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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are second-year into the three-years of our research program into the immunogenetics and drug exposure factors that contribute to chronic pain following spinal cord injury. Administrative advances made in the second year for Study 1 Site 2 (NSW) include submitting a full NEAF after the review of the initial Low – Negligible Risk (LNR) admission was considered to not contain enough information for full review by Human Ethics Research Committee in NSW. Recruitment for Study 1 Site 1 (Royal Adelaide Hospital) includes 173 study invitations letters sent out, 37 consent forms returned & sample/questionnaire mail-outs, 26 buccal samples collected & DNA genotyping completed, 26 participant medical history reports entered into database. We anticipate a quick response from HERC-NSW regarding the NEAF to then begin recruitment from Site 2 in the next 1-2 months.
Study 2: We have initiated study 2 recruitment, screening and testing and data collection in underway.

15. SUBJECT TERMS
Spinal Cord Injury, Immunogenetics, Chronic pain, Opioids

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INTRODUCTION

Spinal cord injury results in significant trauma and inflammation originating at the site of injury as well as from various systemic anatomical compartments. This inflammatory event provides both beneficial activation of repair and clearance systems, but also creates long-term detrimental consequences such as chronic pain. Chronic pain develops after spinal cord injury in more than 65% of the clinical population[1]. However, the reasons why some patients develop chronic pain and others do not remains unknown. Chronic neuropathic pain elicits a number of changes in the activity, properties and transmitter content of pain-pathway neurons[2]. This central sensitization to nociceptive stimuli culminates in profound debilitating pain that serves no adaptive purpose for the sufferer. It is now established that spinal inflammatory events resulting from numerous stimuli initiate and maintain chronic neuropathic pain conditions[3] and may result from a dysregulation of the spinal immunocompetent cells, glia, and their up regulation of pronociceptive (pain) systems. A key event in the initiation of this inflammatory response is the activation of the innate immune system pattern recognition receptor, Toll Like Receptor 4 (TLR4)[4,5]. TLR4 is able to detect the presence of endogenous danger molecular patterns, resulting in the activation of an inflammatory cascade that results in the expression and release of a myriad of inflammatory signals such as proinflammatory cytokines, chemokines, prostaglandins, reactive oxygen species and nitric oxide. Importantly, these same proinflammatory molecular signals also elicit a pronociceptive, or painful, response that contributes indelibly to the chronic pain state[5]. The prototypic opioid, morphine, is capable of TLR4-mediated proinflammation[6-8]. As such, exposure to morphine at the time of injury may result in exacerbated proinflammation and hence produce long-term consequences for the pain susceptibility of the individual. In addition, the immune genes that encode these key inflammatory mediators are highly polymorphic. Hence, an individual may have a genetic predisposition to over respond in a proinflammatory fashion to the spinal cord injury, and/or to experience inflammation in response to opioid exposure. Critically, this genetic variability may significantly impact the long-term health and quality of life of the individual. Thus both genetics and drug exposure at the time of injury may be contributing factors individually and/or interactively that may lead some individuals to develop chronic pain following injury, or may protect others from developing pain pathology. Hence, this project will investigate the impact of both pharmacological agents and genetic variability on the occurrence of chronic pain following spinal cord injury.

BODY

The research management team (Prof Hutchinson, Dr Coller & Dr Clarke) and met between fortnightly to monthly during year 3 of the research project to ensure administrative progress.

Our professional clinical research team has continued in their efforts of subject recruitment, screening and testing. Ms Vicky Staikopoulos is our highly experienced technical research associate (working 3 days a week) who is working on the laboratory side of the team to prepare our subject mail-outs, process DNA samples and receive data back from our analysis center. Vicky has also taken a leadership role in managing the human ethics for our Royal North Shore Hospital (RNSH) recruitment site. This has been a very challenging role owing to significant administrative delays as the RNSH

transitioned to a new national human ethics approval system. This was subsequently compounded by administrative mistakes at the RNSH ethics side out of Vicky's or our control.

