J Neurol. 2002;249(12):1717–22.

16. Orndahl G, Sellden U, Hallin S. Myotonic dystrophy treated with selenium and vitamin E. Acta Med Scand. 1986;219(4):407–14.

17. Orndahl G, Rindby A, Selin E. Selenium therapy of myotonic dystrophy. Acta Med Scand. 1983;213(3):237–9.

18. Orndahl G, Grimby G, Grimby A, Johansson G, Wilhelmsen L. Functional deterioration and selenium-vitamin E treatment in myotonic dystrophy. A placebo-controlled study. J Intern Med. 1994;235(3):205–10.

19. Unlu Z. Myotonic dystrophy. Meandros Med Dent J. 2019;20(2):186–90.

20. Cudia P, Weis L, Baba A, Kiper P, Marcante A, Rossi S, et al. Effects of Functional Electrical Stimulation Lower Extremity Training in Myotonic Dystrophy Type I: A Pilot Controlled Study. Am J Phys Med Rehabil. 2016;95(11):809–17.

21. Roussel MP, Morin M, Gagnon C, Duchesne E. What is known about the effects of exercise or training to reduce skeletal muscle impairments of patients with myotonic dystrophy type 1? A scoping review. BMC Musculoskelet Disord. 2019;20(1):101.

22. Roussel MP, Morin M, Girardin M, Fortin AM, Leone M, Mathieu J, et al. Training program-induced skeletal muscle adaptations in two men with myotonic dystrophy type 1. BMC Res Notes. 2019;12(1):526.

23. Yoon SH, Baek JH, Leem J. Improved grip myotonia in a patient with myotonic dystrophy type 1 following electroacupuncture therapy: A CARE-compliant case report. Medicine (Baltimore). 2020;99(37):e21845.

24. Brady LI, MacNeil LG, Tarnopolsky MA. Impact of habitual exercise on the strength of individuals with myotonic dystrophy type 1. Am J Phys Med Rehabil. 2014;93(9):739–8.

25. Cudia P, Weis L, Baba A, Kiper P, Marcante A, Rossi S, et al. Effects of Functional Electrical Stimulation Lower Extremity Training in Myotonic Dystrophy Type I: A Pilot Controlled Study. Am J Phys Med Rehabil. 2016;95(11):809–17.

26. Nakazora H, Kurihara T. The effect of dehydroepiandrosterone sulfate (DHEAS) on myotonia: intracellular studies. Intern Med Tokyo Jpn. 2005;44(12):1247–51.

27. Moxley RT 3rd. Potential for growth factor treatment of muscle disease. Curr Opin Neurol. 1994;7(5):427–34.

28. Heatwole CR, Eichinger KJ, Friedman DI, Hilbert JE, Jackson CE, Logigian EL, et al. Open-label trial of recombinant human insulin-like growth factor 1/recombinant human insulin-like growth factor binding protein 3 in myotonic dystrophy type 1. Arch Neurol. 2011;68(1):37–44.

29. Vlachopapadopoulou E, Zachwieja JJ, Gertner JM, Manzione D, Bier DM, Matthews DE, et al. Metabolic and clinical response to recombinant human insulin-like growth factor I in myotonic dystrophy--a clinical research center study. J Clin Endocrinol Metab. 1995;80(12):3715–23.

30. Penisson-Besnier I, Devillers M, Porcher R, Orlikowski D, Doppler V, Desnuelle C, et al. Dehydroepiandrosterone for myotonic dystrophy type 1. Neurology. 2008;71(6):407–12.

31. Sugino M, Ohsawa N, Ito T, Ishida S, Yamasaki H, Kimura F, et al. A pilot study of dehydroepiandrosterone sulfate in myotonic dystrophy. Neurology. 1998;51(2):586–9.

32. Griggs RC, Kingston W, Herr BE, Forbes G, Moxley RT 3rd. Lack of relationship of hypogonadism to muscle wasting in myotonic dystrophy. Arch Neurol. 1985;42(9):881–5.

33. Al-Harbi TM, Bainbridge LJ, McQueen MJ, Tarnopolsky MA. Hypogonadism is common in men with myopathies. J Clin Neuromuscul Dis. 2008;9(4):397–401.

34. Griggs RC, Pandya S, Florence JM, Brooke MH, Kingston W, Miller JP, et al. Randomized controlled trial of testosterone in myotonic dystrophy. Neurology. 1989;39(2 Pt 1):219–22.

35. R Core Team. R: A Language and Environment for Statistical Computing. Vienna; 2021.

36. Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Spring-Verlag; 2016.

37. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models. J Stat Softw [Internet]. 2017 [cited 2022 Jun 4];82(13). Available from: http://www.jstatsoft.org/v82/i13/

38. Griggs RC, Kingston W, Herr BE, Forbes G, Moxley RT. Myotonic dystrophy: effect of testosterone on total body potassium and on creatinine excretion. Neurology. 1985 Jul;35(7):1035–40.

39. Haupt HA, Rovere GD. Anabolic steroids: a review of the literature. Am J Sports Med. 1984 Dec;12(6):469–84.

40. Buckley DC, Kudsk KA, Rose B, Koetting CA, Schlatter M, Miller CA. Transcutaneous muscle stimulation

promotes muscle growth in immobilized patients. JPEN J Parenter Enteral Nutr. 1987 Dec;11(6):547–51.

41. Voet NB, van der Kooi EL, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. Cochrane Database Syst Rev. 2019;12(100909747):CD003907.

42. Milner-Brown HS, Miller RG. Muscle strengthening through high-resistance weight training in patients with neuromuscular disorders. Arch Phys Med Rehabil. 1988;69(1):14–9.

43. Gianola S, Pecoraro V, Lambiase S, Gatti R, Banfi G, Moja L. Efficacy of Muscle Exercise in Patients with Muscular Dystrophy: A Systematic Review Showing a Missed Opportunity to Improve Outcomes. PLoS ONE. 2013;8(6):e65414.

44. Missaoui B, Rakotovao E, Bendaya S, Mane M, Pichon B, Faucher M, et al. Posture and gait abilities in patients with myotonic dystrophy (Steinert disease). Evaluation on the short-term of a rehabilitation program. Ann Phys Rehabil Med. 2010;53(6–7):387–98.

45. Gianola S, Castellini G, Pecoraro V, Monticone M, Banfi G, Moja L. Effect of Muscular Exercise on Patients With Muscular Dystrophy: A Systematic Review and Meta-Analysis of the Literature. Front Neurol. 2020;11(101546899):958.

46. Hebert L, Roussel M, Gagnon C, Duchesne E. P.26Strength training in myotonic dystrophy type 1: a promising therapeutic strategy. Neuromuscul Disord. 2019;29(Supplement 1):S49.

47. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. Cochrane Database Syst Rev. 2010;(1):CD003907.

48. Jauvin D, Chretien J, Pandey SK, Martineau L, Revillod L, Bassez G, et al. Targeting DMPK with Antisense Oligonucleotide Improves Muscle Strength in Myotonic Dystrophy Type 1 Mice. Mol Ther Nucleic Acids. 2017;7(101581621):465–74.

49. Bisset DR, Zavaljevski M, Stoltzman C, Chamberlain JR. AAV6-mediated systemic administration of MIR30-based RNAI hairpins impacts muscle disease in the hsalr myotonic dystrophy mouse model. Mol Ther. 2013;21(SUPPL. 1):S173–4.

50. Bassez G, Audureau E, Hogrel JY, Arrouasse R, Baghdoyan S, Bhugaloo H, et al. Improved mobility with metformin in patients with myotonic dystrophy type 1: a randomized controlled trial. Brain J Neurol. 2018;141(10):2855–65.

51. Bargiela A, Sabater-Arcis M, Espinosa-Espinosa J, Zulaica M, Lopez de Munain A, Artero R. Increased Muscleblind levels by chloroquine treatment improve myotonic dystrophy type 1 phenotypes in in vitro and in vivo models. Proc Natl Acad Sci U S A. 2019;116(50):25203–13.

52. Greene E, Thonhoff J, John BS, Rosenfield DB, Helekar SA. Multifocal Noninvasive Magnetic Stimulation of the Primary Motor Cortex in Type 1 Myotonic Dystrophy -A Proof of Concept Pilot Study. J Neuromuscul Dis. 2021;8(6):963–72.

53. Portaro S, Naro A, Leo A, Cimino V, Balletta T, Buda A, et al. Overground exoskeletons may boost neuroplasticity in myotonic dystrophy type 1 rehabilitation: A case report. Medicine (Baltimore). 2019;98(46):e17582.

A novel DOK7 mutation causing autosomal recessive limb-girdle congenital myasthenic syndrome

Adam S. Reynolds MD, Ava Y. Lin MD PhD, Leo H. Wang MD PhD

Department of Neurology, University of Washington Medical Center, Seattle, Washington

ABSTRACT

We report a case series of 5 Latino patients with limb-girdle pattern weakness; four patients are sisters, with one patient unrelated. Repetitive nerve stimulation showed a significant decrement in all cases. Targeted genetic testing for congenital myasthenic syndromes demonstrated a known DOK7 pathogenic mutation in each case, and in all five cases also revealed a novel DOK7 missense mutation in exon 7 with c.94G>A; providing strong evidence this mutation is pathogenic. DOK7-related congenital myasthenic syndrome often lacks significant oculobulbar involvement, and may present with limb-girdle weakness, mimicking limb-girdle muscular dystrophy.

Key words: Congenital myasthenia gravis, Limb-girdle weakness, Downstream of tyrosine kinase 7 (DOK7)

Introduction

Congenital myasthenic syndromes (CMS) are a growing group of rare, genetic disorders affecting neuromuscular transmission at the neuromuscular junction.[1] Clinical features of CMS are highly variable compared to auto-immune-mediated myasthenia gravis, and may not have significant oculobulbar involvement. Limb-girdle CMS is a subgroup that features prominent proximal and sometimes distal appendicular weakness, and can present similarly to limb girdle muscular dystrophy.[2] Limb-girdle CMS can be further subdivided into defects in glycosylation or MUSK-AGRN complex.

DOK7 CMS is characterized clinically by limb-girdle pattern weakness with childhood onset and variable clinical course ranging from pediatric respiratory failure to mild weakness.[3-8] Treatment of DOK7 CMS with anticholinesterase therapy usually results in worsening of symptoms [9,10]. One large case series reported that 94% of adult-onset CMS patients were initially misdiagnosed, most often with myopathy or seronegative myasthenia gravis, with an average delay of 26 years from symptom onset to diagnosis.[11] DOK7 CMS is highly treatable with β-agonist therapy, and to a lesser extent with 3,4-diaminopyridine (3,4-DAP). Herein, we describe a five patient case series with novel DOK7 mutation, characterize their clinical course, and discuss their relevant electrodiagnostic findings, and their subsequent response to albuterol therapy.

Case Report

Patient 1

A 22-year-old right-handed female referred for weakness since childhood. She endorsed normal motor development until age three, when she developed diffuse weakness. Her weakness fluctuated throughout the day and was slightly worse at the end of the day. She denied any falls, dysphagia, ptosis, diplopia, dark colored urine, weight loss, muscle atrophy, or history of respiratory failure.

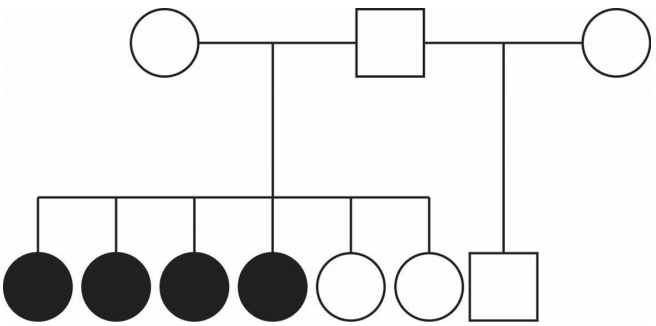


Figure 1. Pedigree of the proband. Circles are female, squares are male. Shaded shapes indicate symptomatic individuals.

She has five sisters and one half-brother. Three of her five sisters had a very similar pattern of weakness. None of her other family members including her parents, grandparents and other siblings were affected.

Her exam was notable for normal facial strength, no ptosis, no diplopia, normal sustained up-gaze, with moderate, diffuse and symmetric weakness, worst proximally.

Repetitive stimulation at 2Hz of the spinal accessory nerve showed a significant decrement. Pyridostigmine was then prescribed empirically at 60mg daily for possible congenital myasthenic syndrome, resulting in a dramatic worsening of weakness within two days, and was subsequently stopped. DOK7 congenital myasthenia was then suspected based on phenotype and worsening with pyridostigmine. Empiric treatment with albuterol 2 milligrams three times a day resulted in significant improvement.

After starting albuterol, she could use the stairs, raise her arms above her head and walk on her toes; none of which she could do prior. A Mayo Congenital Myasthenic Syndrome panel demonstrated a known pathological

heterozygous c.1124_1127dupTGCC frameshift mutation (p.Pro376ProfsX30), which is the most common disease causing mutation in DOK7-related CMS from European studies,[4,6,7,12] in addition to a novel missense c.94G>A mutation that causes a change from valine to methionine in codon 32.

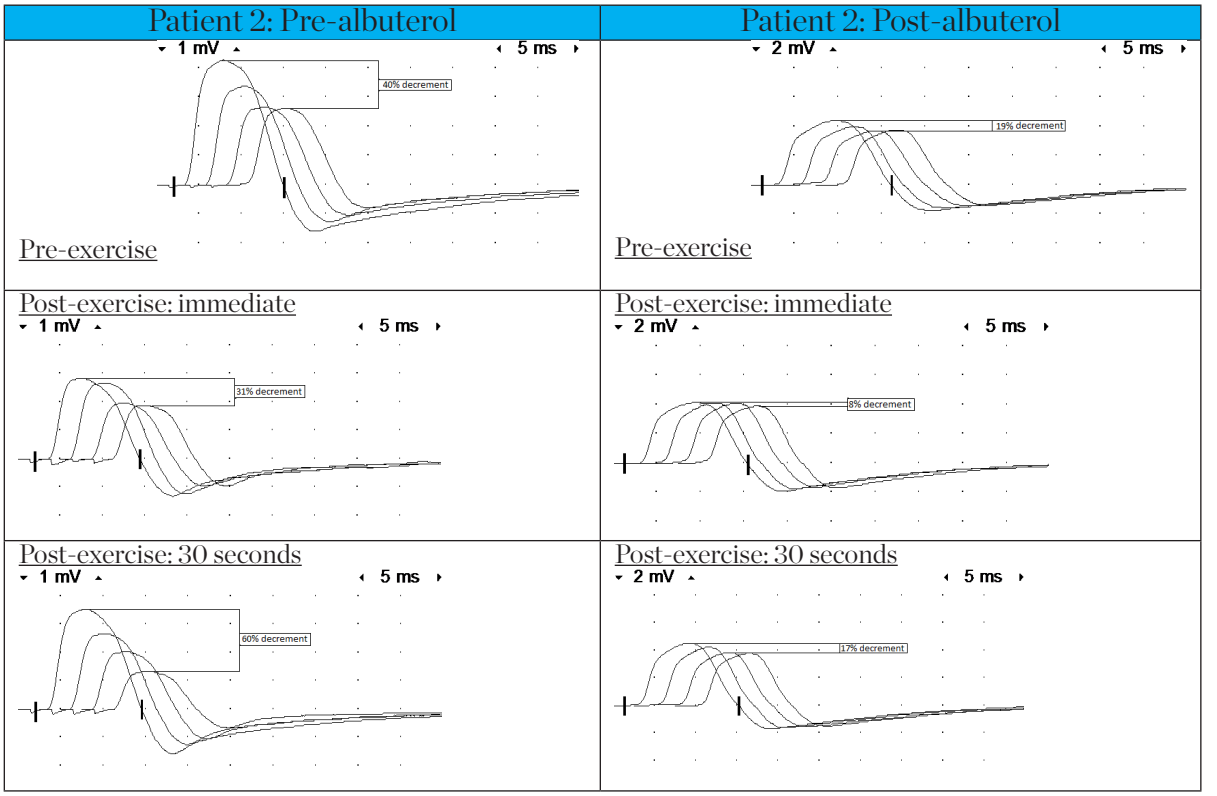
Patients 2-4

All presented similarly to the index patient, with proximal greater than distal weakness, minimal to no fatigable weakness, no oculobulbar involvement, and no history of respiratory failure. All improved substantially after albuterol. Targeted genetic testing for DOK7 in patients 2-4 was the same as in patient 1.

Table 1. Clinical characteristics and treatment responses of 5 patients with DOK7-related CMS in our kinship. SAN: spinal accessory nerve. Rep stim: repetitive stimulation.

	Pre-albuterol treatment							Post-albuterol	
Patient	Age at symptom onset	Ptosis/ diplopia	Distal/ proximal weakness	Fatiguable weakness/ temporal variability	Average grip strength (lbs)	Max % decrement on SAN/SAN max post exercise decrement	Pyridostigmine response	Albuterol response	Average grip strength (lbs)
1	3	-/-	-/+	-/-	N/A	19/23	Worsening	Excellent	72
2	4	-/-	+/+	-/-	25	40/60	N/A	Excellent	80
3	4	-/-	+/+	-/-	26	17/71	N/A	Excellent	35
4	4	-/-	-/+	-/-	57	10/56	N/A	Excellent	N/A
5	1	+/-	+/+	-/-	N/A	6/18	Respiratory arrest	Excellent	87

Figure 2. Patient 2, Electrodiagnostic data pre- and post-albuterol treatment. Note the electrodecrement is significantly lessened post-albuterol.



