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The Design and Methods of Genetic Studies on Acute and Chronic Postoperative Pain in Patients after Total Knee Replacement

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Abstract

Objective—Total knee replacement (TKR) is the treatment option of choice for the millions of individuals whose osteoarthritis pain can no longer be managed through non-invasive methods. Over 500,000 TKRs are performed annually in the United States. Although most patients report improvement in pain and functioning following TKR, up to 30% report persistent pain that interferes with daily function. However, the reasons for poor outcomes are not clear. To best determine which patients are at risk for pain post TKR, a detailed and comprehensive approach is needed. In this article, we present the methodology of a study designed to identify a set of genetic, proteomic, clinical, demographic, psychosocial, and psychophysical risk factors for severe acute and chronic pain post TKR.

Design—Prospective longitudinal observational study.

Setting—University Hospital System.

Subjects—Patients scheduled for unilateral TKR with a target number of 150.

Methods—Prior to surgery, we collect demographic, psychosocial, and pain data. Biological data, including blood samples for genetic analyses, and serum, urine, and joint fluid for cytokine

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assessment are collected intraoperatively. Pain assessments as well as medication use are collected during each of the three days postsurgery. Additionally, pain and psychosocial information is collected 6 and 12 months following surgery.

Conclusions—This study, for the first time, captures the information on both genetic and "environmental" risk factors for acute and chronic pain post-TKR and has the potential to lead to the next step—multicenter large-scale studies on predictors and biomarkers of poor TKR outcomes as well as on tailored interventions and personalized medicine approaches for those at risk.

Keywords

Pain; Total Knee Replacement; Post-surgical; Genetic; Psychosocial; Cytokines

Introduction

Approximately 29 million people in the United States are diagnosed with symptomatic knee osteoarthritis (OA) by age of 60 years [1]. By 2030, 20% of Americans (about 70 million people) >65 years of age will be at risk for OA [2]. Living with this chronic pain condition is difficult and significantly influences physical function and quality of life [3,4]. Total knee replacement (TKR) is the only option for the millions of individuals whose OA pain is no longer relieved by an extended course of non-surgical management. Consequently, the number of joint replacements is also increasing each year. More than 650,000 TKRs were performed in the United States in 2008 (up from 500,000 in 2005) [3,5]; it is projected that by 2030, this number will increase to nearly 3.5 million per year as the population ages [6]. Recent reports have demonstrated that only 70% of patients are satisfied with improvements in function and decreases in pain following TKR [7]. Persistent, function-limiting pain occurs in up to 30% of patients at 1–7 year follow-up [8]. It has been estimated that up to 299,000 new patients each year may experience persistent pain following TKR; this number may increase to between 700,000 and 1.5 million as the frequency of TKR increases [9].

Pain that persists beyond the expected period of healing serves little or no useful purpose and can emerge as a devastating blow to one's sense of well-being. Disabling pain and reduced function have a dramatic impact on quality of life and productivity, including a number of negative effects on mood, daily activities, sleep, cognitive function, and social life [10]. Therefore, it is critical to identify patients at risk for poor TKR outcomes based on individual parameters, develop pain-preventive procedures and implement them in target patients who have no other option than replacement of their deteriorated joint. Although recent studies determined several psychosocial and psychophysical measures as possible predictors of acute postoperative pain after TKR [11], they missed the evaluation of genetic mechanisms underlying variability in human pain perception that have been shown as contributing to risk of chronic pain [12]. Therefore, a more comprehensive approach that includes measures of clinical variables, cyto- and chemokine profiles as well as genetic makeup is needed to understand the entire array of factors that put patients at risk for severe postoperative and, more importantly, chronic pain after TKR.

Identification of patient's environmental and genetic factors associated with risk for severe acute and chronic pain post TKR has the potential to eventually lead to personalized medicine approaches to care. Pain management should be individualized based upon patient's genetic, proteomic as well as clinical variables. It is expected that identification of these variables will lead to development and implementation of novel and more tailored preventive strategies in patients at risk. For example, behavioral interventions tailored to patients' psychosocial, clinical, and demographic presentation as well as genetic makeup may prevent post-TKR pain. Such targeted interventions in patients at risk may reduce the burden of pain and significantly improve quality of life post TKR.

Study Objectives and Hypotheses

This article describes the methodology of the Genetic Studies on Acute and Chronic Post-operative Pain in Patients after Total Knee Replacement conducted in a major medical center in United States. The main objective of the project is to determine a comprehensive set of factors associated with the risk for poor post-TKR outcomes in terms of severe acute or chronic persistent postoperative pain in operated knee, pain-related distress, dysfunction, and impaired quality of life. This objective is based on previous reports showing that higher movement pain, von Frey-evoked pain intensity, and heat pain threshold detected preoperatively predict moderate or severe postoperative movement pain, and higher preoperative resting pain, depression, and younger age predict moderate to severe resting pain after TKR surgery [11], suggesting that it is possible to identify patients at risk via preoperative evaluation. This is in line with a cross-sectional study in a larger sample demonstrating that the presence of chronic widespread pain, depression, higher body mass index (BMI), younger age, and female gender were associated with a higher risk of chronic persistent pain (on average 3.2 years) post TKR [13] Interestingly, individuals with lower preoperative radiographic OA severity grade at the index joint presurgery (defined as tibiofemoral K/L <3 mm) undergoing TKR are more likely to experience high pain post TKR [13]. However, all these and other (e.g., number of years with knee pain, and number of comorbidities) known risk factors are able to explain less than 20% of the variance in pain scores post-TKR [13]. Thus, the risk factors contributing to more severe acute and chronic pain post-TKR remain mostly unknown, and additional efforts should be taken to reveal the hidden sources of interindividual variability in post-TKR outcomes.