Ms Francesca Zappia (nee Alvaro) and Ms Kathy Heyman form the hands on part of our clinical team who are engaged at the Hospital and the Rehabilitation Centre. This has now expanded during year 3 to liaising with the staff at the RNSH to facilitate our expanded subject recruitment drive. Francesca and Kathy liaise with Dr Marshall and Dr Clarke to coordinate the subject recruitment and consenting in South Australia; and with Prof Middleton for activities at the RNSH. Kathy is a registered nurse and Francesca has many years clinical trials experience. Kathy and Francesca have continued the job sharing arrangement at approximately a 3-4 day load during year 3.

This staffing arrangement has continued to work well during year 3 to ensure a balance of strict patient information confidentiality at the hospital side and timely outcomes at the lab side. All staff and investigators have completed NIH Human Research training and their details and credentials have been passed onto the Human Research Protection Office.

STUDY 1

Human research ethics for Study 1 Site 1 (Royal Adelaide Hospital : approval no. 111008) and approved by the Human Research Protection Office (16986.1) has been maintained with required periodic RAH HREC reporting and associated HRPO notifications. Study 1 Site 2 ethics was approved (Royal North Shore Hospital : approval no. SSA/14/HAWKE/71; 1311-372M) and included under HRPO 16986.1. These approvals were received during year 3 after unacceptable delays at the RNSH HREC. The process to receive approval took over 20 months. As outlined previously, these delays were brought about by a change in HREC system and several errors at the RNSH HREC administrative side. These were unavoidable issues as our research team complied with all requirements and resulted in extreme frustration for our staff.

Below is a summary of all the communications with the HREC about Study 1.

Study 1 RAH approval #111008
Study 1 RNSH approval #1311-372M

Initial approval 13/10/2011

- Protocol version 2 (12/10/11)
- PIS-ICF version 2 (12/10/11)

Approval 14/2/2012

- Study protocol, Version 3 dated 26 November 2012
- Telephone screening questions, Version 3, dated 26 November 2012
- Participant questionnaire, Version 3, dated 26 November 2012
- Study Invitation letter, Version 3, dated 13 August 2012

Approval 13/8/2012

- Advertisement, Version 1, dated12 JULY 2012.
- Study contact details for participants dated12 JULY 2012.

Approval 15/10/2012

- Advertisement, Version 2, dated10 October 2012

Approval 12/12/2012

- Participant Information Sheet and Consent form, Version 4, dated13 November 2012
- Note to File #1 Administrative change to the Participant Information Sheet and Consent form Version 3 dated the 6th of February 2012

Approval 26/8/2013

- Access "SNAP" data base for identification of potential participants

Approval 1/10/2013

- Study Advertisement Version 3 dated 27th of September 2013 CURRENT

Approval 11/4/2014

- Northern Sydney Health District HREC approval letter dated 19 December 2013
- Study protocol, SC1.001 – Version 4 dated 21 August 2013, tracked and clean copies
- Participant Questionnaire – SCI.001 – South Australian Version 4 dated 11 March 2014, tracked and clean copies
- Cover letter to participants for the collection of buccal swab samples – SCI.001, Version 1 dated 21 August 2013
- Information Sheet to participants for the collection of buccal swab samples – SCI.001, Version 1 dated 21 August 2013
- Participant Information Sheet and Informed Consent Form, South Australian Version 5 dated 11 March 2014 – tracked and clean copies
- Study Invitation Letter South Australian Version 4 11 March 2014 Clean and tracked copies

Approval 17/4/2014

- Study protocol, SC1.001 – Version 5 dated 8 April 2014 CURRENT
- Participant Questionnaire – SCI.001 – South Australian Version 4 dated 11 March 2014 CURRENT
- Cover letter to participants for the collection of buccal swab samples – SCI.001, Version 1 dated 21 August 2013 CURRENT
- Information Sheet to participants for the collection of buccal swab samples – SCI.001, Version 1 dated 21 August 2013 CURRENT
- Participant Information Sheet and Informed Consent Form, South Australian Version 6 dated 8 April 2014 CURRENT
- Study Invitation Letter South Australian Version 4 11 March 2014 CURRENT

Approval 21/08/2014

- Participant Consent Form (Reason for declining to partake in the study), South Australian Version 1 dated 29 July 2014 CURRENT