Patient 5

A 23-year-old right-handed male referred for weakness. A weak suck was noted at birth. He had mild respiratory insufficiency, diplopia and dysphagia. He walked at age 3, then became non-ambulatory at age 7. He was diagnosed with presumptive congenital myasthenia gravis at age 8. He was treated with empiric pyridostigmine which triggered respiratory failure and received no further pharmacologic treatment. No other family members had weakness, including a son, three half-brothers, and one half-sister.

His exam was notable for bilateral ptosis and moderate diffuse weakness, worst in the proximal upper extremities and distal lower extremities. Repetitive stimulation at 2Hz of the spinal accessory nerve showed a significant decrement.

Genetic testing with the Invitae Comprehensive Neuromuscular Disorders Panel yielded a known pathologic, nonsense, c.957 del (p.Lys320Serfs*136) DOK7 mutation, as well as the same novel missense c.94G>A DOK7 mutation seen in Patients 1-4.

Discussion

There are more than 100 different disease causing mutations in DOK7, with the most common mutation being the c.1124_1127dupTGCC frameshift mutation.[4,6,7,12] The 1124_1127dupTGCC has been shown to result in a truncated C-terminal region. The C-terminal truncations impair activation of MuSK in specific situations. Proteins with these mutations are able to induce MuSK activation during differentiation of C2C12 cells into myotubes but not when the myotubes are fully differentiated.[13]

In our cohort, we report a novel DOK7 mutation that is likely pathogenic. The clinical phenotype in our cohort is similar to that previously reported for a patient carrying mutation from alanine to valine on residue 33, immediately neighboring our unique mutation site. (Figure 3) Residues 30 to 33 were previously shown to be responsible for dimerization of DOK7 to allow appropriate interaction with phosphorylated MuSK to form the Agrin-MuSK-DOK7 complex.[5,14,15]

The novel DOK7 missense c.94G>A mutation seen in our cohort was reported as a variant of unknown significance. Algorithms predicting protein structure disagree on the mutation's effect. PolyPhen-2: benign, SIFT: deleterious. The gnomAD exome allele frequency is rare (0.00003) and 3 of the 4 reported alleles were in Latino patients, with a correspondingly higher frequency in this population. Our cohort of five patients are all Latino. Latino populations have lower rates of genetic testing than other racial groups, and this variant may be under reported as a result.

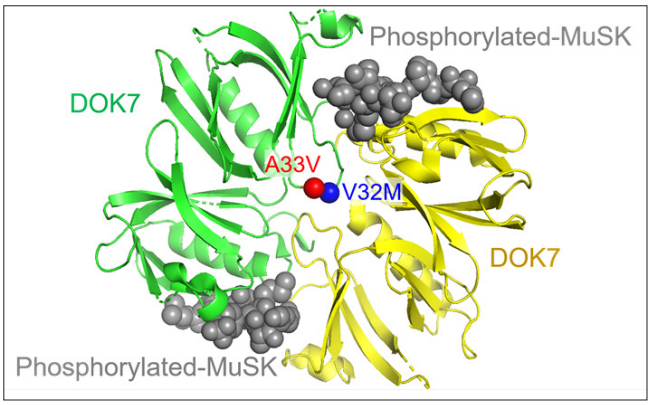


Figure 3. Structural depiction of novel point mutation of V32M (blue dot). DOK7 dimers with molecule 1 in green and molecule 2 in yellow. Phosphorylated tyrosine 553 of its binding partner MuSK identified in grey spheres. A33V is a previously reported disease-causing mutation. Both V32M and A33V are in a highly conserved loop and are key components of proper formation of the DOK7 dimers.[16] This region is proposed to be highly dependent on tight structural coupling and sensitive to steric sizes of these hydrophobic residues. Both mutations of V32M and A33V caused a mutation into a larger hydrophobic residue which would presumably cause steric clash and interference with dimerization.

The functional absence of DOK7 leads to the defective formation of the neuromuscular junction, which has been described in the biopsies of patients with DOK7-related CMS.[13,16-20] We hypothesize that the combination of DOK7 mutations that affect dimerization and activation of MuSK results in a more severe defect in neuromuscular junction formation in mature muscle fibers that results in a more severe CMAP decrement on repetitive nerve stimulation compared with mutations that result in truncation of the C-terminus alone.

Limb-girdle weakness with onset in early childhood may be a form of treatable CMS. Several tests have proven to be helpful in assisting diagnosis of DOK7-related CMS, including electro-microscopic structure of post-synaptic cleft,[21] trial of pyridostigmine,[9,22,23] and repetitive stimulation of proximal nerves. Therefore, repetitive nerve stimulation of proximal nerves should be performed in patients with early-onset unexplained limb-girdle weakness. In a case series of 179 patients with myasthenia gravis with significant decrement on low frequency repetitive nerve stimulation at rest, the average worsening of decrement of the spinal accessory nerve post exercise was 1.9%, and the maximal worsening seen in any patient was 12%.[24] In our series, the post exercise maximal absolute worsening of decrement in the spinal accessory nerve was equal to or more than 12% in patients 2, 3, 4 and 5. (Table 1) This unusually severe pattern of decrement could be related to the combination of the two mutations—missense c.94G>A mutation and nonsense

mutation in the C terminus (c.1124_1127dupTGCC,p.Pro376ProfsX30 or (c.957 del,p.Lys320Serfs*136).

To our knowledge, this is the first published CMS post-exercise repetitive nerve stimulation data, and further study of CMS electrodiagnostic data will be needed to confirm this trend. Our suggestion is that any patient with a post exercise maximal worsening of decrement greater than 10% be considered for CMS; this may have the greatest utility in differentiating CMS from sero-negative myasthenia gravis.

In summary, we report a five patient case series of patients with a limb girdle pattern of weakness, across two unrelated Latino families, all with homozygous DOK7 mutations with a known pathogenic DOK7 mutation in addition to a novel missense c.94G>A variant of unknown significance. This provides strong evidence that DOK7 c.94G>A is pathogenic. Furthermore, this case series illustrates the importance of performing repetitive nerve stimulation in patients with suspected limb-girdle muscular dystrophy, as decrement on repetitive stimulation may indicate limb-girdle CMS.

References

1. Barišić N, Chaouch A, Müller JS, Lochmüller H. Genetic heterogeneity and pathophysiological mechanisms in congenital myasthenic syndromes. Eur J Paediatr Neurol 2011;15:189–196. PubMed PMID: 21498094

2. Evangelista T, Hanna M, Lochmüller H. Congenital Myasthenic Syndromes with Predominant Limb Girdle Weakness. J Neuromuscul Dis 2015; 2(Suppl 2):S21–S29. PubMed PMID: 26870666; PubMed Central PMCID: PMC4746746.

3. Mahjneh I, Lochmüller H, Muntoni F, Abicht A. DOK7 limb-girdle myasthenic syndrome mimicking congenital muscular dystrophy. Neuromuscul Disord 2013;23:36–42. PubMed PMID: 22884442.

4. Srour M, Bolduc V, Guergueltcheva V, Lochmüller H, Gendron D, Schevell M, et al. DOK7 mutations presenting as a proximal myopathy in French Canadians. Neuromuscul Disord 2010;20:453–457. PubMed PMID: 20610155.

5. Müller JS, Herczegfalvi A, Vilchez JJ, Colomer J, Bachinski L, Mihaylova V, et al. Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes. Brain 2007;130:1497–1506. PubMed PMID: 17439981.

6. Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, et al. Clinical features of the DOK7 neuromuscular junction synaptopathy. Brain 2007;130:1507–1515. PubMed PMID: 17452375.

7. Ben Ammar A, Petit F, Alexandri N, Gaudon K, Bauché S, Rouche A, et al. Phenotype genotype analysis in 15 patients presenting a congenital myasthenic syndrome due to mutations in DOK7. J Neurol 2010;257:754–766. PubMed PMID: 20012313.

8. Andreetta F, Rinaldi E, Bartoccioni E, Riviera AP, Bazzigaluppi E, Fazio R, et al. Diagnostics of myasthenic syndromes: detection of anti-AChR and anti-MuSK antibodies. Neurol Sci 2017;38:253–257. PubMed PMID: 29030770.

9. Lozowska D, Ringel SP, Winder TL, Liu J, Liewluck T. Anticholinesterase Therapy Worsening Head Drop and Limb Weakness Due to a Novel DOK7 Mutation. J Clin Neuromuscul Dis 2015;17:72–77. PubMed PMID: 26583494.

10. Witting N, Vissing J. Pharmacologic treatment of downstream of tyrosine kinase 7 congenital myasthenic syndrome. JAMA Neurol 2014;71:350–354. PubMed PMID: 24425145.

11. Kao JC, Milone M, Selcen D, Shen X-M, Engel AG, Liewluck T. Congenital myasthenic syndromes in adult neurology clinic: A long road to diagnosis and therapy. Neurology 2018;91:e1770–e1777. PubMed PMID: 30291185; PubMed Central PMCID: PMC6251603.

12. Cossins J, Liu WW, Belaya K, Maxwell S, Oldridge M, Lester T, et al. The spectrum of mutations that underlie the neuromuscular junction synaptopathy in DOK7 congenital myasthenic syndrome. Hum Mol Genet 2012;21:3765–3775. PubMed PMID: 22661499.

13. Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, et al. Dok-7 mutations underlie a neuromuscular junction synaptopathy. Science 2006;313:1975–1978. PubMed PMID: 16917026.

14. Rodríguez Cruz PM, Palace J, Beeson D. Inherited disorders of the neuromuscular junction: an update. J Neurol 2014;261:2234–2243. PubMed PMID: 25305004.

15.. Bergamin E, Hallock PT, Burden SJ, Hubbard SR. The cytoplasmic adaptor protein Dok7 activates the receptor tyrosine kinase MuSK via dimerization. Mol Cell 2010;39:100–109. PubMed PMID: 20603078; PubMed Central PMCID: PMC2917201.

16. Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, et al. The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. Science 2006;312:1802–1805. PubMed PMID: 16794080

17. Yamanashi Y, Higuch O, Beeson D. Dok-7/MuSK signaling and a congenital myasthenic syndrome. Acta Myol 2008;27:25–29. PubMed PMID: 19108574; PubMed Central PMCID: PMC2859609.

18. Hamuro J, Higuchi O, Okada K, Ueno M, Iemura S-i, Natsume T, et al. Mutations causing DOK7 congenital myasthenia ablate functional motifs in Dok-7. J Biol Chem 2008;283:5518–5524. PubMed PMID: 18165682.

19. Inoue A, Setoguchi K, Matsubara Y, Okada K, Sato N, Iwakura Y, et al. Dok-7 activates the muscle receptor kinase MuSK and shapes synapse formation. Sci Signal 2009;2:ra7. PubMed PMID: 19244212.

20.. Selcen D, Milone M, Shen X-M, Harper CM, Stans AA, Weiben ED, et al. Dok-7 myasthenia: phenotypic and molecular genetic studies in 16 patients. Ann Neurol 2008;64:71–87. PubMed PMID: 18626973; PubMed Central PMCID: PMC2570015.

21. Anderson JA, Ng JJ, Bowe C, McDonald C, Richman D, Wollmann RL, et al. Variable phenotypes associated with mutations in DOK7. Muscle Nerve 2008;37:448–456. PubMed PMID: 18161030.

22. Klein A, Pitt MC, McHugh JC, Niks EH, Sewry CA, Phadke R, et al. DOK7 congenital myasthenic syndrome in childhood: early diagnostic clues in 23 children. Neuromuscul Disord 2013;23:883–891. PubMed PMID: 23831158

23. Kinali M, Beeson D, Pitt MC, Jungbluth J, Simonds AK, Aloysius A, et al. Congenital myasthenic syndromes in childhood: diagnostic and management challenges. J Neuroimmunol 2008;201–202:6–12. PubMed PMID: 18707767

24. Rubin DI, Hentschel K. Is exercise necessary with repetitive nerve stimulation in evaluating patients with suspected myasthenia gravis? Muscle Nerve 2007;35:103–106. PubMed PMID: 17024676.

Steroid Responsive Acute Inflammatory Demyelinating Polyneuropathy Induced by an Immune Check Point Inhibitor

Najwa Al-Bustani, MD; Zulfiqar Hussain, MD.

Department of Neurology, Neurological Institute, Cleveland Clinic Abu Dhabi, UAE

Key words: acute inflammatory demyelinating polyneuropathy, nerve conduction study, intravenous immunoglobulin, steroids, immune check point inhibitors.

Abbreviations:
AIDP, acute inflammatory demyelinating polyneuropathy, ICPs, immune check point inhibitors, NSCLC, non-small cell lung cancer, NCS, nerve conduction study, EMG, electromyography, IVIg, intravenous immunoglobulin.

Introduction

Lung cancer is the second most commonly diagnosed cancer and the leading cause of death worldwide,[1] with most cases presenting at an advanced, inoperable stage of the disease.

Platinum-based chemotherapy is the standard first line therapy for advanced non-small cell lung cancer (NSCLC), however immune check point inhibitors (ICPIs) are considered a major breakthrough in cancer treatment in the last decade. Pembrolizumab, a highly selective anti-PD-1 humanized monoclonal antibody, was approved by the United States Food and Drug Administration (US FDA) in October 2016 for previously untreated metastatic NSCLC patients whose tumors have high PD-L1 expression, as well as for metastatic NSCLC patients progressing on or after platinum-based chemotherapy.[2]

There is a greater focus on side effects of ICPIs [3] as well guidelines by the American Society of Clinical oncology to guide physicians in the management of side effects of ICPIs.[4] There are few case reports describing acute onset inflammatory demyelinating polyneuropathy with use of ICPIs.[5,6,7]

Case Presentation

We report a case of acute inflammatory demyelinating polyneuropathy (AIDP) that developed during platinum-based chemotherapy and pembrolizumab for NSCLC which raised diagnostic dilemma in regards to the cause of the neuropathy in the context of recent diagnosis of cancer, initiation of chemotherapy and ICPIs and a dramatic response to offered therapy.

A 60-year-old gentleman who was diagnosed with stage IV adenocarcinoma of the lung (PD-L1 expression 100%) with extensive infiltrative lymphadenopathy in the mediastinum, supraclavicular region and upper abdomen. He was started on carboplatin/pemetrexed/pembrolizumab on a 21-day cycle. He reported acute onset tingling and numbness in both feet about 10 days after his second cycle; he noted that his symptoms were slowly getting worse and reported them to his treating oncologist who attributed it to side effects of chemotherapy. When he came for his third cycle, it was noted that his symptoms continued to get worse and he started to experience lower extremity weakness and difficulty walking, which necessitated admission for further evaluation.

During his admission he was due for his third cycle which was given but without carboplatin and pemetrexed. Neurology service was consulted for evaluation of his progressive worsening of his symptoms. His neurological examination revealed normal mental status and cranial nerve examination. He had weakness of long forearm flexor muscles MRC grad 4/5, intrinsic hand muscle weakness 3/5, hip flexors 3/5, knee flexion/extension, dorsiflexion and plantar flexion 3/5. Deep tendon reflexes were absent and sensory examination showed reduced light touch and prick up to the level of his knees.

A nerve conduction study (NCS) was done and showed absent right median and ulnar sensory responses with reduced right superficial peroneal sensory nerve action potential (SNAP) amplitude and normal sural sensory study (sural sparring pattern). A motor nerve conduction study showed reduced right median, ulnar, and bilateral peroneal and tibial compound nerve action potential (CMAP) amplitudes with more than 50% partial conduction block of the right ulnar nerve in the forearm segment and tibial nerve in the popliteal fossa with more than 60% temporal dispersion. Distal motor latencies were prolonged along with reduced conduction velocity. Right median and ulnar minimum F-wave latencies were significantly prolonged and bilateral tibial F-waves were absent. Needle electromyography (EMG) showed active denervation changes and reduced recruitment in the leg muscles and the right abductor pollicis brevis with reduced recruitment only noted in the proximal muscles. Based on this study, it was concluded that findings are highly suggestive of acute inflammatory demyelinating polyneuropathy (AIDP). He had extensive blood tests which included a negative paraneoplastic panel and a magnetic resonance imaging (MRI) of the brain and spine with contrast which was negative for metastasis or leptomeningeal enhancement. Lumbar puncture was recommended, however, the patient declined.

He was started on intravenous immunoglobulin (IVIg) 0.4 g/kg/day for 5 days. Initially he had stabilization of his weakness and was discharged to acute rehabilitation center. However, after two weeks, he continued to have worsening of his lower extremity weakness with progression to involve the upper extremity. At this point it was decided to maintain the patient on biweekly IVIg infusion at dose of 1g/kg/day along with IV methylprednisolone 1g with every infusion. After receiving 2 cycles of IVIg and IV methylprednisolone, he had significant improvement of his deficit, as patient started to ambulate with help of a walker.

Discussion

In this case we postulate that AIDP was induced by pembrolizumab, and response to treatment was different than usual cases of AIDP, suggesting the possible role of steroids in such cases to improve prognosis and recovery.

This case also highlights the importance of clinical history in guiding diagnostic work up. Platinum-based chemotherapy is well known to cause neuropathy in a dose-dependent pattern and tends to be predominantly sensory neuropathy caused by damage of the dorsal root ganglion or its axons causing a sensory neuronopathy.[8] Electrodiagnostic study, in this case, played a major role in characterizing the type of neuropathy, as well as guiding the appropriate treatment.