The overarching goal of our study is to utilize a comprehensive set of variables that can prospectively predict the risk of post-TKR pain outcomes in a given patient. We believe that these variables may include demographic (age, gender, BMI, habits, life style, etc.); clinical (e.g., disease-related, surgery-related, medical history-related factors and blood cytokine profile), biobehavioral (sleep patterns and coping styles), psychosocial (mood and personality), psychophysical (pain sensitivity thresholds), and genetic (gene polymorphisms), as all those factors, in different combinations, contribute to variability in other human pain conditions [14].

The primary hypothesis of this study is that genes and psychosocial factors may influence post-TKR outcomes independently, in interaction and/or interplaying with environmental (e.g., demographic and clinical) factors. To address this hypothesis, we apply a range of

techniques that allowed us to collect biological samples (whole blood for DNA isolation; serum, urine, and synovial fluid for cytokine profiling), clinical data (from the subject and the medical record), pain data (from Pain Questionnaires and Quantitative Sensory Testing sessions), and self-report psychosocial data from each patient.

Our secondary hypothesis is that genes may contribute to pain-related psychosocial characteristics (e.g., mood, sleep, and coping behavior) and psychophysical traits (such as individual's thresholds and tolerance to evoked pain), so those traits can be used as intermediate phenotypes in pain genetic studies. To address this hypothesis, we collect psychosocial measurements (from validated surveys) and pain sensitivity assessments (via Quantitative Sensory Testing).

This study, for the first time, captures the information on both genetic and "environmental" risk factors for acute and chronic pain post TKR. In this article, we describe the methodologies used in the Genetic Studies on Acute and Chronic Post-Operative Pain in Patients after TKR project, which includes rigorous pain phenotyping and collection of comprehensive clinical, demographic, psychosocial, psychophysical, cytokine, and genetic data. We believe that this study and developed methodology will lead to the next step—multicenter large-scale studies on predictors and biomarkers of poor TKR outcomes as well as on tailored interventions and personalized medicine approaches for those at risk.

Materials and Methods

Study Design and Overview

This is a prospective longitudinal observational study. Patients scheduled to undergo TKR are evaluated at several time points: presurgery (baseline—visit 1), day of surgery (visit 2), on day 1, 2, and 3 postsurgery (visits 3, 4, and 5, respectively), and 6 and 12 months following surgery (visits 6 and 7, respectively). Evaluations include self-report questionnaires, sensory testing, and collection of clinical data and tissue samples as described below (sections 3.4–3.9) and depicted in Table 1. The protocol for this study is approved by the University of Pittsburgh Institutional Review Board.

At the preoperative (baseline) hospital visit, consented participants complete a demographic form as well as pain and psychosocial questionnaires. Pain intensity at rest is measured and then quantitative sensory tests (QSTs) are performed as described below in section 3.8. Range of motion is then performed, and the subject rates the intensity of pain caused by these movements in the surgical knee. Muscle strength of both knees is also gauged using a handheld dynamometer. On the day of surgery, analgesic medications are recorded and biological samples are obtained.

Postoperatively, the intraoperative anesthesia and analgesia intake is extracted from the electronic medical record (Cerner Powerchart and HPF Document Imaging). Pain intensity at rest and movement (i.e., during range of motion [ROM]) is measured using a 0–10 numeric rating scale (NRS) on postoperative day (POD) 1, 2, and 3, along with the degrees of active movement. The NRS assessments of pain with pressure are also repeated on POD 1, 2, and 3. The NRS assessments of pain with heat are repeated on POD 2. Patients

complete the Situational Pain Catastrophizing Scale (SPCS) [15] following the QST on POD 1, 2, and 3. All postoperative analgesics are recorded.

Pain and psychosocial questionnaires are collected again at 6 and 12 months post TKR over the phone and via postal mail. Telephone interviews are conducted by trained and experienced health professionals with direct web-based data entry.

This study addresses several limitations in previous reports, including cross-sectional or retrospective data collection, lack of disease- and surgery-related data, lack of reliable validated instruments, and lack of genetic data. There are other unique aspects of this study such as examining the effects of cytokine profiling on post-TKR pain and its association with gene polymorphisms. We also describe how chronic post-TKR pain may be correlated with mood, sleep, function, and quality of life as measured through validated patient-reported questionnaires.

Inclusion and Exclusion Criteria

Participants are selected if they meet all inclusion criteria including: 1) 18 years of age or older; 2) undergoing a primary, unilateral total knee arthoplasty for the first time on the respective knee; 3) willing and able to provide informed consent; 4) Caucasian race (to avoid population admixture in genetic data analysis); and 5) non-Hispanic ethnicity (to avoid population admixture in genetic data analysis).

