AUG 10, 2023



**Protocol Citation:** Stephan Frangakis 2023. Study Protocol: The Genetic Markers of Chronic Postsurgical Pain. **protocols.io** https://protocols.io/view/study-protocol-the-genetic-markers-of-chronic-post-crj4v4qw



**Protocol status:** Other
This protocol is our research outline for a planned GWAS

**Created:** Mar 21, 2023

**Last Modified:** Aug 10, 2023

**PROTOCOL integer ID:** 79196

# Study Protocol: The Genetic Markers of Chronic Postsurgical Pain

Stephan Frangakis[1]

[1]Department of Anesthesiology, University of Michigan

Stephan Frangakis
University of Michigan - Ann Arbor

ABSTRACT

This study will investigate the association of genetic variation with chronic postsurgical pain. Patient data will come from two single-center prospective observational cohorts, the Michigan Genomics Initiative (MGI) and the Analgesic Outcome Study (AOS). We will employ a discovery genome-wide association study (GWAS), genome-wide pathway analysis (GWPA), and a targeted replication analysis in our investigation. The primary outcome will be the change in pain scores from baseline to 3 months postoperatively. Secondary outcome will be a pain score ≤4 at 3 months postoperatively (yes/no).

## Abstract

1 This study will investigate the association of genetic variation with chronic postsurgical pain. Patient data will come from two single-center prospective observational cohorts, the Michigan Genomics

Initiative (MGI)[1] and the Analgesic Outcome Study (AOS). We will employ a discovery genome-wide association study (GWAS), genome-wide pathway analysis (GWPA), and a targeted replication analysis in our investigation. The primary outcome will be the change in pain scores from baseline to 3 months postoperatively. Secondary outcome will be a pain score ≤4 at 3 months postoperatively (yes/no).

## Introduction

2 Although there are multiple factors that have been shown to influence pain after surgery, genetic factors have been identified as potential risk factors in the development of postoperative pain. Furthermore, genetic factors have been associated with the development of chronic post-surgical pain (CPSP), affecting up to 30% of postsurgical patients, with estimates of the heritability of CPSP ranging from 30 to 70% [2]. Yet attempts to predict patients that are genetically predisposed to severe postoperative pain or who will progress to develop CPSP, have been largely ineffective. The goal of this work is to identify genetic variants that are associated with the development of acute postoperative pain and/or CPSP. Identifying variants associated with post-surgical pain will allow for targeted studies of other pain cohorts, provide insight into the pathophysiology of pain, and serve as a basis for future investigation into personalized pharmacologic therapies for the prevention and treatment of postoperative pain.

## Aims

3 **Aim 1a:** To test individual genetic variants for association with variability in acute and chronic postoperative pain after surgery throughout the entirety of the human genome using genome-wide association studies (GWAS). The GWAS will be performed in a surgical patient population: >3500 patients for the MGI and AOS datasets. The primary endpoint will be the change in pain scores from baseline to 3 months postoperatively. Secondary endpoint will be pain scores ≤4 at 3 months postoperatively (yes/no).

**Aim 1b:** Using the GWAS data obtained in Aim 1a, but not dependent on its success, this aim will use genome-wide pathway analysis (GWPA) to provide further insight into the genetics of postoperative pain by surveying the cumulative effect of variation in genetic pathways that associate with variability in postoperative pain. GWPA is an agnostic approach that harnesses the wealth of information from GWAS data to identify the additive effects of single variants aggregating in particular gene sets or pathways. We will interpret our GWAS results in the context of gene function and molecular pathways/functions, grouping multiple SNPs into clusters to functionally annotate the observed findings in an integrated manner. This will provide key biological and functional context to the proposed genetic analysis.

4 **Aim 2:** A replication analysis of specific variants of interest based on previously reported

associations with postoperative pain. These specific variants/genes of interest have been compiled through a systematic review of the literature of all genetic variation reported to be associated with postoperative pain. The primary endpoint will be the change in pain scores from baseline to 3 months postoperatively. Secondary endpoint will be a postoperative pain score of 4 or greater at 3 months postoperatively.