Corresponding Author
Najwa Al-Bustani, MD
Cleveland Clinic Abu Dhabi
Abu Dhabi, United Arab Emirates.
albustn@clevelandclinicabudhabi.ae

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA A Cancer J Clin*. 2021;71(3):209-249.

2. Rihawi K, Gelsomino F, Sperandi F, et al. Pembrolizumab in the treatment of metastatic non-small cell lung cancer: a review of current evidence. *Ther Adv Respir Dis*. 2017;11(9):353-373.

3. Zimmer L, Goldinger SM, Hofmann L, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. *European Journal of Cancer*. 2016;60:210-225.

4. Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *JCO*. 2018;36(17):1714-1768.

5. Manam R, Martin JL, Gross JA, et al. Case Reports of Pembrolizumab-induced Acute Inflammatory Demyelinating Polyneuropathy. *Cureus*. Published online September 27, 2018. doi:10.7759/cureus.3371

6. de Maleissye MF, Nicolas G, Saiag P. Pembrolizumab-Induced Demyelinating Polyradiculoneuropathy. *N Engl J Med*. 2016;375(3):296-297.

7. Hashimoto R, Ueda T, Tsuji Y, Otsuka Y, Sekiguchi K, Matsumoto R. Successful treatment of Guillain–Barré syndrome-like acute inflammatory demyelinating polyneuropathy caused by pembrolizumab with a combination of corticosteroid and immunoglobulins: a case report. *Rinsho Shinkeigaku*. 2020;60(11):773-777. 001444

8. Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review: CIPN. *Ann Neurol*. 2017;81(6):772-781.

Non-5q Spinal Muscular Atrophy in a Patient With a Novel BICD2 Missense Variant

Nahee Park BS[1], Michael M Muriello MD[1,2], Donald Basel MD[1,2], Caroline A Kielczewski MS[1,2], Matthew Harmelink MD [1,3]

[1] Medical College of Wisconsin, Milwaukee, WI
[2] Dept of Pediatrics, Section of Medical Genetics, Medical College of Wisconsin, Milwaukee, WI
[3] Dept of Neurology, Section of Child Neurology, Medical College of Wisconsin, Milwaukee, WI

ABSTRACT

Variants in BICD cargo adapter 2 (*BICD2*) cause autosomal dominant spinal muscular atrophy with lower extremity dominance (SMALED2) which is characterized with lower extremity muscle weakness and atrophy. We describe a novel, heterozygous *BICD2* variant (c.1661T>C, [p.Leu554Pro]) in a 21-month-old female patient with a severe phenotypic presentation of the SMALED2 expression including arthrogryposis multiplex congenita, absent deep tendon reflexes, respiratory insufficiency, and cerebral depression. The variant p.Leu554Pro is located just outside of a domain that interacts with the motor protein KIF5A. The detailed neuro-phenotyping as well as a short clinical course is presented here expanding the understanding of *BICD2*-related disease.

Introduction

BICD2 is a highly conserved gene that encodes for protein bicaudal D homolog 2. BICD2 is a cargo adaptor protein involved in the dynein-dynactin motor complex which facilitates binding of transport vesicles and microtubules.[1] Since the identification of *BICD2*, more than 18 publications have been made that describe the disease spectrum.[2-19] Koboldt et al. (2020) published a review describing the phenotypic extremes of *BICD2* variants ranging from the absence of symptoms to arthrogryposis, respiratory failure, and death.[19] This wide range of symptoms suggests variable expressivity and incomplete penetrance.[13] Studies in mouse models suggest genetic modifiers act in a protective fashion which may explain the wide spectrum of symptoms in individuals with *BICD2*-related disease.[20,21]

Of the described *BICD2* variants, most are heterozygous missense variants, although cases of *BICD2* in-frame deletions have been reported.[15,17] Studies have found that individuals carrying heterozygous *BICD2* missense variants typically present with reduced fetal movement and benign or slowly progressive lower extremity muscle weakness and atrophy.[13]

Here we present a five-month-old female with a novel, heterozygous *BICD2* variant (c.1661T>C [p.Leu554Pro]) that has not been reported in ClinVar or GnomAD to date. The patient's presentation of severe arthrogryposis, absent deep tendon reflexes, preserved attentiveness, respiratory insufficiency, feeding difficulties, and cerebral depression is on the severe end of the typical SMALED2 expression spectrum, and we describe a new variant with phenotypic data.[19]

Clinical Report

A 21-month-old African American infant with a history of reduced fetal movement, severe arthrogryposis multiplex congenita (AMC), respiratory insufficiency, and cerebral depression (also known as neonatal encephalopathy) presented at 5 months of age for worsening symptoms. She was born prematurely at 34 weeks and 4 days (birth weight 1.8kg) via cesarean section due to severe joint contractures and hyperextended neck noted prenatally. Postnatal evaluation found severe arthrogryposis (elbows, wrists, fingers, shoulders adducted, knees extended, and hips abducted and dislocated), cervical spine hypermobility, and extreme hyperextension of the neck at rest. At birth, she had dysmorphic features including thin upper lip vermilion, prominent nasolabial fold, ectopic anus, triphalangeal thumb, overlapping fingers, a wide gap between the first and second finger, camptodactyly, and bilateral talipes equinovarus.

At birth, neurologic examination found minimal facial movement with slight myopathic appearance. Gag reflex along with upper and lower extremity stretch reflexes (biceps, supinator, triceps, patella, ankle, plantar) were absent. Muscle tone was low throughout. Overall, movements were limited to minimal distal but not proximal extremity movements. Cranial nerves were intact without tongue fasciculations, and she was responsive to stimuli throughout her extremities and torso. She was unable to be weaned from invasive support due to hypoventilation, and eventually required tracheostomy placement.

Electromyography (EMG) and Nerve Conduction Studies (NCS), done in the intensive care unit, demonstrated mildly prolonged peak latencies in the median and ulnar sensory nerves with profound reduced amplitudes in the right ulnar, right radial, and left sural nerves. The rest of the sensory nerve conduction studies were unremarkable. Additionally, the motor nerve conduction studies on the lower extremity demonstrated reduced amplitudes throughout with normal distal latencies and conduction velocities. Nee-

dle electrode examination of the left lower extremity demonstrated diffuse fibrillation potentials and positive sharp waves in all muscles; during activation, reduced recruitment of mildly increased amplitude motor unit potentials was seen in the tibialis anterior and gastrocnemius. This data is shown in Table 1A, 1B, & 1C.

Table 1: NCS and EMG Report

Nerve/ Sites	**Recording Site**	**Onset Latency (ms)**	**Peak Latency (ms)**	**NP Amplitude (uV)**	**PP Amplitude (uV)**	**Segments**	**Distance (cm)**	**Onset Velocity (m/s)**
Right Median – Index Finger								
Wrist	Index	3.28	4.17	35.6	54.6	Wrist-Index	5	15.2
Right Ulnar – Dig V								
Wrist	Digit V	2.86	3.13	0.56	3.9	Wrist – Digit V	2.4	8.4
Right Radial – Wrist								
Forearm	Wrist	1.51	1.82	3.5	4.1	Forearm – Wrist	3	19.9
Left Sural								
Calf	Lateral Malleolus	1.61	1.93	0.74	11.8	Calf – Lateral Malleolus	3.4	21.1
Left Superficial Fibular (Peroneal)								
Lateral Leg	Ankle	1.72	2.29	75.3	47.9	Lateral Leg – Ankle	3	17.5
Left Medial Plantar								
Medial Sole	Medial Malleolus	1.20	1.56	13.2	10.2	Medial Sole – Medial Malleolus	4.6	38.4

Table 1A: Sensory Nerve Conduction Studies: Demonstrated low amplitudes in the right ulnar, right radial and left sural nerves. Various sides were done to avoid the extremities with the worst contractures as well as support apparatus given the patient was in the ICU and maximize anatomical placement of stimulator and recording electrodes; however, given her overlapping fingers and wrist deviation this was difficult.

Nerve/Sites	Recording Site	Distal Latency (ms)	Amplitude (mV)	Distance (cm)	Velocity (m/s)
Right Median – Abductor pollicis brevis					
Wrist	Abductor pollicis brevis	1.77	2.2	2	
Elbow	Abductor pollicis brevis	3.07	2.5	5	38.4
Right Ulnar – Abductor digiti minimi					
Wrist	Abductor digiti minimi	3.54	1.8	2.6	
Below Elbow	Abductor digiti minimi	4.48	0.7	4.5	48
Above Elbow	Abductor digiti minimi	3.54	0.8	4.5	48
Left Fibular (Common Peroneal) – Extensor digitorum brevis					
Ankle	Extensor digitorum brevis	1.61	1.4	2.5	
Fibular Head	Extensor digitorum brevis	4.17	1.0	7	27.4
Left Tibial – Abductor hallucis					
Ankle	Abductor hallucis	2.5	1.5	2.4	
Knee	Abductor hallucis	5.10	1.1	7	26.9

Table 1B: Motor Nerve Conduction Studies: Reduced amplitudes throughout all muscles for age were seen with normal conduction velocities.

EMG Summary Table									
	Spontaneous/Insertional					Morphology			Recruitment
Muscle	Insertional Activity	Fibrillation	Positive Sharp Waves	Fasciculations	Other	Amplitude	Duration	Polyphasia	Pattern
L. Tibialis Anterior	Normal	1+	None	None	None	1+	Normal	Normal	1-
L. Gastrocnemius (Medial Head)	Normal	1+	None	None	None	1+	Normal	Normal	1-
L. Vastus medialis	Normal	1+	None	None	None	Normal	Normal	Normal	1-
L. Adductor Longus	Normal	1+	None	None	None	Normal	Normal	Normal	1-
L. Iliopsoas	Normal	1+	None	None	None	Normal	Normal	Normal	1-

Table 1C: Needle Electrode Examination: During the needle electrode evaluation, diffuse fibrillation potentials were seen as well as reduced recruitment with large amplitude motor unit potentials in the tibialis anterior and gastrocnemius with other muscle unit morphology being normal.

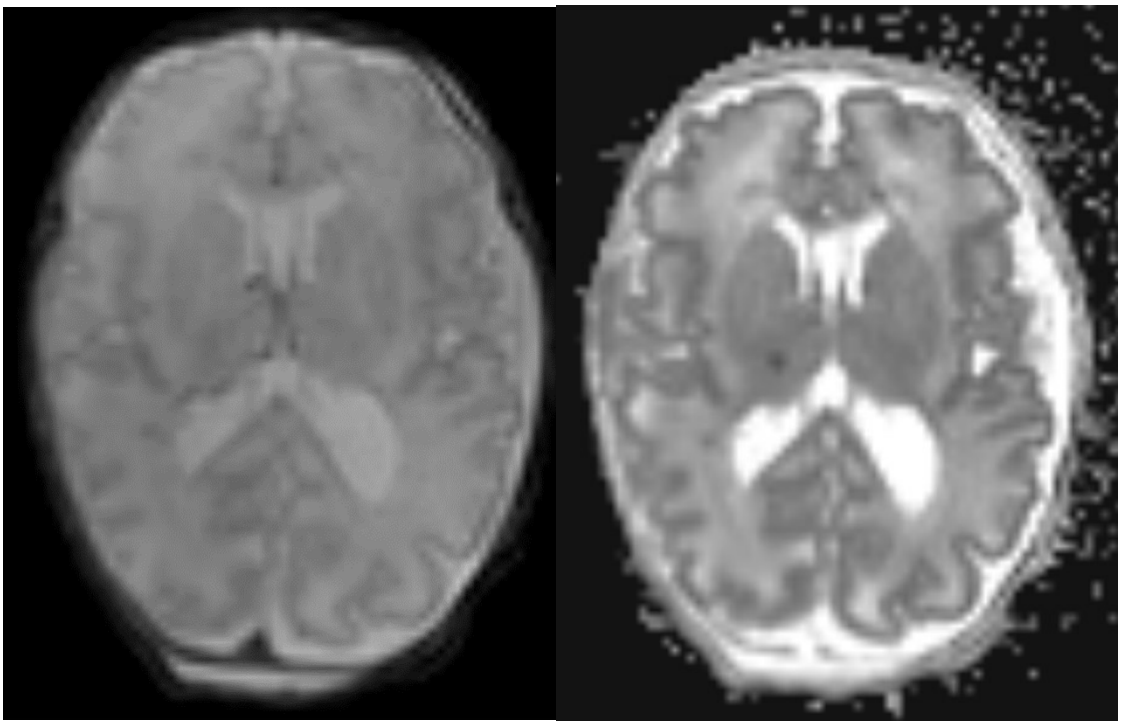


Figure 1: MRI Brain (obtained at age 1 day old) demonstrating mild ventriculomegaly as well as the punctate foci of restricted diffusion. The punctate foci were not felt to be significant to indicate another etiology or birth related injury.

The patient's brain magnetic resonance imaging (MRI) revealed punctate lesions in the right thalamus and left caudate with mild enlargement of the left lateral ventricle (Figure 1). The punctate lesions were thought to be clinically insignificant due to their small size.

The patient underwent genetic analysis which revealed a heterozygous, missense variant in *BICD2* (NM001003800.1, c.1661T>C, p.Leu554Pro). This variant was not present in the ClinVar, GnomAD, or Pubmed databases. Follow up familial testing did not identify the variant in the patient's mother. The father was not available for analysis and his clinical history was unable to be obtained. This variant was classified as a variant of uncertain significance according to ACMG guidelines.[22] There were no clinically relevant copy number variants (CNVs) or structural changes on karyotype. A compilation of further testing done did not reveal significant findings as shown in Table 2.

Supplemental Clinical Report

The patient's clinical progression was tracked with age. At 21-months-old, she had made some improvement without any regressions. Per mother, the patient was able to flex and extend her left elbow but could not move her right elbow. Her contractures were less fixed with slight improvement compared to 1 year prior. However, the patient's motor tone, motor strength, reflexes, and respiratory status were unchanged. Additionally, she started to make non-specific babbling but did not advance cognitively beyond that stage of language. She did not have any history of seizures. Between her 21st and 26th month, she started to say "Hi" without any other developmental advancement despite frequent therapy services.

Methods

Clinical Exome sequencing (WES) assay was used. Next Generation Sequencing (NGS) technologies were used to cover the coding regions of targeted genes. Genomic DNA was extracted from the patient's specimen, captured using Agilent Clinical Research Exome hybridization probes, and sequenced using Illumina's Reversible Dye Terminator (RDT) platform NovaSeq 6000 using 150 by 150 bp paired-end reads (Illumina, San Diego, Ca, USA). Median coverage of the target region was 120X with 98% of target bases covered by at least 20 reads. Data analysis and interpretation were performed using an internally developed software Titanium-Exome. The identified variant was confirmed by targeted Sanger sequencing.

Table 2: Compilation of further testing is shown above with respect to age.

Genetic Testing (Age: 1 day old)	Prevention Genetics Pediatric Disease Panel with exome-wide CNV analysis: - BICD2 c.1661T>C p.Leu554Pro heterozygous variant of unknown significance (VUS). Not maternally inherited. - NEB c.7710T>A p.Asp2570Glu heterozygous variant of unknown significance (VUS). - No copy number variants detected.
Echocardiogram (Age: 1 day old)	Structurally normal heart. - Patent ductus arteriosus with a trivial shunt, left-to-right. - PDA peak gradient = 18 mmHg. - Normal left ventricular cavity size, wall thickness, and systolic function. - Left ventricular ejection fraction (apical 4-chamber) = 66 %. - Normal right ventricular cavity size, wall thickness, and systolic function. - Patent foramen ovale -small interatrial communication -left-to-right shunt. - No pericardial effusion.
Renal Ultrasound (Age: 1 day old)	Normal renal ultrasound including the bladder with trace left pelviectasis, likely physiologic.
FISH Cytogenetics Study Report (Age: 4 days old)	Aneuploidy not detected, female FISH pattern.
Spine MRI (Age: 11 days old)	- Mild prominence of the central canal measuring up to 2.2 mm from T9 through T12. Otherwise unremarkable exam. - Note: This is a limited spine examination performed for detection and follow-up of syringohydromyelia. Other spinal cord, bony spine and soft tissue abnormalities may be missed on this protocol. If there is persistent clinical concern, dedicated MRI of the spine would be of value in further assessment.
Brain MRI and Venogram (Age: 25 days old)	- Stable appearance of small hematoma in left frontal white matter, not significantly changed since study dated 3/16/2020. No evidence of abnormal vascular flow voids in the vicinity of this hematoma to suggest an underlying vascular malformation. No new focus of intracranial hemorrhage is evident. - Punctate foci of T1 hyperintensity and T2 hypointensity in right periatrial white matter appear stable. These are suggestive of foci of nonspecific subacute insult. - Bilateral parieto-occipital convexity subdural fluid collections (larger on left) appear slightly decreased in size since prior study. There is no intracranial mass effect. Basal cisterns are preserved. - Unchanged mild enlargement of the left lateral ventricle. No evidence of transependymal flow. - Normal MR venography of the brain. No evidence of dural venous sinus thrombosis.
XR Legs Bilateral (Age: 39 days old)	Changes of arthrogryposis with bowing deformity. Significant periosteal change is noted involving both femora and both tibia with bowing deformity. The more significant changes to the distal metaphysis of the femur on the left and the left and right distal tibiae and fibulae are suggestive of older posttraumatic change with healing.
Ultrasound Hips (Age: 40 days old)	- Left Hip: Shallow left acetabulum with undercoverage of the left femoral head in the neutral and flexed positions. - Right Hip: Shallow right acetabulum with undercoverage of the right femoral head in the neutral and flexed positions.
Brain MRI (Age: 56 days old)	- No convincing residual subdural collections. - Punctate susceptibility adjacent to left frontal horn is smaller. - Stable left trigone larger than right without CSF flow. - Overall, no acute interval change.
Creatine Kinase (Age: 6 days old)	64 (Within Normal Limits)

The variant was visualized in the 3-dimensional protein structure (Figure 2) using the AlphaFold prediction[28,29] and the YASARA software.[30]

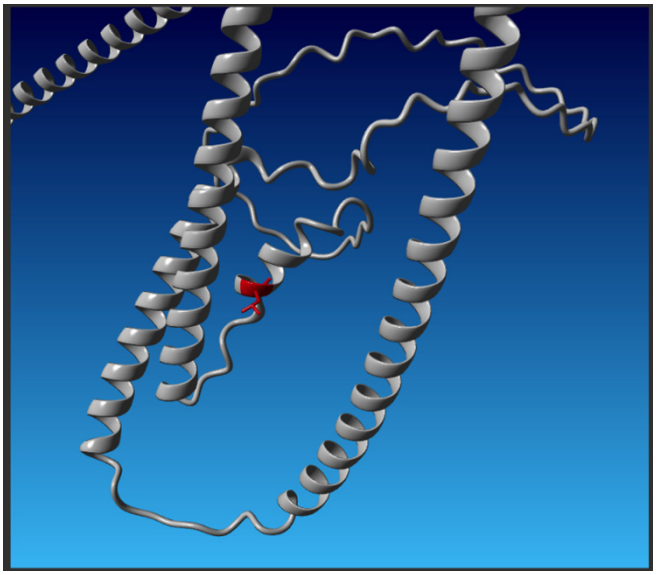


Figure 2: Heterozygous *BICD2* variant (c.1661T>C, p.Leu554Pro) visualized in the 3-dimensional protein structure using the AlphaFold prediction[28,29] and the YASARA software[30]

Discussion

In this case report, we discuss a patient with a novel mutation with a severe phenotypic presentation of the typical SMALED2 expression including severe arthrogryposis, absent deep tendon reflexes, preserved attentiveness, respiratory insufficiency, and cognitive impairment. Genetic testing identified a novel heterozygous variant in *BICD2* (p.Leu554Pro), which fit her phenotype and led to her diagnosis of autosomal dominant spinal muscular atrophy, lower extremity dominant, type 2B. Finally, we demonstrate a 21-month course with minimal improvements but no declines in either motor or cognitive functioning.