Participants are excluded if they do not meet the above inclusion criteria or if they meet the following exclusion criteria: 1) contraindication or refusal for peripheral nerve blocks; 2) any chronic pain condition that may confound the study per investigator's opinion; 3) chronic opioid dependence per investigator's opinion; 4) any diagnosis for total knee arthroplasty other than degenerative joint disease or osteoarthritis; 5) evidence of clinical dementia, dementia, delirium, or any cognitive disorder that inhibits the subject's ability to comprehend and cooperate with researchers; 6) revision or any knee surgery that is not a primary, unilateral, elective total knee arthroplasty being performed for the first time; 7) any issue that, in the investigator's opinion, would hinder the subject's ability to adhere to the protocol; 8) subjects with knee flexion contracture (which is clinically defined for the purpose of our protocol as more than 15 degrees of knee contracture); and 9) pregnancy.

Recruitment

Persons scheduled to undergo a unilateral TKR by one of eight surgeons at the University of Pittsburgh Medical Center's Shadyside Hospital are approached and screened for the study. Interested and eligible persons complete informed consent procedures and baseline assessments prior to the day of surgery or on the day of surgery, preoperatively. We recruit 150 individuals into the study.

The surgical indication for knee replacement is carefully established for each patient during clinical exam, based on patient's history of chronic severe pain due to knee OA with functional impairment.

Medical history and data on comorbidities (such as diabetes, inflammatory diseases, and inflammatory states) is recorded for each study patient and may be used as covariates in data analysis.

Clinical Data Collection

Analgesia and Anesthesia Procedures—Preoperatively, all subjects have femoral and sciatic peripheral nerve block catheters placed. Depending upon the surgeon preference, they may also receive celecoxib, gabapentin, pregabalin, oxymorphone, or additional home analgesics preoperatively. Subjects receive either a spinal or general anesthesia intraoperatively. Postoperatively, all subjects receive continuous femoral and sciatic peripheral nerve blocks under the current guidelines for postoperative analgesia [16]. Additionally, after the Post Anesthesia Care Unit period, all subjects receive either a morphine or hydomorphone patient-controlled analgesia in order to assess opiate consumption. Finally, some subjects receive additional analgesics at the discretion of the Acute Interventional Perioperative Pain Service or the surgical team for the duration of the hospitalization. Data on analgesia and anesthesia variables are collected and will be used as a possible covariate in the analysis as well as a phenotype in genetic association analyses.

Surgical Procedures—Prior to first skin incision, antibiotic prophylaxis is initiated. After induction of anesthesia, the respective leg is prepped and draped from the toes to the umbilicus. A straight anterior incision is made, dissected through the subcutaneous tissue to expose the extensor mechanism, where a medial parapatellar arthrotomy is performed. The arthroplasty is then performed according to the standard practice of the respective surgeon (including using cement in arthroplasty for each study patient). Some subjects receive a surgical drain at the discretion of the surgeon.

Demographic Data Collection

Information collected via self-report includes age, race, ethnicity, natural hair color (to identify red-heads who may have different pain perception depending on underlying genetic mechanisms [17]), employment status, smoking status/history, alcohol usage, exercise frequency, marital status, history of smoking and alcohol use, number of years of education, highest degree obtained, satisfaction with the material standards of life, and household income. Biometric data include weight, height, blood pressure, pulse rate, respirations, temperature, and waist–hip ratio.

Pain Data Collection

Pain is assessed through clinical examination and also via self-report questionnaires at baseline (visit 1), 6 months after surgery (visit 6), and 1 year after surgery (visit 7). Patients complete the Brief Pain Inventory (BPI) [18], which consists of a four-item pain severity scale and a 10-item interference scale, which documents the extent to which pain interferes with activities, mood, self-care, and enjoyment; the Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) [19], which uses a pain scale to further characterize pain as having predominantly neuropathic origin; and the Pain Interference Short Form from the National Institutes of Health (NIH) Initiative Patient-Reported Outcomes Measurement Information System (PROMIS®) [20,21] (http://www.nihpromis.org), which assesses the

impact of pain on day-to-day activities, enjoyment, and concentration during the past week. We use the BPI because it has demonstrated validity in diverse pain groups such as cancer [18], noncancer pain [22], and postsurgical pain [23]. We use PROMIS Pain Interference scale, validated in clinical and community samples of over 14,000 participants [21], as an additional general measure of pain's interference with life. We use S-LANSS in order to assess features of neuropathic pain before and after TKR in light of recent findings that these features are common and present between 8% of subjects with joint pain [24] and >50% of subjects reporting joint pain of at least moderate severity [25].

We also assess pain during a clinical exam, asking the patients to rate their pain using the 11-point NRS anchored at 0: no pain and 10: worst pain imaginable. NRS scores are collected at rest and for three consecutive times with movement during a physical therapy ROM assessment at baseline (visit 1), postoperative day 1 (visit 3), postoperative day 2 (visit 4), and postoperative day 3 (visit 5). The ROM assessment is consistent with the methods of physical therapists at UPMC Shadyside Hospital and is accepted as a standard assessment for functional recovery following TKR. It explores active extension and passive flexion. A cylinder is placed under the knee to be operated on, and the subject is asked to extend their knee (i.e., straighten their leg) maximally for three separate measurements. The angle of flexion is recorded on the goniometer after each extension, and the subject is asked to rate the pain during each movement of the knee using the NRS (pain with movement). NRS core for pain with pressure and NRS score for pain with heat are also collected during the QST session described below.

