Psychiatric and Pain Risk Genes that May Worsen Quality of Life in Chronic Pancreatitis Patients

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Pancreatitis, a fibro-inflammatory disease, can result in debilitating abdominal pain. Pancreatitis pain is difficult to treat even with surgery and opioids. The pain caused by pancreatitis varies in severity and frequency even within patients with similar physical disease states. The purpose of the collection of papers in this dissertation is to identify genetic variation between pancreatitis patients within different patterns of pain in hopes that these results can be used to guide future precision medicine treatments of pancreatitis pain.

At most 1,357 patients with chronic and/or recurrent acute pancreatitis from the North American Pancreatitis Study II of European Ancestry were studied across all three aims. Aim 1 used a GWAS to identify that some genetic risk loci (n=15, p<1e-04) for constant-severe pain in pancreatitis were in genes (n=13, p-value of overlap 0.51) that have previously been identified as associated with unipolar depression from the GWAS Catalog (n=1380). For example, *CTNND2* and *BAIAP2* had loci associated with constant-severe pain. Similarly, using a candidate gene study Aim 2 found loci associated with constant, constant-severe, and severe pain located within genetic risk genes for anxiety and PTSD, such as *CTNND2*, *HTR2A*, *DRD3*, and *BDNF*. A literature review was used to compile the list of 28 anxiety/PTSD candidate genes used in Aim 2. Of those genes, 13 contained 24 lead SNPs (p<0.002) associated with pancreatitis pain. Aim 3 pulled the focus back to genome-wide associations and post-genome-wide association study methods, such as a transcriptome-wide association study (p<2.8e-06, suggestive p<1e-04) and colocalization, to identify associations with constant, constant-severe, and severe pain in patients with chronic and/or

recurrent acute pancreatitis in a hypothesis generating manor. This final aim found that differential expression of *CTRC* in pancreas tissue was associated with the constant pain phenotype. Additionally, differential expression of *HSF2* in skin was associated with the constant-severe phenotype. Finally, differential expression of *DOK6* in nerves was associated with the severe pain phenotype.

These results are new in the field, and necessary for future studies into precision treatments for patients with pancreatitis pain that could replace ineffective pain treatments and increase patients' physical and mental quality of life.

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List of Abbreviations

AA	African Ancestry
AP	Acute Pancreatitis
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel statistic
CP	Chronic Pancreatitis
CRF	Case report form
DM	Diabetes Mellitus
EA	European Ancestry
EPI	Exocrine Pancreatic Insufficiency
eQTL	expression quantitative trait loci
GAD	Generalized anxiety disorder
GWAS	Genome Wide Association Study
HPA	Hypothalamic-pituitary-adrenal
kb	Kilo Bases
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MCS	Mental Component Score
NAPS2	North American Pancreatitis II
NDRI	Norepinephrine-dopamine reuptake inhibitor
OR(s)	Odds Ratio(s)
PCS	Physical Component Score
PTSD	Posttraumatic Stress Disorder
QOL	Quality Of Life
RAP	Recurrent Acute Pancreatitis
SE	Standard Error
SD	Standard deviation
SF-12	Short Form 12
SNP(s)	Single Nucleotide Polymorphism(s)
AA	African Ancestry
ALFA	Allele Frequency Aggregator
AP	Acute Pancreatitis
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BP	Base Pair
Chr	Chromosome
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel statistic
CNS	Central Nervous System
CP	Chronic Pancreatitis
CRF	Case report form
DM	Diabetes Mellitus

DRG	Dorsal Root Ganglia
EA	European Ancestry
EPI	Exocrine Pancreatic Insufficiency
eQTL	Expression Quantitative Trait Loci
FDR	False Discovery Rate
GAD	Generalized anxiety disorder
GWAS	Genome-Wide Association Study(ies)
HPA	Hypothalamic-pituitary-adrenal
IEG	Immediate Early Genes
kb	Kilo Bases
LD	Linkage Disequilibrium
LSD1	Lysine Specific Demethylase 1
MAF	Minor Allele Frequency
MCS	Mental Component Score
MDD	Major Depressive Disorder
NAPS2	North American Pancreatitis II
NAPS2-AS	NAPS2-Ancillary Study
NAPS2-CV	NAPS2-Continuation and Validation Study
NDRI	Norepinephrine-dopamine reuptake inhibitor
NT3	Neurotrophin 3
NT4	Neurotrophin 4
OR(s)	Odds Ratio(s)
PCS	Physical Component Score
PheWAS	Phenome-Wide Association Study(ies)
PP	Posterior Probability
PRO	Patient Reported Outcome
PTSD	Posttraumatic Stress Disorder
QOL	Quality Of Life
QST	Quantitative Sensory Testing
RAP	Recurrent Acute Pancreatitis
Rbfox1	RNA Binding Fox-1 Homolog 1
SAPE	Sentinel Acute Pancreatitis Event
SD	Standard deviation
SE	Standard Error
SF-12	Short Form 12
SNP(s)	Single Nucleotide Polymorphism(s)
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
TPIAT	Total Pancreatectomy with Islet Autotransplantation
TWAS	Transcriptome-Wide Association Study(ies)

Preface

I would like to acknowledge my advisors for this project, Dr. David Whitcomb and Phil Greer. I would be lost in a pile of data without you. I also want to thank my committee members for their invaluable input and instruction. I also must acknowledge the emotional support provided by my parents, significant other, family, friends, and pets. There would be no dissertation without all your support.

In Memory of Sammy, my 15-year-old childhood cat. She was next to me for two theses, most of this dissertation, and all the online learning during Covid-19.

Figure 1-1 Sammy posing for extra credit in Honors Organic Chemistry at USM

1.0 Introduction

1.1 Pancreatitis

Pancreatitis is a fibro-inflammatory syndrome that usually begins with episodes of acute pancreatitis (AP) and recurrent acute pancreatitis (RAP). Continuation and progression of pancreatitis can result in chronic pancreatitis (CP) and irreversible destruction of the pancreas. Other complications of CP include abdominal pain, diabetes mellitus (DM), and exocrine pancreatic insufficiency (EPI) (Mullady et al., 2011; Whitcomb et al., 2016; Whitcomb et al., 2012). The etiology of pancreatitis is complex and variable, resulting in varying diagnostic criteria; criteria used for the North American Pancreatitis Study II (NAPS2) (see section **1.6 North American Pancreatitis Study II**) cohort will be described here (Whitcomb et al., 2016; Whitcomb et al., 2008).

Diagnostic criteria for acute pancreatitis includes having three or more times higher than normal pancreatic enzymes, abdominal pain, and, if available, imaging showing the absence of irreversible damage to the pancreas (Whitcomb et al., 2016; Whitcomb et al., 2008). Recurrent acute pancreatitis (RAP) is classified by the occurrence of two or more episodes of acute pancreatitis (AP) and no evidence from imaging of chronic pancreatitis (CP) (i.e., no irreversible damage could be detected) (Whitcomb et al., 2016; Whitcomb et al., 2008). Finally, CP is distinguished from AP and RAP using histology and/or imaging (CT scan or endoscopic retrograde cholangiopancreatography) to identify irreversible damage to the pancreas (Whitcomb et al., 2008). Some biological markers of the progression to CP include abnormalities in some or all of

the following: duct cells, acinar cells, islet cells, stellate cells, blood vessels, nerves or immune cells (Whitcomb et al., 2016).

One of the most important complications of pancreatitis affecting approximately 90% of CP patients is chronic abdominal pain (Mullady et al., 2011; Whitcomb et al., 2016). However, the experience of this pain is different for each patient—varying in severity (mild to severe) and temporal (intermittent or chronic) pattern. The variability of pain within CP prevents a "one size fits all" approach to pain treatment (Mullady et al., 2011). The level of fibrosis present in the pancreas cannot predict pain in pancreatitis and CP pain does not improve with increased destruction of the pancreas, suggesting that the pain experience is not associated with pancreatic inflammation alone (Mullady et al., 2011; Whitcomb et al., 2016). Additionally, one study using the NAPS2 cohort found no association with the "pain protective" haplotype of *GCH1* in pancreatitis patients (Lazarev et al., 2008). A full genome-wide association study (GWAS) of pancreatitis-associated pain has not yet been published (see Aim 3), but preliminary loci identified in the Whitcomb lab have been used as comparisons in other publications (see **Appendix A**).

Relevant to public health, CP patients with constant abdominal pain are four times more likely to chronically take pain medications and twice as likely to claim disability as CP patients without constant pain (Mullady et al., 2011). Pain in pancreatitis is generally managed with opioids or surgical removal of affected tissue; however, these treatments are not effective in all patients (Phillips, Faghih, Kuhlmann, et al., 2020). When these pain treatment regimens fail, feelings of isolation and hopelessness often arise in patients as evidenced by lower Quality of Life (QOL) in pancreatitis patients with constant pain (Cote et al., 2018; Mullady et al., 2011). Furthermore, chronic pancreatitis patients who experience anxiety and depression show higher levels of pain

(Phillips, Faghih, Drewes, et al., 2020). For an in-depth review of pancreatitis pain and mental health see Dunbar 2021 (see **Appendix A**) (E. K. Dunbar, Saloman, et al., 2021).

Pancreatitis is complex with multiple environmental and genetic risk factors resulting in the same clinical outcome (Whitcomb et al., 2012). Although rare, hereditary pancreatitis often results from gain-of-function mutations in the cationic trypsinogen gene (*PRSS1*) (Whitcomb et al., 1996; Whitcomb et al., 2012; Whitcomb et al., 2008). Lower production of trypsinogen appears to be protective against pancreatic injury (Whitcomb et al., 2012). The single nucleotide polymorphism (SNP) rs10273639 at the *PRSS1-PRSS2* locus shows a reduction of risk of pancreatitis associated with its minor allele (Whitcomb et al., 2012). *CFTR, SPINK1, CASR, CEL, CPA1, CTRC, TRPV6, UBR1* and *CLDN2* are also associated with pancreatitis (Whitcomb et al., 2012; Whitcomb et al., 2008; Zator & Whitcomb, 2017). *CLDN2* has an NFκB (a transcription factor involved in inflammation, memory, depression, and addiction) binding site in the promoter region, and expression of *CLDN2* is upregulated in porcine acinar cells under stress and in other human cells in response to stress or injury (Nennig & Schank, 2017; Whitcomb et al., 2012). Chymotrypsin C, CTRC, normally breaks down trypsinogen and trypsin preventing pancreatic injury (Rosendahl et al., 2008). Loss-of-function mutations in *CTRC* contribute to higher risk for CP (Zator & Whitcomb, 2017).

One of the most common environmental risk factors for pancreatitis is excessive alcohol intake, resulting in alcohol-related pancreatitis (Whitcomb et al., 2012). However, only 3% of patients who abuse alcohol develop pancreatitis. Alcohol use appears to increase effects of both the *PRSS1-PRSS2* and *CLDN2* loci in development of pancreatitis (Whitcomb et al., 2012). Patients reporting disability were more likely to have alcohol related pancreatitis. Additionally, those with alcohol related pancreatitis and constant pain were also more likely to also be current

smokers (Mullady et al., 2011). Fortunately, pain severity is not influenced by continuation of alcohol use (Mullady et al., 2011).

Recurrent acute pancreatitis and CP, which is a more advanced stage of RAP, are complex syndromes affecting multiple cell types and systems. Surprisingly, the complex nature of chronic pancreatic pain does not correlate with pancreatic fibrosis or pancreatic exocrine insufficiency (Wilcox et al., 2014; Zhan et al., 2020). This indicates that the pain experience may involve important variants in the pain processing and control mechanisms, in the stress response, in the psychology of pain, or a combination of extra-pancreatic factors.

1.2 Chronic Pain

Chronic pain (pain lasting more than three months) effects approximately 30% of the population of the world and is a leading cause of disability (Johnston et al., 2019; Meng et al., 2020; Zorina-Lichtenwalter et al., 2016). Pain perception is polygenic, with associations in loci in many genes (Zorina-Lichtenwalter et al., 2016). One genome-wide association study (GWAS) of multisite chronic pain using the UK Biobank found a SNP heritability estimate of 10.2% and 76 independent lead SNPs in 39 loci across 113 genes (Johnston et al., 2019). Although the exact biological mechanism of chronic pain is unknown, some pathways in common to pain include: GABAergic, catecholaminergic, cytokines, growth factors, serotonergic, estrogenic, glutamatergic, proteinases, neurogenesis, nervous-system development, and neural connectivity (Johnston et al., 2019; Tsepilov et al., 2020; Zorina-Lichtenwalter et al., 2016). Affective regions of the brain are involved in chronic pain perception, and structural and functional modifications of

the brain and spinal cord are involved in developing and maintaining chronic pain (Johnston et al., 2019).

1.3 Psychiatric Disorders

Mental health or psychiatric disorders are highly variable, but almost all include disruptions to normal perceptions, emotions, thoughts, behavior and relationships resulting from disruptions in higher cortical functions such as cognition, behavior, perception, and mood (Border et al., 2019; WHO, 2019). Due to the behavioral manifestation of these disorders, diagnosis relies on observation and self-report of behavior and cognition (Border et al., 2019).

Stress-related disorders are associated with the body's stress response system and include anxiety, depression, and post-traumatic stress disorder (PTSD) (Smoller, 2016). Pathways often enriched in psychiatric disorders include: calcium channel signaling, histone methylation, immune function, glial cell function, postsynaptic density, and glutamatergic neurotransmission (Smoller, 2016).

A combination of environmental and genetic risk factors contributes to the variation of psychiatric disorders in the population (Border et al., 2019; Smoller, 2016). However, a potential contributor to the variation is pain. Chronic pain often co-occurs and complicates psychiatric disorders (Bair et al., 2003; Johnston et al., 2019; Nelson & Cunningham, 2020). In the UK Biobank, multisite chronic pain is significantly genetically correlated with depression, depressive symptoms, anxiety, and PTSD (Johnston et al., 2019). Mendelian Randomization indicates that multisite chronic pain has a causal relationship with major depressive disorder (Johnston et al., 2019). Another study using the UK Biobank found that whole body ($r_g = 0.69$) and stomach or

abdominal pain (rg = 0.67) were highly genetically correlated with depression (Meng et al., 2020). This relationship between pain and psychiatric disorders leads us to question if psychiatric genetic risk is involved in pancreatic pain. Physician and patient education of the psychiatric component of pain may allow for mental health focused treatment of pain and reduction of opioid use in pancreatitis patients.

1.4 Public Health Significance

Currently, the variation seen in pancreatitis pain and the subsequent low QOL is not accounted for by physical state of the disease alone, suggesting that the variation has a genetic component (Wilcox et al., 2014; Zhan et al., 2020). However, to the best of my knowledge, the current literature is lacking studies that specifically study that genetic variation. There is a gap in the current knowledge of genetic variation in pancreatitis pain and QOL. However, there is an established connection between psychiatric disorders and chronic pain (see section **1.3 Psychiatric Disorders**). The work compiled here aims to identify if that same connection is present in pancreatitis pain by determining if psychiatric risk loci contribute to the variation of the pain experience and QOL and describing the remaining loci associated with pancreatitis pain. These aims, and subsequent papers, are the first steps in filling the gap in the literature. Knowing if psychiatric genetic risk impacts the outcome of pancreatitis in specific patients, will allow clinicians to incorporate treatment for the psychiatric disorder into each patients' disease management plan. This is especially important and of public health importance since traditional pain management strategies (including opioids) often fail to treat pancreatitis pain (Mullady et al., 2011; Phillips, Faghih, Kuhlmann, et al., 2020).

1.5 Hypothesis and Specific Aims

1.5.1 Hypothesis

Psychiatric disorder loci (depression, anxiety, PTSD) and pancreatitis pain-associated risk loci overlap and are associated with worse quality of life in pancreatitis patients ascertained as part of the North American Pancreatitis Study II (NAPS2) studies (see section **1.6 North American Pancreatitis Study II**) (Conwell et al., 2017; Machicado et al., 2017; Mullady et al., 2011; Whitcomb et al., 2008; Wilcox et al., 2016; Yadav et al., 2009).

1.5.2 AIM 1: To identify loci within depression risk genes that are associated with constant-severe pain within pancreatitis patients

The purpose of Aim 1 is to test the hypothesis that depression risk genes contribute to the variation of the pain experience seen in pancreatitis patients. A genome-wide association study (GWAS) nested within 1,357 RAP+CP pancreatitis patients with and without constant-severe pain was used to identify the overlap between previously reported depression risk genes and risk for pancreatitis pain. Results from this Aim may be useful in future studies identifying individuals who are at a greater risk for higher pain and greater risk for depression which will allow for tailored treatments focusing on mental health.

1.5.3 AIM 2: To identify loci within anxiety and PTSD risk genes that are associated with the different pain categories within pancreatitis patients

The purpose of Aim 2 is to test the hypothesis that psychiatric risk genes contribute to the variation of the pain experience seen in pancreatitis patients. A candidate gene study focusing on previously reported psychiatric risk genes within 818-1,277 pancreatitis patients with differing levels of pain was used to identify overlap between psychiatric genetic risk and risk for pain. Psychiatric disorders with an established association with pain (such as anxiety, and posttraumatic stress disorder [PTSD]) were the primary focus. Sample sizes of pancreatitis patients varied from 818 to 1,277 patients depending on diagnosis (recurrent acute, chronic, or both). Results from this Aim may be useful in future studies identifying individuals who are at a greater risk for higher pain and greater risk for psychiatric disorders which will allow for tailored treatments focusing on mental health.

1.5.4 AIM 3: To identify top loci associated with the different pain categories in pancreatitis patients

The purpose of Aim 3 is to identify and describe the top loci contributing to the variation seen in pancreatitis pain. Aims 1 and 2 tested the hypotheses that psychiatric genetic risk influence pancreatitis pain; Aim 3 is a hypothesis free discovery of loci associated with pancreatitis pain. GWAS methods were used to identify associated loci in patients with constant, constant-severe, or severe pain within 1,254 RAP+CP patients, and post-GWAS analysis was used to further describe those results. Results from this Aim may be useful in generating hypotheses for future

studies identifying pancreatitis patients at risk for a worse pain experience and for future studies understanding the mechanism of pancreatitis pain.

1.6 North American Pancreatitis Study II

Pancreatitis patients and controls were ascertained from the North American Pancreatitis Study II (NAPS2) studies (Conwell et al., 2017; Machicado et al., 2017; Mullady et al., 2011; Whitcomb et al., 2008; Wilcox et al., 2016; Yadav et al., 2009). NAPS2 was launched in 1999 to study known risk factors (alcoholism and smoking) to chronic pancreatitis (CP) and to discover new genetic risk factors (Conwell et al., 2017; Whitcomb et al., 2008; Wilcox et al., 2016). The goal of the study was to prospectively collect 1,000 patients with recurrent acute pancreatitis (RAP) and CP and spouse-friend controls with detailed demographic information and family history to identify pancreatic disease-associated risk factors and disease progression and information on secondary complications including pain, exocrine pancreatic insufficiency (EPI), diabetes (before or after acute pancreatitis (AP)), physical and mental quality of life (QOL), etc. (Whitcomb et al., 2008). Another goal of the NAPS2 study was to test the hypothesis that a Sentinel Acute Pancreatitis Event (SAPE) is required before CP can develop (Stevens et al., 2004; Whitcomb, 1999).

At time of ascertainment, pain was recorded by asking patients with pain to characterize their pain as one of the following: *"A) Episodes of mild to moderate pain, usually controlled by medication, B) Constant mild to moderate pain usually controlled by medication, C) Usually pain free with episodes of severe pain, D) Constant mild pain plus episodes of severe pain, E) Constant severe pain that does not change"* (Mullady et al., 2011). Duration of disease is not associated

with pain pattern (Mullady et al., 2011). Physical and mental QOL was measured using the Short Form-12 (SF-12) health survey. Responses to the SF-12 were used to calculate a physical component score (PCS) and mental component score (MCS) to measure physical and mental QOL respectively (Mullady et al., 2011). Biomarkers were collected from the medical records to determine disease features and stages (e.g., pancreas imaging, secondary diagnoses, special tests, etc.). Blood was collected to measure serum biomarkers and DNA for genetic variants (Whitcomb et al., 2008). Information on psychiatric disorders was not collected.

NAPS2 had three phases, the original NAPS2 cohort (2000-2006) for the ascertainment of 1,000 RAP/CP patients, the NAPS2-continuation and validation study (NAPS2-CV) (2008-2012) to ascertain an additional 500 CP patients for genome-wide association studies (GWAS) studies, and NAPS2-ancillary study (NAPS2-AS) for the ascertainment of 250 CP patients and 250 controls of African Ancestry (AA) (Conwell et al., 2017; Whitcomb et al., 2008; Wilcox et al., 2016). The NAPS2 cohort included 460 RAP patients, 540 CP patients, and 695 controls for a total of 1,695 individuals (Whitcomb et al., 2008). The NAPS2-CV enrolled 521 CP patients (Conwell et al., 2017). Finally, the NAPS2-AS enrolled 248 AA individuals with CP (Wilcox et al., 2016).

Genotyping of NAPS2 data was done on the Illumina HumanOmniExpress BeadChip and Human Core Exome Chip (Whitcomb et al., 2012). The McCarthy Group pre-imputation checking tools were used to prepare genotype data for imputation, which was then imputed against the 1,000 genomes phase-3 reference panel on the Sanger imputation server using the EAGLE2+PBWT pipeline for pre-phasing and imputation (E. Dunbar et al., 2020; Durbin, 2014; Loh et al., 2016; McCarthy et al., 2016).

1.6.1 Phenotypes

As described in the prior section, patients in the NAPS2 characterized their pain as one of 5 severity-frequency options. These options were further grouped into three pain categories: constant, severe, and constant-severe pain. Patients with types B, D, or E were considered as having constant pain. Patients with types C, D, or E were considered as having severe pain, and patients with types D or E were considered as having constant-severe pain. At the time of ascertainment it was recorded if the individual had ever drank, was currently drinking, or had stopped drinking (Whitcomb et al., 2008). Similarly, smoking status (never, ever or current) was also recorded at time of ascertainment. Mental QOL was reported as the MCS, which was calculated using the SF-12. The MCS is a standardized score, with 50 taken to be average and anything lower considered as poor QOL (Whitcomb et al., 2008). The MCS is used as an indicator of poor mental health. Other variables considered were age at ascertainment (years), EPI (yes/no), diabetes (yes/no), body mass index (BMI), and sex (male/female).

Patients and physicians recorded in free text if the patient was taking antidepressants (not for pain) in the case report forms. I used text-mining procedures in R to identify those patients, and generated a binary variable for antidepressant use (yes/no) that was used as proxy for depression in Aim 1 (R Core Team, 2019). Another proxy for depression used in Aim 1 was the "Felt Blue" variable (see Aim 1). This variable was based on an SF-12 question asking participants if they had "felt downhearted and blue?" in the month prior to ascertainment. The question was rated on a Likert scale of 1 "All of the time" to 6 "None of the time" (Mullady et al., 2011). The "Felt Blue" variable was binary with responses 1-3 being "Yes" and responses 4-6 being "No".

1.6.2 Study Sample

The total study sample used to study the genetic variation of pancreatitis pain was pulled from the NAPS2 and NAPS2-CV. There were 2,574 pancreatitis patients and non-pancreatitis controls available for this analysis. However, the main focus of this study is to identify genetic variation within pancreatitis patients (a nested study). The total sample size of pancreatitis cases only is 1,692. The sample size will vary across aims based on which patients have complete genotypic and phenotypic information available. **Table 1-1** contains demographic information for the entire study sample.

Table 1-1 Demographics of Study Sample

	RAP	CP
Total	568	1124
Males	260	595
Females	308	518
Age at Ascertainment (mean yrs ±SD)	45.3±15.6	50.7±15.3
Average MCS (mean±SD)	44.6±11.4	43.1±12.1
BMI at Ascertainment (mean±SD)	27.1±6.7	24.8±5.6
Diabetes (count yes)	78	336
EPI (count yes)	48	406
Alcohol Etiology	117	377

2.0 Aim 1: Constant-Severe Pain in Chronic Pancreatitis is Associated with Genetic Loci for Major Depression in the NAPS2 Cohort

 Journal of Gastroenterology. Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort, Dunbar, E., P. J. Greer, N. Melhem, S. Alkaade, S. T. Amann, R. Brand, G. A. Coté, C. E. Forsmark, T. B. Gardner, A. Gelrud, N. M. Guda, J. LaRusch, M. D. Lewis, J. D. Machicado, T. Muniraj, G. I. Papachristou, J. Romagnuolo, B. S. Sandhu, S. Sherman, C. M. Wilcox, V. K. Singh, D. Yadav and D. C. Whitcomb, 2020 Oct; 55(10):1000-1009. doi: 10.1007/s00535-020-01703-w. Epub 2020 Jul 17. PMID: 32681239; PMCID: PMC9124361.

The original article and supporting information are available online at https://doi.org/10.1007/s00535-020-01703-w.

I contributed to the conceptualization methodology, formal analysis and investigation, and writing of this manuscript (see Author contributions). See **2.1.1 Corrected Table 1** for correction.

See **Appendix B** for full formatted paper.

2.1 Additional Calculations

The following are additional calculations conducted after the publication of Aim 1.

2.1.1 Corrected Table 1

During the course of this dissertation, a typo was discovered which requires the correction of Error! Reference source not found. of the Aim 1 paper. **Table 2-1** below is the corrected table.

Table 2-1 Aim 1 Corrected Table 1

Variable	Level	Not Constant-Severe Pain (n=864)[a]	Constant-Severe Pain (n=493)[b]	Total (n=1357)	p-value[c]
Age	Mean (SD)	50.5 (17)	45.5 (13.6)	48.7 (16)	2.1e-8
Sex	Male	454 (52.5%)[d]	234 (47.5%)	688 (50.7%)	0.08
	Female	410 (47.5%)	259 (52.5%)	669 (49.3%)	
Alcohol	Never	184 (21.4%)	105 (21.3%)	289 (21.4%)	1
	Ever	674 (78.6%)	388 (78.7%)	1062 (78.6%)	
	Missing	6	0	6	
Smoking	Never	328 (38.2%)	129 (26.2%)	457 (33.9%)	9.5e-6
	Ever	530 (61.8%)	363 (73.8%)	893 (66.1%)	
	Missing	6	1	7	
Antidepressant Use	No	439 (77.0%)	158 (65.8%)	597 (73.7%)	1.3e-3
	Yes	131 (23.0%)	82 (34.2%)	213 (26.3%)	
	Missing	294	253	547	
Felt Blue	No	419 (84.8%)	174 (72.5%)	593 (80.8%)	1.1e-4
	Yes	75 (15.2%)	66 (27.5%)	141 (19.2%)	
	Missing	370	253	623	
EPI[e]	No	673 (77.9%)	334 (67.7%)	1007 (74.2%)	5.3e-5
	Yes	191 (22.1%)	159 (32.3%)	350 (25.8%)	
Diabetes	No	667 (77.2%)	364 (73.8%)	1031 (76.0%)	0.2
	Yes	197 (22.8%)	129 (26.2%)	326 (24.0%)	
Mental QOL[f]	Mean (SD)	46.7 (10.9)	39 (11.8)	43.7 (11.9)	2.8e-31
	Missing	109	22	131	

[a]Patients without pain
[b]Patients with pain
[c]Pearson chi-squared for categorical; t test for continuous; two-tailed $p < 0.05$ considered significant
[d]Percentages shown next to counts are column percentages within each variable
[e]Exocrine Pancreatic Insufficiency
[f]Quality of Life

2.1.2 Multiple Testing Considerations

In this aim, I used 1,357 RAP and CP patients from the NAPS2 and NAPS2-CV, of which 493 were cases (constant-severe pain) and 864 were controls (not constant-severe pain). As 9,251,575 SNPs were tested the Bonferroni corrected p-value adjusted for multiple testing burden was $\alpha=(0.05/9{,}251{,}575)=5.4\text{x}10^{-9}$. The alpha value used in the paper was $1\text{x}10^{-04}$ as a higher false positive rate with fewer false negatives was determined to be acceptable. At this alpha, we expected 925 false positives when testing all 9 million SNPs under the null hypothesis. We saw

773 SNPs meeting the alpha of $1x10^{-4}$, of these 219 were LD independent as identified using Plink's "clump" command (Purcell et al., 2007).

In **Table 2-2 Corrected p-values for Aim 1 Top SNPs** the corrected p-values for the 15 independent lead SNPs which fell within depression genes are reported. Adjusted p-values were calculated using the "p.adjust" command in R version 4.2.1 with the number of tests being 9,251,575 (R Core Team, 2022). None of the top SNPs corrected p-values (BH and BY) meet a false discovery rate (FDR) of < 0.05; in fact the FDR is 1 for each SNP. A FDR of 1 suggests that all the results are likely to be false positives and no true positives were detected, and no statistically meaningful conclusions can be drawn from these results. A replication study in a larger sample is needed to confirm the results of this aim, as this study is likely under powered to correctly identify true positives from false positives (see **2.1.3Power**).

Table 2-2 Corrected p-values for Aim 1 Top SNPs

SNP	BONFERRONI	BH[1]	BY[2]	NONE
rs141909432	1	1	1	4.77e-05
rs2968817	1	1	1	7.59e-05
rs113388258	1	1	1	2.35e-05
rs4624600	1	1	1	5.53e-05
rs59442633	1	1	1	2.88e-05
rs458909	1	1	1	9.41e-05
rs11300774	1	1	1	6.13e-05
rs2123323	1	1	1	7.01e-05
rs36106152	1	1	1	7.86e-05
rs71450224	1	1	1	5.06e-05
rs12449867	1	1	1	1.97e-05
rs9898347	1	1	1	9.61e-05
rs34176221	1	1	1	5.93e-05
rs1619323	1	1	1	4.22e-05
rs8137390	1	1	1	4.34e-05

[1]Benjamini & Hochberg (1995)

[2]Benjamini & Yekutieli (2001)

2.1.3 Power

The power of the genetic association analysis at alpha $1x10^{-4}$ to detect an effect (additive genotype relative risk of heterozygotes [RRAa]) across frequencies of the minor allele (MAF in figure) in this aim is represented in the heatmap in **Figure 2-1**. Power was calculated using the "GeneticsDesign" package in R (Duffy et al., 2018). Prevalence of the disease was estimated to be 0.33 since 1 in 3 CP patients have severe pain and a well-studied phenotype-specific prevalence had not yet been reported (Amann et al., 2013; Balliet et al., 2012; Cote et al., 2018; Machicado et al., 2017; Mullady et al., 2011). The heatmap was generated using the "pheatmap" package in R (Kolde, 2019). According to the heatmap, this aim is best powered to detect effects greater than 1.4 in MAF 0.16 to 0.71 as seen in **Figure 2-1**. However, many complex diseases have OR's between 1.08 to 1.16 at similar MAFs (Park et al., 2011) and this aim is underpowered (<0.2) to detect true effects of those sizes and larger effect sizes are not expected under the complex disease model. Replication in a larger sample size is needed to detect true positive SNPs with small effect sizes.

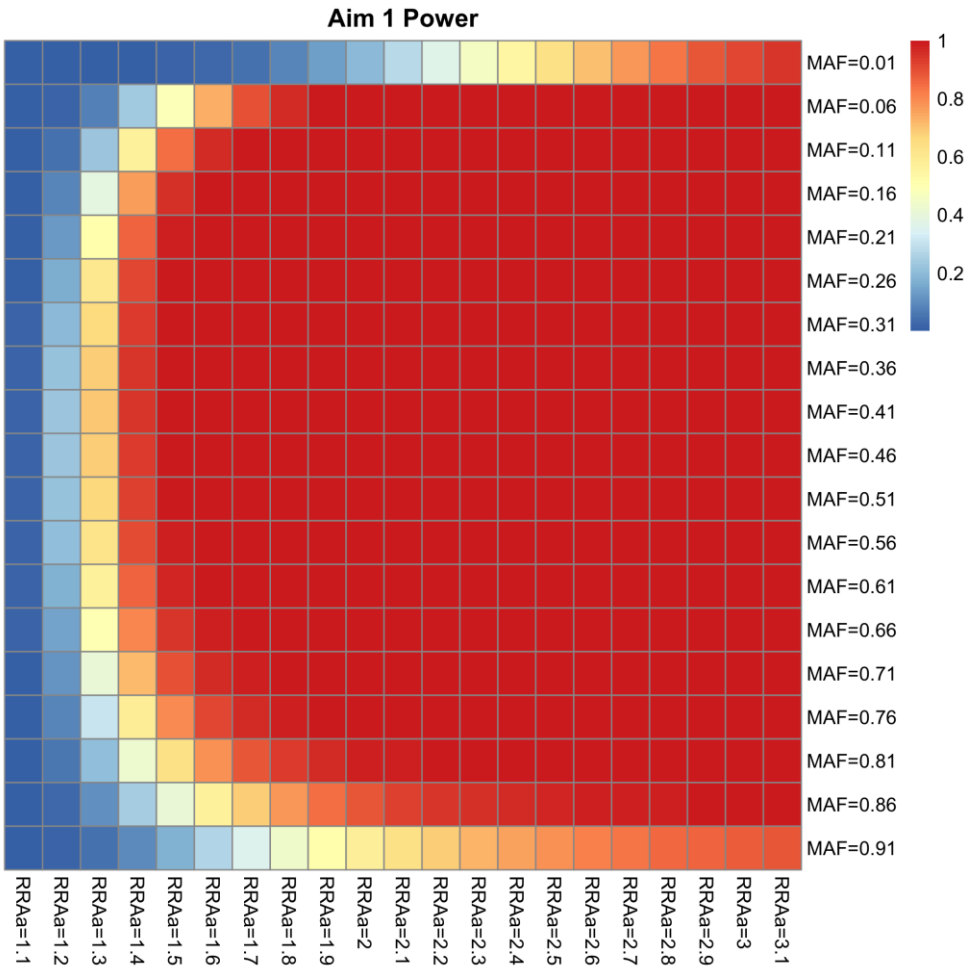

Figure 2-1 Power Heatmap for Aim 1 (RAP+CP, Constant-Severe Pain)

α=1x10^{-4}, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=493, Controls=864.

2.1.4 Permutation

As mentioned in the **limitations** of the published paper in Aim 1 and in the **2.1.3Power** section above, the study is under powered. For Aim 1 the p-value threshold for moving on in the analysis was set to 1x10^{-04}. The significance of the overlap of depression and pancreatitis pain risk was later addressed by permuting the phenotype using the "make-perm-pheno" Plink 1.9 command

and using those randomly permuted phenotypes for the same GWAS, clumping, and checking for overlap of depression genes pipeline used originally (Purcell et al., 2007). The empirical p-value calculated here is testing the significance of the overlap of depression genes (N = 1,380) with pancreatitis pain genes from the GWAS (N = 182) under the null hypothesis of no association of genotypes with pain experience phenotypes. There were N = 10,000 permutations used, with R = 5,086 resulting in an overlap greater than or equal to the original (n = 13). The distribution of overlaps from which the empirical p-value is calculated is represented in **Figure 2-2 Permutation Distribution**. The resultant empirical p-value (R+1/N+1) was 0.51, at an α of 0.05 we failed to reject the null hypothesis. This empirical p-value suggests that the overlap of depression genes with constant-severe pain loci may be due to random chance alone. Put another way, an overlap of 13 or more depression genes with loci associated with a randomized version of the constant-severe pain phenotype was seen 5,086 times out of 10,000. This means our original overlap of depression genes with constant-severe pain associated loci was likely due to random chance. A non-random overlap would have fallen in the ends of the tails of the distribution shown in **Figure 2-2 Permutation Distribution** (approximately 20 and greater for right-sided one-tailed test).

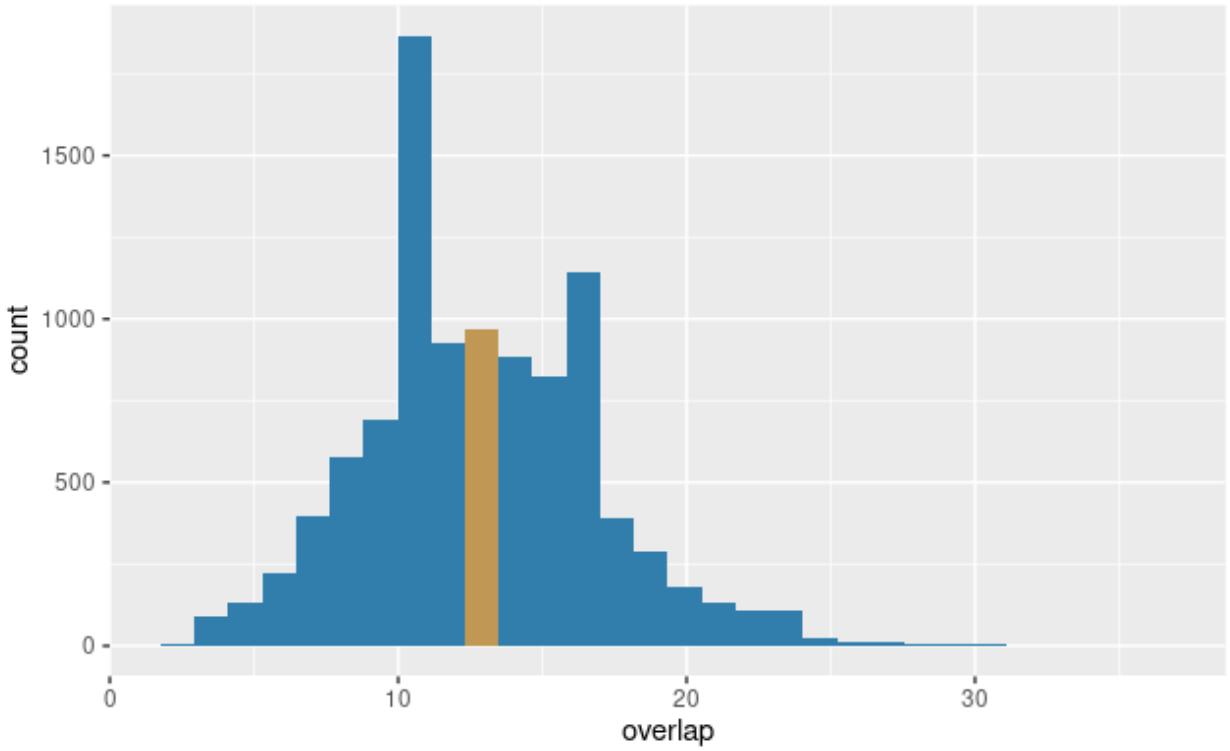

Figure 2-2 Permutation Distribution

Yellow bar corresponds with original overlap (n = 13).