Spinal muscular atrophy (SMA), a leading genetic cause of infant mortality, is a congenital neuromuscular disorder that results in a loss of motor neurons often leading to progressive skeletal muscle weakness and atrophy.[23] However, less data is available for non-SMN1 associated disease. Autosomal dominant SMA with lower extremity dominance (SMALED), which is characterized by skeletal muscle weakness and atrophy in the lower extremities, was later identified to be associated with 2 loci: SMALED1 and SMALED2.[19] In 2013, the molecular basis of SMALED2 was identified to be due to a variant in *BICD2*.[24] Since 2019, the Online Mendelian Inheritance in Man (OMIM) database further classified SMALED2 into two categories: autosomal dominant spinal muscular atrophy type 2A and 2B (OMIM #615290 & 618291).[19] Type 2A describes a milder form of the disease while Type 2B describes a more severe form of the disease.[19]

Previously described *BICD2* variants include heterozygous missense changes and in-frame deletions. With these molecular changes, a wide phenotypic spectrum has been described, ranging from an asymptomatic patient with subclinical findings at the age of 71 years old, to multiple independent families with severe AMC, respiratory insufficiency, and early death.[13]

In a review by Koboldt et al. (2020), all 99 individuals with *BICD2* presented with weakness in distal muscles of the lower limbs. Upper limb (UL) weakness was significant, although distal UL was more prominent than proximal UL. 60% of families had contractures, 49% had hip dislocations, 34% had arthrogryposis multiplex congenita (AMC), 55% had talipes equinovarus, 60% had spinal deformities, 17% had respiratory problems, and 49% showed at least one or more signs of central nervous system involvement including increased reflexes (29%) or brain abnormalities (24%).[19] Phenotypically, our patient exhibited all of these symptoms including severe AMC, hip dislocation, hyperextension of neck, and talipes equinovarus, in addition to respiratory insufficiency.

The patient's brain MRI revealed punctate lesions in the right thalamus and left caudate with mild enlargement of the left lateral ventricle (Figure 1). The punctate lesions were thought to be clinically insignificant due to their small size. Similar cases regarding abnormal cerebral white matter have been identified. For instance, a patient with moderate thinning of the corpus callosum and two minor lesions on the posterior part of bilateral second ventricles ultimately passed away due to respiratory failure.[13] Another patient with complete absence of cerebral white matter and cortex with significant enlargement of the ventricular system developed epilepsy and hydrocephalus requiring shunt placement.[17] Although our patient did not present with epilepsy or hydrocephalus, all three of these patients provide evidence that cerebral atrophy may occur in severe SMALED2B diseases.

Our patient's EMG revealed features consistent with sensorimotor neuropathy (Table 1A, 1B, & 1C). Similar cases regarding EMG results have been identified. For instance, in another case, EMG data revealed fibrillation potentials, positive sharp waves, and motor units with markedly increased amplitude.[6] Additionally, in that case, the patient's muscle biopsy showed neurogenic fiber atrophy. While our patient did not undergo a muscle biopsy for comparison, this example shows the association with

BICD2 variant and chronic neurogenic process with spinal motor neuron involvement. Interestingly, in both the article by Bansagi et al (2015) and in our patient, they report no clinical sensory abnormalities though, in our patient, her age and cognitive impairments may limit this evaluation.

Molecular Analysis

The heterozygous *BICD2* variant (c.1661T>C, p.Leu554Pro) was plotted using UniProtKB entry Q8TD16 and was compared with nearby variants within the mutational hotspot region. This figure displayed three coiled-coil domain structures of the BICD2 protein which were categorized into Coiled Coil 1 (CC1), Coiled Coil 2 (CC2), and Coiled Coil 3 (CC3). The p.Leu554Pro variant is located just outside of the CC2 domain and within a region that has been shown to interact with the motor protein, kinesin-1 (isoform KIF5A) in mice.[25] Mutations in *KIF5A* cause decreased motor neuron survival which led to impaired axonal and dendritic outgrowth and impaired anterograde axonal transport in mice.[26,27] A nearby in-frame 3bp deletion, p.Asn546Del is also just outside of CC2 within a region that interacts with molecular motor kinesin-1.[17] The individual with that variant, like our patient, exhibited extreme phenotypic expression with severe AMC, muscle weakness, cerebral cortical atrophy.

While it has been difficult to predict phenotypic expression of *BICD2* variants, Koboldt et al. attempted to correlate variant location with clinical features. Variants in CC2 and CC3 seemed to associate with severe phenotypes including AMC, brain abnormalities, and respiratory issues.[19] CC2 domain variants tended to exhibit fewer foot deformities and spine deformities. Similar to other patients with variants near domain CC2, our patient exhibited severe phenotypic expressions, supporting the genotype-phenotype conclusion identified by Koboldt et al.

Lastly, although the variant did not meet American College of Medical Genetics criteria for "likely pathogenic," we feel that the data supports the pathogenicity of the variant. This is in alignment with multiple in silico scores which predict a deleterious or damaging effect of the amino acid substitution. Additionally, there was absence of any other notable variants reported on exome analysis with clinical features that could fit the diagnosis of *BICD2*-related disease. Therefore, additional tests such as molecular or metabolic testing were not pursued. However, as with current testing capabilities, it is possible that a variant not detected by exome sequencing or a variant in a gene of uncertain significance was excluded from the analysis and could be contributing to the patient's phenotype.

Conclusion

We describe a novel *BICD2* variant (c.1661T>C, [p.Leu554Pro]) in a patient with a severe phenotypic presentation of the typical SMALED2 expression including arthrogryposis multiplex congenita, respiratory insufficiency, and cerebral depression. While it is challenging to predict how a genotype will present phenotypically, these case studies provide evidence that specific amino acid residues of the BICD2 protein must be essential in organizing and maintaining normal intracellular transport processes.

Future Direction

Since the genetic variant is a VUS, a study on the patient's fibroblasts to evaluate for the presence of Golgi-fragmentation may help us understand the impact of the patient's genetic variation. Additional studies such as muscle or nerve biopsies could provide additional support for a deleterious effect on protein function.

Acknowledgements

We thank the patient and her family for their participation and cooperation in this case report.

Conflict of Interest

The authors report no conflicts of interest.

Author Contributions

NP, MM, DB, CK and MH all participated in the creation, review and submission of the manuscript.

Corresponding Author

Nahee Park
8701 W Watertown Plank Rd, Milwaukee, WI 53226
Phone number: 414-955-8296
npark@mcw.edu

References

1. Martinez Carrera, L, Gabriel, E, Donohoe, C,et al. Novel insights into SMALED2: BICD2 mutations increase microtubule stability and cause defects in axonal and NMJ development. *Human molecular genetics* 2018; *27*(10), 1772–1784.

2. Neveling K, Martinez-Carrera LA, Hölker I, et al. Mutations in BICD2, which encodes a golgin and important motor adaptor, cause congenital autosomal-dominant spinal muscular atrophy. *Am J Hum Genet* 2013; 96: 946–954.

3. Peeters K, Litvinenko I, Asselbergh B, et al. Molecular defects in the motor adaptor BICD2 cause proximal spinal muscular atrophy with autosomal-dominant inheritance. *Am J Hum Genet* 2013; 92: 955–964.

4. Oates EC, Rossor AM, Hafezparast M, et al. Mutations in BICD2 cause dominant congenital spinal muscular atrophy and hereditary spastic paraplegia. *Am J Hum Genet* 2013; 92: 965–973.

5. Rossor AM, Oates EC, Salter HK, et al. Phenotypic and molecular insights into spinal muscular atrophy due to mutations in BICD2. *Brain* 2015; 138: 293–310.

6. Bansagi B, Griffin H, Ramesh V, et al. The p.Ser107Leu in BICD2 is a mutation 'hot spot' causing distal spinal muscular atrophy. *Brain* 2015; 138(pt 11):391.

7. Fiorillo C, Moro F, Brisca G, et al. Beyond spinal muscular atrophy with lower extremity dominance: cerebellar hypoplasia associated with a novel mutation in BICD2. *Eur J Neurol* 2016;23:19–21.

8. Ravenscroft G, Di Donato N, Hahn G, et al. Recurrent de novo BICD2 mutation associated with arthrogryposis multiplex congenita and bilateral perisylvian polymicrogyria. *Neuromuscul Disord* 2016; 26:744–748.

9. Rudnik-Schoneborn S, Deden F, Eggermann K, et al. Autosomal dominant spinal muscular atrophy with lower extremity predominance: A recognizable phenotype of BICD2 mutations. *Muscle Nerve* 2016;54:496–500.

10. Synofzik M, Martinez-Carrera LA, Lindig T, et al. Dominant spinal muscular atrophy due to BICD2: a novel mutation refines the phenotype. *J Neurol Neurosurg Psychiatry* 2014;85:590–592

11. Theunissen TEJ, Sallevelt S, Hellebrekers D, et al. Rapid resolution of blended or composite multigenic disease in infants by wholeexome sequencing. *J Pediatr* 2017;182:371–374.e2

12. Unger A, Dekomien G, Güttsches A, et al. Expanding the phenotype of BICD2 mutations toward skeletal muscle involvement. *Neurology* 2016;87:2235–2243.

13. Storbeck M, Eriksen BH, Unger A, et al. Phenotypic extremes of BICD2-opathies: from lethal, congenital muscular atrophy with arthrogryposis to asymptomatic with subclinical features. *Eur J Hum Genet* 2017;25:1040–1048.

14. Souza PVS, Pinto W, Aivazoglou LU, et al. Distal myopathy due to BICD2 mutations. *Clin Neurol Neurosurg* 2018;165:47–49.

15. Trimouille A, Obre E, Banneau G, et al. An in-frame deletion in BICD2 associated with a non-progressive form of SMALED. *Clin Neurol Neurosurg* 2018;166:1–3.

16. Ahmed AA, Skaria P, Safina NP, et al. Arthrogryposis and pterygia as lethal end manifestations of genetically defined congenital myopathies. *Am J Med Genet A* 2018;176:359–367.

17. Koboldt DC, Kastury RD, Waldrop MA, et al. In-frame de novo mutation in BICD2 in two patients with muscular atrophy and arthrogryposis. *Cold Spring Harb Mol Case Stud* 2018;4:a003160.

18. Yoshioka M, Morisada N, Toyoshima D, et al. Novel BICD2 mutation in a Japanese family with autosomal dominant lower extremitypredominant spinal muscular atrophy-2. *Brain Dev* 2018;40:343–347.

19. Koboldt D, Waldrop M, Wilson R, et al. The Genotypic and Phenotypic Spectrum of BICD2 Variants in Spinal Muscular Atrophy. *Ann Neurol* 2020; 87: 487-496.

20. Hosseinibarkooie S, Peters M, Torres-Benito L et al: The power of human protective modifiers: PLS3 and CORO1C unravel impaired endocytosis in spinal muscular atrophy and rescue SMA phenotype. *Am J Hum Genet* 2016; 99: 647–665.

21. Riessland M, Kaczmarek A, Schneider S et al: Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis. *Am J Hum Genet* 2017; 100: 297–315.

22. Richards, S, Aziz, N, Bale, S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics 2015, 17(5), 405–424.

23. Kolb S, Coffey C, Yankey J, et al. Natural History of Infantile-Onset Spinal Muscular Atrophy. Ann Neurol 2017; 82(6): 883-891.

Neveling K, Martinez-Carrera L, Hölker I, et al. Mutations in BICD2, which encodes a golgin and important motor adaptor, cause congenital autosomal-dominant spinal muscular atrophy. Am J Hum Genet 2013; 92(6):946-954.

Splinter D, Tanenbaum ME, Lindqvist A, et al. Bicaudal D2, dynein, and kinesin-1 associate with nuclear pore complexes and regulate centrosome and nuclear positioning during mitotic entry. PLoS Biology 2010. 8: e1000350.

Karle K, Möckel D, Reid E, et al. Axonal transport deficit in a KIF5A$^{(-/-)}$ mouse model. Neurogenetics 2012; 13: 169–179.

Xia C, Roberts E, Her L, et al. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. J. Cell Biol 2003; 161:55–66.

24. Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature (2021).

25. Varadi M, et al. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Research (2021).

26. Krieger E, Vriend G (2014) Bioinformatics 30, 2981-2982 PMID24996895

27. Frasquet M, et al. Clinical Spectrum of BICD2 Mutations. European Journal of Neurology 2020;27(7):1327-1335

Kansas City Musculoskeletal Diseases Consortium
7th Annual Symposium on Musculoskeletal and Neuromuscular Diseases
UMKC – Pierson Auditorium, 5000 Holmes, Kansas City, MO
Friday, December 2, 2022
10:00 a.m. – 2:00 p.m.

