Biopsychosocial Influence on Shoulder Pain (BISP)

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A. Significance

A1. Public Health Impact

Chronic pain is among the most prevalent, disabling, and costly conditions in the United States, and throughout the world. In 2011 the Institute of Medicine (IOM) Report - *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research* extensively documented chronic pain as a high priority public health problem.[1] Chronic pain affects 100 million people in the United States (U.S.) and produces annual costs up to $635 billion, exceeding the prevalence and costs of heart disease, cancer, and diabetes.[1, 21] These costs are largely driven by musculoskeletal pain conditions. The burden of chronic pain is a global concern; in 2012 the Global Burden of Disease Study identified musculoskeletal pain as a primary contributor to years lived with disability internationally.[22]

Shoulder pain is a common musculoskeletal pain condition. In the IOM report, the shoulder was the 5th most frequently reported pain site (estimated at 9.0% of all US adults over age 18).[1] Other estimates of shoulder pain prevalence document high rates, including 1-year prevalence rates from 5 – 47% [23, 24] and point prevalence rates from 14 – 21%.[25, 26] Shoulder pain is characterized by poor outcomes and resulting disability. For example, in one cohort 40% of the individuals did not report full recovery at 1 year after new onset of shoulder pain.[27] Among those with shoulder pain, 17.7% had difficulty with basic daily activities while 21.4% had difficulty with complex daily activities.[1] ***These data clearly demonstrate the public health significance of musculoskeletal shoulder pain and justify the study of interventions that have the potential to ameliorate shoulder pain and reduce this burden to individuals and society.***

A2. Priority for Pain Relief in the United States

Preventing the development of chronic pain conditions is a high priority initiative for improving patient care. The importance of this issue is highlighted in an NIH Program Announcement (PA-13-118: Mechanisms, Models, Measurement, & Management in Pain Research) and the aforementioned IOM report. Unfortunately, current knowledge of mechanisms involved in the transition to chronic pain is limited, which decreases options for effective treatment of pain. Studies that target validated risk factors that confer increased risk of experiencing chronic pain provide a unique opportunity to vertically advance the field. Indeed, interventions tailored to specific risk factor characteristics (i.e. personalized medicine) hold great promise in reducing the impact of chronic pain.[28, 29] ***The proposed work is significant because it will address this high priority issue by investigating efficacy and mechanisms of a personalized pain intervention that targets a subgroup at high-risk for experiencing chronic shoulder pain.***

A3. Significance of Renewal Application

The ultimate goal of our line of research is to develop personalized interventions for musculoskeletal pain. Personalized medicine via identification of genetic risk factors has been successfully implemented for select areas of cardiac medicine[30-33] and oncology.[34-36] However, similar successes have not been achieved for pain treatment when focusing on genetic risk factors alone.[29] Because of their complex biopsychosocial etiologies, personalized interventions for chronic pain conditions will depend on identification of genetic factors in combination with psychological, environmental, and/or social risk factors.[28] This multiple risk factor approach served as the foundation for our work in the initial funding period. ***We made significant progress toward our ultimate goal by validating a high-risk subgroup comprised of psychological and genetic factors that predicted pain outcomes in pre-clinical and clinical cohorts.***

This renewal application extends and translates this work by determining efficacy and mechanisms of personalized interventions targeting the genetic and psychological factors that make up the high-risk subgroup. We will use a validated pre-clinical model in the renewal application because it controls the injury mechanism and allows for high treatment fidelity. The pre-clinical model also allows us to determine how underlying changes in molecular processes, psychological factors, and pain sensitivity regulation are associated with pain relief. Furthermore this pre-clinical model has an established link to a post-operative clinical model. ***In the renewal application we propose a study that will provide proof of principle that personalized pain interventions favorably alter outcomes and mechanisms for individuals that, without the personalized intervention, would go on to develop chronic shoulder pain.***

B. Innovation

Several aspects of the Biopsychosocial Influence on Shoulder Pain (BISP) design are innovative. BISP follows the structure described by Diatchenko et al[28, 37] for investigating risk factors contributing to the development of chronic musculoskeletal pain conditions. Figure 1 graphically depicts the BISP conceptual model, which focuses on genetic and psychological factors as contributing to pain outcomes in parallel pre-clinical and clinical cohorts. From the outset, genetic and psychological factors were *a priori* selected based on their potential to be targets of personalized interventions in future studies. Thus, the original intent of BISP was to transition from predictive to intervention phases, pending direction provided by data generated in the initial funding period. Additional innovative aspects of BISP are highlighted below.

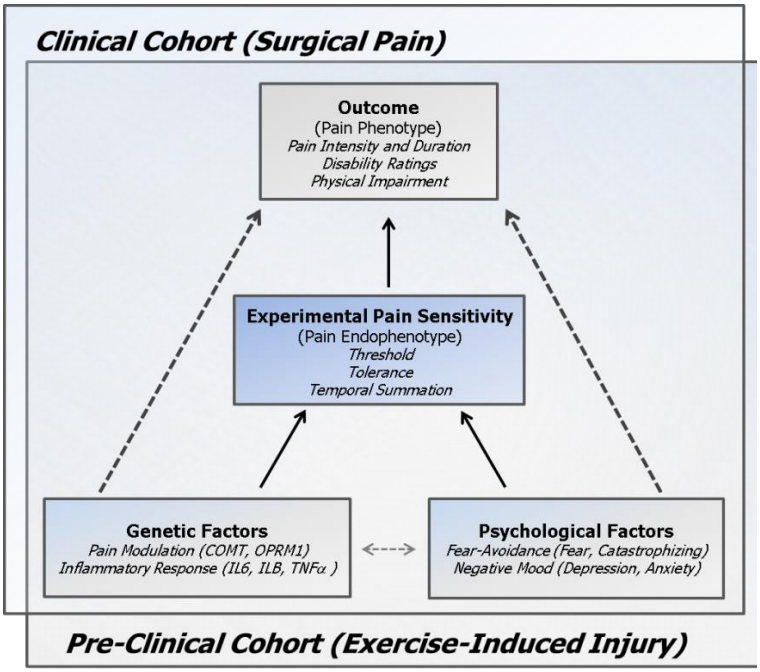


Figure 1. Biopsychosocial Influence on Shoulder Pain Conceptual Model
BISP included: 1) *a priori* determined genetic and psychological risk factors for experimental pain sensitivity and clinical outcomes and 2) parallel investigation in pre-clinical and clinical cohorts. Dark lines represent the hypothesized pathways that the genetic and psychological factors influence. Dotted lines represent direct pathways that were tested as competing hypotheses. The shaded bi-directional line indicates that minimal associations between genetic and psychological factors were observed.

In the initial funding period we identified a human pre-clinical model that successfully translated to a clinical model of pain. This is innovative because nonhuman pre-clinical models often face difficulties in translating to clinical pain populations.[38, 39] We explored an alternate route for translation by inducing shoulder pain in healthy human subjects through controlled muscle injury that follows a fatiguing exercise protocol, referred to as "exercise-induced injury" in this proposal. This pre-clinical model was selected because it had high translational potential for a clinical model of post-operative shoulder pain.[19, 40] This was attributed to: 1) ability to induce muscle injury, inflammation, and pain in a specific anatomical region of the shoulder consistent with common surgical procedures (i.e. rotator cuff) and 2) pain responses monitored over several days which is likely a better clinical proxy than other transient induced pain options.[41-43] Importantly, a high-risk subgroup based on genetic and psychological risk factors predicted heightened pain responses in both the pre-clinical exercise-induced injury model and 12-month outcomes in the clinical post-operative model (in review). This translational success across pain models provides confidence that additional findings from our pre-clinical model will continue to have clinical relevance.