Originally, the list of genes from the GWAS Catalog for unipolar depression was assembled by first downloading the Catalog data file in October of 2019 (Buniello et al., 2019). All 1,380 unique gene names mapped to a SNP associated with unipolar depression were used with no p-value filters with the max p-value being 9e-06. As of July 2022, there are 1,749 total unique gene names mapped to a SNP associated with unipolar depression. After applying a p-value filter of ≤ 5e-08, 651 unique gene names remained. Of the 13 genes reported in Error! Reference source not found., 7 genes were in the filtered list: *DCC*, *BAIAP2*, *CNTN5*, *ROBO2*, *NBAS*, *SGCZ*, and *KSR2*. Using a curated list of genes helps to remove noise from the depression list of genes, increasing confidence that the gene is associated with depression and reduces the chance of an overlap with pancreatitis pain risk by random chance alone.

2.1.5 Clarification

The conclusion drawn from the Cochran-Mantel-Haenszel (CMH) analysis in Aim 1 requires the following clarification: Stratifying patients by antidepressant use using CMH slightly changes the odds ratios (ORs) but does not remove the associations of the loci with the pain phenotype suggesting antidepressant use is not a significant confounder of the pain and genotype relationship.

3.0 Aim 2: Pain Experience in Pancreatitis: Strong Association of Genetic Risk Loci for Anxiety and PTSD in Patients With Severe, Constant, and Constant-Severe Pain

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Title: Pain Experience in Pancreatitis: strong association of genetic risk loci for anxiety and PTSD in patients with severe, constant and constant-severe pain.

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Study Highlights:

WHAT IS KNOWN

- Pancreatitis pain is variable and can be severe, leading to a poor quality of life in some patients
- Current pain treatment strategies are often suboptimal or ineffective
- Depression risk loci overlap pancreatitis pain loci

WHAT IS NEW HERE

- Pancreatitis genetic loci associated with severe pain overlap with generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD) risk loci
- GAD and PTSD are pre-existing risk and are not necessarily only a response to chronic pain.
- Patients who experience constant and severe pancreatic pain may have several overlapping conditions that should be addressed individually as part of a complex disorder

3.1 Abstract

Background: Recurrent acute pancreatitis (RAP) and chronic pancreatitis (CP) are progressive inflammatory syndromes with variable features. Pain is the primary feature that contributes to low physical and mental quality of life with a third of patients reporting severe pain. Pain experience is worsened by depression. Here we tested the hypothesis that genetic risk for the psychiatric conditions of anxiety and post-traumatic stress disorder (PTSD) are associated with pain in CP and RAP+CP subjects.

Methods: The study cohort included phenotyped and genotyped RAP and CP patients from the North American Pancreatitis Study II (NAPS2) of European Ancestry. Candidate genetic association studies were based on the absence of pain versus pain that is constant, constant-severe, or severe. Twenty-eight candidate genetic loci for anxiety and PTSD risk were identified in the literature and were the focus of this study.

Results: We identified 24 significant pain-associated SNPs within 13 loci across the 3 pain patterns in CP and RAP+CP ($p<0.002$). Thirteen anxiety or PTSD genes were within these pain loci indicating non-random associations ($p<4.885x10^{-23}$). *CTNND2* was associated with all pain categories and all pancreatitis etiologies. Implicated systems include Neuronal Signaling *(HTR2A, DRD3, NPY, BDNF)*, Hypothalamic-Pituitary-Adrenal Axis *(NR3C1, FKBP5*) and cell-cell interaction *(CTNND2, THBS2).*

Conclusion: A component of constant and severe pain in patients with RAP and CP is associated with genetic predisposition to anxiety and PTSD. Identification of patients at risk eligible for trials of targeted treatment as a component of a multidisciplinary pain management strategy should be formally evaluated.

Keywords: pain, pancreatitis, anxiety, PTSD, genetics

Abbreviations

AP	Acute Pancreatitis
BMI	Body Mass Index
BP	Base Pair
Chr	Chromosome
CI	Confidence Intervals
CP	Chronic Pancreatitis
EA	European Ancestry
EPI	Exocrine Pancreatic Insufficiency
eQTL	Expression Quantitative Trait Loci
GAD	Generalized Anxiety Disorder
HPA	Hypothalamic-Pituitary-Adrenal
kb	Kilobases
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MCS	Mental Component Summary
NAPS2	North American Pancreatitis Study II
OR	Odds Ratio
PTSD	Posttraumatic Stress Disorder
QOL	Quality Of Life
RAP	Recurrent Acute Pancreatitis
RAP+CP	Variable: RAP and CP Pancreatitis Patients
SD	Standard Deviation
SE	Standard Error
SF-12	Short Form 12
SNP	Single Nucleotide Polymorphism
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor

3.2 Introduction

Pancreatitis is an inflammatory syndrome that can become chronic resulting in irreversible destruction of the pancreas with variable levels of fibrosis, diabetes mellitus, exocrine pancreatic insufficiency (EPI), and abdominal pain (Mullady et al., 2011; Whitcomb et al., 2016; Whitcomb et al., 2012). The complex etiology of acute pancreatitis (AP), recurrent AP (RAP) and chronic pancreatitis (CP) is associated with metabolic and toxic factors such as smoking, alcohol use, hypertriglyceridemia, hypercalcemia, obstructive etiologies, and genetic factors such as variants in or near *CASR, CEL, CFTR, CLDN2, CPA1, CTRC, PRSS1, SPINK1, TRPV6,* and *UBR1* among other genes (Masamune et al., 2020; Whitcomb & North American Pancreatitis Study, 2019; Zator & Whitcomb, 2017). Additional environmental factors and genetic variants also increase patients' risk for secondary complications such as diabetes (Bellin et al., 2017; Goodarzi et al., 2019; Rickels et al., 2013) and pancreatic cancer (F. Chen et al., 2019; Klein et al., 2018; Shelton et al., 2020; Stoffel et al., 2019; Whitcomb et al., 2015). AP and RAP typically occur before progressing to CP (Mullady et al., 2011).

Severe, constant pain, a symptom seen in 1 in 3 CP patients, is the major driver of low quality of life (QOL) in these patients (Amann et al., 2013; Balliet et al., 2012; Cote et al., 2018; Machicado et al., 2017; Mullady et al., 2011). However, even at the early stages of pancreatitis, pain negatively impacts physical and mental health and QOL (Amann et al., 2013; Cote et al., 2018; Machicado et al., 2017). Thus, the detriment in mental QOL in CP is not fully explained by pain alone and may be related, in part, to psychological determinants. Similarly, the reason for the variability of the pain experience by pancreatitis patients is unknown, but it may be influenced by a genetic predisposition to psychiatric disorders, given that psychiatric disorders and pain disorders

often co-occur (Niculescu et al., 2019). In fact, depression and anxiety are common in CP patients (Balliet et al., 2012; Phillips, Faghih, Drewes, et al., 2020).

Both children and adults with chronic abdominal pain commonly report comorbid psychological distress and trauma (Nelson & Cunningham, 2020). It is plausible that pain associated with a pancreatitis attack could be a sufficient stressor to induce psychopathology in genetically at risk patients (Balliet et al., 2012). Existing mental disorders could worsen and be worsened by the pain of the pancreatitis attack in a vicious cycle (Gillman et al., 2020; Niculescu et al., 2019; Smoller, 2016). We have previously identified depression risk genes in pancreatitis patients with constant-severe pain; therefore, the focus of this investigation was on anxiety and post-traumatic stress disorder (PTSD) (E. Dunbar et al., 2020; Smoller, 2016).

The effectiveness of management for pain and poor QOL in patients with pancreatitis is often poor (Anderson et al., 2016; Drewes et al., 2017; Kleeff et al., 2017). Recognition of the role of psychiatric risk in the pain experience may help develop more effective pain management for pancreatitis patients. To test the hypothesis that pain is associated with genetic risk loci for anxiety and PTSD, we investigated patients in the deeply phenotyped and genotyped North American Pancreatitis Study II (NAPS2) cohorts.

3.3 Methods

3.3.1 NAPS2

The NAPS2 cohort represents three sequential, cross-sectional, case-control studies of RAP and CP as previously described (Conwell et al., 2017; Whitcomb et al., 2008; Wilcox et al.,

2016). Standardized questionnaires were used for data collection and single nucleotide polymorphism (SNP) arrays (Illumina HumanOmniExpress BeadChip and HumanCoreExome) were used for genotyping (Whitcomb et al., 2012), with supplemental, targeted genotyping as previously described (E. Dunbar et al., 2020; Phillips et al., 2018). The subset of patients used for this analysis from the NAPS2 cohort was CP (N=818) and RAP+CP (N=1,277) subjects of European ancestry (EA). To reduce heterogeneity, the small sample of NAPS2[1] patients not of EA were excluded.

3.3.2 Pain Categories and Quality of Life

Patterns of pancreatitis pain were defined following Mullady's 6-category severity-frequency classification system with O = no pain; A = episodes of mild pain; B = constant mild to moderate pain; C = episodes of severe pain; D = constant mild and episodes of severe pain; and E = constant-severe pain during the year prior to recruitment (Mullady et al., 2011). Subjects responding with B, D or E were classified as *constant pain*, subjects responding with C, D and E were classified as *severe pain*, and subjects with D and E were *constant-severe pain*.

Anxiety and PTSD were not directly measured in the patient questionnaires; however, a mental component summary (MCS) score was calculated using responses from the Short Form 12 (SF-12) (Amann et al., 2013). The MCS is as a measure of mental QOL, with higher scores correlating with better QOL and a score of 50 representing average health status (Amann et al., 2013; Mullady et al., 2011). The MCS has previously been used as an indicator of mental health

[1] 248 African American Individuals.

and measure of depressive disorders (E. Dunbar et al., 2020; Gill et al., 2007; Vilagut et al., 2013). Thus, we used a lower than average MCS as a proxy indicator of poor mental health as had been done previously for depression (E. Dunbar et al., 2020).

Demographic and phenotypic data for patients in each pain category was compiled and analyzed using R version 3.6.2 (R Core Team, 2019). Univariate comparisons were performed based on demographic variables using Pearson's chi-squared test for categorical data and the *t* test for continuous data. Two-tailed p-values < 0.05 were considered statistically significant (***Tables 1-6***) (R Core Team, 2019).

3.3.3 Variables

Two subsets of patients were tested independently, one group labeled RAP+CP, included both RAP patients and CP patients, and the other comprised of only patients with chronic pancreatitis (CP). All patients were classified as "case" or "control" based on the presence or absence of specific pain endophenotypes. A total of six studies were conducted looking at each of the three pain categories described above within both categories of pancreatitis patients. Both categories were used to compensate for a possible power reduction from assuming similarities of RAP and CP, even though RAP is a part of the CP pathogenesis and to increase sample sizes (Mullady et al., 2011; Whitcomb et al., 2012). A sample of only RAP patients (N=453) from NAPS2, and used in the RAP+CP group, was used to replicate major gene associations (See Tables S1 and S2, which reports results from replication analysis).

3.3.4 Candidate Genes

A literature search was conducted in the summer of 2020 to compile a non-comprehensive list of candidate, autosomal risk genes for anxiety and PTSD (See Table S3, for a list of candidate genes). These are genes implicated in or suggested as being associated with anxiety and/or PTSD, and genes also associated with depression or antidepressant response are labeled in Table S3 (See Table S3, for a list of candidate genes). As a supplemental, the same candidate gene approach was repeated using a list of genes reported for anxiety and PTSD in the GWAS Catalog (See Tables S4 and S5, which reports gene candidate results using GWAS Catalog) (Buniello et al., 2019).

3.3.5 Genetic Data Analysis

The genetic analysis was constructed as a candidate gene review using data from pancreatitis subjects similar to what was done previously with depression (E. Dunbar et al., 2020). This candidate gene review was conducted using PLINK 1.9 software (Purcell et al., 2007). Quality control methods for SNP data have been previously reported (E. Dunbar et al., 2020; Whitcomb et al., 2012). Data was fit to a logistic regression to test for associations. The analysis was restricted to the list of candidate genes with a border of 50 kilobases (kb) added to each gene in PLINK 1.9. Since 28 gene regions instead of the whole genome was tested, the level of significance was relaxed to $p<0.002$ (Dunn, 1961; Neyman & Pearson, 1928). To control for ancestry, the first four principal components of ancestry were included as covariates. Additional

covariates were age, sex, body mass index (BMI), and a variable to control for differences across SNP chips. The minor allele frequency (MAF) threshold was set to 0.01[2].

SNPs meeting the required significance threshold were then combined into groups (likely haplotypes) based on linkage disequilibrium (LD) (± 250 kb from index SNP, $r^2 > 0.5$) in PLINK 1.9 (Purcell et al., 2007). The lead SNPs ($p \leq 0.002$) were annotated with genes within the borders of these LD regions[3] based on genome build GRCh37/hg19.

The MAF for the lead SNPs was calculated using PLINK 1.9 (***Table 7***) (Purcell et al., 2007). Finally, GTEx (https://gtexportal.org/home/) was queried to determine if any of the lead SNPs were also expression quantitative trait loci (eQTLs) (See Table S6, which reports eQTLs) (Lonsdale et al., 2013).

We used an online exact hypergeometric probability calculator to test the probability that the Anxiety/PTSD gene loci were associated with pancreatitis pain loci by chance alone (Lund, 2005).

[2] Minor Allele counts for cases and controls > 600.

[3] Plink 1.9 "clump-range" and the original list of candidate genes was used to physically paste the known candidate gene names to SNPs.

3.4 Results

3.4.1 Patient Characteristics (Tables 1-6)

All six tested categories of disease status and pain pattern show that higher pain levels are all significantly associated with lower average age ($p<1x10^{-5}$). Additionally, higher pain levels are all significantly associated with lower mental QOL scores ($p<1x10^{-5}$). Individually, constant pain is associated with smoking ($p=0.0027$) and EPI ($p=0.0009$) in CP patients, and with sex ($p=0.047$), smoking ($p=6.13x10^{-5}$), EPI ($p<1x10^{-5}$), and diabetes ($p=0.03$) in RAP+CP patients. Constant-severe pain is associated with smoking ($p=0.0018$) and EPI ($p=0.0085$) in CP, and sex ($p=0.028$), smoking ($p=0.0002$), and EPI ($p=2.24x10^{-5}$) in RAP+CP patients. Finally, severe pain in CP is associated only with younger age ($p<1x10^{-5}$) and MCS ($p<1x10^{-5}$), while severe pain in RAP+CP patients is associated with smoking ($p=0.0065$) and EPI ($p=0.022$).

3.4.2 Candidate Anxiety/PTSD Genes Associated with Pain in CP/RAP+CP

Candidate gene studies[4] were conducted within CP and RAP+CP patients across the three pain phenotypes. Resultant odds ratios (OR), 95% confidence intervals (CI), standard error (SE), and p-values for the 24 unique lead SNPs representing 13 loci across the 6 tested categories are reported in ***Table 7***. The biological function of these known Anxiety/PTSD gene products and associated systems is described below.

[4] 28 Candidate genes. CP 17,764 SNPs. RAP+CP 17,747 SNPs.

CTNND2 was the anxiety and/or PTSD candidate gene most commonly associated with the various pain categories and was previously associated with depression (E. Dunbar et al., 2020). Additionally, several genes have multiple loci with different effects. The OR's associated with specific SNPs[5] within different loci suggest that some are protective (OR <1) and others risk (OR >1) for worse pain experience in pancreatitis, suggesting complex gene expression regulatory mechanism. Pain and Anxiety/PTSD risk SNPs in *DRD3* are associated with constant pain in the CP category, but we also identified a SNP that was protective for severe pain in the RAP+CP category.

The probability that these loci for psychiatric disorder genes overlapped with loci for severe pancreatic pain was tested. The probability that the loci were shared by chance alone was very low ($p<4.885x10^{-23}$), indicating a statistically significant association.

Of the 24 lead SNPs, 6 have reported eQTLs from GTEx (Lonsdale et al., 2013) (***Table 7***, See Table S6, which reports eQTLs). The fact that these SNPs are seen in a variety of tissues indicates that the function of these genes is not pancreas-specific and reflects secondary disorders that make the experience of pancreatic disease worse.

3.5 Discussion

The poor QOL experienced by many patients with pancreatitis is linked to the pain experience, which is affected by pain signaling, central processing and the emotional response to those signals (Amann et al., 2013; Cote et al., 2018; E. K. Dunbar, Saloman, et al., 2021;

[5] Determined by which allele is tested.

Machicado et al., 2017; Mullady et al., 2011). We previously noted that symptoms of depression in RAP and CP are associated with constant-severe pain and genetic loci containing depression risk genes (E. Dunbar et al., 2020). We extended the findings of genetic predisposition to depression to investigate genetic predisposition to anxiety and PTSD and identified several candidate genes for anxiety and PTSD that deserve further targeted studies.

Both anxiety and PTSD interfere with daily life and relationships. A common model for understanding the variable etiology of these psychiatric disorders is "diathesis-stress" or rather genes and stress (Gottschalk & Domschke, 2017; Smoller, 2016). This model predicts that after a combination of genes and outside stressors reaches a threshold stress-related psychopathology emerges (Smoller, 2016).

Generalized anxiety disorder (GAD) is characterized by excessive and uncontrolled worry that is not appropriate to the actual risk posed by a stimulus or in the absence of the stimulus (Gottschalk & Domschke, 2017). In addition to exposure to stress early in life, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis also plays a role in anxiety disorders (Gottschalk & Domschke, 2017; Perlis et al., 2013). GAD overlaps phenotypically and is comorbid with other stress related disorders (such as other anxiety disorders, and depression) (Gottschalk & Domschke, 2017). Twin studies produced a heritability estimate of 30-50% (Gottschalk & Domschke, 2017; Smoller, 2016). About two thirds of children experiencing chronic pain also exhibit anxiety, and ~30-60% of patients with chronic pain will experience anxiety (Cunningham et al., 2016; Gillman et al., 2020). Patients with chronic pain and anxiety tend not to respond well to treatment of their pain (Cunningham et al., 2016; Gillman et al., 2020). One study even showed that although children with anxiety and pain were more likely to adhere to cognitive behavioral therapy for their pain, they were less likely to respond to it than other children with pain (Cunningham et al., 2016).

Posttraumatic Stress Disorder typically occurs in some individuals after experiencing traumatic events (Smoller, 2016). PTSD is characterized by four hallmark symptoms: hyper-arousal or reactivity, re-experiencing of the trauma, poor mood and thoughts related to the trauma, and avoidance of stimuli related to the trauma (Smoller, 2016). Twin studies have shown that both exposure to trauma (combat) and the symptoms of PTSD are heritable (Smoller, 2016). Additionally, PTSD can increase pain perception (Nikbakhtzadeh et al., 2020).

3.5.1 Clinical Implications

These findings further expand the opportunities to improve patient care through precision medicine (Whitcomb, 2019). Clinicians typically find it difficult to effectively treat CP pain due to the lack of precise therapies to relieve the different etiologies and severity patterns of pain in pancreatitis patients. In addition, the regulatory pressure to avoid opiates adds another challenge. The possibility of identifying pain-predominant symptoms linked to genetic risk for GAD, PTSD or depression at the point-of-care (including rural communities) provides a new precision medicine option for selecting specific medications for individual patients, educating them about how these psychological tendencies affect pain perception and QOL, and referring them for adjunctive therapy(ies) such as cognitive behavioral therapy that targets the specific aspect of pain. However, randomized, double blind, placebo-controlled trials are needed to determine the correlation between the genetic predictions and the utility of specific psychotropic medications and the magnitude of the effects, with and without additional psychiatric interventions.

3.6 Limitations

The limitations include relatively small sample size, including only people of EA, and lack of psychiatric phenotypic data (E. Dunbar et al., 2020). An additional limitation of this study may be a non-exhaustive candidate gene list (Border et al., 2019). The candidate gene list was intended to capture the more established loci for anxiety and PTSD. However, we used a tool using exact hypergeometric probability[6] to determine that the overlap (n=15)[7] of our candidate genes (n=28) with pain genes (n=315)[8] is not by random chance alone ($p<4.885x10^{-23}$, 30,000 total genes[9]) (Lund, 2005). Refer to the Tables S4 and S5, which reports gene candidate results using GWAS Catalog for more exhaustive results using genes reported in the GWAS Catalog as being associated with anxiety and/or PTSD (Buniello et al., 2019).

3.7 Conclusion

Several established genes associated with anxiety and PTSD are also associated with pain in pancreatitis. Many of these genes are involved with dopamine biology: *DRD3*, *BDNF*, *SLC6A3*,

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[6] See **3.11.3Exact Hypergeometric Probability**

[7] 15 genes from lab identified expected pancreatitis pain genes, 6 of which had significant SNPs, overlapped with anxiety/PTSD gene list

[8] Unpublished expected pancreatitis pain experience genes assembled and used internally by Whitcomb Lab based on a literature search.

[9] Estimate from the Human Genome Project (https://www.genome.gov/human-genome-project/Completion-FAQ)

and *NPY*. Other pathways that these candidate genes are associated with include neuronal signaling, prepulse inhibition, HPA axis, G protein-coupled receptor signaling, and cell-cell interaction (See Table 8 and **3.10.1Candidate Genes**, for a discussion of the significant candidate genes). The cell-cell interaction gene *CTNND2* has shown significant associations across all pain categories in CP and RAP+CP patients. These associations to pain phenotypes were also replicated in our cohort, using only RAP patients (See Tables S1 and S2, which reports results from replication analysis). Pain in pancreatitis is subjective and a complex symptom. It is not predictably responsive to current therapies, and has a significant impact on QOL. As we showed previously with depression, identifying patients at risk for psychiatric disorders may be beneficial in recommending alternative pain therapies (E. Dunbar et al., 2020). Further studies into genotypic and phenotypic associations of pain and mental health are warranted.

3.8 Acknowledgements

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3.9 Tables

Table 3-1 Aim 2 Table 1

Variable	Level	Controls[1] (N=443)	Cases[2] (N=375)	Total (N=818)	p-value
Age at Ascertainment	Mean (SD)	54.3 (16.7)	47.4 (13.2)	51.1 (15.6)	< 1e-05
Sex	Male	247 (55.8%)	185 (49.3%)	432 (52.8%)	0.08
	Female	196 (44.2%)	190 (50.7%)	386 (47.2%)	
Mental QOL	Mean (SD)	47.8 (10.5)	38.5 (11.8)	43.3 (12)	< 1e-05
	Missing	63	13	76	
Drinking	Never	90 (20.5%)	68 (18.1%)	158 (19.4%)	0.46
	Ever	350 (79.5%)	307 (81.9%)	657 (80.6%)	
	Missing	3	0	3	
Smoking	Never	143 (32.4%)	85 (22.7%)	228 (27.9%)	0.0027
	Ever	299 (67.6%)	290 (77.3%)	589 (72.1%)	
	Missing	1	0	1	
EPI	No	308 (69.5%)	218 (58.1%)	526 (64.3%)	0.00091
	Yes	135 (30.5%)	157 (41.9%)	292 (35.7%)	
Diabetes	No	308 (69.5%)	263 (70.1%)	571 (69.8%)	0.91
	Yes	135 (30.5%)	112 (29.9%)	247 (30.2%)	

Table 1. Association of phenotypes within CP patients with constant pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without constant pain. [2]Patients with constant pain.

CP, chronic pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

Table 3-2 Aim 2 Table 2

Variable	Level	Controls[1] (N=488)	Cases[2] (N=330)	Total (N=818)	p-value
Age at Ascertainment	Mean (SD)	53.6 (16.4)	47.5 (13.4)	51.1 (15.6)	< 1e-05
Sex	Male	271 (55.5%)	161 (48.8%)	432 (52.8%)	0.068
	Female	217 (44.5%)	169 (51.2%)	386 (47.2%)	
Mental QOL	Mean (SD)	46.8 (10.9)	38.7 (11.9)	43.3 (12)	< 1e-05
	Missing	66	10	76	
Drinking	Never	102 (21.0%)	56 (17.0%)	158 (19.4%)	0.18
	Ever	383 (79.0%)	274 (83.0%)	657 (80.6%)	
	Missing	3	0	3	
Smoking	Never	156 (32.0%)	72 (21.8%)	228 (27.9%)	0.0018
	Ever	331 (68.0%)	258 (78.2%)	589 (72.1%)	
	Missing	1	0	1	
EPI	No	332 (68.0%)	194 (58.8%)	526 (64.3%)	0.0085
	Yes	156 (32.0%)	136 (41.2%)	292 (35.7%)	
Diabetes	No	337 (69.1%)	234 (70.9%)	571 (69.8%)	0.63
	Yes	151 (30.9%)	96 (29.1%)	247 (30.2%)	

Table 2. Association of phenotypes within CP patients with constant-severe pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without constant-severe pain. [2]Patients with constant-severe pain.

CP, chronic pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

Table 3-3 Aim 2 Table 3

Variable	Level	Controls[1] (N=312)	Cases[2] (N=506)	Total (N=818)	p-value
Age at Ascertainment	Mean (SD)	55.4 (15.3)	48.5 (15.1)	51.1 (15.6)	< 1e-05
Sex	Male	170 (54.5%)	262 (51.8%)	432 (52.8%)	0.5
	Female	142 (45.5%)	244 (48.2%)	386 (47.2%)	
Mental QOL	Mean (SD)	46.2 (11.3)	41.8 (12.1)	43.3 (12)	< 1e-05
	Missing	61	15	76	
Drinking	Never	61 (19.7%)	97 (19.2%)	158 (19.4%)	0.91
	Ever	248 (80.3%)	409 (80.8%)	657 (80.6%)	
	Missing	3	0	3	
Smoking	Never	99 (31.8%)	129 (25.5%)	228 (27.9%)	0.06
	Ever	212 (68.2%)	377 (74.5%)	589 (72.1%)	
	Missing	1	0	1	
EPI	No	203 (65.1%)	323 (63.8%)	526 (64.3%)	0.78
	Yes	109 (34.9%)	183 (36.2%)	292 (35.7%)	
Diabetes	No	208 (66.7%)	363 (71.7%)	571 (69.8%)	0.15
	Yes	104 (33.3%)	143 (28.3%)	247 (30.2%)	

Table 3. Association of phenotypes within CP patients with severe pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without severe pain. [2]Patients with severe pain.

CP, chronic pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

Table 3-4 Aim 2 Table 4

Variable	Level	Controls[1] (N=770)	Cases[2] (N=507)	Total (N=1,277)	p-value
Age at Ascertainment	Mean (SD)	51.3 (16.9)	46 (13.4)	49.2 (15.8)	< 1e-05
	Missing	14	0	14	
Sex	Male	408 (53.0%)	239 (47.1%)	647 (50.7%)	0.047
	Female	362 (47.0%)	268 (52.9%)	630 (49.3%)	
Mental QOL	Mean (SD)	47.3 (10.7)	38.8 (11.6)	43.7 (11.8)	< 1e-05
	Missing	113	24	137	
Drinking	Never	156 (20.8%)	112 (22.1%)	268 (21.3%)	0.62
	Ever	595 (79.2%)	395 (77.9%)	990 (78.7%)	
	Missing	19	0	19	
Smoking	Never	288 (38.3%)	138 (27.3%)	426 (33.9%)	6.13e-05
	Ever	463 (61.7%)	368 (72.7%)	831 (66.1%)	
	Missing	19	1	20	
EPI	No	600 (79.4%)	332 (65.5%)	932 (73.8%)	< 1e-05
	Yes	156 (20.6%)	175 (34.5%)	331 (26.2%)	
	Missing	14	0	14	
Diabetes	No	589 (77.9%)	367 (72.4%)	956 (75.7%)	0.03
	Yes	167 (22.1%)	140 (27.6%)	307 (24.3%)	
	Missing	14	0	14	

Table 4. Association of phenotypes within RAP+CP patients with constant pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without constant pain. [2]Patients with constant pain.

CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

Table 3-5 Aim 2 Table 5

Variable	Level	Controls[1] (N=810)	Cases[2] (N=453)	Total (N=1,263)	p-value
Age at Ascertainment	Mean (SD)	50.9 (16.6)	46.1 (13.5)	49.2 (15.8)	< 1e-05
Sex	Male	431 (53.2%)	211 (46.6%)	642 (50.8%)	0.028
	Female	379 (46.8%)	242 (53.4%)	621 (49.2%)	
Mental QOL	Mean (SD)	46.6 (10.9)	39 (11.8)	43.7 (11.8)	< 1e-05
	Missing	104	19	123	
Drinking	Never	169 (21.0%)	99 (21.9%)	268 (21.3%)	0.77
	Ever	636 (79.0%)	354 (78.1%)	990 (78.7%)	
	Missing	5	0	5	
Smoking	Never	303 (37.6%)	123 (27.2%)	426 (33.9%)	0.00023
	Ever	502 (62.4%)	329 (72.8%)	831 (66.1%)	
	Missing	5	1	6	
EPI	No	630 (77.8%)	302 (66.7%)	932 (73.8%)	2.24e-05
	Yes	180 (22.2%)	151 (33.3%)	331 (26.2%)	
Diabetes	No	625 (77.2%)	331 (73.1%)	956 (75.7%)	0.12
	Yes	185 (22.8%)	122 (26.9%)	307 (24.3%)	

Table 5. Association of phenotypes within RAP+CP patients with constant-severe pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without constant-severe pain. [2]Patients with constant-severe pain.

CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

Table 3-6 Aim 2 Table 6

Variable	Level	Controls[1*] (N=443)	Cases[2*] (N=375)	Total[*] (N=818)	p-value
Age at Ascertainment	Mean (SD)	52 (16.1)	47.1 (15.2)	49.2 (15.8)	< 1e-05
Sex	Male	265 (49.9%)	377 (51.5%)	642 (50.8%)	0.61
	Female	266 (50.1%)	355 (48.5%)	621 (49.2%)	
Mental QOL	Mean (SD)	46.7 (11)	41.8 (12)	43.7 (11.8)	< 1e-05
	Missing	90	33	123	
Drinking	Never	105 (20.0%)	163 (22.3%)	268 (2.3%)	0.36
	Ever	421 (80.0%)	569 (77.7%)	990 (78.7%)	
	Missing	5	0	5	
Smoking	Never	202 (38.3%)	224 (30.7%)	426 (33.9%)	0.0065
	Ever	326 (61.7%)	505 (69.3%)	831 (66.1%)	
	Missing	3	3	6	
EPI	No	410 (77.2%)	522 (71.3%)	932 (73.8%)	0.022
	Yes	121 (22.8%)	210 (28.7%)	331 (26.2%)	
Diabetes	No	407 (76.6%)	549 (75.0%)	956 (75.7%)	0.54
	Yes	124 (23.4%)	183 (25.0%)	307 (24.3%)	

Table 6. Association of phenotypes within RAP+CP patients with severe pain. Percentages shown next to the counts are column percentages within each variable. [1]Patients without severe pain. [2]Patients with severe pain.

CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; SD, standard deviation; QOL, quality of life; EPI, exocrine pancreatic insufficiency.

*These case-control numbers were a typo; the correct numbers are Controls (N = 531) and Cases (N = 732) and Total (N = 1263)(E. K. Dunbar, Greer, et al., 2021a).

Table 3-7 Aim 2 Table 7

	Pain	Chr	SNP	OR (95% CI)	SE	P	Minor Allele	MAF	Gene
CP	Constant	3	rs79626250	2.97 (1.49, 5.92)	0.35	$1.9x10^{-3}$	A	0.036	*DRD3*
		5	rs111759924	0.51 (0.34, 0.75)	0.2	$7.7x10^{-4}$	T	0.054	*CTNND2*
		5	rs16901689	0.63 (0.47, 0.83)	0.15	$1.4x10^{-3}$	T	0.12	*CTNND2*
		5	rs59442633	2.01 (1.41, 2.88)	0.18	$1.3x10^{-4}$	C	0.12	*CTNND2*
		5*	rs72802806	1.59 (1.24, 2.03)	0.13	$2.3x10^{-4}$	A	0.26	*NR3C1*
		11*	rs1491851	1.38 (1.12, 1.69)	0.1	$2.0 x10^{-3}$	T	0.49	Upstream *BDNF*
	Constant-Severe	2*	rs62132337	3.15 (1.55, 6.42)	0.36	$1.5x10^{-3}$	T	0.039	*CAMKMT*
		3	rs79626250	2.94 (1.51, 5.73)	0.34	$1.6x10^{-3}$	A	0.038	*DRD3*
		5	rs10054369	0.48 (0.31, 0.73)	0.22	$6.3x10^{-4}$	T	0.048	*CTNND2*
		5	rs12513857	1.41 (1.14, 1.75)	0.11	$1.8x10^{-3}$	T	0.35	*NR3C1*
		5	rs16901689	0.62 (0.46, 0.83)	0.15	$1.5x10^{-3}$	T	0.12	*CTNND2*
		5	rs59442633	2.23 (1.56, 3.18)	0.18	$1.0 x10^{-5}$	C	0.13	*CTNND2*
		5	rs6865292	1.45 (1.17, 1.81)	0.11	$8.1x10^{-4}$	C	0.31	*NR3C1*
		6*	rs56977771	3.26 (1.56, 6.79)	0.37	$1.6x10^{-3}$	T	0.035	Downstream *FKBP5*
		13	rs1328677	0.69 (0.55, 0.86)	0.12	$1.3x10^{-3}$	A	0.24	*HTR2A*
		13*	rs731245	1.44 (1.18, 1.76)	0.1	$4.1x10^{-4}$	G	0.52	Upstream *HTR2A*
	Severe	2	rs189479791	0.2 (0.079, 0.51)	0.47	$7.1x10^{-4}$	C	0.0068	*PDE1A*
		5	rs142199704	0.37 (0.19, 0.69)	0.32	$1.9x10^{-3}$	A	0.018	*SLC6A3*
		5	rs76003244	3.83 (1.78, 8.24)	0.39	$6.0x10^{-4}$	A	0.05	*CTNND2*
		13	rs73175516	0.58 (0.41, 0.81)	0.17	$1.6x10^{-3}$	C	0.081	*HTR2A*
RAP+ CP	Constant	5	rs10054369	0.53 (0.38, 0.73)	0.17	$1.3x10^{-4}$	T	0.054	*CTNND2*
		5	rs16901689	0.63 (0.5, 0.80)	0.12	$1.1x10^{-4}$	T	0.13	*CTNND2*
		5	rs59442633	1.73 (1.3, 2.31)	0.15	$2.0x10^{-4}$	C	0.1	*CTNND2*
		5*	rs72802806	1.4 (1.16, 1.7)	0.099	$5.9x10^{-4}$	A	0.24	*NR3C1*
		6*	rs9294969	1.82 (1.29, 2.56)	0.17	$5.8x10^{-4}$	A	0.078	Downstream *THBS2*
		7	rs148812933	2.15 (1.34, 3.46)	0.24	$1.5x10^{-3}$	T	0.043	Downstream *NPY*
		17	rs541569598	0.75 (0.63, 0.89)	0.088	$1.2x10^{-3}$	T	0.35	Upstream *SHMT1*
	Constant-Severe	5	rs10054369	0.51 (0.36, 0.72)	0.17	$1.2x10^{-4}$	T	0.052	*CTNND2*
		5	rs16901689	0.62 (0.49, 0.8)	0.12	$1.4x10^{-4}$	T	0.12	*CTNND2*
		5	rs59442633	1.82 (1.36, 2.44)	0.15	$6.0 x10^{-5}$	C	0.11	*CTNND2*

	5*	rs72802806	1.37 (1.13, 1.67)	0.1	$1.6x10^{-3}$	A	0.24	*NR3C1*
	6*	rs9294969	1.87 (1.33, 2.64)	0.18	$3.6x10^{-4}$	A	0.079	Downstream *THBS2*
	13*	rs731245	1.32 (1.12, 1.55)	0.082	$8.9x10^{-4}$	G	0.51	Upstream *HTR2A*
Severe	2	rs78195040	2.84 (1.47, 5.47)	0.34	$9.3x10^{-4}$	G	0.029	*CAMKMT*
	2	rs189479791	0.33 (0.17, 0.64)	0.33	$1.9x10^{-3}$	C	0.01	*PDE1A*
	3	rs111466137	0.52 (0.35, 0.76)	0.2	$8.8x10^{-4}$	T	0.036	*DRD3*
	5	rs56825733	0.48 (0.3, 0.75)	0.23	$1.4x10^{-3}$	T	0.024	*CTNND2*
	7	rs7357103	1.32 (1.11, 1.57)	0.089	$1.9x10^{-3}$	G	0.37	*NPSR1*

Table 7. Lead SNPS. Candidate gene association results for lead SNPs for each group of pancreatitis and pain from NAPS2 data. *SNP has an eQTL as reported in GTEx (Lonsdale et al., 2013).

CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; Chr, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence intervals; SE, standard error; MAF, minor allele frequency.

Table 3-8 Aim 2 Table 8

Pathway	Candidate Genes	Protein Information
Neuronal Signaling	*HTR2A*	Serotonin receptor
	DRD3	Dopamine receptor
	NPY	Neuropeptide
	BDNF	Nerve growth factor
Prepulse Inhibition	*SLC6A3*	Dopamine transporter
	SHMT1	Cytosolic serine hydroxylmethyltransferase
HPA Axis	*NR3C1*	Glucocorticoid receptor
	FKBP5	Glucocorticoid receptor co-chaperone
G Protein-Coupled Receptor Signaling	*CAMKMT*	Class I protein methyltransferase
	PDE1A	Cyclic nucleotide phosphodiesterase
	NPSR1	G Protein-coupled receptor
Cell-Cell Interaction	*CTNND2*	Adhesive junction
	THBS2	Tumor growth inhibitor

Table 8. Summary of Significant Candidate Genes.

HPA, Hypothalamic-Pituitary-Adrenal.

3.10 Supplemental

Table 3-9 Aim 2 Table S1

Pain	Chr	SNP	OR (95% CI)	SE	P	Minor Allele	MAF	Gene
Constant	1	rs563109615	4.062 (1.75, 9.44)	0.43	1.13E-03	A	0.059	Downstream *NGF*
	1	rs141581625	2.15 (1.33, 3.49)	0.25	1.91E-03	C	0.14	*DISC1*
	2	rs653231	1.68 (1.25, 2.24)	0.15	4.94E-04	G	0.58	*CAMKMT*
	2*	rs186372019	3.61 (1.65, 7.91)	0.4	1.33E-03	T	0.059	Upstream *PDE1A*
	5	rs10078516	1.74 (1.23, 2.47)	0.18	1.83E-03	T	0.29	Downstream *CTNND2*
	7	rs1419793	4.49 (1.87, 10.78)	0.45	7.91E-04	C	0.051	*NPSR1*
Constant-Severe	1	rs563109615	4.23 (1.82, 9.83)	0.43	8.02E-04	A	0.064	Downstream *NGF*
	1	rs145897450	4.97 (1.81, 13.7)	0.52	1.92E-03	A	0.06	*NGF-AS1*
	1	rs78658433	9.24 (2.27, 37.71)	0.72	1.94E-03	A	0.04	*TSNAX-DISC1*
	2	rs80192418	5.0 (1.88, 13.33)	0.5	1.29E-03	A	0.044	*CAMKMT*
	2*	rs186372019	4.062 (1.83, 9.013)	0.41	5.66E-04	T	0.064	Upstream *PDE1A*
	5	rs10078516	1.93 (1.35, 2.76)	0.18	3.27E-04	T	0.3	Downstream *CTNND2*
	5	rs4702765	2.94 (1.49, 5.83)	0.35	1.95E-03	T	0.076	Downstream *CTNND2*
	5	rs13184818	3.14 (1.53, 6.45)	0.37	1.78E-03	C	0.072	*CTNND2*
	7	rs1419793	4.95 (2.021, 12.12)	0.46	4.65E-04	C	0.056	*NPSR1*
	22	rs141779732	2.86 (1.47, 5.54)	0.34	1.91E-03	T	0.088	*TANGO2*, Downstream *COMT*
Severe	2	rs4245799	1.89 (1.31,2.73)	0.19	6.37E-04	C	0.23	Downstream *CAMKMT*
	5	rs258834	0.093(0.027, 0.32)	0.63	1.83E-04	T	0.0065	*CTNND2*

Table S1. Lead SNPS. Candidate gene association results for lead SNPs for recurrent acute pancreatitis and pain from NAPS2 data. *SNP has an eQTL as reported in GTEx (Lonsdale et al., 2013). Chr, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence intervals; SE, standard error; MAF, minor allele frequency.

Table 3-10 Aim 2 Table S2

SNP	Gene	eQTL Tissue	Link
rs186372019	*SSFA2*	Artery-Tibial	https://www.gtexportal.org/home/snp/rs186372019

Table S2. eQTL loci for lead SNP as reported from GTEx (Lonsdale et al., 2013).

SNP, single nucleotide polymorphism; eQTL, expression quantitative trait loci.

Table 3-11 Aim 2 Table S3

Chr	BP Start	BP End	Gene	Sources
1	231762561	232177019	*DISC1***	(Smoller, 2016)
1	115828537	115880857	*NGF*	(Duncan et al., 2018)
1	160765864	160798045	*LY9*	(Niculescu et al., 2019)
1	89517987	89531043	*GBP1*,**	(Niculescu et al., 2019)
2	44589043	44999731	*CAMKMT**	(Gottschalk & Domschke, 2017)
2	183004762	183387572	*PDE1A*	(Gottschalk & Domschke, 2017)
3	113847557	113897899	*DRD3*	(Gottschalk & Domschke, 2017)
3	101659703	101716770	*LOC152225*	(Gottschalk & Domschke, 2017)
4	34312045	34312045	*cg09242288*	(Daskalakis et al., 2018)
5	10971952	11904110	*CTNND2*,**	(Smoller, 2016)
5	63255875	63258119	*HTR1A*	(Gottschalk & Domschke, 2017)
5	142657496	142784045	*NR3C1*	(Gottschalk & Domschke, 2017)
5	1392905	1445543	*SLC6A3*	(Gottschalk & Domschke, 2017)
5	153418519	153437014	*MFAP3*	(Niculescu et al., 2019)
6	35541362	35656719	*FKBP5*	(Daskalakis et al., 2018)
6	78171948	78173120	*HTR1B*	(Gottschalk & Domschke, 2017)
6	169615875	169654137	*THBS2*	(Gottschalk & Domschke, 2017)
7	31092076	31151093	*ADCYAP1R1**	(Daskalakis et al., 2018)
7	34697897	34917944	*NPSR1*	(Gottschalk & Domschke, 2017)
7	24323807	24331484	*NPY*	(Gottschalk & Domschke, 2017)
11	27676442	27722600	*BDNF*	(Duncan et al., 2018; Gottschalk & Domschke, 2017; Smoller, 2016)
11	113280317	113346001	*DRD2**	(Gottschalk & Domschke, 2017; Smoller, 2016)
11	637305	640705	*DRD4*	(Gottschalk & Domschke, 2017)
13	47405677	47471211	*HTR2A*	(Gottschalk & Domschke, 2017)
17	43861646	43913194	*CRHR1**	(Gottschalk & Domschke, 2017)
17	28521337	28562986	*SLC6A4*	(Gottschalk & Domschke, 2017)
17	18231187	18266856	*SHMT1*	(Niculescu et al., 2019)
22	19929263	19957498	*COMT*	(Gottschalk & Domschke, 2017; Niculescu et al., 2019)

Table S3. Candidate Genes. Genes extracted from a literature review as being associated with anxiety and/or PTSD. Base pair regions are for the gene itself using hg19 from the UCSC Genome Browser (Kent WJ et al., 2002). *Associated with depression in GWAS Catalog. **Associated with antidepressants in GWAS Catalog (Buniello et al., 2019).

Chr, chromosome; BP, base pair.

Table 3-12 Aim 2 Table S4

	Pain	Chr	SNP	OR (95% CI)	SE	P	Minor Allele	MAF	Gene
CP	Constant	1*	rs7417320	1.54 (1.26, 1.9)	0.11	3.69E-05	G	0.47	*MAD2L2*
		3	rs1154373	0.64 (0.52, 0.78)	0.11	1.40E-05	G	0.38	*GRM7*
		3	rs3804883	1.58 (1.27, 1.98)	0.11	5.69E-05	G	0.34	*GRM7*
		5	rs1895360	7.96 (3.03, 20.95)	0.49	2.64E-05	T	0.04	*PPP2R2B*
		5	rs465409	6.32 (2.57, 15.58)	0.46	6.16E-05	A	0.04	*PPP2R2B*
		6	rs4454135	1.58 (1.28, 1.95)	0.11	2.31E-05	C	0.42	Upstream *TNFRSF21*
		6	rs803406	0.62 (0.49, 0.79)	0.12	6.99E-05	T	0.24	*PLEKHG1*
		8	rs62480128	2.21 (1.54, 3.17)	0.19	1.77E-05	G	0.13	*CSMD1*
		9	rs2761694	0.65 (0.52, 0.8)	0.11	7.87E-05	C	0.33	*PTPRD*
	Constant-severe	1*	rs3767300	0.66 (0.54, 0.81)	0.11	7.14E-05	C	0.41	*MAD2L2*
		5	rs1895360	8.17 (3.32, 20.1)	0.46	4.83E-06	T	0.045	*PPP2R2B*
		5	rs465409	5.64 (2.51, 12.7)	0.41	2.95E-05	A	0.044	*PPP2R2B*
		6	rs803406	0.6 (0.47, 0.76)	0.12	3.10E-05	T	0.23	*PLEKHG1*
		8	rs62480128	2.37 (1.66, 3.39)	0.18	2.03E-06	G	0.14	*CSMD1*
		16	rs6500947	1.63 (1.3, 2.06)	0.12	3.09E-05	G	0.32	*RBFOX1*
	Severe	5	rs331201	2.24 (1.5, 3.34)	0.21	8.98E-05	T	0.11	Downstream *CDH10*
		7*	rs4397289	1.57 (1.26, 1.95)	0.11	6.55E-05	G	0.4	*THSD7A*
		7	rs4129040	0.62 (0.49, 0.79)	0.12	8.76E-05	G	0.23	*DPP6*
		8*	rs7463086	0.63 (0.51, 0.77)	0.11	1.02E-05	G	0.35	*ERICH1-AS1*
		11	rs7479307	0.41 (0.27, 0.62)	0.21	2.13E-05	T	0.046	*GRM5*
		16	rs7202500	2.62 (1.66, 4.15)	0.24	3.99E-05	T	0.095	*RBFOX1*
		20*	rs75501185	0.4 (0.26, 0.62)	0.22	3.20E-05	A	0.045	*SALL4*
RAP+ CP	Constant	1	rs41332551	2.33 (1.53, 3.54)	0.21	7.29E-05	T	0.055	*C1orf94*
		4	rs2870322	0.54 (0.39, 0.73)	0.16	9.40E-05	C	0.06	*CCSER1*
		5	rs1895360	3.77 (1.95, 7.28)	0.34	8.24E-05	T	0.031	*PPP2R2B*
		5	rs2011893	0.25 (0.12, 0.49)	0.36	7.82E-05	G	0.012	*TENM2*
		6	rs4129326	1.4 (1.19, 1.65)	0.083	5.26E-05	T	0.44	Upstream *TNFRSF21*
		6	rs803411	0.68 (0.56, 0.82)	0.095	4.04E-05	T	0.24	*PLEKHG1*
		9	rs77948918	0.62 (0.49, 0.78)	0.12	5.64E-05	C	0.11	*PTPRD*
		9	rs7858684	0.69 (0.58, 0.83)	0.089	4.17E-05	T	0.28	*PTPRD*
		9	rs191106810	0.55 (0.41, 0.74)	0.15	9.55E-05	A	0.067	*GABBR2*
		12	rs759764	1.48 (1.22, 1.79)	0.098	7.24E-05	C	0.27	*ANKS1B*
		1	rs41332551	2.32 (1.54, 3.51)	0.21	6.44E-05	T	0.058	Upstream *TNFRSF21*

Table 3-12 Aim 2 Table S4

	2*	rs17635425	2.32 (1.54, 3.51)	0.21	6.13E-05	C	0.061	*CNNM3*
	5	rs10079739	1.41 (1.19, 1.67)	0.087	7.51E-05	C	0.42	*HCN1*
	5	rs1895360	4.28 (2.2, 8.31)	0.34	1.77E-05	T	0.034	*PPP2R2B*
Constant-severe	7	rs757323	1.4 (1.18, 1.66)	0.086	9.76E-05	G	0.53	Downstream *COBL*
	12	rs759764	1.5 (1.23, 1.83)	0.01	4.73E-05	C	0.27	*ANKS1B*
	18	rs77567232	3.35 (1.83, 6.13)	0.31	9.09E-05	T	0.034	*DCC*
	18	rs4801075	2.77 (1.71, 4.49)	0.25	3.52E-05	T	0.047	*LINC-ROR*
	18	rs181903213	4.69 (2.21, 9.96)	0.38	5.82E-05	T	0.027	*WDR7*
	2	rs139971969	2.66 (1.63, 4.35)	0.25	9.90E-05	C	0.052	*EFHD1*
	4	rs12646702	0.71 (0.6, 0.84)	0.084	5.58E-05	A	0.45	*SMAD1*
	7	rs10270255	0.69 (0.58, 0.82)	0.09	2.71E-05	A	0.26	*THSD7A*
	7	rs757323	1.48 (1.25, 1.75)	0.085	3.58E-06	G	0.52	Downstream *COBL*
Severe	8*	rs12550299	0.63 (0.5, 0.79)	0.12	9.86E-05	C	0.12	*MCPH1*
	11*	rs7125204	1.5 (1.25, 1.8)	0.093	1.26E-05	G	0.32	*ELP4*
	16	rs67176054	2.45 (1.7, 3.53)	0.19	1.53E-06	A	0.09	*RBFOX1*
	16	rs34009260	2.19 (1.56, 3.08)	0.17	5.96E-06	A	0.098	*RBFOX1*
	16	rs74011978	0.17 (0.073, 0.4)	0.43	4.16E-05	G	0.0047	*RBFOX1*

Table S4. Lead SNPS. GWAS Catalog (Buniello et al., 2019) gene association results for lead SNPs for each group of pancreatitis and pain from NAPS2 data. *SNP has an eQTL as reported in GTEx (Lonsdale et al., 2013).

CP, chronic pancreatitis; RAP, recurrent acute pancreatitis; Chr, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence intervals; SE, standard error; MAF, minor allele frequency.

Table 3-13 Aim 2 Table S5

SNP	Gene(s)	eQTL Tissue(s)	Link
rs7417320	*MAD2L2*	Testis	https://www.gtexportal.org/home/snp/rs7417320
	FBXO6	Muscle-Skeletal Artery-Aorta Whole Blood Heart-Left Ventricle Artery-Coronary Heart-Atrial Appendage Artery-Tibial	
rs3767300	*MAD2L2*	Testis Heart-Left Ventricle	https://www.gtexportal.org/home/snp/rs3767300
	FBXO6	Muscle-Skeletal Artery-Aorta Heart-Atrial Appendage Artery-Tibial Heart-Left Ventricle Whole Blood Artery-Coronary Esophagus-Mucosa Adipose-Subcutaneous Skin-Sun Exposed(Lower leg)	
rs4397289	*THSD7A*	Brain-Cerebellum	https://www.gtexportal.org/home/snp/rs4397289
	VWDE	Cells-Cultured fibroblasts	
rs7463086	*ERICH1*	Whole Blood	https://www.gtexportal.org/home/snp/rs7463086
rs75501185	*SALL4*	Thyroid Nerve-Tibial Pancreas	https://www.gtexportal.org/home/snp/rs75501185
rs17635425	*LMAN2L*	Skin-Sun Exposed(Lower leg) Skin-Not Sun Exposed(Suprapubic) Muscle-Skeletal	https://www.gtexportal.org/home/snp/rs17635425
	CIAO1	Testis	
	ADRA2B	Skin-Sun Exposed(Lower leg)	
rs12550299	*MCPH1*	Nerve-Tibial Colon-Sigmoid Brain-Cerebellum Brain-Cerebellar Hemisphere Esophagus-Muscularis Brain-Caudate(Basal ganglia)	https://www.gtexportal.org/home/snp/rs12550299

Table S5. eQTL loci for lead SNPs as reported from GTEx (Lonsdale et al., 2013).

SNP, single nucleotide polymorphism; eQTL, expression quantitative trait loci.

Table 3-14 Aim 2 Table S6

SNP	Gene(s)	eQTL Tissue(s)	Link
rs72802806	*NR3C1*	Esophagus-Mucosa	https://www.gtexportal.org/home/snp/rs72802806
rs1491851	*LIN7C* *LIN7C* *LIN7C* *BDNF-AS*	Esophagus-Muscularis Artery-Tibial Lung Thyroid	https://www.gtexportal.org/home/snp/rs1491851
rs62132337	*LRPPRC* *LRPPRC*	Skin-Not Sun Exposed (Suprapubic) Pancreas	https://www.gtexportal.org/home/snp/rs62132337
rs56977771	*ZNF76* *TEAD3*	Artery-Tibial Cells-Cultured fibroblasts	https://gtexportal.org/home/snp/rs56977771
rs731245	*HTR2A* *HTR2A*	Testis Artery-Aorta	https://gtexportal.org/home/snp/rs731245
rs9294969	*RP11-417E7.2* *RP11-417E7.2* *RP11-417E7.2* *RP11-417E7.2* *RP11-417E7.2* *LINC01615* *LINC01615*	Artery-Tibial Heart-Artrial Appendage Thyroid Adipose-Subcutaneous Skin-Sun Exposed (Lower leg) Cells-Cultured fibroblasts Esophagus-Mucosa	https://gtexportal.org/home/snp/rs9294969

Table S6. eQTL loci for lead SNPs as reported from GTEx (Lonsdale et al., 2013).

SNP, single nucleotide polymorphism; eQTL, expression quantitative trait loci.

3.10.1 Candidate Genes

3.10.1.1 Neuronal Signaling (HTR2A, DRD3, NPY, BDNF)

The 5-hydroxytryptamine receptor 2A gene (*HTR2A*) codes for a serotonin receptor (Murphy et al., 2006). SNPs in *HTR2A* have been associated with risk of schizophrenia, obsessive compulsive disorder, and response to citalopram (Murphy et al., 2006).The serotonin 2A receptor also alters expression of *BDNF* in several limbic neurocircuits (Tsybko et al., 2020). *HTR2A* is the target of many drugs in clinical trials for the treatment of GAD, social anxiety disorder, PTSD, and obsessive compulsive disorder (Ochoa et al., 2020). The SNP rs731245 associated with constant-severe pain in CP and RAP+CP has two eQTLs in *HTR2A* in the testis and aorta (See Table S5, which reports eQTLs) (Lonsdale et al., 2013).

Dopamine receptor D3 (*DRD3*) codes the high affinity D3 subtype of the five dopamine receptors and is regulated by G proteins (Murphy et al., 2006). *DRD3* is expressed mainly in the areas of the brain (limbic) associated with emotional, cognitive, reward sensitivity, impulsivity, and endocrine functions (Montoya et al., 2019; Murphy et al., 2006; Worhunsky et al., 2020). The *DRD3* receptor is involved in several signaling pathways, including inhibiting the formation of cAMP via G protein coupling (Arango-Lievano et al., 2016). Many anti-Parkinsonian and antipsychotic drugs target *DRD3* and *DRD2* (Arango-Lievano et al., 2016). Levodopa, which targets *DRD3*, has been through clinical trials for the treatment of PTSD (Ochoa et al., 2020). Individuals with low stress resilience appear to have overexpression of dopamine receptor genes, including *DRD3*, and lower levels of dopamine degradation resulting in higher levels of dopamine in their brains (Azadmarzabadi et al., 2018). Variants in *DRD3* in combination with variants in *BDNF* (below) are associated with anxiety that is comorbid with bipolar disorder (Chang et al., 2013).

Pro-neuropeptide Y gene (*NPY*) is expressed in the central nervous system, is a target for anxiolytic drugs, and is associated with decreased endogenous μ-opioid response to pain (Gottschalk & Domschke, 2017; Murphy et al., 2006). *NPY* is involved with food motivation response in mesolimbic dopamine circuits (West & Roseberry, 2017). *NPY* also shows some environmental interactions and is associated with stress resilience (Azadmarzabadi et al., 2018; Gottschalk & Domschke, 2017).

Low levels of brain-derived neurotropic factor (*BDNF*), a nerve growth factor, are strongly associated with major depressive disorder, possibly through disturbed neuronal plasticity and impaired neurogenesis (Y. Shi et al., 2020). *BDNF* has several alternative splice sites; one of these proteins increases survival of neurons in the brain (Murphy et al., 2006). *BDNF* is involved in the stress response and mood disorders, and is associated with "anticipatory worry", depression, and anxiety (Azadmarzabadi et al., 2018; Chang et al., 2013; Gottschalk & Domschke, 2017; Murphy et al., 2006). BDNF can be produced by anti-inflammatory M2 microglia (Montoya et al., 2019). The protein is a target of dopamine and serotonin, and regulates expression of *DRD3* (Azadmarzabadi et al., 2018; Zai et al., 2015). Our SNP associated with constant pain in CP, rs1491851, has eQTLs in both *LIN7C* and *BDNF-AS* in multiple tissue types (See Table S5, which reports eQTLs) (Lonsdale et al., 2013).

3.10.1.2 Prepulse Inhibition (SLC6A3, SHMT1)

The solute carrier family 6 member 3 (*SLC6A3*) gene encodes a dopamine transporter that removes dopamine from the synapse (Murphy et al., 2006; Yin et al., 2015). A variable number tandem repeat in the 3' untranslated region has been associated with attention deficit hyperactivity disorder, epilepsy, alcohol and cocaine dependence, Parkinson disease, prepulse inhibition, and reduced nicotine dependence (Murphy et al., 2006; Notzon et al., 2017). *SLC6A3* has been the

target of a clinical trial treating PTSD with methylphenidate (Ochoa et al., 2020). It is suggested that variation in *SLC6A3* is associated with response to antidepressants; however, more studies are needed (Yin et al., 2015).

Serine hydroxymethyltransferase 1 (*SHMT1*) codes for the cytosolic version of serine hydroxymethyltransferase, which is mainly expressed in the kidney and liver (Murphy et al., 2006). However, SHMT1 has higher expression in schizophrenic human brains (Maekawa et al., 2010). SHMT1 converts glycine (a NMDA receptor co-chaperone) to L-serine; a process implicated in abnormal prepulse inhibition, which is involved in many psychiatric disorders (Maekawa et al., 2010; Notzon et al., 2017). *SHMT1* is a predictor for emergency department visits associated with female PTSD (Niculescu et al., 2019).

3.10.1.3 HPA Axis (NR3C1, FKBP5)

The nuclear receptor subfamily 3 group C member 1 (*NR3C1*) gene codes for a glucocorticoid receptor that acts as a transcription factor, and is a part of inflammation (Murphy et al., 2006). Glucocorticoids are stress hormones involved in the HPA axis; dysregulation of this axis is often associated with stress related disorders (Gerritsen et al., 2017). Clinical trials have tested whether variants in the *NR3C1* locus affect PTSD response to Mifepristone, Dexamethasone, Prednisone, and Hydrocortisone and suggest some predictive value (Ochoa et al., 2020). The SNP associated with constant pain in CP and both constant and constant-severe pain in RAP+CP patients (rs72802806) also has an eQTL associated with *NR3C1* measured in the esophagus (See Table S5, which reports eQTLs) (Lonsdale et al., 2013).

The FKBP prolyl isomerase 5 gene (*FKBP5*) codes for a protein in the immunophilin family that is involved in immunoregulation, protein folding and trafficking expressed mainly in fat (Murphy et al., 2006). The SNP rs56977771 associated with constant-severe pain in CP

presently also has eQTLs in *ZNF76* and *TEAD3* (See Table S5, which reports eQTLs) (Lonsdale et al., 2013). *FKBP5* shows a gene-by-environmental association with childhood trauma and increased risk of the stress disorders PTSD and depression (Daskalakis et al., 2018; Smoller, 2016). Some variants in *FKBP5* affect HPA axis activity, as the FKBP5 protein is a co-chaperone of the glucocorticoid receptor (Daskalakis et al., 2018; Gerritsen et al., 2017). Previous research found an association between lower response to psychotherapy in PTSD and DNA methylation in the promoter region of this gene (Daskalakis et al., 2018).

3.10.1.4 G Protein-Coupled Receptor Signaling (CAMKMT, PDE1A, NPSR1)

Calmodulin-lysine N-methyltransferase (*CAMKMT*) codes for a class I protein methyltransferase that is involved in trimethylation of lysine 115 in calmodulin (Murphy et al., 2006) and influences the activator properties of calmodulin with target enzymes (Magnani et al., 2010). High expression of *CAMKMT* is seen in the testis, thyroid, and brain (Murphy et al., 2006) and is required for somatosensory development and brain function (Haziza et al., 2015). The lead SNP (rs62132337) associated with constant-severe pain in CP patients in our study contains an eQTL in *LRPPRC*, a gene linked to mitochondrial function (See Table S5, which reports eQTLs) (Lonsdale et al., 2013). *CAMKMT* has been associated with overall latent anxiety disorder factor scores (Gottschalk & Domschke, 2017). Phosphodiesterase 1A (*PDE1A*) is a cyclic nucleotide phosphodiesterase involved in signal transduction and is activated by calmodulin when Ca^{2+} is present (Murphy et al., 2006). *PDE1A* is associated with response to antidepressants in individuals with GAD (Gottschalk & Domschke, 2017) but we are not aware of studies linking beneficial clinical responses of patients with variants in the *CAMKMT* locus and leading SNPs in our study.

Neuropeptide S receptor 1 (*NPSR1*) codes for a vasopression/oxytocin subfamily of G protein-coupled receptors, a membrane protein that binds neuropeptide S (NPS) (Murphy et al.,

2006). The NPS/NPSR system affects anxiety, food intake, memory, arousal, locomotion, and drug addiction (Guerrini et al., 2010). SNPs in *NPSR1* are associated with rheumatoid arthritis, asthma, inflammatory bowel disease, panic disorders, PTSD and a gene-by-environment interaction with childhood trauma highlighted an association with anxiety within the functional neuropeptide S receptor (Gottschalk & Domschke, 2017; Haxhibeqiri et al., 2019; Murphy et al., 2006). In one small 8-week case-control study from China of patients with GAD, the *NPSR1* rs324981 genotypes appeared to predict response to escitalopram, a selective serotonin reuptake inhibitor (SSRI) and to a lesser degree venlafaxine, a serotonin-norepinephrine reuptake inhibitor (SNRI), further studies are needed to verify the observation (He et al., 2018).

3.10.1.5 Cell-Cell Interaction (CTNND2, THBS2)

Catenin delta 2 (*CTNND2*) codes for an adhesive junction protein in the armadillo/β-catenin family, which promotes cell spreading (Murphy et al., 2006). δ-catenin interacts with glutamate receptors in neurons and is implicated in brain processes involving synaptic regulation such as emotion and learning (Lu et al., 2016) δ-catenin is also involved in maintaining neurons in the mature cortex and the developing hippocampus (Lu et al., 2016). *CTNND2* has been associated with anxiety (Nivard et al., 2014; Smoller, 2016).

Thrombospondin 2 (*THBS2*) is expressed mainly in the gallbladder and endometrium (Murphy et al., 2006). This protein is part of the thrombospondin family and regulates cell-to-cell and cell-to-matrix interactions. Additionally, the protein is a tumor growth inhibitor (Murphy et al., 2006). Previously, a SNP within the *THBS2* gene was associated with anxiety in individuals of Hispanic and Latin American ancestry; although, a meta-analysis failed to replicate the association (Gottschalk & Domschke, 2017). However, we did find an association between a different SNP in *THBS2* and constant-severe and constant pain in RAP+CP patients. This SNP, rs9294969, has an

eQTL associated with *RP11-417E7.2* and *LINC01615* (See Table S5, which reports eQTLs) (Lonsdale et al., 2013).

SNPs in *DRD3, NR3C1, and PDE1A* have previously been associated with treatment of anxiety with duloxetine (Gottschalk & Domschke, 2017). Additionally, SNPs in *NR3C1* and *FKBP5* are associated with response to treatment in patients with depression (Perlis et al., 2013). Although SNPs in *BDNF* and *SLC6A3* show associations with response to venlafaxine for treating depression, they do not show a response for anxiety (Gottschalk & Domschke, 2017). SNPs in *HTR2A* respond well to treatment of anxiety with venlafaxine (Gottschalk & Domschke, 2017). Drug targets of *DRD3*, *HTR2A*, *NR3C1*, and *SLC6A3* have been investigated in clinical trials for the treatment of PTSD, social anxiety, and GAD (Ochoa et al., 2020). The SNPs associated with treatment in candidate genes are not the same individual SNPs associated with pancreatitis pain in this study; however, there remains potential for locus-based therapies that should be investigated further.

3.11 Additional Calculations

The following are additional calculations conducted after the publication of Aim 2.

3.11.1 Multiple Testing Considerations

For this aim, I used 818 to 1,277 RAP and CP patients and 818 CP only patients for the main analysis and 453 RAP only patients for replication of genetic results, all of European Ancestry (EA). All three pain categories were tested across both groups of patients, resulting in

six groupings. The six tested categories were: CP and constant pain (cases = 375, controls = 443), CP and severe pain (cases = 506, controls = 312), CP and constant-severe pain (cases = 330, controls = 488), RAP+CP and constant pain (cases = 507, controls = 770), RAP+CP and severe pain (cases = 375, controls = 443), and RAP+CP and constant-severe pain (cases = 453, controls = 810). The alpha level used here was 0.002 as there where 28 gene regions tested, and a higher false positive rate was determined to be acceptable. At this alpha, testing all 17 thousand SNPs was expected to result in 36 false positive results on average under the null hypothesis.

The Bonferroni corrected α adjusted for the multiple testing burden of testing 17,764 SNPs in CP was $\alpha=(0.05/17{,}764)=2.81\text{x}10^{-6}$ and for 17,747 SNPs in RAP+CP was $\alpha=(0.05/17{,}747)=2.82\text{x}10^{-6}$. None of the most significant independent SNPs reported (see **Table 3-7 Aim 2 Table 7**) meet this threshold suggesting no involvement of the 28 anxiety/PTSD candidate genes with pancreatitis pain. Although this method is too strict given LD (O. Cinar & W. Viechtbauer, 2022). Therefore, the poolr package for R (version 3.6.0) was used to calculate the effective number of tests given LD (Ozan Cinar & Wolfgang Viechtbauer, 2022; R Core Team, 2019). For CP with 17,764 SNPs the effective number of tests was 5,762 which increases the multiple testing correction to $\alpha=(0.05/5{,}762)=8.68\text{x}10^{-6}$. For RAP+CP with 17,747 SNPs the effective number of tests was 5,791 which increases the multiple testing correction to $\alpha=(0.05/5{,}791)=8.63\text{x}10^{-6}$. Even at this threshold corrected for effective number of tests none of the most significant independent SNPs reported (see **Table 3-7 Aim 2 Table 7**) meet the threshold suggesting no involvement of the 28 anxiety/PTSD candidate genes with pancreatitis pain.

3.11.2 Power

Power for these studies were estimated using the same procedure as in Aim 1, and using the same disease prevalence (0.33). Heatmaps for each study are in **Figure 3-1**, **Figure 3-2**, **Figure 3-3**, **Figure 3-4**, **Figure 3-5**, and **Figure 3-6**. These studies are best powered to detect effects greater than 1.3 and at MAFs between 0.1 and 0.6. Unfortunately, many complex diseases have OR's between 1.08 to 1.16 at similar MAFs (Park et al., 2011) and this aim is underpowered (<0.2) to detect true effects of those sizes and larger effect sizes are not expected with complex diseases. Replication in a larger sample size is needed to detect true positive SNPs with small effect sizes.

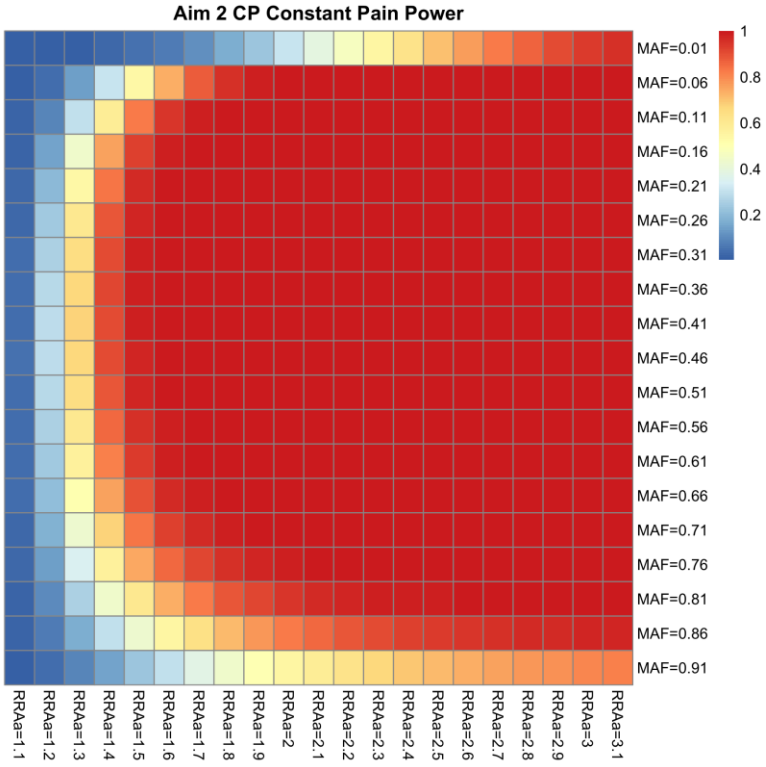

Figure 3-1 Power Heatmap for CP Constant Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=375, Controls=443.

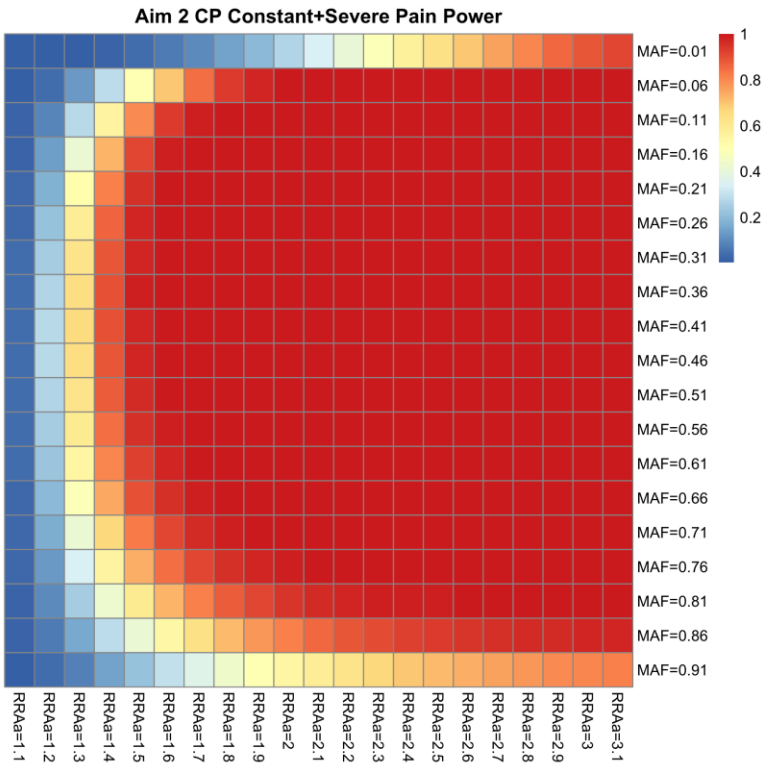

Figure 3-2 Power Heatmap for CP Constant-Severe Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=330, Controls=488.

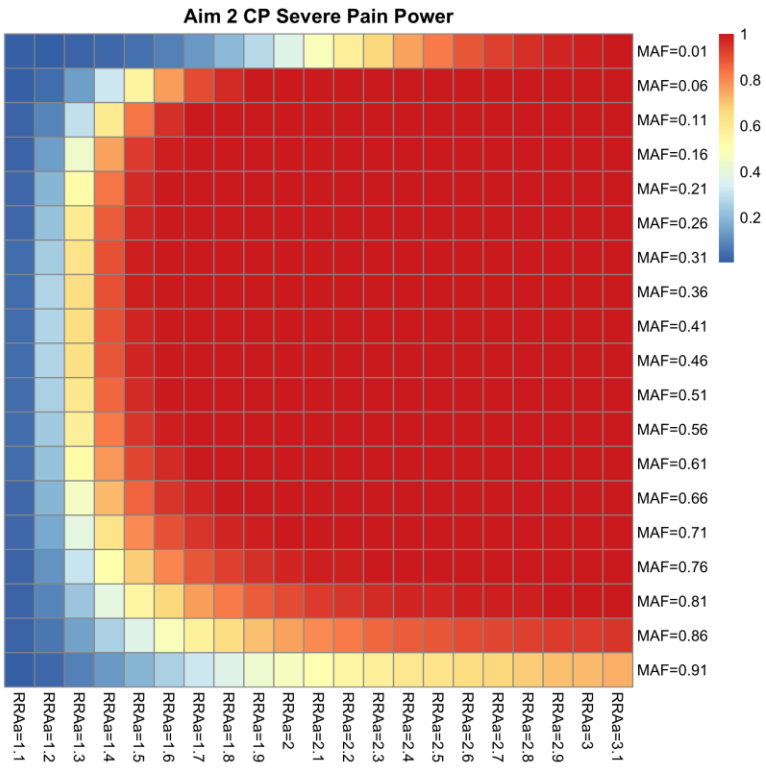

Figure 3-3 Power Heatmap for CP Severe Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=506, Controls=312.