10:00 am	**WELCOME** **Edward R. O'Connor,** PhD, MBA, FACHE, Executive Director, KCMD Consortium, Provost and Executive Vice President for Academic, Research and Student Affairs, Kansas City University
10:05 am	**INTRODUCTION OF KEYNOTE SPEAKER** **Richard J. Barohn,** MD, Executive Vice Chancellor for Health Affairs, University of Missouri – Columbia
10:10 am	**KEYNOTE SPEAKER** **W. David Arnold**, MD, Executive Director, NextGen Precision Health Initiative, University of Missouri – Columbia Sarcopenia has a lot of nerve! Neurobiological Mechanisms of Age-related Loss of Physical Function
11:00 am	**PREVIOUS KCMD AWARD WINNER PRESENTATIONS** Moderated by **Dr. O'Connor**
11:05 am	Erin Bumann, UMKC and Pamela Tran, KUMC, *"Genetic interaction between two ciliary paralogs regulates postnatal skeletal bone growth"*
11:20 am	Mazen Dimachkie, KUMC, *"Inclusion Body Myositis Treatment with Celution Processed Adipose Derived Regenerative Cells"*
11:35 am	Charlotte Phillips and Brittany Lafaver, MU, "*Characterization of Heart Abnormalities in Pre-translational Models of Osteogenesis Imperfecta"*
11:50 am	Hillary McGraw, UMKC, "*Foxg1a regulates cranial facial development in the zebrafish*" (Poster Presentation)
12:05 pm	**Working Lunch**
12:20 pm	Wen Liu, KUMC, "*Time trajectory of joint pain and compliance to an interval or continuous walking exercise in people with Knee Osteoarthritis*" (Virtual)

RRNMF Neuromuscular Journal 2023;4(1):42-73

12:35 pm Heather Wilkins, KUMC, "*Functional Biomarkers for ALS*"

12:50 pm Sarah Dallas, UMKC, "*Live Cell and Intravital Imaging of Bone Resorption Dynamics and Osteocyte Fate During Osteoclastic Bone Resorption*"

1:05 pm **Group Q&A –** Dr. O'Connor, Moderator

1:20 pm **POSTER PRESENTATIONS**

Ryan Anderson, UMKC, "*Maternal dietary vitamin A levels as a determinant of penetrance and severity of cleft lip/palate in a Wnt9b model*"

Kennedy Davis, KCU and Lisa Stehno-Bittel, KUMC, "*Joint Lubricants as Delivery Methods for Multipotent Stromal Cells*"

Andrew Heim, KUMC, "*Trial of Oxaloacetate in ALS (TOALS)*"

Claire Houchen, UMKC, *"A Novel PCR-based Strategy for Investigating Sexual Dimorphism in Avian Embryos: An Example in Bone"*

Stefan Lohfeld and Roland Klar, UMKC, "*3D printed multi-gradient microsphere scaffolds for guided osteochondral tissue engineering*"

Nuria Lara, UMKC, "*Role of Estrogen Receptor α in Bone-Muscle Crosstalk*"

Loretta Laughrey, UMKC, "*Multiscale, 3D finite element analysis using Micro-CT and confocal multiplexed images for correlation of osteocyte B-catenin signal pathway activation with predicted lacunar wall strain*"

Soumya Rao, UMKC, "*New insights into the genetic basis of the Oculo-Auriculo-Vertebral Spectrum (OAVS)"*

Maria Spletter, UMKC, "*Bruno1 Is Required Throughout Drosophila Indirect Flight Muscle Development to Regulate Dynamics Of Sarcomere Assembly And Growth*"

Jacob Thomas, MU, "*Preliminary Results from a Novel Movement-Based Concussion Screening Too*l"

Julian Vallejo, UMKC, "*Tibia Mechanical Loading Acutely Decreases Resting Heart Rate in Mice Likely via the Sympathetic Autonomic Nervous System*"

Sam Weiss, MU, "*Serial Subtraction Dual-Task Alters Lateral Step-Down Tibiofemoral Kinematics in Healthy Adults*"

Yixia Xie, UMKC, "*Confocal and Tissue Clearing/3D Imaging in Transgenic Mice Expressing a Membrane-GFP targeted to Mineralizing Cell Types*"

Padmini Giri, KUMC, "*An Interesting Case of Anti-Mi-2-Antibody Associated with Paraneoplastic Dermatomyositis in a Patient with Cervical Cancer*" (Virtual)

Richard Sherwood, MU, "*Maturation-based Prediction of Craniofacial Growth*" (Virtual)

Kun Wang, UMKC, "*Extracellular Vesicle-Mediated Communication Between Cells in Bone*"

2:00 pm **Closing Remarks: Dr. O'Connor**

The Executive Committee of the Kansas City Musculoskeletal Diseases Consortium:

- Edward R. O'Connor, PhD, MBA, FACHE, Provost and Executive Vice President for Academic, Research, and Student Affairs, Kansas City University, and KCMD Executive Director
- Richard J. Barohn, MD, Executive Vice Chancellor for Health Affairs, University of Missouri – Columbia
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- Jeffrey M. Statland, MD, Professor, Department of Neurology, University of Kansas Medical Center

Previous KCMD Award Winner Presentation Speakers:

Kansas City University (KCU)

Kennedy Davis, Research Graduate Student, KCU College of Biosciences

University of Kansas Medical Center (KUMC)

Mazen M. Dimachkie, MD, Vice Chairman for Research Programs & Executive Vice Chairman, Department of Neurology

Wen Li, PhD, Associate Professor, Director of Neuromuscular Research Laboratory, Department of Physical Therapy & Rehabilitation Science

Lisa Stehno-Bittel, BSPT, PhD, Clinical Professor, Department of Physical Therapy, Rehabilitation Science, and Athletic Training

Pamela V. Tran, PhD, Associate Professor, Anatomy and Cell Biology, Department of Cell Biology & Physiology

Heather Wilkins, PhD, Assistant Professor, Department of Neurology; Assistant Director, Biomarker Core, University of Kansas Alzheimer's Disease Center

University of Missouri – Columbia (MU)

Charlotte L. Phillips, PhD, Professor, Director of Graduate Studies, Department of Biochemistry

University of Missouri – Kansas City (UMKC)

Erin E. Bumann, DDS, PhD, MS, Assistant Professor, Department of Oral and Craniofacial Sciences

Sarah L. Dallas, PhD, UM Curator's Distinguished Professor, Lee M. and William Lefkowitz Endowed Professor, School of Dentistry, Department of Oral and Craniofacial Sciences

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Genetic interaction between two ciliary paralogs regulates postnatal skeletal bone growth

Erin E. Bumann[1] and Pamela V. Tran[2]

[1] Department of Oral and Craniofacial Sciences, School of Dentistry, University of Missouri-Kansas City
[2] Department of Cell Biology and Physiology, Jared Grantham Kidney Institute, University of Kansas Medical Center

Primary cilia are signaling organelles that receive mechanical and chemical cues in the extracellular environment, and are potent modifiers of skeletal growth. Mutation of ciliary genes causes syndromic disorders termed ciliopathies, which can manifest osteochondrodysplasias. We have generated a new mouse model of osteochondrodysplasia, generated by combinatorial deletion of two ciliary paralogs, *Thm1* (also known as *Ttc21b*) and *Thm2* (*Ttc21a*). While *THM1* mutations have been identified in patients with the ciliary osteochondrodysplasia, Jeune Syndrome, *THM2* is less characterized. We have found that in mice, $Thm2^{-/-};Thm1^{+/-}$ triple allele deletion causes small stature and micrognathia, and disrupts chondrocyte and osteoblast differentiation. Genetic downregulation of Hedgehog (Hh) signaling exacerbates the skeletal phenotype in mice, while Hh agonist, SAG, rescues the osteoblast differentiation defect *in vitro*. To determine the etiology of the bone defects and the clinical significance, future studies will investigate the osteoblast differentiation defect and bone remodeling in mouse, and co-occurrence of *THM2* and *THM1* rare variants in children with skeletal anomalies.

Inclusion Body Myositis Treatment with Celution Processed Adipose Derived Regenerative Cells

Heim, AJ; Soder, R; Bhavsar, D; Agbas, A; Kosa, E; Ciersdorff, A; Pasnoor, M; Jawdat, O; Jabari, D; Farmakidis, C; Chandrashekhar, S; Dimachkie, MM

Abstract

Background: Inclusion Body Myositis (IBM) is a progressive, debilitating disease causing both proximal and distal muscle weakness, characteristically most prominent in the quadriceps and finger flexors. Over time it can lead to severe disability, including loss of hand function, falls due to quadriceps muscle weakness and foot drop, dysphagia, and eventually respiratory muscle weakness. The Celution System is a closed, automated system intended to digest adipose tissue in order to further extract, wash, and concentrate stromal stem cells and other associated progenitor cells intended for autologous reimplantation in a real-time bedside manner and has been tested in vascular disease and wound healing.

Objectives: The primary objective of this study is to assess the safety in IBM of an autologous graft consisting of adipose-derived regenerative cells (ADRCs) derived from the Celution. Secondary efficacy measures are included. This study is conducted under IDE 25043.

Methods: ADRCs were isolated and purified from human abdominal subcutaneous fat tissue using the Celution 800/CRS. ADRCs were processed aseptically, had a mean viability of 90% and were suspended in lactated Ringer's solution for infusion. Enrollment is staggered into 3 groups. Nine IBM subjects are randomized 2:1 in groups of 3 to late (Part 1) versus early (Part 2) ADRC autologous graft injections. Stem cell injections will occur unilaterally at 2 sites in the flexor digitorum profundus muscle and 6 sites in the quadriceps group of muscles. Subjects are injected on the side of the body that muscle strength is graded between 6 and 9 for finger flexion and knee extension using the Kandell 0-10 scale at the screening visit. A total of 30 million cells is injected into the subject between the 8 injection sites. Subjects are followed up every 3-6 months for two years after the stem cell injections for safety and efficacy measures.

Results: All 9 IBM subjects have been enrolled in the trial. In totality 3 subjects have received stem cell injections. On October 26, 2022, the third group participant received the ADRC autologous graft injections via EMG-guidance. The remaining 6 subjects will receive injections in 2023 with study completion estimated for 2025. In collaboration with Dr. Abdulbaki Agbas, we are collecting whole blood samples for assessing the profile of biomarker protein TDP-43 at various visits in Part 1 and Part 2.

Discussion: While study results are not yet available, the stem cell injections have been so far well-tolerated. The study-related adverse events have been limited to expected transient effects of the liposuction procedure. TDP-43 assessment data is being collected and will be analyzed by capillary electrophoresis Immunoassay (CEI).

Acknowledgements: This study received funding from the Kansas City Musculoskeletal Consortium and the Vice Chancellor of Research at the University of Kansas Medical Center.

Characterization of Heart Abnormalities in Pre-translational Models of Osteogenesis Imperfecta - KCMD Research Progress Update

Charlotte L. Phillips PhD[1,2], Brittany Lafaver[1]*, Departments of Biochemistry[1], and Child Health[2], University of Missouri, Columbia MO 65211 (*poster presenter)

Lixin Ma PhD[3,4], Li Lee PhD[3,4], Departments of Radiology[3], University of Missouri, Columbia MO 65211, VA-Biomolecular Imaging Center[4], Truman VA Hospital, Columbia, MO 65201

Maike Krenz MD[5,6], Departments of Medical Pharmacology and Physiology[5] University of Missouri, Dalton Cardiovascular Research Center[6], Columbia, MO 65211

Michael Wacker PhD[7], Krish Sardesai[7], Julian Vallejo MS[7], Evan Pierce[7], Department of Biomedical Sciences[7], School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108

Osteogenesis Imperfecta (OI) is a heritable connective tissue disease that affects 1:10,000 births. OI patients often present with brittle bones and scoliosis. Recent investigations have begun to elucidate the presence of inherent muscle weakness and cardiopulmonary complications. Previously cardiopulmonary complications were attributed to thoracic spinal deformities, however recent evidence demonstrates that patients with low grade or no scoliosis also exhibit cardiopulmonary manifestations. Echocardiographic clinical studies suggest that diastolic dysfunction in the OI patient population is common, often accompanied by valvular regurgitation. Of the extracellular matrix in the normal heart myocardium, approximately 85% of total myocardial collagen is type I, which is important in maintaining the structural integrity of the myocardium. Whether the presence of reduced or abnormal collagen levels alters cardiovascular health in OI patients remains to be fully elucidated, as cardiovascular complications are thought to be the second leading cause of death in OI.

The osteogenesis imperfecta murine (*oim*) mouse models severe type III human OI in its homozygous (*oim/oim*) form. In the present study 4-month-old wildtype (Wt) and *oim/oim* littermates underwent functional studies, followed by sacrifice, where the hearts were weighed and flash frozen. Analyses of the wet weights of male and female *oim/oim* and Wt hearts demonstrated increased cardiac mass in *oim/oim* compared to age and sex matched Wt hearts. Preliminary cardiac MRI analyses demonstrated that *oim/oim* hearts exhibited increased left ventricular volume (end-diastole and end-systole) and decreased ejection fraction relative to Wt hearts. Introductory echocardiography studies show that 2 out of 5 *oim/oim* males and 2 out of 5 *oim/oim* females have significantly increased peak blood flow velocities in the ascending aorta. This indicates that a subset of *oim/oim* mice developed aortic valve stenosis. Initial electrocardiography studies suggest the conduction pathway in *oim/oim* hearts is not altered. Cardiac tissue evaluated by RT-qPCR showed elevated Brain Natriuretic Peptide (BNP) expression in the female *oim/oim* heart, as well as a myosin heavy chain (MHC) isoform switch, represented by lowered α-MHC and raised β-MHC in males. Overall, these investigations suggest *oim/oim* mice demonstrate altered cardiac function as well as gene expression of BNP, indicating potential cardiac stress. Further investigations are needed to elucidate the mechanisms in the pathogenesis of the cardiac manifestations in the *oim/oim* heart and its implications to cardiovascular health in patients with OI.

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Foxg1a regulates cranial facial development in the zebrafish

Nusaybah Ibrahim, Laylah Liwaru and Hillary McGraw
University of Missouri-Kansas City, School of Science and Engineering

Foxg1 is a forkhead transcription factor with a critical role in neural development. Foxg1 regulates many cellular behaviors including proliferation, differentiation and mitochondrial function. In humans, Foxg1 mutations are linked to Foxg1 syndrome, which is defined by defects in neural development, intellectual disability, language deficits, movement disorders, disrupted circadian rhythm, and social withdrawal. Additionally, Foxg1 mutations are linked to cranial facial defects in some human patients, thought a specific role during development has not been described. Our work focuses on a zebrafish *foxg1a*^{a266} mutant line, which was generated using CRISPR-Cas9 genome editing (Thyme et al. 2019). Preliminary analysis of the *foxg1a*^{a266} mutants reveals defects in cartilage elements of the developing jaw. The mutants have abnormal joint development, narrow heads, and early lethality. Understanding these connections of cranial facial defects and the *foxg1a* mutation will help in uncovering how the mutation affects the development of other vertebrates and aspects of Foxg1 syndrome.

Time trajectory of joint pain and compliance to an interval or continuous walking exercise in people with knee osteoarthritis

Wen Liu, Ph.D. [1]
Batool Alkhamis PT, Ph.D.[1,2]
Yaqiong Wang, DPT, Ph.D.[3]
Mingui Fu, Ph.D. [4]
Irina Smirnova, Ph.D. [1]

[1] Department of Physical Therapy, Rehabilitation Science, and Athletic Training, University of Kansas Medical Center, Kansas City, KS, USA

[2] Department of Medical Rehabilitation Sciences, King Khalid University, Saudi Arabia

[3] SERC Physical Therapy, Lakewood, Lee's Summit, MO, USA

[4] Department of Biomedical Science, University of Missouri Kansas City, Kansas City, MO, USA

Abstract

Introduction People with knee osteoarthritis (KOA) often complain of worsening joint pain post-exercise and fail to meet the recommended physical exercise. A past study showed that one bout of interval walking (IW) may reduce pain level while one bout of continuous walking (CW) may raise pain level in people with KOA, but it is unknown how the joint pain will respond to IW or CW in a long-term. **Purpose** In this pilot randomized clinical trial, we examined the changes of joint pain after each session of IW or CW exercise over 6 weeks and changes of compliance to the IW or CW exercise in people with KOA. **Methods** Twenty-two participants with KOA were enrolled and randomly assigned to either an IW or CW group. The intervention involved 30 minutes of walking exercise, 3 times/week over 6 weeks. The IW was a 30-minute walking in 2 bouts (15 minutes each) with 30-40 minutes of a resting interval, while the CW was a 30-minute walking in one continuous bout. The joint pain level of each participant was assessed using a visual analogue scale (VAS) prior to and immediately after each walking session. The data of compliance to walking exercise was collected using an exercise diary from each participant. **Results** Nine participants in each group completed the intervention. The joint pain pre and post walking exercise showed a similar trend of decreasing over time in both IW and CW groups. However, after each walking session, the joint pain decreased in the IW group but increased in the CW group. The difference in changes of joint pain after each walking session was significant between the two groups. The compliance to walking exercise was consistently high in the IW group throughout the study but declined significantly in the CW group after the first four weeks due to worsening of the joint pain. **Conclusion** The results of this study indicated that the regulation of the joint pain at resting status or during exercise might involve different systems, specifically local versus central system. The IW may be a better form of a walking exercise than the CW for people with KOA.

Functional Biomarkers for ALS

Whited K[1], Lillig K[1], Gerringer C[1], Higgs K[1], Colgan S[1], Saavedra L[1], Roath K[1], Walker M[1], Clay R[1], Taylor N[2], Bright A[2], Kosa E[3], Swerdlow RH[1], Jawdat MD[1], Jabari D[1], Agbas A[3], Statland JM[1], Barohn RJ[2], Wilkins HM[1]
[1]University of Kansas Medical Center, Kansas City, KS USA
[2]University of Missouri, Columbia, MO, USA
[3]Kansas City University, Kansas City, KS, USA

Background: Amyotrophic lateral sclerosis (ALS) pathological features include mitochondrial dysfunction and loss of proteostasis (protein aggregation). Mitochondrial dysfunction and proteostasis are intricately linked biological modalities which are key targets for upcoming clinical trials in ALS. These clinical trials will benefit from functional blood-based biomarkers. Our overall goal is to clinically validate blood based mitochondrial and proteostasis biomarker protocols for use as indices of target engagement in future clinical trials.
Objectives: We are comparing mitochondrial and proteostasis biomarkers within 150 ALS and 50 control subjects over eight months.
Methods: We have currently enrolled 20 ALS subjects from KUMC and MU-Columbia neuromuscular clinic. Each subject will undergo four blood draws at 2–4-month intervals. We measure lymphocyte mitochondrial biomarkers, including mitochondrial membrane potential (TMRE), mitochondrial superoxide (MitoSox), mitochondrial mass (MitoTracker), and apoptosis (Annexin V). Using these biomarkers, we employ an algorithm to determine a mitochondrial health index (MHI). We also measure plasma neurofilament light (NfL) values and phosphorylated TDP43 in plasma and platelets. Data are compared with clinical assessments including forced vital capacity (FVC) and the ALS functional rating scale (ALSFRS).
Results: Current data suggest that lymphocyte MHI values decrease at each visit, while NfL levels are relatively stable but elevated. Lymphocyte MHI does not correlated with NfL. However, individual biomarkers do correlate with clinical assessment. Mitochondrial superoxide in lymphocytes correlates with FVC. Lymphocyte mitochondrial membrane potential correlates with plasma NfL levels.
Discussion: Overall, our data show that lymphocyte mitochondrial function could be a biomarker of ALS progression.