Finally, during the QST session (described in section 3.8), subjects complete one additional pain questionnaire related to their testing experience: the SPCS [15] (i.e., catastrophizing measured during or directly after the administration of noxious stimulation) that provides information distinct from that obtained by standard, or "dispositional" measures (which assess individuals' recall of pain catastrophizing in daily life) [26]. Most of the patient-reported measures are collected using a web-based format, thus reducing the probability of data entry error.

Psychosocial Data Collection

We assess several domains of psychosocial functioning through self-report questionnaires. Anxiety during the past week is assessed using the seven-item anxiety short form from the NIH PROMIS (http://www.nihpromis.org). The PROMIS measures have been calibrated on approximately 15,000 respondents and the short forms demonstrate construct validity comparable with much longer conventional measures [27]. The eight-item PROMIS depression short form [28] and eight-item PROMIS Sleep Disturbance short form, which has demonstrated greater measurement precision than longer sleep questionnaires [29], are completed to characterize depressive symptoms and sleep problems over the previous week. The 10-item Perceived Stress Scale [30], validated on a probability sample of over 2,300 US adults, is used to describe the extent to which patients feel overwhelmed by difficulties. Additionally, patients complete the six-item Somatization Scale from the Brief Symptom Index 18 [31] to measure tendency to be somatically focused, the Pain Catastrophizing Scale [32] to measure dysfunctional or catastrophic thinking associated with pain; and the 10-item

Neuroticism/Emotional Stability scale from the Big 5 Personality Index [33–35]. Positive affect is assessed by the 10-item scale of the Positive and Negative Affect Scale [36]. The psychosocial domains of anxiety, distress, sleep problems, somatization, stress, and neuroticism are associated with development of chronic pain [37] and postsurgical pain [38–40]. The questionnaires are chosen for their brevity as well as their validation in pain patients [41,42]. This set of brief psychosocial instruments minimizes subject burden while maximizing information on important conceptual domains. The questionnaires require approximately 20 minutes completing and most were administered via computer tablet, which minimizes data entry error.

We conduct the psychosocial assessment at baseline to evaluate the predictive value of psychological and biobehavioral variables postsurgery and to determine if their use in a larger-scale study could be predictive of the intensity or chronicity of pain in post-TKR patients. We collect psychosocial data at 6 and 12 months postoperatively to determine if these variables correlate with postoperative pain intensity and/or chronicity as well as patient's functional impairment. As psychological assessment is linked to the previous week or month, and is time and labor-consuming, it is not feasible to collect these data on postoperative day 1–3 during hospitalization.

QST

During the QST session, subjects are seated comfortably in a chair or in a bed in a supine position. At the beginning of the testing session, the subject is asked to rate pain at rest at that particular moment using the NRS pain, after which participants undergo the psychophysical testing procedures described below. The quantitative sensory measurements assessed in the QST session, and described below, have been previously used to determine increased sensitivity to pain [11] and have been shown to be reliable [43–45].

Von Frey Hair Filament Threshold—The subject is asked to close their eyes, the researchers touch each Von Frey filament (Stoelting, Wood Dale, IL, USA) to the three areas on each knee (medial to the incision, 1–2 cm away from incision, and lateral to the incision) and on the volar forearm. The subject is asked whether he/she can feel the light touch sensation. The filament where the subject cannot feel the sensation or the last filament able to be felt is reported for this assessment. This test is performed at baseline (visit 1), postoperative day one (visit 3), postoperative day two (visit 4), and postoperative day three (visit 5).

Pressure Pain Tolerance—Using a pressure algometer (FDN200, Wagner Instruments, Greenwich, CT, USA) with a flat TOUCH transducer and a probe area of 0.785 cm^2, mechanical force is applied over the subject's trapezius muscle at the upper back, approximately 5 cm lateral to the C8 spinous process bilaterally. It is also applied to the three areas on each knee (medial to the incision, 1–2 cm away from incision, and lateral to the incision). Subjects indicate the pressure at which the pain is no longer tolerable, or until the device's maximum range of 20 kg pressure is reached. After each anatomical location, the subject is asked to rate the pain with pressure using NRS for seven distinct pain scores

and pressure results. This test is performed at baseline (visit 1), postoperative day one (visit 3), postoperative day two (visit 4), and postoperative day three (visit 5).

Heat Threshold and Tolerance—Contact heat stimuli are delivered using a 1.6 × 1.6 cm square (2.56 cm^2) contact thermode of Thermal Sensory Analyzer (Medoc Advanced Medical Systems, Ramat Yishai, Israel; U.S. Food and Drug Administration-approved instrument). In order to measure the heat pain threshold, the thermode is applied to the volar aspect (nonhairy skin) of the forearm and to the three areas on each knee (medial to the incision, 1–2 cm away from incision, and lateral to the incision). In each trial, the temperature of the probe begins at 32°C and increases at a rate of 0.5°/s. For safety, a cut-off temperature of 50°C is used. The subject clicks a mouse to indicate when they first feel the stimulus as painful (thermal pain threshold), which triggers an immediate decrease in temperature back to baseline, or they max out the highest temperature without ever noticing pain. After each time the subject clicks the mouse, they are asked to rate the pain with heat using NRS.

