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The Genetics of Pain: An exploration of gene-by-environment interactions and their effects on pain

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Abstract

The findings presented in this dissertation are part of the bigger SYMBIOME project which aims to use the biopsychosocial model of pain to develop a prognostic clinical phenotype for people that experience musculoskeletal (MSK) trauma. Chapter 2 presents an exploratory analysis to assess the relationships between genetic polymorphisms and pain severity and interference. Early childhood trauma was also explored as a moderator between genetic polymorphisms and pain outcomes. For pain severity, major allele carriers (A/A and G/A) of *FKBP5* rs9394314 reported significantly higher scores than minor allele carriers (G/G). Further, major allele carriers who had at least one adverse childhood experience (ACE) reported significantly higher scores than minor allele carriers with at least one ACE. For pain interference, minor allele carriers (G/G) of *CNR2* rs2501431 scored significantly higher than major allele carriers (A/A and G/A). Chapter 3 presents a cluster analysis that combines genotypes of *FKBP5* rs9394314 and *CNR2* rs2501431 to explore meaningful relationships with pain and trauma-related distress. ACE was also explored as a moderator of these relationships. Three clusters were identified where the second cluster characterized by major allele carriers of rs9394314 and minor allele carriers of rs2501431 reported significantly higher pain-related functional interference scores. Participants in the second cluster with at least one ACE reported higher pain interference and traumatic distress scores compared to the third cluster, while participants in the first cluster with at least one ACE reported higher pain severity compared to the first cluster. Chapter 4 presents genomic structural equation models (SEM) that explore the relationships of genotypes with trauma-related distress using the traumatic injuries distress scale (TIDS), ACE, and recovery outcomes. The results demonstrate a relationship between TIDS and recovery outcomes, and

an indirect relationship between *FKBP5* rs9394314 and recovery outcomes exist which is mediated by TIDS. Major allele carriers of *FKBP5* rs9394314 reported higher TIDS scores, which was also demonstrated for participants that had at least one ACE. Major allele carriers that scored higher on the TIDS were predicted to be in the none-recovered category. These results support the notion that gene-x-environment interactions may play an important role in pain and recovery.

Keywords

Pain, chronic, musculoskeletal, trauma, genetics, SNP, recovery, childhood adversity

Summary for Lay Audience

Pain is a complicated experience that involves different processes within the body. When one or more of these processes fail to respond, pain can become a long-term problem that begins to affect quality of life. People struggling with chronic pain start to suffer in other areas of life including work, mental and physical health, financial burdens, and relationships. Understanding and treating pain can be difficult because of how unique it is to each person. To improve our understanding of pain and how to treat it effectively, we must consider the biological, psychological, and societal factors involved. The purpose of this project is to explore how genetic variations and environmental factors together play a role in a person's pain and recovery outcomes by collecting blood samples and using questionnaires from a sample of people that suffered an acute traumatic injury. After one year of tracking our participants, some people developed chronic pain while others fully recovered. By comparing the differences in genetic variants and psychological responses between these people, we have a better understanding of why some people recover while others develop chronic pain. Factors such as early childhood trauma may also contribute to how your genes develop, causing differences in how you respond to a traumatic injury later in life and your ability to recover. Studying different genetic variants along with environmental influences may provide more insight on the types of new treatments that need to be developed to help treat people with pain before their pain becomes a chronic issue.

Co-Authorship Statement

The three studies presented in this dissertation were co-designed, analyzed, co-interpreted and written by Mohamad Fakhereddin with invaluable guidance and collaboration from Dr. David Walton. Other collaborators including Dr. Joshua Lee, Dr. Frank Beier, and Dr. Michele Battie provided essential feedback into the study design and interpretation to strengthen each chapter. Dr. Amjed Abojedi and Dr. Paul Tremblay provided valuable feedback and guidance regarding the statistical analyses used in all three studies. Adam McIntyre, John Robinson, and David Carter shared their valuable knowledge and expertise regarding the genotyping techniques I carried out in Dr. Robert Hegele's lab. The data used in these studies were collected by Dr. Joshua Lee, Paul Phares, and Ryan Power, with logistical help provided by Helen Phan.

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Dedication

To my loving parents, I cannot thank you enough for all you have done for me. My achievements would not be possible without your faith in me. Thank you for always believing in me, even when I did not believe in myself.

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Chapter 1

Introduction

The intention of this dissertation is to explore relationships between genes and psychosocial variables to gain a better understanding of why some people transition from acute to chronic pain after a traumatic musculoskeletal injury. Using a biopsychosocial approach, the studies outlined in this dissertation may provide further insight into the complexity of pain and promote future research to enhance strategies for early detection. This chapter will highlight the global impact of chronic pain and the current methods of assessing and managing pain, discuss the role of genetics and genes relevant to pain research, and touch on the potential for genetic testing as a screening tool for chronic pain.

Global Impact and Managing Pain

Although pain is a unique experience and an important response for survival, its persistence has a hugely negative impact on overall quality of life. The burden of pain resonates in multiple areas of life such as social and financial[1]. Along with the physical effects of chronic pain, those suffering tend to also struggle with depression, anxiety, and feelings of isolation which lead to detrimental consequences in their personal and professional lives[1,2]. This has led to negative stigma directed towards those suffering from chronic pain[3]. As a result, people dealing with chronic pain feel a decrease in effectiveness and productivity and are less motivated to seek treatment[4]. On a global scale, pain that is undertreated is causing people to suffer regardless of their sex, gender, socioeconomic status, age, or race/ethnicity[5]. In 2011, it was reported by the

National Academy of Medicine that over 100 million Americans suffer from chronic pain with costs of more than $600 billion USD annually. This is more than the number of people with diabetes, heart disease and cancer combined. Similar numbers have been reported in Canada, with around one-third of the population affected by chronic pain and more than $43 billion CAD in annual costs associated with lost wages, productivity, and medical care[6]. Opioid use as a treatment for pain increased by 347% in the United States[7], a therapy that has been shown to be unsuccessful as a long-term answer for chronic, non-cancer pain. The use of opioid drugs has led to risks of abuse and addiction which has become more apparent with the opioid crisis resulting in opioid-related overdose and death[8–10]. The complexity of pain encompasses biology, environment and mental health and has proven to be resistant to typical pharmaceutical treatment options that are meant to remove symptoms[11].

As evidenced through research, pain is both an emotional and sensory experience, causing the sensation to be highly variable among different people[12]. This leaves the field with a lack of solid pain management strategies and generalizable treatment options. Therefore, current pain research focuses more on understanding the many mechanisms of pain and how it is experienced by each person. Although there is an abundance of literature highlighting the psychological and social implications of chronic pain, understanding how these variables are affected by biological mechanisms has become a growing area of interest for pain researchers. Biological systems influence cognitive function and vice versa, making it crucial to understand the interactions between the biological, psychological, and societal aspects of pain. Understanding the multifaceted nature of pain will lead to development of more accurate

measurements to assess and manage people with chronic pain and is the next step in the field of pain research.

A Multidisciplinary Approach

The International Classification of Functioning, Disability and Health (ICF) was developed as a framework for describing health and health-related states by using standard and integrative language[13]. The introduction of the ICF has helped clinicians and researchers better define the usual problems in functioning for patients with chronic pain by considering environmental factors and using language for health and functioning that has been globally-agreed-upon[14]. Although the ICF includes a biological perspective for health in its model, there is a heavier emphasis on psychological and social perspectives[13]. As research continues to grow for biological mechanisms involved in the development of chronic pain, the biopsychosocial model becomes more important to highlight that understanding the interactions of each component of the model is necessary for a better understanding of pain.

The biopsychosocial model views pain as a dynamic interaction within biological, societal and psychological factors unique to each person[15]. The earliest concepts regarding pain focused more on understanding the biological and pathophysiological associations of pain. Separation of the body and mind, also known as Cartesian Dualism, first came to be theorized by Rene Descartes in the 17th century as a process exclusive to the sensory nervous system[16]. The experience of pain was considered to be directed to the brain from the skin without any psychosocial interaction. In the 1960's, a new model was developed termed the Gate Control

Theory of Pain by Melzack and Wall that described potential mechanisms through which the underlying mechanisms of pain could involve the interaction of physiological and psychosocial processes[17].

The emphasis of the Gate Control Theory was the important role that psychosocial factors can play in the perception of pain. The reason behind the term 'gate control' is because of the process found within dorsal horn of the spinal cord which Melzack and Wall claimed works like a gate-like function, regulating the amount of afferent impulses from the periphery to the T-cells of the dorsal horn[17]. It was believed that higher cortical functions influenced this process, allowing psychological phenomena to directly impact the experience of pain. From a clinical standpoint, gate control theory opened avenues through which psychological and social interventions could be included with the process of assessing and treating patients with pain. Psychological difficulties usually enhance the intensity of the sensory input, which was found to be exacerbated by negative habits that included smoking, eating poorly, and lack of adequate sleep and exercise; therefore, techniques that focused on stress reduction and coping mechanisms helped "close the gate" for these types of patients[16].

Although the gate control theory is widely viewed as one of the more robust models for explaining how the central nervous system interacts with cognitive processes, an extension to this theory is the Neuromatrix Model of Pain[18]. This model, also put forth by Melzack, involves three systems of cognitive functioning that interact during the experience of pain: motivational-affective (emotional responses), cognitive-evaluative (expecting a noxious stimulus), and

sensory-discriminative (location/intensity of nociceptive input)[18]. Based on this new theory, each person's distinctive neuromatrix influences the overall understanding of their pain experience[19].

Stress Response System

A major system involved in responding to stress is the Hypothalamic-Pituitary-Adrenal (HPA) axis which connects impulses in the brain with endocrine glands that help control hormones in the body[20]. This pathway is acting as an emotional nerve centre for anxiety, depression and chronic pain[21]. During a stressful incident, the HPA axis normally releases corticotropin releasing hormone (CRH) from the hypothalamus which then acts on structures such as amygdala and the pituitary[22]. Upon pituitary stimulation, adrenocorticotropic hormone (ACTH) is released into the bloodstream, resulting in the production and release of glucocorticoids (GCs) from the adrenal cortex[21,22]. The release of GCs triggers immune response systems, elevates blood pressure and blood glucose levels, and activates the Central Nervous System (CNS)[23]. Further, GC release causes the amygdala to release CRH which is associated with heightened fear and anxiety[24]. One of the main GC products from the HPA axis is Cortisol, a steroid hormone that has been shown to be elevated during chronic and experimentally-induced pain[25].

Activity of the HPA axis can be affected by early life stress and trauma, resulting in long-lasting consequences. Maniam et al. suggest that adverse experiences early in life are associated with hypersensitivity to stress, elevated levels of GCs, and increased anxiety and depression-like

behaviours later in life[26]. GC levels that remain consistently high can potentially damage the hippocampus since this would reduce its neurological structure and interfere with plasticity[27,28]. This is believed to happen because of a decrease in brain-derived neurotropic factor (BDNF), which is normally active in the hippocampus and is important for neuronal plasticity and the forming of long-term memory[29]. A decrease in BDNF in the hippocampus has been linked to major mood disorders, stress, and experimental pain[30–32]. Early life trauma may influence physiological responses to stressors later in life and contribute to the development of chronic pain after a traumatic injury.

Genetics in Pain Research

Today, pain research has focused more on using the biopsychosocial model to understand the multifaceted nature of the pain experience. A model developed by Turk, termed the diathesis-stress model, suggests that certain elements of a person's psychosocial and physiological character may increase their vulnerability to pain after a traumatic incident[33]. When a person is injured, intricate physiological processes occur that influence pain sensations. More recently, it has been suggested that these processes may be due to the interaction of a person's genetic makeup and psychological or social factors, such as early childhood trauma[34–37]. Early childhood trauma has been shown to be a risk-factor for developing other chronic health diseases later in life[38]. Although a traumatic childhood experience may lead to difficulties with other life stressors in adult years, this is certainly not true for everyone; in fact, some people become high-functioning and successful members of society[39]. The varying outcomes in people after a traumatic event or injury may be explained in part by their genetics.

Many genes are being researched for their roles in modulating biological and cognitive responses to important pathways involved in the experience of pain. Single Nucleotide Polymorphisms (SNPs) are genetic variations in DNA represented by a change in a single DNA base pair and are the main genetic components of interest in pain research. For example, the *catechol-o-methyltransferase (COMT)* gene, that codes for an enzyme that catabolizes catecholamines that are released after stressful incidents, has been found to have alleles associated with persistent post-traumatic musculoskeletal pain[40]. In their study, McLean et al. combined *COMT* single nucleotide polymorphisms present at rs6269, rs4680, rs4633 and rs4818 based on previous findings linking these SNPs with experimental pain sensitivity[41,42], and vulnerabilities to both anxiety disorders[43] and chronic pain[44]. This *COMT* pain vulnerable genotype showed an association with an increased duration of pain and pain severity following motor vehicle collisions, as well as for survivors of sexual assault[40].

Other genetic polymorphisms have been suggested to play a role in the physical and psychological experience of pain. Bortsov et al. found that SNPs rs3800373, rs9380526, rs9394314, rs2817040 and rs2817032 of the *FKBP Prolyl Isomerase 5* (*FKBP5*) gene were associated with pain severity symptoms after a motor vehicle collision and sexual assault[45], while Ulirsch et al. showed that carriers of *FKBP5* rs2817038 associated with worse musculoskeletal pain outcomes were also of lower socioeconomic status[46]. *FKBP5* is responsible for encoding a family of proteins involved in regulating glucocorticoid receptor sensitivity in the immune system and HPA axis, both systems playing crucial roles in response to pain. With

respect to genes responsible for the function of the immune system, the *IL-1ß* gene that encodes the Interleukin-1 beta (IL-1ß) cytokine, which is involved in the development of hyperalgesia, has been shown to be elevated during injury and pain[47,48], including sensations related to pain sensitivity[49]. Further, the *TGFß1* gene encodes Transforming Growth Factor-beta (TGF-ß1), an anti-inflammatory cytokine that counters the actions of IL-1ß during a painful experience[50,51]. Another important marker that has genetic polymorphisms found to be involved in chronic pain sensitization is BDNF rs6265[52], a neuropeptide encoded by the *BDNF* gene[53,54]. These genes have been studied in multiple animal models of injury and stress[32], and their roles in their respective systems are now being observed more closely in humans. The variation between people's vulnerability to pain suggests that genetics along with psychosocial factors such as early life trauma and other life stressors are important to study as they are likely the underlying reasons for the development of chronic pain.

Genetic Testing for Pain

Genetic testing is one promising prognostic tool for pain since it provides objective data unique to each person, and this can be complimented with psychosocial data to aid in pain management. Advancements in genetic research have provided us the tools to easily identify variations among people that make them unique. Genome-wide association studies are the most common approach to look for genetic polymorphisms involved in human diseases. Thousands of SNPs can be investigated at the same time in one study, generating data that helps researchers and clinicians identify genetic variants that may contribute to a person's risk

of developing a particular disease. This technology has helped us understand the human genome and the traits of individual people in a way that can improve quality of life for many.

Although genetic testing for pain is still in its infancy, genetic testing has potential to be a useful tool to identify potential risk factors for the development of chronic pain after an acute injury. The ability to determine who is likely to recover or not is appealing to clinicians, researchers, and, most importantly, patients. However, this work has been undertaken under a broader lens of social justice that requires us to consider the potential for unintended harms that may arise as a result of incidental findings. Accordingly, the work described herein should be considered exploratory rather than confirmatory, and any ongoing work in the field is similarly encouraged to consider the ethical and societal implications of genetic testing and genetic privacy.

Thesis Outline

The initial findings presented in this dissertation demonstrate an ambitious exploration of the genetic and psychosocial mechanisms believed to be involved in transitioning from acute to chronic pain. While pain is certainly a complex and unique sensation for everyone, the theme of this work emphasizes the importance of bridging psychological influences with clinical genotyping to highlight the overlapping systems involved in properties of the pain experience. Therefore, this dissertation will be represented by three core chapters, followed by a summary chapter.

The objective of chapter 2 is to identify associations between a panel of genetic polymorphisms of interest and pain severity and interference, while also considering the potential moderating effects of early childhood trauma. In this chapter, I outline bivariate analyses to test the hypothesis that meaningful associations exist between pain and genetics in a sample of people that have suffered from an acute non-catastrophic musculoskeletal traumatic injury. Furthermore, I explore whether adverse childhood experiences moderate these associations to give a more inclusive perspective at the interaction between psychological and physiological systems to go beyond simple relationships between pain and genetics.

The objective of chapter 3 is to explore the hypothesis that there may be clusters of genetic polymorphisms from different biological pathways that interact to better explain the relationship between people's genetics and their experience with pain and trauma-related distress. Upon identifying genotype clusters, general linear modeling is used to determine whether SNP clusters have a relationship with pain and trauma-related distress, and again to explore the degree to which these associations could be moderated by early childhood trauma. Another objective of this chapter is to explore bivariate associations between individual genetic polymorphisms of interest and trauma-related distress, and if adverse childhood experiences moderate these associations.

The objective of chapter 4 is to explore the potential of using the genetic polymorphisms and psychosocial variables as prognostic tools to predict recovery outcomes in participants with acute MSK injuries. I considered genotypes that previously showed an association with pain and

trauma-related distress in the prior studies to test *a-priori* hypotheses. Using genomic structural equation modeling, I developed *a-priori* models to test potential pathways and mediator effects among genotypes, psychosocial variables, and recovery outcomes.

The overall objective of these projects and this dissertation is to add to the existing knowledge of pain within the biopsychosocial model while also exploring novel pathways that have yet to be investigated in the field to help explain and predict the transition from acute to chronic musculoskeletal pain. By bridging genetics with the more personal elements of pain, we may enhance our understanding of this complex phenomena and take a step forward in developing new therapeutic strategies for it.

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Chapter 2

Exploring interaction effects through gene-x-environment relationships on pain severity and interference scores in people with acute musculoskeletal injuries

Introduction

Pain is a unique, complex, and highly subjective experience[1]. The complexity of this experience makes it hard for a person to explain their pain with friends, family, employers, or healthcare providers. The subjectivity of pain also makes it difficult to diagnose, which leads to poor – or even lack of – access to proper treatments[2]. As a result, people feel a decrease in effectiveness and productivity and are less motivated to seek treatment[3]. On a global scale, undertreated pain is causing people to suffer regardless of their sex, gender, socioeconomic status, age, or race/ethnicity[4]. Chronic pain conditions such as low back, neck and osteoarthritis are amongst the highest global burdens of disease[5].

Pain research has largely embraced a bio-psycho-social model as an approach to understand the multiple components of the pain experience. Turk's diathesis-stress model proposes that certain components of a person's physiological and psychosocial makeup may make them more vulnerable to pain in the event of a traumatic incident[6]. Accordingly, understanding how the psychological and social implications of pain interact with biological mechanisms has become an area of interest.

A cascade of physiological processes occurs when a person is injured, of which some may contribute to the experience of pain. Considerable evidence has accrued to indicate that these processes are also influenced by genetic and epigenetic factors[7–9], as well as psychological and societal contributors such as early childhood trauma[10]. Prior work has shown an association with early childhood trauma being a risk-factor to development of other chronic health disorders later in life, such as depression[11]. For some, an adverse childhood experience makes it harder to deal with other stressors as adults. However, this is not the case for everyone; some end up as high-functioning resilient people[12]. Contrasting responses to stressful life situations, such as an adverse childhood experience, a traumatic injury, or dealing with chronic pain, may be explained in part by genetics.

A host of genes have been identified and continue to be researched for their important roles in regulating the biological and cognitive responses to distress. Genetic variations known as Single Nucleotide Polymorphisms (SNPs) are mutations in DNA represented by a change in a single DNA base pair, such as a substitution mutation. The normal occurrence of a SNP results in the most common type of genetic variation among people. For example, the *catechol-o-methyltransferase (COMT)* gene codes for an enzyme that catabolizes catecholamines, such as those released during periods of stress[13]. Different alleles of the *COMT* gene have been associated with the experience and persistence of post-traumatic musculoskeletal pain. McLean and colleagues found that a *COMT* 'pain vulnerable' genotype was associated with greater pain severity and longer pain duration in people following motor vehicle collisions or sexual assault survivors. They subsequently found that the effect of COMT on pain is moderated by

socioeconomic status[14]. This type of work raises the possibility that there may be genetic drivers of different pain experiences, but that sole reliance on genetic variants in the absence of socio-contextual factors risks over- or under-estimating the effect.

