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TITLE: Restless Legs Syndrome and the Melanocortin System

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CONTRACTING ORGANIZATION: Yale University

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14. ABSTRACT

The objective of this proposal is to study the underlying biological mechanisms of **restless legs syndrome (RLS)**. We propose to study the hormonal **melanocortin (MC)** system in humans with RLS, based upon similarities between the MC hormones and features of RLS. Central to RLS are an urge to move associated with sensory discomfort and increased movement to alleviate symptoms. The MC hormones are well known to mediate increased locomotion and pain sensitivity in rats and cause motor restlessness when administered in humans. In this proposal, we will evaluate MC biology in humans by measuring MC hormone levels in blood and CSF of persons with RLS compared to persons without RLS. We hypothesize that MC hormone levels will be higher in persons with RLS and will correlate to the severity of RLS.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

The biological mechanisms underlying restless legs syndrome (RLS) are largely unknown. The melanocortin (MC) hormones are a family of hormones produced in the brain which modulate numerous essential life functions such as metabolism, sleep, eating, movement, and mood. Many properties of these hormones bear resemblance to core features of RLS, such that the hormones given to rodents produce excessive movement and a state of hyperalgesia, similar to the central sensorimotor hallmark of RLS. Furthermore, the hormones are released in a diurnal pattern and have negative effects on mood, similar to the nighttime character of RLS and that RLS is tightly tied with depression. Also, the main MC agonist, alpha-melanocyte stimulating hormone, administered intravenously in humans causes an intense feeling of motor restlessness. Based upon these lines of evidence, our central hypothesis is that the MC hormone levels are higher in the serum and cerebrospinal fluid of persons with RLS than non-RLS controls. The purpose of this research project is to measure levels of MC hormones in the blood and CSF of persons with RLS and controls without RLS and in the RLS cohort, to determine if MCn hormone levels correlate to the severity of RLS.

2. Keywords

Restless legs syndrome, RLS; hormones, melanocortin, sleep, cerebrospinal fluid

3. Accomplishments

Statement of Work

4. **Specific Aim 1: To evaluate MC biology by measuring α-MSH, ACTH, and POMC in serum and CSF of RLS patients and age-sex matched controls in the nighttime.** **<u>Specific Aim 2:</u> To characterize RLS by measuring severity of RLS symptoms, physiology, and neurobehavior; and to correlate these changes to MC levels in RLS patients.**	**Timeline**	**Personnel**
Major Task 1: Complete Regulatory Requirements for Study and Administrative Tasks	Months	
DoD award-specific IRB protocol (submitted 10/16/19)	1	NA
HRPO approval	2-4	
Submitting ammendments, adverse events, protocol deviations	1-24	Koo
Annual IRB Continuing review	1-24	Koo
Major Task 2: Set up Infrastructure for Research		
Admitting research subjects to Hospital Research unit: Dr. Koo has set up	Done	

protocol for admitting subjects and several research subjects have already successfully gone through the protocol		Koo
Blood draw and lumbar puncture infrastructure	Done	Koo
Sleep and behavior assessment infrastructure	Done	Koo
Laboratory assays for measuring hormones: Dr. Koo has worked closely with Yale's core lab and also the lab of Dr. Sabrina Diano to optimize assays to measure hormones	Done	Koo/Diano
Major Task 3: Research implementation		
Recruiting research subjects	1-20	Koo/team
Biospecimen processing	1-20	Koo/team
Hormone measurements	6-24	Koo/Diano Wardlaw
Data collection and handling	1-24	Koo/team
Interim data analysis	13-15	Li/Koo
Final data analysis	20-24	Li/Koo
Manuscript preparation and completion	23-24	Koo

	Year 1				**Year 2**			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Site 1	8	8	8	8	8	8	8	
Site 2		4	4	4	4	4	4	
Target Enrollment (cumulative)	**8**	**20**	**32**	**44**	**56**	**68**	**80**	

Major task #1, completing regulatory and administrative requirements for the study, and #2, setting up research infrastructure, have been completed. Within the first month of the award, a Yale IRB protocol specific to this DoD grant was approved. Much of the research infrastructure had been set up in advance of this award period, but minor changes were instituted in the first 1-2 months of the award.

Our recruitment goal is to include 40 persons with RLS and 40 controls over 2 years. This recruitment has been somewhat slowed by the Covid pandemic and as a result we were able to get a 1 year no-cost extension for this award.

To date, starting from December of 2019, 29 persons with RLS and 28 non-RLS controls have completed our research protocol. We were able to get CSF in 25 subjects with RLS and 21 non-RLS controls. In these subjects, we have blood from 3 times points, CSF, and data regarding sleep, leg movements during sleep, and RLS severity. We have carried out measurement of hormones, including POMC, ACTH, alpha MSH,

beta endorphin, and beta MSH for many of these samples. We are working with Sharon Wardlaw to complete remaining CSF sample measurement for POMC, beta MSH and beta endorphin in early 2022.

We have also been working with the Yale Center for Molecular Discovery to create separate viable cell lines which express either MC3 or MC4 receptors and now have both these cell lines. We are in the process of testing these cell lines against known standard of MC hormone (alpha-melanocyte stimulating hormone).

5. Impact

This research is critical as the biological mechanisms underlying RLS are not known. Our poor knowledge of RLS pathobiology is reflected in the problems which have arisen due the widespread use of dopamine medications to treat RLS. RLS is exquisitely responsive to dopamine medications initially, but over time, usually years, and as doses escalate, the dopamine medicines commonly cause a paradoxical worsening of RLS, termed augmentation. Given the initial exquisite response of RLS to dopamine agonist medication, it was thought early on that dopamine was deficient in RLS, but more recent evidence has shown that dopamine is actually in excess in those with RLS. The MC hormone system has not been studied to any degree in humans with RLS. If found to be involved in the pathophysiology of RLS, the MC system would represent a novel target for more precisely directed and hopefully effective treatments for RLS.

6. Changes/Problems

Our recruitment is a little more than half of what we projected. We have underperformed largely because of the Covid-19 pandemic. Non-essential clinical research was placed on hold from the months of March through mid-September 2020 at Yale University. In addition, even while allowed to continue with clinical research in recent months, we were only able to recruit persons from CT (Yale guidelines) until early in 2021. The majority of our subjects with RLS actually come from NY, so this was a major setback. More recently, we have been able to include persons outside of CT which has helped. We are interested in getting samples from 40 RLS and 40 controls including CSF. Given that not all LPs are successful, we believe that we will have to recruit about 20 more RLS subjects and 25 more control subjects which we are confident that we will complete in the next year.

7. Products

Nothing to report

8. Participants & Other Collaborating Organizations

Brian Koo: PI

Dr. Koo is a co-investigator on the VA grant I01 HX002324-01A2 (PI Sico), Addressing Sleep Apnea Post-Stroke for 1.2 calendar months per year (10/1/2020 – 9/30/2025).

Dr. Koo is a collaborator on a Michael J Fox grant (PI Hafler), Tracing Origin of Parkinson's Disease through Neuro-Immune Interactome, for 1.2 calendar months per year (10/1/2020 – 9/30/2023).

Jung Dae Kim has since left Yale University and will not be receiving salary support from this grant.

9. Special Reporting Requirements

Nothing to report

10. Appendices

Nothing to report

11. Adverse Events and Medical Monitor

We have had no adverse events to report. Our medical monitor is Sule Tinaz.