The methodology in the renewal application represents an innovative approach to investigating personalized interventions for pain relief. Personalized pain relief is often evaluated by identifying responder subgroups in a post-hoc manner and determining baseline characteristics predictive of a positive outcome.[44] These studies lack experimental design features (e.g. randomization, precise treatment application) that allow for causality and typically fail to include *a priori* hypotheses regarding variables that will predict treatment response. In the renewal application we will include an *a priori* identified high-risk subgroup validated during the initial funding period. We will use an experimental design to determine the efficacy of a personalized intervention matched to the genetic and psychological characteristics of the high-risk subgroup. Additional innovation comes from comparing combined personalized interventions (i.e. pharmaceutical and psychological) to each individual intervention (i.e. pharmaceutical or psychological) and a combined placebo condition. In clinical studies pharmaceutical and psychological interventions are not typically investigated together in such a systematic manner, yet knowing the efficacy of a combined personalized intervention compared to different non-personalized conditions is imperative to drive understanding of what the "active" ingredients of the personalized intervention are. In addition we will be measuring key processes that contribute to pain relief to determine underlying mechanisms within the same study. Moreover, use of our pre-clinical model makes it feasible to prescreen participants to identify and target the high risk subgroup for enrollment. This provides far greater efficiency in the research design and is directly relevant to subsequent clinical investigations that will focus on improving post-operative outcomes for this high risk subgroup.

In summary, the renewal application for BISP offers many innovative aspects that make it likely to produce novel and important findings regarding efficacy and mechanisms of personalized interventions for pain relief. The information generated will vertically advance the field and also be vital in planning a follow-up clinical trial investigating personalized interventions to improve post-operative shoulder pain outcomes.

Progress During the Initial Funding Period

We completed pre-clinical exercise-induced shoulder injury (n = 190) and clinical post-operative shoulder pain (n = 150) cohort studies. We have presented findings at national (n = 4) and international (n = 3) scientific conferences and disseminated results in high quality journals (n = 11 published, with n = 3 review). We made notable progress towards our overall goal of developing personalized musculoskeletal pain interventions by identifying and validating a high-risk subgroup comprised of *COMT* genotype and pain catastrophizing. This high-risk subgroup predicted: a) heightened pain responses following exercise-induced shoulder injury, and b) poor post-operative shoulder pain outcomes. Below is a summary of key findings supporting our renewal:

Exercise-Induced Shoulder Injury as a Pre-Clinical Model for Clinical Post-Operative Shoulder Pain: The use of exercise-induced injury in humans was motivated by the difficulties and challenges with non-human animals as pre-clinical models in pain research.[38, 39] One feature enhancing the translational potential of exercise-induced injury is that it produces prolonged pain (mean days = 6.1, sd = 1.8), which is unique among experimental methods of inducing pain in humans (where duration is measured in seconds or minutes).[41-43] This is important because long duration is the defining characteristic of chronic pain. Furthermore, there was overlap in pain intensity and disability reports between the pre-clinical and clinical participants' pre-operative ratings (Figure 2). Additional evidence supporting this pre-clinical model came from analyses reported in the peer-review literature regarding similarities in endogenous pain modulation[45, 46] and that a specific psychological factor, pain catastrophizing, was a predictor of pain outcomes for both cohorts.[16, 47] These data supported the validity of this pre-clinical model and provided direction for future analyses.

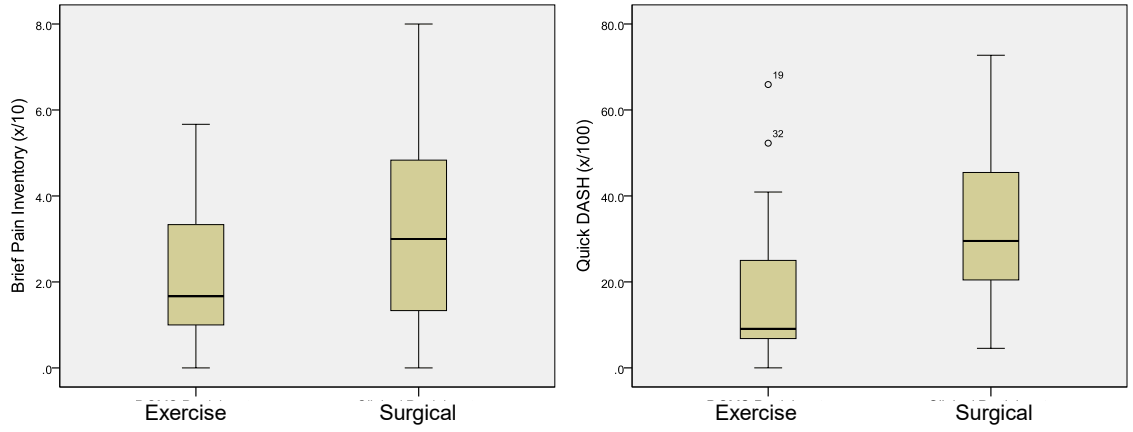


Figure 2. Pain Intensity and Disability Overlap for Pre-Clinical (Exercise) and Clinical (Surgical) Shoulder Pain Models
Pain (left panel) and upper-extremity disability ratings generated following exercise-induced injury were comparable with lower levels of pre-operative pain and disability ratings from the surgical cohort when means are compared. The entire range of pain and disability ratings demonstrated approximately 75% overlap between the two cohorts.

Interactions Between Genetic and Psychological Factors: A primary aim in the initial funding period was to examine whether pain modulatory or pro-inflammatory genes interacted with pain-related psychological factors to predict phenotypes in the pre-clinical cohort (see previous Figure 1 for details). The specific shoulder phenotypes studied were average and peak pain intensity, average and peak upper-extremity disability, and duration of shoulder pain. Our findings revealed multiple interactions between genetic and psychological factors that improved prediction beyond either factor alone.[19, 40] Two examples from these analyses are depicted in Figure 3 below. ***These findings represent important progress during the initial funding period because they: 1) supported the conceptual foundation and primary aims of BISP, and 2) identified specific interactions between genetic and psychological factors that were further investigated as risk subgroups.***