Prior research has suggested that there may be several other gene variants that may influence the physical and/or emotional experience of pain. Other candidate genes include *Solute Carrier Family 6 Member 4* (*SLC6A4*) that encodes a key serotonin transporter protein in cell membranes and has previously been associated with depression-susceptibility in people experiencing emotional trauma and may have epigenetic implications for vulnerabilities to pain in people who have had an early traumatic incident[8,15]; *FKBP Prolyl Isomerase 5* (*FKBP5*) has certain variants that encode a family of proteins involved in inflammatory response by regulating glucocorticoid receptor sensitivity in the immune system, which have been shown to influence the severity of musculoskeletal pain symptoms after a motor vehicle collision and sexual assault[16–18]; *IL-1ß* encodes the Interleukin-1 beta (IL-1ß) cytokine which is greatly elevated during injury and pain[19], and is also involved in development of allodynia and hyperalgesia, as well as extending the sensitivity to pain[19–21]; the anti-inflammatory cytokine Transforming Growth Factor-beta (TGF-ß1), encoded by the *TGFß1* gene, is also crucially involved in the pain experience by its ability to counter pain produced from IL-1ß[22,23]; BDNF is a neuropeptide encoded by the *BDNF* gene which has variants that appear to play a role in chronic pain sensitization[24,25]. However, understanding how these genes interact with environmental factors remains underdeveloped. Exploring potential gene-x-environment interactions may provide a better understanding on the mechanisms of pain.

Research conducted from a biopsychosocial perspective requires simultaneous consideration of biological vulnerability combined with psychological and social factors, such as early trauma or other lifestyle stressors[26]. However, it is still unclear why these changes lead to a transition from acute to chronic pain in some people but not others. The purpose of this paper is to explore potential relationships and interaction effects between genetic polymorphisms, early childhood trauma, and pain severity and interference ratings in patients that have suffered an acute traumatic musculoskeletal injury.

Methods

Participant recruitment

Data for this study were drawn from the SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) longitudinal acute trauma database (clinicaltrials.gov ID no. NCT02711085). Participants were recruited from an Urgent Care Centre in Ontario, Canada. Eligible participants were those seeking medical attention for pain-related symptoms arising from acute (within 3 weeks from onset) non-catastrophic MSK trauma (injuries that did not require hospitalization or surgery) such as whiplash, low back injuries, sports or slip and fall-type injuries that result in sprain/strain of muscle, tendon, ligament, or other such soft tissues. Other inclusion criteria considered for eligibility was the ability to speak and understand conversational English and between 18-66 years old. Excluded from the study were those with significant neuromuscular or systemic comorbidities that may affect physiological response to trauma or recovery (e.g. active cancer, rheumatic conditions or other systemic inflammatory

processes), significant organ disease, those with immunocompromised conditions (e.g. HIV/AIDS) or taking immunomodulatory drugs (e.g. high-dose steroids or disease- modifying anti-rheumatic drugs).

Interested participants were approached after being medically discharged. Upon receiving permission, a member of the research team described the study and answered any questions. Enrollment occurred before the participant left the centre. A package of self-report questionnaires was given to the participants and their serum samples were collected into two 4mL K2 EDTA BD vacutainer tubes from the median cubital vein by a phlebotomy-trained research team member. Pain severity was captured through the Brief Pain Inventory (BPI, severity subscale[27]) questionnaire and functional interference related to pain was captured using the BPI Interference subscale[28]. The BPI is a commonly used and well-recognized pain measurement tool[29] and has sufficient validity across various clinical populations including musculoskeletal pain[30]. The pain severity subscale asks participants to rate their pain out of 10 (10 being extreme pain and 0 being no pain) to determine the severity of their pain. The pain interference subscale asks participants to rate how their pain has interfered with routine things in their lives such as their sleep, mood, ability to do normal things and their enjoyment of life. Questions from the BPI are specifically asking about pain over the past 24 hours. Other questionnaires included participant metadata such as age, sex, BMI, education level, household income, employment status, pre-existing health conditions and medicolegal status (whether a participant obtained an insurance claim or sought a personal injury lawyer). Amongst others, the Adverse Childhood Experience Questionnaire (ACEQ) was used to capture the presence or

absence of early childhood trauma upon inception as it has been determined to be a relevant outcome in MSK trauma[31]. The ACEQ was dichotomized into two categories to determine whether a person had experienced at least one early life (prior to age 18) adverse experience or none to explore the effect of any adversity regardless of the nature of that adversity as we did not have the statistical power to compare the relative effects of any one type over any other type. The ACEQ is a commonly used measurement tool that has adequate validity in studies assessing early life trauma[32]. Prior to participation, all participants provided informed, written consent and before its initiation, the study was approved by the local institutional review board.

Genotyping analysis for candidate SNPs

Blood samples collected from participants were transferred on ice to a local wet lab for analysis. The samples were centrifuged for 10 minutes at 2000*g* and the plasma was then pipetted into 50µL aliquots, and both supernatant and pellets were stored in a -80^{o}C freezer until genotyping. A panel of 39 SNPs (Table 2) were chosen for this study because of previously demonstrated associations with pain, inflammatory response, drug metabolism and mental distress as discussed in the introduction. All SNP sequences are available on the dbSNP database (National Centre for Biotechnology Information) where the major and minor alleles were highlighted on the positive (5' -> 3') and negative (3' -> 5') strands. Genotyping was carried out using TaqMan Assays (ThermoFisher Scientific). Each DNA sample was diluted to 5-50ng/µL. In a 384 well plate, 1.5 µL of DNA was pipetted into wells that contained a mixture of TaqMan Master Mix, 40X SNP Assay and distilled water, and a negative control was prepared

that contained the master mix without DNA. The prepared plate was then placed in the Applied Biosystems ViiA 7 Real-Time PCR (qPCR – ThermoFisher Scientific) to run for approximately 2 hours. Upon completion, the samples were amplified and quantified into three genotyped categories: 1) homozygotes containing major alleles, 2) heterozygotes containing one major and one minor allele, and 3) homozygous minor alleles.

Analysis

Data checking

Hardy-Weinberg equilibrium was assessed prior to any statistical analyses using an online Hardy-Weinberg calculator (Gene Calculators, Jesse Hayesmoore; www.genecalculators.net/pq-chwe-check). Three genetic models to determine likely mode of inheritance were explored: an additive model (genotype is coded as the number of minor alleles, i.e. 0, 1, 2), a recessive model (homozygous for major allele vs other), and a dominant model (homozygous for minor allele vs other). Normality (skew and kurtosis) of the primary dependent variables was statistically checked through Kolmogrov-Smirnov test to confirm the assumptions for each statistical analysis. Descriptive statistics for participants genotyping data and metadata were analyzed and reported (mean, median, range).

Bivariate associations

The dependent variables were BPI pain severity and BPI pain interference, both collected within 24 hours of the blood samples. The results for the Adverse Childhood Experience Questionnaire

(ACEQ) were dichotomized into two categories (having experienced at least one adverse childhood experience or none) to further explore how prior trauma effected pain outcomes compared to those who never experienced early childhood trauma. Each individual SNP served as the independent variable. One-way analysis of variance (ANOVA) was used to explore mean differences in pain severity and pain interference between genotypes.

Interaction effects

A two-way ANOVA was performed to explore potential interaction effects of having at least one adverse childhood experience on the pain-related findings for each of the individual 39 SNPs. This was done by building in the dichotomized ACEQ (at least one ACE vs none) as another independent variable along with the SNP being tested against either BPI pain severity or interference scores. As a discovery-based analysis we accepted $p < 0.05$ to indicate potentially significant differences between groups, not correcting for multiple comparisons thereby accepting an increased risk of alpha error. All statistical analyses were performed with IBM SPSS Statistics 25.0 software.

Sample size estimation

Previous studies investigating genetic polymorphisms and pain by McLean and colleagues calculated a modest sample size for their study[14]. A power analysis was performed using G*Power v3.1[33] showing that a total sample of 100 participants would be required to indicate moderate effects ($f^2 = 0.10$) with ß = 80% using multiple ANOVA with $\alpha = 0.05$.

Results

Blood samples were collected from 108 participants, while data for the ACEQ variable were available for 95 of the participants. Participant demographics and baseline data are shown in Table 1. The sample included slightly more female participants (54.1%), mean age of 43.7 years (± 14.6), with the participants being marginally overweight (BMI = 26.7 kg/m^2, ± 6.2). Participants described a range of non-catastrophic MSK injuries where 70.8% affected the peripheral regions (lower/upper extremities including strains, sprains, or non-displaced fractures) and the remaining affecting the axial regions (neck and lower back injuries). After splitting the sample based on mean adverse childhood experience, 36 participants (37.9%) were in the group that had no adverse childhood experiences, while 59 participants (62.1%) were in the group that had at least one.

Table 1: Baseline values and characteristics for SYMBIOME participants

N= 108	**Proportion or mean**
Sex, female (%)	54.1%
Age, mean (SD)	43.7 (±14.6)
BMI, mean (SD)	26.7 kg/m^2 (±6.2)
Spinal injury	29.2%
Post-secondary education	66.7%
Household income (less than $80K/year)	43.5%
Unemployed	22.2%
Mean BPI pain severity (out of 10)[+]	4.5 (±1.9)
Mean BPI pain interference (%)[+]	28.4 (±17.0)
Mean TIDS (range)[+]	5.7 (0 to 19)
Any adverse childhood experience, at least one (%)[++]	62.1%
Recovery outcomes, recovered (%)[+++]	85.1%

[+]Data available for this variable relied on n=96 participants.
[++] Data available for this variable relied on n=95 participants.
[+++] Data available for this variable relied on n=101 participants.

Genotyping results

All SNPs were confirmed to be in Hardy-Weinberg equilibrium and had successful call rates (>95%). Based on our findings, a dominant model best fit the data and was used for all subsequent analyses. Proportions of alleles for each SNP found in our sample are displayed in Table 2 where bolded alleles are the major alleles and those not bolded are minor.

Table 2: Proportion of alleles for each SNP in participant data

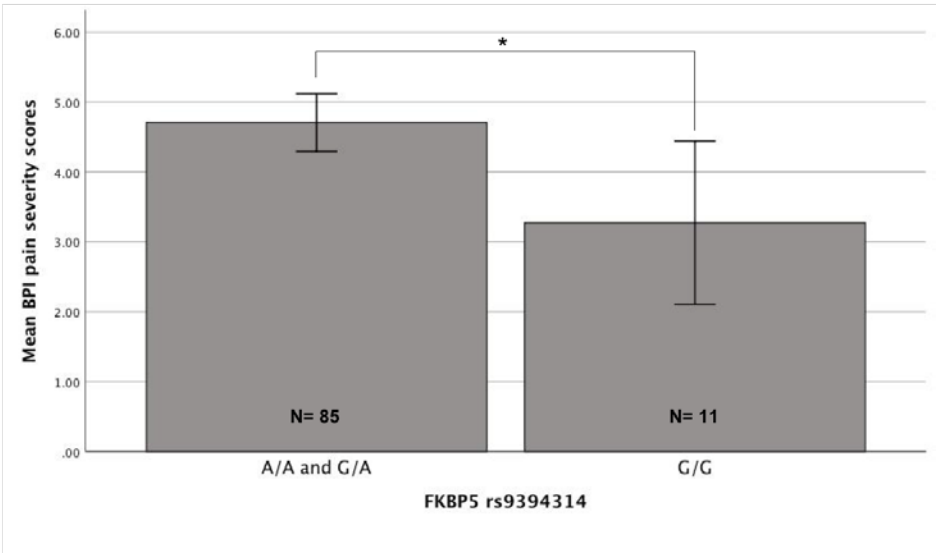
SNP	Proportion (%)	SNP	Proportion (%)	SNP	Proportion (%)
SLC6A4_rs1042173		**FKBP5_rs3800373**		**TGFß1_rs4803455**	
C/C	20 (19%)	**A/A**	54 (50%)	**A/A**	30 (28%)
A/C	54 (50%)	**C/A**	44 (41%)	**C/A**	52 (48%)
A/A	34 (31%)	C/C	10 (9%)	C/C	26 (24%)
COMT_rs6269		**FKBP5_rs9380526**		**TGFß1_rs2241719**	
G/G	17 (16%)	**T/T**	45 (42%)	T/T	76 (70%)
A/G	54 (50%)	**C/T**	50 (46%)	**T/A**	29 (27%)
A/A	37 (34%)	C/C	13 (12%)	**A/A**	3 (3%)
COMT_rs4680		**FKBP5_rs9394314**		**TGFß1_rs1982072**	
G/G	26 (24%)	**A/A**	52 (48%)	**A/A**	59 (55%)
G/A	54 (50%)	**G/A**	45 (42%)	**T/A**	38 (35%)
A/A	28 (26%)	G/G	11 (10%)	T/T	11 (10%)
COMT_rs2020917		**FKBP5_rs2817032**		**TGFß1_rs1800469**	
C/C	56 (52%)	**C/C**	9 (8%)	**G/G**	59 (55%)
C/T	43 (40%)	**T/C**	45 (42%)	**A/G**	38 (35%)
T/T	9 (8%)	T/T	54 (50%)	A/A	11 (10%)
COMT_rs737865		**NTRK3_rs7180942**		**TGFß1_ rs1800470**	
A/A	56 (52%)	T/T	32 (30%)	**A/A**	46 (42%)
A/G	43 (40%)	**T/C**	55 (51%)	**G/A**	45 (42%)
G/G	9 (8%)	**C/C**	21 (19%)	G/G	17 (16%)
COMT_rs1544325		**NTRK3_rs2059588**		**IL1ß_rs16944**	
G/G	35 (32%)	T/T	32 (30%)	A/A	13 (12%)
A/G	57 (53%)	**T/C**	55 (51%)	**A/G**	47 (44%)
A/A	16 (15%)	**C/C**	21 (19%)	**G/G**	48 (44%)
COMT_rs4633		**NTRK3_rs1110306**		**IL1ß_rs1143643**	
C/C	26 (24%)	G/G	33 (31%)	C/C	41 (38%)
C/T	54 (50%)	**G/A**	55 (51%)	**C/T**	47 (44%)
T/T	28 (26%)	**A/A**	20 (18%)	**T/T**	20 (18%)
COMT_rs4818		**NTRK3_rs3784406**		**OPRM1_rs1799971**	
C/C	42 (39%)	C/C	36 (33%)	A/A	92 (85%)
C/G	50 (46%)	**C/T**	55 (51%)	**A/G**	15 (14%)
G/G	16 (15%)	**T/T**	17 (16%)	**G/G**	1 (1%)
COMT_rs165774		**BDNF_rs6265**		**OPRM1_rs1799972**	
G/G	48 (44%)	C/C	77 (71%)	C/C	107 (99%)
G/A	49 (46%)	**C/T**	28 (26%)	**C/T**	1 (1%)
A/A	11 (10%)	**T/T**	3 (3%)		
COMT_rs174697		**BDNF_rs2203877**		**CNR1_rs806369**	
G/G	96 (89%)	T/T	34 (32%)	**C/C**	62 (58%)
A/G	11 (10%)	**T/C**	50 (46%)	**T/C**	38 (35%)

A/A	1 (1%)	**C/C**	24 (22%)	T/T	8 (7%)
COMT_rs165599		**BDNF_rs7124442**		**CNR1_rs1049353**	
A/A	52 (48%)	**T/T**	47 (44%)	C/C	59 (55%)
G/A	47 (44%)	**C/T**	51 (47%)	**C/T**	40 (37%)
G/G	9 (8%)	C/C	10 (9%)	**T/T**	9 (8%)
BDNF_rs7103411		**BDNF_rs2049045**		**CNR1_rs4707436**	
T/T	73 (67%)	G/G	79 (73%)	G/G	59 (55%)
C/T	30 (28%)	**G/C**	27 (25%)	**G/A**	41 (38%)
C/C	5 (5%)	**C/C**	2 (2%)	**A/A**	8 (7%)
CNR2_rs2501431		**CNR1_rs7766029**		**CNR1_rs806366**	
A/A	41 (38%)	T/T	23 (21%)	**T/T**	24 (22%)
G/A	48 (44%)	**T/C**	47 (44%)	**C/T**	58 (54%)
G/G	19 (18%)	**C/C**	38 (35%)	C/C	26 (24%)

Bivariate associations

The results of the ANOVA tests are presented in Table 3 and Table 4 displaying mean BPI scores and 95% confidence intervals. The analyses without ACEQ as an interaction term indicated carriers of the *FKBP5* rs9394314 A/A and G/A major alleles (n = 85) reported a significantly higher BPI pain severity score (mean = 4.7, 95%CI 4.3 to 5.1) than carriers of the homozygous minor G/G allele (n = 11, mean = 3.3, 95%CI 2.1 to 4.4; $F(1,95)=5.53$, $p=0.02$, $\eta^2=0.056$). Mean BPI pain interference scores were significantly higher for carriers of the *CNR2* rs2501431 homozygous minor G/G allele (n = 16, mean = 36.6, 95%CI 28.6 to 44.6) than carriers of the major A/A and G/A alleles (n = 80, mean = 26.9, 95%CI 23.1 to 30.7; $F(1,95)=4.28$, $p=0.04$, $\eta^2=0.044$). These are shown in Figures 1A and B, respectively.

A



B

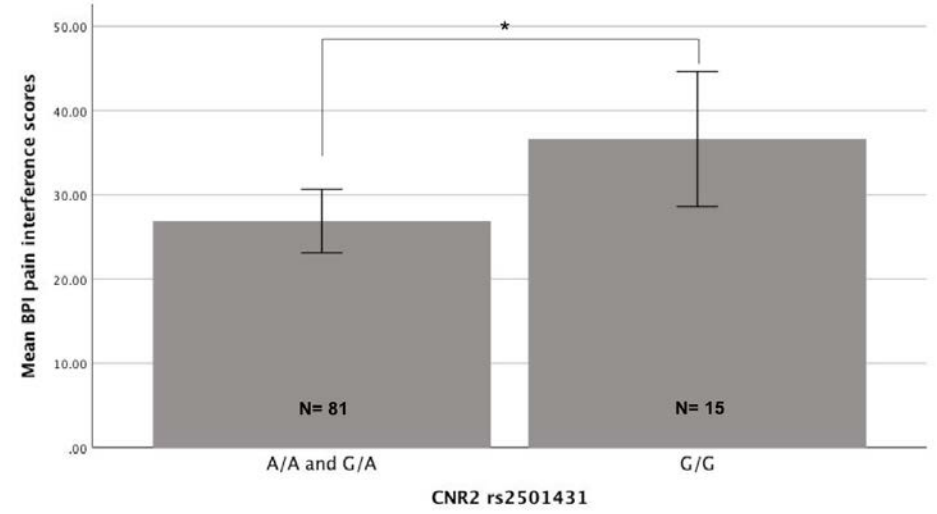


Figure 1 – Graphical representation of SNPs with significant differences between major and minor alleles for BPI pain severity and BPI pain interference scores. Error bars represent 95% CI and an asterisk indicates significance between alleles at $p<0.05$.