Live Cell and Intravital Imaging of Bone Resorption Dynamics and Osteocyte Fate During Osteoclastic Bone Resorption

Sarah L. Dallas[1], Valerie Kirtley[1], Anthony Meljanac[1], Yixia Xie, LeAnn Tiede-Lewis[1], Eleanor Ray[1], David S. Moore[1]

[1]University of Missouri-Kansas City

Abstract

Live cell imaging allows us to gain novel insight, from a dynamic perspective, into the fundamental processes controlling bone formation and resorption during bone development, maturation, repair and aging. Using transgenic mice with fluorescently tagged osteoclasts, osteocytes and collagen, we have performed timelapse live cell and intravital imaging to visualize the dynamic and resorptive behavior of osteoclasts in their natural bone environment and determine the fate of osteocytes after bone resorption.

Transgenic lines, including mice with red fluorescently tagged osteoclasts (LysM-Cre/tdTomato), green or red fluorescently tagged osteocytes (Dmp1-AcGFPmem) or (Dmp1-Cre/tdTomato) or green fluorescently tagged type I collagen (COL1A2-GFP*tpz*), were crossed in various 2-color combinations. Intravital multiphoton imaging was performed in 4-6wk old mice and confocal or widefield epifluorescence live cell imaging was performed using calvarial explants from 10-14 day old mice in the presence or absence of stimulators and inhibitors of osteoclastic resorption. Live imaging in bone explants provided resolution sufficient to visualize osteoclast dynamics as well as the dynamics of their sealed zones (actin rings). Dual imaging of osteoclasts with GFP-collagen has enabled us to correlate osteoclast cell dynamics with collagen resorption kinetics. The osteoclasts had a multi-lobed morphology and actively resorbing osteoclasts were morphologically distinct from inactive ones, exhibiting a more compact, less branched morphology. Over long-term timelapse imaging (up to 96h), osteoclast morphology was continuously changing, with amoeboid-like streaming of the cell, repeated extension and retraction of cell processes and formation of varying numbers of transient sealed zones rings (up to 3-4 per osteoclast) that correlated with bone resorption activity. Osteoclasts showed complex cell dynamics including fission, fusion and recycling. In control bones osteoclast maximum velocities were up to 52.6±5.7μm/h, with an average of 13.5±1.4μm/h. Treatment with PAM3CSK4, an inflammatory stimulator of bone resorption, decreased the maximum and mean velocities to 38.3±2.1 and 8.8±0.4μm/h but increased the directionality of osteoclast motion. PAM3CSK4 also increased the mean number of actin rings per osteoclast from 0.27±0.11 to 1.4±0.25 and decreased osteoclast fusion, while increasing osteoclast survival. Dual imaging of osteoclasts/osteocytes showed that the majority (>90%) of osteocytes in areas of bone that were resorbed underwent cell death/apoptosis, a small fraction (~5%) were internalized within vacuoles and only in rare cases did osteocytes appear to survive osteoclastic resorption by migrating out of their lacunae. These data provide new insight into the dynamic behavior of osteoclasts in their natural bone environment, how these dynamics are altered in inflammatory bone resorption, and can be used to enhance our understanding of the mechanisms of action of bone therapeutics.

Maternal dietary vitamin A levels as a determinant of penetrance and severity of cleft lip/palate in a Wnt9b model.

Ryan Anderson[1], Akiko Suzuki[1], Zaib Malik[1], Liza Cox[1], & Timothy Cox[1,2]

Departments of [1]Oral & Craniofacial Sciences and [2]Pediatrics, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Vitamin A (VA) and its derivatives (retinoids) play essential roles during embryogenesis and particularly facial development, with both severe VA deficiency and vast excess of VA derivatives having been linked to CL/P risk. Previous studies have shown that knockout of *Retinol binding protein 4* (*Rbp4)* severely limits the mobilization of liver retinol stores and that elevated dietary intake is required to ensure sufficient retinol supply to peripheral tissues. This dietary-dependence underpins the *Rbp4* KO line as a sensitive and tunable model to study the effects of VA on development and disease.

To study the interaction between maternal dietary VA and specific cleft susceptibilities, we have crossed the *Wnt9b* KO – the best-characterized single gene CL/P model - on to the *Rbp4* KO background. Pregnant dams were switched, post conception, from a sufficient diet (23IU/g) to either a low VA (4IU/g) or high VA (40IU/g) diet. Embryos were then imaged using optical projection tomography for qualitative and quantitative facial phenotyping. Our data reveal a window of optimal maternal dietary VA, where the incidence of CL/P was reduced to <20%. Notably, doubling maternal VA levels increased both CL/P severity and incidence (to ~65%), whereas vitamin A insufficiency led to a switch from bilateral clefting to midline facial clefts and repatterning of the frontonasal tissue. These findings suggest that optimization of maternal dietary VA during the early stages of pregnancy may reduce the chances of a child being born with CL/P. Ongoing work is determining whether this holds true for other CL/P risk alleles.

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Joint Lubricants as Delivery Methods for Multipotent Stromal Cells

Kennedy Davis, Megan Hamilton, Douglas Bittel, Michael Filla,
Lisa Stehno-Bittel, PhD
Kansas City University, University of Kansas Bioengineering Program, and Likarda, LLC, Kansas City, Missouri, USA

Multipotent Stromal Cells (MSC) are utilized as therapeutic agents for addressing tissue regeneration for musculoskeletal conditions, including knee osteoarthritis (OA). Currently, cell therapies lack FDA-approval for injections to alleviate joint OA. To overcome this barrier, some clinicians are utilizing autologous stem cell transplants that are not regulated. Yet, the results are mixed with a majority of patients indicating little or no relief. Major challenges in the clinical application include poor MSC viability after isolation, extreme shear stress of the injections, maintenance of the cells in the joint capsule, and the harsh inflammatory environment of knee OA. As hyaluronic acid (HA) is an innate polymer of synovial joints that maintains cartilage viscoelastic integrity, HA-based cell delivery systems are of interest. While MSCs could be delivered in these uncrosslinked gels, it is hypothesized that they will not trap the cells in the knee joint long enough to have an effect. The aims of this study were: 1) to determine whether a commercial HA joint lubricant (Monovisc) could maintain MSC viability under different conditions, and 2) to determine the ability of cells delivered in Monovisc to reverse knee joint degeneration in an OA rat model.

Trial of Oxaloacetate in ALS (TOALS)

Heim, AJ; Lillig, K; Statland, J; Jabari, D; Wilkins, H; Agbas, A; Choi, I;
Lee, P; Zhang, N; Gerringer, C; Dimachkie, MM; Swerdlow, R; Barohn, RJ; Jawdat, O

Abstract

Background: Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease. ALS affects nerve cells in the brain and spinal cord which control muscle movement. Death of the motor neurons leads to denervation of skeletal muscles resulting in muscle atrophy, weakness, fatigue, fasciculations, respiratory failure, and death. The majority of patients with ALS die within three to five years from symptom onset. The exact cause of motor neurodegeneration remains uncertain; however, mitochondrial dysfunction has been implicated having a role in motor neuron death in ALS. Oxaloacetate (OAA) is a Krebs cycle and gluconeogenesis intermediate, which enhance glycolysis flux, supports oxidative phosphorylation, and modifies bioenergetics-related infrastructures as supported by both human and animal model studies.

Objectives: The primary objective of this study is to determine safety of OAA administration and if reducing mitochondrial stress is a viable treatment strategy for ALS. The study design includes a dose escalation which will determine the maximal tolerated dose of OAA and whether OAA improved biomarkers of mitochondrial stress.

Methods: Enrollment is staggered into a 3+3 dose escalation of oral oxaloacetate for 28 days. First patient's cohort, consisting of three subjects, has received 1000mg OAA twice daily. If no dose limiting toxicity observed, second patient cohort has received 1500 mg OAA twice daily. Finally, third cohort has received maximum 2500 mg OAA twice daily. Subjects have three onsite visits and weekly phone call visits throughout the duration of the study to evaluate safety and efficacy. Subjects undergo an MR spectroscopy of brain glutathione at the Hoglund Biomedical Imaging Center at Screening and Day 28. In collaboration with Dr. Abdulbaki Agbas and Dr. Heather Wilkins, platelet TDP-43 and pTDP-43, and mitochondrial biomarker blood sample data were collected at screening, baseline, and day 28 for all cohorts.

Results: To date, 13 subjects have enrolled and completed the 28-day dosing period. The final cohort evaluating the 2500 mg twice daily dose is currently enrolling 5 more subjects. Related adverse events have been limited to gastrointestinal upset, which is an expected side effect of OAA.

Discussion: OAA has been well tolerated and enrollment has continued into the final cohort of 2500 mg twice daily. Reported adverse events have been limited and expected with ALS progression. Safety and biomarker blood samples are being collected for analyzing platelet TDP-43 and pTDP-43 by employing capillary electrophoresis Immunoassay (CEI).

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A Novel PCR-based Strategy for Investigating Sexual Dimorphism in Avian Embryos: An Example in Bone

Claire J. Houchen[1], Maria Gonzalez[1], Erin E. Bumann[1]

[1]Department of Oral and Craniofacial Sciences, University of Missouri-Kansas City School of Dentistry

Sex is known to influence human skeletal development, making it critical to consider sex as a biological variable in bone development research. We use embryonic duck (*Anas platyrhynchos*) and quail (*Coturnix japonica*) to answer questions about the development of bones, such as the lower jaw. There is known sexual dimorphism in adult duck skeletons, but not in quail. However, the impact of sex on embryonic lower jaw bone gene expression is unknown in both our avian models and in humans. Such studies are challenging because sex is morphologically unidentifiable in embryos of most avian species, therefore molecular techniques are required for sex identification. Molecular sex identification of embryonic birds can be difficult, particularly in species outside of the widely-studied chick (*Gallus gallus*). To improve upon a polymerase chain reaction (PCR) technique in chick that identifies histidine triad nucleotide binding protein W (*HINTW)*, which is located on the female-specific W chromosome, we developed a novel quantitative PCR (qPCR)-based technique to identify the sex of avian embryos using *HINTW* in multiple species, such as chick, duck, and quail. Messenger RNA was extracted from embryonic quail and duck lower jaw tissue at two developmental timepoints: one just before bone calcification begins in the facial complex and one when it is largely calcified. Expression of eight genes related to bone development were analyzed ($n=3$-4/stage/sex, $p<0.05$). Statistically significant sex differences were noted only in duck matrix extracellular phosphoglycoprotein (*MEPE)* with expression 4-fold higher in males, and duck receptor activator of nuclear factor κ B (*RANK)* with expression 2-fold higher in males. None of the other genes analyzed demonstrated sexual dimorphism, including those with large fold changes between stages (matrix metalloproteinase-9 (*MMP9)*, osteoprotegrin (*OPN),* and *MMP13 (*in quail)), those with mild fold changes (collagen type I alpha 1 chain (*COL1A1*), fibroblast growth factor-23 (*FGF23), MEPE* (in quail), and *MMP13* (in duck)), and those with biologically minor changes (runt-related transcription factor 2 (*RUNX2)*, and *RANK* (in quail)). None of the genes analyzed in quail demonstrated sexual dimorphism, which aligns with the lack of sexual dimorphism in adult quail skeletons. In contrast, our data suggest possible sexual dimorphism in the mRNA expression of some bone-related genes in embryonic duck, whose adult skeletons are dimorphic. This study demonstrates our novel *HINTW* qPCR technique to identify the sex of avian embryos could be a useful tool for including sex as a biological variable in analysis of a variety of tissues and cells used in developmental biology research.

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3D printed multi-gradient microsphere scaffolds for guided osteochondral tissue engineering

Roland M. Klar & Stefan Lohfeld

Department of Oral and Craniofacial Sciences, School of Dentistry, University of Missouri-Kansas City

Osteochondral joint regeneration is one of the most challenging tissue engineering endeavors. It involves the recapitulation of two distinct and yet similar biological processes, which are joint chondrogenesis versus subchondral osteogenesis, requiring proper temporal and spatial signaling coupled with guiding mechanical stimulation and correct geometrical configurations. These factors have made it difficult for tissue engineers to design biomaterials that can replicate nature's designs. A promising solution to this issue are synthetically derived microspheres, which can be used to encapsulate therapeutic substances to provide a temporally controlled release mechanism. 3D printing techniques that could be used to create gradient scaffolds to control the spatial release of signaling biomolecules from load-bearing scaffold applications as needed for joints, currently cannot distribute microspheres in the desirable fashion.

We have recently overcome this issue and have generated a 3D printed scaffold comprised solely of PLA microspheres with varying porosities and geometrical alterations that better mimic the architectural foundation of bone. Not only are we now able to generate such a solely microsphere comprised scaffold but have successfully generated the first 3D printed microsphere scaffold with a 3D material gradient simulating the structure of an osteochondral joint matrix that would foster possible cellular migration, differentiation and tissue formation into cartilage and bone.

Our theory is that with the ability to precise control release of signaling biomolecules temporally and spatially within one scaffold, we can better study cell response on certain stimuli, e.g. the amount of growth factors released at any time, or cascading different factors in a certain order, while also providing preferred architectural environments for cell invasion and proliferation. This research will then enable us to create scaffolds that better mimic the natural process and hence create superior tissue-engineering based therapies to heal bone and cartilage defects.

Role of Estrogen Receptor α in Bone-Muscle Crosstalk

Nuria Lara-Castillo[1], Julian Vallejo[2], Mark R. Dallas[1], Yixia Xie[1], Alexandra Ledbetter[3], Jennifer Rosser[1]
Eleanor Ray[1], Sarah L. Dallas[1], Ganesh Thiagarajan[3], Michael Wacker[2], Mark L. Johnson[1]

[1,] School of Dentistry, [2,] School of Medicine, [3,] School of Engineering, University of Missouri – Kansas City

Estrogen plays critical roles in bone and muscle health by signaling through its two main receptors, ERα and ERβ. To investigate the role of ERα in bone-muscle crosstalk, we deleted this receptor in skeletal muscle by crossing ERα$^{f/f}$ mice with the tamoxifen inducible HSA-MCM-Cre mouse to generate ERα$^{-/-sm}$ mice. Cre^{-} littermates were used as controls. Mice were injected with tamoxifen one month prior to sacrifice, at either 5 mo old, or 11 mo old (n=3-8/group). Extensor digitorum longus (EDL) and soleus muscles were used for *ex-vivo* contractility analysis. Femurs were used for μCT analysis and biomechanical analysis.

At 6 months of age, muscle specific deletion of ERα impacted several muscle properties. Male ERα$^{-/-sm}$ EDL weight was lower (16.2±2.9 vs 13.3±0.4 mg, $p<.05$), produced lower absolute force (267.8±35.8 vs 214.3±22.7 mN, $p<.05$), and had lower relaxation rate (3100±407 vs 2485±257 mN/s, $p<.05$) at submaximal frequencies, and had improved fatigue resistance ($p<.05$). Muscle deletion of ERα also resulted in altered trabecular bone parameters only in male mice. Femurs of ERα$^{-/-sm}$ males had higher Tb.BMD (0.39±0.06 vs 0.46±0.05 g.cm^{-3}, $p<.05$), Tb.BV/TV (13.8±2.6 vs 20.8±4.4%, $p<.01$) and an increase in Tb.N (4±1.1 vs 5.7±1.0 1/mm, $p<.05$). ERα$^{-/-sm}$ male tibias had an increase in Tb.Th (0.054±0.01 vs 0.062±0.01, $p<.05$). ERα$^{-/-sm}$ female mice had improved fatigue resistance in EDL muscle compared to control ($p<.05$), but bone parameters were not altered.

At 12 mo, there were no differences between ERα$^{-/-sm}$ and control male mice in muscle and bone properties. ERα$^{-/-sm}$ female EDL muscles produced lower absolute and specific force (54.1±2.8 vs 40.5±3.9 N/cm^2, $p<.01$) at high frequencies. However, there were no differences after a fatigue regime and exposure to caffeine, suggesting alterations in excitation contraction coupling after repetitive stimulation which restored force to control levels. There were no changes in bone properties in the femurs of ERα$^{-/-sm}$ female mice.

In summary, deletion of ERα after development reduced ex vivo contractility in EDL, while improving fatigue properties. ERα expression in skeletal muscle in 6-month-old male mice suppresses trabecular bone formation, but these changes were not present in 12-month-old mice. These data support an important role of ERα-mediated signaling in bone to muscle crosstalk at the biochemical level that changes with aging.

Multiscale, 3D finite element analysis using Micro-CT and confocal multiplexed images for correlation of osteocyte β-catenin signal pathway activation with predicted lacunar wall strain.

Loretta Laughrey, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences, Department of Civil and Mechanical Engineering
Nuria Lara-Castillo, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
Yixia Xie, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
David Moore, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
Mark Dallas, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
Mark Johnson, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
Sarah Dallas, University of Missouri - Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences
Thiagarajan Ganesh, University of Missouri - Kansas City, Department of Civil and Mechanical Engineering

Abstract:

It is well known that mechanical loading of bone activates β-catenin signaling pathways in osteocytes, initiating an osteogenic response. The mechanotransduction process involved is not fully understood, although it is hypothesized that strain in the lacunocanalicular walls surrounding osteocytes is correlated with osteocyte activation. We have previously shown that cyclic compression loading results in heterogeneous activation of β-catenin signaling in neighboring osteocytes which are thought to experience similar global (whole tissue) strains. Determining whether this heterogeneity is a result of heterogeneity in the local (lacunocanalicular) bone strains experienced by osteocytes will improve our understanding of the mechanisms involved in initiating the osteogenic response, possibly leading to better treatments or therapies for bone diseases like osteoporosis.