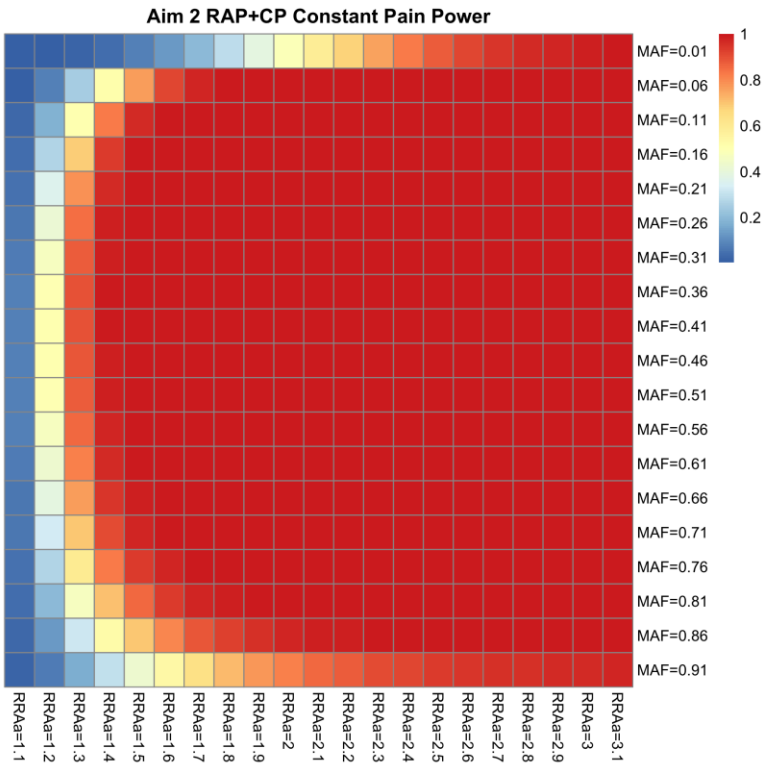

Figure 3-4 Power Heatmap for RAP+CP Constant Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=507, Controls=770.

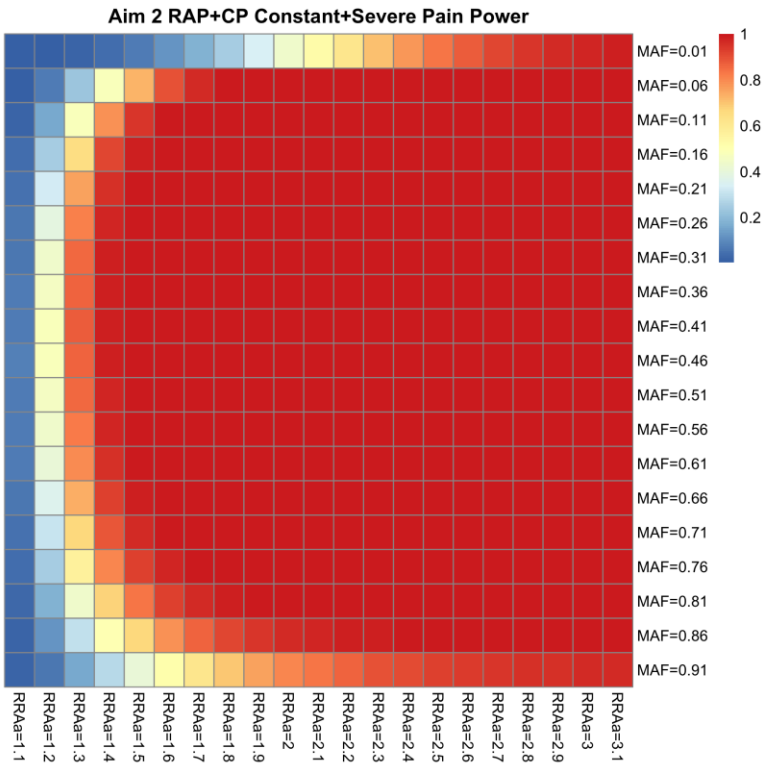

Figure 3-5 Power Heatmap for RAP+CP Constant-Severe Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=453, Controls=810.

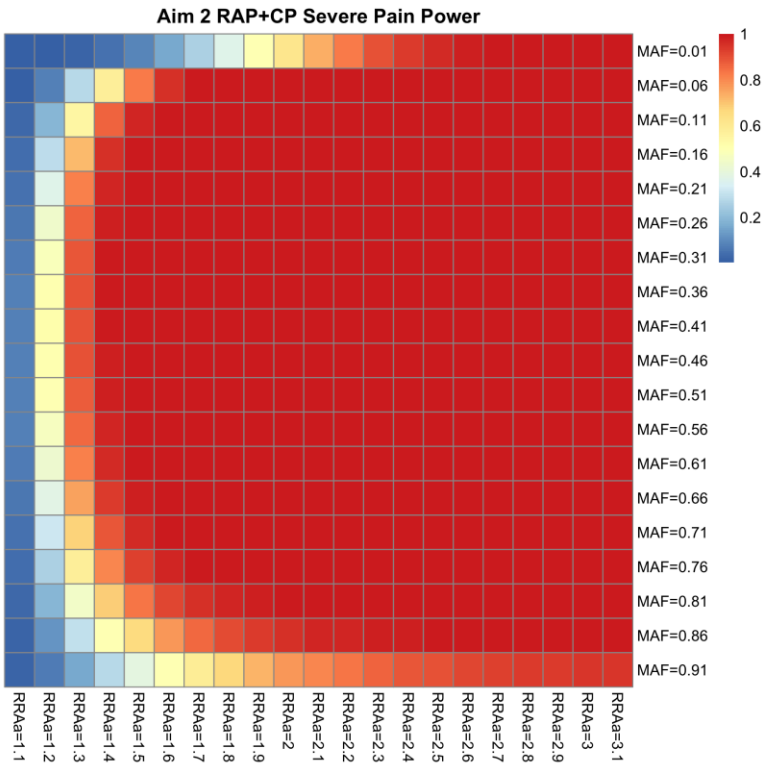

Figure 3-6 Power Heatmap for RAP+CP Severe Pain

α=0.002, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=732, Controls=531.

3.11.3 Exact Hypergeometric Probability

In the above paper, I used an online exact hypergeometric probability calculator (Lund, 2005) to roughly assess and demonstrate the chance of 28 anxiety/PTSD genes overlapping with genes expected to be associated with any pancreatitis pain (n = 315) from a literature search conducted by former members of the lab. There were 15 genes in both the candidate gene list and the list of expected pancreatitis pain genes. This calculator was designed specifically to test the significance of overlap of two gene lists drawn from the whole genome by calculating a representation factor and exact hypergeometric probability (Lund, 2005).

At the time of this calculation (circa winter 2020), I used 30,000 as the estimated number of genes in the genome as reported by the Human Genome Project (see https://www.genome.gov/human-genome-project/Completion-FAQ). However, the most recent estimate of the number of protein coding genes is 19,969 (Nurk et al., 2022). Using the updated number of genes to repeat the hypergeometric probability slightly raises the p-value to $p < 2.078x10^{-20}$ from $p < 4.885x10^{-23}$, but the new p-value is still significant at $p < 0.05$. Fewer total genes in the genome makes it easier to randomly overlap gene sets; therefore, reducing the significance of the overlap that was seen.

This type of test is often referred to as and calculated as a one-sided Fisher's Exact test (Rivals et al., 2007). Methods using Fisher's Exact and hypergeometric tests are most often used to calculate enrichment or depletion of GO categories in a list of differentially expressed genes (Rivals et al., 2007). However, an assumption of these tests is that the gene sets are independent of each other, and most of the available tools to calculate these tests make that assumption (Tamayo et al., 2016). While it is very difficult to meet this assumption biologically (Tamayo et al., 2016), given the interaction of genes and proteins with each other and pleiotropy, I was not aware of any published literature associating anxiety/PTSD genetic risk specifically with pancreatitis pain at the time this paper was written. Additionally, it is not known if different types of pain are genetically similar or if a connection between one type of pain and a comorbidity—like anxiety—is applicable to other types of pain (Meng et al., 2020). I argue that the assumption of independence is met here simply because there is not published literature stating otherwise. I do recognize that this is a tenuous argument at best, and that going forward it no longer holds given the results of this paper. However, using tests like this and assuming that the independence assumption is met is so common as to be an "industry standard" despite the known issues with this assumption. In fact, the popular

tool PANTHER uses the Fisher Exact test (Mi et al., 2019). In the future, a more appropriate test may be developed to test the significance of the overlap of anxiety/PTSD genes and loci associated with pancreatitis pain.

One potential approach may be permutation. The null hypothesis was that none of the anxiety/PTSD genes would contain associations to pancreatitis pain. A potential permutation method would be to select 28 random genes with similar sizes to the original candidate genes and associate those random genes with a permuted pain phenotype. From each permutation the number of genes with loci meeting the original p-value threshold of 0.002 would be recorded. The empirical p-value of seeing the original number of candidate genes with loci meeting the significance threshold (n = 13) for association with the pain phenotype or more would be calculated from that distribution. This method considers SNP density, gene size, and sample size by using random genes of the same size and p-values simulated using the original sample data. Whereas the hypergeometric probability only accounts for number of genes meeting the criteria for "success" (overlap between two gene sets). The results for the permutation are more reliable as they are directly applicable to our results and data, while the hypergeometric probability addresses the general "for example" probability of seeing results like ours.

4.0 Aim 3: Nested GWAS For Constant And/Or Severe Pain in The NAPS2 Cohort Identifies Multiple Candidate Genes Associated With Specific Pain Phenotypes in Chronic and Recurrent Acute Pancreatitis

The following manuscript is ready to be submitted for publication.

Title: **Nested GWAS For Constant And/Or Severe Pain in The NAPS2 Cohort Identifies Multiple Candidate Genes Associated With Specific Pain Phenotypes in Chronic and Recurrent Acute Pancreatitis.**

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4.1 Abstract

Background: Pancreatitis is an inflammatory disease with pain as a hallmark symptom. However, pancreatitis pain is highly variable, even in patients with similar physical disease states. The purpose of this paper is to identify preliminary genetic associations with constant, constant-severe, and severe pancreatitis pain.

Methods: Individuals with recurrent acute pancreatitis and chronic pancreatitis from the North American Pancreatitis Study II (NAPS2) of European Ancestry were included in genome-wide association studies (GWAS) based on their pain categories to identify genetic associations with pain. The GWAS results were then used in transcriptome-wide association studies (TWAS) to identify any predicted differential expression of genes associated with pancreatitis pain. Finally, the GWAS results from TWAS identified genes were colocalized with expression quantitative trait loci (eQTLs) from GTEx data to confirm that the predicted differential expression and GWAS signals were from the same loci.

Results: Predicted differential expression of *CTRC* was identified by TWAS in pancreatitis patients with constant (p-value 2.45×10^{-5}) and constant-severe pain (p-value 4.5×10^{-5}), and colocalized with eQTLs in pancreas tissues (PP.H3 0.02, PP.H4 0.73) in constant pain. *DOK6* was identified as being associated with severe pain from the GWAS (p-value 8.75×10^{-6}), associated with differential expression in patients with severe pain from the TWAS (p-value 7.5×10^{-5}), and colocalized in nerve tissue (PP.H3 0.01, PP.H4 0.98). Finally, *HSF2* was differentially expressed in patients with constant-severe pain (p-value 5.85×10^{-6}) and colocalized in the skin (PP.H3 0.06, PP.H4 0.70).

Conclusion: Using TWAS and colocalization combines biological data with GWAS results, producing a more informed result than GWAS results alone. The appearance of *CTRC* in

the TWAS and colocalization is a replication of previous studies and *CTRC* should be the focus of future studies. Additionally, TWAS and colocalization identified new associations with pancreatitis pain in *HSF2* and *DOK6*.

4.2 Introduction

Pancreatitis is an inflammatory disorder of the pancreas with many etiologies that may irreversibly damage the tissue leading to organ dysfunction or failure (Mullady et al., 2011; Whitcomb et al., 2016; Whitcomb et al., 2012; Whitcomb & North American Pancreatitis Study, 2019). Pancreatitis often starts with episodes of acute pancreatitis (AP) that variably progresses to recurrent acute pancreatitis (RAP) and finally chronic pancreatitis (CP) (Ahmed Ali et al., 2016; Mullady et al., 2011; Whitcomb et al., 2016; Whitcomb et al., 2012). All stages of disease are accompanied by diminished physical and mental quality of life (QOL) and the major driver of these debilitating outcomes is pain (Amann et al., 2013; Cote et al., 2018; Machicado et al., 2017).

The most debilitating symptom of pancreatitis is severe, constant pain, which is seen in 1 out of 3 CP patients (Amann et al., 2013; Cote et al., 2018; Machicado et al., 2017; Mullady et al., 2011; Nordaas et al., 2022). Pancreatitis pain is especially challenging to manage as treatments using medications, endoscopy or surgery are often ineffective (E. K. Dunbar, Saloman, et al., 2021; Wilcox et al., 2014). Additionally, the pain experienced by RAP and CP patients varies in frequency, character, severity and chronicity even within patients with similar disease states (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021b; E. K. Dunbar, Saloman, et al., 2021; Mullady et al., 2011; Wilcox et al., 2014). Furthermore, pancreatitis pain does not correlate with abdominal imaging, exocrine pancreatic insufficiency (EPI) or other common features, suggesting

genetic and environmental risks affecting the immune system, the nervous system, psychosocial systems or complex combinations of multiple factors in multiple systems (Frøkjær et al., 2013; Olesen et al., 2021; Steinkohl et al., 2020; Wilcox et al., 2016). Some systems that may be involved in pancreatitis pain, include GABAergic, catecholaminergic, cytokines, growth factors, serotonergic, estrogenic, glutamatergic, proteinases, neurogenesis, nervous-system development, and neural connectivity (Johnston et al., 2019; Tsepilov et al., 2020; Zorina-Lichtenwalter et al., 2016).

Pain and suffering are complex concepts that are difficult to measure and phenotype because both sensory and emotional components contribute to the individual patient's experience. Although pain and poor QOL in pancreatitis are the most important clinical consideration for patients, insights into the underlying mechanisms of pancreatitis pain have been difficult to study beyond endoscopic or surgical drainage procedures for obstructed pancreatic ducts or more extensive surgeries including pancreatic resection and total pancreatectomy with islet autotransplantation (TPIAT). More systematic approaches include new patient reported outcomes (PRO) such as the COMPAT-SF (Kuhlmann et al., 2022) and quantitative sensory testing (QST) (Faghih et al., 2022; Phillips, Faghih, Kuhlmann, et al., 2020), but they alone do not adequately address the underlying mechanisms of aberrant pain responses, as they may have a significant genetic component.

Challenges to determining the effects of genetic variants on pain severity, quality, persistence and stress-associated psychiatric disorders include the availability of a limited number of well phenotyped patients with genetic material, and inclusion of adequate PROs and QST in population studies. Thus, the phenotype(s) are imprecise and subjective, the number of cases

within pancreatitis cohorts with or without pain features is limited, lowering study power, and there are few, if any comparison groups at the present time.

We have attempted to overcome these challenges using the following approaches. First, there are multiple genetic pain association studies *not* in the pancreas that can be leveraged to identify risk loci for both local and central nervous system (CNS) effects in pancreatitis-associated pain. Second, based on these studies, a candidate gene approach can be used to replicate pain phenotype loci in the pancreas. Candidate gene methods, as done previously for major depressive disorder (MDD), generalized anxiety disorder (GAD), and post-traumatic stress disorder (PTSD), can have less stringent significance thresholds since only small portions of the genome are being analyzed compared to genome-wide association studies (GWAS) (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021b). For example, threshold association levels for depression (MDD) genes overlapping with pancreatitis constant-severe pain was set at $p<0.0001$ (E. Dunbar et al., 2020), and for a formal analysis of GAD and PTSD candidate gene loci being associated with severe, constant-severe and constant pain loci was $p<0.002$ based on the number of genes/loci preselected as candidates (E. K. Dunbar, Greer, et al., 2021b). Third, genomic approaches including transcriptome-wide association studies (TWAS) and expression quantitative trait loci (eQTL)-tissue colocalization can be used, as demonstrated here, to leverage information about altered pain systems biology rather than relying only on the agnostic and study size-dependent statistical methods of GWAS.

4.3 Methods

4.3.1 Data

The North American Pancreatitis Study II (NAPS2) cohorts served as the primary data source. These cohorts included three consecutive cross-sectional, case-control studies of individuals with RAP, CP, and phenotyped controls (Conwell et al., 2017; Whitcomb et al., 2008; Wilcox et al., 2016). Phenotypes were recorded with standardized questionnaires and DNA was genotyped using Illumina Human-OmniExpress BeadChip and HumanCoreExome (E. Dunbar et al., 2020; Phillips et al., 2018; Whitcomb et al., 2012). The McCarthy Group pre-imputation checking tools were used to prepare data for imputation against the 1,000 genomes phase-3 reference panel on the Sanger imputation server using the EAGLE2+PBWT pipeline (E. Dunbar et al., 2020; Durbin, 2014; Loh et al., 2016; McCarthy et al., 2016). The genotypes were mapped on genome build CRCh37/hg19. Patients with RAP or CP of European Ancestry (EA) were analyzed as the initial genotyping array included variants from European Ancestry cohorts (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021b). Demographic data for patients are in Table 1 and Supplemental Tables S1, S2, and S3. These and other tables in this paper were generated using R (version 4.0.4) and the flextable package version 0.7.0 (Gohel, 2022; R Core Team, 2021).

4.3.2 Pain Categories

Pancreatitis pain patterns in the year prior to recruitment were described using Mullady's 6 severity-frequency patterns where O = no pain; A = episodes of mild pain; B = constant mild to moderate pain; C = episodes of severe pain; D = constant mild and episodes of severe pain; E =

constant severe pain (Mullady et al., 2011). As done previously, individuals responding with B, D, or E were categorized as *constant pain*, individuals responding with C, D, or E were categorized as *severe pain*, and individuals responding with D or E were *constant-severe pain* (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021a, 2021b). Sample sizes are reported in Table 1.

4.3.3 GWAS

Genome-wide association studies were performed using Plink 1.9 (Purcell et al., 2007). Since these are case-control studies, the data was fit to a logistic regression to test for associations. Covariates included age, sex, body mass index (BMI), principal components of ancestry 1-4, and a variable to control for differences across SNP chips. The threshold for minor allele frequency (MAF) was set to 0.01 and calculated with Plink 1.9 leaving 7,745,456 SNPs in the analysis (Purcell et al., 2007). The standard genome-wide levels of significance of $5x10^{-8}$ and suggestive significance of $1x10^{-5}$ were applied. Manhattan and QQ plots were generated in R (version 3.6.0) using the ggfastman package version 1.2 (R Core Team, 2019; Tremmel, 2021).

4.3.4 FUMA

Annotation of the GWAS results from Plink was done using FUMA online (Watanabe et al., 2017). The original GWAS results based on build hg19 were used for compatibility with FUMA. FUMA uses linkage disequilibrium (LD) at $r^2 \geq 0.6$ to identify candidate lead SNPs within candidate genomic loci. The reference panel used to calculate LD was 1000 Genomes Project Phase 3 European. Independent lead SNPs—and their genomic loci—with a p-value less than $1x10^{-5}$ are reported (Tables 2, 3, 4; Supplemental Tables S4, S5, S6).

4.3.5 TWAS

TWAS is a post-GWAS method that uses eQTLs to identify the genes that are predicted to have differential expression associated with the phenotype (Wainberg et al., 2019). Unlike GWAS which only captures information about *cis* loci, TWAS can capture *trans* effects and biological information of loci (Robinson et al., 2018).

TWAS was conducted using the MetaXcan family of tools (see https://github.com/hakyimlab/MetaXcan) (Barbeira et al., 2019). Auxiliary files necessary for the TWAS were downloaded from Zendo (Barbeira & Im, 2019). The calculations were conducted in a Python environment provided by the authors of MetaXcan. We used methods described in the GitHub Wiki to perform full harmonization with liftover to build hg38 from build hg19, imputation of summary statistics with GTEx-v8, S-PrediXcan on the provided 49 tissues using MetaMany, and finally S-MultiXcan to produce aggregated TWAS results across all tested tissues. All available tissues were used as pain receptors are in all tissues and pain induced expression changes may be found in the blood (Institute of Medicine Committee on Pain & Chronic Illness, 1987). The summary versions of the calculations were used as we used summary statistics from our GWAS. MASHR-M prediction models were used as these models include more biological information than prior models (Barbeira et al., 2021; Barbeira et al., 2019). The significance level of 2.8×10^{-6} used for the final step of the TWAS (S-MultiXcan) was Bonferroni corrected based on the tissue with the highest number of genes tested (Testis, 17,867 genes) as in the Barbeira et al 2019 paper (Barbeira et al., 2019). Associations with a p-value smaller than 1×10^{-4} were considered suggestive significant due to LD misspecification from using summary statistics and comparing to a reference set that may not match perfectly (Barbeira et al., 2019). Graphical representations of

results (Figures 1, 2, and 3) were generated in R (version 3.6.0) using the ggfastman package version 1.2 (R Core Team, 2019; Tremmel, 2021).

4.3.6 Colocalization

Colocalization is a statistical method to determine if a disease-associated phenotype and expression phenotype are due to the same SNPs within a locus (Wallace, 2021). We used Coloc for colocalization (Wallace, 2021) following instructions on the GitHub Wiki (https://chr1swallace.github.io/coloc/index.html). The colocalization was conducted in R (version 3.6.0) using the coloc version 5.1.1 package (R Core Team, 2019; Wallace, 2021). Coloc was performed on the significant gene-tissue pairs from S-MultiXcan. GTEx data downloaded from the GTEx Portal was used, specifically GTEx_Analysis_v8_eQTL_EUR.tar (https://storage.googleapis.com/gtex_analysis_v8/single_tissue_qtl_data/GTEx_Analysis_v8_eQTL_EUR.tar). This data included eQTLs per tissue using European Ancestry samples, which matches the ancestry of the NAPS2 data used here and the data used for the MASHR-M models from the TWAS (Barbeira et al., 2021). We used sample sizes per tissue reported in Supplementary Table 8 of a 2020 paper by the GTEx Consortium (GTEx Consortium, 2020). Samples sizes for our GWAS are reported in Table 1. The GWAS data formatted for the TWAS was used as it matches naming conventions of the GTEx data and contains all the SNPs included in the TWAS. SNPS from the GWAS were annotated to genes using Gencode v26, and SNPs within 1 Mbp up and downstream of the gene were included (Frankish et al., 2021; Kho et al., 2021). The function "coloc.abf" was used to conduct the colocalization using default priors. Coloc tests five hypotheses at any given locus using an Approximate Bayes Factor: 0) the null of no association with either trait (GWAS association signal and eQTL), 1) association with GWAS only, 2) association with

eQTL only, 3) association with both traits in two independent SNPs, and 4) association with both traits in one shared SNP (Giambartolomei et al., 2014). The coloc procedure produces posterior probabilities (PP) for each hypothesis, with the larger probability, closer to 1, lending more support for the hypothesis (Giambartolomei et al., 2014). Significant evidence of colocalization was a PP.H4 > 0.5, PP.H3 < 0.5, and PP.H0+PP.H1+PP.H3 < 0.3 (Giambartolomei et al., 2014). Significant colocalizations were visualized using locuscompare in R version (version 3.6.0) (Boxiang et al., 2019; R Core Team, 2019).

4.4 Results

The goal of this analysis pipeline is to identify genetic variants that alter expression of genes that have a biologically plausible mechanism of causing a more severe pain experience. Supplemental information from several intermediate steps in the analysis is provided here for quality assessment purposes. Several lead SNPs from the initial GWAS/FUMA analysis are highlighted here as possible candidate genes while further analysis highlights genomic predictions.

4.4.1 GWAS/FUMA

Manhattan plots and lead SNPs are given for the GWAS results for constant pain (Table 2; Supplemental Figure S1 and Table S4), constant-severe pain (Table 3; Supplemental Figure S3 and Table S5) and severe pain (Table 4; Supplemental Figure S5 and Table S6). Although none of the 7,745,456 SNPs tested reached independent genome-wide significance ($p<5x10^{-8}$), there were many suggestive significant hits with $p<1x10^{-5}$. The lower threshold was chosen as a screening

tool for *cis*-acting elements (e.g. genes within the same locus) noting that the closest gene to a SNP is correct about 70% of the time (Backman et al., 2021; Nasser et al., 2021; Pietzner et al., 2021) and that *post-hoc* candidate gene selection would be applied using a literature search. The lower-than-expected tails in the QQ plots suggests that the data contains false negatives or that any dominant genetic effect was undetectable (see Supplemental Figures S2, S4, and S6).

4.4.1.1 Constant Pain

GWAS/FUMA identified 13 genomic loci with 13 independent lead SNPs meeting suggestive significance in constant pain (Table 2). A review of the nearest gene(s) revealed multiple candidate genes associated with the constant pain phenotype (*SYNPR, NTF3, SLITRK6*).

The variant rs2060757C>T (MAF T=0.364 Allele Frequency Aggregator [ALFA] European (Phan et al., 2020)) is on chromosome 3 and intronic to *SYNPR* which codes for synaptoporin, an intrinsic membrane protein of small presynaptic vesicles in neuron projections (Chung et al., 2019; Safran et al., 2021; Sherry et al., 2001; Sun et al., 2006). It is known that there is central sprouting of nociceptive afferents in response to neural injury which enhances excitability of nociceptive pathways causing pain. Central expression of synaptophysin consistently represents synaptic terminations of projecting afferents, at least in part, including nociceptive Aδ- and C-fibers projecting to the dorsal horn (Chung et al., 2019; Sun et al., 2006). Thus, synaptoporin represents a plausible candidate gene for future studies relevant to constant pancreatitis pain in humans.

A chromosome 12 loci defined by rs10492094G>T (MAF T=0.324 ALFA European) is upstream to *NTF3* which codes for neurotrophin 3 (NT3). NT3 is a nerve growth factor that regulates the development and repair of the nervous system (Omar et al., 2022), is upregulated in the presence of inflammatory cytokines such as IL-1-β or TNF-α, and causes significant nerve

growth in cell cultures (Gruber et al., 2017). NT3 binds to the TrkC tyrosine kinase receptor; whereas, nerve growth factor (NGF) binds to TrkA and brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT4) bind to the TrkB receptor (Khan & Smith, 2015). Another lower affinity receptor, p75 neurotrophin receptor (p75NTR), is also expressed on some cells, including Schwann cells. TrkB and TrkC are co-expressed in most neurons in the CNS as well as neurons in the dorsal root ganglia (DRG) (Ateaque et al., 2022). Of note, a subset of chronic pancreatitis patients who undergo surgical resection of the pancreas have marked neural hypertrophy on histology that is associated with severe pain, while other CP patients do not for unknown reasons (Ceyhan et al., 2009; Demir et al., 2015).

The integrated role of NT3 in neurobiology is complex, as NT3 binds to TrkA and TrkB, and with higher affinity to TrkB than BDNF (Ateaque et al., 2022). In rodents, elevated NGF and BDNF are associated with neuropathic pain; whereas, NT3 generally appears to alleviate neuropathic pain (Khan & Smith, 2015). Furthermore, we previously identified variants associated with *BNDF* linked to constant pancreatic pain and general anxiety disorders using a candidate gene approach (E. K. Dunbar, Greer, et al., 2021b). The SNP rs1491851T>C has an eQTL for *BDNF-AS*, a long noncoding antisense RNA transcript with highest expression in the spinal cord, followed by brain and peripheral nerves (Lonsdale et al., 2013), that is a negative regulator of *BDNF* (Modarresi et al., 2012). Thus, genetic variants near *NTF3* are plausible candidates for differences in patient pain experience linked to variant neuronal response to recurrent and chronic pancreatitis possibly due to dysfunction of the TrkB/BDNF neuropathic pain control mechanisms.

A SNP on chromosome 13, rs117027346C>T (MAF T=0.036 ALFA European) is near *SLITRK6*, which codes for SLIT and NTRK-like protein 6 precursor. rs117027346 is a member of a very large haplotype that spans the *SLITRK6* gene. The protein shares homology with Trk

neurotrophin receptors (noted above) and appears to be associated with hearing and vision. There are no eQTLs listed on GTEx, but *SLITRK6* may be part of a co-expression network involved in voluntary movement and associated with neuropsychiatric phenotypes in mice (Chunduri et al., 2022). In neuronal cell cultures made from human iPS cells, *SLITRK6* expression responded to Zonisamide, an antiseizure drug being evaluated for neuropathic pain (Koshimizu et al., 2020), and survival of dopaminergic neurons was associated with *SLITRK6* expression levels (Miyawaki et al., 2020). We interpret this as a hypothesis generating finding, but several steps away from a strong candidate gene.

4.4.1.2 Constant-Severe Pain

In constant-severe pain, GWAS/FUMA identified 13 genomic loci and 14 independent lead SNPs associated with the phenotype (Table 3). In this analysis, we identified *SYNPO* as a plausible candidate gene associated with the constant-severe pain phenotype.

A chromosome 5 SNP, rs11745888C>T (MAF T=0.439 ALFA European), is annotated to the *SYNPO* gene that codes for synaptopodin. The splicing QTL for rs11745888C>T in GTEx is for *SYNPO* with $p=7.4x10^{-6}$ in tibial nerve (https://www.gtexportal.org/home/snp/rs11745888). Synaptopodin has known functions in kidney pseudopodia and the nervous system where it is essential for the formation of spine apparatuses in spines of telencephalic neurons involved in synaptic plasticity (Mundel et al., 1997). It also plays a role in epithelial cell apical stress biology (Morris et al., 2022). Elramah et al. recently demonstrated that, in a mouse model of cancer pain, upregulation of synaptopodin by downregulation of miR-124, an endogenous specific inhibitor of synaptopodin, resulted in severe cancer pain which was alleviated by intrathecal miR-124 infusion (Elramah et al., 2017). While miR-124 may have additional targets (J. Shi et al., 2022) the role of synaptopodin remains intriguing, especially based on the current association study. These data

suggest that altered expression of *SYNPO* should be a candidate mechanism for different pain experiences in pancreatitis patients.

4.4.1.3 Severe Pain

Severe pain had 11 genomic loci identified by GWAS/FUMA with 12 independent lead SNPs meeting suggestive significance (Table 4). Possible candidate genes for severe pain based on GWAS results are *COBL, RP11-37N22.1, RBFOX1, DOK6* and *LDLR*.

The chromosome 7 SNP rs757323G>A (MAF G=0.484 ALFA European) is 6kb 3' of or intronic to *COBL* (reverse direction) coding for the cordon-bleu WH2 repeat protein, a protein regulating intestinal microvilli (Grega-Larson et al., 2015) and neuron morphogenesis and increases branching of axons and dendrites (Ahuja et al., 2007; Hou et al., 2015; Qualmann & Kessels, 2021). *COBL* is highly expressed in the brain, muscles and peripheral nerves with low expression in the pancreas. An eQTL for rs757323 links to *COBL* (Lonsdale et al., 2013). One GWAS study of subjects of EA identified several SNPs near *COBL* associated with PTSD (Xie et al., 2013), but it has not previously been associated with pain making it an interesting gene to follow up on.

Chromosome 8 loci are tagged by rs12548675T>C (MAF T=0.233 ALFA European) that is intronic to uncharacterized *RP11-37N22.1*(*LOC101927588*). The SNP has no eQTLs on GTEx or HaploReg and is not part of a haplotype block with regulatory SNPs (Lonsdale et al., 2013; Ward & Kellis, 2016). However, the closest protein-coding gene, *TMEM65*, is about 95kb downstream of rs12548675. *TMEM65* codes for transmembrane protein 65. In a GWAS study *TMEM65* was associated with "fear of pain" (Randall et al., 2017) and was differentially methylated in chronic widespread pain syndrome (Burri et al., 2016) making it an interesting candidate for future studies.

RBFOX1 is a strong candidate gene on chromosome 16 linked to two independent SNPs. rs34109083A>G (MAF G=0.086 ALFA European) is a tag-SNP for a large haplotype spanning the entire *RBFOX1* gene (Sherry et al., 2001; Ward & Kellis, 2016). In addition, rs67176054G>A (MAF A=0.0017 ALFA European) is an intronic variant in *RBFOX1*. There are no eQTLs for SNPs in the tagged haplotype but extensive changes in DNA motifs at promoter and enhancer histone marks, but no DNAse, or protein binding site motif changes are described (HaploReg V4.1) (Ward & Kellis, 2016). Likewise, there are no eQTLs for rs67176054, but it does change the SMAD3 binding motif (Ward & Kellis, 2016).

RBFOX1 codes for RNA Binding Fox-1 Homolog 1 (Rbfox1) an RNA binding protein that regulates alternative splicing events by binding to 5'-UGCAUGU-3' elements. *RBFOX1* is highly expressed in brain (especially frontal cortex), muscle, heart and other tissues such as the kidney (Lonsdale et al., 2013). Rbfox1 appears to modify the post-transcriptional landscape of gene splice variants in response to stress as demonstrated in human renal proximal tubular epithelial cells (HK-2 cells) where exogenous Rbfox1 inhibited inflammation and oxidative stress to reduce hypoxia/reoxygenation-induced apoptosis of HK-2 cells (Lin et al., 2022). In the brain, Rbfox1 modifies the activity of synaptic regulators in response to neuronal activity, keeping excitability within healthy domains (Forastieri et al., 2022; Prashad & Gopal, 2021). For example, it modifies expression of the TrkB isoform, reducing binding of BDNF (Tomassoni-Ardori et al., 2019). Rbfox1 also modifies the transcriptional corepressor Lysine Specific Demethylase 1 (*LSD1/KDM1A*) isoforms. *LSD1* is a homeostatic immediate early genes (IEGs) regulator which plays a relevant part in the environmental stress-response (Forastieri et al., 2022). Based on several genetic associations of the alternative splicing regulator *RBFOX1* with psychiatric conditions and biological connections with *LSD1* and IEGs, Forastieri et al concluded that homeostatic unbalance

linked to these factors provides a neuronal signature of stress-associated psychiatric conditions (Forastieri et al., 2022). Indeed, genetic variants linked to *RBFOX1* have been associated with nicotine dependence (J. Chen et al., 2016; J. Chen et al., 2020), addiction to cocaine in mice (Feng et al., 2014), neuroticism and MDD (Zhang et al., 2021),and both autism (Bacchelli et al., 2020; Fernàndez-Castillo et al., 2020) and schizophrenia (J. Chen et al., 2016). To our knowledge, our study is the first to associate variants that are associated with *RBFOX1* with severe pain experience in pancreatitis.

The last two candidate genes, *DOK6* and *LDLR* are discussed below.

4.4.2 TWAS

There was one gene that reached Bonferroni corrected significance (p-value < $2.8x10^{-6}$) from the TWAS S-MultiXcan in constant (Supplemental Table S10) and constant-severe pain (Supplemental Table S11), *MAML1* (p-value 2.07e-7, and 4.99e-8 respectively), while *CTRC* (p-value 2.45e-5) and *NEURL3* (p-value 9.28e-6) met suggestive significance (p-value < $1x10^{-4}$) for constant pain (Figure 1 and 2). *CTRC* (p-value 4.5e-5), *HSF2* (p-value 5.85e-6) and *ZNF385D* (p-value 8.25e-5) met suggestive significance for constant-severe pain (Figure 2, Supplemental Table S11) and *LDLR* (p-value 6.53e-5) and *DOK6* (p-value 7.5e-5) met suggestive significance for severe pain (Figure 3, Supplemental Table S12). Below is a discussion of each of the TWAS identified genes including information aggregated from a post hoc literature search supporting the candidacy of each gene. Each reported gene shows differential expression across many tissues associated with the pain phenotype, also reported is the single tissue with the best p-value.

The results from our TWAS showed that *MAML1* was predicted to be differentially expressed in constant pain subjects (greatest effect seen in the heart), and in constant-severe pain

subjects (greatest effects seen in the cerebellar hemisphere of the brain). *MAML1*, mastermind like transcriptional coactivator 1, codes for the human version of the *Drosophila* mastermind protein, which is involved with Notch signaling (Murphy et al., 2006). *MAML1* is heavily involved in protein transcription and regulation in humans including the NOTCH signaling pathway, Hippo signaling, NF-κB, and Sonic Hedgehog (Hamidi et al., 2022; Safran et al., 2021). Differential expression of *MAML1* can result in changes in regulation of NOTCH which can then result in human cancer including gastric cancer (Hamidi et al., 2022).

Our TWAS suggests that *CTRC* is differentially expressed in patients with constant pain and constant-severe pain with the greatest effect seen in the pancreas. *CTRC* codes for chymotrypsin C, a pancreatic digestive enzyme that plays an important role in protecting the pancreas from trypsin-associated injury by cooperating in the proteolytic destruction of the trypsin molecule (Rosendahl et al., 2008). *CTRC* is expressed almost exclusively in the pancreas. Loss of function or lowered expression of *CTRC* is a major risk factor for chronic pancreatitis, with the most commonly seen risk haplotype defined by rs497078C>T (p.G60G) (MAF T=0.092 ALFA European) that is strongly associated with reduced function ($p = 3.2x10^{-14}$) (Lonsdale et al., 2013; Sherry et al., 2001; Zator & Whitcomb, 2017). Thus, it is plausible that constant and constant-severe pancreatic pain are associated with continued, subclinical, trypsin-associated inflammation. It is interesting that differential expression of *CTRC* is suggestively associated with both constant and constant-severe pain, whereas variants altering *SPINK1* expression (haplotype tagged by rs17107315T>C [MAF C=0.0098 ALFA European] p.Asn34Ser/N34S) coding for another trypsin inhibitor, are not. Our study is likely underpowered to detect effects of altered *SPINK1* expression because the MAF of the common risk haplotype of *SPINK1* is 10% of the common *CTRC* risk haplotype mentioned above.