Table 3: ANOVA results for BPI pain severity and BPI pain interference

SNP	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)	SNP	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)
SLC6A4_rs1042173					**FKBP5_rs3800373**				
C/C and A/C	66	4.5 (4.1, 5.0)	66	30.0 (25.6, 34.5)	A/A and C/A	86	4.6 (4.2, 5.0)	86	28.3 (24.7, 32.0)
A/A	30	4.5 (3.8, 5.3)	30	24.9 (19.7, 30.0)	C/C	10	4.2 (2.6, 5.8)	10	28.7 (16.0, 41.4)
COMT_rs6269					**FKBP5_rs9380526**				
G/G and A/G	63	4.6 (4.2, 5.1)	63	28.5 (24.3, 32.7)	T/T and C/T	83	4.6 (4.2, 5.1)	83	29.0 (25.3, 32.7)
A/A	33	4.4 (3.6, 5.2)	33	28.2 (21.9, 34.6)	C/C	13	3.9 (2.7, 5.2)	13	24.8 (14.4, 35.3)
COMT_rs4680					**FKBP5_rs9394314***				
A/A and G/A	74	4.4 (4.0, 4.9)	73	29.0 (25.1, 33.0)	A/A and G/A	85	**4.7 (4.3, 5.1)**	85	29.3 (25.6, 32.9)
G/G	22	4.9 (3.9, 5.8)	23	26.4 (18.8, 34.0)	G/G	11	**3.3 (2.1, 4.4)**	11	21.8 (11.5, 32.2)
					Mean difference		**1.4 (0.3, 2.5)***		
COMT_rs2020917					**FKBP5_rs2817032**				
C/C	51	4.5 (3.9, 5.1)	51	27.8 (23.1, 32.5)	C/C and T/C	48	4.4 (3.8, 4.9)	47	30.1 (25.4, 34.8)
C/T and T/T	45	4.6 (4.0, 5.2)	45	29.1 (23.9, 34.3)	T/T	48	4.7 (4.1, 5.3)	49	26.8 (21.7, 31.9)
COMT_rs737865					**NTRK3_rs7180942**				
A/G and G/G	45	4.6 (4.0, 5.2)	45	29.1 (23.9, 34.3)	T/C and C/C	65	4.5 (4.0, 5.0)	65	27.8 (23.5, 32.1)
A/A	51	4.5 (3.9, 5.1)	51	27.8 (23.1, 32.5)	T/T	31	4.7 (4.0, 5.4)	31	29.7 (23.7, 35.8)
COMT_rs1544325					**NTRK3_rs2059588**				
G/G and A/G	83	4.5 (4.1, 5.0)	82	29.7 (26.0, 33.5)	T/C and C/C	65	4.5 (4.0, 5.0)	65	28.4 (24.1, 32.6)
A/A	13	4.5 (3.4, 5.5)	14	20.6 (13.0, 28.1)	T/T	31	4.6 (3.9, 5.3)	31	28.5 (22.2, 34.7)
COMT_rs4633					**NTRK3_rs1110306**				
C/T and T/T	74	4.5 (4.0, 4.9)	73	28.9 (25.0, 32.9)	G/A and A/A	64	4.5 (4.0, 5.0)	64	27.6 (23.3, 32.0)
C/C	22	4.8 (3.9, 5.7)	23	26.7 (19.0, 34.4)	G/G	32	4.7 (4.1, 5.4)	32	30.0 (24.1, 35.8)
COMT_rs4818					**NTRK3_rs3784406**				
C/G and G/G	58	4.6 (4.1, 5.1)	58	28.0 (23.7, 32.3)	C/T and T/T	62	4.5 (4.0, 5.0)	62	27.7 (23.2, 32.2)
C/C	38	4.5 (3.8, 5.2)	38	29.1 (23.2, 35.0)	C/C	34	4.6 (4.0, 5.2)	34	29.7 (24.1, 35.3)
COMT_rs165774					**OPRM1_rs1799971**				
G/A and A/A	53	4.5 (3.9, 5.0)	53	28.5 (23.8, 33.2)	A/G and G/G	14	4.6 (3.6, 5.7)	14	27.6 (17.1, 38.1)
G/G	43	4.6 (4.0, 5.2)	43	28.3 (23.0, 33.6)	A/A	82	4.5 (4.1, 5.0)	82	28.5 (24.8, 32.3)
COMT_rs165599					**OPRM1_rs1799972**				
A/A and G/A	88	4.5 (4.1, 4.9)	88	28.1 (24.6, 31.6)	C/C	95	4.5 (4.1, 4.9)	95	28.4 (24.9, 31.9)
G/G	8	4.9 (3.4, 6.3)	8	31.4 (12.2, 50.5)	**C/T**	1	8.0	1	31.0
COMT_rs174697					**CNR2_2501431***				
G/G and A/G	95	4.6 (4.2, 5.0)	95	28.6 (25.2, 32.1)	A/A and G/A	80	4.5 (4.1, 5.0)	81	**26.9 (23.1, 30.7)**
A/A	1	3.0	1	8.0	G/G	16	4.6 (3.5, 5.7)	15	**36.6 (28.6, 44.6)**
					Mean difference				**-9.7 (-17.9, -1.5)***
BDNF_rs6265					**CNR1_rs806369**				
C/T and T/T	28	4.6 (3.7, 5.3)	27	29.1 (21.9, 36.4)	C/C and T/C	89	4.5 (4.1, 4.9)	90	28.1 (24.6, 31.7)

C/C	68	4.5 (4.1, 5.0)	69	28.1 (24.2, 32.1)	T/T	7	4.9 (3.1, 6.7)	6	32.5 (16.2, 48.8)
BDNF_rs2203877					**CNR1_rs1049353**				
T/C and C/C	64	4.5 (4.0, 5.0)	65	28.3 (24.0, 32.6)	C/T and T/T	46	4.6 (4.1, 5.2)	47	29.6 (24.8, 34.4)
T/T	32	4.6 (3.9, 5.4)	31	28.7 (22.7, 34.7)	C/C	50	4.5 (3.9, 5.1)	49	27.3 (22.2, 32.3)
BDNF_rs7124442					**CNR1_rs4707436**				
T/T and C/T	86	4.6 (4.2, 5.1)	86	29.0 (25.4, 32.7)	G/A and A/A	46	4.6 (4.1, 5.2)	47	29.8 (25.0, 34.7)
C/C	10	3.8 (2.5, 5.1)	10	23.0 (11.6, 34.4)	G/G	50	4.5 (3.9, 5.1)	49	27.1 (22.1, 32.1)
BDNF_rs2049045					**CNR1_rs806366**				
G/C and C/C	26	4.5 (3.7, 5.3)	25	29.2 (21.5, 36.9)	T/T and C/T	72	4.4 (3.9, 4.9)	73	27.1 (23.2, 31.1)
G/G	70	4.6 (4.1, 5.0)	71	28.1 (24.2, 32.0)	C/C	24	5.0 (4.3, 5.7)	23	32.4 (25.2, 39.7)
BDNF_rs7103411					**CNR1_rs7766029**				
T/T and C/T	92	4.5 (4.1, 4.9)	92	27.8 (24.3, 31.3)	T/C and C/C	77	4.5 (4.0, 4.9)	78	27.7 (23.7, 31.8)
C/C	4	5.8 (2.2, 9.3)	4	42.5 (14.4, 70.7)	T/T	19	4.8 (3.9, 5.7)	18	31.3 (24.8, 37.8)
IL1ß_rs16944					**TGFß1_rs4803455**				
A/G and G/G	86	4.6 (4.2, 5.0)	86	28.4 (24.8, 32.0)	A/A and C/A	75	4.5 (4.0, 5.0)	75	27.9 (23.8, 31.9)
A/A	10	3.8 (2.3, 5.3)	10	28.7 (14.2, 43.2)	C/C	21	4.6 (4.0, 5.3)	21	30.4 (23.4, 37.4)
IL1ß_rs1143643					**TGFß1_rs2241719**				
C/T and T/T	59	4.7 (4.2, 5.2)	59	29.3 (24.9, 33.78)	T/T	69	4.6 (4.1, 5.1)	69	27.8 (23.5, 32.2)
C/C	37	4.2 (3.6, 4.9)	37	27.0 (21.3, 32.6)	T/A and A/A	27	4.4 (3.7, 5.1)	27	29.9 (24.3, 35.5)
TGFß1_ rs1800470					**TGFß1_rs1982072**				
A/A and G/A	83	4.5 (4.1, 4.9)	83	27.4 (23.5, 31.2)	A/A and T/A	88	4.5 (4.0, 4.9)	88	27.6 (23.9, 31.2)
G/G	13	4.8 (4.0, 5.7)	13	35.1 (28.2, 42.0)	T/T	8	5.4 (4.4, 6.4)	8	37.8 (29.2, 46.3)
					TGFß1_rs1800469				
					G/G and A/G	88	4.5 (4.0, 4.9)	88	27.6 (23.9, 31.2)
					A/A	8	5.4 (4.4, 6.4)	8	37.8 (29.2, 46.3)

*indicates significance at $p<0.05$ and the mean difference for significant values

Interaction effects

Table 4 presents the results of the ANOVA analyses with ACEQ included as an interaction term. A significantly higher mean BPI pain severity score was reported for *FKBP5* rs9394314 major alleles (n = 53) in those who reported at least one ACE (mean = 4.9, 95%CI 4.4 to 5.4) compared to the homozygous minor alleles (n = 6, mean = 2.7, 95%CI 1.2 to 4.2; $F(1,57)=8.77$, $p=0.004$, $\eta^2=0.08$). These findings are demonstrated in Figure 2. There were no other significant findings.

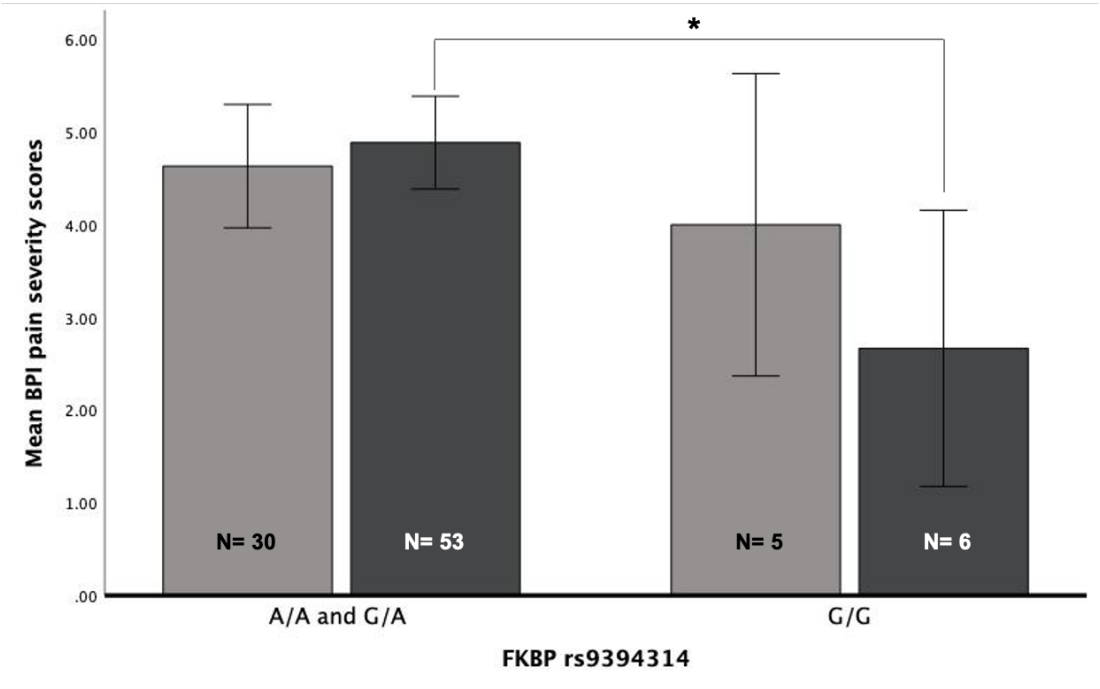


Figure 2 – Graphical representation of significant differences between the minor and major alleles of *FKBP5* rs9394314 with at least one ACE for BPI pain severity scores. Bars shaded light grey represent no adverse childhood experience and bars shaded dark grey represent at least one or more adverse childhood experience. Error bars represent 95% CI and an asterisk indicates significance between alleles at $p<0.05$.

Table 4: ANOVA with ACEQ as interaction term for BPI pain severity and BPI pain interference

SLC6A4_rs1042173	ACE total	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)	FKBP5_rs3800373	ACE total	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)
C/C and A/C	None	24	4.7 (3.9, 5.4)	25	29.7 (23.0, 36.4)	A/A and C/A	None	29	4.4 (3.8, 5.1)	30	25.2 (19.0, 31.3)
	At least one	40	4.7 (4.1, 5.3)	40	30.7 (25.4, 36.0)		At least one	55	4.8 (4.3, 5.3)	55	30.5 (25.9, 35.0)
A/A	None	11	4.3 (3.1, 5.4)	11	19.3 (9.2, 29.4)	C/C	None	6	5.0 (3.5, 6.5)	6	33.2 (19.4, 46.9)
	At least one	19	4.7 (3.8, 5.6)	19	28.1 (20.4, 35.8)		At least one	4	3.0 (1.1, 4.9)	4	22.0 (5.1, 38.9)
COMT_rs6269						**FKBP5_rs9380526**					
G/G and A/G	None	26	4.8 (4.1, 5.6)	27	28.0 (21.5, 34.5)	T/T and C/T	None	28	4.5 (3.8, 5.2)	29	25.8 (19.5, 32.0)
	At least one	36	4.6 (4.0, 5.2)	36	28.9 (23.2, 34.5)		At least one	53	4.8 (4.3, 5.4)	53	31.1 (26.5, 35.7)
A/A	None	9	3.8 (2.5, 5.0)	9	22.0 (10.7, 33.3)	C/C	None	7	4.7 (3.3, 6.1)	7	29.6 (16.9, 42.3)
	At least one	23	4.8 (4.0, 5.6)	23	31.5 (24.4, 38.5)		At least one	6	3.0 (1.5, 4.5)	6	19.3 (5.6, 33.0)
COMT_rs4680						**FKBP5_rs9394314***					
A/A and G/A	None	25	4.5 (3.8, 5.3)	25	29.5 (22.8, 36.2)	A/A and G/A	None	30	4.6 (4.0, 5.3)	31	26.8 (20.7, 32.8)
	At least one	47	4.6 (4.0, 5.1)	47	29.2 (24.3, 34.1)		At least one	53	**4.9 (4.4, 5.4)**	53	31.1 (26.5, 35.7)
G/G	None	10	4.6 (3.4, 5.8)	11	19.6 (9.5, 29.8)	G/G	None	5	4.0 (2.4, 5.6)	5	24.8 (9.8, 39.8)
	At least one	12	5.1 (4.0, 6.2)	12	32.6 (22.9, 42.3)		At least one	6	**2.7 (1.2, 4.2)**	6	19.3 (5.6, 33.1)
							Mean difference		**2.2 (0.7, 3.7)***		
COMT_rs2020917						**FKBP5_rs2817032**					
C/C	None	15	4.3 (3.3, 5.3)	16	25.8 (17.2, 34.3)	C/C and T/C	None	18	4.5 (3.6, 5.4)	18	27.3 (19.3, 35.3)
	At least one	34	4.8 (4.1, 5.5)	34	29.3 (23.4, 35.1)		At least one	29	4.4 (3.7, 5.1)	29	31.9 (25.6, 38.2)
C/T and T/T	None	20	4.8 (3.9, 5.6)	20	27.1 (19.5, 34.7)	T/T	None	17	4.6 (3.7, 5.5)	18	25.7 (17.7, 33.7)
	At least one	25	4.5 (3.7, 5.2)	25	30.7 (23.9, 37.5)		At least one	30	4.9 (4.2, 5.6)	30	28.0 (21.8, 34.2)
COMT_rs737865						**NTRK3_rs7180942**					
A/G and G/G	None	20	4.8 (3.9, 5.6)	20	27.1 (19.5, 34.7)	T/C and C/C	None	26	4.6 (3.8, 5.3)	27	26.7 (20.2, 33.2)
	At least one	25	4.5 (3.7, 5.2)	25	30.7 (23.9, 37.5)		At least one	38	4.4 (3.8, 5.1)	38	28.5 (23.0, 34.0)
A/A	None	15	4.3 (3.3, 5.3)	16	25.8 (17.2, 34.3)	T/T	None	9	4.4 (3.2, 5.7)	9	25.9 (14.6, 37.2)
	At least one	34	4.8 (4.1, 5.5)	34	29.3 (23.4, 35.1)		At least one	21	5.0 (4.2, 5.9)	21	32.3 (24.9, 39.8)
COMT_rs1544325						**NTRK3_rs2059588**					
G/G and A/G	None	30	4.6 (3.9, 5.3)	30	26.8 (20.8, 32.9)	T/C and C/C	None	26	4.6 (3.8, 5.3)	27	26.7 (20.1, 33.3)
	At least one	51	4.7 (4.2, 5.2)	51	31.9 (27.2, 36.5)		At least one	38	4.5 (3.9, 5.1)	38	29.6 (24.1, 35.1)
A/A	None	5	4.4 (2.7, 6.1)	6	25.0 (11.5, 38.5)	T/T	None	9	4.4 (3.2, 5.7)	9	25.9 (14.5, 37.3)
	At least one	8	4.5 (3.2, 5.9)	8	17.3 (5.5, 29.0)		At least one	21	4.9 (4.1, 5.7)	21	30.4 (23.0, 37.9)
COMT_rs4633						**NTRK3_rs1110306**					
C/T and T/T	None	25	4.5 (3.8, 5.3)	25	29.5 (22.8, 36.2)	G/A and A/A	None	26	4.6 (3.8, 5.3)	27	26.7 (20.2, 33.2)
	At least one	47	4.6 (4.0, 5.1)	47	29.0 (24.2, 33.9)		At least one	37	4.4 (3.8, 5.1)	37	28.3 (22.7, 33.9)
C/C	None	10	4.6 (3.4, 5.8)	11	19.6 (9.5, 29.7)	G/G	None	9	4.4 (3.2, 5.7)	9	25.9 (14.6, 37.2)
	At least one	12	5.0 (3.9, 6.1)	12	33.2 (23.5, 42.8)		At least one	22	5.0 (4.2, 5.9)	22	32.5 (25.3, 39.8)