## Methods

5 **Study Population**

Data for this study come from two single-center prospective observational cohorts, the Michigan Genomics Initiative (MGI) and the Analgesic Outcome Study (AOS). All participants completed a full written informed consent, and the use of the data for this study was approved by the Institutional Review Board (University of Michigan, Ann Arbor, MI). The consent specifically allowed for future uses of phenotypic and genotypic data after IRB approval, hence additional participant approval was not required.

Patients were recruited prior to surgery and completed a brief battery validated, self-report measures, including pain severity using the 0-10 Likert scale pain questions from the Brief Pain Inventory, which has been validated in various pain disorders.[3] DNA was extracted from a blood sample collected at consent. Patients that underwent surgical intervention and had baseline and 3-month data pain outcomes data were included in the analysis. Patients were excluded from analysis if they had one or more datapoints that were missing (age, gender, baseline pain, pain at 3 months, and self-reported race). Data for Aims 1 and 2 will be derived from this portion of MGI/AOS with existing prospectively collected patient-reported outcomes and pain data at baseline and 3 months after surgery (n >3500). The use of the data for this study was approved by the Institutional Review Board (IRB No. HUM00071298, University of Michigan, Ann Arbor, MI).

In addition to the above eligibility criteria, individuals had to meet the broad inclusion criteria of MGI and AOS. Included participants were 18 years of age or older and had a procedure done with an IV for blood draw at a University of Michigan surgical site (University Hospital, Mott Children's Hospital, Cardiovascular Center, Cardiac Procedures Unit, or East Ann Arbor). Exclusion criteria were: current pregnancy, history of allogenic bone marrow transplant, enrollment in another study, language barrier, cognitive impairment, and lack of consent.

6 **Patient Reported Outcomes**

Participants completed validated patient-reported outcomes prior to surgery and again after surgery at set time points, as previously described. These surveys were designed to identify changes from baseline psychological and physical health characteristics. Patients were initially contacted via mail followed by two follow-up attempts via phone and one via email. Subjects were able to complete either a paper form of the survey or an electronic form via Qualtrics survey.

7 **Data Collection**

After enrollment, participants completed a brief battery of validated self-report measures of pain,

mood, function, and medication use, along with demographics and medical history. Preoperative survey data for this analysis will include: pain score at (1) the site of surgery and (2) overall body pain in the preceding week using the 11-point numeric rating scale from the Brief Pain Inventory (average pain score and pain score at its worst, on a 10-point scale with 0 = no pain and 10 = worst pain imaginable); demographics including age, sex, and self-reported race/ethnicity.

8 **Genetic Data**

600K variants were directly genotyped on either the Illumina Infinium CoreExome array or the Global Screening Array depending on time of recruitment. Participant genotypes underwent rigorous QC and were subsequently imputed to > 51M variants passing standard imputation quality metrics using the Michigan Imputation Server[4] with the Trans-Omics for Precision Medicine (TopMED) reference panel.[5]

9 **Outcomes**

The primary outcome for Aims 1 and 2 is change in patient-reported surgical site pain (scaled from 0 to 10, with 0 equal to no pain) at baseline and 3 months postoperatively (Δ = postsurgical – baseline). This CPSP outcome definition is the same as the A2CPS: Acute to Chronic Pain Signatures program, which is the largest ever NIH funded study of postsurgical pain.[6] We define our secondary outcome as a postoperative pain score of 4 or greater (TRUE IF postop ≥ 4).

10 **Statistical Analyses**

For GWAS (Aim 1a), we will perform genome-wide association analyses using SAIGE software.[7] We will control for the first 10 principal components (PC) and chip and/or study version. Our primary outcome is the difference in pre- and postsurgical pain scores (Difference). Since our primary outcome is quantitative, we will perform its association analysis using a linear mixed model. Prior to inputting our phenotype into SAIGE, we will perform a linear regression on our Difference data in the R statistical software, and using the covariates of age, gender, patient reported race, and preoperative (baseline) pain score. The post-regression residual values will be normalized using the ordered quantile normalization method in R, and subsequently utilized for the GWAS.