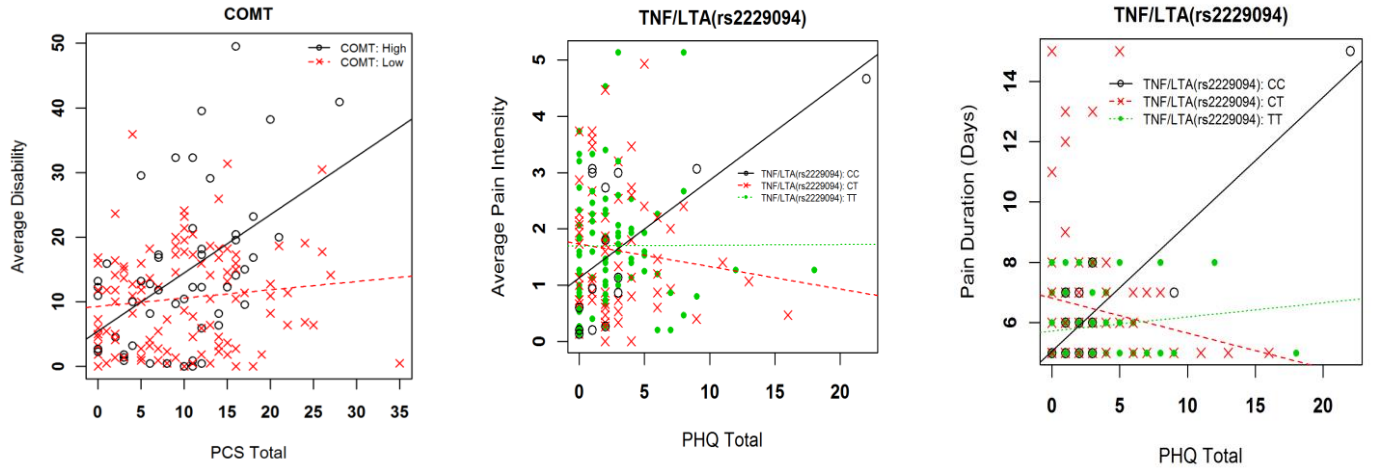


Figure 3. Genetic and Psychological Interactions for Shoulder Pain
Left Panel - for individuals with the high pain sensitive *COMT* diplotype, pain catastrophizing was a stronger predictor of average shoulder disability compared to those with the low pain sensitive diplotype
Center and Right Panels - Analysis of the pro-inflammatory genes identified that the combination of a high-risk *TNF/LTA* region SNP and depressive symptoms improved prediction of average pain intensity and shoulder pain duration compared to those with the low risk SNP.

Risk Subgroup Identification and Validation: The data presented in this section are currently in review.
We *a priori* selected SNPs from *COMT, AVPR1A, IL1B*, and *TNF/LTA* region genes and psychological constructs (fear of pain, catastrophizing) represented as interaction terms in the previous analyses, and investigated whether risk subgroups comprised of these factors had prognostic value. A subgroup approach was taken because it was more amenable to identifying individuals for personalized interventions in comparison to regression focused approaches. In these analyses, we first identified the high-risk subgroups in the pre-clinical model and then validated these subgroups in the clinical model. ***Hence, any risk subgroup that was predictive in both models would be considered a robust marker for identifying increased risk for the development of persistent shoulder pain, and an indication that the risk subgroup was appropriate for developing personalized pain interventions.*** In the pre-clinical model, subgroups were identified based on prediction of heightened pain responses to muscle injury. A "heightened response" was operationally defined as a peak pain intensity rating of ≥ 7/10 or pain that continued for at least 7 days (expected response is 4-5 days). Eight genetic and psychological combinations were considered from the selected potential risk factors. Only the subgroups comprised of a *COMT* SNP for high pain sensitivity (rs6269) and 1) pain catastrophizing or 2) fear of pain predicted heightened pain response in the pre-clinical model (Table 1, indicated in bold font).

Table 1. Risk Subgroups for Heightened Pain Responses follow Exercise-Induced Injury

	High Risk Subgroup	**High Risk**		**Low Risk**		**P-Value**
		N	**Percent Outcome**	**N**	**Percent Outcome**	
Gene	*Outcome = Duration ≥ 7 days*					
COMT	**rs6269 = 'AA' and FPQ≥15**	**52**	**36.5%**	**130**	**21.5%**	**0.037**
	rs6269 = 'AA' and PCS≥5	**42**	**40.5%**	**140**	**21.4%**	**0.013**
AVPR1A	rs1042615 = 'AA' and FPQ≥15	28	35.7%	158	24.1%	0.194
	rs1042615 = 'AA' and PCS≥5	22	36.4%	164	24.4%	0.228
TNF/LTA	rs2229094 = 'CT' and FPQ≥15	64	31.3%	110	23.6%	0.272
	rs2229094 = 'CT' and PCS≥5	50	32.0%	124	24.2%	0.291
IL1B	rs1143627 = 'GG' and FPQ≥15	41	19.5%	142	26.8%	0.346
	rs1143627 = 'GG' and PCS≥5	72	20.8%	111	27.9%	0.280
Gene	*Outcome = Peak BPI ≥ 7/10*					
COMT	**rs6269 = 'AA' and FPQ≥15**	**52**	**48.1%**	**130**	**23.1%**	**0.001**
	rs6269 = 'AA' and PCS≥5	**42**	**52.4%**	**140**	**23.6%**	**<0.001**
AVPR1A	rs1042615 = 'AA' and FPQ≥15	28	25.0%	158	29.7%	0.610
	rs1042615 = 'AA' and PCS≥5	22	27.3%	164	29.3%	0.847
TNF/LTA	rs2229094 = 'CT' and FPQ≥15	64	25.0%	110	34.5%	0.189
	rs2229094 = 'CT' and PCS≥5	50	24.0%	124	33.9%	0.203
IL1B	rs1143627 = 'GG' and FPQ≥15	41	31.0%	142	29.3%	0.834
	rs1143627 = 'GG' and PCS≥5	72	31.5%	111	31.6%	0.735

In the clinical model these same high-risk subgroups were predictive of operative shoulder pain outcomes. Further analysis of the high risk subgroups accounting for age, sex, race, comorbid depressive symptoms, rotator cuff tear size, medication status and diplotype for COMT indicated that ***the high-risk subgroup of pain catastrophizing and COMT was the stronger predictor in the clinical model (Table 2).***

Table 2. Risk Subgroup Prediction of 12 Month Post-Operative Pain Recovery Outcome

	FPQ Subgroup				PCS Subgroup		
	Hazard Ratio	95% CI	p-value		Hazard Ratio	95% CI	p-value
	COMT rs 6269						
Unadjusted model	0.69	0.44 – 1.07	0.096		0.51	0.31 – 0.84	0.009
Partially adjusted model	0.66	0.42 – 1.05	0.077		0.53	0.32 – 0.88	0.013
Fully adjusted model	0.61	0.37 – 1.00	0.050		0.44	0.25 – 0.77	0.004
	COMT Diplotype						
Unadjusted model	0.69	0.45 – 1.07	0.095		0.52	0.32 – 0.83	0.006
Partially adjusted model	0.68	0.43 – 1.08	0.100		0.56	0.34 – 0.92	0.021
Fully adjusted model	0.64	0.40 – 1.04	0.072		0.46	0.27 – 0.80	0.006

Partially adjusted model was adjusted for demographics (age, sex, race); Fully adjusted model was adjusted for demographics, depressive symptoms, medications, and rotator cuff tear size.