COMT_rs4818						**NTRK3_rs3784406**					
C/G and G/G	None	25	4.8 (4.0, 5.5)	26	26.9 (20.3, 33.6)	C/T and T/T	None	25	4.6 (3.8, 5.3)	26	27.1 (20.5, 33.8)
	At least one	32	4.5 (3.9, 5.2)	32	28.8 (22.8, 34.9)		At least one	36	4.6 (3.9, 5.2)	36	28.1 (22.5, 33.8)
C/C	None	10	4.0 (2.8, 5.2)	10	25.4 (14.6, 36.2)	C/C	None	10	4.5 (3.3, 5.7)	10	24.9 (14.2, 35.6)
	At least one	27	4.8 (4.1, 5.5)	27	31.1 (24.6, 37.7)		At least one	23	4.8 (4.0, 5.6)	23	32.7 (25.6, 39.7)
COMT_rs165774						**OPRM1_rs1799971**					
G/A and A/A	None	18	4.3 (3.4, 5.2)	18	25.5 (17.5)	A/G and G/G	None	3	4.3 (2.1, 6.5)	3	20.7 (1.0, 40.3)
	At least one	34	4.7 (4.0, 5.3)	34	30.6 (16.7)		At least one	11	4.7 (3.6, 5.9)	11	29.5 (19.2, 39.7)
G/G	None	17	4.8 (3.8, 5.7)	18	27.5 (19.5, 35.5)	A/A	None	32	4.6 (3.9, 5.2)	33	27.0 (21.1, 33.0)
	At least one	25	4.6 (3.9, 5.4)	25	28.9 (22.1, 35.7)		At least one	48	4.6 (4.1, 5.2)	48	30.0 (25.1, 34.9)
COMT_rs165599						**CNR2_2501431**					
A/A and G/A	None	32	4.5 (3.8, 5.2)	33	26.1 (20.2, 32.1)	A/A and G/A	None	33	4.5 (3.8, 5.2)	34	25.9 (20.1, 31.6)
	At least one	54	4.6 (4.1, 5.2)	54	29.7 (25.1, 34.3)		At least one	46	4.7 (4.1, 5.2)	46	28.0 (23.1, 32.9)
G/G	None	3	5.0 (2.8, 7.2)	3	30.7 (11.0, 50.3)	G/G	None	2	5.5 (2.8, 8.2)	2	37.0 (13.3, 60.7)
	At least one	5	4.8 (3.1, 6.5)	5	31.8 (16.6, 47.0)		At least one	13	4.6 (3.6, 5.7)	13	36.5 (27.3, 45.8)
COMT_rs174697						**CNR1_rs806369**					
G/G and A/G	None	34	4.6 (3.9, 5.2)	35	27.0 (21.3, 32.7)	C/C and T/C	None	32	4.5 (3.8, 5.1)	33	26.3 (20.4, 32.2)
	At least one	59	4.7 (4.2, 5.2)	59	29.9 (25.5, 34.3)		At least one	56	4.6 (4.1, 5.1)	56	29.5 (25.0, 34.1)
A/A	None	1	3.0 (-0.8, 6.8)	1	8.0 (-25.7, 41.7)	T/T	None	3	5.3 (3.1, 7.5)	3	28.7 (9.0, 48.3)
							At least one	3	5.3 (3.1, 7.5)	3	36.3 (16.7, 56.0)
BDNF_rs6265						**CNR1_rs1049353**					
C/T and T/T	None	8	5.1 (3.8, 6.5)	8	29.4 (17.4, 41.4)	C/T and T/T	None	15	5.1 (4.1, 6.0)	16	32.0 (23.6, 40.4)
	At least one	19	4.4 (3.5, 5.2)	19	29.0 (21.2, 36.8)		At least one	31	4.4 (3.8, 5.1)	31	28.4 (22.4, 34.4)
C/C	None	27	4.4 (3.6, 5.1)	28	25.7 (19.3, 32.1)	C/C	None	20	4.2 (3.3, 5.0)	20	22.1 (14.6, 29.6)
	At least one	40	4.8 (4.2, 5.4)	40	30.3 (24.9, 35.7)		At least one	28	4.9 (4.2, 5.6)	28	31.6 (25.3, 37.9)
BDNF_rs2203877						**CNR1_rs4707436**					
T/C and C/C	None	23	4.4 (3.6, 5.2)	24	26.3 (19.3, 33.2)	G/A and A/A	None	15	5.1 (4.1, 6.0)	16	32.0 (23.6, 40.4)
	At least one	40	4.7 (4.1, 5.3)	40	29.9 (24.5, 35.3)		At least one	31	4.4 (3.8, 5.1)	31	28.7 (22.7, 34.7)
T/T	None	12	4.8 (3.7, 5.9)	12	27.0 (17.2, 36.8)	G/G	None	20	4.2 (3.3, 5.0)	20	22.1 (14.6, 29.6)
	At least one	19	4.7 (3.8, 5.6)	19	29.8 (22.0, 37.6)		At least one	28	4.9 (4.2, 5.6)	28	31.2 (24.9, 37.5)
BDNF_rs7124442						**CNR1_rs806366**					
T/T and C/T	None	31	4.6 (4.0, 5.3)	32	27.7 (21.7, 33.6)	T/T and C/T	None	25	4.3 (3.6, 5.1)	26	25.7 (19.1, 32.4)
	At least one	53	4.8 (4.2, 5.3)	53	30.2 (25.6, 34.9)		At least one	46	4.5 (4.0, 5.1)	46	28.3 (23.3, 33.3)
C/C	None	4	3.8 (1.9, 5.6)	4	17.3 (0.4, 34.2)	C/C	None	10	5.1 (3.9, 6.3)	10	28.5 (17.8, 39.2)
	At least one	6	3.8 (2.3, 5.4)	6	26.8 (13.0, 40.6)		At least one	13	5.2 (4.1, 6.2)	13	35.5 (26.1, 44.8)
BDNF_rs2049045						**CNR1_rs7766029**					
G/C and C/C	None	7	5.6 (4.2, 7.0)	7	31.3 (18.5, 44.1)	T/C and C/C	None	27	4.4 (3.7, 5.1)	28	26.1 (19.7, 32.5)
	At least one	18	4.2 (3.3, 5.1)	18	28.3 (20.3, 36.3)		At least one	49	4.6 (4.1, 5.2)	49	29.0 (24.2, 33.9)
G/G	None	28	4.3 (3.6, 5.0)	29	25.3 (19.1, 31.7)	T/T	None	8	5.0 (3.7, 6.3)	8	27.9 (15.9, 39.9)
	At least one	41	4.9 (4.3, 5.4)	41	30.6 (25.3, 35.9)		At least one	10	4.9 (3.7, 6.1)	10	34.0 (23.3, 44.7)
BDNF_rs7103411						**TGFß1_rs4803455**					

T/T and C/T	None	34	4.6 (4.0, 5.2)	35	26.5 (20.9, 32.2)	A/A and C/A	None	26	4.4 (3.7, 5.2)	27	24.7 (18.2, 31.3)
	At least one	56	4.6 (4.1, 5.1)	56	28.9 (24.4, 33.4)		At least one	47	4.7 (4.2, 5.3)	47	30.0 (25.1, 35.0)
C/C	None	1	3.0 (-0.7, 6.7)	1	25.0 (-8.4, 58.4)	C/C	None	9	4.9 (3.6, 6.2)	9	31.8 (20.5, 43.1)
	At least one	3	6.7 (4.5, 8.8)	3	48.3 (29.1, 67.6)		At least one	12	4.4 (3.3, 5.5)	12	29.3 (19.6, 39.1)
IL1ß_rs16944						**TGFß1_rs2241719**					
A/G and G/G	None	32	4.7 (4.0, 5.4)	33	27.2 (21.2, 33.1)	T/A and A/A	None	9	4.2 (3.0, 5.5)	9	29.2 (17.9, 40.6)
	At least one	52	4.7 (4.2, 5.3)	52	29.5 (24.8, 34.2)		At least one	17	4.8 (3.8, 5.7)	17	31.4 (23.2, 39.7)
A/A	None	3	3.0 (0.8, 5.2)	3	19.3 (-0.3, 38.9)	T/T	None	26	4.7 (3.9, 5.4)	27	25.6 (19.1, 32.1)
	At least one	7	4.1 (2.7, 5.6)	7	32.7 (19.9, 45.5)		At least one	42	4.6 (4.0, 5.2)	42	29.3 (24.0, 34.5)
IL1ß_rs1143643						**TGFß1_rs1982072**					
C/T and T/T	None	21	4.6 (3.8, 5.4)	22	26.6 (19.4, 33.8)	A/A and T/A	None	32	4.4 (3.7, 5.1)	33	25.1 (19.3, 31.0)
	At least one	36	5.0 (4.4, 5.6)	36	31.5 (25.8, 37.2)		At least one	54	4.6 (4.1, 5.1)	54	29.4 (24.8, 33.9)
C/C	None	14	4.4 (3.4, 5.4)	14	26.4 (17.3, 35.4)	T/T	None	3	6.0 (3.8, 8.2)	3	41.7 (22.3, 61.0)
	At least one	23	4.1 (3.4, 4.9)	23	27.3 (20.3, 34.4)		At least one	5	5.0 (3.3, 6.7)	5	35.4 (20.4, 50.4)
TGFß1_rs1800470						**TGFß1_rs1800469**					
A/A and G/A	None	31	4.5 (3.8, 5.1)	32	25.1 (19.2, 31.1)	G/G and A/G	None	32	4.4 (3.7, 5.1)	33	25.1 (19.3, 31.0)
	At least one	50	4.7 (4.1, 5.2)	50	29.1 (24.4, 33.9)		At least one	54	4.6 (4.1, 5.1)	54	29.4 (24.8, 33.9)
G/G	None	4	5.3 (3.4, 7.2)	4	37.5 (20.7, 54.3)	A/A	None	3	6.0 (3.8, 8.2)	3	41.7 (22.3, 61.0)
	At least one	9	4.7 (3.4, 5.9)	9	34.0 (22.8, 45.2)		At least one	5	5.0 (3.3, 6.7)	5	35.4 (20.4, 50.4)

*indicates significance at $p<0.05$ and the mean difference for significant values.

Discussion

This study sought to explore potential associations between 39 genetic polymorphisms with prior evidence of an association with pain or distress, and pain severity and pain interference. Another purpose of this study was to determine if early childhood trauma moderates these relationships. Prior studies in the field have already demonstrated the meaningful relationships that exist between genetics and adverse childhood experiences, though we are aware of no others that explore these relationships within the context of musculoskeletal pain severity and pain interference. The results from our study showed that *FKBP5* rs9394314 may have an association with pain severity, and that *CNR2* rs2501431 may have an association with pain interference. Further, when considering the presence of an adverse childhood experience, carriers of the *FKBP5* rs9394314 major alleles tended to report higher ratings of pain severity compared to minor allele carriers. No associations were found for the other 37 SNPs with pain severity and pain interference scores, even when including the presence or absence of an adverse childhood experience as an interaction term.

Prior work on musculoskeletal trauma focusing on motor vehicle collisions found a significant association between *FKBP5* polymorphisms and the severity of pain symptoms, where the results were also replicated in a cohort of women who suffered from sexual assault[16]. Our findings for *FKBP5* rs9394314 using the BPI pain severity subscale demonstrate similar effects for the alleles of this polymorphism on the severity of musculoskeletal pain symptoms. More recent studies have highlighted the potential role of *FKBP5* variants in modulating neural activity in response to stress[18]. Our study showed that early childhood trauma may play a role

in the relationship between *FKBP5* polymorphisms and pain severity. Our findings indicate that major allele carriers of rs9394314 may be predisposed to vulnerabilities related to pain severity and those that have suffered an adverse childhood experience are even more likely to present greater pain. Further investigation is needed on how glucocorticoid pathways may influence persistent post-traumatic pain and stress response.

CNR2 has been shown to be part of a dynamic system involved in processing nociceptive signals[34,35]. However, the relationship between *CNR2* polymorphisms and musculoskeletal pain is still being explored and does not have a lot of research to support its significance. One previous study looking at *CNR2* genetic expression and genotype in people with low back pain and chronicity found that there was no significant associations with *CNR2* rs2501431 and any of their measures[36]. However, they found that *CNR2* mRNA expression was significantly elevated in all of their patients with lower back pain. As they included participants that reported chronic (rather than acute) pain in the lower back region only and excluded participants that reported pain anywhere else, this may suggest that other factors are important to consider, such as location/type of injury, when determining the significance of our findings between *CNR2* rs2501431 and pain interference. Furthermore, in a study by Peiro and colleagues, the G-allele of *CNR2* rs2501431 was found to play a protective role against panic disorder in male carriers [37], which may be the reason why the relationship between this SNP and pain was no longer significant when including early childhood experiences as a potential moderator of pain in our study. As most research on *CNR2* has focused primarily on mental health disorders, further

investigation is needed to understand the impact of these genes on musculoskeletal pain in conjunction with psychosocial variables.

Limitations

Although we were able to demonstrate associations between two SNPs and pain, the true significance of these is still unclear. Our sample size was likely underpowered within each SNP considering the proportionally uneven representation of alleles, though data normality and equality of variance was satisfied in our analyses. This is also reflected in the uneven proportions of alleles within each SNP and the further reduction in their sample size numbers when incorporating the effect of ACEQ as a variable. Further, we explored only a single potential interaction term, ACEQ, while there are very likely many other such variables that may show stronger effects. With so many analyses already in a single report we were sensitive to adding even more variables, though this is a reasonable direction for further work. Overall, more research is needed with larger independent samples to further investigate these findings.

Conclusion

This exploratory study has demonstrated a relationship between two genetic polymorphisms and musculoskeletal pain severity and interference to help further understand the biological mechanisms of pain. Our results show that a significant difference in mean pain severity scores exists between carriers of major and minor alleles for *FKBP5* rs9394314, and carriers of these alleles who reported at least one adverse childhood experience also showed a significant difference in mean pain severity scores. Further, a significant mean difference in pain

interference scores was found between carriers of major and minor alleles for *CNR2* rs2501431. This study adds to existing knowledge that genetics alone and psychosocial factors alone cannot fully capture the effects each has on pain. Future research may investigate some of these SNPs synergistically and identify if a stronger relationship exists with pain. Including more psychosocial variables such as socioeconomic status may provide more insight on which associations can help predict recovery or development of chronic pain after an acute traumatic injury.

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Chapter 3

Cluster analysis of genetic polymorphisms and their relationship with pain and distress in people with acute musculoskeletal trauma

Introduction

The significant burden of chronic pain on patients and healthcare systems stems from its complex nature, making it difficult to adequately treat those suffering[1]. Successful pain management techniques continue to be a challenge despite the progress made in improved research for interdisciplinary care[2]. Due to these challenges and the growing burden of chronic pain, healthcare researchers and clinicians are placing a stronger focus on understanding mechanisms for early detection and intervention strategies[3,4].

Previous longitudinal studies have found pain and recovery trajectories that generally suggest 15-25% of participants report long-term persistent pain and functional interference after suffering musculoskeletal (MSK) trauma[5–8]. A prior study using our longitudinal cohort identified a 3-trajectory model of functional recovery from MSK trauma. The three trajectories represented rapid recovery (32.0% of the sample), delayed recovery (26.7%), and minimal or no recovery (41.3%)[9]. These findings are similar to those of a study conducted by Sterling and colleagues who also found a 3-trajectory model that best fit their data after following post-traumatic stress outcomes[8]. The ability to predict recovery trajectories such as these offers new avenues to explore potential predictive mechanisms of pain and recovery.

Pain and trauma-related distress are likely influenced by several factors, one of which could be genetics and the interaction between genes. Classifying people into outcome groups can be adopted for genetic polymorphisms. Single nucleotide polymorphisms (SNPs) are mutations in DNA represented by a change of a single DNA nucleotide, resulting in genetic variation among people. Currently, research in the field of genetics has focused a great deal on the involvement of multiple SNPs after a musculoskeletal (MSK) injury or trauma-related experiences. Bortsov et al. found some polymorphisms of the *FKBP Prolyl Isomerase 5* (*FKBP5*) gene contain risk alleles that may help predict persistent pain in people after exposure to trauma[10]. Another study showed that SNPs of *FKBP5* played a role in moderating chronic MSK pain development after a motor vehicle collision among people of lower socioeconomic status[11]. More recently, Linnstaedt et al. found that when variants of the *FKBP5* and *Corticotropin Releasing Hormone Binding Protein* (*CRHBP)* genes were explored together, their interaction showed a substantial increase in MSK pain after a motor vehicle collision in people carrying the risk allele for both genes compared to that of either gene alone[12]. These studies support the importance of exploring genetic variants as potential predictors or moderators of pain and/or distress, as well as the value of clustering variants from different genes to explore relationships of these outcomes.

In a prior study (see Chapter 2), we sought to explore potential associations and interaction effects between relevant SNPs, adverse childhood experiences, and pain severity and interference scores among people that had suffered an acute traumatic injury. We found that two genetic polymorphisms, *FKBP5* rs9394314 and *Cannabinoid receptor 2 (CNR2)* rs2501431,

showed an effect on pain severity and pain interference, respectively. The purpose of this study was to explore whether these two genetic polymorphisms have meaningful associations with distress-related outcomes after trauma and if these associations also exist when the polymorphisms are considered as clusters rather than single bivariate relationships. Potential associations between clusters and pain-related outcomes were also explored. Further, we sought to explore the interacting effect of any gene clusters with adverse childhood experiences in explaining pain severity and functional interference outcomes, as well as distress- and trauma-related outcomes.

Methods

Participant recruitment

Data for this exploratory study were drawn from the longitudinal SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) database (clinicaltrials.gov ID no. NCT02711085). The study was approved by the office of Human Research Ethics at Western University and the Lawson Health Research Institute. Eligible participants were recruited from an urgent care centre in London, ON, Canada seeking medical attention for pain-related symptoms arising from acute non-catastrophic MSK trauma (injuries that did not require hospitalization or surgery) such as whiplash, low back injuries, sports or slip and fall-type injuries that result in sprain/strain of muscle, tendon, ligament, or other such soft tissues. Interested participants were approached after being medically discharged. Excluded from the study were those with significant neuromuscular or systemic comorbidities that may affect physiological response to trauma or recovery (e.g. active cancer, rheumatic conditions or other

systemic inflammatory processes), significant organ disease, those with immunocompromised conditions (e.g. HIV/AIDS) or taking immunomodulatory drugs (e.g. high-dose steroids or disease- modifying anti-rheumatic drugs).

Upon receiving permission, a member of the research team described the study and answered any questions. Enrollment occurred before the participant left the centre. A package of self-report questionnaires was given to the participants. Blood samples were collected into two 4mL K2 EDTA BD vacutainer tubes from the median cubital vein by a phlebotomy-trained research team member. Samples were transferred on ice to a local wet lab for analysis. The samples were centrifuged for 10 minutes at 2000*g* and the plasma was then pipetted into 50µL aliquots, and both supernatant and pellets were stored in a -80^{o}C freezer until genotyping.

Psychometric variables and metadata

Pain intensity and functional interference were captured through the Brief Pain Inventory (BPI)[13]. The BPI is a commonly used and well-recognized pain measurement tool[14] and has sufficient validity across various clinical populations including musculoskeletal pain[15]. The pain interference subscale asks participants to rate how their pain has interfered with routine things in their lives such as their sleep, mood, ability to do normal things and their enjoyment of life. The pain severity subscale asks participants to rate their pain out of 10, with 10 being extreme pain and 0 being no pain, to determine the severity of their pain. Questions from the BPI are specifically asking about pain over the past 24 hours. The Traumatic Injuries Distress Scale (TIDS) is a 12-item self-report tool that was used to measure acute post-traumatic distress

following MSK injury and has demonstrated sound structural and prognostic validity across samples of acutely-injured participants[16]. Other questionnaires included participant metadata (age, sex, BMI, education level, household income, and employment status) and pre-existing health conditions. Adverse childhood experiences were captured upon inception as it has been determined to be an influential outcome in MSK trauma[17]. The Adverse Childhood Experiences questionnaire (ACEQ) asks respondents to indicate whether they had experienced any of 10 different adversities prior to age 18. The ACEQ is a commonly used measurement tool that has adequate validity in studies assessing early life trauma[18].

Genotyping analysis

The SNPs rs9394314 (*FKBP5*) and rs2501431 (*CNR2*) were chosen for this study as they had previously demonstrated bivariate associations with pain in a prior study (see Chapter 2). All SNP sequences are available on the dbSNP database (National Centre for Biotechnology Information) where the major and minor alleles were highlighted on the positive (5' -> 3') and negative (3' -> 5') strands. Genotyping was carried out using TaqMan Assays (ThermoFisher Scientific). Each DNA sample was diluted to 5-50ng/µL. In a 384 well plate, 1.5 µL of DNA was pipetted into wells that contained a mixture of TaqMan Master Mix, 40X SNP Assay and distilled water, and a negative control was prepared that contained the master mix without DNA. The prepared plate was then placed in the Applied Biosystems ViiA 7 Real-Time PCR (qPCR – ThermoFisher Scientific) to run for approximately 2 hours. Upon completion, the samples were amplified and quantified into three genotyped categories: 1) homozygotes containing major

alleles, 2) heterozygotes containing one major and one minor allele, and 3) homozygous minor alleles.

Analysis

Data fidelity

Hardy-Weinberg equilibrium was assessed prior to any statistical analyses via an online Hardy-Weinberg calculator (Gene Calculators, Jesse Hayesmoore; www.genecalculators.net/pq-chwe-check). Based on findings from our previous study (see Chapter 2), a dominant model (homozygous for minor allele vs other) was used for all analyses. Normality (skew and kurtosis) of the dependent variables (TIDS and BPI) was statistically checked through Kolmogrov-Smirnov test to confirm the assumptions for each statistical analysis. Participant characteristics were descriptively analyzed and reported (mean, median, range).

Association and cluster analyses

Mean differences in the dependent variable (TIDS) were assessed using one-way analysis of variance (ANOVA) analysis. Two-way ANOVA was used to explore the main effects of each SNP with ACEQ being dichotomized (at least one ACE vs none) and included as an interaction effect. The results for the Adverse Childhood Experience Questionnaire (ACEQ) were dichotomized into two categories to better understand how prior trauma effected pain outcomes compared to those who never experienced early childhood trauma. Significant main effects and

interaction effects for the dependent variable were explored with Tukey's post-hoc test to conduct pairwise comparisons.

A four-way crosstab table was created to determine the possible cluster combinations between the genotypic groups of the two SNPs. Mean differences were explored between the SNP clusters and the 3 dependent variables (BPI pain severity, BPI pain interference and TIDS) and mean differences in scores were reported. Two-way ANOVAs were performed for the SNP clusters with ACEQ serving as an interaction variable to explore to significant main affects and interaction affects for the dependent variables using pairwise comparisons. All statistical analyses were performed with IBM SPSS Statistics 27.0 software.

Sample size estimation

There is minimal support in the literature regarding optimal sample size to perform cluster analyses for only two genetic polymorphisms that were genotyped using a TaqMan Assay approach. Between the two SNPs, we know that there will be a maximum of four clusters possible. We also know that we have two ACEQ groups. Therefore, a power analysis was performed using G*Power v3.1[19] to estimate a sample size of 158 participants with a small to medium effect size (f=0.25) and ß = 80% using ANOVA with main and interaction effects with α = 0.05.