For GWPA (Aim 1b), we will use several different methods to capture an array of potential associated genetic signals and obtain robust pathway results, based on the recommendation of Mooney and Wilmot (2015),[8]. Pathway-level assessment can be competitive or self-contained depending on our null hypothesis. If multiple significant SNPs are found in the GWAS analysis in Aim 1a, the SNPs will be mapped to corresponding gene loci and we will utilize *overrepresentation analysis* to assess for an enrichment of significant SNPs in specific pathways. As this compares a single pathway with all others, it is a competitive assessment with a null hypothesis that the enrichment of said pathway is not more than the average of all pathways. We will additionally use *set-based methods* (regardless of the results of the GWAS analysis in Aim 1a) that combine data from all SNPs and genes within each pathway into one aggregated value. Each value will be analyzed as to whether it is larger than what would be expected under the null hypothesis that there is no association between

genes in the specified pathway and our measured outcome. We will use software such as IPA,[9] MetaCore,[10] PathVisio 3,[11]and to identify and visualize potential genetic pathways contributing to our primary and secondary outcomes. We will use three gene-set references that each group genes based on functional-annotation (as opposed to disorder-based or high-throughput-data-based): Gene Ontology (GO),[12] Kyoto Encyclopedia of Genes and Genomes (KEGG),[13] and Synapse Gene Ontology (SynGO).[14]

For the replication analysis (Aim 2), we will perform gene-based association analyses using SAIGE-GENE+,[15] or similar software. Our analysis will focus on detecting associations in our dataset with genes previously identified as being associated with postoperative pain. Our completed literature review of genetic variation associated with post-operative pain suggests targeted gene testing of
156 variants in 79 genes (full list provided in Appendix). Threshold for significance will be calculated using a Bonferroni correction based on the number of variants ($p< 3.2 \times 10^{-4}$ [0.05/156]) or genes ($p<6.3 \times 10^{-4}$ [0.05/79]).

11

## Results

12

Genetic and survey data from a total of 3,137patients from the AOS study were included in our analyses. From the MGI study, 447 patients had pre- and 3-month postoperative pain surveys and were also included in our analyses, for a combined total of 3,584 patients.

We anticipate that novel genes and variants that are associated with CPSP will be identified through our genome-wide association analysis (Aim 1a). We also anticipate that several variants previously reported to be associated with CPSP will be replicated in our cohort through targeted gene analysis (Aim 2).

We anticipate that GWPA (Aim 1b) will show a significant enrichment of SNPs in several genetic pathways, specifically signal transduction, neurotransmission, inflammatory response and signaling, and drug metabolism. Using three different gene pathway datasets, each with its own unique advantages, we expect to find multiple pathways associated with the development of chronic postsurgical pain. Pathways identified with more than one dataset will reinforce the validity of our results.

