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14. ABSTRACT

We are in the 5th year of our research program into the immunogenetics and drug exposure factors that contribute to chronic pain following spinal cord injury. Recruitment for Study 1 has continued to be slow. However, we have continued to progress with the expansion of Study 1 recruitment sites by broadening our collaborative network. Owing to the delay in recruitment an extension without funds request has recently been submitted as has a revised statement of work.

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 is hypothesised 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 is investigating the impact of both pharmacological agents and genetic variability on the occurrence of chronic pain following spinal cord injury.

KEYWORDS
Pain, spinal cord injury, opioid, glia, innate immunology, single nucleotide polymorphism, genetics

ACCOMPLISHMENTS

The research management team (Prof Hutchinson, Dr Coller & Dr Clarke), laboratory research (Vicky Staikopoulos) and the clinical unit team (Kathy Heyman & Francesca Zappia) met between fortnightly to monthly during year 6 of the research project to ensure administrative and recruitment progress. Francesca Zappia has worked tirelessly to drive recruitment and facilitate the addition of a new site Austin Health in Victoria Australia. Significant delays were experienced in receiving approval from CDMRP to allow us to start the recruitment from Victorian site. We received approval to continue the trial on the 13 of February 2017 (as there was some hold up for funding), and a reply to our request for a new site on the 11 of April but we did not get final approval until the 8th of September 2017. This means that much of this year has been lost to hold ups in the HRPO process that was out of our control. Since this time, we have already recruited 18 people from the new Austin Health Victorian site and we are working overtime to get new subjects into the project. Prof Nunn and his professional team are working very well in this role to support our recruitment efforts.

Our professional clinical research team has continued in their efforts of subject recruitment, screening and testing at the other sites. They have continued to liaise with the spinal cord injury unit at site 1 (Royal Adelaide Hospital) to get new spinal cord injury patient information. Ms Vicky Staikopoulos is our highly experienced technical research associate who is working on the laboratory side of the team to process DNA samples and receive data back from our analysis center. Vicky also has the leadership role in managing the human ethics for our Royal North Shore Hospital (RNSH) recruitment site. This continues to be a very challenging role owing to significant administrative delays at the RNSH. Dr Coller and Francesca Zappia have taken a joint role in preparing our subject mail-outs and direct phone contact.

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. As highlighted previously we expanded our recruitment sites during year 6 to 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 of clinical trials experience. Kathy as a registered nurse has taken a leadership role in reviewing the participants medical records.

This staffing arrangement has continued to work well during year 6 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 (16986.1a) has been maintained with required periodic RAH HREC reporting and associated HRPO notifications. The Royal Adelaide Hospital Ethics Committee has approved the addition a new site, Site 3 Austin Health in Victoria Australia. The Research Governance Office of the Austin Health site has finally been approved after the delays outlined above. Study 1 Site 2 ethics has maintained approval (Royal North Shore Hospital, in New South Wales, Australia: approval no. SSA/14/HAWKE/71; 1311-372M) and included under HRPO 16986.1b.

The clinical team at Site 1 (Hampstead Rehabilitation Centre, Royal Adelaide Hospital) has identified over 900 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 month. To date, recruitment from the RAH site remains considerably lower than anticipated as detailed in our request for an extension without funds and revised statement of work documentation. We are continuing to make follow-up phone calls to all participants who have been sent study invitations letters but have not responded. To date we have recruited 61 people at the RAH site 1.

It continues to be clear from the dialogue with the SCI population that other concurrent trials, such as the very large cold therapy trial, are perceived (but not mandated) as an exclusion from all other clinical trials such as ours. We are continuing to work with the population to improve the communication of these key messages.

Site 2 at RNSH has been sensing sending out recruitment information packs with 800 packs sent to date with 19 patients recruited. This is an unprecedentedly low response rate.

Site 3 at the Austin have sent out 180 invitations and have 18 subjects recruited. This is proving to be a much more productive recruitment site which we will continue to use through 2018.

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.

COLLABORATION

Despite these set backs, the process of drawing together the broader range of clinicians, health workers and life scientists around our project has triggered a range of new and very exciting collaborative projects outside the remit of this funding.

The clinical trial of Dr Clarke and Prof Hutchinson on a novel blood tests with predictive ability of motor recovery upon discharge continues through the collaboration built upon the $42M Australian Research Council Centre of Excellence for Nanoscale BioPhotonics and the ability to use advanced BioPhotonic imaging and sensing to provide point of care devices in the future.

IMPACT

The inherent nature of the study is that all our results are back ended and as such the scientific impact will be associated with this long timeline.

CHANGES/PROBLEMS

As outlined above, recruitment has been unprecedentedly slow. As has the approval for the new study site. As such, we have sought and had approved a no cost extension and a revision of the statement of work.

Included below is a copy of the revised statement of work

Revised Statement of work for W81XWH-11-1-0806

All work will be carried out at the University of Adelaide, Medical School (L5 North Wing) Frome Rd, and the Pain and Anaesthesia Research Clinic, Royal Adelaide Hospital, North Tce, Adelaide, South Australia.

SPECIFIC AIMS 1

TASK 1 – STUDY 1
This task involves recruiting 225 spinal cord injury (SCI) patients and 450 healthy individuals for immunogenetic analysis and collation of demographic parameters and clinical outcome data.
1a. Submit human ethics protocol and receive approval (months 1-6, University of Adelaide Human Research Ethics Committee meets every 2 months)
COMPLETED APROVAL
Added RNSH and Austin health as recruitment sites

1b. Recruitment of study participants and nation-wide mail out of buccal swabs (commercially available foam-tip swab) for collection of cell samples. Genomic DNA will be extracted from the brushes upon receipt in Adelaide using the modified manufacturer protocol. Concentration and purity of isolated DNA will be determined using standard techniques measuring absorption at 260 and 280 nm on a spectrophotometer. DNA will be diluted to specific concentrations using a QIAgility pipetting robot and shipped in batches of 500 to AGRF for genotyping (turn-around of 4-6 weeks), with the remaining sample archived at -80°C.
ONGOING

1c. Demographic data and frequency of clinical outcome measures of pain and current opioid use will be collated from clinical case notes and study information report forms and entered for each participant into a computer database, with appropriate coding for use in bioinformatic analysis.
ONGOING

1d. Bioinformatic analysis: Raw data for individual SNPs will be analysed with regard to allele and genotype Data will use initial data batches to design a pipeline for multiple regression analysis that will include demographic factors and clinical outcome data collected in task 1c as covariates, with α-levels adjusted with false discovery rate calculations to account for multiple testing. As more data becomes available this analysis will be refined and updated.
ONGOING

1f. Final conclusions from study outcomes completed and presented at an appropriate national or international scientific meeting, and milestone manuscripts prepared for publication, in 12 months (Dec 2018).
ONGOING

PRODUCTS

Not applicable

PARTICIPANTS AND OTHER COLLABORATING ORGANISATIONS

Roster for past year

Prof Mark Hutchinson (University of Adelaide)
Dr Janet Coller (University of Adelaide)
Dr Jillian Clarke (University of Adelaide)
Dr Ruth Marshal (Royal Adelaide Hospital)
Dr James Middleton (Royal North Shore Hospital)
Dr Paul Rolan (University of Adelaide)
Prof Paul Nunn (Austin Health)

Ms Vicky Staikopoulos (University of Adelaide)
Mrs Francesca Zappia (University of Adelaide)
Mrs Kathy Hayman (University of Adelaide)

SPECIAL REPORTING REQUIRMENTS

Not applicable

APPENDICES

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