The TWAS conducted in our study suggestively predicted differential expression of *NEURL3* in patients with constant pain with greatest effect in the substantia nigra of the brain. *NEURL3*, neuralized E3 ubiquitin protein ligase 3 formally known as *LINCR*, is involved in protein ubiquitination and is primarily expressed in salivary glands and pancreas (Lonsdale et al., 2013; Murphy et al., 2006; Safran et al., 2021). NEURL3 is involved in cellular mechanisms involved in spinal assembly (Ashburner et al., 2000; Consortium, 2021). Increased expression of NEURL3 has been seen in lung tissue in response to inflammation from endotoxemia (Hu et al., 2005). Involvement of *NEURL3* in response to inflammation may be important in constant pancreatitis pain.

Our TWAS identified suggestive differential expression of *HSF2* in patients with constant-severe pain with the best reported tissue from the TWAS being skin not sun exposed suprapubic. *HSF2* codes a heat shock factor (HSF) protein, heat shock transcription factor 2, and is highly expressed in the brain (Murphy et al., 2006). HSF2 is a transcription factor involved in chromatin condensing especially during miosis; therefore, *HSF2* is involved in the regulation of the cell cycle (Tokunaga et al., 2022). HSF2 is activated by hemin rather than heat (Tokunaga et al., 2022). Additionally, HSF2 also activates the transcription of some genes in response to oxidative stress, similar to what is seen in acinar and duct cells (Himanen et al., 2022) making it an interesting candidate for follow up studies into pancreatitis pain.

The TWAS conducted from our study predicted suggestive differential expression of *ZNF385D* in patients with constant-severe pain most significantly in the aorta. *ZNF385D* codes for zinc finger protein 385D and is mostly expressed in the brain (Murphy et al., 2006; Safran et al., 2021). In a GWAS of placebo and duloxetine response in major depressive disorder, suggestive associations with *ZNF385D* (rs4261893; β=-0.46, $p=1.55\times10^{-5}$) was observed within the

duloxetine-treated subsample (Maciukiewicz et al., 2018). *ZNF385D* may be a viable candidate for future studies into drug response within pancreatitis patients.

Differential expression of *LDLR* was suggestively predicted in patients with severe pain by the current TWAS with greatest effect seen in the artery. *LDLR* codes the low density lipoprotein receptor which is normally a cell surface protein (Murphy et al., 2006; Safran et al., 2021). Mutations in this gene are associated with familial hypercholesterolemia (Rodríguez-Nóvoa et al., 2020).

Differential expression of *DOK6* was suggestively predicted in patients with severe pain in pancreatitis—greatest effect in nerve tissue. *DOK6*, docking protein 6, is involved in the RET signaling cascade and is primarily expressed in the brain (Murphy et al., 2006). RET signaling is key to axon guidance and neuron development (Enomoto et al., 2001). Lowered expression of *DOK6* within gastric cancer tissues was associated with higher survivability (Leong et al., 2017). The RET signaling cascade is a good candidate system for follow up studies in pancreatitis pain.

4.4.3 Colocalization

Colocalization was performed on the gene-tissue pairs identified from the TWAS (see Table 5, Supplemental Tables S10, S11, S12). Results are in Table 5. For constant pain, *CTRC* the non-significant GWAS signals colocalized with the eQTL in pancreas tissue (Supplemental Figure S7). In constant pain, *CTRC* had a PP.H4 0.73 and a PP.H3 0.02, suggesting that the signals colocalize to one SNP. Additionally, the sum of PP.H0-PP.H2 (0.25) was less than 0.3, suggesting strong evidence of colocalization meaning that even though the GWAS signals for *CTRC* were not significant these signals were likely due to the same SNP as the eQTL. The eQTL and constant-severe pain GWAS signals colocalized in *HSF2* (PP.H4 0.7, PP.H3 0.06, PP.H0+PP.H1+PP.H2

0.24) in skin not sun exposed suprapubic (Supplemental Figure S8). Finally, the signals from *DOK6* (PP.H4 0.98, PP.H3 0.01, PP.H0+PP.H1+PP.H2 0.01) colocalized in nerve tibial tissue in severe pain (Supplemental Figure S9).

4.5 Discussion

Pancreatitis pain can be devastating both mentally and physically, and difficult to treat (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021b; E. K. Dunbar, Saloman, et al., 2021; Mullady et al., 2011; Wilcox et al., 2014). Therefore, precision treatments based on individual patients' genetics is needed. This work adds new loci to the previously identified psychiatric loci, moving a step closer to new precision treatments (E. Dunbar et al., 2020; E. K. Dunbar, Greer, et al., 2021b; E. K. Dunbar, Saloman, et al., 2021).

Although the exact biological mechanism of chronic pain is unknown, some pathways in common to pain include: GABAergic, catecholaminergic, cytokines, growth factors, serotonergic, estrogenic, glutamatergic, proteinases, neurogenesis, nervous-system development, and neural connectivity (Johnston et al., 2019; Tsepilov et al., 2020; Zorina-Lichtenwalter et al., 2016). Many of the candidate genes discussed above are involved in nervous-system development, growth, and connectivity (*NFT3*, *DOK6*, *COBL*, *SLITRK6*, *SYNPO*), which focuses attention on these pathway candidates for the severe pancreatitis pain experience. The *BDNF* pathway also appears to be important for pancreatitis pain as well as anxiety (E. K. Dunbar, Greer, et al., 2021b). The genes and pathways identified here should be the focus of future research into precision treatments of pancreatitis pain.

Taking GWAS results and incorporating biological information using TWAS increases the power of the results and ability to identify genetic findings that would be underpowered in GWAS alone. The final addition of statistical colocalization tests and confirms that the overlap of the GWAS signal and the eQTL in a locus is not random and that they are not independent. To our knowledge, this is the first application of TWAS and colocalization to pancreatitis pain.

4.6 Limitations

The limitations of this work include using only individuals of European Ancestry. Additionally, this study is slightly underpowered due to small sample sizes. In prior studies, candidate gene methods were used to alleviate low power issues. Here the extensive post-GWAS methods, TWAS and colocalization, were used to provide additional biologically informed results using the data available to us.

The TWAS uses expression data from GTEx (Lonsdale et al., 2013) to predict which genes may be differentially expressed in patients with more severe pancreatitis pain. GTEx uses tissues harvested postmortem to study gene expression (Lonsdale et al., 2013). The “normal” expression that the prediction models use is therefore limited to the biological conditions of the tissues when they were harvested which may not be an accurate representation of the expression profile of our patients. This is one reason that candidate genes from the GWAS are not identified by the TWAS. However, given the incorporation of biological information TWAS is better suited to predict candidate genes than an underpowered GWAS was able to detect.

4.7 Conclusion

Of all the candidate genes identified and discussed above *CTRC*, *DOK6*, and *HSF2* survived the entire analysis pipeline. *CTRC* was not identified as significant in the GWAS for constant pain; however, it was identified as being suggestively differentially expressed across many tissues with the most significant tissue being pancreas tissue via TWAS. Additionally, the eQTL and GWAS signals colocalize, confirming the importance of *CTRC* in constant pancreatitis pain. Conversely, *DOK6* was identified in the GWAS of severe pain as suggestively significant. It was then identified as being differentially expressed across many tissues with the best evidence in nerve tissue by the TWAS. Finally, the eQTL signal from *DOK6* does colocalize with the GWAS signal. The final gene that colocalized was *HSF2* in constant-severe pain. *HSF2* did not have a suggestive significant GWAS signal; however, it is located within the *PKIB* locus identified by FUMA. The TWAS found that *HSF2* was differentially expressed across many tissues but most significantly in skin. Next steps are to confirm these findings in models of pancreatitis, replicate the results in larger sample sizes, and use individuals of non-European ancestry.

4.8 Tables and Figures

Table 4-1 Aim 3 Table 1 Sample Sizes of Pain GWAS

Pain	Variable	Cases	Controls	Total
Constant	**Sample Size**	504	750	1,254
	Etiology: Alcohol Alone	165	149	314
	Etiology: Alcohol Plus	47	92	139
	Etiology: Genetic	56	64	120
	Etiology: Idiopathic	117	232	349
	Etiology: Obstructive	46	81	127
	Etiology: Autoimmune	10	16	26
	Etiology: Hyperlipidemia	23	21	44
	Etiology: Gallstone	10	31	41
	Etiology: Medications	3	5	8
	Etiology: Other	26	56	82
	Etiology: Missing	1	3	4
	Sex: Male	238	398	636
	Sex: Female	266	352	618
Constant-Severe	**Sample Size**	450	804	1,254
	Etiology: Alcohol Alone	149	165	314
	Etiology: Alcohol Plus	42	97	139
	Etiology: Genetic	51	69	120
	Etiology: Idiopathic	100	249	349
	Etiology: Obstructive	44	83	127
	Etiology: Autoimmune	10	16	26
	Etiology: Hyperlipidemia	20	24	44
	Etiology: Gallstone	9	32	41
	Etiology: Medications	2	6	8
	Etiology: Other	22	60	82
	Etiology: Missing	1	3	4
	Sex: Male	210	426	636
	Sex: Female	240	378	618
Severe	**Sample Size**	727	527	1,254
	Etiology: Alcohol Alone	219	95	314
	Etiology: Alcohol Plus	73	66	139
	Etiology: Genetic	81	39	120
	Etiology: Idiopathic	173	176	349
	Etiology: Obstructive	67	60	127
	Etiology: Autoimmune	12	14	26
	Etiology: Hyperlipidemia	29	15	44
	Etiology: Gallstone	19	22	41
	Etiology: Medications	3	5	8
	Etiology: Other	50	32	82
	Etiology: Missing	1	3	4
	Sex: Male	374	262	636
	Sex: Female	353	265	618

Table 4-2 Aim 3 Table 2
FUMA Independent Lead SNPS for Constant Pain

GenomicLocusRegion[a]	rsID	chr	pos	p[b]	nGWASSNPs[c]	nearestGene
1:40814528-40823404	rs4660406	1	40,823,404	3.69e-06	5	*SMAP2*
3:63441514-63455599	rs2060757	3	63,455,599	9.01e-06	5	*SYNPR:SYNPR-AS1*
4:184484425-184505515	rs10009455	4	184,494,883	8.80e-06	16	*ING2*
5:49435222-49435222	rs149312484	5	49,435,222	3.05e-06	1	*EMB*
7:47565793-47575970	rs334527	7	47,567,227	1.51e-06	13	*TNS3*
8:138803133-138806916	rs66890414	8	138,803,658	8.64e-06	3	*FAM135B*
12:5441541-5487932	rs10492094	12	5,478,148	3.52e-06	3	*NTF3*
13:20847066-20866839	13:20855444[C,T]	13	20,855,444	3.17e-06	35	*GJB6*
13:86362179-86565426	rs117027346	13	86,362,179	7.69e-06	100	*SLITRK6*
13:103580361-103606829	rs701545	13	103,580,541	1.51e-07	3	*METTL21EP*
16:81238750-81264177	rs111271001	16	81,259,428	6.19e-06	21	*PKD1L2*
19:295231-295295	rs734885	19	295,231	7.50e-06	2	*PPAP2C (PLPP2)*
20:62200860-62263747	rs6062978	20	62,256,590	8.20e-06	6	*GMEB2*

[a]chr:start-end based on hg19
[b]GWAS p value
[c]Number of GWAS SNPs in LD with lead SNP

Table 4-3 Aim 3 Table 3
FUMA Independent Lead SNPs for Constant-Severe Pain

GenomicLocusRegion[a]	rsID	chr	pos	p[b]	nGWASSNPs[c]	nearestGene
1:54896755-54922021	rs4927113	1	54,902,861	5.08e-06	15	*SSBP3*
3:148698474-148876261	rs58186391	3	148,839,366	1.54e-06	53	*HPS3*
5:49435222-49435222	rs149312484	5	49,435,222	9.35e-06	1	*EMB*
5:149954864-149990727	rs11745888	5	149,968,929	3.91e-06	40	*SYNPO*
6:122429305-122921183	rs9388097	6	122,885,461	9.60e-06	97	*PKIB*
6:122429305-122921183	rs76046919	6	122,903,206	2.73e-06	6	*PKIB*
7:47565793-47575970	rs334527	7	47,567,227	2.59e-07	13	*TNS3*
8:138803133-138806916	rs66890414	8	138,803,658	6.27e-06	3	*FAM135B*
11:116519655-116519655	rs516226	11	116,519,655	6.39e-06	1	*AP000770.1*
12:5284122-5315245	rs645410	12	5,301,847	4.71e-06	17	*RP11-319E16.1*
12:12963744-12990341	rs17394079	12	12,990,341	8.41e-06	10	*DDX47*
14:46976743-46986881	rs7161256	14	46,976,743	1.68e-06	2	*LINC00871*
15:93892942-93908051	rs7167068	15	93,893,035	7.14e-07	4	*RGMA*
19:11221180-11232696	rs35878749	19	11,229,765	7.26e-06	10	*LDLR*

[a]chr:start-end based on hg19
[b]GWAS p value
[c]Number of GWAS SNPs in LD with lead SNP

Table 4-4 Aim 3 Table 4
FUMA Independent Lead SNPs for Severe Pain

GenomicLocusRegion[a]	rsID	chr	pos	p[b]	nGWASSNPs[c]	nearestGene
1:213685950-213755621	rs530848	1	213,732,214	4.45e-06	27	*RPL31P13*
2:78764895-78837866	rs1915703	2	78,832,777	4.70e-06	11	*CYCSP6*
3:109525798-109681921	rs75623530	3	109,672,395	4.33e-06	30	*MIR4445*
6:155022713-155160128	rs7771767	6	155,038,479	8.18e-06	76	*SCAF8*
7:51035899-51079151	rs757323	7	51,077,759	4.54e-06	6	*COBL*
8:125224719-125224719	rs12548675	8	125,224,719	1.39e-06	1	*RP11-37N22.1*
9:139614170-139642961	rs2275160	9	139,621,168	6.76e-06	10	*SNHG7*
16:7353976-7417955	rs67176054	16	7,371,066	6.34e-07	17	*RBFOX1*
16:7353976-7417955	rs34109083	16	7,380,549	1.12e-06	53	*RBFOX1*
18:67306031-67327598	rs11663004	18	67,324,345	8.75e-06	26	*DOK6*
19:11221180-11232696	rs35878749	19	11,229,765	9.45e-07	10	*LDLR*
19:27947716-28309577	rs62111935	19	27,992,394	7.79e-06	93	*LINC00662*

[a]chr:start-end based on hg19
[b]GWAS p value
[c]Number of GWAS SNPs in LD with lead SNP

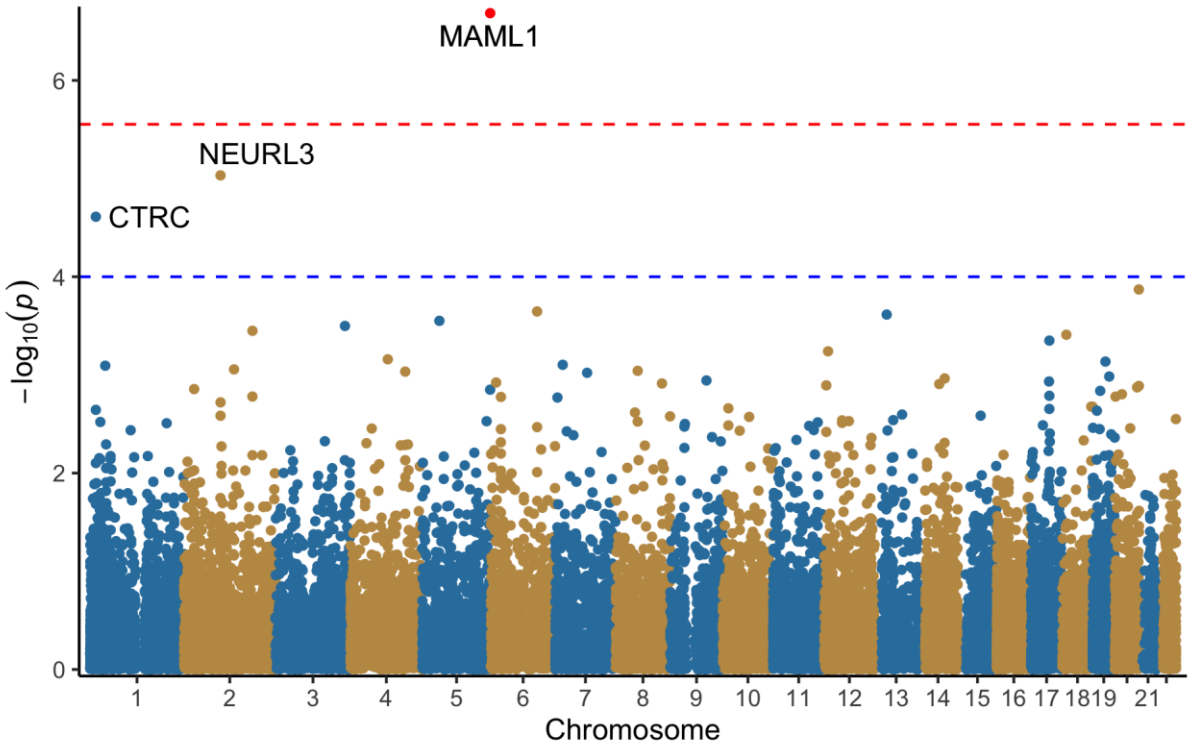

Figure 4-1 Aim 3 Figure 1

S-MultiXcan Results for Constant Pain. Red line: p=2.8e-06. Blue line: p=1.0e-04.

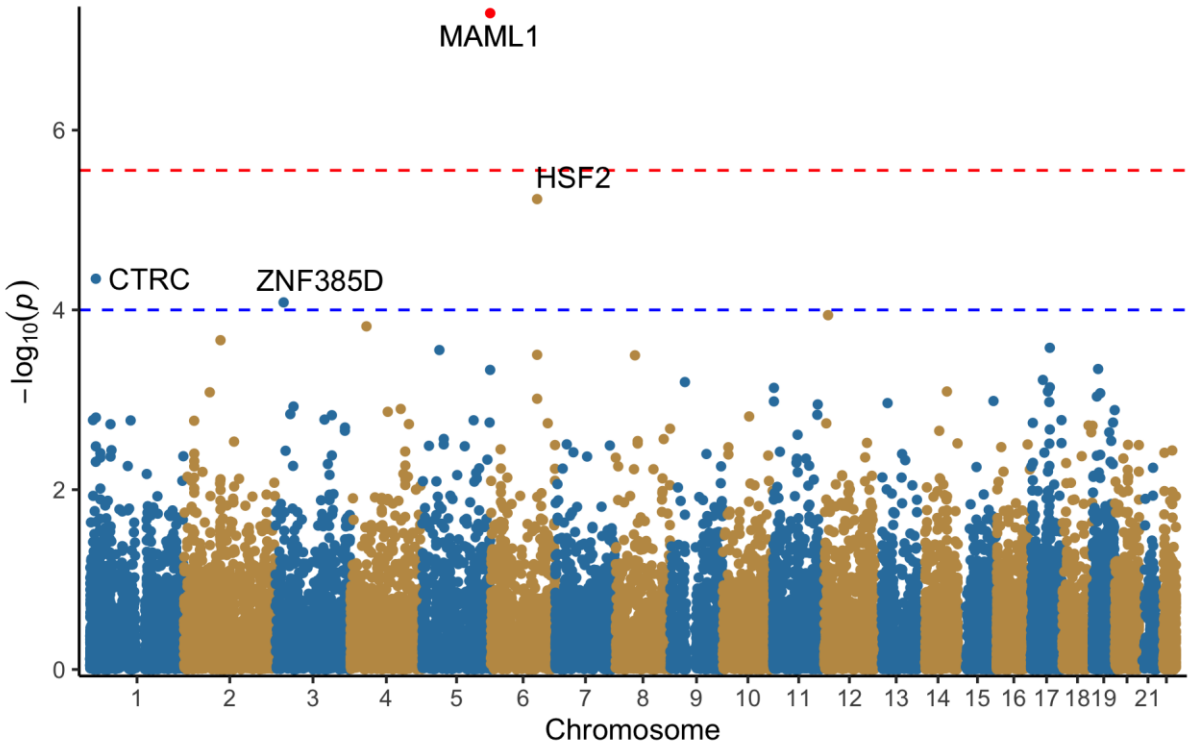

Figure 4-2 Aim 3 Figure 2

S-MultiXcan Results for Constant-Severe Pain. Red line: p=2.8e-06. Blue line: p=1.0e-04.

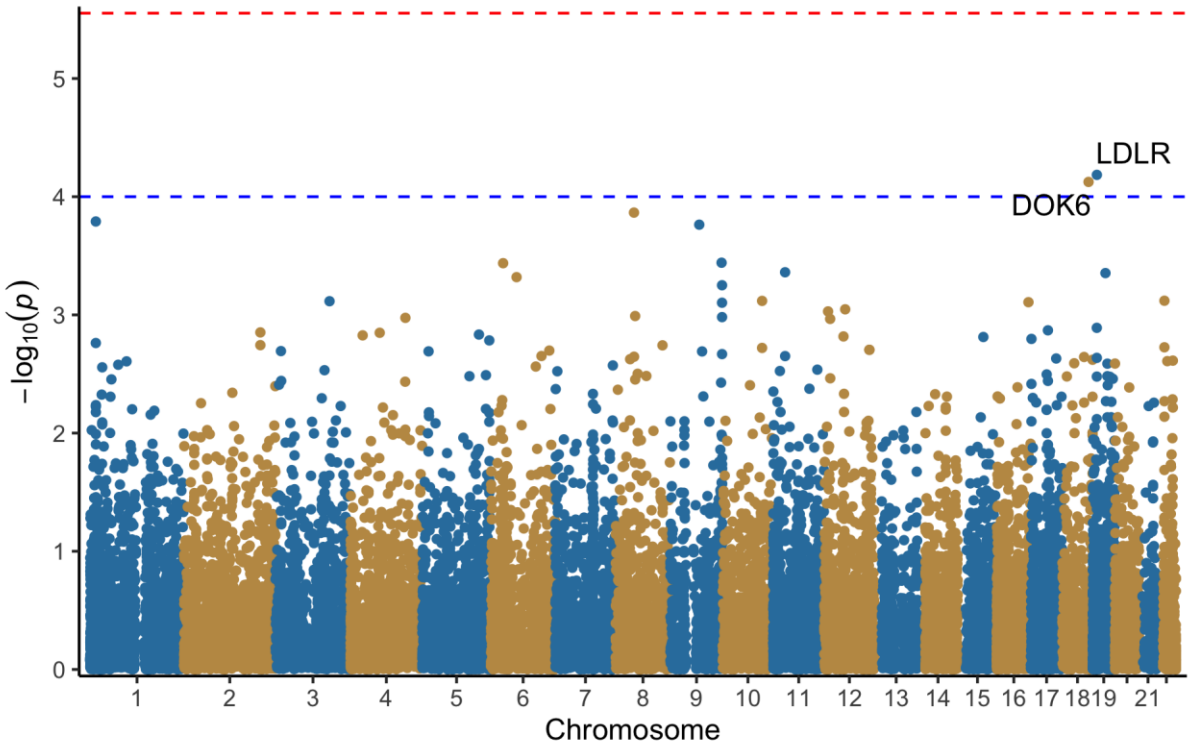

Figure 4-3 Aim 3 Figure 3

S-MultiXcan Results for Severe Pain. Red line: p=2.8e-06. Blue line: p=1.0e-04.

Table 4-5 Aim 3 Table 5
Coloc Results for Pain GWAS

Pain		Gene	Tissue	nsnps	PP.H0	PP.H1	PP.H2	PP.H3	PP.H4	Colocalization[1]
	Constant	*MAML1*	Heart Left Ventricle	16	0.73	1.59e-03	0.24	4.87e-04	0.03	No
		NEURL3	Brain Substantia Nigra	1	1.00	3.38e-04	2.40e-03	0	8.13e-04	No
		CTRC	Pancreas	30	5.45e-03	3.60e-04	0.25	0.02	0.73	**Yes**
	Constant-Severe	*MAML1*	Brain Cerebellar Hemisphere	1	1.00	1.24e-04	2.50e-04	0	3.10e-05	No
		HSF2	Skin Not Sun Exposed Suprapubic	69	0.05	0.01	0.18	0.06	0.70	**Yes**
		ZNF385D	Artery Aorta	51	6.52e-03	1.44e-04	0.69	0.02	0.29	No
		CTRC	Pancreas	30	0.01	2.70e-04	0.46	0.01	0.51	No
	Severe	*LDLR*	Artery Tibial	1	1.00	1.16e-04	6.02e-04	0	6.97e-05	No
		DOK6	Nerve Tibial	17	2.07e-03	2.77e-03	9.00e-03	0.01	0.98	**Yes**

[1]Evidence of Colocalization taken to be PP.H4 > 0.5 and PP.H3 <0.5, PP.H0+PP.H1+PP.H2<0.3.

4.9 Supplemental Tables and Figures

Table 4-6 Aim 3 Table S1
Demographics of Constant Pain

Variable	Level	Controls (n=750)[a]	Cases (n=504)[b]	Total (n=1254)	p-value[c]
Age at Ascertainment	Mean (SD)	51.3 (16.9)	46 (13.4)	49.2 (15.8)	2.81e-09
Sex	Male	398 (53.1%)[d]	238 (47.2%)	636 (50.7%)	0.05
	Female	352 (46.9%)	266 (52.8%)	618 (49.3%)	
Diagnosis	CP	441 (58.8%)	373 (74.0%)	814 (64.9%)	4.44e-08
	RAP	309 (41.2%)	131 (26.0%)	440 (35.1%)	
BMI[e]	Mean (SD)	26 (6.1)	25.7 (6)	25.9 (6.1)	0.35
Mental QOL[f]	Mean (SD)	47.3 (10.6)	38.8 (11.7)	43.7 (11.8)	9.17e-37
	Missing	96	24	120	

[a]Patients without pain
[b]Patients with pain
[c]Pearson chi-squared for categorical; t test for continuous; two-tailed $p < 0.05$ considered significant
[d]Percentages shown next to counts are column percentages within each variable
[e]Body Mass Index
[f]Quality of Life

Table 4-7 Aim 3 Table S2
Demographics of Constant-Severe Pain

Variable	Level	Controls (n=804)[a]	Cases (n=450)[b]	Total (n=1254)	p-value[c]
Age at Ascertainment	Mean (SD)	51 (16.7)	46 (13.5)	49.2 (15.8)	8.08e-08
Sex	Male	426 (53.0%)[d]	210 (46.7%)	636 (50.7%)	0.04
	Female	378 (47.0%)	240 (53.3%)	618 (49.3%)	
Diagnosis	CP	486 (60.4%)	328 (72.9%)	814 (64.9%)	1.26e-05
	RAP	318 (39.6%)	122 (27.1%)	440 (35.1%)	
BMI[e]	Mean (SD)	26 (6.1)	25.7 (6.1)	25.9 (6.1)	0.37
Mental QOL[f]	Mean (SD)	46.6 (10.9)	39 (11.8)	43.7 (11.8)	5.37e-28
	Missing	101	19	120	

[a]Patients without pain
[b]Patients with pain
[c]Pearson chi-squared for categorical; t test for continuous; two-tailed $p < 0.05$ considered significant
[d]Percentages shown next to counts are column percentages within each variable
[e]Body Mass Index
[f]Quality of Life

Table 4-8 Aim 3 Table S3
Demographics of Severe Pain

Variable	Level	Controls (n=527)[a]	Cases (n=727)[b]	Total (n=1254)	p-value[c]
Age at Ascertainment	Mean (SD)	52.1 (16.1)	47.1 (15.2)	49.2 (15.8)	1.68e-08
Sex	Male	262 (49.7%)[d]	374 (51.4%)	636 (50.7%)	0.58
	Female	265 (50.3%)	353 (48.6%)	618 (49.3%)	
Diagnosis	CP	311 (59.0%)	503 (69.2%)	814 (64.9%)	2.46e-04
	RAP	216 (41.0%)	224 (30.8%)	440 (35.1%)	
BMI[e]	Mean (SD)	26.1 (6.2)	25.7 (6)	25.9 (6.1)	0.29
Mental QOL[f]	Mean (SD)	46.7 (11)	41.8 (12)	43.7 (11.8)	4.03e-12
	Missing	88	32	120	

[a]Patients without pain
[b]Patients with pain
[c]Pearson chi-squared for categorical; t test for continuous; two-tailed $p < 0.05$ considered significant
[d]Percentages shown next to counts are column percentages within each variable
[e]Body Mass Index
[f]Quality of Life

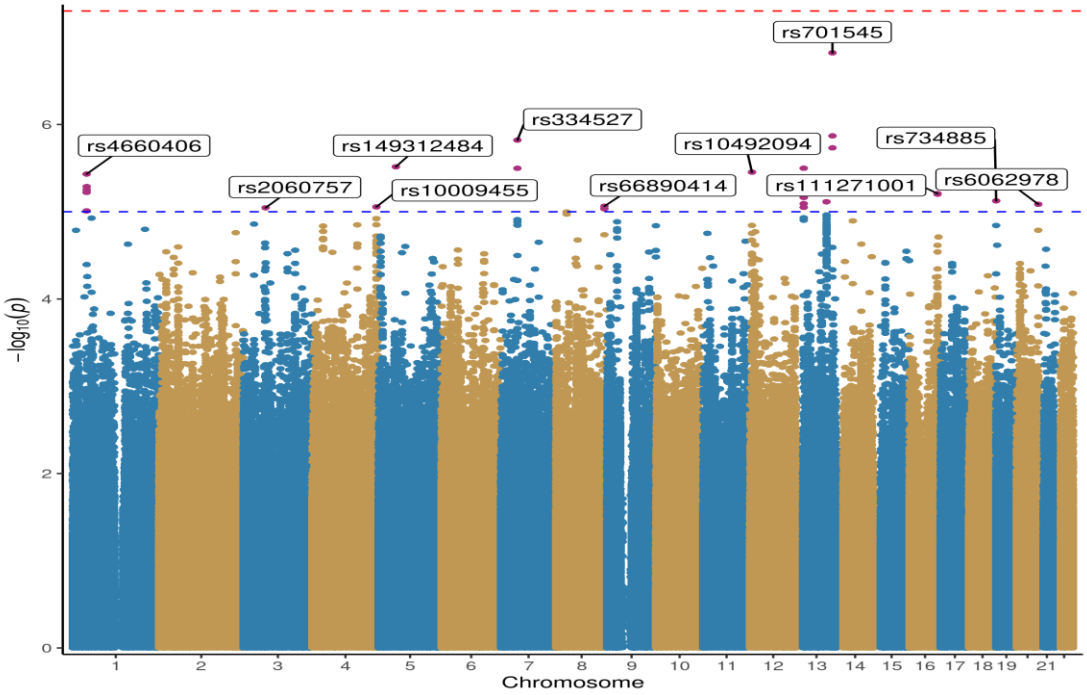

Figure 4-4 Aim 3 Figure S1

Manhattan Plot for Constant Pain.

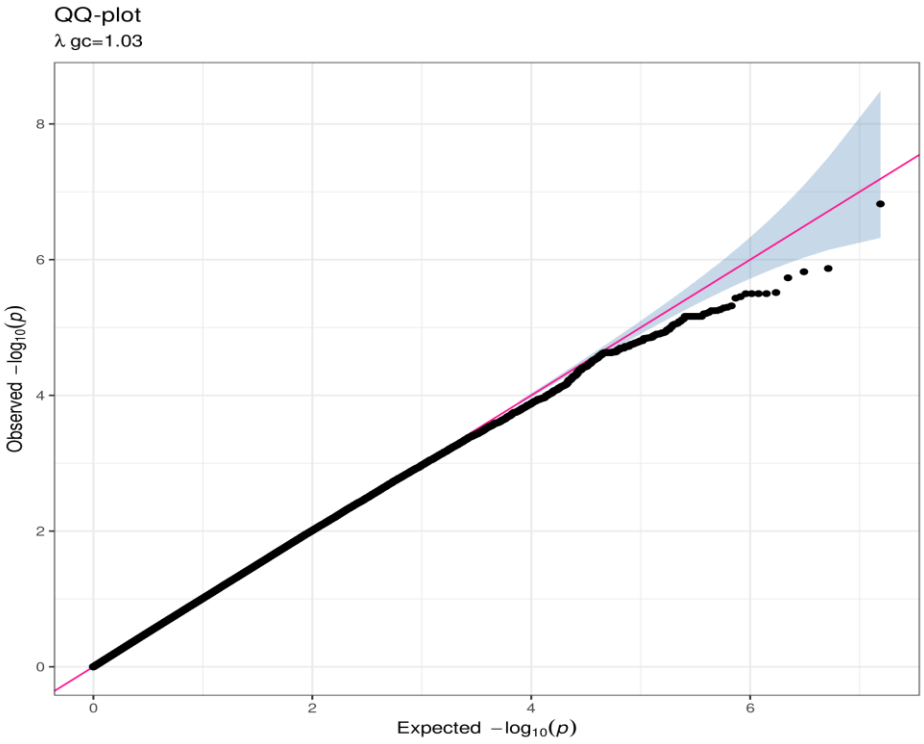

Figure 4-5 Aim 3 Figure S2

QQ plot for Constant Pain.