The right ulna of a TOPGAL β-galactosidase (β-Gal) LacZ reporter mouse was subjected to cyclic compression loading, and the left ulna was used as a non-loaded control. The β-Gal substrate DDAOG was used to stain activated osteocytes with the fluorescent product DDAO whose signal is related to the level of β-catenin pathway signaling activity in cells. Using the loaded bone, Micro-CT scans and 40x confocal images were analyzed to measure relative osteocyte activation levels and predict the bone strain distribution in 5 finite element models with similar FE characteristics. Strain values surrounding the lacunae were filtered to look at average strains, the average of strains greater than the overall average + 3 standard deviations, and the average of strains in the top 5% of all values. Linear regression was used to evaluate the correlation between strain and signal intensity.

DDAO signal was observed in all osteocytes in the non-loaded and loaded samples, indicating a basal level of β-catenin pathway signaling regardless of loading history. The average DDAO signal intensity in osteocytes in the non-loaded bone was 1.2×10^6 ranging from 1.2×10^4 to 2.3×10^6, while in loaded bone the average was 3.6×10^7 ranging from 3.4×10^6 to 9.1×10^7, demonstrating the anticipated increase in osteocyte activation due to loading, and heterogeneity of activation between neighboring osteocytes.

The average effective strain distribution in the 5 models was ~2500 $\mu\epsilon$, with a maximum strain of ~70,000 $\mu\epsilon$. Comparing DDAO signal intensity with average strain in FE elements near the lacunar walls (see Figure) indicated an increase of osteocyte activation with increasing strain overall. However, when strain data was filtered to include only elements with strains that were significantly higher than average, the linear regression was less likely to show a positive relationship between activation and strain. R^2 values were only significant in one

of the 5 models examined.

The results suggest that strain in the lacunar walls is not the most significant factor in determining osteocyte activation levels. A possible alternative factor that cannot be addressed by this study is the effect of fluid flow shear stress, which is also widely believed to influence osteocyte activation.

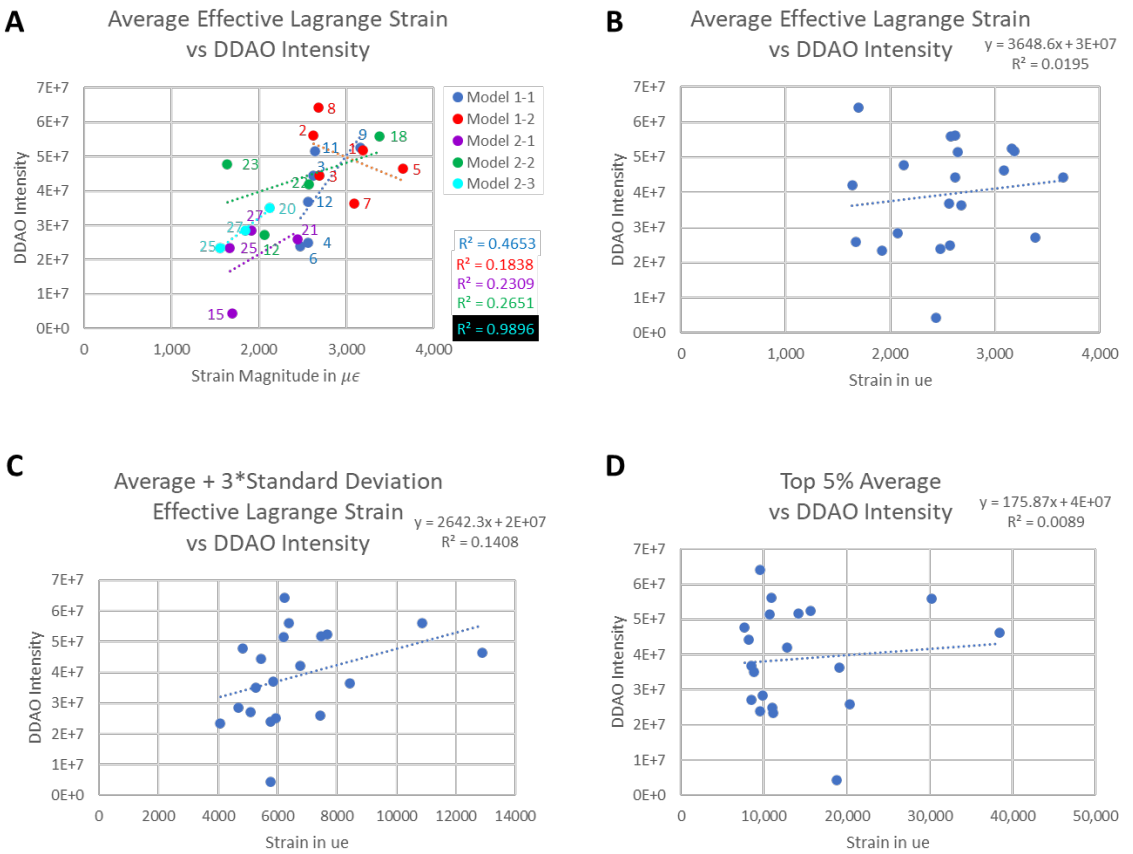


Figure: Linear regression of DDAO signal intensity (relative osteocyte activation) and effective Lagrange strain in loaded ulna. A) Average strain of elements surrounding each lacuna with each model depicted separately. Data labels indicate lacuna number. Note that overlapping models have similar results (lacunae 3, 25, and 27). Model 1-2 has a negative slope. Only model 2-3 has a significant R^2 value. B) Average strain of elements surrounding each lacuna for all 5 models. C) Filtered data representing only elements whose strain is higher than the average plus 3 standard deviations. D) Filtered data representing only elements whose strain is in the top 5% of values. Note in figures B, C, and D that the slope of the regression line lessens as elements with lower strain are eliminated from the data.

New insights into the genetic basis of the Oculo-Auriculo-Vertebral Spectrum (OAVS)

Soumya Rao[1], Kristin Watt[2], Emily Farrow[3], Lyndon Gallacher[4], Hong Ky Ho[5], Susan M. White[4,5], John Christodoulou[4,5], Tomi Pastinen[3], Tiong Y Tan[4,5], Paul A. Trainor[2], Timothy C Cox[1,6.]

[1]*Department of Oral & Craniofacial Sciences, University of Missouri-Kansas City, Kansas City, MO, USA,* [2]*Stowers Medical Research Institute, Kansas City, MO, USA,* [3]*Children's Mercy Hospital, Kansas City, MO, USA,* [4]*Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Parkville VIC Australia,* [5]*Department of Paediatrics, University of Melbourne, Parkville VIC Australia, and* [6]*Department of Pediatrics, University of Missouri-Kansas City, Kansas City, MO, USA*

Oculo-Auriculo-Vertebral Spectrum (OAVS) represents a wide range of symptoms involving three intimately related, rare disorders involving, but not limited to, eyes, ears and spine. It is recognized by its frequently asymmetric facial presentation that can variably include microtia, maxillary and mandibular hypoplasia, pre-auricular or lateral facial tags, and cervical vertebral defects. The phenotypic spectrum shares notable overlap with, but distinct from, the mandibulofacial and acrofacial dysostoses and other disorders of branchial arch development.

The prevailing view is that OAVS is genetically heterogeneous with significant environmental contributions: the most convincing being exposure to elevated levels of retinoids. Further support for disrupted retinoid signaling as a contributing factor has come from our characterization of a mouse mutant that displays an OAVS-like phenotype and carries an inversion/deletion mutation that results in elevated expression of two adjacent retinol dehydrogenase genes. In patient studies, three missense variants have been described in *MYT1* – which encodes a transcriptional suppressor of retinoid signaling - in a cohort of 225 patients. Array CGH studies have also implicated a number of loci including 14q22 (involving craniofacial genes *OTX2*, *SIX1* and *SIX6*) and 4p16 (involving *HMX1*).

In an effort to identify additional single gene and structural causes of OAVS, we have employed a combined exome- and whole genome sequencing approach on a sub-cohort of OAVS patients that were selected from a larger clinically-phenotyped cohort of 80 patients. The initial sub-cohort is comprised of 9 sporadic cases and 2 families exhibiting autosomal dominant inheritance. We have identified a number of candidate genes and will present findings using a zebrafish model of one of these candidates, which supports it as a bona fide OAVS gene.

Bruno1 Is Required Throughout Drosophila Indirect Flight Muscle Development to Regulate Dynamics Of Sarcomere Assembly And Growth

Elena Nikonova[1], Tobias Straub[2], Maria Spletter[1,3]

1. Physiological Chemistry, Biomedical Center, Ludwig-Maximilians-Universität München, München, Germany. 2. Bioinformatics Core Unit, Biomedical Center, Ludwig-Maximilians-Universität München, München, Germany. 3. Biological and Biomedical Systems, School of Science and Engineering, University of Missouri Kansas City, Kansas City, MO USA.

Abstract:
The differential expression of structural protein isoforms influences cytoskeletal assembly and contractile properties. CELF family RNA binding proteins are important regulators of RNA processing, but we do not fully understand how misregulation of CELF proteins leads to defects in sarcomere assembly, growth and function. Bruno1 (Bru1, Arrest) encodes a CELF1/2 homolog in *Drosophila* that regulates flight muscle specific alternative splicing. Here we show that Bru1 is required throughout muscle development to regulate cytoskeletal assembly and growth dynamics. During early myofibril formation before 48h APF, using both temporally-restricted RNAi knockdown and overexpression, we show that misexpression of Bru1 leads to disorganization of the actin cytoskeleton, aberrant myofiber compaction and defects in pre-myofibril formation. Transcriptomic and proteomic analyses revealed misexpression and isoform switches in diverse structural proteins regulating sarcomere growth and actomyosin interactions. Live-imaging assays confirmed aberrant contractility of *bru1* mutant myofibers. By monitoring incorporation of fluorescent actin and myosin proteins during myofibril maturation after 56h APF in *bru1* mutant IFM, we show that lateral sarcomere growth is dramatically misregulated, leading to exacerbation of pre-existing defects, myofibril fusion and formation of hollow myofibrils. A progression in the severity of cellular and molecular phenotypes from 80h APF to adult distinguishes hypercontraction from earlier growth defects, and temporally restricted rescue can partially alleviate hypercontraction in late pupal and adult stages. Taken together, our data indicate that Bru1 regulates cytoskeletal growth and remodeling throughout myogenesis, including cytoskeletal rearrangement necessary for myofibril formation as well as the balance in length versus lateral growth of the sarcomere. Defective RNA processing due to misexpression of CELF proteins thus causes wide-reaching structural defects and progressive malfunction of affected muscles.

Preliminary Results from a Novel Movement-Based Concussion Screening Tool

Jacob Thomas[1], Samantha Weiss[2], Rebecca Bliss[3], Trent M. Guess[3,4]
[1]School of Health Professions, University of Missouri, Columbia, MO
[2]Department of Engineering, University of Missouri, Columbia, MO
[3]Department of Physical Therapy, University of Missouri, Columbia, MO
[4]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

Background: Following concussion, individuals are 2-3 times more likely to sustain a subsequent lower extremity injury. Current methods used to screen for concussions are subjective and not based on movement tasks which simulate the demands of sport.
Research Question: The purpose of this study was to assess the ability of a novel movement-based concussion screening tool (MPASS) to quantify differences between individuals with and without concussion.
Methods: 17 individuals (22.59±1.66 yrs., females=12) participated in this study. Five participants suffered a concussion within 1-month before data collection. Twelve had not suffered a concussion within the past year. All participants completed the same battery of functional tasks: walking (control, serial subtraction by seven dual-task, and head-shaking), Romberg balance tests (firm-surface and foam-surface with eyes-open, eyes-closed, and eyes-closed head-shaking), and reaction time. During walking tasks, lower extremity spatiotemporal parameters were collected via Kinect depth-sensing camera. During balance tasks, a force plate recorded center of pressure and Kinect recorded center of mass. Reaction time was recorded using an Arduino-based reaction board while center of pressure was simultaneously recorded via force plate. This resulted in 133 unique variables for each participant. Principal component analysis (PCA) was used to reduce the dimensionality of the data. All statistical analyses were complete in RStudio (v4.2.0).
Results: Five principal components (PCs) were retained using Horn's Parallel Analysis for component retention. These 5 PCs explained 65.35% of dataset variance. Visual analysis of the first 3 PCs demonstrated clear separation between the two groups(concussion and control). Analysis of each variable's contribution to retained PCs revealed significant contributions from center of mass data during balance tasks, stride and step length during walking, and step length during head-shaking walking. This suggests results from these tasks are most vital in distinguishing individuals with concussion from those without concussion. However, further samples will be required before this can be determined.
Significance: Results demonstrate promising initial results for concussion screening using MPASS.
Acknowledgements: This study was funded in part by the University of Missouri Coulter Biomedical Accelerator.

References:

Howell, D. R., Buckley, T. A., Lynall, R. C., & Meehan III, W. P. (2018). Worsening dual-task gait costs after concussion and their association with subsequent sport-related injury. *Journal of neurotrauma, 35*(14), 1630-1636.

Quatman-Yates, C. C., Hunter-Giordano, A., Shimamura, K. K., Landel, R., Alsalaheen, B. A., Hanke, T. A., ... & Silverberg, N. (2020). Physical therapy evaluation and treatment after concussion/mild traumatic brain injury: clinical practice guidelines linked to the international classification of functioning, disability and health from the academy of orthopaedic physical therapy, American Academy of sports physical therapy, academy of neurologic physical therapy, and academy of pediatric physical therapy of the American Physical therapy association. *Journal of Orthopaedic & Sports Physical Therapy*, *50*(4), CPG1-CPG73.

Tibia Mechanical Loading Acutely Decreases Resting Heart Rate in Mice Likely via the Sympathetic Autonomic Nervous System

[1,2]Julian A. Vallejo, [1]Mark A. Gray, [1]Andrew Wacker, [1]Evan Peirce, [1]Jackson Klump, [1]Makayla Ayres, [2]Mark Dallas, [2]Mark L. Johnson, [1]Michael J. Wacker.
[1]University of Missouri-Kanas City, School of Medicine, Department of Biomedical Sciences
[2]University of Missouri-Kanas City, School of Dentistry, Department of Oral & Craniofacial Sciences

Bone adapts to mechanical forces by altering its overall structure and mass. Sensation of applied load in bone occurs in osteocytes via fluid flow shear stress generated throughout the lacunocanalicular system during mechanical strain. This results in the release of intracellular signaling molecules including prostaglandin E2 which ultimately leads to the activation of bone formation and suppression of bone resorption. In addition, the response to bone loading is blunted during the setting of osteoporosis and aging. Previous studies from our group have demonstrated that osteocyte-secreted factors enhance muscle contractility and improve muscle cell proliferation and differentiation. Moreover, recent reports have suggested that bone may directly regulate heart function and the acute stress response. We therefore hypothesized that bone mechanical loading would elicit an acute increase in cardiac muscle functional output. To test this hypothesis, we performed acute *in vivo* mechanical loading (2 Hz, 300 cycles, 9.25 N) on the right leg tibias of anesthetized young adult (2-6 months old) CD-1 mice while simultaneously monitoring heart depolarization/repolarization parameters, heart rate and heart rate variability (HRV) using lead II electrocardiogram (ECG). In both male and female mice, tibia loading resulted in an immediate and transient reduction in resting heart rate (0.93 fold decrease from baseline, $n=6\text{-}7$, $p<0.01$) accompanied by an increase in HRV (1.23 fold increase from baseline, $n=6\text{-}7$, $p<0.01$) which returned to baseline levels upon completion of the loading process. ECG intervals including QRS and corrected QT (QTc) were not altered during tibia loading nor during a 30 minute period subsequent to loading ($p>0.05$). The speed at which the heart responded to loading suggested that this response could be mediated neuronally. We therefore injected lidocaine (2.5mg/kg) into the hindlimb near the tibia to inhibit local neuronal afferent activity prior to loading and found that the decrease in heart rate was significantly attenuated (vehicle: 0.90 vs. lidocaine: 0.97 fold change from baseline, $n=7\text{-}8$, $p<0.05$). To further delineate the specific arm of the nervous system which mediates the heart rate changes we observed during loading, we injected mice with muscarinic acetylcholine receptor antagonist atropine (2mg/kg) or $\beta1/\beta2$ receptor antagonist propranolol (10mg/kg) and found that propranolol (vehicle: 0.88 vs. propranolol: 0.98 fold change from baseline, $n=7\text{-}8$, $p<0.05$) but not atropine (vehicle: 0.89 vs. atropine: 0.94 fold change from baseline, $n\text{-}8\text{-}11$, $p>0.05$) significantly inhibited the heart rate decrease suggesting a likely role for changes in sympathetic autonomic tone on the heart during bone loading. In conclusion, our study has uncovered a novel bone-heart neural reflex likely involving afferent neurons in the hindlimb and changes to sympathetic tone. In the future, it will be important to determine how this bone-neural-heart reflex contributes to heart physiology during the settings of exercise, osteoporosis and aging.