Cytokine Profile Assessment

Collection of Serum—Approximately 10 mL of blood is collected in one sterile 10 mL vacutainer (BD Vacutainer® Serum 67820, red top; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at baseline or intraoperatively (visit 1 or 2) and once again on postoperative day one (visit 3). Specimens are inverted, left upright on the bench at room temperature for approximately 1.5 hour to allow a clot to form. Samples are then centrifuged at 1,500 × *g* (3,000 rpm) for 10 minutes. Sera are aliquoted into eight 0.5 mL polypropylene microcentrifuge vials and frozen at −80°C at the Clinical Laboratory Improvement Amendments (CLIA) and The College of American Pathologists (CAP) certified laboratory at UPMC Shadyside Hospital until transfer to the Luminex Core Facility of the University of Pittsburgh Cancer Institute (UPCI) at the Hillman Cancer Center.

Collection of Urine—Subjects are asked to provide a urine sample at baseline or intraoperatively (visit 1 or 2) and once again on postoperative day one (visit 3). Urines are then aliquoted into nine 1.0 mL Nunc™ CryoTube™ (Sigma-Aldrich Co., St. Louis, MO, USA) and frozen at −80°C at the CLIA and CAP certified laboratory at UPMC Shadyside Hospital until transfer to the Luminex Core Facility of UPCI.

Synovial Fluid Collection—Using a sterile needle, intraoperatively, the surgical team transarticularly obtains a sample of synovial fluid from the knee to be replaced. The aspirated fluid from the syringe is transferred into a storage vial, and the intra-articular fluid is frozen in a −80°C degree freezer.

Luminex Analysis—Cytokine profiling is conducted on serum, synovial fluid and urine samples at the UPCI Luminex Core Facility (http://www.upci.upmc.edu/cbf/luminex.cfm) using the BioSource™ Invitrogen Hu cytokine Panel 30-plex immunoassay (Life Technologies, Grand Island, NY, USA). The use of a multiplex bead-based cytokine immunoassay and Luminex technology enables simultaneous measurement of representative 1) proinflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor,

interleukin (IL)-1b, interleukin 1 receptor antagonist, IL-6, IL-8, and tumor necrosis factor alpha; 2) T helper cells (Th)1/Th2 distinguishing cytokine, interferon (IFN), IL-2, IL-2R, IL-4, IL-5, and IL-10; 3) nonspecific acting cytokines, IFNa, IL-7, IL-12p40/p70, IL-13, IL-15, and IL-17; and 4) chemokines, eotaxin, (IFN-γ)-inducible protein-10, macrophage chemotactic protein-1, macrophage inflammatory protein (MIP)-1a, MIP-1b, IFN-gamma, and regulated on activation, normal T cell expressed and secreted [46–48]. The xMAP assays are done in 96-well microplate format according to the protocol provided by EMD Millipore (Billerica, MA, USA). A filter-bottom, 96-well microplate (Millipore) is blocked for 10 minutes with phosphate buffered saline/bovine serum albumin. To generate a standard curve, fivefold dilutions of appropriate standards are prepared in serum diluent. Standards and patient sera are pipetted at 25 μL per well and mixed with 25 μL of the bead mixture. The microplate is incubated for 1 hour at room temperature on a microtiter shaker. Wells are then washed thrice with washing buffer using a vacuum manifold. Phycoerythrin-conjugated secondary antibody is added to the appropriate wells, and the wells are incubated for 45 minutes in the dark with constant shaking. Wells are washed twice, the assay buffer is added to each well, and the samples are analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA, USA). Analysis of experimental data is done using five-parametric-curve fitting.

Genetic Data Collection

DNA Collection—Whole blood sample (10 cc) is collected from each subject and placed in ethylenediaminetetraacetic acid-coated tubes prior to surgery during the routine blood work at the University of Pittsburgh's Shadyside Hospital. Genomic DNA is isolated using Qiagen DNA Extraction Kit (Qiagen, Foster City, CA, USA) according to the manufacturer's protocol. Quality and quantity of the DNA sample is measured using a NanoDrop Machine (Thermo Scientific, USA). Then each sample is diluted to 10 ng/uL and plated into optical plates (Applied Biosystems Foster City, CA, USA).

Gene/Marker Selection—Genes are selected based on their reported role in human pain perception in general, and in acute and chronic postsurgical pain in particular. In addition, the selected candidate genes have to have polymorphic loci (single-nucleotide polymorphisms [SNPs]) previously used in genetic association studies; with evidence on sufficient frequency in Caucasian population (e.g., minor allele frequency [MAF] of 5% or more); and functional consequence (e.g., change the encoded amino acids [translation] or influence promoter activity [gene expression], messenger RNA (mRNA) conformation (stability), and subcellular localization of mRNAs and/or proteins) [49].

We select five candidate genes (*GCH1*, *KCNS1*, *SCN9A*, *COMT*, and *OPRM1*, Table 2) that have been shown by us to be associated with multiple acute and chronic pain conditions, including in postsurgical pain populations. These genes have common functional alleles (SNPs, or their haplotypic combinations) associated with human experimental and/or clinical chronic pain phenotypes and have been validated in independent cohorts. *GCH1:* GTP cyclohydrolase (*GCH1*), the rate-limiting enzyme for tetrahydrobiopterin (BH4) synthesis, is a key modulator of peripheral neuropathic and inflammatory pain [50]. BH4 is an essential cofactor for catecholamine, serotonin, and nitric oxide production. In humans, a

haplotype of the *GCH1* gene (allele frequency 15.4% in Caucasians) was significantly associated with less pain following standard discectomy for persistent radicular low back pain [50] and better clinical postsurgical outcomes in patients with disk degenerative disease [51]. In addition, healthy women homozygous for this haplotype exhibited reduced experimental pain sensitivity; and forskolin-stimulated immortalized leukocytes from haplotype carriers upregulated *GCH1* less than controls [50]. BH4 is therefore, an intrinsic regulator of pain sensitivity and chronicity, and the *GCH1* haplotype is a marker of these traits. *GCH1* is currently one of the most reproducible genes associated with human pain. We also documented that the *GCH1* pain-protective haplotype may be identified by genotyping just three SNPs while maintaining the reliability, specificity, and sensitivity of the genetic diagnosis [52]. These SNPs span the entire sequence range of the haplotype: rs8007267 in the 5′ untranslated region, rs3783641 in intron 1, and rs10483639 in the 3′ untranslated region. Therefore, these three SNPs are selected for this study.