Transition to Renewal Application: ***The validation of a robust high-risk subgroup comprised of COMT variation for high pain sensitivity and pain catastrophizing was the culminating finding from the initial funding period and provided clear direction for the renewal application.*** Now BISP will transition to an intervention phase (Figure 4), using the pre-clinical model to determine the mechanisms and efficacy of personalized pain interventions matched to the genetic and psychological characteristics of the high-risk subgroup. Our ultimate goal is to conduct a randomized clinical trial in patients undergoing post-operative shoulder pain. However, we believe a clinical trial would be premature at this point, as there remains a clear need for a pre-clinical study to establish proof of principle for efficacy and identify mechanisms of pain relief in this high risk subgroup before a highly resource-intensive clinical trial could be justified. Very few treatment models for personalized pain interventions exist[28, 29]; therefore, this renewal application represents a significant advance in moving towards the reality of personalized treatments for musculoskeletal shoulder pain based on established genetic and psychological risk factors. This study will strongly impact the field by determining if individuals at high genetic and psychosocial risk for heightened response from shoulder injury respond favorably to personalized interventions matched to the characteristics of the risk subgroup. Moreover, we will determine the relevant psychological, physiological, and pain sensitivity regulation mechanisms involved in pain relief.

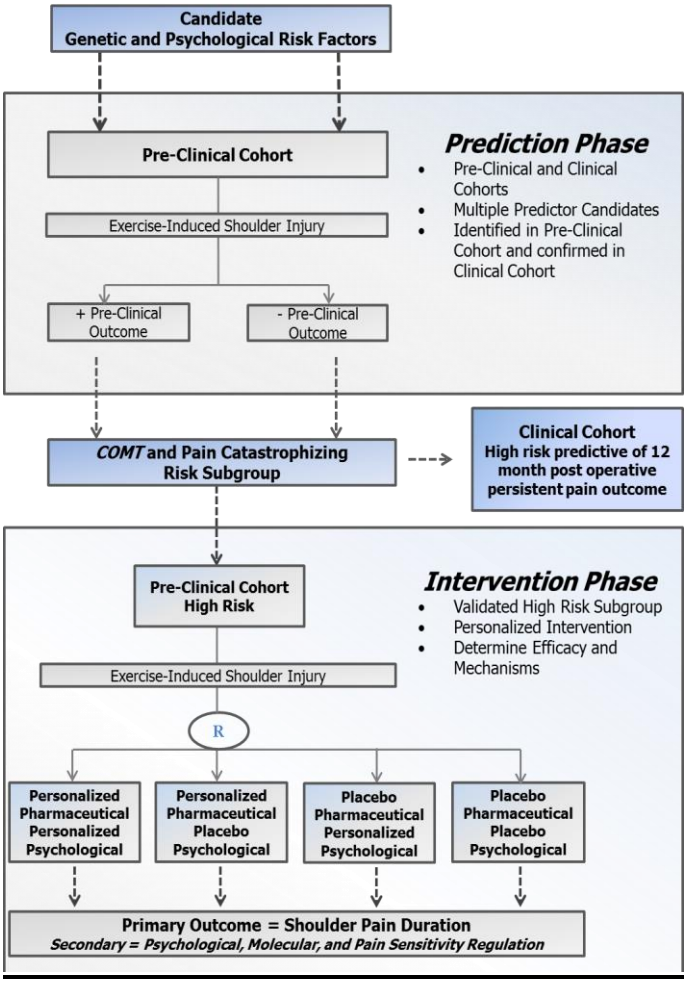


Figure 4. Biopsychosocial Influence on Shoulder Pain (BISP): Transition to Intervention Phase. The top panel depicts the approach implemented during the initial funding period. The bottom panel depicts the proposed approach in this application.

C. Approach

C1. Rationale

The optimal theorized match for the identified high-risk subgroup is a combination of personalized pharmaceutical and personalized psychological interventions. This combined intervention versus a combined placebo condition is the primary comparison of interest for this study. We also will evaluate the individual effect of both the pharmaceutical and the psychological intervention. Such comparisons will provide important information on whether the active portion of the combined personalized intervention is truly BOTH pharmaceutical and psychological components, or whether one component is sufficient for effective pain relief to occur.

The high-risk subgroup is characterized by *COMT* genotype. COMT is a ubiquitously expressed detoxifying enzyme involved in a number of important biochemical pathways, particularly metabolism of catecholamines.[48] *COMT* encodes a

membrane-bound form that is 50 residues longer at the amino terminus than the soluble form and the relative levels of each appear to have temporal and tissue specificity. In the initial funding period we found evidence for SNP rs6269 as a risk group predictor, consistent with a previous study that documented reduced COMT enzyme activity and increased pain sensitivity with the "A" allele at rs6269.[49] In additional analyses of the clinical cohort we considered the established *COMT* 4-SNP pain sensitivity haplotype[2, 50] for the genetic subgroup component and similar results were obtained (Table 4). However the rs6269 SNP was the stronger predictor in the pre-clinical cohort which may have been due to the diplotype grouping, underlying stratification in the subject cohort, or it might indicate that this SNP is carrying more of the effect in this risk subgroup. Specifically, rs6269 is in the promoter region of the soluble isoform of the gene, and thus could theoretically affect expression of the soluble form. Alternatively, SNP rs6269 can be a genetic marker that differentiates COMT haplotypes coding for high activity enzyme variants from lower activity variants, consistent with other studies reporting an association with rs6269 SNP in general neurological[51, 52] and pain-specific[53] phenotypes. Regardless of the specific mechanisms involved, the genetic contribution of this risk subgroup appears to be diminished ability to modulate pain due to low COMT enzyme activity. Consistent with a biopsychosocial framework for pain we also considered psychological factors and pain catastrophizing as the other part of the validated high-risk subgroup. Pain catastrophizing is a maladaptive coping style comprised of magnification, rumination, and helplessness beliefs that perpetuate the experience of musculoskeletal pain.[12] In studies from the initial funding period a genetic predisposition for low COMT enzyme activity and a coping approach consistent with pain catastrophizing was predictive of heightened pain responses and poor clinical outcomes. The consistent nature of these findings in separate cohorts lends credence to the notion that this particular risk subgroup is robust because it has strong theoretical and empirical evidence for its relevance in predicting chronic shoulder pain. ***Therefore, we will address these risk factors by implementing a pharmaceutical intervention to counter effects of decreased COMT enzyme and a psychological intervention to address the impact of pain catastrophizing.***