The clinical team at Site 1 (Hampstead Rehabilitation Centre, Royal Adelaide Hospital)

has identified over 800 patients in their database who fulfill the inclusion criteria for Study 1. They have been processing 20-30 recruitment and questionnaire/sample mail-outs per week. To date, recruitment from the RAH site remains lower than hoped as detailed in our request for an extension without funds. We are continuing to make follow-up phone calls to those participants who have been sent study invitations letters but have not responded, in the anticipation of increasing the participation rate of those initially contacted. Additionally, we have begun a parallel exercise to understand why we have experienced such low response rates compared to our historical recruiting successes in this patient population.

Now that Site 2 at RNSH has come on line we have begun sending out during September, 100 recruitment information packs a week to a list of approximately 800 to 1200 eligible spinal cord injury patients on their records. The clinical team has already begun receiving the phone calls from interested subjects.

Buccal sample processing and DNA genotyping has continued as the samples have been returned by the participants. This data has been entered into our database, along with the audit entries from the participant's medical histories.

Parallel to these efforts in the spinal cord injury patient population we have expanded the collection of the DNA samples from the healthy control reference sample population.

To proactively address these recruitment issues, the Investigator team have used aggressive marketing techniques in the past months, within Human Research Ethics Committee (HREC) approval guidelines, including a national TV, radio and print news presence that had an estimated reach of over 1.4 million people (equivalent to AUD350k free advertising). This national media presence was specifically timed to occur along side the initiation of the RNSH recruitment efforts. This has spurred significant new interest in the study and is resulting in new recruitment. As such we have requested an extension without funds (EWOF) for this award to enable us to complete our recruitment initiatives.

STUDY 2

Study 2 institutional Ethics approval has been maintained (Approval no. 111035) and appropriate Human Research Protection Office (HRPO) notifications have continued (16986.2). Study 2 recruitment has also been slow, mirroring the issues experienced in Study 1. However, we have continued screening and testing spinal cord injury subjects. As these patients have been recruited the healthy control and spinally intact chronic pain patients have been recruited. An additional unforeseen issue is the relatively recent increase in opioid use amongst the spinal cord injury population, which has excluded several promising participants from the study.

Below is a summary of all the communications with the HREC about Study 2.

Study 2 RAH approval #111035

Approval 8 October 2012

- Protocol SCI.002, Version 3 (24 September 2012)
- Participant Information Sheet & Informed Consent Form - Spinal Cord Injury,

Version 3 (24 September 2012) CURRENT
- Participant Information Sheet & Informed Consent Form - Chronic Pain, Version 3 (24 September 2012) CURRENT
- Participant Information Sheet & Informed Consent Form - Healthy Participants, Version 3 (24 September 2012) CURRENT
- Spinal Cord Injury Patient Study Invitation Letter (24 September 2012) CURRENT
- Chronic Pain Patient Study Invitation Letter (24 September 2012) CURRENT
- Healthy Control Study Invitation Letter (24 September 2012) CURRENT
- Spinal Cord Injury Patient Questionnaire Q4 September 2012)

- Chronic Pain Patient Questionnaire Q4 September 2012
- Treatment Algorithm for Autonomic Dysreflexia (Hypertensive Crisis) in Spinal Cord (10 April2006) CURRENT
- Treatment Algorithm for Autonomic Dysreflexia (Hypertensive Crisis) in Spinal Cord Injury (2010) CURRENT

Approval 20/2/2013
- Protocol Version 4 dated 12 February 2013

Approval 14/8/2013
- Protocol Version 4 – Administrative change - dated 12 February 2013 CURRENT

Approval 26/8/2013
- Access "SNAP" data base for identification of potential participants

Approval 09/04/2014
- SCI-002 Participant Questionnaire South Australian Version 2; 25MAR2014 CURRENT
- The processing data pipeline has been constructed and is being employed in all data collection to date.

KEY RESEARCH ACCOMPLISHMENTS

Human research ethics approval maintained and obtained for Study 1 at 2 sites.
Human research ethics approval maintained for Study 2.
Ongoing recruitment efforts combined with significant national media presence promoting the study.