Table 4-9 Aim 3 Table S4
SNPs Meeting Suggestive Significance (1e-5) for Constant Pain

CHR	BP[1]	SNP[2]	OR	L95[3]	U95[4]	SE	P	A1[5]	A2[6]	MAF[7]
1	40,814,528	rs7522271	1.52	1.269	1.821	0.0922	5.62e-06	C	T	0.347
1	40,815,975	rs2036197	1.505	1.261	1.796	0.0903	5.99e-06	G	T	0.368
1	40,819,094	rs4660162	1.491	1.249	1.78	0.0903	9.78e-06	G	A	0.366
1	40,822,421	rs4660405	1.511	1.265	1.804	0.0905	5.15e-06	C	T	0.367
1	40,823,404	rs4660406	1.52	1.273	1.814	0.0904	3.69e-06	C	T	0.368
3	63,455,599	rs2060757	0.6704	0.5619	0.7999	0.0901	9.01e-06	T	C	0.317
4	184,494,883	rs10009455	0.453	0.3195	0.6423	0.178	8.80e-06	G	A	0.0466
5	49,435,222	rs149312484	0.6283	0.5169	0.7637	0.0996	3.05e-06	A	G	0.205
7	47,565,793	rs734899	0.6631	0.5578	0.7882	0.0882	3.18e-06	G	A	0.375
7	47,567,227	rs334527	0.649	0.5442	0.774	0.0899	1.51e-06	C	T	0.326
7	47,571,488	7:47571488[A,C]	0.6729	0.5678	0.7974	0.0866	4.80e-06	A	C	0.373
8	138,803,133	rs36014323	0.5803	0.4562	0.7381	0.123	9.24e-06	G	A	0.123
8	138,803,658	rs66890414	0.5786	0.4546	0.7363	0.123	8.64e-06	T	C	0.122
12	5,478,148	rs10492094	0.6594	0.553	0.7862	0.0898	3.52e-06	T	G	0.282
13	20,850,871	rs945371	0.5864	0.4638	0.7413	0.12	8.09e-06	G	C	0.125
13	20,853,876	rs7327840	0.5876	0.466	0.7408	0.118	6.85e-06	C	G	0.13
13	20,853,886	rs7326549	0.5876	0.466	0.7408	0.118	6.85e-06	G	A	0.13
13	20,853,986	rs7333853	0.5876	0.466	0.7408	0.118	6.85e-06	T	G	0.13
13	20,854,161	rs9552126	0.5876	0.466	0.7408	0.118	6.85e-06	C	T	0.13
13	20,854,191	rs9552127	0.5876	0.466	0.7408	0.118	6.85e-06	A	G	0.13
13	20,854,299	rs9552128	0.5876	0.466	0.7408	0.118	6.85e-06	G	A	0.13
13	20,854,384	rs9552129	0.5876	0.466	0.7408	0.118	6.85e-06	A	G	0.13
13	20,854,763	rs2050500	0.5846	0.4643	0.7362	0.118	5.03e-06	A	G	0.13
13	20,854,910	13:20854910[C,T]	0.5777	0.4586	0.7277	0.118	3.17e-06	C	T	0.13
13	20,855,039	rs2050498	0.5876	0.466	0.7408	0.118	6.85e-06	T	C	0.13
13	20,855,095	rs4594101	0.5876	0.466	0.7408	0.118	6.85e-06	C	T	0.13
13	20,855,131	rs2050497	0.5876	0.466	0.7408	0.118	6.85e-06	A	G	0.13
13	20,855,428	rs9552130	0.5777	0.4586	0.7277	0.118	3.17e-06	C	T	0.13
13	20,855,444	13:20855444[C,T]	0.5777	0.4586	0.7277	0.118	3.17e-06	C	T	0.13
13	20,857,149	rs7329495	0.5836	0.4627	0.7361	0.118	5.44e-06	C	G	0.129
13	20,866,631	rs2152451	0.5921	0.4698	0.7461	0.118	8.90e-06	C	G	0.131
13	20,866,838	rs2152446	0.585	0.4641	0.7374	0.118	5.65e-06	A	C	0.131
13	20,866,839	rs2152445	0.585	0.4641	0.7374	0.118	5.65e-06	C	G	0.131
13	86,362,179	rs117027346	0.2706	0.1526	0.4798	0.292	7.69e-06	T	C	0.0149
13	103,580,361	13:103580361[A,G]	0.6597	0.5561	0.7827	0.0872	1.85e-06	G	A	0.357
13	103,580,541	rs701545	0.6397	0.5415	0.7558	0.0851	1.51e-07	C	G	0.395
13	103,606,829	rs766223	1.503	1.274	1.773	0.0843	1.35e-06	G	A	0.529
16	81,259,428	rs111271001	0.559	0.4344	0.7194	0.129	6.19e-06	G	A	0.106
16	81,260,855	rs113101650	0.5571	0.4322	0.7181	0.13	6.29e-06	T	G	0.103
19	295,231	rs734885	1.927	1.446	2.567	0.146	7.50e-06	A	G	0.12
20	62,256,590	rs6062978	1.618	1.31	1.999	0.108	8.20e-06	A	G	0.226

[1]hg19 Base Pair
[2]Blue SNPs were Identified as Lead SNPs by FUMA
[3]Lower 95% Confidence Interval
[4]Upper 95% Confidence Interval
[5]Minor Allele
[6]Major Allele
[7]Minor Allele Frequency

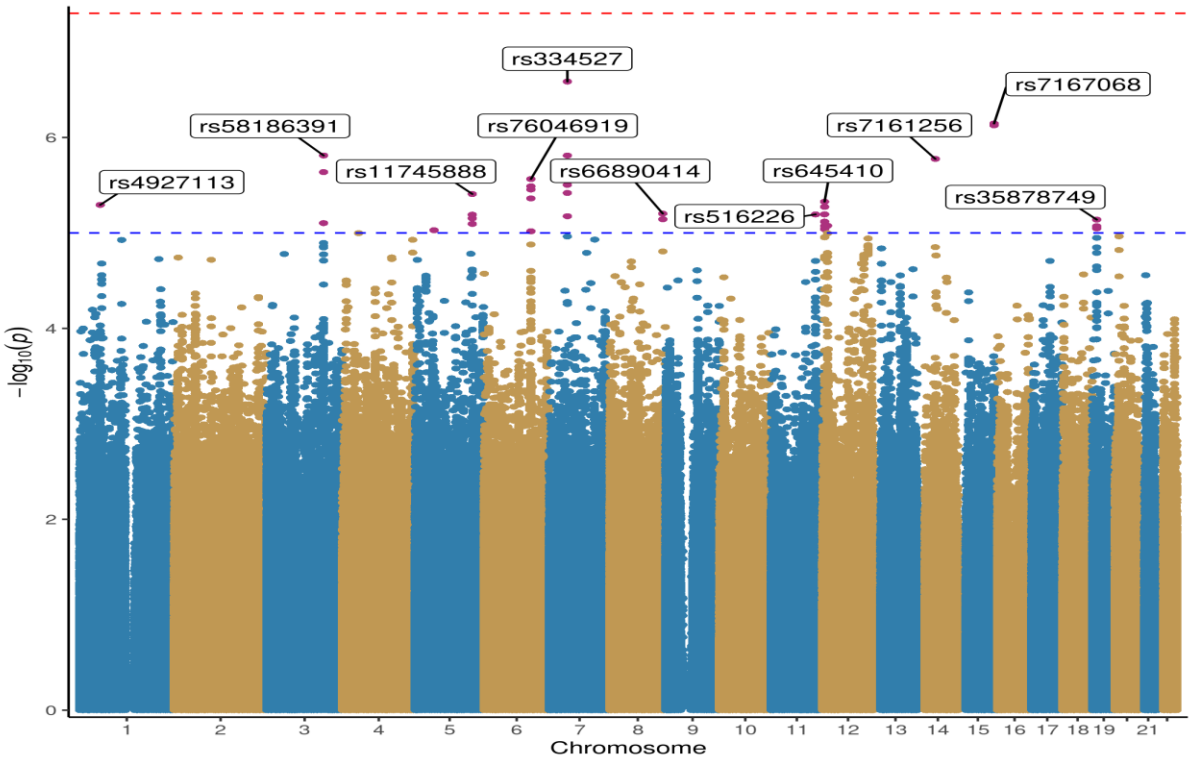

Figure 4-6 Aim 3 Figure S3

Manhattan Plot for Constant-Severe Pain.

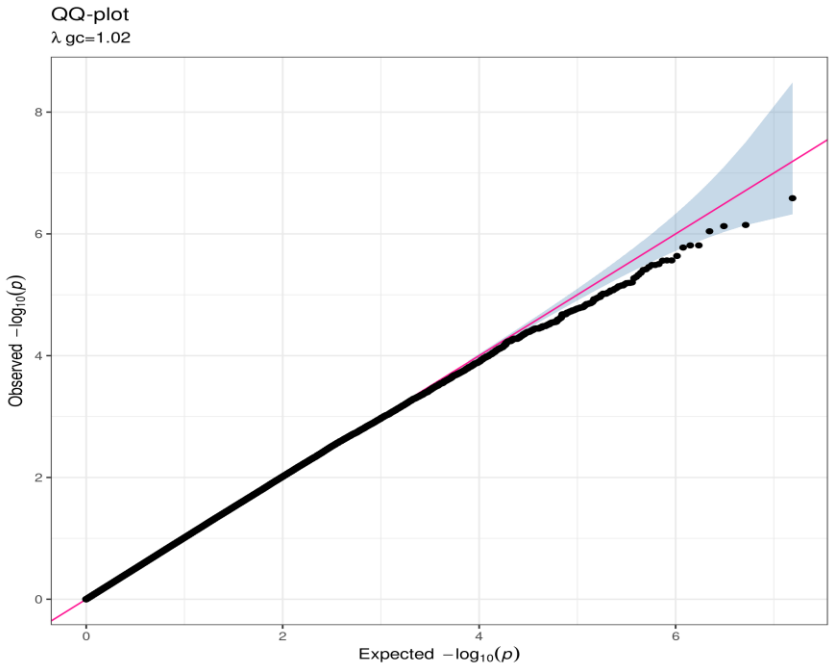

Figure 4-7 Aim 3 Figure S4

QQ Plot for Constant-Severe Pain.

Table 4-10 Aim 3 Table S5
SNPs Meeting Suggestive Significance (1e-5) for Constant-Severe Pain

CHR	BP[1]	SNP[2]	OR	L95[3]	U95[4]	SE	P	A1[5]	A2[6]	MAF[7]
1	54,902,861	rs4927113	0.6421	0.5308	0.7768	0.0971	5.08e-06	C	T	0.253
3	148,837,122	rs772805	1.481	1.247	1.76	0.0879	7.89e-06	A	G	0.408
3	148,839,366	rs58186391	1.547	1.295	1.848	0.0907	1.54e-06	T	A	0.383
3	148,848,923	rs78985718	1.538	1.287	1.839	0.0912	2.30e-06	G	A	0.381
5	49,435,222	rs149312484	0.6356	0.5201	0.7766	0.102	9.35e-06	A	G	0.203
5	149,965,575	rs10058260	1.492	1.253	1.776	0.089	7.02e-06	G	A	0.486
5	149,966,193	rs1363181	1.496	1.256	1.781	0.0892	6.44e-06	A	G	0.483
5	149,966,690	rs10044420	1.488	1.25	1.771	0.089	8.04e-06	A	G	0.484
5	149,967,587	rs6579794	1.492	1.253	1.776	0.089	7.02e-06	G	A	0.486
5	149,968,929	rs11745888	1.512	1.268	1.802	0.0895	3.91e-06	T	C	0.483
6	122,878,836	rs197687	1.48	1.244	1.761	0.0886	9.60e-06	A	G	0.392
6	122,885,419	rs9388096	1.48	1.244	1.761	0.0886	9.60e-06	C	T	0.392
6	122,885,461	rs9388097	1.48	1.244	1.761	0.0886	9.60e-06	C	T	0.392
6	122,897,790	rs9482255	1.48	1.244	1.761	0.0886	9.60e-06	G	A	0.392
6	122,903,206	rs76046919	0.4441	0.3163	0.6234	0.173	2.73e-06	C	T	0.0522
6	122,906,639	rs56304640	0.4509	0.3221	0.6313	0.172	3.50e-06	A	G	0.0533
6	122,908,557	rs9490487	0.4441	0.3163	0.6234	0.173	2.73e-06	C	A	0.0522
6	122,918,372	rs4554346	0.45	0.3201	0.6327	0.174	4.35e-06	A	G	0.0522
6	122,919,311	rs2082196	0.4451	0.3166	0.6259	0.174	3.24e-06	C	G	0.0522
6	122,921,183	rs75083052	0.4451	0.3166	0.6259	0.174	3.24e-06	G	T	0.0522
7	47,565,793	rs734899	0.6479	0.5428	0.7734	0.0903	1.55e-06	G	A	0.368
7	47,566,276	rs334525	0.6507	0.5423	0.7808	0.093	3.81e-06	C	T	0.314
7	47,567,227	rs334527	0.6212	0.5182	0.7446	0.0924	2.59e-07	C	T	0.317
7	47,569,436	rs10265512	0.653	0.5464	0.7803	0.0909	2.75e-06	G	A	0.331
7	47,571,370	rs7786087	0.6628	0.5576	0.7879	0.0882	3.13e-06	A	G	0.381
7	47,571,488	7:47571488[A,C]	0.6462	0.5429	0.7692	0.0889	9.06e-07	A	C	0.363
7	47,573,637	rs6964063	0.6585	0.549	0.7898	0.0928	6.69e-06	A	C	0.298
8	138,803,133	rs36014323	0.5647	0.44	0.7248	0.127	7.19e-06	G	A	0.119
8	138,803,658	rs66890414	0.5618	0.4374	0.7215	0.128	6.27e-06	T	C	0.118
11	116,519,655	rs516226	1.65	1.327	2.05	0.111	6.39e-06	T	C	0.218
12	5,289,170	rs7314052	1.584	1.299	1.931	0.101	5.33e-06	C	A	0.276
12	5,290,113	rs12316588	1.578	1.294	1.923	0.101	6.38e-06	T	C	0.276
12	5,290,114	rs12296611	1.565	1.284	1.908	0.101	9.14e-06	G	A	0.276
12	5,294,347	rs2291095	1.566	1.286	1.908	0.101	8.48e-06	T	G	0.278
12	5,294,806	rs2291094	1.57	1.288	1.912	0.101	7.61e-06	T	C	0.279
12	5,301,847	rs645410	1.581	1.299	1.923	0.1	4.71e-06	C	T	0.283
12	12,990,341	rs17394079	0.6038	0.4836	0.7539	0.113	8.41e-06	T	C	0.156
14	46,976,743	rs7161256	2.035	1.521	2.721	0.148	1.68e-06	A	G	0.121
15	93,892,942	rs7164857	0.6465	0.5439	0.7684	0.0882	7.46e-07	T	G	0.389
15	93,893,035	rs7167068	0.646	0.5435	0.7678	0.0882	7.14e-07	T	A	0.393
19	11,229,765	rs35878749	0.6654	0.5569	0.795	0.0908	7.26e-06	A	G	0.289
19	11,229,850	rs34444274	0.6682	0.5593	0.7984	0.0908	8.97e-06	G	C	0.29
19	11,230,402	rs12611067	0.6678	0.5591	0.7977	0.0907	8.51e-06	T	G	0.29

[1]hg19 Base Pair
[2]Blue SNPs were Identified as Lead SNPs by FUMA
[3]Lower 95% Confidence Interval
[4]Upper 95% Confidence Interval
[5]Minor Allele
[6]Major Allele
[7]Minor Allele Frequency

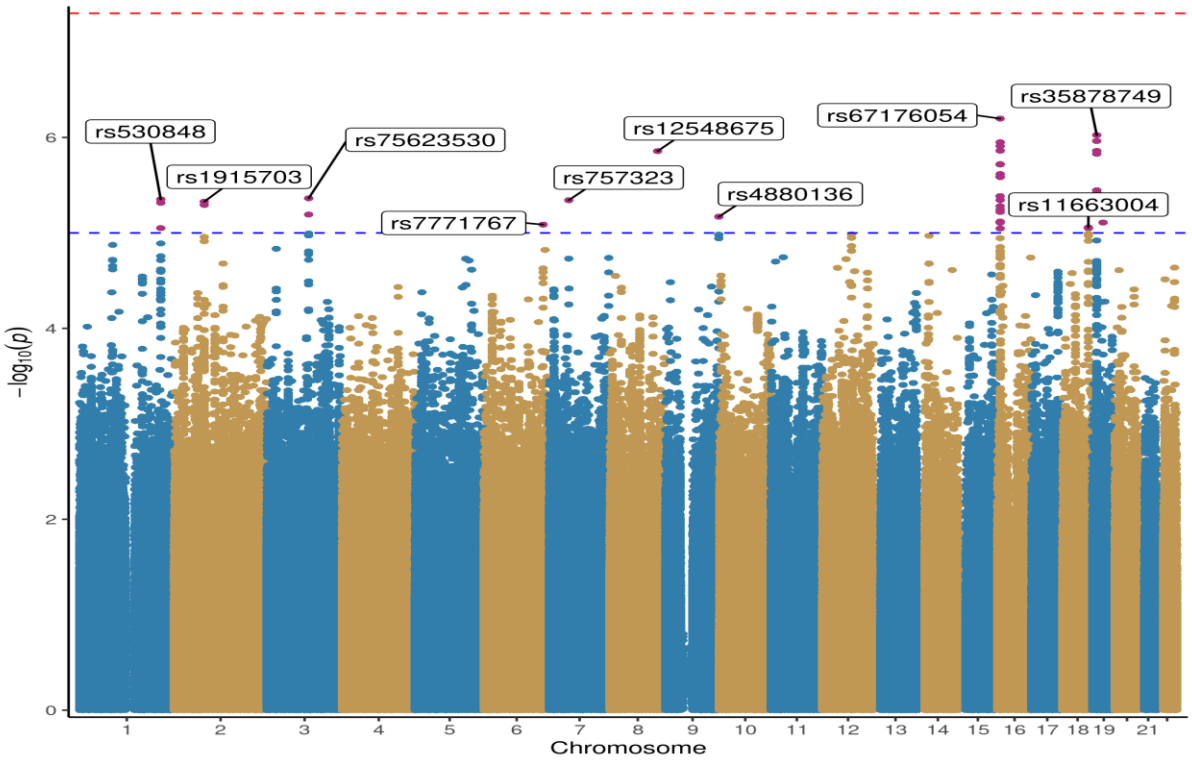

Figure 4-8 Aim 3 Figure S5

Manhattan Plot for Severe Pain.

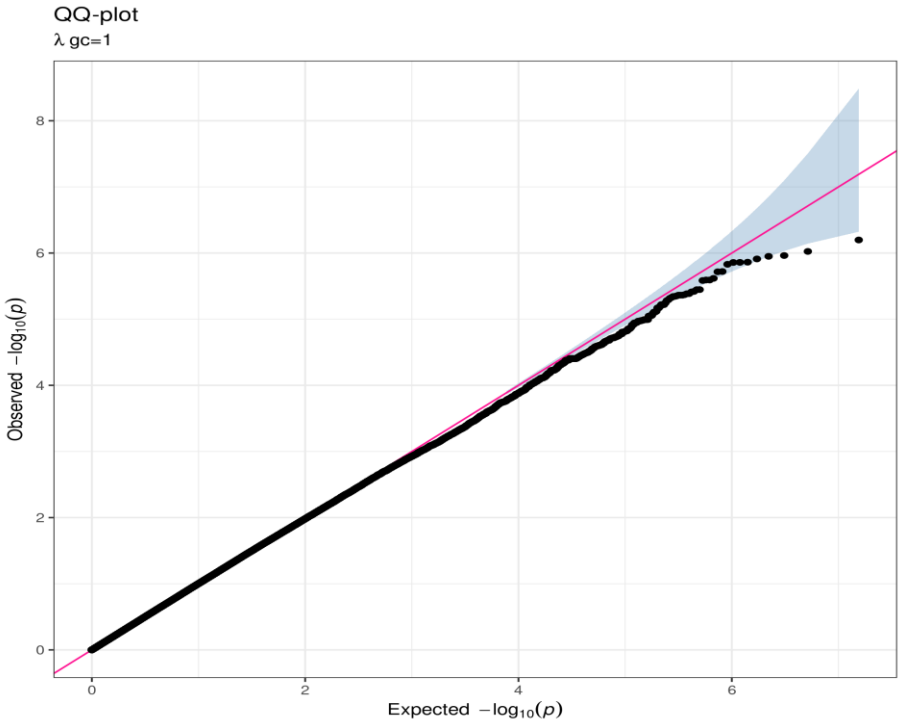

Figure 4-9 Aim 3 Figure S6

QQ plot for Severe Pain.

Table 4-11 Aim 3 Table S6
SNPs Meeting Suggestive Significance (1e-5) for Severe Pain

CHR	BP[1]	SNP[2]	OR	L95[3]	U95[4]	SE	P	A1[5]	A2[6]	MAF[7]
1	213,732,037	rs431573	0.606	0.4858	0.7558	0.113	8.90e-06	A	G	0.144
1	213,732,214	rs530848	0.6072	0.4907	0.7514	0.109	4.45e-06	C	G	0.155
1	213,749,065	rs6682832	0.5843	0.4641	0.7357	0.117	4.84e-06	C	T	0.129
2	78,830,744	rs1239078	1.525	1.272	1.828	0.0925	5.08e-06	G	A	0.325
2	78,832,777	rs1915703	1.527	1.274	1.83	0.0924	4.70e-06	A	G	0.325
3	109,644,774	rs59868665	0.1839	0.0881	0.3837	0.375	6.41e-06	C	A	0.00688
3	109,672,395	rs75623530	0.1795	0.0863	0.3735	0.374	4.33e-06	A	G	0.00688
3	109,673,273	rs116440411	0.1795	0.0863	0.3735	0.374	4.33e-06	T	C	0.00688
3	109,675,891	rs140099063	0.1795	0.0863	0.3735	0.374	4.33e-06	G	C	0.00688
6	155,038,479	rs7771767	1.451	1.232	1.709	0.0834	8.18e-06	A	G	0.547
7	51,077,759	rs757323	1.476	1.249	1.742	0.0848	4.54e-06	G	A	0.514
8	125,224,719	rs12548675	0.6237	0.5149	0.7555	0.0978	1.39e-06	T	C	0.202
9	139,620,311	rs4880136	0.6762	0.5703	0.8018	0.0869	6.76e-06	A	G	0.447
9	139,621,168	rs2275160	0.6762	0.5703	0.8018	0.0869	6.76e-06	G	A	0.447
16	7,362,235	rs28591292	2.241	1.601	3.137	0.172	2.60e-06	C	T	0.102
16	7,365,355	16:7365355[A,G]	2.274	1.616	3.199	0.174	2.42e-06	A	G	0.0997
16	7,367,240	rs12918524	2.169	1.554	3.027	0.17	5.24e-06	C	A	0.102
16	7,367,453	rs34265202	2.187	1.568	3.052	0.17	4.11e-06	G	A	0.102
16	7,368,357	rs11864520	2.073	1.52	2.828	0.158	4.13e-06	G	A	0.115
16	7,369,787	rs35999696	2.012	1.481	2.732	0.156	7.59e-06	G	C	0.116
16	7,369,880	rs35767895	2.041	1.498	2.78	0.158	6.03e-06	G	A	0.115
16	7,370,244	rs55870430	2.041	1.498	2.78	0.158	6.03e-06	C	T	0.115
16	7,370,418	rs36095768	2.041	1.498	2.78	0.158	6.03e-06	G	T	0.115
16	7,370,953	rs8052441	2.06	1.513	2.805	0.158	4.52e-06	G	C	0.116
16	7,371,066	rs67176054	2.585	1.779	3.757	0.191	6.34e-07	A	G	0.0894
16	7,380,549	rs34109083	2.371	1.675	3.356	0.177	1.12e-06	G	A	0.099
16	7,381,299	rs17739067	2.329	1.645	3.297	0.177	1.90e-06	G	A	0.0977
16	7,383,759	rs67729500	2.257	1.608	3.168	0.173	2.56e-06	T	A	0.1
16	7,384,352	rs4362406	2.298	1.632	3.236	0.175	1.92e-06	A	G	0.0997
16	7,384,503	rs4516245	2.257	1.608	3.168	0.173	2.56e-06	C	G	0.1
16	7,385,103	rs17143464	2.348	1.663	3.315	0.176	1.23e-06	T	C	0.0997
16	7,385,942	rs34009260	2.355	1.663	3.334	0.177	1.37e-06	A	G	0.0984
16	7,396,323	rs72769295	2.237	1.572	3.185	0.18	7.80e-06	T	C	0.0915
16	7,405,611	rs7202500	2.263	1.59	3.22	0.18	5.67e-06	T	C	0.0922
16	7,417,955	rs35508038	2.253	1.574	3.224	0.183	8.98e-06	T	C	0.0887
18	67,322,660	rs11151522	0.6873	0.5824	0.8109	0.0844	8.91e-06	A	G	0.411
18	67,324,345	rs11663004	0.6872	0.5825	0.8108	0.0844	8.75e-06	A	G	0.411
19	11,221,180	rs892116	0.685	0.5835	0.8042	0.0818	3.80e-06	A	G	0.362
19	11,224,265	rs5930	0.6863	0.5846	0.8058	0.0819	4.26e-06	A	G	0.358
19	11,227,070	rs2738445	0.6863	0.5853	0.8047	0.0812	3.57e-06	C	T	0.382
19	11,227,480	19:11227480[A,C]	0.6859	0.5848	0.8045	0.0813	3.58e-06	A	C	0.372
19	11,228,745	rs2569550	0.6871	0.586	0.8058	0.0812	3.87e-06	T	C	0.369
19	11,229,765	rs35878749	0.6576	0.5561	0.7775	0.0855	9.45e-07	A	G	0.307
19	11,229,850	rs34444274	0.6591	0.5574	0.7794	0.0855	1.09e-06	G	C	0.308
19	11,230,362	rs12609673	0.6649	0.5631	0.7851	0.0848	1.48e-06	C	A	0.321
19	11,230,402	rs12611067	0.6622	0.5601	0.7828	0.0854	1.38e-06	T	G	0.309
19	27,992,394	rs62111935	0.572	0.4478	0.7307	0.125	7.79e-06	A	G	0.105

[1]hg19 Base Pair
[2]Blue SNPs were Identified as Lead SNPs by FUMA
[3]Lower 95% Confidence Interval
[4]Upper 95% Confidence Interval
[5]Minor Allele
[6]Major Allele
[7]Minor Allele Frequency

Table 4-12 Aim 3 Table S7
FUMA Genomic Loci for Constant Pain

GenomicLocusRegion[1]	rsID[2]	chr	pos	p[3]	nGWASSNPs[4]	nIndSigSNPs[5]	IndSigSNPs[6]	nearestGene
1:40814528-40823404	rs4660406	1	40,823,404	3.69e-06	5	1	rs4660406	*SMAP2*
3:63441514-63455599	rs2060757	3	63,455,599	9.01e-06	5	1	rs2060757	*SYNPR:SYNPR-AS1*
4:184484425-184505515	rs10009455	4	184,494,883	8.80e-06	16	1	rs10009455	*ING2*
5:49435222-49435222	rs149312484	5	49,435,222	3.05e-06	1	1	rs149312484	*EMB*
7:47565793-47575970	rs334527	7	47,567,227	1.51e-06	13	1	rs334527	*TNS3*
8:138803133-138806916	rs66890414	8	138,803,658	8.64e-06	3	1	rs66890414	*FAM135B*
12:5441541-5487932	rs10492094	12	5,478,148	3.52e-06	3	1	rs10492094	*NTF3*
13:20847066-20866839	13:20855444[C,T]	13	20,855,444	3.17e-06	35	1	13:20855444[C,T]	*GJB6*
13:86362179-86565426	rs117027346	13	86,362,179	7.69e-06	100	1	rs117027346	*SLITRK6*
13:103580361-103606829	rs701545	13	103,580,541	1.51e-07	3	1	rs701545	*METTL21EP*
16:81238750-81264177	rs111271001	16	81,259,428	6.19e-06	21	1	rs111271001	*PKD1L2*
19:295231-295295	rs734885	19	295,231	7.50e-06	2	1	rs734885	*PPAP2C*
20:62200860-62263747	rs6062978	20	62,256,590	8.20e-06	6	1	rs6062978	*GMEB2*

[1]chr:start-end based on hg19; [2]rsID of top lead SNP; [3]GWAS p value; [4]Number of unique GWAS SNPs in locus; [5]Number of LD independent SNPs; [6]rsID of independent SNPs

Table 4-13 Aim 3 Table S8
FUMA Genomic Loci for Constant-Severe Pain

GenomicLocusRegion[1]	rsID[2]	chr	pos	p[3]	nGWASSNPs[4]	nIndSigSNPs[5]	IndSigSNPs[6]	nearestGene
1:54896755-54922021	rs4927113	1	54,902,861	5.08e-06	15	1	rs4927113	*SSBP3*
3:148698474-148876261	rs58186391	3	148,839,366	1.54e-06	53	1	rs58186391	*HPS3*
5:49435222-49435222	rs149312484	5	49,435,222	9.35e-06	1	1	rs149312484	*EMB*
5:149954864-149990727	rs11745888	5	149,968,929	3.91e-06	40	1	rs11745888	*SYNPO*
6:122429305-122921183	rs76046919	6	122,903,206	2.73e-06	103	2	rs9388097;rs76046919	*PKIB*
7:47565793-47575970	rs334527	7	47,567,227	2.59e-07	13	1	rs334527	*TNS3*
8:138803133-138806916	rs66890414	8	138,803,658	6.27e-06	3	1	rs66890414	*FAM135B*
11:116519655-116519655	rs516226	11	116,519,655	6.39e-06	1	1	rs516226	*AP000770.1*
12:5284122-5315245	rs645410	12	5,301,847	4.71e-06	17	1	rs645410	*RP11-319E16.1*
12:12963744-12990341	rs17394079	12	12,990,341	8.41e-06	10	1	rs17394079	*DDX47*
14:46976743-46986881	rs7161256	14	46,976,743	1.68e-06	2	1	rs7161256	*LINC00871*
15:93892942-93908051	rs7167068	15	93,893,035	7.14e-07	4	1	rs7167068	*RGMA*
19:11221180-11232696	rs35878749	19	11,229,765	7.26e-06	10	1	rs35878749	*LDLR*

[1]chr:start-end based on hg19; [2]rsID of top lead SNP; [3]GWAS p value; [4]Number of unique GWAS SNPs in locus; [5]Number of LD independent SNPs; [6]rsID of independent SNPs

Table 4-14 Aim 3 Table S9
FUMA Genomic Loci for Severe Pain

GenomicLocusRegion[1]	rsID[2]	chr	pos	p[3]	nGWASSNPs[4]	nIndSigSNPs[5]	IndSigSNPs[6]	nearestGene
1:213685950-213755621	rs530848	1	213,732,214	4.45e-06	27	1	rs530848	*RPL31P13*
2:78764895-78837866	rs1915703	2	78,832,777	4.70e-06	11	1	rs1915703	*CYCSP6*
3:109525798-109681921	rs75623530	3	109,672,395	4.33e-06	30	1	rs75623530	*MIR4445*
6:155022713-155160128	rs7771767	6	155,038,479	8.18e-06	76	1	rs7771767	*SCAF8*
7:51035899-51079151	rs757323	7	51,077,759	4.54e-06	6	1	rs757323	*COBL*
8:125224719-125224719	rs12548675	8	125,224,719	1.39e-06	1	1	rs12548675	*RP11-37N22.1*
9:139614170-139642961	rs2275160	9	139,621,168	6.76e-06	10	1	rs2275160	*SNHG7*
16:7353976-7417955	rs67176054	16	7,371,066	6.34e-07	54	2	rs67176054;rs34109083	*RBFOX1*
18:67306031-67327598	rs11663004	18	67,324,345	8.75e-06	26	1	rs11663004	*DOK6*
19:11221180-11232696	rs35878749	19	11,229,765	9.45e-07	10	1	rs35878749	*LDLR*
19:27947716-28309577	rs62111935	19	27,992,394	7.79e-06	93	1	rs62111935	*LINC00662*

[1]chr:start-end based on hg19; [2]rsID of top lead SNP; [3]GWAS p value; [4]Number of unique GWAS SNPs in locus; [5]Number of LD independent SNPs; [6]rsID of independent SNPs

Table 4-15 Aim 3 Table S10
S-MultiXcan Results for Constant Pain (see Table S13: Legend for S-MultiXcan Variables)

gene	gene_name	pvalue	n	n_indep	p_i_best	t_i_best	p_i_worst	t_i_worst	eigen_max	eigen_min	eigen_min_kept	z_min	z_max	z_mean	z_sd
ENSG00000161021.11	*MAML1*	2.07e-07	44	4	3.39e-02	Heart_Left_Ventricle	0.75	Spleen	29.2	2.86e-15	1.44	-2.12	1.81	-1.14	1.19
ENSG00000163121.9	*NEURL3*	9.28e-06	11	3	1.22e-03	Brain_Substantia_nigra	0.761	Brain_Nucleus_accumbens_basal_ganglia	9.36	3.55e-18	0.701	-3.23	3.23	0.678	2.8
ENSG00000162438.11	*CTRC*	2.45e-05	8	6	5.51e-04	Pancreas	0.358	Testis	3.72	2.78e-17	0.387	-3.45	3.4	0.323	2.39

Table 4-16 Aim 3 Table S11
S-MultiXcan Results for Constant-Severe Pain (see Table S13: Legend for S-MultiXcan Variables)

gene	gene_name	pvalue	n	n_indep	p_i_best	t_i_best	p_i_worst	t_i_worst	eigen_max	eigen_min	eigen_min_kept	z_min	z_max	z_mean	z_sd
ENSG00000161021.11	*MAML1*	4.99e-08	44	4	6.19e-02	Brain_Cerebellar_Hemisphere	0.837	Spleen	29.2	2.86e-15	1.44	-1.87	1.77	-0.882	1.23
ENSG00000025156.12	*HSF2*	5.85e-06	46	5	3.09e-05	Skin_Not_Sun_Exposed_Suprapubic	0.469	Artery_Aorta	29.8	1.01e-15	1.21	-3.22	4.17	0.79	2.38
ENSG00000162438.11	*CTRC*	4.5e-05	8	6	2.29e-03	Pancreas	0.316	Whole_Blood	3.72	2.78e-17	0.387	-3.05	2.99	0.488	2.3
ENSG00000151789.10	*ZNF385D*	8.25e-05	7	7	4.85e-03	Artery_Aorta	0.814	Spleen	1.74	0.439	0.439	-2.82	2.61	0.456	1.7

Table 4-17 Aim 3 Table S12
S-MultiXcan Results for Severe Pain (see Table S13: Legend for S-MultiXcan Variables)

gene	gene_name	pvalue	n	n_indep	p_i_best	t_i_best	p_i_worst	t_i_worst	eigen_max	eigen_min	eigen_min_kept	z_min	z_max	z_mean	z_sd
ENSG00000130164.13	*LDLR*	6.53e-05	3	2	2.16e-05	Artery_Tibial	0.259	Pancreas	2.36	2.3e-17	0.643	-4.25	-1.13	-2.17	1.8
ENSG00000206052.10	*DOK6*	7.5e-05	14	10	8.75e-06	Nerve_Tibial	0.864	Brain_Hypothalamus	3.58	4.87e-17	0.137	-4.45	2.25	-0.554	1.84

Table 4-18 Aim 3 Table S13
Legend for S-MultiXcan Variables

gene: a gene's id.
gene_name: gene name.
pvalue: p-value of S-MultiXcan association.
n: number of tissues available for gene.
n_indep: number of independent components of variation kept among the tissues' predictions.
p_i_best: best p-value of single-tissue S-PrediXcan association.
t_i_best: name of best single-tissue S-PrediXcan association.
p_i_worst: worst p-value of single-tissue S-PrediXcan association.
t_i_worst: name of worst single-tissue S-PrediXcan association.
eigen_max: In the SVD decomposition of predicted expression correlation matrix: eigenvalue (variance explained) of the top independent component.
eigen_min: In the SVD decomposition of predicted expression correlation matrix: eigenvalue (variance explained) of the last independent component.
eigen_min_kept: In the SVD decomposition of predicted expression correlation matrix: eigenvalue (variance explained) of the smallest independent component that was kept.
z_min: minimum z-score among single-tissue S-PrediXcan associations.
z_max: maximum z-score among single-tissue S-PrediXcan associations.
z_mean: mean z-score among single-tissue S-PrediXcan associations.
z_sd: standard deviation of the mean z-score among single-tissue S-PrediXcan associations.

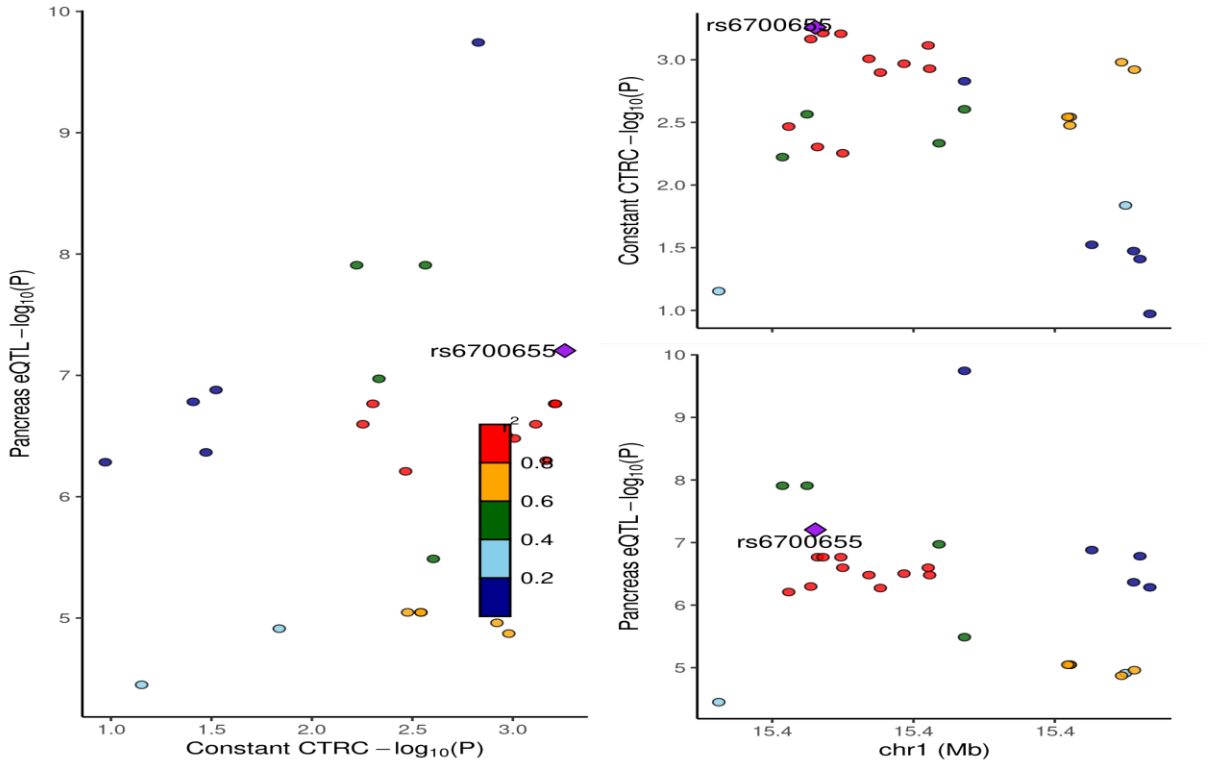

Figure 4-10 Aim 3 Figure S7

Locuscompare Plot for Constant Pain CTRC and Pancreas eQTL. Top right: regional scatter plot for GWAS. Bottom right: regional scatter plot for eQTL. Left: joint distribution of p-values from GWAS and eQTL. Color represents LD with lead SNP.