# Appendix

## 13 References


1. Zawistowski, M., et al., *The Michigan Genomics Initiative: A biobank linking genotypes and electronic clinical records in Michigan Medicine patients.* Cell Genomics, 2023. **3**(2): p. 100257.
2. Clarke, H., et al., *Genetics of chronic post-surgical pain: a crucial step toward personal pain medicine.* Canadian Journal of Anesthesia/Journal canadien d'anesthésie, 2015. **62**(3): p. 294-303.
3. Keller, S., et al., *Validity of the brief pain inventory for use in documenting the outcomes of patients with noncancer pain.*Clin J Pain, 2004. **20**(5): p. 309-18.
4. Das, S., et al., *Next-generation genotype imputation service and methods.* Nature Genetics, 2016. **48**(10): p. 1284-1287.
5. Taliun, D., et al., *Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program.* Nature, 2021. **590**(7845): p. 290-299.
6. Berardi, G., et al., *Multi-Site Observational Study to Assess Biomarkers for Susceptibility or Resilience to Chronic Pain: The Acute to Chronic Pain Signatures (A2CPS) Study Protocol.* Front Med
(Lausanne), 2022. **9**: p. 849214.
7. Zhou, W., et al., *Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies.* Nature Genetics, 2018. **50**(9): p. 1335-1341.
8. Mooney, M.A. and B. Wilmot, *Gene set analysis: A step-by-step guide.* Am J Med Genet B Neuropsychiatr Genet, 2015. **168**(7): p. 517-27.
9. Analysis, I.o.Q.s.I.P., *Calculating and Interpreting the p-values for Functions, Pathways and Lists in IPA.* 2016.
10. Song, G.G. and Y.H. Lee, *Pathway analysis of genome-wide association studies for Parkinson's disease.* Mol Biol Rep, 2013. **40**(3): p. 2599-607.
11. Kutmon, M., et al., *PathVisio 3: an extendable pathway analysis toolbox.* PLoS Comput Biol, 2015. **11**(2): p. e1004085.
12. *The Gene Ontology resource: enriching a GOldmine.* Nucleic Acids Res, 2021. **49**(D1): p. D325-d334.
13. Kanehisa, M. and S. Goto, *KEGG: kyoto encyclopedia of genes and genomes.* Nucleic Acids Res, 2000. **28**(1): p. 27-30.
14. Koopmans, F., et al., *SynGO: An Evidence-Based, Expert-Curated Knowledge Base for the Synapse.* Neuron, 2019. **103**(2): p. 217-234.e4.
15. Zhou, W., et al., *Set-based rare variant association tests for biobank scale sequencing data sets.* medRxiv, 2021: p. 2021.07.12.21260400.


## 14 Previously reported genes/alleles with significant association with postsurgical pain

| | A | B |
|---|---|---|
| | Gene | allele/variant |
| | ABCB1 | rs1045642 |
| | ABCB1 | rs1128503 |

| | A | B |
|---|---|---|
| | ABCB1 | rs2032582 |
| | ADIPOR1 | rs12045862 |
| | ADRA2A | rs1800035 |
| | ADRA2A | rs201376588 |
| | ADRA2A | rs775887911 |
| | ADRB1 | rs1801253 |
| | ADRB2 | rs1042713 |
| | ASTN2 | rs7858836 |
| | ASTN2 | rs958804 |
| | ATXN1 | rs179997 |
| | BDNF | rs1491850 |
| | BDNF | rs6265 |
| | CACNA1E | rs3845446 |
| | CALCA | rs145837941 |
| | CALCA | rs3781719 |
| | CCL2 | rs4586 |
| | CHRNA6 | rs7828365 |
| | CNR1 | (AAT)n |
| | COMT | rs165656 |
| | COMT | rs4633 |
| | COMT | rs4680 |
| | COMT | rs4818 |
| | COMT | rs6269 |
| | COMT | rs740603 |
| | CREB1 | rs2952768 |
| | CRP | rs2794521 |
| | CRTC3 | rs117119665 |
| | CTSG | rs2070697 |
| | CTSG | rs2236742 |
| | CX3CL1 | rs614230 |

| | A | B |
|---|---|---|
| | CXCL8 | rs4073 |
| | CYP2C9 | rs1799853 |
| | CYP2D6 | rs1065852 |
| | CYP2D6 | rs28371725 |
| | CYP2D6 | rs35742686 |
| | CYP2D6 | rs3892097 |
| | CYP2D6 | rs5030655 |
| | CYP2D6 | rs5030865 |
| | CYP2D6 | rs5030867 |
| | CYP3A4 | rs2242480 |
| | CYP3A4 | rs28371759 |
| | CYP3A5 | rs10264272 |
| | CYP3A5 | rs776746 |
| | DQB1 | DQB1 hla |
| | DRB1 | DRB1 hla |
| | DRD2 | rs12364283 |
| | DRD2 | rs4648317 |
| | DRD4 | DRD4 VNTR |
| | FAAH | rs324420 |
| | FKBP5 | rs3800373 |
| | GCH1 | rs10483639 |
| | GCH1 | rs3783641 |
| | GCH1 | rs4411417 |
| | GCH1 | rs8007267 |
| | GDF5 | rs143384 |
| | GRIN2A | rs3219790 |
| | HCRTR2 | rs2653349 |
| | HTR1B | rs6296 |
| | HTR2A | rs1923886 |
| | HTR2A | rs2770298 |

