A murine model of CCSVI is associated with mild but significant impairment of gait as assessed by neurobehavioral testing

Authors & Affiliation: *Porama Thanaporn, Nicholas Dantzker, Jerry Lee, Evan Shannon, Joerg Herold, Janet Okogba, Michael Dake, John Cooke Stanford University School of Medicine*

Introduction: A murine model of chronic cerebrospinal venous insufficiency (CCSVI) would greatly accelerate investigations into this newly described disease and would serve as a platform for understanding the pathophysiology of CCSVI and the development of endovascular therapies as well as immunomodulatory treatments. Here we propose a model for CCSVI in C57/Bl6 mice.

Materials & Methods: Institutional protocol approvals were obtained prior to initiation to ensure compliance with APLAC regulations and guidelines. Intensive animal husbandry was implemented given potential for marked morbidity and mortality. Animals were monitored daily. C57/Bl6 mice aged 15-16 weeks underwent bilateral external jugular vein ligation or sham operation while under isoflurane anesthesia. Sham mice underwent exposure and manipulation of the neck vessels but did not undergo ligation. Subsequently, the mice had weekly neurobehavioral testing including Rotarod, gait analysis, and footfault performed during the dark cycle. Observers were blinded to assigned treatment arm.

Quantitative gait analysis was performed with the Catwalk apparatus and software. Gait analysis included swing speed, stand index, standing time, duty cycle, footprint contact area, footprint contact time, and stride length. Footfault determination was made with horizontal ladder (Columbus Instruments). Rotarod was performed weekly using a 4-40 rpm accelerating protocol over 5 minutes.

Results: No mice developed gross neurological defects after surgerization. There were no observed cases of retinal vein occlusion within the test group. On the Catwalk, there were no significant differences between the two male groups in observed changes in hindlimb duty cycle. By six weeks right hindlimb swing speed was significantly increased in ligated animals versus unligated controls (mean swing speed 452.0 mm/sec vs. 305.4 mm/sec at Week 6, p=0.0006 by two-way, repeated measures ANOVA, n=5

for each group, Bonferroni correction for significance p<0.00135 for 37 different comparisons). Left hindlimb swingspeed was also significantly increased (mean swing speed 488.5 mm/sec vs 332.8 mm/sec at Week 6, p=0.0004 by two way, repeated measures ANOVA, n=5 for each group). Other measures of quantitative gait analysis assessed by Catwalk were not found to be significantly different in animals having undergone bilateral jugular vein ligation. Footfault approached statistical significance in males that underwent ligation as compared to sham males (p=0.067, n=5). Decrease in Rotarod times in ligated mice was not significantly different in comparison to sham controls.

Discussion & Conclusion: This model for CCSVI demonstrates small functional differences that are seen between ligated and unligated animals that occur within one week of ligation and persisted for the study period. Further investigation is needed to determine the contribution of chronic cerebrospinal venous insufficiency to morbidity.

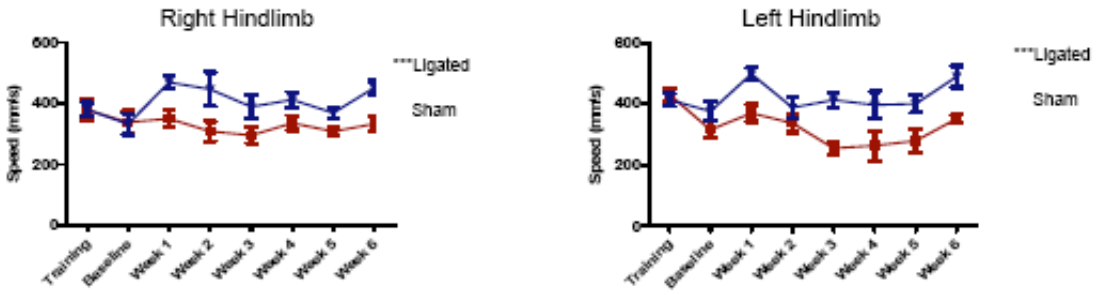

Figure 1. Swingspeed of right and left hindlimb as assessed by gait analysis. Differences appeared within one week of bilateral external jugular ligation and were maintained until completion of testing at Week 6. ***p=0.0006 for right hindlimb and ***p=0.0004 for left hindlimb swingspeed.