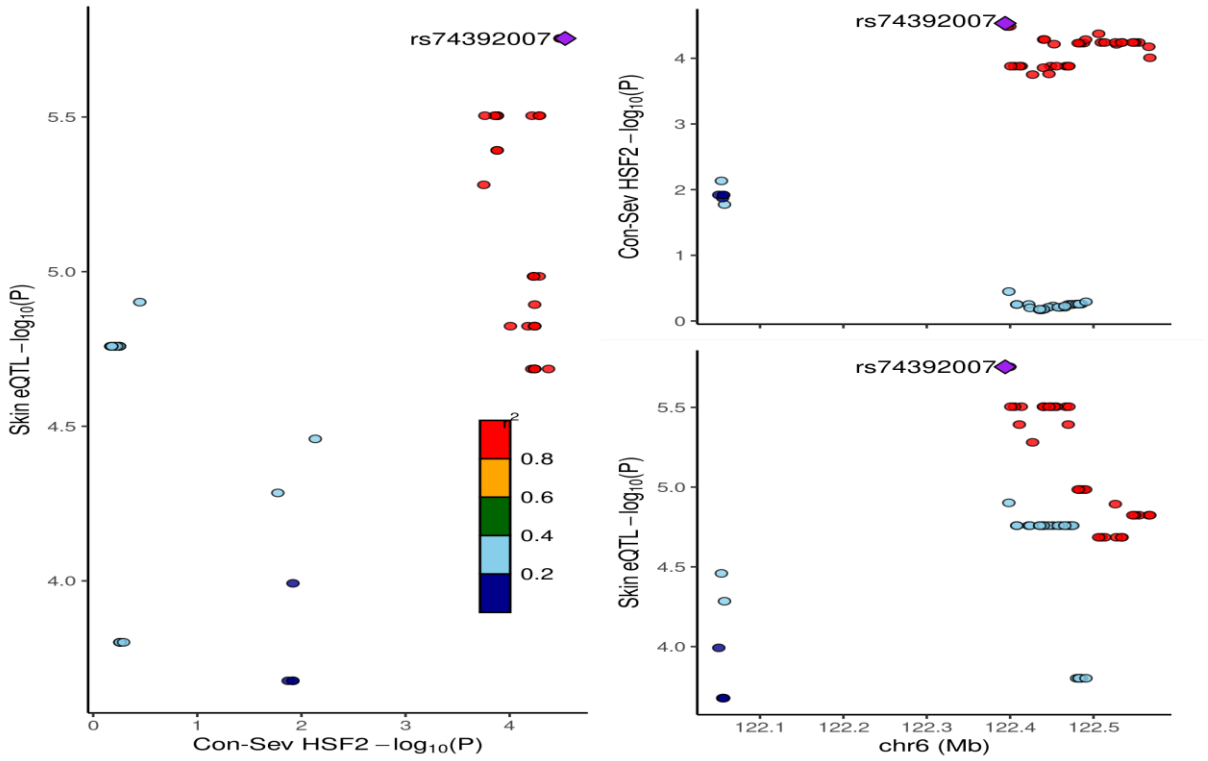

Figure 4-11 Aim 3 Figure S8

Locuscompare Plot for Constant-Severe Pain HSF2 and Skin Not Sun Exposed Suprapubic eQTL. Top right: regional scatter plot for GWAS. Bottom right: regional scatter plot for eQTL. Left: joint distribution of p-values from GWAS and eQTL. Color represents LD with lead SNP.

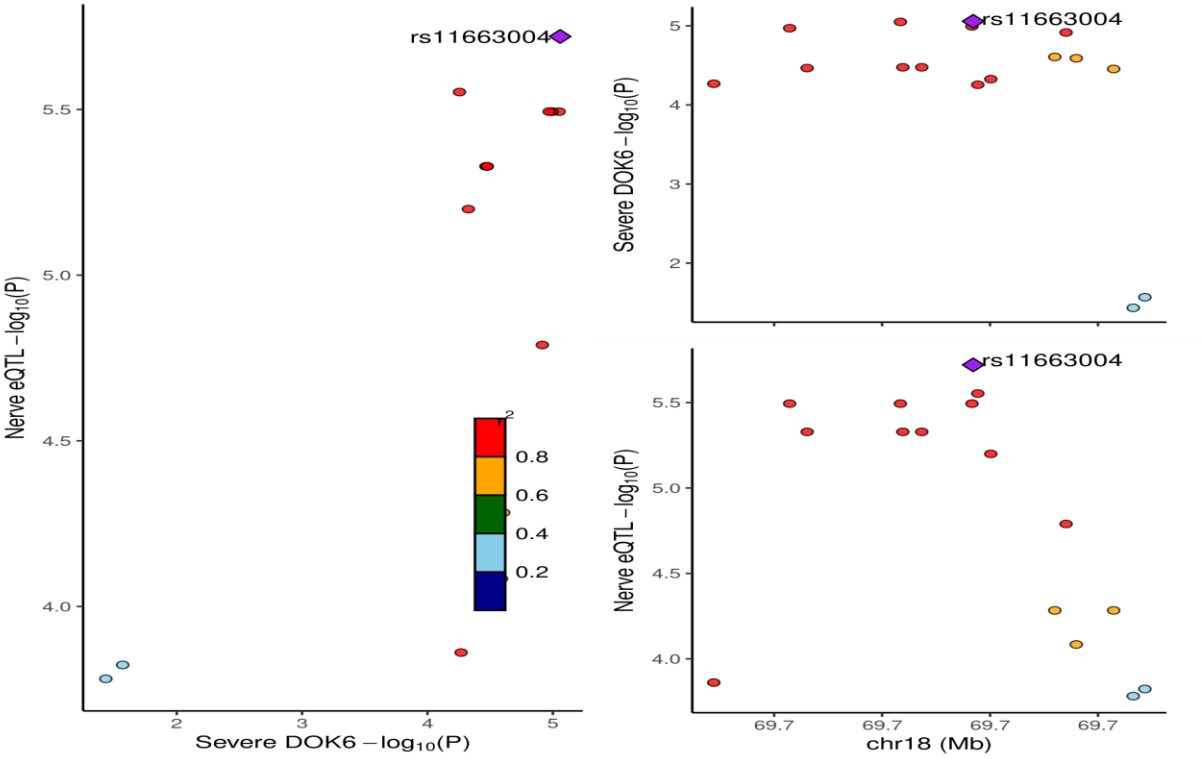

Figure 4-12 Aim 3 Figure S9

Locuscompare Plot for Severe Pain DOK6 and Nerve Tibial eQTL. Top right: regional scatter plot for GWAS. Bottom right: regional scatter plot for eQTL. Left: joint distribution of p-values from GWAS and eQTL. Color represents LD with lead SNP.

4.10 Additional Calculations

The following calculations were not included in the manuscript for Aim 3.

4.10.1 Power

All three pain categories were tested in 1,254 RAP+CP patients. Expected power at an alpha level of $5x10^{-8}$ for these studies is reported in **Figure 4-13**, **Figure 4-14**, and **Figure 4-15**. Independent loci reaching suggestive significance ($1x10^{-5}$) were also reported. At the suggestive threshold we would expect to observe 77 false positive SNPs on average under the null hypothesis after testing all 7,745,456 SNPs. The GWAS portion of these studies are best powered to detect true effects at effect sizes greater than 1.6 in MAF between 0.21 and 0.56. Many complex diseases have OR's between 1.08 to 1.16 at similar MAFs (Park et al., 2011) and this aim is underpowered (<0.2) to detect true effects of those sizes. Replication in a larger sample size is needed to detect true positive SNPs with small effect sizes. However, using post-GWAS tools like TWAS and colocalization helps to interpret GWAS results by including biological information.

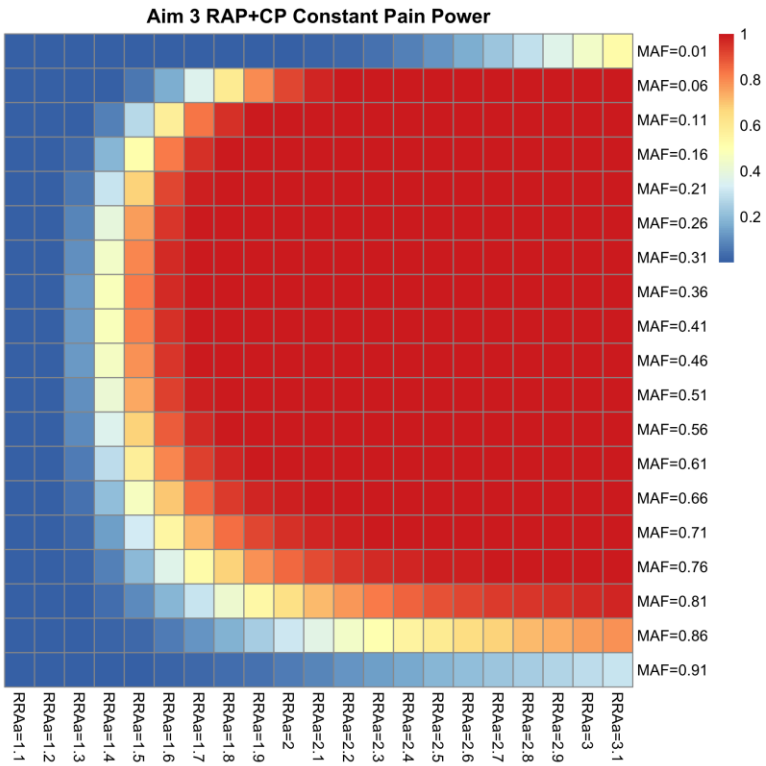

Figure 4-13 Power Heatmap for RAP+CP Constant Pain

α=5x10^{-8}, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=504, Controls=750.

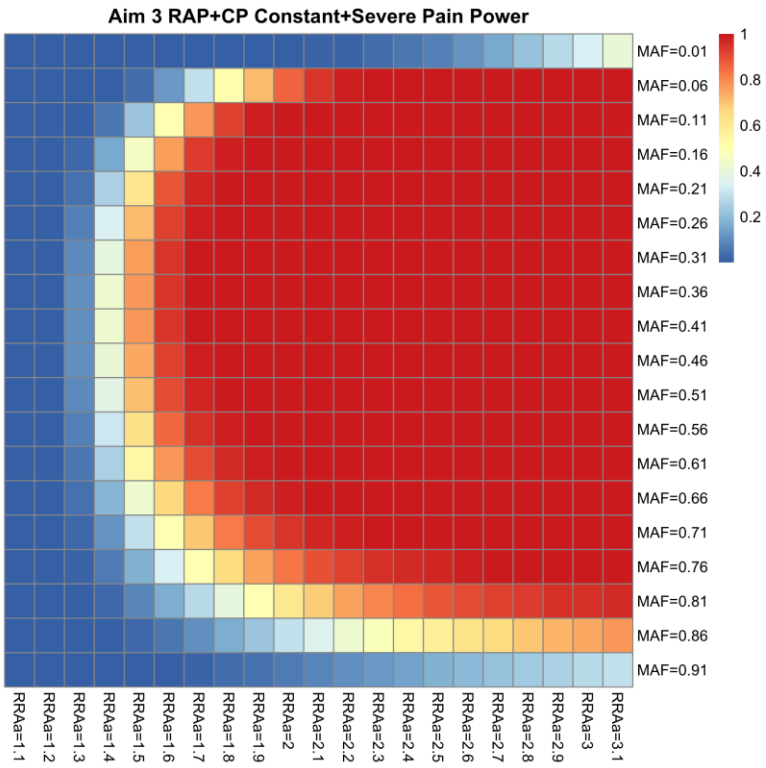

Figure 4-14 Power Heatmap for RAP+CP Constant-Severe Pain

α=5x10^{-8}, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=450, Controls=804.

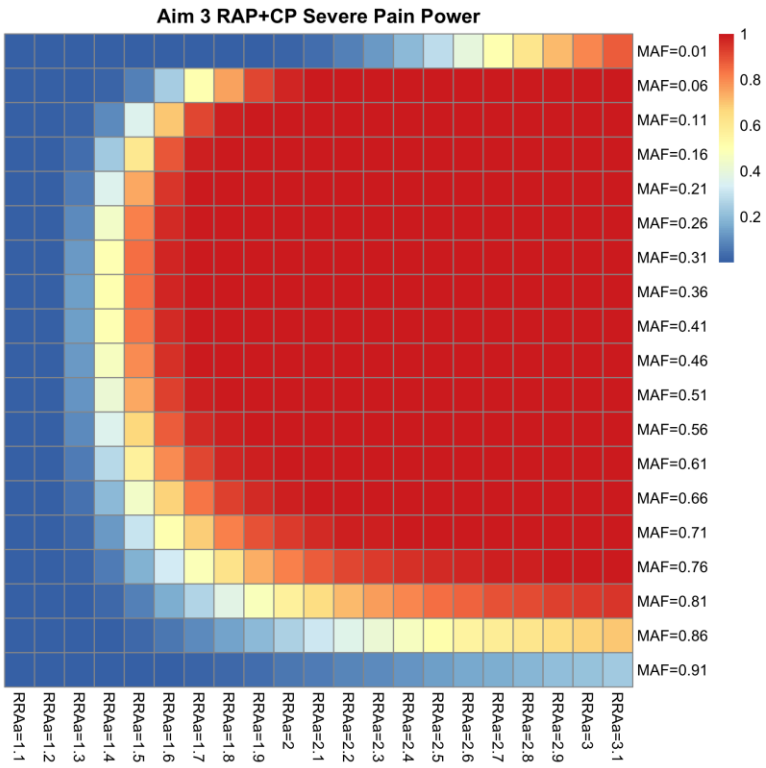

Figure 4-15 Power Heatmap for RAP+CP Severe Pain

α=5x10^{-8}, MAF= Minor Allele Frequency, RRAa=genotypic relative risk heterozygote. Cases=727, Controls=527.

5.0 Conclusion

5.1 Summary of Results

The core purpose of this dissertation was to begin to develop an understanding of why the pain experience varies so much across pancreatitis patients. This variance is not explained by disease duration, physical state of the pancreas, or even if the pancreas has been removed (Mullady et al., 2011; Whitcomb et al., 2016). Some environmental risk factors (smoking, drinking) are associated with higher levels of pain; however, the temporal relationship between these and pancreatitis pain is muddy given that many individuals continue these behaviors to dull the pain (E. K. Dunbar, Saloman, et al., 2021; Jeon et al., 2019; Mullady et al., 2011). The question about the nature of genetic variability between pancreatitis patients with different pain experiences remained unanswered. Additionally, the question of the involvement of psychiatric disorders with pancreatitis pain was beginning to circulate among the field.

In order to address both questions, I designed an original method (see Aim 1) which checked if loci associated with constant-severe pain in RAP+CP patients fell within genes that had previously been associated with depression. I used the largest group of patients available from the NAPS2, and the pain category predicted to be most the most debilitating for this aim. I identified 15 independent SNPs (p-value < $1x10^{-4}$) from 13 genes suggestively associated with constant-severe pain in RAP+CP patients that are located in genes known to be associated with depression. These depression genes included *ROBO2*, *CTNND2*, *SGCZ*, *CNTN5*, and *BAIAP2*. *ROBO2*, *CTNND2*, and *SGCZ* are also associated with response to antidepressants, which could have future implications in developing precision treatments for pancreatitis pain once these results are

validated. This aim served as a proof of concept, suggesting that genetic variability between patients could potentially contribute to the differences in pancreatitis pain experiences, and further study in higher powered samples using established methods was merited.

A logical next step was to address other stress disorders, including anxiety and PTSD. Therefore, in Aim 2, I broadened my perspective to check if genetic risk for anxiety and PTSD contributed to the pain experience in pancreatitis patients using a more traditional candidate gene approach. I found 24 independent lead SNPs (p-value < 0.002) suggestively associated with constant, constant-severe, and severe pain experiences within candidate genes for anxiety and PTSD. Interestingly, Aim 2 tested CP and RAP+CP patients separately, and used constant, constant-severe, and severe pain categories in an attempt to capture all interesting findings across the combinations. As a result, I was able to observe that loci associated with different pain fall in different psychiatric risk loci. Specifically different SNPs in or near *CTNND2* were associated with all categories of pain in both groups of patients. Conversely, SNPs assigned to *BDNF* were only associated with constant pain in CP patients (see **Table 3-7 Aim 2 Table 7)**.

A downfall of studies driven by candidate genes as Aims 1 and 2, is that other equally or more important loci associated with the pain experience will be missed. It is also easy to construct a story based on interesting findings from genetic association tests alone that may not be relevant to the primary trait (Biedrzycki et al., 2019). Which is why in Aim 3 I stepped completely back from candidate genes to look at genome-wide and post-GWAS associations with pancreatitis pain across constant, constant-severe, and severe pain in RAP+CP patients. This is important because in Aim 2 all patients with more severe pain experiences had a lower mental quality of life than those without that worse pain experience and I did not want to restrict my findings to known psychiatric risk. However, I still only had access to the small sample sizes, which result in low

powered studies. I used the larger sample of RAP+CP patients for this reason. As a countermeasure to false positives from a low powered GWAS, I used TWAS to incorporate biological information about gene expression into the GWAS results to help identify potential candidate genes. The TWAS predicts genes and tissues likely to be differentially expressed in our sample based on our pain phenotypes. As an additional measure, I colocalized the GWAS signals in genes from the TWAS and expression signals to determine if the signals were the result of a single SNP or if the signals were just near each other. Aim 3 found interesting loci associated with each pain experience. Predicted differential expression of *CTRC* in the pancreas is associated with constant and constant-severe pain and colocalized in constant pain—albeit colocalization was with non-significant GWAS signals that would have been missed if we had not conducted the TWAS. *HSF2* colocalized in skin and predicted differential expression is associated with constant-severe pain. Finally, predicted differential expression of *DOK6* in nerve tissue colocalized with severe pain.

The purpose of this dissertation is to provide preliminary details about the genetic variation of the pain experience in pancreatitis patients, using a small subset of the patients from the well described NAPS2 data. To that end, Aims 1 and 2 focus on identifying any loci that happen to be within candidate genes for depression, anxiety, and/or PTSD. Aim 3 focused on finding any genes that may contribute to the variation seen in pancreatitis pain. From these Aims I have presented many genes implicated in the pain experience in pancreatitis; several of which are biologically plausible being involved in axon guidance (*ROBO2*), neuronal signaling (*BDNF*), and pancreatic digestive enzymes (*CTRC*) to name a few. My results, while considering the limitations discussed later, provide reasonable suspicion that future research into these genes, or genes involved in similar biological processes and/or psychiatric disorders, may provide valuable and clinically

actionable information about the pain experience in pancreatitis. Future work validating my results will also reinforce that physicians should consider patients' mental health during pain management.

5.2 Strengths and Limitations

As mentioned in each Aim, these studies are underpowered due to small sample sizes. The genetic tests here are best suited to detect larger effects (1.3-1.6) (see sections **2.1.33.11.2**and **4.10.1**). Unfortunately, most complex diseases have effect sizes smaller than this range (Park et al., 2011). It is also very difficult to find true positives ranked highly among SNPs with the lowest p-values without high genome-wide power when testing SNPs individually as was done here. Multiple testing corrections only slightly help to rank true positives higher than false positives (Zaykin & Zhivotovsky, 2005). Therefore, given the low power of our tests, most of the high-ranking results are statistically likely to be false positives, with the true positives ranking lower—potentially in the region that we reported.

The candidate gene approach used in Aim 2 and the post-GWAS analysis used in Aim 3 help to counter the low sample size by reducing the multiple testing burden by not testing the entire genome (1.7e+4 vs 9.3e+6 SNPs) and incorporating biological information into the results respectively. Furthermore, the small sample sizes are due to the “nested” study design of these studies where both cases and controls are pancreatitis patients which controls for the disease and reduces heterogeneity between cases and controls. Reducing the heterogeneity of the sample increases the power of the study (Heidel, 2016). Additionally, using controls without pancreatitis would detect associations with pancreatitis itself rather than pancreatitis pain.

These studies only used patients of European Ancestry, which limits the generalizability of these results to other populations. The pain phenotypes used are well studied (Mullady et al., 2011) but are still self-reported and subject to exaggeration and under reporting, limiting the ability to correctly distinguish between cases and controls (see **5.3 Future Directions** for methods to address this limitation).

The approach to handling the false discovery rate in Aims 1 and 2 were less strict. The limitation to less strict corrections is that the results are likely to have more false positives than true positives; however, the interpretation of my results presented in my published papers (see **Appendix A**) is appropriate given the target audience to whom false negatives are more egregious than false positives and the consequences of the follow up of a false positive is minimal. The multiple testing burden correction used in Aim 3 is more stringent, using standard genome-wide significance ($5x10^{-8}$) and Bonferroni corrections and suggestive thresholds suggested by the authors of TWAS (**4.3 Methods**) (Barbeira et al., 2019). More stringent corrections for multiple testing help to control for Type 1 error (false positives) resulting in the initial reporting of fewer to no false positives while simultaneously missing less significant true positives. Suggestive significance thresholds mark results that may be true, but need further validation, such as colocalization.

A possible limitation of Aims 1 and 2 is that gene annotation was based on *cis* location relative to the gene. Annotating genes using this method can possibly miss *trans* regulatory elements important for that gene (Mountjoy et al., 2021). However, annotating a SNP to the closest gene is correct about 70% of the time (Backman et al., 2021; Nasser et al., 2021; Pietzner et al., 2021). It is therefore possible that about 30% of my results from Aim 1 and 2 are annotated to the incorrect genes, and these genes have nothing to do with pancreatitis pain or the candidate

depression, anxiety, or PTSD genes making them false positives. SNP specific pleiotropy tests could be done to address the issue of annotating to nearest genes.

A limitation to the retrospective interpretation of the conclusions of Aim 1 is the non-significant overlap of depression genes with loci associated with constant-severe pain in pancreatitis (see **2.1.4Permutation**). While this result does not mean that the individual loci are not associated with constant-severe pancreatitis pain, it does suggest that the overlap of genetic risk for depression with these loci associated with constant-severe pain in our sample was due to random chance rather than due to a true "enrichment" of depression risk as we had expected and originally concluded (see Introduction and Discussion of Aim 1 paper). It is important to note that this result does not statistically support our conclusion of the importance of depression in the pancreatitis pain experience. Nor does non-significance of the overlap mean that an overlap does not exist—we just cannot make any conclusions on the importance of depression in the pancreatitis pain experience based on this overlap alone. However, an international study evaluating the pain experience in pancreatitis has come to the same conclusion that depression is important in the pancreatitis pain experience (Phillips, Faghih, Drewes, et al., 2020).

Another limitation of Aims 1 and 2 is that gene specific tests where gene specific p-values were not calculated. Gene specific p-values could have been used to test the hypotheses: 1) a candidate gene is not associated with the pancreatitis pain experience and not associated with depression, anxiety, or PTSD; 2) a candidate gene is not associated with the pancreatitis pain experience and is associated with depression, anxiety, or PTSD; 3) a candidate gene is associated with the pancreatitis pain experience but not with depression, anxiety, or PTSD, and finally the alternative; 4) a candidate gene is associated with the pancreatitis pain experience and with

depression, anxiety, or PTSD. However, these tests will still be limited by the small sample size available.

5.3 Future Directions

The genetic risk loci identified in this dissertation and the knowledge that some psychiatric risk loci may contribute to the pain experience can be used to develop precision treatments for pancreatitis patients. The loci can eventually be incorporated into genetic tests used by physicians to help identify patients that may benefit from mental health treatments and to help identify patients who may have a worse pain experience earlier in the disease. The identified loci can immediately direct replication studies in larger sample sizes and samples of different ancestry.

Future analysis to be done includes checking for depression risk loci in the other pain categories using improved methodology similar to Aim 2. Future analysis for Aim 2 could be to apply a permutation approach to generate an empirical p-value of the overlap of anxiety/PTSD genes with loci associated with pancreatitis pain. Alternative methods for investigating if pancreatitis pain risk loci are associated with psychiatric risk loci (Aims 1 and 2) could be phenome-wide association studies (PheWAS) or bidirectional Mendelian Randomization. The PheWAS would test if a SNP associated with pancreatitis pain is also associated with the psychiatric disorder using electronic health record data (Robinson et al., 2018). Multiple testing corrections, non-independence of diseases, and availability of data are limitations to PheWAS, the strength of a PheWAS using electronic health records or epidemiologically-defined phenotypes is the unbiased recording (without aiming to collect a specific phenotype) of phenotypes (Robinson et al., 2018). Bidirectional Mendelian Randomization could be used to test the causal relationship

between pancreatitis pain and psychiatric disorders; however, bidirectional Mendelian Randomization cannot parse a true causal effect from confounding by pleiotropy (Tang et al., 2022). Both PheWAS and Mendelian Randomization assess the relationship between pancreatitis pain and psychiatric disorders, while existing methods in Aims 1 and 2 identify a physical overlap of candidate loci. The methods of Aims 1 and 2 cannot directly address the phenotypic relationship between the pancreatitis pain experience and psychiatric disorders, which is why PheWAS and/or Mendelian Randomization would provide valuable additional information in the future. Other methods detecting and distinguishing between types of pleiotropy, such as fine mapping or Pleiotropy Regional Identification Method, would also be beneficial as these would identify if the observed overlapping pancreatitis pain experience loci and psychiatric loci are actually effecting both phenotypes (Solovieff et al., 2013).

Additionally, replication of all Aims in larger sample sizes and in patients of other ancestry groups needs to be completed. Finally, replication using more accurate and quantitative measures of pancreatitis pain from quantitative sensory testing needs to be done (Faghih et al., 2022; Phillips, Faghih, Kuhlmann, et al., 2020). Using these measures removes the recall and other bias associated with the existing self-report measures of pain.

Appendix A Published Papers

These papers were published during the course of this dissertation work. I contributed to the conceptualization, methodology, formal analysis and investigation, writing the original draft preparation, and reviewing, editing, and approval of the final drafts of the following papers.

Background: Dunbar, E. K., Saloman, J. L., Phillips, A. E., & Whitcomb, D. C. (2021). Severe Pain in Chronic Pancreatitis Patients: Considering Mental Health and Associated Genetic Factors. *J Pain Res, 14*, 773-784. doi:10.2147/jpr.S274276

Aim 1: Dunbar, E., Greer, P. J., Melhem, N., Alkaade, S., Amann, S. T., Brand, R., Coté, G. A., Forsmark, C. E., Gardner, T. B., Gelrud, A., Guda, N. M., LaRusch, J., Lewis, M. D., Machicado, J. D., Muniraj, T., Papachristou, G. I., Romagnuolo, J., Sandhu, B. S., Sherman, S., Wilcox, C. M., Singh, V. K., Yadav, D., & Whitcomb, D. C. (2020). Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort. *J Gastroenterol*. doi:10.1007/s00535-020-01703-w

Aim 2: Dunbar, E. K., Greer, P. J., Amann, S. T., Alkaade, S., Banks, P., Brand, R., Conwell, D. L., Forsmark, C. E., Gardner, T. B., Guda, N. M., Lewis, M. D., Machicado, J. D., Muniraj, T., Papachristou, G. I., Romagnuolo, J., Sandhu, B. S., Sherman, S., Slivka, A., Wilcox, C. M., Yadav, D., & Whitcomb, D. C. (2021). Pain Experience in Pancreatitis: Strong Association of Genetic Risk Loci for Anxiety and PTSD in Patients With Severe, Constant, and Constant-Severe Pain. *Am J Gastroenterol*. doi:10.14309/ajg.0000000000001366

Dunbar, E. K., Greer, P. J., Amann, S. T., Alkaade, S., Banks, P., Brand, R., Conwell, D. L., Forsmark, C. E., Gardner, T. B., Guda, N. M., Lewis, M. D., Machicado, J. D., Muniraj, T., Papachristou, G. I., Romagnuolo, J., Sandhu, B. S., Sherman, S., Slivka, A., Wilcox, C. M., Yadav, D., & Whitcomb, D. C. (2021). Correction to: Pain Experience in Pancreatitis: Strong Association of Genetic Risk Loci for Anxiety and PTSD in Patients With Severe, Constant, and Constant-Severe Pain. *Am J Gastroenterol*. doi:10.14309/ajg.0000000000001549

Dunbar, E., & Whitcomb, D. C. (2021). Response to Liu et al. *Am J Gastroenterol*. doi:10.14309/ajg.0000000000001556

Appendix B Aim 1 Paper

 Journal of Gastroenterology. Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort, Dunbar, E., P. J. Greer, N. Melhem, S. Alkaade, S. T. Amann, R. Brand, G. A. Coté, C. E. Forsmark, T. B. Gardner, A. Gelrud, N. M. Guda, J. LaRusch, M. D. Lewis, J. D. Machicado, T. Muniraj, G. I. Papachristou, J. Romagnuolo, B. S. Sandhu, S. Sherman, C. M. Wilcox, V. K. Singh, D. Yadav and D. C. Whitcomb, 2020 Oct; 55(10):1000-1009. doi: 10.1007/s00535-020-01703-w. Epub 2020 Jul 17. PMID: 32681239; PMCID: PMC9124361.

The original article and supporting information are available online at https://doi.org/10.1007/s00535-020-01703-w.

I contributed to the conceptualization methodology, formal analysis and investigation, and writing of this manuscript (see Author contributions). See **2.1.1 Corrected Table 1** for correction.

J Gastroenterol
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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort

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Abstract

Background Pain is the most debilitating symptom of recurrent acute pancreatitis (RAP) and chronic pancreatitis (CP) and often requires chronic opioids or total pancreatectomy with islet autotransplantation to manage. Pain is a complex experience that can be exacerbated by depression and vice versa. Our aim was to test the hypothesis that depression-associated genes are associated with a constant-severe pain experience in RAP/CP patients.

Study A retrospective study was done using North American Pancreatitis Study II (NAPS2) genotyped RAP and CP patients with completed case report forms (n = 1,357). Subjects were divided based on pattern of pain and pain severity as constant-severe pain (n = 787) versus not constant-severe pain (n = 570) to conduct a nested genome-wide association study. The association between

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reported antidepressant medication use and depression gene loci was tested.
Results Constant-severe pain was reported in 58% (n = 787) of pancreatitis patients. No differences in sex or alcohol consumption were found based on pain severity. Antidepressant use was reported in 28% (n = 223), and they had lower SF-12 mental quality of life (MCS, $p < 2.2 \times 10^{-16}$). Fifteen loci associated with constant-severe pain ($p < 0.00001$) were found to be in or near depression-associated genes including *ROBO2, CTNND2, SGCZ, CNTN5* and *BAIAP2*. Three of these genes respond to antidepressant use (*SGCZ, ROBO2,* and *CTNND2*).
Conclusion Depression is a major co-factor in the pain experience. This genetic predisposition to depression may have utility in counseling patients and in instituting early antidepressant therapy for pain management of pancreatitis patients. Prospective randomized trials are warranted.
Clinical trials registration Clinicaltriasl.gov.# NCT01545167

Keywords Pain chronic · Chronic pancreatitis · Depression · Antidepressants · Human genetics

Abbreviations

AP	Acute pancreatitis
BAIAP2-AS1	The BAIAP2 divergent transcript gene (or BAIAP2-DT)
BMI	Body mass index
CMH	Cochran–Mantel–Haenszel statistic
CP	Chronic pancreatitis
CRF	Case report form
CTNND2	The catenin delta 2 gene (or GT24; NPRAP)
DM	Diabetes mellitus
EPI	Exocrine pancreatic insufficiency
EA	European ancestry
GWAS	Genome-wide association study
LD	Linkage disequilibrium
MAF	Minor allele frequency
MCS	Mental component summary
NAPS2	North American Pancreatitis Study II
NDRI	Antidepressant drug class of norepinephrine-dopamine reuptake inhibitors
OR	Odds ratio
QOL	Quality of life
RAP	Recurrent acute pancreatitis
ROBO2	The roundabout guidance receptor 2 gene (or *SAX3*)
SGCZ	The sarcoglycan zeta gene (or ZSG1)
SF-12	Short form 12
SNP	Single-nucleotide polymorphism
SSNRI	Antidepressant drug class of serotonin and norepinephrine reuptake inhibitors
TIGAR-O	Common risk and etiology list for pancreatitis including Toxic, Idiopathic, Genetic, Autoimmune, Recurrent Acute and Severe Acute pancreatitis, and Obstructive

Introduction

Chronic pancreatitis (CP) is a syndrome of inflammatory destruction of the pancreas ending in irreversible damage with variable degrees of exocrine pancreatic insufficiency (EPI), diabetes mellitus (DM) and constant-severe pain [1–3]. Progression to CP typically begins with acute pancreatitis (AP) and recurrent AP (RAP), but even at these earlier stages, there are negative effects on physical and mental health and quality of life (QOL) [4–6]. The etiology of AP, RAP and CP is complex and associated with toxic and metabolic factors, such as alcohol, smoking, hypercalcemia, hypertriglyceridemia, and genetic factors, such as DNA sequence variants in or near *CASR, CEL, CFTR, CLDN2, CPA1, CTRC, PRSS1, SPINK1, TRPV6, UBR1* and others, and obstructive etiologies [7–9]. Additional genetic variants and environmental factors predispose to secondary complications, such as diabetes [10–12] and pancreatic cancer [13–17]. The major disabling feature that drives low quality of life is severe, constant pain, a condition that develops in 1 in 3 CP patients [1, 5, 6]. The reason for variability of this feature among pancreatitis patients is unknown, but may include genetic factors.

Pain is a subjective experience that encompasses the generation of pain signals from the injured organ, the physiological and pathological reflex responses, and higher brain responses linked to regions associated with emotion and motivation (prefrontal region, limbic system, and midbrain periaqueductal gray) to the pain signals [18]. Depression is recognized as a comorbid diagnosis in a variety of pain disorders and antidepressants have been empirically used in patients with CP to manage pain [19]. However, the effects of depression on the pain experience may be overlooked, and it is unclear as to who is likely to respond to antidepressant medications or other therapies in the management of the constant-severe pain experience in CP and the mechanisms implicated in treatment response.

Major depressive disorder (e.g. ICD-10 F33.2) is characterized by recurrent episodes of depression lasting at least two weeks and can include lowered energy and enjoyment of activities, depressed mood, different patterns of sleeping and eating, anxiety, lack of focus, guilt, and

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medically unexplainable symptoms [20]. Depression can be influenced by environmental factors—such as illness, financial instability, and childhood trauma—and genetic factors across many loci [21, 22]. The heritability of depression has been estimated to be between 30 and 40% based on twin studies [21, 23]. Individuals of both sexes with depression are also at an increased risk for certain physical disorders, such as diabetes mellitus, stroke, and heart disease [21, 24]. Patients with depression tend to have higher inflammatory immune responses; similarly, cytokines can induce depression and depression-like symptoms in patients [25]. Therefore, it is possible that some RAP/CP patients that are genetically prone to depression are also susceptible to altered inflammation and more severe pain.

We conducted an exploratory study in a well-phenotyped and genotyped cohort of patients with RAP and CP to determine whether genetic loci that have previously been demonstrated to be associated with depression were over-represented in the pancreatitis patients who also reported the most constant-severe pain. Our findings of associations between the constant-severe pain experience and several depression risk loci that are known to be responsive to antidepressant medications, and may respond to other therapies, establish this approach as a pathway toward more effective, personalized treatment of the most disabling complication of pancreatitis.

Materials and methods

Study population

CP and RAP patients and controls were ascertained from the North American Pancreatitis II (NAPS2) studies (NCT01545167) [1, 6, 26–29]. NAPS2 was launched in 1999 to determine the contribution of known risk factors (alcoholism and smoking) to CP and to discover new genetic risk factors [26, 28, 29]. The concept was to prospectively ascertain 1000 subjects with RAP and CP and spouse–friend controls with detailed demographic information and family history to define and quantify pancreatic disease-associated risk factors and exposures (based on TIGAR-O [30]), disease onset and progression to test the Sentinel Acute Pancreatitis Event (SAPE) hypothesis, [31, 32] and the prevalence and timing of secondary complications including pain, exocrine pancreatic insufficiency (EPI), diabetes (before or after AP), quality of life, etc. Biomarker results from the medical records were recorded to determine disease features and stages (e.g. pancreas imaging, list of secondary diagnoses, special tests, etc.) and blood was collected to measure serum biomarkers and DNA for genetic variants.

NAPS2 occurred in three phases, the original NAPS2 cohort (2000–2006) designed to ascertain 1000 RAP/CP patients, the NAPS2-continuation and validation study (NAPS2-CV) (2008–2012) designed to ascertain an additional 500 CP patients for GWAS studies, and NAPS2-ancillary study (NAPS2-AS) designed to ascertain 250 CP patients and 250 controls of African ancestry. Between sub-studies, minor changes were made to the case report forms to clarify ambiguities or uncertainties in previous versions (e.g. the physician was asked whether the patient was on pancreatic enzyme replacement therapy, but initially they were not asked if it was for EPI or pain). The result was over 50 direct and secondary publications including defining the role of alcohol and smoking in CP [27, 33, 34], pain patterns and their effect on quality of life [1, 6, 35], differences among patients based on age [1, 6, 35], sex [36] and ancestry [11, 28, 37], and multiple genetic findings including the first CP genome-wide association study (GWAS) identifying *PRSS1-2* and *CLDN1* risk loci [2], a new cystic fibrosis related syndrome [38], complex genetic risks [37–40], establishing the foundation for a new mechanistic definition of CP [3] and providing the rationale for precision medicine for pancreatic diseases [41–43].

The final subset of the NAPS2 cohort used in this study included RAP and CP subjects (n = 1,357). The diagnosis of RAP was based on two or more documented episodes of acute pancreatitis. CP was diagnosed based on validated imaging studies or histology [26, 35]. The study was constructed as a cross-sectional study including questionnaires described later. Only patients of European ancestry (EA) were included in the analysis to reduce heterogeneity since the majority of subjects recruited for NAPS2 were of EA. Previous genotyping was done on the Illumina HumanOmniExpress BeadChip [2]. Genotype data were prepared for imputation using the McCarthy Group pre-imputation checking tools and imputed against the 1000 genomes phase-3 reference panel on the Sanger imputation server using EAGLE2 for pre-phasing and PBWT for imputation [44–46]. Resultant imputed files were filtered based on the INFO score ≥ 0.5, renamed based on position, filtered for biallelic positions only, and filtered for genotype completeness at 90% leaving a total of 9,251,575 SNPs with a MAF > 0.01 for our analysis.

Questionnaires

Two sets of questionnaires were used to collect detailed information: one administered to patients by a trained research coordinator and the other completed by the enrolling physician. The patient questionnaires collected information on demographics (including current and maximal weight and height to calculate body mass index (BMI)), diabetes, EPI, personal and family history,

exposure to alcohol and tobacco, medication use, mental and physical quality of life using Short Form-12 (SF-12), pain experience using a visual analogue scale, pain frequency and severity, and McGill pain score. [1, 6]. The questionnaires in NAPS2 original and NAPS2-CV were identical in the core elements, but additional questions on the use of specific medication for pancreatic disease (rather than a list of all medicines) and their perceived utility were used in NAPS2-CV and NAPS2-AS so that not all information was available in all patients, resulting in missing information on antidepressants from those individuals (n = 547).