Results

Table 1 in Chapter 2 presents the characteristics of the sample population at baseline. There were 108 participants from the SYMBIOME database that provided blood samples. Data for the BPI and TIDS variables were available for 96 of those participants and data for the ACE variable were available for 95 of the participants who formed the sample for these analyses. Mean age for the full sample was 43.7 years, of which 54.1% were female. Pain severity and interference were moderate at baseline (mean severity = 4.5/10, SD = 1.9; mean interference = 28.4/70, SD = 17.0). From the data available for ACE, 36 participants (37.9%) reported no adverse childhood experiences, while 59 participants (62.1%) reported at least one.

Genotyping results and bivariate associations

Both SNPs were confirmed to be in Hardy-Weinberg equilibrium and had successful call rates (>95%). Minor allele frequency was 0.31 for *FKBP5* rs9394314 and 0.40 for *CNR2* rs2501431. Table 5 shows the results of the bivariate (unmoderated) analyses for *FKBP5* rs9394314 and *CNR2* rs2501431 and their associations with mean TIDS scores and 95% confidence intervals after adjusting for multiple comparisons. *FKBP5* rs9394314 showed a significantly higher TIDS score among carriers of the major alleles (n = 85, mean = 6.0, 95%CI 5.1 to 6.9) compared to the homozygous minor alleles (n = 11, mean = 3.3, 95%CI 1.5 to 5.0; $F(1,95)=4.35$, $p=0.04$, $\eta^2=0.044$). These findings are shown in Figure 3.

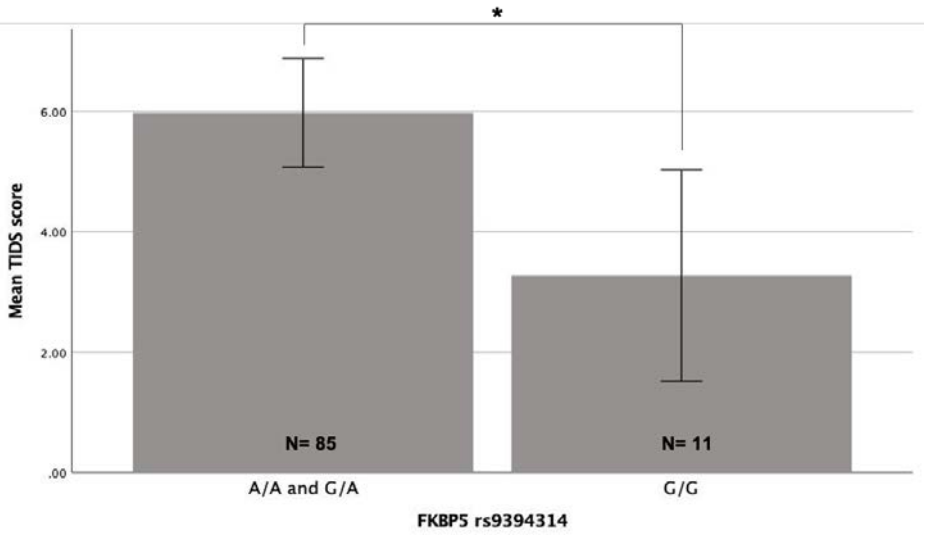


Figure 3 – Graphical representation of significant differences between the major and minor alleles of *FKBP5* rs9394314 for mean TIDS scores. Error bars represent 95% CI and an asterisk indicates significance between alleles at $p<0.05$.

Table 5: ANOVA results for TIDS scores

FKBP5 rs9394314*	**TIDS mean scores (CI)**
A/A and G/A	**6.0 (5.1, 6.9)**
G/G	**3.3 (1.5, 5.0)**
Mean difference	**2.7 (0.9, 4.5)***
CNR2 rs2501431	
A/A and G/A	5.5 (4.5, 6.4)
G/G	6.7 (4.7, 8.7)

*indicates significance at $p<0.05$ and the mean difference for significant value

Table 6 presents the results of the ANOVA analyses with ACEQ included as an interaction term. A significantly higher mean TIDS score was reported for *FKBP5* rs9394314 major alleles (n = 53) when there was at least one ACE present (mean = 6.8, 95%CI 5.7 to 7.9) compared to the homozygous minor alleles (n = 6, mean = 1.8, 95%CI 0.03 to 3.6; $F(1,57)=8.83$, $p=0.004$,

η^2=0.086). These findings are demonstrated in Figure 4. There were no other significant findings.

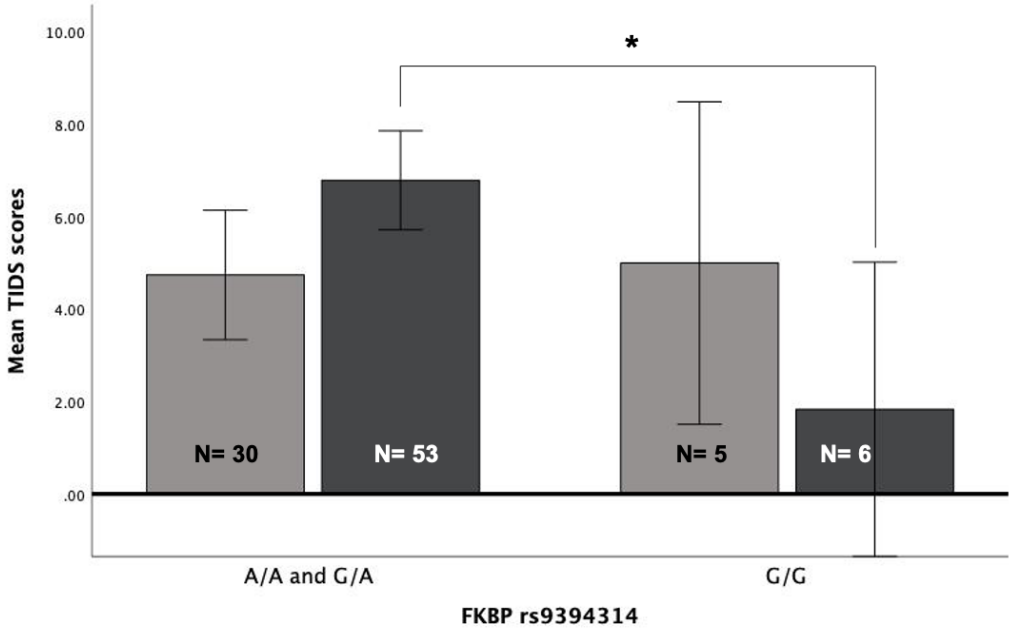


Figure 4 – Graphical representation of significant differences between the major and minor alleles of *FKBP5* rs9394314 with at least one ACE for mean TIDS scores. Bars shaded light grey represent no adverse childhood experience and bars shaded dark grey represent at least one or more adverse childhood experience. Error bars represent 95% CI and an asterisk indicates significance between alleles at p<0.05.

Table 6: ANOVA results for TIDS scores with ACEQ included as an interaction term

FKBP5 rs9394314*	ACE	N	TIDS mean scores (CI)
A/A and G/A	None At least one	31 53	4.7 (3.3, 6.1) **6.8 (5.7, 7.9)**
G/G Mean difference	None At least one	5 6	5.0 (1.5, 8.5) **1.8 (-1.4, 5.0)** **5.0 (3.3, 6.7)***
CNR2 rs2501431			
A/A and G/A	None At least one	34 46	4.6 (3.3, 6.0) 6.2 (5.0, 7.4)
G/G	None At least one	2 13	7.0 (1.3, 12.8) 6.7 (4.4, 8.9)

*indicates significance at p<0.05 and the mean difference for significant values

Cluster results

The final clusters chosen from the four-way crosstab table are summarized in Table 7. The four-way crosstab table produced 4 classes: 1) *FKBP5* major alleles and *CNR2* major alleles (n = 79); 2) *FKBP5* major alleles and *CNR2* minor alleles (n = 18); 3) *FKBP5* minor alleles and *CNR2* major alleles (n = 10); and 4) *FKBP5* minor alleles and *CNR2* minor alleles (n = 1). The fourth cluster was not included since its sample size (n = 1) was smaller than 5% of the total sample (n = 108). Table 8 shows the results of the unmoderated mean differences between the clusters and mean BPI and TIDS scores with 95% confidence intervals. The second cluster (*FKBP5* major and *CNR2* minor, n = 14) reported a significantly higher mean BPI pain interference score (mean = 35.8, 95%CI 27.3 to 44.2) than the third cluster (*FKBP5* minor and *CNR2* major; n = 10, mean = 19.2, 95%CI 9.6 to 28.8; $F(2,94)=2.93$, $p=0.047$, $\eta^2=0.06$) which is displayed in Figure 5.

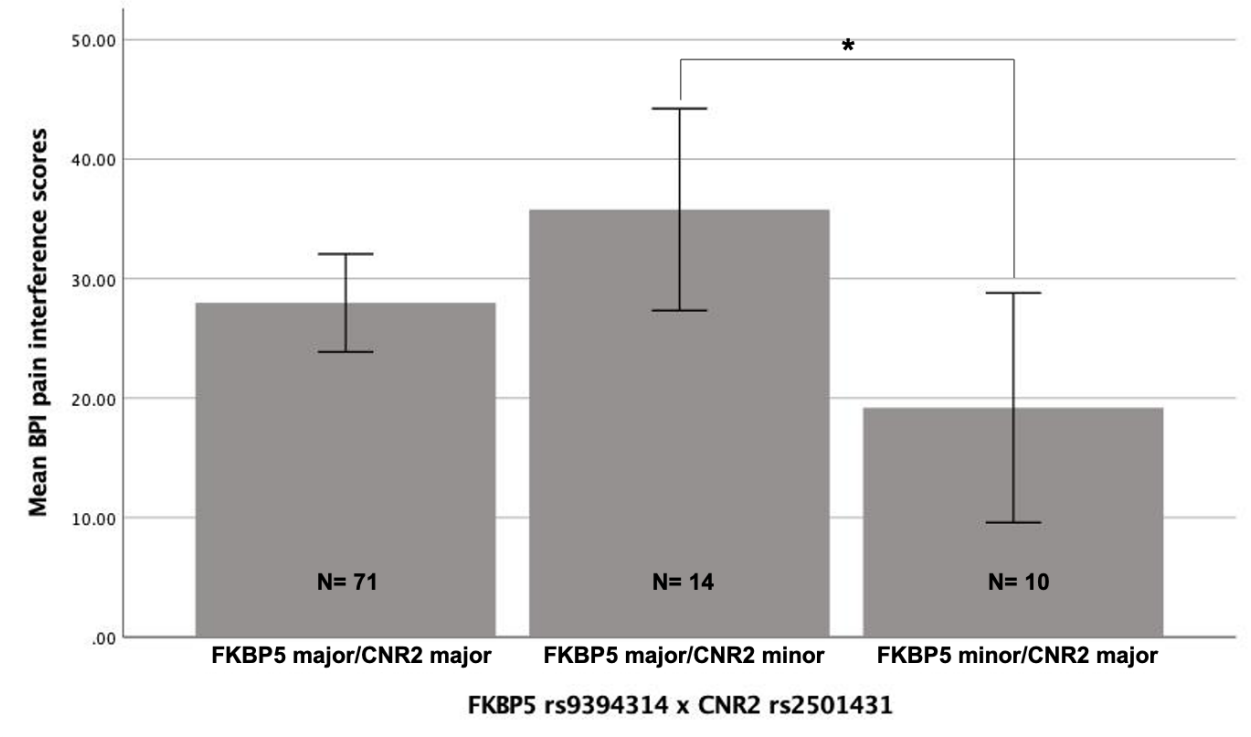


Figure 5 – Graphical representation of significant differences between the second and third clusters for BPI pain interference scores. Error bars represent 95% CI and an asterisk indicates significance between clusters at $p<0.05$.

Table 7: Final SNP clusters

Clusters	N
1 = FKBP5 major alleles and CNR2 major alleles	79
2 = FKBP5 major alleles and CNR2 minor alleles	18
3 = FKBP5 minor alleles and CNR2 major alleles	10
Total	107

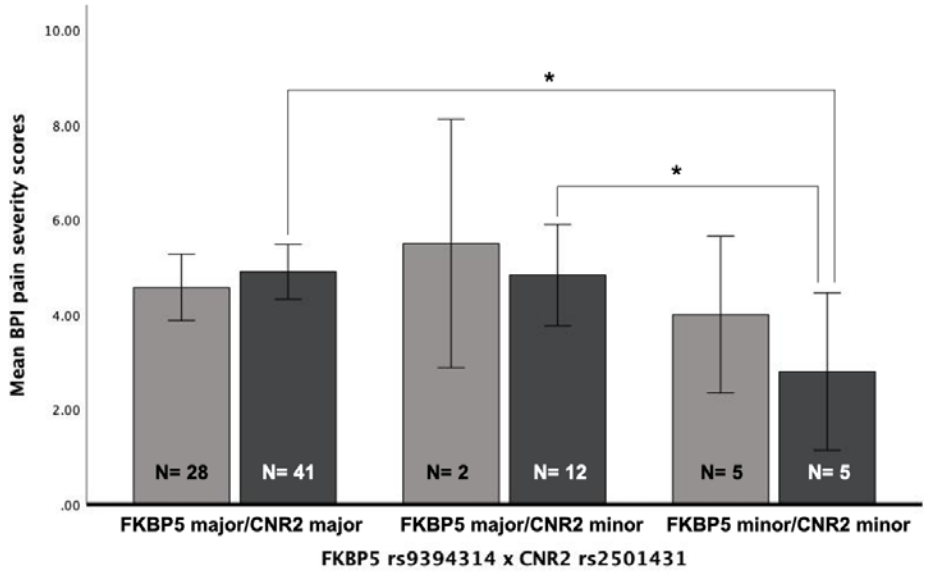
Table 8: ANOVA results of clusters and BPI and TIDS scores

Clusters	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)	TIDS mean scores (CI)
FKBP5 major alleles/CNR2 major alleles	70	4.7 (4.2, 5.2)	71	28.0 (23.9, 32.1)	5.7 (4.7, 6.8)
FKBP5 major alleles/CNR2 minor alleles	15	4.7 (3.6, 5.9)	14	**35.8 (27.3, 44.2)**	7.1 (5.2, 9.1)
FKBP5 minor alleles/CNR2 major alleles	10	3.4 (2.1, 4.7)	10	**19.2 (9.6, 28.8)**	3.5 (1.6, 5.4)
Mean difference				**16.6 (5.3, 27.9)***	

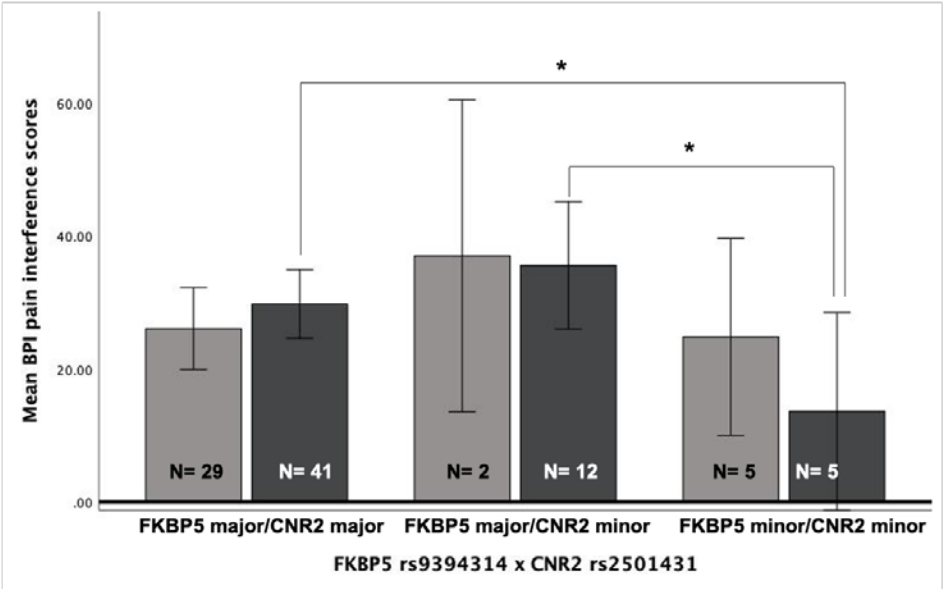
*indicates significance at $p<0.05$ and the mean difference for significant values

When at least one ACE was present (Table 9), mean BPI pain severity scores were significantly higher for the first cluster (n = 41, mean = 4.9, 95%CI 4.3 to 5.5) and the second cluster (n = 12, mean = 4.8, 95%CI 3.8 to 5.9) compared to the third cluster (n = 5, mean = 2.8, 95%CI 1.1 to 4.5; $F(2,57)=3.19$, $p=0.049$, $\eta^2=0.062$). Mean BPI pain interference scores were significantly higher for the first cluster (n = 41, mean = 29.8, 95%CI 24.6 to 34.9) and second cluster (n = 12, mean = 35.6, 95%CI 26.0 to 45.2) compared to the third cluster (n = 5, mean = 13.6, 95%CI -1.2 to 28.4; $F(2,57)=3.63$, $p=0.03$, $\eta^2=0.065$). People in the first (n = 41) and second clusters (n = 12) reported significantly higher mean TIDS scores with at least one ACE present (mean = 6.7, 95%CI 5.4 to 7.9 and mean = 7.2, 95%CI 4.9 to 9.4, respectively) compared to the third cluster (n = 5, mean = 2.0, 95%CI -1.5 to 5.5; $F(2,57)=3.46$, $p=0.04$, $\eta^2=0.071$). These findings are presented in Figure 6.

A



B



C

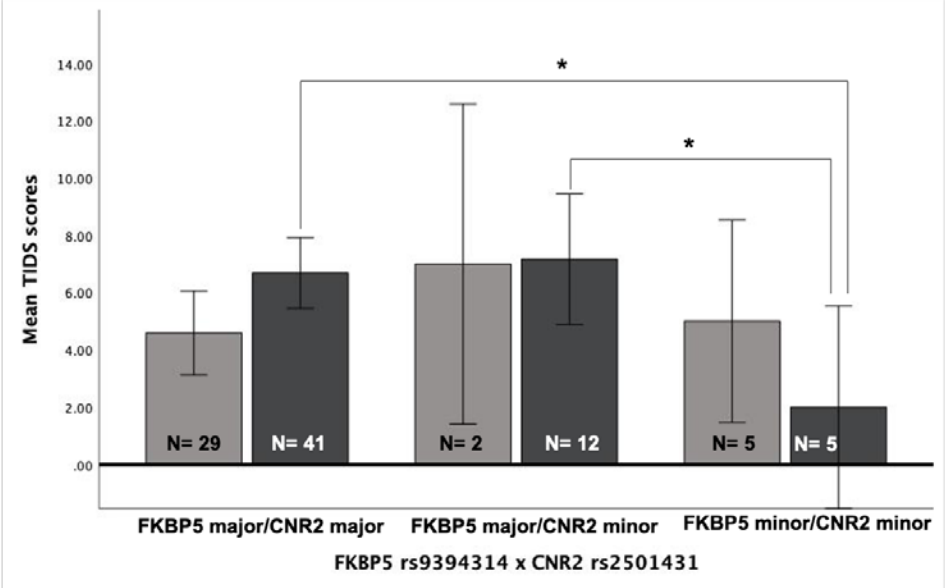


Figure 6 – Graphical representation of significant differences between clusters of SNPs with at least one ACE for BPI pain severity (A), BPI pain interference (B) and TIDS (C) scores. Bars shaded light grey represent no adverse childhood experience and bars shaded dark grey represent at least one or more adverse childhood experience. Error bars represent 95% CI and an asterisk indicates significance between clusters at $p<0.05$.