KCNS1: This gene encodes the potassium channel alpha subunit *KCNS1* (also called Kv9.1), which is involved in neuronal excitability. It is constitutively expressed in sensory neurons and markedly downregulated following nerve injury [53]. Recently, we have shown the *KCNS1* allele to be a marker of human pain sensitivity and chronicity [53]. A common single amino acid changing SNP, rs734784, significantly associated with higher pain scores in patients with persistent postsurgical lumbar root pain. This valine “pain risk” allele (frequency = 18–22% in Caucasians) is also associated with hypersensitivity in healthy women and in independent clinical pain cohorts comprising limb amputees and sciatica patients from three different countries. This SNP marker is selected for this study.

The *SCN9A* gene, encoding the α-subunit of the voltage-gated sodium channel Nav1.7, is responsible for three human pain disorders: rare nonsense mutations cause a complete absence of pain, whereas rare activating mutations cause severe episodic pain in the Paroxysmal Extreme Pain Disorder and in Primary Erythromelalgia [54]. We have reported that a common SNP in *SCN9A*, rs6746030, is associated with increased pain scores in five independent clinical pain cohorts, including 1,277 patients in total; some were chronic postoperative pain patients as well as women with chronic orofacial pain). This SNP (MAF = 12.5% in Caucasians) was also associated with altered pain threshold, an effect mediated through C-fiber activation [54]. This SNP is selected for this study.

The *COMT* gene encodes the enzyme Catechol-O-methyltransferase (*COMT*) that metabolizes the catechol neurotransmitters dopamine, noradrenaline, and adrenaline, which are involved in various psychiatric diseases as well as several psychological functions in normal participants, including mood, cognition, and stress response [55]. We have identified three functional common haplotypes in *COMT* gene that are associated with increased risk for chronic temporomandibular pain in healthy women [56] and chronic postoperative pain in patients with vertebral disk degenerative disease 1 year after surgery [57]. Genetic variations in *COMT* were also associated with experimental pain [58,59], fibromyalgia pain [60], headaches [61], and other pain conditions [62]. Therefore, four common SNPs (rs6269 with MAF = 37%, rs4633 with MAF = 39%, rs4818 with MAF = 32%, and rs4680 with MAF = 39%) that represent the functional *COMT* haplotypes are selected for this study.

The *OPRM1* gene encodes the mu-opioid receptor that is the principal receptor target for endogenous opioids and exogenous opiate analgesics. We have documented that the SNP rs563649 (MAF = 6%), which is located within 5′-UTR of exon 13 and coding novel *OPRM1* isoform, was associated with individual variations in pain perception of healthy women [63]. This SNP marker as well as the well-established functional A118G SNP (rs1799971 with MAF = 19%) that also influence pain intensity and analgesia [64] are selected for this study.

Genetic data from this study may provide evidence on the contribution of genetic factors to pain phenotypes related to TKR and could lead to a larger multicenter study that would use the developed methodology and collect comprehensive pain phenotypes in a very large cohort powered for Genome-Wide Association Study (GWAS), the only unbiased approach to identify novel genetic risk factors [65].

Genotyping—Genotyping is done using TaqMan method [66] and predesigned SNP Genotyping Assays (e.g., two allele-specific TaqMan® MGB probes containing distinct fluorescent dyes and a PCR primer pair to detect specific SNP targets, product of Applied Biosystems, CA, USA). Genotyping error rate is directly determined by regenotyping 25% of the DNA samples, randomly chosen, for each gene locus.

All the SNPs are checked for Hardy–Weinberg equilibrium (HWE) by the exact test [67]. Only those SNPs that pass the quality check (i.e., HWE *P* value > 0.05) are included into genetic association analyses.

Statistical Analysis

The primary analysis is focused on the outcome of day two postoperative pain. This outcome is selected as primary outcome for both clinical and pragmatic reasons: on postoperative day 2 most of post-TKR patients meet the functional criteria for discharge (e.g., full functional recovery after anesthesia) but experience substantial pain and are still available for full pain assessment in clinical setting. Thus, pain outcome on postoperative day 1 would not be representative of clear functional end point while pain outcome assessment on postoperative day 3 maybe unavailable as many of these patients would have already been discharged.