The enrolling physician provided information on age at onset and diagnosis of CP, exocrine and endocrine insufficiency, disease etiology, TIGAR-O risk factors, imaging findings, treatments tried and their perceived effectiveness [30]. The detailed assessment of alcohol and smoking in the NAPS2 cohort has been previously reported [26]. "Never drank" is less than 20 drinks in a lifetime. Smoking history was obtained from the patients' case report forms (CRF) with "never smoked" being less than 100 cigarettes in a lifetime.

Pain, depressive symptoms, and antidepressant use

Patterns of pain were defined following Mullady [1] using a 6-category severity–frequency classification system with O = no pain; A = episodes of mild pain; B = constant mild to moderate pain; C = episodes of severe pain; D = constant mild and episodes of severe pain; E = constant-severe pain [1]. For this study, subjects responding with D or E were classified as *constant-severe pain*, while subjects responding with O, A, B, or C were classified as *not constant-severe pain*. This combination of constant and severe pain had the highest impact on QOL.

Depression was not directly measured by the SF-12; however, a self-reported symptom of depression and a mental component summary (MCS) was gathered and reported as proxies in this study. The depressive symptom ("Felt Blue") was assessed using the SF-12 question "Have you felt downhearted and blue?" with reference to the previous 4 weeks and rated on a Likert scale of 1 "All of the time" to 6 "None of the time." The "Felt Blue" variable was a dichotomized version of the Likert responses with responses 1, 2, or 3 corresponding to "Yes" and 4, 5, or 6 corresponding to "No." The MCS was used as a measure of mental QOL, with a higher score indicating a greater QOL [1]. The MCS has been used previously as a measure of mental health and depressive disorders [47, 48].

Antidepressant use was reported in free-text format in the patient and/or physician case report forms. Text mining procedures in R were used to extract the antidepressants [49]. A list of the queried drugs, both brand name and corresponding generic names, can be found in Supplemental Table 1. The antidepressant variable was binary, with 1 meaning antidepressants were reported in the CRF and not used as pain medication and 0 meaning no antidepressants were reported. The majority of CP and RAP patients from the original NAPS2 cohort that were taking antidepressants were taking selective serotonin reuptake inhibitors (SSRIs, 56%), followed by tricyclic antidepressants (TCAs, 19%), serotonin and norepinephrine reuptake inhibitors (SNRIs, 16%), and norepinephrine–dopamine reuptake inhibitor (NDRIs, 10%) (Supplemental Table 1).

Genetic data analysis

The genetic analysis was constructed as a candidate gene review within a nested genome-wide association study (GWAS) data set of subjects with RAP/CP subjects based on pain patterns. The RAP and CP patients were combined, then classified as one of two groups: *constant-severe pain* (cases) or *not constant-severe pain* (controls). The nested GWAS was conducted using PLINK 1.9 software [50]. Quality control methods have been previously reported [2]. Data were fit to a logistic regression to test for associations. To control for ancestry, the first 4 principle components of ancestry were included as covariates. The minor allele frequency (MAF) was set to 0.01. Single-nucleotide polymorphisms (SNPs) with a p-value of less than 1×10^{-4} were chosen to continue in the analysis.

SNPs meeting the required significance threshold were then clumped into groups based on linkage disequilibrium (LD) ($\pm$ 250 kb from index SNP, $r^2 > 0.5$) using the "clump" command in PLINK [50]. The lead SNPs (p-value ≤ 0.0001) for each clump were annotated with gene names based on build GRCh37/hg19. These genes were then compared to a list of genes associated with unipolar depression and antidepressant response obtained from the GWAS Catalog in October 2019 [51]. Odds ratios (OR) for the lead-pain SNPs stratified by the binary antidepressant use variable were calculated with Cochran–Mantel–Haenszel (CMH) statistics using the "within" and "mh" commands in PLINK [50].

Demographic data were compiled and analyzed using R version 3.6.0. Univariant comparisons were performed based on the demographic variables using Pearson's chi-squared test for categorical data. Two-tailed p-values < 0.05 were considered statistically significant (***Table 1***) [49]. Zoom plots were created using the online platform LocusZoom, and exported using Gapplin (Supplemental Figs. 1–5) [52, 53].

Appendix Table 1 Aim 1

Table 1 Association of demographic and depression phenotypes with constant-severe pain

Variable	Level	Not Constant-severe Pain (n = 570)	Constant-severe Pain (n = 787)	Total (n = 1357)	*p-value*
Age[a]	Mean (sd)	51.7 (16.3)	46.5 (15.4)	48.7 (16.0)	***
Sex	Male	281 (49.3%)	407 (51.7%)	688 (50.7%)	
	Female	289 (50.7%)	380 (48.3%)	669 (49.3%)	
Alcohol	Never	118 (20.9%)	171 (21.8%)	289 (21.4%)	
	Ever	447 (79.1%)	615 (78.2%)	1062 (78.6%)	
	Missing	5	1	6	
Smoking	Never	220 (38.8%)	237 (30.3%)	457 (33.9%)	
	Ever	347 (61.2%)	546 (69.7%)	893 (66.1%)	**
	Missing	3	4	7	
Antidepressant Use	No	300 (75.2%)	287 (69.8%)	587 (72.5%)	
	Yes	99 (24.8%)	124 (30.2%)	223 (27.5%)	
	Missing	171	376	547	
"Felt Blue"	No	281 (86.5%)	312 (76.3%)	593 (80.8%)	
	Yes	44 (13.5%)	97 (23.7%)	141 (19.2%)	***
	Missing	245	378	623	
EPI	No	335 (71.9%)	501 (69.6%)	836 (70.5%)	
	Yes	131 (28.1%)	219 (30.4%)	350 (29.5%)	
	Missing	104	67	171	
Diabetes	No	354 (72.7%)	568 (74.6%)	922 (73.9%)	
	Yes	133 (27.3%)	193 (25.4%)	326 (26.1%)	
	Missing	83	26	109	
Mental QOL	Mean (sd)	46.8 (11)	41.8 (12)	43.7 (11.9)	***
	Missing	96	35	131	

Percentages shown next to the counts are column percentages within each variable

Stars indicate level of significance within each variable and with pain severity (*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$)

[a]Age of ascertainment

Results

Patient characteristics

The characteristics of the RAP and CP subjects classified as *constant-severe pain* or *not constant-severe pain* and the associations between constant-severe pain and general risk factors or depression-associated features are summarized in Table 1. Age, smoking, "Felt Blue", and MCS are all associated with *constant-severe pain*. Antidepressant use was associated with lower MCS (Wilcoxon rank sum test with continuity correction, W = 208,590, $p < 2.2 \times 10^{-16}$).

Genetic associations between constant-severe pain and depression genes.

There were a total of 1357 genotyped individuals of European ancestry with pancreatitis and core pain/depression information in the dataset. Candidate chromosomal loci for depression-associated genes in patients with a *constant-severe pain* phenotype were identified using PLINK by comparing *not constant-severe pain* with *constant-severe pain*. Candidate loci were identified by lead SNPs ($p < 0.0001$), clumped with other SNPs within 250 kb and with r^2 greater than 0.5 with the lead SNP [50]. Genes associated with the lead SNPs of the clumps were compared to genes associated with depression and reported in the GWAS Catalog identifying 15 pain loci containing depression genes [51]. The SNP most significantly associated with pain and with a depression-associated gene was rs12449867 on chromosome 17 near *BAIAP2-AS1* (OR 1.44, $p = 2.0 \times 10^{-5}$ for pain) (***Table 2***). Additionally, three genes (*ROBO2*, *CTNND2*, and *SGCZ* (***Table 2***)) were also reported in the GWAS Catalog as being associated with antidepressant response [51]. Individual zoom plots of the leading loci are in Supplemental Figs. 1–5. The LD regions shown in the zoom plots were based on EA.

Appendix Table 2 Aim 1

Table 2 GWAS results for 15 depression SNPs associated with pain in the NAPS2 data

Chr	Location (bp)	SNP	P	A1/A2[a]	OR (A1)	95% CI	Freq. A1	Gene
2	15,686,142	rs141909432	4.8×10^{-5}	A/G	3.97	2.04, 7.71	0.02	NBAS
2	55,331,982	rs2968817	7.6×10^{-5}	A/G	0.69	0.57, 0.83	0.29	RTN4
3	12,816,143	rs113388258	2.4×10^{-5}	C/T	2.03	1.46, 2.82	0.06	CAND2, TMEM40
3	77,151,787	rs4624600	5.5×10^{-5}	C/T	1.36	1.17, 1.59	0.35	ROBO2*
5	11,187,984	rs59442633	2.9×10^{-5}	C/T	1.81	1.37, 2.38	0.08	CTNND2*
5	146,034,762	rs458909	9.4×10^{-5}	A/C	3.44	1.85, 6.39	0.02	PPP2R2B
8	14,471,243	rs11300774	6.1×10^{-5}	T/TA	0.68	0.56, 0.82	0.25	SGCZ*
11	100,059,361	rs2123323	7.0×10^{-5}	T/C	1.39	1.18, 1.64	0.46	CNTN5
11	100,126,103	rs36106152	7.9×10^{-5}	G/GA	0.71	0.59, 0.84	0.33	CNTN5
12	118,024,434	rs71450224	5.1×10^{-5}	A/AAAAG	0.68	0.56, 0.82	0.26	KSR2
17	79,004,271	rs12449867	2.0×10^{-5}	C/T	1.44	1.22, 1.71	0.34	BAIAP2-AS1
17	79,031,825	rs9898347	9.6×10^{-5}	A/G	0.71	0.60, 0.84	0.35	BAIAP2
17	79,036,107	rs34176221	5.9×10^{-5}	AT/A	1.39	1.19, 1.64	0.45	BAIAP2
18	49,961,950	rs1619323	4.2×10^{-5}	C/T	0.66	0.54, 0.81	0.22	DCC
22	45,353,108	rs8137390	4.3×10^{-5}	G/A	1.50	1.24, 1.82	0.20	PHF21B

Chromosomal locations are based on build GRCh37/hg19

*Indicates regions associated with antidepressant response

[a]A1 is the minor allele, A2 is the major allele

Appendix Table 3 Aim 1

Table 3 CMH results for lead SNPs when genotype and pain are grouped by antidepressant use

Chr	SNP	A1	MAF	A2	CHISQ	P	OR	SE	L95	U95
2	rs141909432	A	0.016	G	15.16	9.89×10^{-5}	3.221	0.327	1.698	6.113
2	rs2968817	A	0.291	G	16.32	5.36×10^{-5}	0.691	0.092	0.578	0.828
3	rs113388258	C	0.059	T	18.73	1.51×10^{-5}	2.077	0.17	1.489	2.898
3	rs4624600	C	0.347	T	18.13	2.06×10^{-5}	1.435	0.085	1.215	1.695
5	rs59442633	C	0.082	T	18.43	1.76×10^{-5}	1.84	0.144	1.388	2.439
5	rs458909	A	0.017	C	17.48	2.90×10^{-5}	3.581	0.323	1.9	6.748
8	rs11300774	T	0.246	TA	21.09	4.38×10^{-6}	0.636	0.099	0.524	0.771
11	rs2123323	T	0.461	C	11.96	5.44×10^{-4}	1.326	0.082	1.13	1.555
11	rs36106152	G	0.329	GA	16.44	5.03×10^{-5}	0.699	0.089	0.588	0.831
12	rs71450224	A	0.262	AAAAG	16.62	4.56×10^{-5}	0.677	0.096	0.561	0.817
17	rs12449867	C	0.336	T	16.68	4.43×10^{-5}	1.415	0.085	1.197	1.672
17	rs9898347	A	0.355	G	12.4	4.30×10^{-4}	0.737	0.087	0.622	0.874
17	rs34176221	AT	0.445	A	11.19	8.23×10^{-4}	1.315	0.082	1.12	1.543
18	rs1619323	C	0.217	T	17.35	3.10×10^{-5}	0.653	0.103	0.534	0.799
22	rs8137390	G	0.202	A	15.99	6.37×10^{-5}	1.485	0.099	1.223	1.804

The use of antidepressants in patients with pain was assessed using Cochran–Mantel–Haenszel analysis. The result for each SNP associated with constant-severe pain was correlated with antidepressant use (Table 3). The most significantly associated locus after stratification by antidepressant use is at Chromosome 8:14,471,243, rs11300774 (OR 0.636, $p = 4.38 \times 10^{-6}$). Genotype counts for each SNP in cases and controls are reported in Table 4.

Discussion

Depression is a major, worldwide problem. It is estimated that at least 1 in every 6 individuals worldwide will experience depression during their lifetime, making depression the top cause of disability in the world [23]. Depression often starts during the teen years, persisting into adulthood. Like most psychiatric disorders, depression

Appendix Table 4 Aim 1

Table 4 Genotypic distribution among cases and controls. Counts represent number of individuals

Chr	rsID	Gene	A1 (minor)	A2 (major)	Total	*Not Constant-severe Pain*				*Constant-severe Pain*			
						A1/A1	A1/A2	A2/A2	Control	A1/A1	A1/A2	A2/A2	Cases
2	rs141909432	NBAS	A	G	1355	0	9	560	569	0	33	753	786
2	rs2968817	RTN4	A	G	1356	47	274	248	569	59	303	425	787
3	rs113388258	CAND2,TMEM40	C	T	1357	0	51	519	570	4	100	683	787
3	rs4624600	ROBO2	C	T	1347	72	193	300	565	155	287	340	782
5	rs59442633	CTNND2	C	T	1355	1	74	495	570	11	123	651	785
5	rs458909	PPP2R2B	A	C	1357	0	10	560	570	0	36	751	787
8	rs11300774	SGCZ	T	TA	1356	43	301	225	569	46	478	263	787
11	rs2123323	CNTN5	T	C	1356	97	300	173	570	182	392	212	786
11	rs36106152	CNTN5	G	GA	1357	65	231	274	570	82	381	324	787
12	rs71450224	KSR2	A	AAAAG	1354	41	234	295	570	45	303	436	784
17	rs12449867	BAIAP2-AS1	C	T	1355	49	239	281	569	99	376	311	786
17	rs9898347	BAIAP2	A	G	1356	72	296	202	570	91	340	355	786
17	rs34176221	BAIAP2	AT	A	1354	88	284	197	569	177	391	217	785
18	rs1619323	DCC	C	T	1355	31	195	343	569	36	260	490	786
22	rs8137390	PHF21B	G	A	1356	20	177	372	569	35	262	490	787

is a syndrome diagnosed using observation and self-report methods [22].

Pain and depression are comorbid and reciprocal, with the severity of one increasing the severity of the other [54, 55]. Severe depression affects an estimated 85% of patients with chronic pain [56]. Chronic pain has a heritability of approximately 30% [55]. Patients with depression and a chronic pain disorder were less likely to recover from their pain, and future episodes of pain were predicted by the presence of severe depression. Patients with severe depression before surgery suffered more pain after surgery than patients without depression. Conversely, antidepressants may not be as effective at treating depression in patients with higher pain levels at baseline, with 94% of relapsing depression occurring in patients with mild to moderate pain. Fortunately, when treatment of depression is successful, some symptoms of pain are also alleviated [54].

Pain is the most important clinical feature associated with disability and poor mental and physical quality of life in patients with RAP and CP [1, 6]. The connection between pain in chronic pancreatitis and depression is known. For example, one study found that in patients with chronic pancreatitis, not caused by alcoholism, severe depression was associated with higher intensity of pain [57]. Additionally, depression was a predictor of hospital readmissions at 30 days in patients with chronic pancreatitis [58]. Pain is a predictor of relapsing depression, it is therefore reasonable to suggest that a longer duration of pancreatic pain would influence the severity of depression, although duration was not associated with the "Felt Blue" variable (Supplemental Table 2) [29]. Thus, this is a major problem that must be addressed. Here, we evaluated the possible role of underlying genetic risk of depression to the experience of constant-severe pain. Our approach was an exploratory study to determine whether depression-associated genes that were already discovered and well characterized were within regions associated with constant-severe pain in patients with RAP and CP.

Pain and depression genes in pancreatitis

Fifteen candidate loci with known depression-associated genetic risk variants that were associated with constant-severe pain phenotype loci ($p < 0.00001$) were identified in our NAPS2 cohort. Additionally, the use of antidepressant medications indicates that over a quarter of patients were being treated with a trend toward higher use in constant-severe pain (30.2% constant-severe vs. 24.8% not constant-severe), but we could not determine if some patients initially had constant-severe pain that was improved with antidepressants and were, therefore, in the *not constant-severe pain* category at the time of ascertainment. We also saw an association with constant-severe pain and smoking (70% *constant-severe* vs. 61% *not constant-severe*, $p < 0.01$); however, this result may be confounded by some subjects smoking to control pain [33]. Alcohol use was not significantly different between pain categories. However, patients in the *constant-severe* pain category had a lower MCS, indicative of a lower QOL

associated with their pain. These data clearly indicate that depression is a major problem in patients with RAP and CP and that genetic loci with known depression-associated genetic risk variants are significantly associated with constant-severe pain.

Depression-associated genes

Multiple well-established depression-associated genes were associated with pain in pancreatitis patients including *ROBO2, CTNND2, SGCZ, CNTN5,* and *BAIAP2*. Three of these genes are also associated with response to antidepressants (*ROBO2*, *CTNND2*, *SGCZ*) [51]. Further information on these genes can be found in the supplemental information. These findings may also provide mechanistic support to the empiric observation that some patients with CP respond to antidepressant medications [19].

Clinical implications

The identification of genetic risk variants for depression associated with the severe-constant pain phenotype may have important clinical implications. The data presented here are a retrospective, cross-sectional, observational cohort study that are not designed to study depression per se. Furthermore, genetic risks for complex conditions, such as depression, are not independently causal as multiple genetic, epigenetic, environmental, emotional and other contextual factors (e.g. childhood abuse) also contribute to the phenotype. Thus, further longitudinal studies that are designed to address issues of pain and depression in pancreatitis—including interventional studies—are still needed. Nevertheless, the associations identified here were strong, and some immediate applications should be considered.

Recognition by the physician that a patient with pancreatitis and pain has genetic risks for depression can be useful for providing patient education that they are more likely to have biology-based components to depression, that depression makes pain worse, and that addressing these symptoms may augment pain treatment. Furthermore, there may be benefit in early interventions, such as cognitive behavioral therapy and, in some cases, detection of genetic risks in specific genes, such as *SGCZ, ROBO2,* and *CTNND2*, may provide rationale for a trial of specific medications that are known to improve symptoms in patients with depression from other etiologies.

Limitations and future directions

This nested study has a small sample size, which reduces power to discover genetic variants associated with a phenotype using the accepted genome-wide significance threshold of 5×10^{-8}.[59] Although no SNPs reached genome-wide significance as an independently associated factor, the study design was to identify previously validated depression genes within loci that were marginally associated with pain in RAP/CP. Thus, we do not believe that stringent thresholds for new genetic associations with correction for genome-wide associations apply here. Since depression analysis was not a goal of the NAPS2 studies, the depression phenotype(s) were not well defined, and slight changes in the CRFs may have affected study precision and power (e.g. missing data). The text mining process used to identify antidepressants is limited by spelling errors present in the case report forms, recall bias, as well as the number of subjects that contained full pharmacologic data. However, the NAP2 phenotypes and CRFs were completed by expert clinicians so the accuracy of the overall data set is fundamentally superior to administrative data sets and most of the unclear text data were easily resolved. The temporal relationship between severe pain and use of antidepressants was also not ascertained. Thus, the relationships between pain, depression, and antidepressants could not be fully assessed in this study, but should be addressed in future studies.

Summary and conclusion

Pain is the most debilitating symptom of chronic pancreatitis and one of the most difficult to treat [1, 35]. Approximately 18% of patients with chronic pancreatitis also experience depression [58]. Pain can increase the severity of depression, and vice versa [54]. GWAS data have been used to study the comorbidity and overlapping risk alleles of depression in diseases like type-2 diabetes, metabolic syndrome, and inflammatory bowel disease, and now in pancreatitis [23, 24, 60]. Our findings suggest that there is an overlap of depression-associated genes and constant-severe pancreatic pain.

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Author contributions Conceptualization: ED, PJG, DCW; Methodology: ED, PJG, JL, DCW, DY; Formal analysis and investigation: ED, PJG, NM, SA, STA, RB, GAC, CEF, TBG, AG, NMG, JL, MDL, JDM, TM, GIP, JR, BSS, SS, CMW, DY, DCW; Writing—original draft preparation: ED, DCW; Writing—review and editing: all authors; Funding acquisition: DCW; Resources: Supervision: DCW.

Compliance with ethical standards

Conflict of interest None of the authors had any financial relationship with any organization that sponsored the research.

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Supplemental Information.

1. Gene Function
2. Supplemental Figures
3. Supplemental Table

1. *Gene Functions*

The *ROBO2* (roundabout guidance receptor 2) gene is located at 3p12.3. The roundabout homolog 2 protein is an immunoglobulin transmembrane receptor for slit homolog 2 and plays a role in axon guidance and cell migration. *ROBO2* is primarily expressed in the brain and lungs, but mutations are associated with vesicoureteral reflux.(35) Additionally, ROBO2 also regulates dopamine in the midbrain and insulin in the pancreas. Anbalagan et al. also recently showed that ROBO2 plays a role in synaptic oxytocin levels (2019).(36) Oxytocin and ROBO2 also have been associated with Autism spectrum disorders.(36) Interestingly, ROBO2 has been shown to associate with BAIAP2.(37)

The *CTNND2* (catenin delta 2, 5p15.2) codes a protein that is part of the armadillo/beta-catenin superfamily and is an adhesive junction associated protein.(35) *CTNND2* was first associated with anxiety-related phenotypes in the Rat Genome Consortium, and was associated with hippocampal volume and dysfunctional synapses in mice. Nivard et al. replicated the association of *CTNND2* and anxiety and depression in humans (2014).(38)

SGCZ (sarcoglycan zeta, 8p22) codes a protein that is part of the sarcoglycan complex. These proteins are members of the dystrophin-associated glycoprotein complex, and are primarily

expressed in the ovaries and brain.(35) *SGCZ* is associated with antidepressant response and paliperidone efficacy in schizophrenia.(39, 40)

Conatctin 5 (*CNTN5*, 11q22.1) is part of the immunoglobulin superfamily and contactin family that is involved with nervous system development. The gene is expressed mainly in the placenta, thyroid, and brain.(35) There is evidence suggesting that ASD phenotypes may be the result of increased activity of glutamatergic neurons lacking a single copy of *CNTN5*.(41)

The *BAIAP2* (BAR/IMD domain containing adaptor protein 2, 17q25.3) gene codes the brain-specific angiogenesis inhibitor 1-associated protein 2, which is involved G-protein coupling. BAIAP2 is also an insulin receptor tyrosine kinase substrate and is involved in lamellipodia and filopodia formation.(35) In mice deletion of the gene homologous to *BAIAP2* leads to increased activity of NMDA receptors and abnormal behaviors. These behaviors are reversed in mice treated with memantine, which is an uncompetitive antagonist of NMDA receptors. BAIAP2 is also implicated in psychiatric disorders such as ADHD, autism spectrum disorders, and schizophrenia in humans.(42)

2. Supplemental Figures.

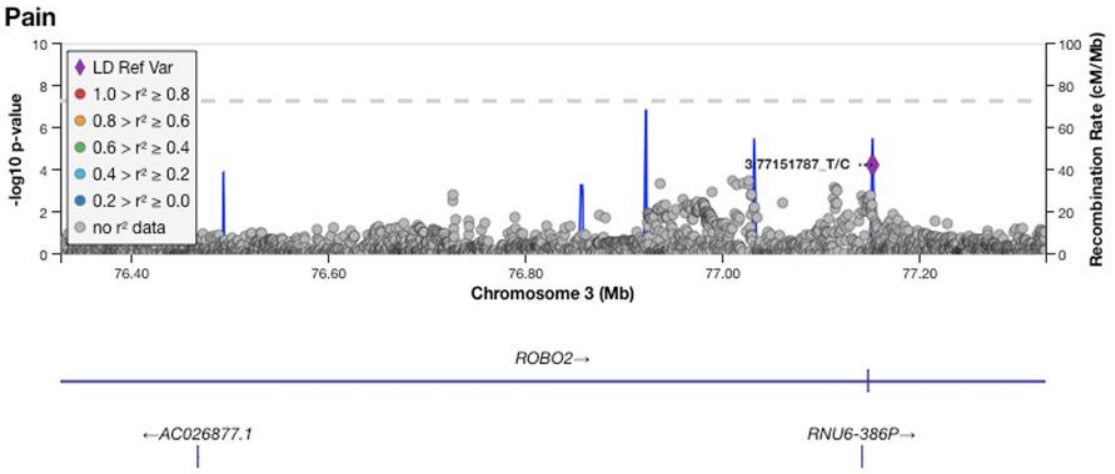

Figure S1. Zoom plot of SNP rs4624600 on chromosome 3. The locus covers the ROBO2 gene. The X axis is the chromosomal position in megabases. Y axis left, LOD score of individual SNPs (circles) colored by probability that they are associated with pain by chance. Y axis right, frequency of recombination of alleles between chromosomes (vertical blue lines).

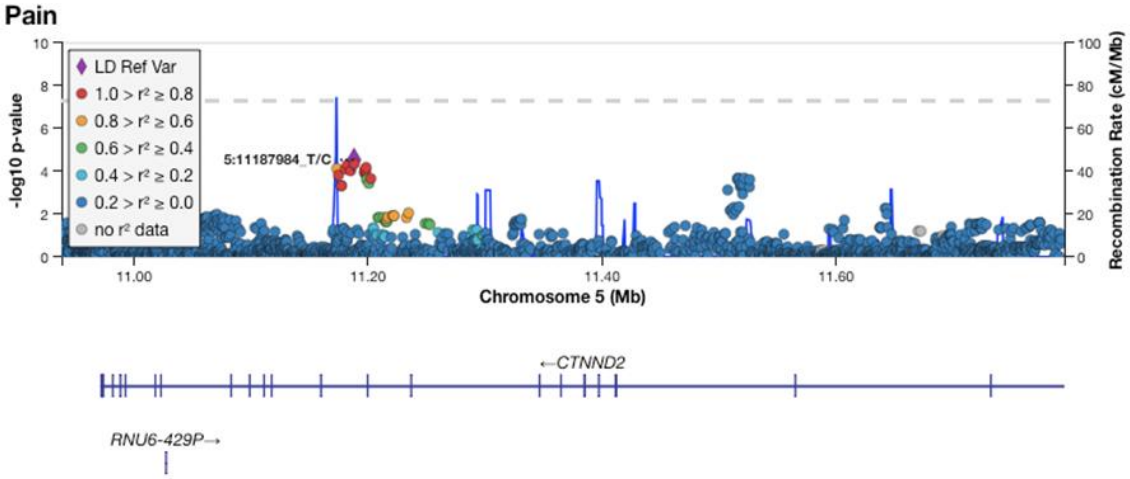

Figure S2. Zoom plot of SNP rs59442633 on chromosome 5. The locus covers the CTNND2 gene. The X axis is the chromosomal position in megabases. Y axis left, LOD score of individual SNPs (circles) colored by probability that they are associated with pain by chance. Y axis right, frequency of recombination of alleles between chromosomes (vertical blue lines).

Figure 5-1 Aim 1 Figures S1 and S2

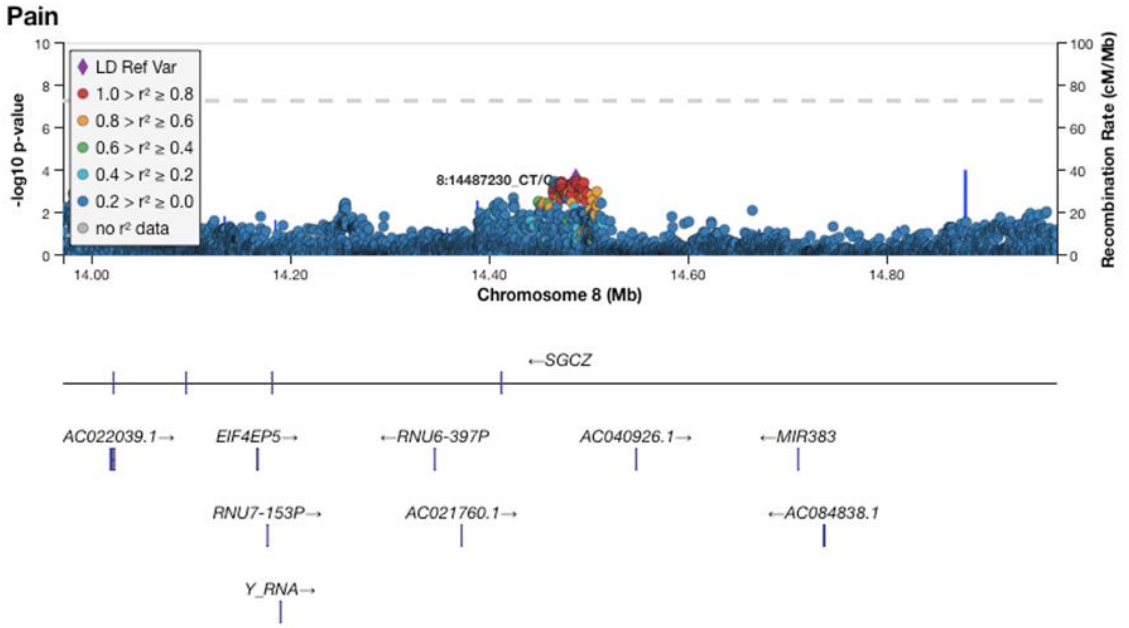

Figure S3. Zoom plot of SNP rs11300774 on chromosome 8. The exact location of the lead SNP is not visible but is within the cluster (red). The locus covers the *SGCZ* gene. X axis is the chromosomal position in mega bases. Y axis left, LOD score of individual SNPs (circles) colored by probability that they are associated with pain by chance. Y axis right, frequency of recombination of alleles between chromosomes (vertical blue lines).

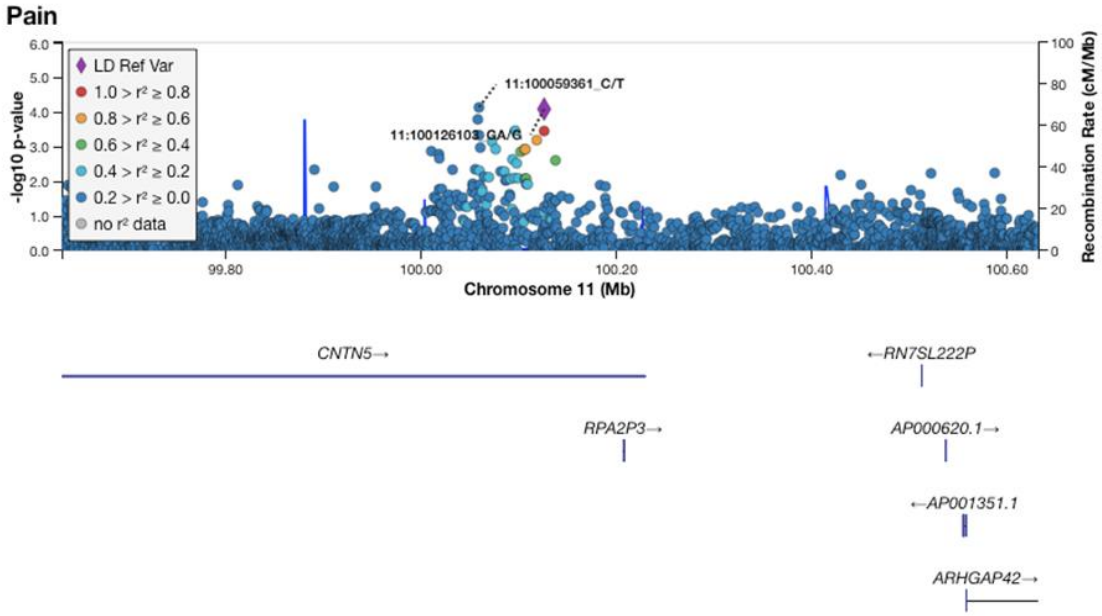

Figure S4. Zoom plot of SNP rs2123323 on chromosome 8. The exact location of the lead SNP is not visible but is within the cluster (red). The locus covers the *CNTN5* gene. X axis is the chromosomal position in mega bases. Y axis left, LOD score of individual SNPs (circles) colored by probability that they are associated with pain by chance. Y axis right, frequency of recombination of alleles between chromosomes (vertical blue lines).

Figure 5-2 Aim 1 Figures S3 and S4

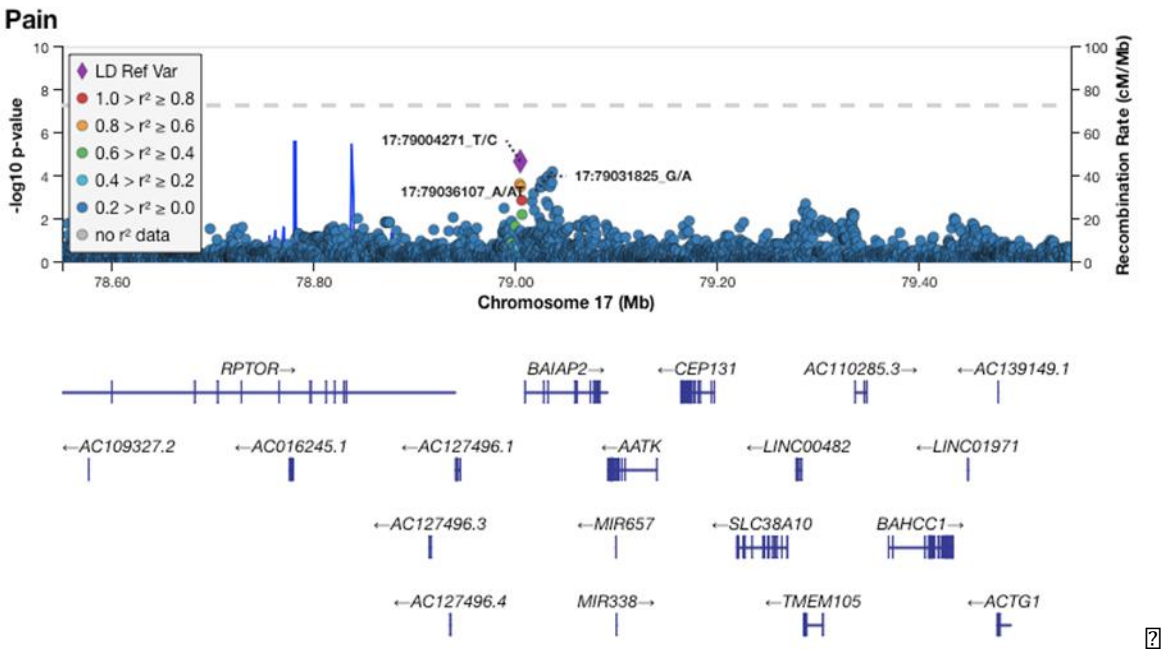

Figure S5. Zoom plot of SNPs rs12449867 (purple diamond, *BAIAP2-AS1*), rs9898347 (17:79031825) and rs34176221 (17:79036107) on chromosome 17. The locus covers the *BAIAP2* gene. X axis is the chromosomal position in megabases. Y axis left, LOD score of individual SNPs (circles) colored by probability that they are associated with pain by chance. Y axis right, frequency of recombination of alleles between chromosomes (vertical blue lines).

Figure 5-3 Aim 1 Figure S5

Appendix Table 5 Aim 1 Table S1

Brand names	Generic names	Class	n
Wellbutrin Wellbutrin XL Wellbutrin SR Forfivo Aplenzin	Bupropion	NDRI	31
Effexor Effexor XR	Venlafaxine	SSNRI	42
Cymbalta	Duloxetine	SSNRI	8
Fetzima	Levomilnacipran	SSNRI	0
Pristiq Khedezla	Desvenlafaxine	SSNRI	0
Zoloft	Sertraline	SSRI	47
Lexapro	Escitalopram	SSRI	46
Prozac Sarafem	Fluoxetine	SSRI	33
Paxil Paxil CR Pexeva Brisdelle	Paroxetine	SSRI	31
Celexa	Citalopram	SSRI	19
Luvox	Fluvoxamine	SSRI	3
Brintellix	Vortioxetine	SSRI	0
Viibryd	Vilazodone	SSRI/5HT1A receptor partial agonist	0
Elavil	Amitriptyline	TCA	36
Pamelor	Nortriptyline	TCA	14
Quitaxon Aponal Sinequan	Doxepin	TCA	6
Norpramin	Desipramine	TCA	2
Tofranil	Imipramine	TCA	2
Anafranil	Clomipramine	TCA	0
Vivactyl	Protriptyline	TCA	0
Surmontil	Trimipramine	TCA	0
Asendin	Amoxapine	TeCA	0

Supplemental Table 1. Medications queried in CRF. *n* is based on total pancreatitis patients, prior to sorting by availability of genotype data.

Appendix Table 6 Aim 1 Table S2

Variable	Level	Not Blue (n=593)	Felt Blue (n=141)	Missing (n=623)	Total (n=1357)	p-value
Duration	mean (sd)	6.5 (7.5)	6.8 (6.8)	7.6 (8.4)	7 (7.8)	
	missing	6	0	80	86	ns

Supplemental Table 2 Association of the average duration of pancreatitis with "Felt Blue." Duration reported in years. (ns, * p<0.05; ** p<0.01, *** p<0.001)

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