Table 9: ANOVA results of clusters and BPI and TIDS scores with ACEQ interaction

Clusters	ACE	N	BPI pain severity mean scores (CI)	N	BPI pain interference mean scores (CI)	TIDS mean scores (CI)
FKBP5 major alleles/CNR2 major alleles Mean difference	None At least one	28 41	4.6 (3.9, 5.3) **4.9 (4.3, 5.5)** **2.1 (0.1, 4.1)***	29 41	26.1 (19.9, 32.2) **29.8 (24.6, 34.9)** **16.2 (5.9, 26.5)***	4.6 (3.1, 6.1) **6.7 (5.4, 7.9)** **4.7 (2.6, 6.8)***
FKBP5 major alleles/CNR2 minor alleles Mean difference	None At least one	2 12	5.5 (2.9, 8.1) **4.8 (3.8, 5.9)** **2.0 (-0.2, 4.2)***	2 12	37.0 (13.5, 60.5) **35.6 (26.0, 45.2)** **22.0 (9.3, 34.7)***	7.0 (1.4, 12.6) **7.2 (4.9, 9.4)** **5.2 (2.5, 7.9)***
FKBP5 minor alleles/CNR2 major alleles	None At least one	5 5	4.0 (2.3, 5.7) **2.8 (1.1, 4.5)**	5 5	24.8 (10.0, 39.6) **13.6 (-1.2, 28.4)**	5.0 (1.5, 8.5) **2.0 (-1.5, 5.5)**

*indicates significance at $p<0.05$ and the mean difference for significant values

Discussion

This study sought to explore the interaction between two genetic variants of different molecular pathways and their combined effects on pain, distress, and early childhood trauma. Through an exploration of possible allele frequencies, a 4-cluster solution was determined for the two genetic polymorphisms. However, one cluster was excluded due to a small sample size (n=1). Most participants fell into the first cluster which was characterized by the major alleles of both polymorphisms, while the second cluster was characterized by *FKBP5* major alleles and *CNR2* homozygous minor alleles and the third cluster was described by *FKBP5* homozygous minor alleles and *CNR2* major alleles. Participants assigned to the second cluster rated higher on self-reported scales of pain-related functional interference. Further, when accounting for the presence of at least one adverse childhood experience, participants in the first cluster demonstrated higher pain severity scores, participants assigned to the second cluster reported higher pain interference scores, and participants in both the first and second clusters scored higher on trauma and distress-related scales when in the presence of at least one adverse

childhood experience. To our knowledge, this is the first time that these two genetic polymorphisms have been shown to interact in a way that may demonstrate clinical utility; however, due to the small sample, we exercise caution until the results can be replicated in a larger independent sample.

FKBP5 encodes a binding protein that is involved in the activity of the hypothalamic-pituitary adrenal (HPA) axis through its regulatory role of the glucocorticoid receptor. When *FKBP5* is highly expressed, there is an increase in negative feedback of the HPA axis, resulting in a decrease of stress hormone system activation following experience to stress[20]. This may create risk factors for stress-related incidents and psychiatric disorders[21]. A paper by Zannas et al. assessing genetic and epigenetic roles of *FKBP5* polymorphisms in various psychological outcomes indicated that carriers of minor alleles derived from a haplotype of *FKBP5* polymorphisms were at greater risk of developing psychiatric disorders as adults following early childhood trauma compared to carriers of major alleles[22]. In another study by Binder et al. on patients that had experienced depressive episodes, genetic variants of *FKBP5* were shown to be associated with greater episodes of depression[23]. Carriers of the associated polymorphisms had less HPA-axis hyperactivity during depressive episodes compared to non-carriers. Further, Halldorsdottir et al. conducted a study that explored the interaction between childhood trauma and polymorphisms of *FKBP5* in students aged 12-17 and their parents to determine if they can predict rumination and catastrophizing in adolescents. They found that *FKBP5* polymorphisms moderated the association between childhood trauma and abnormal emotional regulation[24]. In our previous study (see Chapter 2), *FKBP5* rs9394314 was associated with pain severity and

when considering the presence of an adverse childhood experience, carriers of the rs9394314 major alleles also tended to report higher ratings of pain severity. In this study, we showed that rs9394314 may also be associated with trauma and distress in our sample of participants reflected by major allele carriers reporting higher mean TIDS scores compared to homozygous minor allele carriers. This was also the demonstrated when considering the presence of an adverse childhood experience. These findings indicate that major allele carriers of rs9394314 may be predisposed to vulnerabilities related to distress following a traumatic injury, and those that have suffered an adverse childhood experience are even more likely to present greater distress.

Polymorphisms of endocannabinoid genes are commonly associated with psychiatric disorders such as major depression and post-traumatic stress disorder[25]. Morena et. al. have demonstrated that the endocannabinoid system plays an integral role in stress response which involves activation of the HPA axis, causing an increase in anxiety-related behaviour during stress[26]. Since the HPA axis is heavily involved in stress responses and can be responsible for the outcome of stress-related disorders, it is no surprise that genes playing a role in the HPA axis system may interact and contribute to these stress-related disorders. A study conducted by Ishiguro et. al. found that inducing anxiety-like behaviour led to reduction of *Fkbp5* expression in brains of the mice where *Cnr2* heterozygotes were knocked out compared to the *Cnr2* wild-type mice, indicating a cross-talk may exist between the two markers[27]. Other studies have explored the potential interactive roles of genes involved in the endocannabinoid system and the stress response system[28–30] such as *FKBP5* and *CNR2*; however, to our knowledge no study

has investigated the interaction between *FKBP5* rs9394314 and *CNR2* rs2501431 in the context of pain following a traumatic injury or the effects of an adverse childhood experience. In our prior study, pain interference scores were only significantly higher for homozygous minor allele carriers of rs2501431 while rs9394314 major allele carriers reported slightly higher pain interference scores but were not significantly different from scores reported by homozygous minor allele carriers. Therefore, it is not surprising to see that in the present study, combining both polymorphisms resulted in a higher mean score for pain-related functional interference for participants in the second cluster compared to the third cluster. Similar findings were reported for pain interference between the second and third clusters when considering the presence of at least one adverse childhood experience, which was also the case for participants' mean TIDS scores. It is possible that carriers of rs9394314 major alleles and rs2501431 homozygous minor alleles are predisposed to vulnerabilities of pain interference and traumatic distress following a traumatic injury, and the combined effect of both polymorphisms demonstrates an increase in these vulnerabilities especially with the presence of an adverse childhood experience.

Limitations

As this was an exploratory study, there are important limitations to consider. First, our sample was underpowered based on our sample size estimation and the actual number of participants involved in our study. This is also reflected in the uneven proportions of alleles within each SNP and the further reduction in their sample size numbers when incorporating the effect of ACEQ as a variable. Second, we did not try to include more complex multivariate models such as BMI,

sex, or pre-existing psychopathology or pre-existing pain. As demonstrated in our prior study, as well as other genetic studies focused on post-injury pain and trauma, associations may be moderated by other important psychosocial variables that require larger datasets for further exploration. Overall, more research is needed in larger independent samples to build on our early findings.

Conclusion

We have demonstrated an association between *FKBP5* rs9394314 and trauma-related distress by use of the TIDS tool, as well as an association that also exists when at least one adverse childhood experience was present in a sample of people that suffered an acute non-catastrophic musculoskeletal trauma. We have also presented an exploratory study of clustering SNPs to better understand the interaction of genetic polymorphisms from stress response and endocannabinoid systems. We identified three clusters where the second cluster characterized by major allele carriers of rs9394314 and homozygous minor allele carriers of rs2501431 reported significantly higher pain-related functional interference scores. Further, participants in the second cluster reporting at least one adverse childhood experience tended to report higher pain interference and traumatic distress. These findings, along with the consideration of other self-report measures, may provide a framework for future biopsychosocial studies aiming to better understand the interactions of genetic polymorphisms and their roles in clinical pain and distress-related outcomes.

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Chapter 4

Exploring the roles of genetic polymorphisms and early childhood trauma in predicting recovery outcomes in people that suffered from acute musculoskeletal trauma

Introduction

Acute and chronic pain following musculoskeletal trauma are complicated experiences for people who suffer from such incidents. Current models present the phenomena of pain as a very subjective experience that is influenced by the involvement of psychological, social and biological interactions[1]. The ability to sense, interpret, and react to potentially harmful stimuli is universally recognized as a necessity for survival and adaptation in humans and many other organisms[2]. Pain that goes unresolved and transitions into chronic pain tends to become a burden on people and intrudes on many aspects of their lives[3]. Lack of adequate care has led pain to be considered a serious pathological condition[4]. In Canada and the United States, the prevalence of chronic pain in adults is approximately 20% and has led to an overwhelming social and economic burden for many[5–7]. Why some people recover while others develop chronic pain continues to be an area of great interest for healthcare providers and researchers as the ability to predict recovery can help implement early intervention strategies to avoid or minimize severity of chronic pain.

Previous attempts have been made to develop screening tools and protocols for patients with general musculoskeletal (MSK) trauma with the purpose of identifying early symptoms to prevent chronic pain development[8,9]. Many of the screening tools available were designed for a

specific sample population or for specific regions of injury, such as those with whiplash associated disorder[10] or low back pain[11]. For example, the screening tool developed by Lentz et al. is one of the few multi-region tools used for MSK injuries that shows good association with other variables of psychological distress. Another screening tool that is not dependent on region of injury is the Traumatic Injuries Distress Scale (TIDS). The TIDS is a self-report tool meant to capture the likelihood and reasons of not recovering in all cases of MSK trauma. Walton et al. found that the TIDS displayed sound validity in acutely injured participants and showed strong associations with 5 different outcomes 12 weeks later in the initial development of the TIDS tool[12]. A later study that included mixed cohorts from Canada and the United States found that the TIDS tool was able to predict recovery outcomes with ≥76% accuracy for patients that had a non-catastrophic MSK injury[13]. The recovery outcome trajectories that were used by Walton et al. were identified in a previous study by Lee et al. that used the same cohort of patients[14]. Lee et al. used pain interference outcomes over the course of 12 months from initial trauma to discover 3 classes of recovery: rapid recovery represented by 34.9% of the sample, delayed recovery (full recovery still reached by 12 months) represented by 19.2% of the sample, and minimal to no recovery at all which was represented by 45.9% of the sample.

Although there are many early screening tools intended to predict chronicity, none have explored the possibility of interactions between genetics and psychosocial factors, and how they may influence potential recovery outcomes within the context of MSK trauma. One gene that has been heavily studied for its associations with pain and psychological distress is *FKBP Prolyl Isomerase 5* (*FKBP5*) which is responsible for encoding a binding protein involved in the

activity of the hypothalamic-pituitary adrenal (HPA) axis through its regulatory role of the glucocorticoid receptor. One study found that genetic polymorphisms of *FKBP5* contained risk alleles that may help predict persistent pain in people after trauma exposure, while another study demonstrated that *FKBP5* polymorphisms moderated chronic MSK pain development after a motor vehicle collision among people of lower socioeconomic status only[15,16]. More recently, a study using our cohort of patients found an association between the *FKBP5* polymorphism rs9394314 and pain severity as well as an association between rs9394314 and the TIDS tool where carriers of major alleles reported higher mean scores compared to minor allele carriers (see Chapters 2 and 3). These associations were also demonstrated when considering the presence of an adverse childhood experience. Further, we previously identified 3 clusters of genotypes using two different genetic polymorphisms (*FKBP5* rs9394314 and *Cannabinoid receptor 2 (CNR2)* rs2501431). The SNPs clusters demonstrated an association with pain interference alone, and an association with pain interference and TIDS in those also reporting an adverse childhood experience (see Chapter 3). The relationships identified between the genetic polymorphisms and TIDS in our prior studies have provided a framework to continue exploring downstream mechanisms that may help better predict recovery outcomes.

Longitudinal outcomes introduce a different and important perspective for early identification and intervention of trauma-related pain outcomes. Therefore, using the findings from our previous cross-sectional studies (see Chapters 2 and 3), the purpose of this study was to explore potential pathways that link *FKBP5* rs9394314 with adverse childhood experiences to help

predict recovery outcomes in patients that suffered an acute non-catastrophic MSK trauma, and if these pathways can be mediated by the TIDS tool to help predict recovery vs non-recovery patterns. Further, the same pathways were explored for the genotype clusters identified from combining *FKBP5* rs9394314 and *CNR2* rs2501431.

Methods

Participants

Data were drawn from the longitudinal SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) database (clinicaltrials.gov ID no. NCT02711085). The study was approved by the local institutional review board. Eligible participants were 18 years or over and recruited from an urgent care centre in London, ON, Canada seeking medical care for a non-catastrophic MSK injury such as whiplash, low back injuries, sports or slip and fall-type injuries that result in sprain/strain of muscle, tendon, ligament, or other such soft tissues. Excluded from the study were those with significant neuromuscular or systemic comorbidities that may affect physiological response to trauma or recovery (e.g. active cancer, rheumatic conditions or other systemic inflammatory processes), significant organ disease, those with immunocompromised conditions (e.g. HIV/AIDS) or taking immunomodulatory drugs (e.g. high-dose steroids or disease- modifying anti-rheumatic drugs).

After being medically discharged, a member of the research team approached interested participants and described the study and answered any questions. Enrollment occurred before

the participant left the centre. A package of self-report questionnaires was given to the participants. Blood samples were collected into two 4mL K2 EDTA BD vacutainer tubes from the median cubital vein by a phlebotomy-trained research team member. Samples were transferred on ice to a local wet lab for analysis. The samples were centrifuged for 10 minutes at 2000*g* and the plasma was then pipetted into 50µL aliquots, and both supernatant and pellets were stored in a -80^{o}C freezer until genotyping.

Recovery trajectories were identified in a prior study using data from the SYMBIOME database[14]. Functional recovery was assessed using the pain interference subscale of the Brief Pain Inventory (BPI). The BPI is a well-recognized pain measurement tool[17,18] and has sufficient validity across various clinical populations[19]. The Traumatic Injuries Distress Scale (TIDS) is a 12-item self-report tool measuring acute post-traumatic distress following MSK injury. The TIDS has demonstrated sound prognostic validity across samples of participants that suffered an acute injury[12]. Information regarding early childhood trauma was also captured using the Adverse Childhood Experiences Questionnaire (ACEQ) as it has been shown to be an important outcome in MSK trauma[20]. The ACEQ asks participants to indicate whether they had experienced any of 10 different adversities prior to age 18. The ACEQ is a widely used measurement tool that has acceptable validity in studies assessing early life trauma[21]. Participant metadata (age, sex, BMI, education level and household income) and pre-existing health conditions were also captured upon inception.

Genotyping

FKBP5 rs9394314 was chosen for this study as it had previously demonstrated an association with traumatic distress in our prior study (see Chapters 3). SNP sequences are available on the dbSNP database (National Centre for Biotechnology Information) where the major and minor alleles were highlighted on the positive (5' -> 3') and negative (3' -> 5') strands. Genotyping was carried out using TaqMan Assays (ThermoFisher Scientific). Each DNA sample was diluted to 5-50ng/µL. In a 384 well plate, 1.5 µL of DNA was pipetted into wells that contained a mixture of TaqMan Master Mix, 40X SNP Assay and distilled water. A negative control was prepared that contained the master mix without DNA. The prepared plate was placed in the Applied Biosystems ViiA 7 Real-Time PCR (qPCR – ThermoFisher Scientific) to run for approximately 2 hours. Upon completion, the samples were amplified and quantified into three genotyped categories: 1) homozygotes containing major alleles (A/A), 2) heterozygotes containing one major and one minor allele (G/A), and 3) homozygous minor alleles (G/G). Further, a genotype cluster (given the name SNPs Clusters for this study) identified from combining two SNPs in our prior study was explored in this study as it previously demonstrated associations with traumatic distress (see Chapter 3).

Analysis

Preliminary analysis

Prior to any statistical analyses, preliminary analyses were performed to refine our data and inform subsequent steps. Hardy-Weinberg equilibrium was assessed via an online Hardy-

Weinberg calculator (Gene Calculators, Jesse Hayesmoore; www.genecalculators.net/pq-chwe-check). Normality (skew and kurtosis) for TIDS was statistically checked through Kolmogrov-Smirnov test to confirm the assumptions for each statistical analysis. Analysis of variance tests (ANOVA) were used to evaluate the differences in mean TIDS scores between the carriers of genotypes of *FKBP5* rs9394314 and the SNPs clusters. Participants were also categorized based on ACEQ responses into none (no ACEs endorsed) or at least one ACE endorsed, and a two (SNP) by two (ACE) ANOVA was conducted considering ACE category as an interaction term. The results for the Adverse Childhood Experience Questionnaire (ACEQ) were dichotomized into two categories to investigate how prior trauma effected pain outcomes compared to those who never experienced early childhood trauma. Significant main affects and interaction affects for the dependent variable were explored with Tukey's post-hoc test to conduct pairwise comparisons. Participant characteristics were descriptively analyzed and reported (mean, median, range). These data are available in Chapter 3. Recovery trajectories of acute non-catastrophic MSK pain were based on pain interference outcomes over the course of 12 months from the time of initial trauma. Three trajectories were identified: rapid recovery, delayed recovery (full recovery still reached by 12 months), and minimal to no recovery at all. Recovery outcomes for this study were dichotomized into two levels (recovered vs no recovery). It is important to note that only recovery outcomes of participants who provided blood for genotyping were included in the analyses.

Genomic structural equation modelling

Based on theory and the results of our previous study (see Chapters 3), two *a priori* structural equation models (SEM) were tested – one for recovery and *FKBP5* rs9394314 and one for recovery and our SNPs Clusters. Both models had an interaction included that combined the SNPs with ACEQ as one independent variable. The hypothetical models were assessed through path analyses and were designed for the SNPs that showed significant associations with the TIDS. We fully assessed our first SEM model while additionally testing three mediational analyses for recovery: ACEQ → TIDS → Recovery pathway, *FKBP5* rs9394314 → TIDS → Recovery pathway and *FKBP5* rs9394314xACEQ → TIDS → Recovery pathway. Our second SEM model was fully assessed along with two mediational analyses: SNPs Clusters → TIDS → Recovery pathway and SNPs Clusters-x-ACEQ → TIDS → Recovery pathway. Standardized and unstandardized direct and indirect effects were estimated for our models through AMOS v27 for IBM SPSS Statistics 27.0 software. P-values and bootstrapped 95% confidence intervals were used for determining the significance of the effects through *the PROCESS macro* for SPSS. The pathway was considered significant where zero was not included in the confidence intervals.

In mediation analyses, when an indirect (mediated) effect exists but no direct effect exists, mediation is present. When an indirect effect does not exist, but other direct effects do exist, it is considered a direct-only (non-mediation) effect. Model fit was interpreted through standard goodness of fit indicators according to the following criteria: Chi-square, where smaller values indicate better fit; Comparative Fit Index (CFI) > 0.95; Normed Fit Index (NFI) > 0.95; Root Mean Squared Error of Approximation (RMSEA) ≤ 0.05.

Sample size estimation

Soper algorithms[22] estimated a minimum sample size of 90 participants with an effect size of 0.3, an alpha error of 0.05 and 80% power.

Results

Preliminary analysis

There were 108 participants from the SYMBIOME database that provided blood samples. Table 1 in Chapter 2 presents the characteristics of the sample population. Data for TIDS were available for 96 of those participants, data for the ACEQ variable were available for 95 of the participants and data for recovery outcomes were available for 101 participants. Mean age for the full sample was 43.7 years, of which 54.1% were female. From the data available for ACEQ, 36 participants (37.9%) reported no adverse childhood experiences, while 59 participants (62.1%) reported at least one. Of the data available for participant's recovery outcomes, 86 participants (85.1%) recovered, while 15 (14.9%) did not.

Genomic SEM for recovery

The proposed *a-priori* statistical model for *FKBP5* rs9394314 and Recovery is demonstrated in Figure 7. Good model fit was established by a RMSEA value ≤ 0.05. The regression coefficients for three of the hypothesized paths were significant: the path from *FKBP5* rs9394314 to TIDS (ß=0.22, *p*=0.03), the path from *FKBP5* rs9394314xACEQ to TIDS (ß=0.20, *p*=0.03), and the path from TIDS to Recovery (ß=-0.23, *p*=0.03). Table 10 presents the results of the three mediation analyses. The mediation analyses revealed that only the indirect effect of *FKBP5* rs9394314 →

TIDS → Recovery pathway was significant (Standardized indirect effect = -0.05, Unstandardized indirect effect = -0.12 (SE=0.07), 95% CI -0.3 to -0.02).