Serial Subtraction Dual-Task Alters Lateral Step-Down Tibiofemoral Kinematics in Healthy Adults

Sam Weiss[1], Jacob Thomas[1], Rebecca Bliss[1], Trent Guess[1]
[1]University of Missouri – Columbia

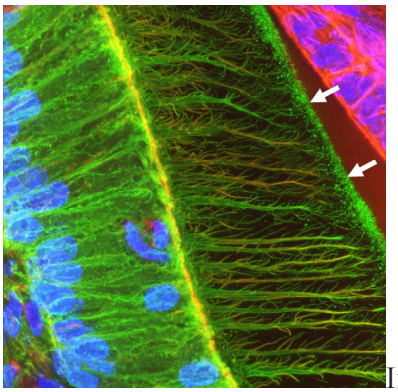
INTRODUCTION: Prior research has shown cognitive dual tasking can have negative impacts on gait[1]. Less is known regarding the effects of dual tasking on lower extremity patterns of movement. However, it is known cognitive dual tasking can lead to increased injury risk while performing dynamic limb movements[2]. Greater understanding of these injuries, and factors which may contribute to them, is necessary to improve treatment and prevention efforts. The purpose of this study was to use the Mizzou Knee Arthrometer Testing System (MKATS) to assess, one aspect of lower limb movement, differences in tibiofemoral motion related to dual tasking during activities.
METHODS: The MKATS uses an electromagnetic motion tracking system (Polhemus Patriot System, Polhemus, Colchester, VT) attached to a 3D printed housing which covers the femoral epicondyles with a separate clamp on the tibia. This configuration accurately captures knee motion across three planes of motion (frontal, sagittal, and transverse) after proper calibration determining the knee axis of rotation and anatomical axes. Following calibration, with every task, the participant performed 5 repetitions of the lateral step-down (LSD) on each leg. The first trial was performed without cognitive dual tasking. Second and third trials consisted of the Stroop test followed by Serial Sevens (SS) dual task.
RESULTS: 19 healthy individuals (22.05±1.61 yrs., 173.92±9.21 cm, 67.99±12.65 kg) participated in this study. At maximum knee flexion, significant kinematic differences were found between control and SS conditions, but not between control and Stroop. Knee abduction angle was significantly lower (right side: M = -1.35, SD = 3.54, left side: M = -2.14, SD = 2.88) during SS than during control (right: M = -1.04, SD = 3.61; left: M = -1.42, SD = 2.90). Transverse plane rotation of the left knee (M = -1.86, SD = 4.45) was externally rotated significantly higher during SS than control (M = -2.53, SD = 4.30). The transverse plane rotation of the right knee did not show that significance. No variables showed significant differences between control and Stroop conditions (all $p > 0.05$). 18/19 participants were right leg dominant.
DISCUSSION: The results indicate differences in tibiofemoral kinematics dependent on the type of cognitive dual task being done. Specifically, the SS dual task led to greater results regarding tibiofemoral kinematics while performing a LSD than the Stroop test. In addition, the nondominant left leg also yielded a greater number of significant results than the right dominant leg. This suggests an increased rate of cognitive dual tasking on nondominant limb kinematics during LSD compared to the dominant limb. More research should follow to explore more varied populations simulating other everyday dynamic movements.
ACKNOWLEDGEMENTS: Data collection possible with resources from the University of Missouri Motion Analysis Center and funding by the University of Missouri Coulter Biomedical Accelerator Program.
REFERENCES: 1. Beurskens, R., & Bock, O. (2012). 2. Imai, S., Harato, K. et.al. (2022).

Confocal and Tissue Clearing/3D Imaging in Transgenic Mice Expressing a Membrane-GFP targeted to Mineralizing Cell Types

Yixia Xie[1], David S. Moore[1], Eleanor Ray[1], Lisa Le[1], Sarah L. Dallas[1]
University of Missouri, Kansas City

High resolution 3D imaging of cells in mineralized tissues is challenging due to the light scattering properties of mineral and extracellular matrix. Imaging fine detail of dendritic cells, such as osteocytes, cementocytes and odontoblasts requires cell-specific staining methods that target the membrane or substructures within cell processes. Using transgenic mice expressing a membrane-targeted GFP driven by the dentin matrix-1 promoter (Dmp1-mGFP mice), we have used 3D confocal imaging and tissue clearing/3D imaging to examine tissue expression of the mGFP reporter and fine cellular detail of mineralizing cells in the mandible and dentition of Dmp1-mGFP transgenic mice.

Mandibles were prepared from 7-10 day and 2 month old Dmp1-mGFP mice or transgenic mice co-expressing Dmp1-mGFP and a LysM-Cre/tdTomato reporter to target osteoclasts. Samples were decalcified and 50m cryosections were counterstained with alexa555-phalloidin to visualize F-actin and DAPI to visualize nuclei. Alternatively, samples were processed intact using the PEGASOS tissue clearing method [Jing et al: Cell Res. 28(8):803-818 2018], with and without decalcification. Specimens were imaged by confocal, multiphoton or lightsheet microscopy.



In 7 day mandibles the Dmp1-mGFP transgene was expressed in osteocytes, odontoblasts, some pulpal cells adjacent to odontoblasts and a subset of late osteoblasts on the bone surface. The membrane-targeted-GFP enabled visualization of fine detail and branching of odontoblast processes (fig.1) and osteocyte dendrites. In 2 month mice, Dmp1-mGFP expression was also observed in cementocytes. The Dmp1-mGFP transgene labeled abundant extracellular vesicle (EV)-like particles (~ 80-500nm), found at the distal tips of odontoblast processes at the dentin-enamel junction (Fig 1, arrowheads) and throughout the mineralized matrix surrounding osteocytes and cementocytes, which likely represent matrix vesicles associated with initiation of mineralization. Confocal imaging of intact mandibles without tissue clearing did not allow imaging deeper than the alveolar bone. In contrast, PEGASOS clearing allowed deep tissue imaging throughout the alveolar bone and teeth, with imaging depth limited primarily by the objective working distance. Lightsheet imaging enabled 3D reconstruction of Dmp1-mGFP and LysM-Cre/td tomato reporters in the entire mandible and teeth and virtual sectioning in any plane. This also revealed expression of Dmp1-GFP in a subset of cells within blood vessels supplying the cervical loop region of the incisor, consistent with vascular pericytes.

In summary, the Dmp1-mGFP mouse is a valuable model for studying differentiation and imaging the fine structure of mineralizing cell types in craniofacial tissues and is compatible with high resolution confocal imaging, tissue clearing/3D lightsheet imaging and live cell imaging.

An Interesting Case of Anti-Mi-2-Antibody Associated with Paraneoplastic Dermatomyositis in a Patient with Cervical Cancer

Padmini Giri, MD, Rheumatology Fellow PGY4

Introduction: Dermatomyositis (DM) is a type of idiopathic inflammatory myositis (IIM). DM is often associated with malignancies. Myositis associated antibodies (MSAs), have been helpful to stratify patients with IIM and have been used as a marker for cancer associated myositis. MSAs such as anti-TIF1- γ and anti-NXP2 antibodies, are known to be associated with cancer more frequently than other MSAs. Anti-mi-2 antibodies have historically been less likely to be associated with malignancy. It is more commonly associated with classic cutaneous findings of dermatomyositis (1). Some cancers that are more commonly associated with dermatomyositis include breast, lung, rectum, and kidney (3). It is uncommon for cervical cancer to be associated with dermatomyositis. We present a case of paraneoplastic dermatomyositis with anti-mi-2 Antibodies, associated with inoperable cervical cancer.

Case: A 53-year-old female of Hispanic descent, with history of steatohepatitis and diabetes who presented to the outpatient clinic for dermatomyositis. She developed proximal muscle weakness 6 months prior which became progressive and further workup by her primary physician revealed an elevated Creatine kinase (CK) level over 5000 U/L, mildly elevated ESR 29 mm/hr and CRP 5.4 mg/dL. She was initially evaluated for rhabdomyolysis but there was no significant improvement in CK (improved to over 3000 in one month). Biopsy of the right biceps muscle active myopathic process with muscle fiber necrosis, elevated MHC-1 staining, and accentuated perifascicular atrophy, all suspicious for dermatomyositis. The patient endorsed symptoms of mechanics hands for two years prior to this presentation, for which her general practitioner reportedly was treating her with various ointments for dry/cracked skin but was unhelpful. The patient was placed on high dose, long term prednisone taper with improvement of skin findings and myopathy. Age-appropriate cancer screening was pursued, which revealed moderately differentiated cervical squamous cell carcinoma, which was deemed inoperable requiring radiation and chemotherapy. Myomarker panel demonstrated a weakly positive anti-mi-2 antibody only. Other serology was positive for ANA 1:320 with homogenous pattern. She is currently following with concerned specialties for treatment of dermatomyositis and cervical cancer.

Discussion: Dermatomyositis is an uncommon inflammatory myositis. It may present with cutaneous features and muscle inflammation. This condition is already distressing and can be debilitating if not treated promptly, but it also acts as warning signal for malignancy. Anti-mi-2-antibodies are commonly seen MSAs in DM. They are known to be associated with typical cutaneous findings and mild myositis (7). Dermatomyositis can precede neoplasm in 40% of cases; both conditions may occur together (26%) or cancer may occur first (34%). The incidence of carcinoma in association with DM varies from 15 to 34% (3). The majority of DM is idiopathic, but 15-30% may present as paraneoplastic syndromes (4). In our patient, she had features of Mechanic's Hands about 2-years prior to her overt clinical picture of DM and diagnosis of cervical cancer. Given that cervical cancer is known to be slow growing, can takes 3-5 years to progress, and generally easily treatable if caught early, it is likely that this patient had a paraneoplastic manifestation of DM a couple of years ago with the development of cervical cancer. The feature of Mechanic's Hands was not properly identified by the patient's general practitioner. It was thought to be a feature of dry skin. Her condition eventually progressed to an inoperable stage requiring treatment with Methotrexate and IVIG for DM as well as radiation and chemotherapy for cervical cancer. This case also highlights the uncommon anti-mi-2-antibody positivity in a DM patient and malignancy. There could even be a consideration for ethnicity. In a PubMed search for 'Dermatomyositis and Hispanic' turned out less

than 20 relevant results. One study (6) conducted specifically in Mexico, reported a high prevalence of anti-Mi-2 antibodies, in Mexican DM, however they related it to environmental factors, as they have also mentioned a lower prevalence of Mi-2-antibodies in Mexican Americans compared to patients in Mexico. There are large scale studies on IIM conducted in Europe and Asia, but not many in America.

Conclusion: There are very few studies that report anti-mi-2-antibodies associated with cancer. Paraneoplastic DM associated with cervical cancer is also rare. This case serves to underline the vast differences in presentation of patients with IIM and MSAs, although in some cases can be helpful for predictive outcome, can vary in different patient populations. Given that our country is fortunate to have a melting pot of ethnicities, more studies analyzing MSAs in IIM and associated development of malignancy should be pursued. More research on this topic in our own country will lead to better cancer surveillance guidelines and improved patient care in this patient population.

References:

1. Alenzi FM. Myositis Specific Autoantibodies: A Clinical Perspective. Open Access Rheumatol. 2020 Jan 14; 12:9-14. doi: 10.2147/OARRR.S231195. PMID: 32021502; PMCID: PMC6969688.
2. dos Passos Carvalho, M.I.C., Shinjo, S.K. Frequency and clinical relevance of anti-Mi-2 autoantibody in adult Brazilian patients with dermatomyositis. *Adv Rheumatol* **59**, 27 (2019). https://doi.org/10.1186/s42358-019-0071-y
3. Kumar S, Mahajan BB, Kaur S, Singh A. Paraneoplastic dermatomyositis with carcinoma cervix: a rare clinical association. Case Rep Dermatol Med. 2014; 2014:836246. doi: 10.1155/2014/836246. Epub 2014 Dec 18. PMID: 25587465; PMCID: PMC4281437.
4. Luu X, Leonard S, Joseph KA. Dermatomyositis presenting as a paraneoplastic syndrome with resolution of symptoms following surgical management of underlying breast malignancy. J Surg Case Rep. 2015 Jul 7;2015(7):rjv075. doi: 10.1093/jscr/rjv075. PMID: 26157052; PMCID: PMC4495251.
5. Pacheco M, Ferreira H, Silva C, Silva J, Matias E, Antunes T, Almeida JS. Antinuclear Antibody (ANA) and Anti-Mi-2-Alpha Positive Dermatomyositis Hinting a Cancer Diagnosis. Cureus. 2022 Feb 2;14(2):e21844. doi: 10.7759/cureus.21844. PMID: 35291538; PMCID: PMC8896541.
6. Petri, M.H., Satoh, M., Martin-Marquez, B.T. *et al.* Implications in the difference of anti-Mi-2 and -p155/140 autoantibody prevalence in two dermatomyositis cohorts from Mexico City and Guadalajara. *Arthritis Res Ther* **15**, R48 (2013). https://doi.org/10.1186/ar4207
7. Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EK. A Comprehensive Overview on Myositis-Specific Antibodies: New and Old Biomarkers in Idiopathic Inflammatory Myopathy. Clin Rev Allergy Immunol. 2017 Feb;52(1):1-19. doi: 10.1007/s12016-015-8510-y. PMID: 26424665; PMCID: PMC5828023.
8. Yang, H., Peng, Q., Yin, L. *et al.* Identification of multiple cancer-associated myositis-specific autoantibodies in idiopathic inflammatory myopathies: a large longitudinal cohort study. *Arthritis Res Ther* **19**, 259 (2017). https://doi.org/10.1186/s13075-017-1469-8

Maturation-based Prediction of Craniofacial Growth

Richard J. Sherwood[1,2,3], Dana L. Duren[2], Kevin M. Middleton[4]

[1]Craniofacial Research Center, University of Missouri School of Medicine, Columbia, MO 65212
[2]Dept. of Orthopaedic Surgery, University of Missouri School of Medicine, Columbia, MO 65212
[3]Dept. of Orthodontics, School of Dental Medicine, Case Western Reserve University, Cleveland, OH 44106
[4]Division of Biological Sciences, University of Missouri, Columbia, MO 65211

Objectives: To examine differences in timing of ontogenetic parameters for craniofacial measures in untreated pediatric subjects, age 3 to 25 years, using chronological age and skeletal age.
Methods: Double logistic models of craniofacial growth were fit to 158 male and 145 female participants of the Fels Longitudinal Study (Yellow Springs, OH) with longitudinal lateral cephalograms (minimum 5 time points/participant) and matching skeletal maturity assessments from hand/wrist radiographs. Separate models were fit based on chronological age and skeletal age. Age at peak growth velocity (aPGV) was calculated for these individuals based on their individual growth velocity curves (first derivative of the predicted trait measure).
Results: Comparison of the two models clearly demonstrate how prediction of the timing of aPGV is improved by the use of skeletal age rather than chronological age. When chronological age is used, aPGV has a 95% CI of 8.9-13.1 years (females) and 11.1-15.1 years (male). When skeletal age is used aPGV has a 95% CI of 10.05-13.3 years (females) and 13.41-14.2 years (male). These maturity estimates effectively shift the "age" axis for each individual allowing it better match with the patient's actual pace of growth. The consideration of skeletal age in place of chronological age when evaluating growth could have a distinct clinical advantage resulting in optimized treatment timing and reduced patient (and physician) burden.
Conclusions: The discovery of measurable differences in growth trajectories in children demonstrating different patterns of skeletal maturity continues our long-term aim of providing individualized predictions of craniofacial growth, ultimately improving decisions in treatment timing.

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Extracellular Vesicle-Mediated Communication Between Cells in Bone

Kun Wang[1], Andrew Keightley[1], LeAnn Tiede-Lewis[1], Lynda Bonewald[1], Sarah Dallas[1]
[1]University of Missouri-Kansas City, [2]University of Vermont; [3]University of Kansas Medical Center, USA

Abstract
Cell shedding of exosomes/extracellular vesicles (EV) provides an important mechanism for cell-cell communication. These EV deliver their cargo of proteins, mRNAs and miRNAs to target cells, thereby altering their differentiation and function. Using transgenic mice expressing a membrane targeted GFP in osteocytes, we have previously shown that osteocytes and other mineralizing cell types deposit EV in the bone matrix and that GFP-positive EV can be found in the circulation. EV from osteocyte-enriched IDG-SW3 cells or primary calvarial cells promoted osteoblast-to-osteocyte differentiation and their cargo was altered by treatment with the bone regulatory hormone, PTH. To further characterize the cargo of osteocyte EV and their function in bone, multiple approaches were used. Western blotting, combined with proteomic analysis of EVs from osteocyte-enriched IDG-SW3 cells by LC/MS/MS using Tandem Mass Tag labeling technology revealed a proteome of > 3000 proteins that was enriched for exosome markers CD81, ALIX, and RAB5. The osteocyte EV contained all of the top 20 and 93 of the top 100 exosome markers listed in the Exocarta database. Osteocyte markers PHEX, MEPE, E11, RANKL and sclerostin were present in the EV as well as proteins playing a role in biomineralization, membrane fusion/exocytosis, motility/neurite outgrowth, extracellular matrix assembly, and Wnt/β-catenin, FGF and TGFβ signaling. Treatment of undifferentiated IDG-SW3 cells with EV from osteocyte enriched IDG-SW3 cell cultures induced expression of early osteocyte marker genes and induced mineralization, suggesting that they promote osteoblast to osteocyte transition and have overlapping functions with matrix vesicles, known to play a role in bone mineralization. EV from PTH-treated IDG-SW3 cells also induced osteoclast formation in whole marrow cell cultures. Live cell and intravital imaging showed that osteocytes shed EV from their cell membrane and dendrite tips and that osteocytes adjacent to blood vessels may release EV into the circulation. Together, our data suggest that EV shed by osteocytes may play an important role in regulation of both osteoblast and osteoclast function in bone and may potentially communicate with distant target cells via the circulation to regulate their function.