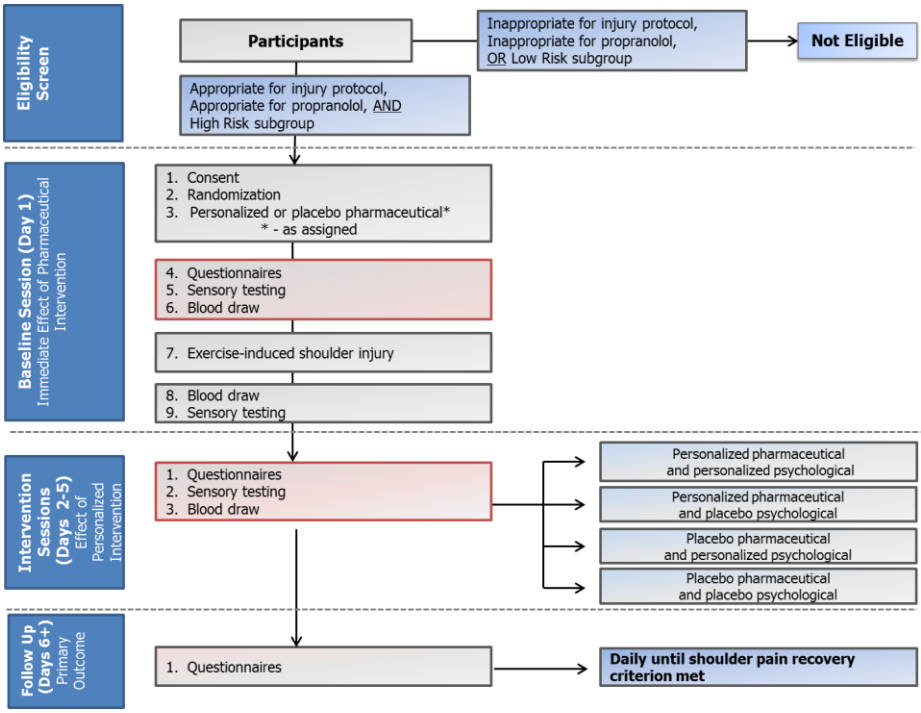
C2. Overview

Potential subjects will be screened and those meeting the high-risk criteria will be randomized into intervention groups. Treatment conditions will be administered for four consecutive days and statistical analysis will determine whether the combined personalized intervention group experienced shorter shoulder pain duration, lower peak pain intensity, or decreased upper-extremity disability (Aim #1) and determine which molecular, psychological, and pain sensitivity regulation mechanisms are associated with pain relief (Aim #2).

C3. Procedures

An overview of BISP study procedures is presented in Figure 5, and then described in more detail.

Figure 5. BISP Timeline and Study Procedures



Risk Subgroup Screening. We propose to enroll 448 high-risk participants, which will require prescreening approximately 3,000 people. In order to accomplish this we will conduct quarterly large-scale screenings on campus and in the local community (e.g. at local shopping malls, supermarkets, and community events) on a quarterly basis. We anticipate screening at least 200 people at each event for the first 4 years of the award (15 events total). At each screening, after providing informed consent, the participant will complete a brief screening questionnaire to determine eligibility and will be asked for permission to recontact. Members of the BISP team (Drs. Diatchenko and Fillingim) have prior experience on screening this volume of participants

through their work on the Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) study.[54] Eligibility will be determined from established inclusion and exclusion criteria from our initial funding period that establish appropriateness for the exercise-induced injury protocol, now modified to account for the risks of administering propranolol. Details of the inclusion and exclusion criteria are reported in the Human Subjects section. If eligible, each participant will provide a saliva sample and complete the Pain Catastrophizing Scale (PCS), a 13-item, 4-point rating scale.[55, 56] A small incentive ($5 gift card) will encourage participation. We have had success with similar approaches in the past for OPPERA.

High-risk subgroup status will be based on parameters predictive of poor pre-clinical and clinical shoulder pain outcomes in the initial funding period. Specifically, subjects with PCS scores of 5 or greater and *COMT* genotype indicative of high pain sensitivity by rs6269 (i.e. "AA") and will be designated as high-risk. ***Those familiar with the PCS will notice that a score of 5 is not elevated for general or clinical populations. However, this cut-off is specific for those with the COMT high pain sensitivity variation, so it is lower than if a general cut-off score independent of the genotype was used.*** To control costs, genotyping will be performed only in those individuals who exceed the PCS cutoff. All genotyping will be performed by Dr. Wallace's laboratory using techniques described in the initial funding period.[19, 40] DNA for the screening will be initially extracted from saliva (buccal swab, using Gentra PureGene system) and quality and quantity will be verified with Nanodrop spectrophotometry. Screening will focus on the rs6269 genotype based on our outcome from the initial funding period. Dr. Wallace's lab has developed a rapid, inexpensive in-house genotyping system for this SNP, using PCR followed by BstBI restriction digestion of the PCR product, to specify alleles based on simple gel electrophoresis (with appropriate known-genotype controls for partial digestion). Based on the lab's extensive experience, DNA extraction and genotyping will be completed for each sample within 3 days, so that eligible participants can be recontacted as soon as possible. For participants in the full study, additional DNA will be extracted from leukocytes from blood (also established in the lab) and re-genotyped for rs6269 for quality control. In addition, the other three established, linked, *COMT* SNPs will be genotyped (rs4633, rs4818, rs4680) to investigate the full range of individual SNP and haplotypes that previously been associated with pain sensitivity in other studies,[2, 57] and our own studies.[17-19, 40] This genotyping will be done in batches through the UF Pharmacogenomics Core using established TaqMan Genotyping assayswith 5% duplicates for quality control. Any samples failing TaqMan will be genotyped in the Wallace lab by restriction digest or sequencing. Hardy-Weinberg equilibrium analysis will be done, as well as *COMT* haplotyping. Subject DNA (and RNA extracted from leukocytes) will be stored in Dr. Wallace's lab, in an IRB-approved tissue/data bank, available for future analyses per consent of each subject. Screening is necessary because the personalized intervention is designed for genetic and psychological makeup of the high-risk subgroup. Participants in the low risk subgroup are not further eligible, and collected data will be destroyed. In the initial funding period 42/182 (23.1%) subjects were designated as high-risk. Therefore, screening 3000 individuals gives us an expected 690 eligible to recruit 448 participants (64.9%). The enrollment rate for eligible subjects was much higher than 65% in the initial funding period, so we think this is a conservative estimate of the numbers that will need to be screened.

Randomization. There are reported sex differences in pain conditions,[58, 59] and we have observed that females report higher pain sensitivity in our studies of shoulder pain.[60] Sex differences in how *COMT* impacts pain sensitivity has also been described, with females having a stronger association with pain sensitivity for certain genetic variants.[61] Therefore randomization will be balanced to ensure equal allocation for males and females in the different intervention groups (i.e. sex stratified). The randomization scheme will be prepared by computer and completed prior to the start of the study. After the randomization list is generated treatment assignments will be contained in sealed, numbered envelopes. Envelopes will be opened in sequential order as each participant enters the study by research staff blinded to application of interventions (i.e. treatment assignment and allocation will be by different research personnel). We will randomize prior to muscle injury to ensure propranolol is absorbed by the system before muscle injury. Propranolol administration before injury allows for any immediate pre-emptive effects on the molecular or pain sensitivity regulation measures to be detected during the baseline session (Figure 5). This administration also matches when propranolol would be administered in a clinical model (i.e. pre-operatively) so that translational component will be maintained.