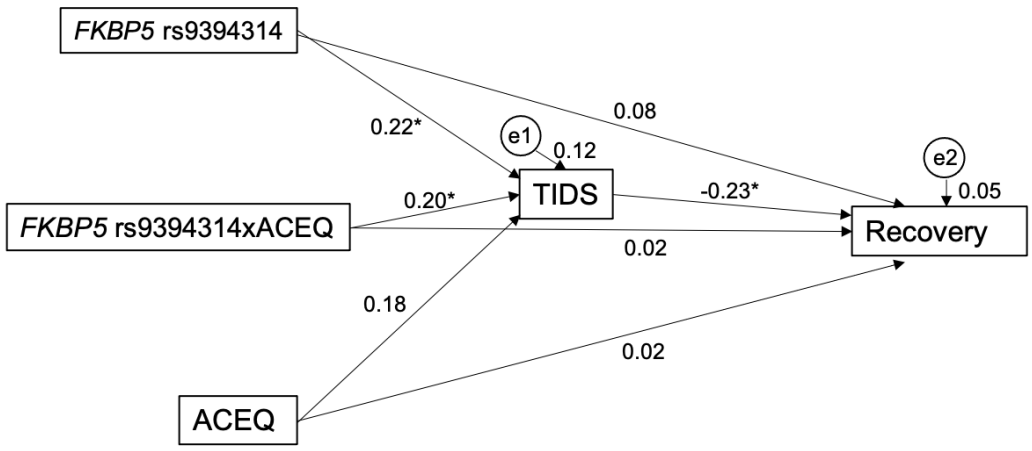

Figure 7 – *a-priori* model of *FKBP5* rs9394314 for Recovery. An asterisk (*) indicates $p<0.05$; e = error. Path loadings are standardized coefficients.

Table 10: Direct and indirect effects for mediator path models

Path	Standardized Effect[1]	Unstandardized Effect (SE), (95% LLCI, ULCI)[2]
ACEQ→TIDS→Recovery	Direct effect = 0.02	Direct effect = 0.04 (0.30), P=0.89 (-0.6, 0.6)
	Indirect effect = -0.04	Indirect effect = -0.10 (0.06), (-0.2, 0.03)
FKBP5 rs9394314 →TIDS→Recovery	Direct effect = 0.08	Direct effect = 0.24 (0.27), P=0.38 (-0.3, 0.8)
	Indirect effect = **-0.05***	Indirect effect = **-0.12* (0.07), (-0.3, -0.02)**

FKBP5 rs9394314xACEQ →TIDS→Recovery	Direct effect = 0.02	Direct effect = -0.01 (0.30), P=0.97, (-0.6, 0.6)
	Indirect effect = -0.05	Indirect effect = -0.08 (0.07), (-0.3, 0.0)
SNPs Clusters→TIDS→Recovery	Direct effect = -0.01	Direct effect = -0.07 (0.30), P=0.82, (-0.7, 0.5)
	Indirect effect = 0.03	Indirect effect = 0.05 (0.06), (-0.1, 0.2)
SNPs Clusters-x-ACEQ →TIDS→Recovery	Direct effect = 0.004	Direct effect = 0.04 (0.29), P=0.89, (-0.5, 0.6)
	Indirect effect = 0.03	Indirect effect = 0.07 (0.06), (-0.0, 0.2)

*Indicates significance

[1]Standardized effect values were assessed in AMOS v27 for IBM SPSS Statistics 27.0

[2]Significance was determined through unstandardized effects which were assessed through *PROCESS macro* for SPSS

The proposed *a-priori* statistical model for SNPs Clusters and Recovery is presented in Figure 8. Good model fit was established by a RMSEA value ≤ 0.05. The regression coefficient for only one of the proposed paths was significant, the path from TIDS to Recovery (ß=-0.21, *p*=0.04). The results of mediation analyses that were tested are displayed in Table 10. None of the mediation pathways were significant.

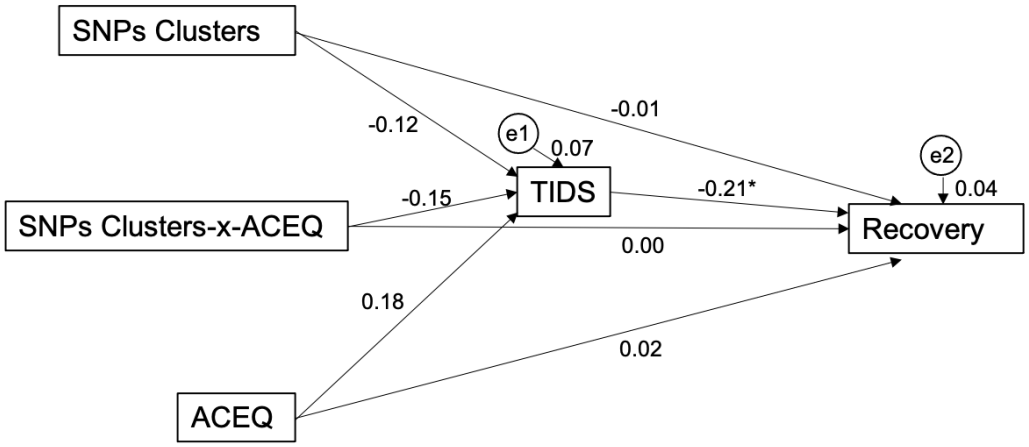

Figure 8 – *a-priori* model of SNPs Clusters for Recovery. An asterisk (*) indicates $p<0.05$; e = error. Path loadings are standardized coefficients.

Discussion

The focus of this study was to explore the relationships and pathways between genetic polymorphisms, self-reported adverse childhood experiences and TIDS scores, and longitudinal recovery outcomes for people that experienced an acute traumatic MSK injury. Our findings from this study showed that people carrying major alleles (A/A and G/A) of *FKBP5* rs9394314 reported higher TIDS scores, and that TIDS indirectly mediated the relationship between *FKBP5* rs9394314 and recovery, where major allele carriers reporting high TIDS scores fell in the none-recovery category. Major allele carriers that experienced at least one or more adverse childhood events were found to report higher TIDS scores, which implies ACEQ as a moderator between *FKBP5* rs9394314 and TIDS, as was expected based on our prior study (see Chapter 3); however, there was no direct or indirect relationship between these people and recovery

outcomes. There was no significant relationship between pathways involving our SNP clusters, ACEQ, TIDS, and recovery outcomes. To our knowledge, this is the first study that demonstrates a predictive pathway to recovery outcomes using TIDS as a mediator between *FKBP5* rs9394314 and recovery.

Our previous study (see Chapter 3) demonstrated a significant association between *FKBP5* rs9394314 and mean TIDS scores, where major allele carriers reported higher scores compared to minor allele (G/G) carriers. Major allele carriers that reported having at least one adverse childhood experience had significantly higher mean TIDS scores compared to minor allele carriers. As a key regulator of the glucocorticoid receptor through the binding protein it encodes, *FKBP5* plays a major role in stress response via the HPA-axis. Varying expression levels caused by associated genetic polymorphisms of *FKBP5* may alter the way the HPA-axis responds to stress, creating potential risk factors for stress-related experiences[23,24,25]. Further, a study by Ising et al. revealed that certain *FKBP5* polymorphisms are associated with elevated Cortisol levels after exposure to stress[26], while Binder et al. discovered that carriers of *FKBP5* risk alleles were associated with more frequent episodes of depression and showed smaller activity levels in their HPA-axis during these episodes of depression compared to non-carriers[27]. Based on another study by Halldorsdottir et al. which found that *FKBP5* polymorphisms moderated the association between childhood trauma and abnormal emotional regulation in adolescents[28], we hypothesized that the interaction between rs9394314 and a prior adverse childhood experience would show a significant relationship with TIDS scores for our study participants. The results of our current study support the relationship between *FKBP5* rs9394314 and TIDS scores, as well

as the interaction between early childhood trauma and rs9394314 on TIDS scores that were hypothesized based on our previous studies and findings in prior literature.

Hyperalgesia induced by non-habituating sound stress in a rat model showed that both elevated catecholamines and elevated glucocorticoids after exposure to stress were required as the cause for persistent generalized hyperalgesia[29,30]. This hyperalgesia development was delayed onset and resulted from action of catecholamines and glucocorticoids on primary sensory, which were mediated by stress-induced changes in second messenger signalling pathways[31]. Based on these findings, we hypothesize that major allele carriers of *FKBP5* rs9394314 in our study that scored higher on the TIDS questionnaire experienced increased pain and fell into the non-recovered category due, at least in part, to the peripheral effects of elevated glucocorticoids on sensory afferent neurons. Furthermore, glucocorticoid systems have an important influence on the function of the immune system[32,33]; therefore, carriers of the *FKBP5* rs9394314 major alleles may influence post-stress outcomes which lead to elevated pro-inflammatory mediators such as cytokines. These mediators may encourage continued hyperalgesia through sensitizing peripheral and central afferents directly, and by sensitizing neurons of the central nervous system through an afferent feedback mechanism[34–39]. Although our previous study showed an association between our SNP clusters and mean TIDS scores when there was at least one adverse childhood experience (see Chapter 3), our current study did not reveal any relationships between our SNP clusters and TIDS or recovery outcomes, even when considering the presence or absence of an adverse childhood experiences. This may be due to *CNR2* rs2501431 not having an association with mean TIDS

scores in our previous study and was therefore influencing our SNP cluster genotypes. Further, the association between our SNP clusters and TIDS when patients experienced at least one adverse childhood event was based on uneven proportions of alleles within each SNP which was further reduced in sample size numbers when considering the presence of ACEQ. It is possible that the difference in proportionality was accounted for better in our genomic structural equation model in this study compared to the ANOVA models tested in our previous study.

Limitations

There are a few important limitations to consider. First, although path analysis is well-established methodology for exploring hypothetical relationships between variables and theoretical constructs, interpretation must be done with caution since structural equation modelling has been referred to as causal modelling, and implying causation requires more evidence. This is particularly true for genomic SEM which is still a relatively new area of interest and requires large sample sizes. Second, we must consider errors typical of self-report questionnaires such as external bias and how it can affect the responses of the participant. Finally, we did not include more multivariate models such as sex or pre-existing pain. As demonstrated in our prior studies, associations may be moderated by other important psychosocial variables that require further exploration. To better understand pain, recovery, and inform pain management, other psychosocial variables should be considered, and a larger, more socio-cultural diverse sample should be the focus for future research.

Conclusion

We have presented a genomic structural equation model that demonstrates a relationship between *FKBP5* rs9394314 and TIDS scores and the interaction between ACEQ and *FKBP5* rs9394314 having a relationship with TIDS scores in participants that suffered an acute MSK trauma. Further, we have revealed a relationship between TIDS and recovery outcomes, and the indirect relationship between *FKBP5* rs9394314 and recovery outcomes which is mediated by TIDS. Major allele carriers (A/A and G/A) of *FKBP5* rs9394314 reported higher scores on the TIDS questionnaire, which was also demonstrated when considering the presence of at least one adverse childhood experience. Further, major allele carriers that scored higher on the TIDS were predicted to be in the non-recovered category. Our findings may provide a framework for future genetic studies aiming to better understand the biopsychosocial interactions of pain and distress-related outcomes, and their roles in predicting recovery outcomes after MSK trauma.

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Chapter 5

Summary

Genetic testing is becoming increasingly popular in healthcare because of its ability to identify many diseases and can be used to predict the risk of developing a health condition. Research in genetics has progressed across many health conditions, with mutations being identified that can predict or modify the onset of disease. In some cases, these genetic profiles are also being used to identify personalized intervention strategies to prevent or slow disease onset. With respect to chronic pain, a condition that is best conceptualized through a biopsychosocial framework, genetic testing is being looked at with a lens of cautious optimism. There is no clear-cut treatment for chronic pain. Surely people *can* recover from chronic pain, but even the path to recovery remains ambiguous. For these reasons, chronic pain can be difficult to treat and may cause people with chronic pain to be frustrated with their situation. For some people, genetic testing may provide more information about the presence or absence of risk alleles for genes associated with chronic pain and help indicate whether they are on the path of recovery and their pain is temporary; or it may give them (along with their clinician and loved ones) an answer for their continued pain that can perhaps be managed differently.

The purpose of this dissertation was to present the findings of our efforts to combine genetic polymorphisms with psychological and social factors to better understand the experience of pain. These chapters highlight my attempts to reconcile biological characteristics with personal

lived experiences to further our knowledge of the biopsychosocial model of pain and facilitate continued research in hopes of improved early intervention strategies to prevent chronic pain.

Chapter 2 demonstrates the results of an exploratory analysis of a variety of single nucleotide polymorphisms (SNPs) and their relationship with pain severity and pain interference outcomes. Taking an adverse childhood experience into consideration as a moderator was also explored to determine the effect it has on the relationships between SNPs and pain. In relation to pain severity, *FKBP5* rs9394314 showed a significant difference in pain severity scores between major allele carriers and minor allele carriers. This difference was also significant for people who experienced at least one adverse childhood event. For pain interference, a significant difference in scores was found between carriers of major and minor alleles for *CNR2* rs2501431. This association was not moderated by adverse childhood experiences. These results support the existing knowledge that psychosocial factors and genetics may be tied to pain through more than simple bivariate associations and alone cannot fully capture the effects each has on pain. The greater implication of this work is that it encourages further exploration of interactions between psychosocial factors and genotypes of people who have experienced recent or prior musculoskeletal trauma.

Chapter 3 demonstrates the results of combining the two genetic polymorphisms from the prior analysis, *FKBP5* rs9394314 and *CNR2* rs2501431, to identify potential for genotype clusters that were further explored in the context of pain and trauma-related distress. Three clusters were identified where the second cluster characterized by major allele carriers of

rs9394314 and homozygous minor allele carriers of rs2501431 reported significantly higher pain-related functional interference scores. Further, participants in that cluster who also reported at least one adverse childhood experience tended to report higher pain interference and traumatic distress scores compared to the third cluster, while participants in the first cluster reporting at least one adverse childhood experience tended to report higher pain severity scores compared to the third cluster. Chapter 3 also demonstrated that *FKBP5* rs9394314 major allele carriers reported significantly higher scores on the Traumatic Injuries Distress Scale (TIDS) compared to minor allele carriers, which was also the case for participants who experienced at least one adverse childhood event. The implications of this work are that although genetic polymorphisms from different biological systems may lead to a better understanding of pain and distress individually, exploring their effects together along with psychosocial factors may be more useful in creating holistic profiles of people that have suffered from musculoskeletal trauma to better understand and predict their pain outcomes. Future research may wish to combine other genetic polymorphisms to determine if a panel of genotypes can be identified in a context of pain and distress.

The first two studies relied exclusively on cross-sectional data. In Chapter 4 I used genomic structural equation models that explored the relationships between genotypes, trauma-related distress scores, adverse childhood experiences and prospective recovery trajectories. The findings from this chapter revealed a relationship between TIDS and recovery outcomes that has been previously shown in other work, with the added findings of an indirect relationship between *FKBP5* rs9394314 and recovery outcomes that is mediated by TIDS. Major allele

carriers of *FKBP5* rs9394314 reported higher TIDS scores than minor allele carriers, as did major allele carriers that had at least one adverse childhood experience compared to those reporting no ACEs. Further, major allele carriers that scored higher on the TIDS were predicted to be in the none-recovered category. These results suggest that genetic polymorphisms along with the TIDS questionnaire may provide a useful prognostic tool in predicting recovery outcomes for people that experience musculoskeletal trauma. Further research should focus on the relationship of other genetic polymorphisms and TIDS, as well as their ability to predict recovery outcomes.

There are some important limitations of this dissertation to consider. First, a panel of 39 SNPs was statistically explored leading to multiple comparisons where each set of analyses may produce a potential discovery. In future, it would be beneficial to prespecify hypotheses for less SNPs to investigate to avoid chance findings. Second, by choosing one genetic model that groups alleles together for all analyses makes it possible that important information is lost such as the strength of an association between one allele and an outcome of the dependent variable. Further, one genetic model was chosen for all SNPs which may have been appropriate for some of the SNPs but not others may have benefited from a different genetic model. To avoid this, fewer SNPs should be selected and investigated that fall under the same genetic model. Lastly, loss of important information was also possible by dichotomizing the ACEQ variable. Associations between SNPs and specific types of traumas listed in the ACEQ may tell us more about why people respond differently to certain types of traumatic injuries and how prior traumatic events influence these differences. Future research should explore all individual

traumatic experiences listed in the ACEQ to determine the relationship between each event and SNPs to gain a better understanding of the gene-x-environment interactions that can affect people's response to pain and trauma.

This dissertation provides further support for the notion that a broader view is necessary for a better understanding of pain experiences and pain research. These findings represent our efforts to lay a framework for clinical genotyping of pain in a biopsychosocial model. By continuing to learn and understand the type of interactions between different systems and the relevant threats therein, it may be possible to develop genetic screening tools to accurately identify those people who are at greater risk of developing chronic pain. This information can also be used to develop better individualized therapeutic strategies for people already suffering from chronic pain by implementing a more holistic and interdisciplinary pain management approach.

Critical Reflexivity

As research continues to uncover the roles of genetics in our health, the biobank for mutations being identified via genetic testing grows. However, the growth of genetic research and genetic testing itself needs to be matched with implementation of ethical guidelines and regulations that will protect people who choose to perform a genetic test. Researchers, clinicians, and industries providing genetic tests must be held accountable for the data they are retrieving from people as it can greatly impact the lives of many if mishandled or miscommunicated. Education, knowledge translation, and collaborations between leaders in the world of politics,

healthcare, and academia are critical to ensure continued success for genetic testing at the clinical, system and research levels, which in turn will allow for satisfactory testing at the patient level. It is important that a great level of respect is shown to the person choosing to conduct a genetic test and that there continues to be stringent policies made to ensure genetic privacy.

Genetic testing as a prognostic tool to help predict chronic pain has valuable promise for the field of pain as discussed above. On the other hand, genetic test results may lead a patient to feel marginalized by being labelled as a chronic pain patient. It may also lead to genetic discrimination. People with chronic pain may find it harder to seek proper treatment if their healthcare provider is not prioritizing them or providing them with enough care. There is potential for genetic testing to do harm to people with chronic pain or people identified to be at high risk of developing chronic pain. More research needs to be conducted to investigate clinical and patient perspectives on the use of genetic testing for pain prognosis and diagnosis to determine how this modality may affect this population of people.

There is still a lot of grey area to be explored regarding the ethics of genetic testing and its use for people suffering from pain. Arguments can be made for and against its use that need to be considered before implementing genetic tests for patients with pain. By acknowledging both the promise and limitations that genetic testing shows for healthcare purposes, and by emphasizing the complexity of pain and the global burden of chronic pain, we hope genetic testing can be a potential tool to aid in decreasing this immense burden. The purpose of this

section is to provoke reflexivity among the population of people involved in trying to understand and treat pain at the many levels of healthcare which can involve government policy makers, insurance providers, academics, and clinicians. It is important to ensure the ethical considerations of genetic testing are understood and to highlight the seriousness and potential of using genetic testing as a tool to help advance the field of pain and the population of people suffering from pain.

Future Directions

As mentioned earlier, this project is the initial step in identifying pain-associated genotypes. To better understand and develop these prognostic genotypes, future research should look to explore epigenetic modifications in relation to development of pain. Given the need to research pain within a biopsychosocial framework, it would be beneficial to incorporate epigenetic research within this model to understand gene-x-environment impacts on pain outcomes. There already exists a vast amount of epigenetic research regarding psychological disorders and how epigenetic modifications are associated to early childhood trauma and trauma-related distress[1–3]. More recently, epigenetic pain research has emerged as way to further understand how histone modification and DNA methylation of immune and nervous system markers influence pain outcomes[4–8]. Although these studies have shown the important involvement of epigenetic modifications on the expressions of pain, they do not account for the equally important impact of psychosocial factors. This area represents another dimension of bridging epigenetic pain research into the biopsychosocial model of pain.