Secondary analyses focus on a range of the main threshold measures from Section 3.8. Secondary analyses are also conducted for these outcomes at other time points. The primary statistical models are robust linear models for continuous pain measures and logistic models for moderate or severe vs. mild pain. Robust linear models are necessary to capture the non-normal skewed distributions typically associated with pain scores (which are not amiable to achieving normality through any transformations). Multivariable models adjust for demographics and other possible confounders. Different multivariable models are fit for each type of variable (e.g., genetic factors vs. psychosocial variables); the specific modeling strategies vary depending on the specific focus for a given set of variables. For instance, modeling psychosocial variables may require adjusting for certain factors *a priori* and/or including different moderating factors in the analysis that are not specifically relevant to other variable types. In general, variables which are at least marginally significant at *P* <

0.20 are included in the multivariable regression model. The specific modeling strategies will be addressed individually within follow-up manuscripts with findings from this study.

Cytokine Analysis—Analysis of cytokine data includes several main types of analyses. First, the level of agreement in cytokine profile is evaluated between serum, urine and synovial fluid. Second, associations between individual cytokines and pain variables are evaluated with both linear robust models and logistic models in the same manner as the other analyses (see below). Finally, associations between individual cytokines and psychosocial variables are evaluated with the same models.

Genetic Data Analysis—To assess whether common functional polymorphisms affect TKR-related pain (baseline, acute postoperative, and chronic postoperative), we employ two main statistical models, which include 1) robust linear regression [68] of continuous pain or functioning scores (utilizing the previously described instruments and scales such as pain severity and pain interference from the BPI); and 2) logistic regression [69] of pain persistence based on a dichotomized measure of whether or not moderate to severe pain persists at the given time points. For each outcome, we initially fit models for a single polymorphism adjusted for key demographic and clinical factors (specified *a priori*, or retained after variable selection), e.g., age, gender, and BMI. The additive genetic inheritance (i.e., gene-dosage effect) models for each SNP is considered, in which three possible genotypes are coded as (0, 1, 2) based on the numbers of variant (minor) alleles. In other words, the homozygote of the wide-type or major allele is always used as the reference category for statistical comparison. As SNPs may affect pain individually or in combination, each possible two-way and three-way interaction is also tested (controlling key clinical factors) for each outcome.

The effect size is quantified using the beta coefficient or odds ratio representing the risk or protection incurred by the additive increase of each minor allele for a SNP. Both unadjusted *P* values and adjusted *P* values using the false-discovery rate method [70] for adjustment of multiplicity or multiple comparisons are reported. A final adjusted *P* value of less than 0.05 is considered to be statistically significant.

Females and males respond to pain in different ways; and sex-specific effects of pain candidate gene like *COMT* have been reported [71]. Our genetic association analyses, therefore, are performed in the whole sample, female-only sample, and male-only sample, respectively. It is worth noting that these scientifically justifiable analyses may suffer from the loss of power due to decreased sample size as we split the data, as well as the additional multiplicity that have to be adjusted for.

Sample Size Consideration—This study protocol is initially proposed to assess the feasibility of conducting a larger multicenter GWAS aiming to identify risk factors of post-TKR pain outcomes. That is, we intend to assess whether we are able to enroll the targeted number of 150 TKR patients within the specified 2-year study period and to test the sensitivity and validity of inclusion–exclusion or eligibility criteria for the larger scale study. Second, we intend to assess whether we are able to successfully conduct a complete phenotypic and genetic profiling of those who are enrolled into this study, and how well we

can follow-up with them to assess their long-term post-TKR outcomes (e.g., 6- or 12-month pain outcomes).

With a sample size of 150 patients, however, any “hypothesis-free” genetic testing of association between a large number of SNPs and pain outcomes would quickly suffer from loss of power because of required adjustment for multiple comparisons to avoid inflated false positive rates. Therefore, we only intend to genotype a few common functional alleles from preselected validated pain-associated candidate genes (Table 2), aiming to identify similar effects of these genetic polymorphisms as shown in previous reports in other pain models. Point estimates and their precision (e.g., 95% CI) of these effects, instead of formal statistical inference of association, will be reported and highlighted in follow-up manuscripts.

Nevertheless, for a SNP with a minor allele frequency of 0.20, assuming an additive genetic effect model, our power estimation using Quanto (University of Southern California Los Angeles, CA, USA) [72] finds that a sample size of 150 results in 80% power to detect a genetic effect of 0.05 (i.e., a quantitative trait locus specific variance of 0.05, or the SNP explains for 5% of total variation of outcome) at a two-sided alpha level of 0.05.

Discussion

Pain and pain-related dysfunction are the main clinical outcomes of OA, and the major reason for seeking surgical treatment [73]. TKR is the only currently available surgical option, and the number of TKR annually perfumed in the United States and worldwide is growing [6]. Nevertheless, a substantial proportion of patients report little improvement in pain and functional outcomes post-TKR: 44% of patients have persistent pain in the operated knee years after surgery, with 15% of TKR cases reporting severe to extreme pain [24]. There is an urgent need to identify all the factors that contribute to poor post-TKR outcomes, predict patients at risk, and develop novel strategies to modify these factors and prevent bad outcomes.

It is important to acknowledge the limitations of this study. Despite efforts to include comprehensive sets of psychosocial, psychophysical, genetic, clinical, and demographic contributors to pain post-TKR, it is possible that other, unassessed factors may play important roles. In particular, the exploration of determinants of chronic pain post TKR is complex, due to the multitude of environmental and personal factors that may influence the development of pain over many months. Recognizing that a balance between participant burden and comprehensive data collection is necessary, we chose to use brief but precise measures and a relatively small number of assessment time points.