Exercise-Induced Shoulder Injury. Research personnel performing the muscle injury protocol will be blinded to randomization results. Subjects will undergo exercise-induced shoulder injury to the dominant arm. The

specific eccentric exercise fatigue protocol uses isokinetic equipment[62-66] and is an established protocol from the initial funding period[16, 19, 40] and other prior published studies.[18, 67] The fatigue protocol will be performed prior to randomization to maintain blinding to prevent bias. Briefly, shoulder fatigue will be induced using a Kin-Com (Chattanooga, TN) isokinetic dynamometer. Subjects will be placed in a seated position, with shoulder straps applied to support the torso. Then, the dominant shoulder will be placed in the scapular plane because this position has been associated with high test-retest reliability and has decreased impingement of the greater tuberosity under the acromion.[62, 68] Maximum voluntary isometric contraction (MVIC) will be determined by having the subjects perform 5 repetitions of isometric shoulder external rotation. Subjects will be asked to perform the contractions with maximal effort and given verbal encouragement during the contractions. The MVIC will be determined by averaging peak force from the middle 3 repetitions.[69, 70]

After MVIC is determined, subjects will complete eccentric/concentric external rotation repetitions to induce muscle fatigue and microtrauma. The first set of repetitions will be completed at 100 degrees/second to familiarize the subjects with the testing apparatus. Then, the speed will be lowered to 60 degrees/second for 3 sets of 10 repetitions that constituted the fatigue protocol. After completing those repetitions, subjects will be re-tested to determine if they can generate more than 50% of their respective MVIC. Previous research has indicated the inability to achieve 50% of initial peak MVIC is an indicator of muscle fatigue.[63-66] If they are unable to achieve at least 50% of their MVIC, the fatigue protocol is terminated. If they are able to generate more than 50% of their MVIC, subjects will perform additional sets of 10 repetitions at speeds of 45 degrees/second. This will be repeated until their peak force is less than 50% of the initial MVIC. Subjects will be allowed to rest 30 seconds between sets and the total amount of work performed to reach muscle fatigue will be recorded. The goal of the injury protocol is to induce delayed onset muscle soreness (DOMS) in the rotator cuff musculature. Shoulder DOMS is a clinically relevant model because subjects experience increased pain intensity, loss of range of motion, inflammatory responses, altered proprioception, and the use of self-care behaviors.[63, 64, 66, 71-75] Additional relevance of this model for post-operative shoulder pain was confirmed in the aforementioned studies from our initial funding period.

Personalized Interventions. Pharmaceutical: Increasing evidence implicates β-adrenergic drive in the pathophysiology of chronic pain conditions. Indeed, musculoskeletal pain conditions are associated with heightened catecholamine levels and increased sympathetic responses to stressors.[76-78] Also, in rodents epinephrine produced a β-adrenergic receptor-mediated mechanical hyperalgesia.[79] Additional evidence suggests that these pronociceptive effects of catecholamines can be reversed by blocking beta-adrenergic receptors. For example, a single infusion of propranolol temporarily reduced clinical pain among individuals with temporomandibular disorder and fibromyalgia.[80] Another study showed that pindolol, a medication that blocks both β-adrenergic and serotonin 1A receptors, reduced pain and tenderness in patients with fibromyalgia.[81] Moreover, in rodents, propranolol has been found to decrease inflammation-evoked hyperalgesia in joint and muscle.[82, 83] Catechol-O-methyltransferase (COMT), the enzyme encoded by *COMT*, metabolizes catecholamines, including epinephrine and norepinephrine.[84] *COMT* genotypes associated with lower COMT activity have been associated with increased risk of musculoskeletal pain[3, 85] and greater pain sensitivity.[2, 57] Preclinical work from the laboratory of Dr. Diatchenko (a consultant to the current project) showed that COMT inhibition produced robust thermal and mechanical hyperalgesia, which was blocked by propranolol.[9] Furthermore, clinical work from Dr. Diatchenko demonstrated that the analgesic effect of propranolol in people with orofacial pain was dependent on the subject's COMT genotype – with greater analgesia observed in patients with a haplotype conferring low COMT activity.[8] Our personalized pharmaceutical intervention is designed to be consistent with that finding. ***Thus, lower COMT activity leads to increased catecholaminergic activation of beta-adrenergic receptors, thereby increasing risk for and severity of musculoskeletal pain. Therefore, we hypothesize that due to its blockade of beta-adrenergic receptors, propranolol will be particularly effective at reducing pain in individuals with low COMT activity, such as for our high-risk group.***

The UF Investigational Drug Service will prepare long-acting propranolol (Propranolol LA) 60 mg to be administered orally in the Pain Clinical Research Unit once daily for the five days of the protocol. This dose will provide a bioequivalent dose to that recently reported in a clinical study examining responses to propranolol among patients with TMD pain.[8] The first dose will be administered prior to the exercise-induced shoulder injury to mimic pre-operative settings and to allow for immediate effects to be observed during the baseline session. Subsequent doses will be applied at the beginning of each session. Cardiovascular response (i.e. ECG, HR and BP) will be monitored 60 minutes after drug administration by a research nurse. The purpose of

this monitoring is for safety (early identification of potential adverse events) and efficacy (demonstrate medication absorbed). These measures will be recorded by the research nurse in a blinded manner, and not made available to any other research personnel performing testing within that session, unless subject safety is a concern. These data will be used by the investigator team as a manipulation check to assure that propranolol absorption is occurring, and allow for adjustments to be made early in the protocol if not.

Psychological: Dr. George has been involved with design of psychological interventions used in several low back pain clinical trials[86-89] and co-authored a perspective on key principles in psychologically informed interventions for low back pain.[20] These principles are not specific to the anatomical region of pain, for example there is a clinical trial of cognitive behavioral treatment for reducing catastrophizing in individuals with chronic headache.[90] These same principles were used to design a psychological intervention for shoulder pain. There is consistent evidence that psychological influence on exercise-induced shoulder injury comes from pain-related fear, kinesiophobia, and pain catastrophizing,[15, 16, 67] as has been reported for individuals with pain in other body regions.[13, 14, 91, 92] Therefore, the psychological intervention will reduce these factors with special emphasis on pain catastrophizing since that was the factor in the subgroup and it has been established as an important therapeutic target for cognitive based interventions.[93] Additional justification for a separate psychological intervention comes from an indication that propranolol alone did not improve psychological status for subjects with orofacial pain.[8] The psychological intervention will be administered on Days 2-5 of the protocol since the intervention is predicated on the individual experiencing pain. This approach also allows for immediate effects of propranolol to be determined in the baseline session. ***The first principle relates to cognitive restructuring through psycho-education,[20]*** which will encourage activation by: a) reducing the threat of muscle injury; b) encouraging normal use of the shoulder and arm; and c) addressing specific concerns or misapprehensions expressed by the subject (e.g. concern that pain experienced with shoulder motion is a sign of re-injury). The education approach will be scripted and structured so it is provided in a standardized manner for all subjects. ***The second principle relates to cognitive restructuring through activity[20]*** which will be graded exposure. Briefly, graded exposure is a behavioral treatment appropriate for individuals that avoid activity due to fear of pain and catastrophizing. Graded exposure involves identifying an activity the subject is fearful of or threatened by, and then devising a movement program that gradually leads to being able to perform the activity.[94] The progression of the movement program is hierarchically based so that when subjects have lessening of fear, more difficult movements are added. Successful application of graded exposure for low back pain has been reported in pre-clinical settings[95] and in our previous clinical trials.[86, 96] There will be modifications made for shoulder pain. For example for low back pain reaching or twisting are commonly avoided activities,[95, 96] but subjects with shoulder pain have reported avoiding washing their hair. In this case the graded exposure program would initially encourage shoulder external rotation and elevation, first in protected ranges, and then in more elevated ranges that mimic washing hair. Although the activity identified as avoidant will differ between subjects, the graded exposure approach to address this activity limitation will be scripted and structured so it is provided in a standardized manner for all subjects.