Our early findings showed the association of a genetic polymorphism for a cannabinoid receptor gene with pain interference, and then its combined interaction with a gene responsible for regulating glucocorticoid receptors. In terms of intervention, cannabinoids are potential modulating compounds for key processes related to pain as shown through their modulating effects on immune[9,10] and nervous systems[11]. Endocannabinoid neurotransmitters and cannabinoid receptors accommodate for external cannabinoids to have an effect[12,13] since cannabinoid receptors are found throughout the body due to the endocannabinoid system being closely associated with other major physiological systems[14]. Upon activation, these receptors affect a variety of tissues which influence pain, anxiety and inflammation[15]. Therefore, future research should focus on how further interactions between genetic polymorphisms of cannabinoid receptor genes interact with variants of genes that are responsible for regulating inflammation and immune response systems that are heavily linked with pain. Understanding how these interactions influence the efficacy of cannabinoid metabolism in different people can lead to the development of personalized medicine through pharmacogenetics[16] which screens genes associated with a drug's activity to determine how a person will metabolically respond to the drug[17]. Understanding the interactions between cannabinoid receptor genes and genetic variants of other associated systems of pain will further the utility of genetic profiles in clinical settings through genetic screening tools such as pharmacogenetics. Using this technology along with psychometric questionnaires and known recovery groups in musculoskeletal pain may help find gene-trait associations and aid in treatment strategies involving cannabinoids. It is important to maintain a holistic approach in

researching pain within a biopsychosocial model to identify strategies to treat acute pain and prevent development of chronic pain.

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Appendices

Appendix A: Ethics approval for the ongoing SYMBIOME project

Western Research

Date: 4 November 2019

To: Dr. Dave Walton

Project ID: 106140

Study Title: Modeling post-traumatic pain and recovery: The SYMBIOME longitudinal cohort study

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 19/Nov/2019

Date Approval Issued: 04/Nov/2019

REB Approval Expiry Date: 17/Nov/2020

Dear Dr. Dave Walton,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Page 1 of 1

Western HealthSciences

May 28, 2015

Principal Investigator: Dr. David Walton

Funding source: Western internal funding, CIHR bridge grant, Canadian Pain Society

Letter of Information

Modeling recovery after traumatic injuries

Dear Sir/Madam,
Thank you for your time in reviewing this letter of information and for considering participation in our study. Please be sure to read this letter in its entirety and have any questions you may have answered to your satisfaction before consenting to participate.

Why am I being invited to participate?

You are being invited to participate because you have indicated that you are a male/female between the ages of 18-65 and are seeking care from emergency, medical or rehabilitation services for a recent accident or injury to your muscles, bones or ligaments, or because you have responded to one of the posted advertisements for this study.
You are not eligible for this study if any of the following apply to you. Please tell the research coordinator if any of these apply:

1. Severe gingivitis, periodontal disease, active dental caries (tooth decay), or any other active oral condition
2. Actively undergoing cancer treatment
3. Are currently experiencing an infection or illness (cold, flu, fever, etc.)
4. Are currently taking antibiotics or have taken antibiotics within the past week
5. You are a smoker or have been a smoker within the past year
6. You have Diabetes, either Type I or Type II
7. You currently have stomach ulcers, Celiac or Inflammatory Bowel Disease (Ulcerative colitis or Crohn's Disease)

What is this study about?

We are trying to understand the process of recovery over the 6 months following a traumatic injury, and to identify things (factors) that may explain why people differ in how they recover after these events. We will be collecting information including the nature of your injury, your biology, psychology, and past experiences all in the same period. Our goal is to not only improve understanding of *how* people recover following different types of injuries, but what factors influence that recovery. By identifying important factors, we will start to work on developing new ways to treat those factors and eventually improve the likelihood of successful recovery for people injured in the future.

What will I be asked to do?

If you agree to participate, you will be provided with a package that includes almost all of the data collection instruments that you will be asked to complete on your own at home starting at least 48 hours after your injury. The procedures include questionnaires for you to complete and different vials into which you will provide saliva and a stool sample. Once collected, the samples can be stored in your home freezer until a member of the research team comes to pick them up. The questionnaires will be repeated monthly for 6 months after your injury and the biological sampling will be repeated after 3 and 6 months. After the 6th month, your participation in the study will be complete. Below you will find more detailed information on the types of data instruments in this study.

1. **A set of questionnaires** that will ask you about a variety of different things. These include: i) your age, sex, work and educational status, ii) the nature of your injury (type of injury, when it occurred, how long ago it occurred, a brief description of the injury itself), iii) your medical and legal involvement (if any), iv) experiences from your childhood, including bullying and home environment, v) recent stressors you may have experienced, vi) the stress you have experienced as a result of your injury, vii) the type and amount of symptoms and interference you have experienced as a result of your injury.
2. **Drool/Saliva (part 1)** – You will receive 3 specialized test tubes with sterile cotton swabs in each. You will start on a day that is convenient to you, preferably within 5 days of completing your questionnaires. A pamphlet explaining all procedures is included with the instruments. This pamphlet should be read in its entirety. The tubes with the cotton swabs are to be used 3 times during the same day – once immediately upon waking, again 20-30 minutes after waking, and again mid-afternoon between 2pm and 4pm. This will require you to chew the cotton swab for about 10 seconds before returning it to the test tube, sealing it and placing it in your freezer.
3. **Drool/Saliva (part 2):** You will receive a specialized test tube into which you will spit or drool a small amount of saliva BEFORE your nightly (bedtime) routine, before brushing but at least 2 hours after eating. Once completed, this and the other samples can be stored in your residential freezer until retrieved by a member of the research team.
4. **Serum:** A trained researcher will draw 3cc of blood from the vein on the front of your elbow.

The following two components are optional.

5. **Stool**: This is an optional part of the study. You will provide a sample of stool using a specialized, sterile tube with a Q-tip type cotton swab. This will simply require you to twirl the end of the swab in a piece of used bathroom tissue, sealing it in the test tube and placing in your freezer. Only a small sample is required, and this can be collected at any time of day.
6. **Hair:** This is an optional part of the study for which you will be compensated if you choose to participate. As long as you have at least 3cm of hair on your head, we will cut approximately 100 hairs from the back of your scalp in a manner that minimizes any obvious physical change in your hair style using sterile scissors. This will be done by a member of the research team, and will only be done once at the beginning of the study.

We are collecting saliva samples in order to analyze the levels of specific proteins, which we are calling "biomarkers", that are typically present in the body and that may change during times of stress. Specifically, these are classed broadly as the stress hormone *cortisol*, the gonadal hormone *testosterone*, and immune or inflammatory markers that are referred to as '*cytokines*'. Stool samples, on the other hand, will provide us with specific information regarding the different bacterial populations that inhabit your intestines. The types of bacteria in your intestines may be influenced as a result of significant stressors, such as trauma or injury. We will be looking to see if any major shifts in the types of bacteria occur in your system as you are

recovering. There is some research that suggests certain genes play a role in the speed and effectiveness of recovery from an injury. The blood is being drawn primarily for exploratory and data redundancy reasons. If the other tissues/fluids fail for any reason, the blood will allow us to evaluate the same chemical markers without having to reconnect with you to collect more data. Finally, from your hair we will be able to determine the presence of different hormones that have been stored in your hair from the time before your injury. It is important to understand that everyone's body is different and it's currently difficult to say what is 'good' or 'bad' in these analyses. For that reason, these tests should not be considered diagnostic of any specific diseases or conditions.

Once all samples have been collected, contact the research team at Western University. These samples will then be retrieved from you by a member of the research team at a day, time and location that is convenient for you. A subset of the questionnaires will be completed again at 1 month intervals (approximately 10 minutes to complete), and the biological samples will be collected at 3 and 6 months. After the 6th month, your participation in the study is complete.

What are the risks and benefits of participating?

There are no immediate anticipated benefits to you from participating in this study. If you request it, we will provide you with the results of the different system tests that we conduct, although they may be difficult to interpret in isolation until the rest of our data have been collected. However, if our predictions are correct and we are able to identify dysfunction in key systems that can explain at least part of the pain experience, this may open new avenues to treatment that may have benefit to you or others in the future.
All participants may receive a final report of the study in which the results (using only group data) will be presented. If you wish to receive this report, you will need to indicate this on the consent form and include contact information to which the report should be sent. Those participants who wish to receive their own individual results will be required to contact the Lead Researcher Dr. David Walton directly to make that request. His contact information can be found at the end of this letter. Keep in mind that the data associated with this study <u>is not a medical record</u> and shouldn't be used as such. We will keep the Master List that links your name with your ID number for 6 months after your completion of the study after which it will be shredded for confidentiality and privacy protection reasons. This means that we will not be able to provide your individual results beyond 1 year from your injury.

The risks to participation are minimal and are largely inconveniences due to time. The salivette (saliva collection tube with cotton swab) samples must be performed at three separate times throughout a single day which may be a mild disruption to your daily routine for that day. Improper collection and handling of stool samples MAY pose a risk of bacterial contamination/infection, however, if carefully performed (including washing your hands afterwards), this risk is quite minimal. The blood will be drawn using a standard protocol that you have likely experienced before in a doctor's office or the Red Cross.
Completion of the questionnaires may lead to some people experiencing emotional distress, especially those that ask you to recall and reflect upon childhood experiences if yours were not positive. We have provided suggestions for managing emotional distress, should you experience it, at the end of this letter. We will do everything in our power to ensure your data, including the biological specimens and your questionnaires, are kept secure and confidential. However, we cannot guarantee against a data breach regardless of how good our physical and virtual security is. Your data will be stored with only a random ID number in order to mitigate any potential risk, nonetheless the risk of data breach or loss is possible and we want to ensure you're aware of this. Should this happen you will be quickly informed.

Will I be compensated for my participation?

You have different options for the degree to which you wish to participate in this study. The minimum level of participation is to complete the paper forms, saliva, and blood draw. This would be done once when you enter the study, then at 1, 2, 3, 6 and 12 months later. Each follow-up will likely take about 45 minutes of your time, and you will receive $30 total for participating in this level of the study. The hair and stool are optional components, and for each one you will receive an additional $15 ($30 for both). We recognize that collecting these samples is no small commitment, but can be completed in its entirety in a single day and a total anticipated time commitment of approximately 1 hour at each collection period. Out of respect for your time, you will be therefore be reimbursed a minimum of $180 total for participating in each phase of this study (intake and 1, 2, 3, 6, and 12 months). If you complete the two additional components you are eligible for an additional $30 per session, up to an additional $180 for the entire study.

Who will have access to my information?

A unique randomly-generated 6-digit ID number will appear on all forms belonging to you for the sole purpose of connecting all of the data you provide at each period. The lead researcher at Western University, Dr. David Walton, will collect all of the data provided by all participants and will analyze it as an anonymous group. Once transcribed, all data are stored on the secure, password protected and firewalled server of Western University and the paper forms are shredded. Western University's REB and representatives from Lawson's Quality Assurance and Education Program will have access to participant's data to ensure that it is following the proper laws and regulations. Outside of these groups, your specific information will not be shared with anyone without your express written consent to do so.

Note some of the tools to be completed are meant to measure severity of symptoms related to depression or anxiety. *IF* your responses lead to a score that is suggestive of either depression or anxiety, your family doctor will be contacted to inform him/her of the results of the scale and what they may mean. It will ultimately be up to your family physician to decide how and when he/she should follow up with you if at all.

Data will be retained in anonymous form indefinitely as an ongoing database.

Voluntary participation

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time. If you choose to withdraw from the study, you may request to have your contributions to that point removed, at any time up until 6 months after you are done the study.
Withdrawal from the study or refusal to participate is your decision, and may be done without the requirement of explanation on your part. Withdrawal will in no way affect your current or future relationship with any of the research team or clinicians associated with the study.

What if I want more information?

You may contact the lead researcher, Dr. David Walton, at Western University (London, Canada) if you require any further clarification. His contact information can be found below. If you have any questions about your rights as a research participant or the conduct of the study you may

contact the Office of Research Ethics at (519) 661-3036 or by email at ethics@uwo.ca. You are encouraged to keep this letter of information for your own records.

If you wish to receive a summarized copy of the results of this study and/or your individual results, you may leave your email address on a separate sheet. The sheet will be held by the research coordinator, and the email addresses will only be used to provide the results, after which the list will be destroyed. We thank you in advance for considering participation in this study. *You do not waive any legal rights by signing this consent form*.

If you are experiencing emotional distress:

This research study does NOT include treatment recommendations. However, while completing the questionnaires about your emotional state or past experiences, you may find that you experience emotional distress (e.g. sadness or anxiety) by virtue of thinking about and answering the questions. If this should happen, it is most commonly short-lived and may be a sign to take a break from the questionnaires until you settle down enough to come back to them.

However, in the distress can last longer than a day or can be quite severe in some people. If this happens to you, you are encouraged to seek professional assistance to help deal with your emotional state. The Canadian Mental Health Association includes several resources on their website as a good place to start: http://www.cmha.ca/mental-health/find-help/. TeleHealth Ontario can also offer support or direction, they can be reached 24 hours, 7 days per week at 1-866-797-0000. The London Mental Health Crisis Service offers 24-hour, 7 days per week support to those in acute mental distress. They can be reached at 519-433-2023. Finally, if you feel you are in significant emotional distress and require more immediate help, you can call your family doctor or emergency services (9-1-1). In that case you should refrain from completing any further questionnaires and let the researchers know that you are unable to continue.

January 9, 2015

Consent form

Modeling recovery from traumatic injuries

Principal Investigator: Dr. David M. Walton PT PhD

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I also consent to being contacted by the Lead Researcher in the case any of my scale scores suggest possible problems with depression or anxiety.

Please indicate the level of study participation to which you are consenting by placing a check in the appropriate circle:

◯ Paper forms only (monthly forms, approximately 10 minutes each, $25 total compensation)

◯ Paper forms *and* biological specimens *but not hair* (saliva, stool, serum) at intake, 3 and 6 months (approximately 1 hour each), paper forms at 1, 2, 4, and 5 months (approximately 10 minutes each). $100 total compensation

◯ Paper forms *and* biological specimens *including a sample of about 100 hairs from the back of your head at intake*. Other data and intervals as described directly above (approximately 10 minutes in months 1, 2, 4 and 5, approximately 1 hour in months 3 and 6). $125 total compensation.

_____

Participant name (print)

_____ _____

Participant signature Date

_____

Person obtaining consent (print)

_____ _____

Signature of person obtaining consent Date

Request for Summary of Results

◯ I would like to receive a copy of the group average results from this project (Note: these results will not have any clinical application and will not affect your medical treatment)

If you would like to receive a copy of the group results, please provide your preferred method of delivery:

◯ Electronic (email); Email address: _____

OR

◯ Postal mail; Mailing address (incl. Street, City, and Postal code):

_____

_____

_____

Curriculum Vitae

Name: Mohamad Fakhereddin

Post-secondary Education and Degrees:

University of Windsor
Windsor, Ontario, Canada
2014-2017 MSc. Molecular and Developmental Biology

University of Windsor
Windsor, Ontario, Canada
2009-2013 BSc. Biological Sciences

Honors and Awards

- **Jan 2020 –** IASP Financial Aid Award to attend the IASP 18th World Congress on Pain in Amsterdam, Netherlands. Valued at $800 USD
- **Oct 2018 –** Health and Rehabilitation Sciences Graduate Conference Travel Award for my poster abstract presentation at the IASP 17th World Congress on Pain. Valued at $500
- **Oct 2018** – Faculty of Health Sciences Graduate Conference Travel Award for my poster abstract presentation at the IASP 17th World Congress on Pain. Valued at $250
- **June 2018** – Institutionally nominated for the 2018/2019 Vanier Canada Graduate Scholarship by the Faculty of Health Sciences Graduate Chair. Valued at $50,000
- **Mar 2016** – A.R. and E.G. Ferris Award. Valued at $1,000
- **Jan 2012** – University of Windsor Recognition of Achievement Scholarship (Bursary). Valued at $1,000
- **Jan 2011** – University of Windsor Recognition of Achievement Scholarship (Bursary). Valued at $1,000
- **Jan 2010** – University of Windsor Recognition of Achievement Scholarship (Bursary). Valued at $1,000
- **Sept 2009** – University of Windsor Entrance Scholarship. Valued at $10,000

Related Work Experience

- **Hematology Research Associate – London Health Sciences Centre**
  Dec 2021 – present
- **Clinical Research Coordinator – KGK Science Inc.**
  Jan 2018 – Dec 2021
- **PhD Research Trainee – Western University**
  Sept 2017 – present
- **Graduate Teachers Assistant – Western University**
    - Health Science and Kinesiology Practicum: Sept 2019 – April 2021

    - Advanced Quantitative Research Methods: Sept 2019 – Dec 2019
    - Human Anatomy: Sept 2017 – April 2019
- **MSc Research Trainee**
  Jan 2014 – Feb 2017
- **Graduate Teachers Assistant – University of Windsor**
    - Embryology: Sept 2014 – Dec 2016
    - Biotechnology: Jan 2014 – April 2016

Professional Development

Memberships

- International Association for the Study of Pain (IASP) – Since Dec 2017

Certifications

- Canada Basic Social and Behavioral Research Ethics Course – Sept 2021
- Canada GCP – Since Sept 2021
- Health Canada Division 5 – Drugs for Clinical Trials Involving Human Subjects – Sept 2021
- RCR Life Sciences – Sept 2021
- Transportation of Dangerous Goods TDG/IATA – Sept 2021
- Biosafety – Oct 2018
- Laboratory Safety and Hazardous Waste Management – Oct 2018
- Accessibility in Service (AODA) – Sept 2017
- Safe Campus Community Certificate – Sept 2017
- Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics (TCPS 2: CORE) – Sept 2017
- WHMIS – Sept 2017
- Worker Health and Safety Awareness – Sept 2017

Review and Evaluation

- Health Science Inquiry – Scientific peer reviewer
  Dec 2017 – Feb 2020

Publications

Papers

- Lee, J.Y, **Fakhereddin, M.**, MacDermid, J.C., Elliott, J.M., Schabrun, S.M., Walton, D.M. (2021), An Exploration of Blood Marker-x-environment Interaction Effects on Pain Severity and Interference Scores in People with Acute Musculoskeletal Trauma. Clin J Pain
- Walton, D.M., Elliott, J.M., Lee, J., **Fakhereddin, M.**, Seo, W. (2021), Identification of clinically-useful cut scores of the Traumatic Injuries Distress Scale (TIDS) for predicting rate of recovery following musculoskeletal trauma. PLoS ONE, 16(3)
- **Fakhereddin, M. F.** (2018), The Genetics of Pain: A Bio-Psycho-Social Approach for Understanding Pain. HSI, 9(1): 23-24.

- Lee, J.Y., Guy, S.D., Lukacs, M.J., Letwin, Z.A., **Fakhereddin, M.F.**, Al-Nasri, I.J., Salim, S. (2018), Management of Fibromyalgia Syndrome: Cognitive-Behavioral Therapy (CBT) for Healthcare Professionals. UWOMJ, 87(1): 34-37.
- Hooker, L.N., Smoczer, C., Abbott, S., **Fakhereddin, M.**, Hudson, J.W. and Crawford, M.J. (2017), Xenopus pitx3 target genes lhx1 and xnr5 are identified using a novel three-fluor flow cytometry–based analysis of promoter activation and repression. Dev. Dyn., 246: 657–669. doi:10.1002/dvdy.24532

Peer-reviewed abstracts/presentations

- Joshua Y. Lee, **Mohamad F. Fakhereddin**, Maryam Ghodrati, David M. Walton. 2018. Exploring non-linear and interactive relationships between psychological and physiological markers of post-traumatic pain and distress: toward a biopsychosocial model of pain. Biannual Canadian Bone and Joint Conference. London, ON, Canada.
- **Mohamad F. Fakhereddin**, Joshua Y. Lee, Maryam Ghodrati, David M. Walton. 2018. Exploring Recovery Trajectories and Predicting Outcomes of Acute Musculoskeletal Trauma: Further Exploration of the Prognostic Validity of the Traumatic Injuries Distress Scale (TIDS). Abstract and poster presentation. Biannual Canadian Bone and Joint Conference. London, ON, Canada.
- **Mohamad F. Fakhereddin**, Joshua Y. Lee, Maryam Ghodrati, David M. Walton. 2018. Exploring Recovery Trajectories and Predicting Outcomes of Acute Musculoskeletal Trauma: Further Exploration of the Prognostic Validity of the Traumatic Injuries Distress Scale (TIDS). Abstract and poster presentation. IASP 17th World Congress on Pain. Boston, MA, USA.
- **Mohamad F. Fakhereddin.** 2018. The Genetics of Pain. Oral presentation. Annual Health & Rehabilitation Sciences Graduate Research Conference. London, ON, Canada.
- **Mohamad F. Fakhereddin**, Saqib Sachani, Michael J. Crawford. 2016. Exploring the Role of *rax1* in Eye Development. Abstract and poster presentation. The 8th Canadian Developmental Biology Conference. Banff, AB, Canada.