| | A | B |
|---|---|---|
| | HTR2A | rs6313 |
| | HTR2A | rs7330636 |
| | HTR2A | rs9534511 |
| | HTR3A | rs1985242 |
| | IL17A | rs2275913 |
| | IL1B | rs1143634 |
| | IL1R2 | rs11674595 |
| | IL1RN | IL1RN VNTR |
| | IL2 | rs2069762 |
| | IL6 | rs1800795 |
| | IL6 | rs2069840 |
| | IL6R | rs2228145 |
| | IQGAP1 | rs1145324 |
| | KCNA1 | rs4766311 |
| | KCND2 | rs1072198 |
| | KCND2 | rs17376373 |
| | KCNJ3 | rs12995382 |
| | KCNJ3 | rs17641121 |
| | KCNJ6 | rs1543754 |
| | KCNJ6 | rs1787337 |
| | KCNJ6 | rs2070995 |
| | KCNJ6 | rs2211843 |
| | KCNJ6 | rs2835859 |
| | KCNJ6 | rs2835925 |
| | KCNJ6 | rs2835930 |
| | KCNJ6 | rs858003 |
| | KCNJ6 | rs858035 |
| | KCNJ6 | rs928723 |
| | KCNJ6 | rs9981629 |
| | KCNK4 | rs2286614 |

| | A | B |
|---|---|---|
| | KCNK9 | rs2014712 |
| | KCNK9 | rs2542424 |
| | KCNK9 | rs2545457 |
| | KCNS1 | rs734784 |
| | LAMB3 | rs2076222 |
| | MAOA | rs1800659 |
| | MAOA | rs2064070 |
| | MAOA | rs2283724 |
| | MAOA | rs3788862 |
| | MAOA | rs979605 |
| | MAOB | rs1799836 |
| | NAV3 | rs118184265 |
| | NFKB1A | rs696 |
| | NFKBIA | rs8904 |
| | OPRK1 | rs6985606 |
| | OPRM1 | rs1323040 |
| | OPRM1 | rs1799971 |
| | OPRM1 | rs2075572 |
| | OPRM1 | rs499796 |
| | OPRM1 | rs548646 |
| | OPRM1 | rs634479 |
| | OPRM1 | rs679987 |
| | OPRM1 | rs9322447 |
| | OPRM1 | rs9384179 |
| | P2RX7 | rs1718125 |
| | P2RX7 | rs208294 |
| | P2RX7 | rs208296 |
| | P2RX7 | rs7958311 |
| | P2RY12 | rs3732765 |
| | PENK | rs3138832 |

| | A | B |
|---|---|---|
| | PRKCA | rs887797 |
| | PTGS2 | rs20417 |
| | PTGS2 | rs2206593 |
| | RETN | rs3745367 |
| | SCN10A | rs6795970 |
| | SCN11A | rs11709492 |
| | SCN11A | rs13080116 |
| | SCN11A | rs33985936 |
| | SCN9A | rs12619987 |
| | SCN9A | rs12994338 |
| | SCN9A | rs16851799 |
| | SCN9A | rs4286289 |
| | SCN9A | rs4369876 |
| | SCN9A | rs4387806 |
| | SCN9A | rs6724623 |
| | SCN9A | rs6739404 |
| | SCN9A | rs6746030 |
| | SCN9A | rs6754031 |
| | SCN9A | rs9646772 |
| | SLC22A1 | rs12208357 |
| | SLC22A1 | rs34059508 |
| | SLC22A1 | rs34130495 |
| | SLC22A1 | rs55918055 |
| | SLC22A1 | rs72552763 |
| | SLC6A4 | 44 bp in/del |
| | TGFB1 | rs1800469 |
| | TH | rs2070762 |
| | TLR2 | rs3804100 |
| | TLR4 | rs4986790 |
| | TNF | rs1800610 |

| | A | B |
|---|---|---|
| | TNF | rs1800629 |
| | TRPC3 | rs1465040 |
| | UGT2B7 | rs7439366 |
| | ZNF429 | rs2562456 |

Variants reported to be significantly associated with postsurgical pain in at least one study.