Placebo Interventions. Pharmaceutical: Placebo pharmaceutical capsules will be prepared by the UF Investigational Drug Service to be visually indistinguishable from the active medication. Placebo administration will be done in the same fashion as was described in the personalized pharmaceutical section to maintain blinding. This includes the same timing for each session and monitoring of cardiovascular responses.

Psychological: The placebo psychological intervention will match the structure and administration of the personalized intervention with the participant remaining blinded to what is received. These components consist of general education and activity principles that did not change psychological measures in our low back clinical trials.[87, 88] Since these principles are not specific to anatomical region, they are appropriate for use in this study of shoulder pain. The first principle relates to education which will enhance understanding of shoulder anatomy by reviewing: a) structure and arthrokinematics of the shoulder joint; b) muscle anatomy of the shoulder with emphasis on the rotator cuff; and c) potential pain generators in the shoulder. The placebo component is an neducation approach that accounts for time spent with research staff and dissemination of knowledge relevant to the mechanism of muscle injury, but is devoid of the cognitive restructuring that characterizes the active psychological intervention. ***Thus, this education is not specific to issues that perpetuate pain catastrophizing and can be considered a placebo.*** The second principle relates to activity which will be a general exercise program that encourages gradual return of shoulder movement. Briefly, subjects will be instructed in a 24-hour rest period following the injury, then range of motion exercises, and finally muscle

activation will be resumed through isometric exercise. The progression of the exercise will be based on the time from muscle injury, with a progression each day anticipated. ***The placebo component is that it is a generic, protocol based exercise program not specific to activity being avoided due to pain catastrophizing.*** In this manner it accounts for time spent with research staff for general exercise or activity recommendations. The placebo psychological intervention will be scripted and structured so it is provided in a standardized manner for all subjects.

Primary Outcome Measures. The primary outcome measures were selected based on relevance to the clinical model and successful use in the initial funding period. Pain intensity ratings and self-report of upper-extremity disability will be used as primary outcome measures to determine efficacy for shoulder pain duration, peak shoulder pain intensity, and peak upper-extremity disability. ***These constructs have a conceptual link to chronic pain[1] and therefore can be used to characterize the presence of persistent or continued pain following exercise-induced injury.*** The Brief Pain Inventory (BPI) will be used to measure pain intensity as it has been found to have good test-rest reliability over short intervals.[97] The BPI consists of rating pain intensity on an 11-point numerical rating scale ranging from 0 (no pain) to 10 (worst pain intensity imaginable). The BPI asks subjects to rate current pain and pain at its worst, best and average over the past 24 hours. ***To determine recovery, subjects will complete the BPI daily until they rated their current pain at 0/10 and their worst pain was rated less than 2/10. The number of days it took to reach this recovery criterion will be recorded as duration of shoulder pain.*** The highest worst pain intensity recorded during recovery will be recorded as the peak shoulder pain intensity. The Disabilities of the Arm, Shoulder, and Hand Questionnaire (DASH) will be used to assess upper-extremity disability and we will continue to use a validated abridged version of the DASH (the QuickDASH) which consists of 11 functional items, with total scores ranging from 0 (not disability) to 100 (complete disability).[98] We will use the QuickDASH because shoulder pain can also affect distal function of the arm and hand, and we wanted to obtain a global upper-extremity assessment. Similar to the BPI ratings, QuickDASH scores will be recorded daily until recovery and the highest score during this period will be recorded as peak upper extremity disability. Another advantage of these measures is they are widely accepted as primary outcome measures in clinical studies, and were used as such in the initial funding period. Therefore, these measures will allow for specific effect size estimates for a subsequent randomized clinical trial in post-operative shoulder pain.

Secondary Outcome Measures. These measures represent underlying mechanisms that we hypothesize to be related to how the personalized interventions affect pain relief. These measures will be obtained at the same time each day (relative to the time of the initial shoulder injury) to avoid unwarranted variation.

Molecular: These measures will capture relevant inflammatory biomarkers. The low COMT activity of our high-risk group results in increased catecholamine activity, which augments release of proinflammatory cytokines under conditions of stress.[99-101] This catecholamine-evoked cytokine release can be attenuated by propranolol.[99-102] Moreover, catastrophizing, which also characterizes our high-risk group, has been associated with greater increases in circulating proinflammatory cytokines following both acute pain[103] and induction of pain-related negative emotions.[104] Thus, we hypothesize that personalized pharmaceutical or psychological intervention will significantly attenuate cytokine levels, with the greatest reduction observed in the combined personalized intervention condition. Moreover, because the increased pain evoked by COMT inhibition has been found to be mediated by increased circulating cytokines and reversed by blockade of beta-adrenergic (β2 and β3) receptors,[7] we further expect that the attenuation of cytokine release will be associated with the efficacy of our combined personalized intervention for reducing pain and disability. Thus, we plan to perform assays for several inflammatory cytokines, including IL1B, IL6, IL8, and TNFα at baseline, immediately after the exercise-induced injury, and at regular intervals (Figure 5). All assays will be performed by the Metabolism and Translational Science Core of the Claude D. Pepper Older Americans Independence Center using commercially available kits with which they have extensive experience.

Psychological: In addition to the aforementioned PCS, the Tampa Scale of Kinesiophobia which is an 11-item (TSK-11), 4 point rating scale to quantify avoidance and re-injury beliefs[105, 106] and the Fear of Pain Questionnaire (FPQ-III) which is a 30-item, 5-point rating scale to quantify fear about specific situations that normally produce pain[107-109] will be used to capture psychological processes. Consistent with a fear-avoidance model of musculoskeletal pain,[110] we hypothesize that the personalized psychological intervention will reduce these levels significantly via cognitive restructuring providing subsequent decreases in disability and pain.

Pain Sensitivity Regulation: These measures characterize nervous system processing of standard stimuli so that central or peripheral sensitization states indicative of pain amplification can be detected.[41, 43, 111] Pain amplification is important to account for separately because it is hypothesized as a precursor to chronic musculoskeletal pain conditions that can occur with or independent of the molecular and psychological measures.[28] The potential contributors to pain amplification are multifactorial, therefore we hypothesize that the combined personalized intervention group will show the largest reduction in measures indicative of pain amplification. All pain sensitivity measures will be obtained by psychophysical sensory testing as per established protocols established from the initial funding period.[45-47, 111] All stimuli will be delivered to bilateral upper extremities to allow for side to side comparisons. Stimulation sites will be varied to prevent carryover effects due to local sensitization. The stimuli are to be applied by a research assistant blinded to intervention status who ensures proper application and the range of stimulus intensities to be used (30-54°C) will be presented beforehand to each subject. All subjects will undergo a brief training with the stimuli to be tested. We have found this procedure to be useful because it familiarizes subjects with the stimulus range, tends to obviate range effects in psychophysical scaling, and helps alleviate subject anxiety about the upper limit of stimulus intensities to be used. The research assistant will record patient visual analogue scale (VAS) response to each stimulus used. The VAS will consist of a 10 cm line whose endpoints are designated as 'no pain sensation' and 'the most intense pain sensation imaginable'. In order to standardize the scaling instructions, standard instructions[112] will be used for all subjects. The specific pain sensitivity regulation measures to be collected include suprathreshold heat pain responses, pressure pain threshold, and conditioned pain modulation. Exact parameters for these measures are explained in more detail in publications from the initial funding period.[45-47, 111] These measures were selected because we expected them to be responsive to the combined personalized intervention and associated with pain relief. For example, elevated suprathreshold heat pain responses normalized post-operatively in the clinical cohort[46] and its changes were associated with improvements in post-operative shoulder pain and disability.[113]