Studies of human postsurgical pain show that certain risk factors correlate with pain persistence [74]. Likewise, several demographic (e.g., BMI, age, and gender) psychological (e.g., presence of depression), and clinical (e.g., preoperative radiographic OA severity) factors predict chronic pain experience post TKR [13]. However, a comprehensive array of risk factors is currently unknown and understudied. There is gap of knowledge on genetic mechanisms underlying the development of severe acute and/or chronic pain after standard

surgeries. Here we present in detail the design and methodology of a study that aims to identify genetic determinates of post-TKR outcomes, via candidate gene association study using deep prospective phenotyping of OA patients undergoing TKR. We realize that this study may provide only limited genetic data due to the nature of candidate gene approach [75]. Still, this study is the first one to address genetically post-TKR pain, and we believe that our methods are applicable for a multicenter large-scale GWAS on acute and chronic postsurgical pain after TKR.

Genetic data from this study may provide evidence on the contribution of genetic factors to pain phenotypes related to TKR and could lead to a larger multicenter study that would use the developed methodology and collect comprehensive pain phenotypes in a very large cohort powered for GWAS, the only unbiased approach to identify novel genetic risk factors [49,65]. A large-scale study that includes GWAS and deep prospective phenotyping of patients undergoing TKR is expected to lead to the development of personalized clinical interventions to reduce the significant burden of chronic knee pain in the years following TKR.

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Table 1

Study flow chart

Assessment Cluster	Visit 1 (Screening/Baseline)	Visit 2 (Day of Surgery)	Visit 3 (Postop. Day 1)	Visit 4 (Postop. Day 2)	Visit 5 (Postop. Day 3)	Visit 6 (Day of Discharge)	Visit 7 (6 Months Postop)	Visit 8 (12 Months Postop)
Informed consent	X							
Demographics form	X							
Patient questionnaires								
Brief Pain Inventory (BPI) pain severity and interference	X			X		X	X	X
Pain interference: PROMIS	X					X	X	X
Neuropathic pain: S-LANSS	X						X	X
Anxiety: PROMIS	X						X	X
Depression: PROMIS	X						X	X
Sleep disturbance: PROMIS	X						X	X
Perceived stress: PSS	X							X
Somatization: BSI-S	X							X
Positive affect: PANAS	X						X	X
Catastrophizing: PCS	X						X	X
Neuroticism/emotional stability	X							X
Clinical assessments								
Pain at rest: NRS	X		X	X	X			
Range of motion	X		X	X	X		X	X
Pain with movement: NRS	X		X	X	X		X	X
Pressure algometry threshold	X		X	X	X			
Pain with pressure algometry: NRS	X		X	X	X			
Touch threshold: Von Frey hair	X		X	X	X			
Muscle strength: hand held dynamometer	X		X	X	X			
Situational pain catastrophizing	X		X	X	X			
Pain thermal threshold: medoc TSA-II	X			X				
Pain with heat: NRS	X			X				
Laboratory assessments								
DNA testing: blood sampling		X						

Assessment Cluster	Visit 1 (Screening/Baseline)	Visit 2 (Day of Surgery)	Visit 3 (Postop. Day 1)	Visit 4 (Postop. Day 2)	Visit 5 (Postop. Day 3)	Visit 6 (Day of Discharge)	Visit 7 (6 Months Postop)	Visit 8 (12 Months Postop)
Cytokine panel: joint fluid (N = 75)		X	X[§]					
Cytokine panel: Urine (N = 75)		X						
Medication assessments								
Patient-controlled analgesia		X		X[dc]				
Concomitant medications	X	X	X	X	X		X	X
Femoral and sciatic nerve blocks (start and d'c)		X		X[s]	X[f]			

[§] When the drain is removed, the joint fluid will be collected.

[dc] PCA (patient-controlled analgesia) discontinued.

[s] Sciatic block discontinued.

[f] Femoral block discontinued.

Abbreviations: BSI-S = brief symptom inventory-somatization scale; NRS = numeric rating scale; PANAS = positive and negative affect scale; PCS = pain catastrophizing scale; PROMIS SF = Patient-Reported Outcomes Management Information System—Short Form; PSS = Perceived Stress Scale (4 item version); S-LANSS = Leeds Assessment of Neuropathic Symptoms and Signs.

Table 2

Selected genetic markers

Gene/Gene Product	Allele	Function/Phenotypes	Reference[#]
COMT/catechol-O-methyltransferase	High pain sensitivity, average pain sensitivity and low pain sensitivity haplotypes (rs6269, rs4633, rs4818, and rs4680)	Affects anxiety, depression, pain sensitivity, chronic pain, post-surgical pain, analgesic response	[55–62]
OPRM1/mu-opioid receptor	SNP rs563649 A118G SNP rs1799971	Encodes alternative isoform (6 trans-membrane receptor); affects pain and response to opioids Affects incidence, intensity, or duration of chronic pain and opioid consumption	[63] [64]
GCH1/GTP cyclohydrolase	3-SNP haplotype (rs8007267, rs3783641, rs10483639)	Protected from chronic post-surgical pain and experimentally-induced pain	[50,51]
KCNS1/potassium channel alpha subunit	SNP rs734784	Val allele increased risk for chronic post-surgical pain	[53]
SCN9A/α-subunit of the voltage-gated sodium channel Nav1.7	SNP rs6746030	A allele was associated with increased pain scores in osteoarthritis, sciatica, phantom pain and lumbar discectomy patients	[54]