REPORTABLE OUTCOMES

Prof Hutchinson
- Keynote presentation at the Faculty of Pain Medicine Spring meeting October 2013, Byron Bay
- Parliamentary presentation during SMP2014, March 2014, Canberra
- Invited Topical workshop presentation at AIM2014, March 2014, Sydney
- Symposia presentation at Australian Pain Society, March 2014, Hobart
- Invited seminar presentation at University of WA, Perkins, July 2014, Perth
- Invited Keynote at a Medical Education Event, Pfizer, July 2014, Perth
- Invited seminar presentation at University of Melbourne, August 2014, Melbourne

- Invited Keynote at ANZSPM, Sept 2014, Gold Coast
- Invited Trainee event at ANZSPM, Sept 2014, Gold Coast
- Invited Keynote at ANZLAA, Sept 2014, Adelaide
- Invited Keynote at ANZSOM, Oct 2014, Adelaide

Dr Coller

- Symposia presentation at Australian Pain Society 2014, March Hobart

Dr Clarke's work related to the project but not funded by the work. Oral presentations acknowledged the collective funding sources including CDMRP.

- Clark J, Marshall R, Sharkey D. Prognostic value of neutrophil to leukocyte ratio and cytokine signatures in patients presenting with spinal cord injury (SCI) JCSM Vol 20 Suppl 1:12
- Clark J, Marshall R, Sharkey D. Prognostic value of neutrophil to leukocyte ratio and cytokine signatures in patients presenting with spinal cord injury (SCI) 40th Annual Scientific Meeting of the American Spinal Injury Association May 14th-17th, 2014, San Antonio, Texas, USA
- Clark J, Marshall R, Sharkey D. Prognostic value of neutrophil to leukocyte ratio and cytokine signatures in patients presenting with spinal cord injury (SCI) 14th Annual Adelaide Spinal Research Centre Meeting, Adelaide, South Australia
- Clark J, Marshall R, Sharkey D. Prognostic value of neutrophil to leukocyte ratio and cytokine signatures in patients presenting with spinal cord injury (SCI) 5th Australian Neurotrauma Symposium 2014, Oct 16-18th, Adelaide, South Australia ECR Award Finalist
- Marshall R, Clark JM, Dunlop SA, Galea MP The International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI): Consensus between Expert Examiners and Clinicians 53rd International Spinal Cord Society Annual Scientific Meeting, Sept 2014, Mastrich, Netherlands.
- Marshall R, Clark JM, Dunlop SA, Galea MP The International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI): Consensus between Expert Examiners and Clinicians. 14th Annual Adelaide Spinal Research Centre Meeting, Adelaide, South Australia
- Clark J, Marshall R, Sharkey D, Wilkinson M, Clifton-Bligh R. Evidence for altered bone and skeletal muscle interactions in spinal cord injured (SCI) patients 40th Annual Scientific Meeting of the American Spinal Injury Association May 14th- 17th, 2014, San Antonio, Texas, USA – Award Eligible Paper
- Clark J, Marshall R, Sharkey D, Wilkinson M, Clifton-Bligh R. Evidence for altered bone and skeletal muscle interactions in spinal cord injured (SCI) patients JCSM Vol 20 Suppl 1:56-57

CONCLUSION

Since the awarding of this grant, publications of additional parallel basic mechanistic research by our group and others around the world continues to support the core hypothesis underlying this research program, exposure to drugs, such as opioid analgesics at the time of spinal cord injury may result in profound alterations in the proinflammatory environment within the central nervous system that may produce long-term consequences for the pain susceptibility of the individual.

Thus, genetic polymorphisms in the immune genes responsible for this immune response may have a genetic predisposition to over respond in a proinflammatory fashion to the spinal cord injury and/or to experience inflammation in response to opioid exposure. Critically, this genetic variability may significantly impact the long-term health and quality of life of the individual. Thus both genetics and drug exposure at the time of injury may be contributing factors individually and/or interactively that lead some individuals to develop chronic pain following injury or may protect others from developing a pain pathology. The data collected from the 2 studies being carried out here will provide critical foundational clinical evidence to support these hypotheses.

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APPENDICES n/a