C4. Statistical Analysis Plan

All statistical analyses will be performed using the SAS software, version 9 (SAS Institute Inc, 1996). Summary statistics will be provided for baseline measures by intervention groups to determine if randomization produced balanced groups. Any group imbalance will be investigated further to determine if covariates should be considered. For Specific Aim #1, our primary analysis for efficacy will compare shoulder pain duration (i.e. *number of days until subject rated their current pain at 0/10 and their worst pain was rated less than 2/10*) across the four randomly assigned groups with the use of logistic regression. Consistent with the initial funding period, the primary outcome variable will be dichotomized based on duration of at least 7 days or not. There is an *a priori* plan to include age, sex, and race as covariates in this analysis, additional variables will be added as covariates only if imbalanced across groups and correlated with outcome measures. We anticipate very little missing data because this is a pre-clinical study, but any missing outcomes will be predicted by subject pain intensity trajectory plus baseline demographic factors. The primary comparison between the combined personalized intervention and the combined placebo condition will be tested at the 0.05 significance level, while the other five between group contrasts will be tested using a step-down procedure. For the other primary outcomes (peak pain intensity and upper-extremity disability as continuous measures) we will perform a similar analysis process (i.e. same considerations for post randomization imbalance, covariates, and missing data) with linear regression analysis to compare the four intervention groups. For Specific Aim #2, we will fit path models with predetermined orders to investigate the direct (intervention group) and indirect effects (through the mediating psychological, molecular, and pain sensitivity paths) of the randomly assigned condition on pain relief. The combined placebo intervention will serve as reference group for these analyses. The total, direct and indirect mechanistic effects on pain duration (dichotomous) and peak pain intensity and upper-extremity disability (continuous) will be estimated and tested at the 0.05 significance level for the comparison of assigned conditions. Specifically, the total effect will be estimated and tested through a logistic regression for pain duration and a general linear model for peak pain intensity, both of which include the assigned intervention conditions as independent variables while controlling for participant age, sex, and race. The direct effect will be estimated and tested with the use of the same models including the same independent variables, but controlling for mediating molecular, psychological, and pain sensitivity variables in addition to age, sex and race. The two regression models in the second step will reveal the contributions of the mediating psychological, molecular, and pain sensitivity variables on pain relief. On the other hand, the indirect effect will be calculated based on the difference between the total and direct effects. In addition, we will conduct a third

set of regression analyses to evaluate the effect of the assigned condition on the mediating variables which will determine which indirect paths are statistically significant.

C4. Sample Size Estimate and Study Timeline

A total of 448 high-risk subjects will be recruited. In the initial funding period we found that 40.5% of high risk subjects had pain duration ≥ 7 days while 21.4% of the low risk group had long duration (Table 1). In our power analysis, we assumed that 40% of the combined placebo group will have long pain duration (rate for high risk subgroup), while the rate for the combined personalized intervention group will be 20% (rate for low risk group). Those with one personalized intervention were assumed to have 30% chance of having duration ≥ 7 days. The planned sample size will enable us to have 80% power to detect the assumed differences across the four intervention groups and 91% power for the primary comparison between the combined personalized and the combined placebo interventions at a type-I error level of 0.05. The overall BISP timeline is in Table 3.

Table 3. Timeline and Key Periodic Events for BISP

Year 1		***Year 2***		***Year 3***		***Year 4***		***Year 5***	
Start Up	Quarterly Meetings with Research Team Annual Progress Reports to Institutional Review Board, Data Safety Monitoring Body, and NIH								
	Screening, Recruitment (annual rate = 96-120 participants), Intervention, and Follow Up								
	Scientific Conference		Scientific Conference		Scientific Conference		Scientific Conference		Scientific Conference
On Site Consult		On Site Consult		On Site Consult		On Site Consult		On Site Consult	Analysis, Dissemination

C5. Potential Pitfalls and Alternative Approaches

1) Feasibility of Proposed Sample Size. In the initial funding period we recruited 340 subjects. There is potential for higher recruitment rate in this proposal because it involves only the exercise-induced injury cohort of healthy volunteers. Recruiting for the clinical cohort was much more time intensive. Drs. Diatchenko and Fillingim have experience in large scale recruitment from OPPERA[54] and our planned screenings will generate sufficient high-risk participants. The protocol requires at least five days of participation from participants and we had an outstanding completion rate (189/190) during the initial funding period. Therefore, retention and missing data are not expected to be a concern. If there is more missing data than anticipated we will account for it in our statistical analysis. *2) Lack of Shoulder Specific Intervention Pilot Data.* Pilot data demonstrating effect sizes will be vital when proposing a clinical trial in the post-operative setting. This renewal proposal includes established pharamaceutical[8] and psychological[86] interventions that have demonstrated effects in clinical populations (orofacial and low back pain respectively) from studies conducted by members of this research team. We will monitor intervention responses for expected results on process variables and adjust the intervention components if indicated. *3) Low Risk Subgroup*. Intervention effects for low risk subjects will be unknown, and these effects potentially have scientific interest. However the pragmatic advantages of greater research design efficiency and direct relevance to clinical investigations justified focus on the high risk subgroup. *4*) *Exploratory Analyses*. We intentionally presented a focused statistical analysis plan to determine the efficacy (Aim #1) and mechanisms (Aim #2) of the personalized interventions described in this proposal. We do acknowledge that response to these interventions may not be as predictable as we have hypothesized. In the unlikely event that the Aim #1 analyses indicate no group differences we will perform additional analyses to inform future research in this area. For example as already mentioned, there is potential for sex differences in pain sensitivity[58-60] and *COMT* variant influence on pain sensitivity[61] to impact study results. We have accounted for this with stratified randomization based on sex and by *a priori* including sex as a covariate in primary analyses. In the case of null findings, however, a specific exploratory analysis will determine if sex-specific intervention effects occurred. For Aim #2 other analyses will be more exploratory in nature (e.g. post-hoc treatment responder) and will be enhanced by the storage of excess DNA, plasma, and RNA so additional genetic predictors and molecular mechanisms can be considered (assuming subjects consent for that use).

C6. Summary and Future Research Directions

Chronic musculoskeletal pain is a significant public health problem. Since 2006 this talented, multidisciplinary team has been working to develop personalized musculoskeletal pain management options. Renewal of this application will allow the important predictive work from the initial funding period to be transitioned to a high impact pre-clinical intervention study. Ultimately, we hope that completion of the proposed study provides foundational data to substantially improve standard of care by identifying personalized interventions that offer pain relief for those most at risk for developing chronic shoulder pain.