GENETICALLY INFORMED APPROACHES TO DISENTANGLE RELATIONSHIPS OF CHRONIC PAIN TO EXECUTIVE FUNCTIONING AND SUBSTANCE USE

by

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Genetically informed approaches to disentangle relationships of chronic pain to executive functioning and substance use

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Chronic pain is pain that persists for 3 or more months, often without discernible cause. There are many risk factors associated with chronified pain, including cognitive dysfunction and substance use. This dissertation uses multiple genetically informed methods to examine questions about genetic and environmental influences on chronic pain and its risk factors. Overall, these studies examine the phenotypic, genetic, environmental, and epigenetic relationships of chronic pain with executive functions and substance use. **Study I** found that chronic pain in young adults specifically relates to updating working memory, rather than to common executive function or mental set shifting, and that the association was entirely due to genetic effects. **Study II** demonstrates that the relationship between chronic pain and smoking may be due to underlying genetic risk, rather than a direct relationship, and that the reward pathway in the brain does not appear to be a significant mediator of the association. **Study III** shows that general chronic pain risk genetically relates to substance use disorder risk, and also shares additional associations with cigarette consumption and alcohol frequency. This dissertation establishes the importance of using genetically informed methods to interrogate epidemiological questions of interest.

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CHAPTER I: INTRODUCTION

Chronic Pain

Pain is defined as an ‘unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’ (IASP Task Force on Taxonomy, 1994). The perception of pain is theorized to be constructed from sensory-discriminative, cognitive-evaluative, and affective-motivational neuronal pathways (Melzack & Wall, 1965), emphasizing the interplay of both biological and psychological mechanisms in constructing the pain experience. While pain likely evolved to alarm of bodily injury and inflammation (Walters & Williams, 2019), chronic pain, defined as pain which has persisted for at least 3 months and often without discernable cause (Treede et al., 2019), is one of the primary causes of disability, with epidemiologic studies estimating a prevalence of 18-34.5% worldwide (King et al., 2011; Yong et al., 2022). The biopsychosocial model of chronic pain states that both biological (e.g., genetics, brain) and psychosocial risk factors (e.g., cognitive control, substance use, exercise, mental health) influence pain development, and investigating these pathways may help elucidate pain pathophysiology, inform beneficial lifestyle modifications, and idealistically, provide personalized healthcare (Gatchel et al., 2007).

Executive Functions and Pain

One component of the biopsychosocial risk factor model of chronic pain are deficits in cognitive control. Executive functions (EFs) encompass many facets of cognitive abilities that are important in goal-directed behavior, such as inhibitory control, working memory, and cognitive flexibility (Diamond, 2013; Friedman & Miyake, 2017). Poorer performance on a wide variety of EF tasks have been repeatedly associated with chronic pain status (Berryman et al., 2013, 2014) and pain patients show brain anomalies in parts of the cortex related to EF

(Smallwood et al., 2013; Smolker et al., 2018). EFs have been proposed to be mechanistically important in pain chronification, as poorer EF performance prospectively predicts chronic pain development (Attal et al., 2014). However, deconstructing which facets of EF are uniquely related to pain through the use of EF tasks has yielded conflicting results (Berryman et al., 2013, 2014). EF tasks are susceptible to noise and often tap multiple types of EF simultaneously (Friedman & Miyake, 2017). Latent variable modeling can be used to partition variance into common factors that capture a shared signal, and into residual variance that is independent of the factors (Bollen, 2002). Hence, multiple EF tasks can be partitioned into separable EF factors (e.g., response inhibition, working memory updating, mental set shifting) and then tested in relationship to pain. Furthermore, if the study is genetically informed, that can be used to model the degree of shared genetic and environmental risk, which is yet to be explored.

Substance Use and Pain

Another prominent risk factor in the biopsychosocial model of pain are substance use and substance use disorders as they co-occur with chronic pain at a much higher rate than the general population (Dassieu et al., 2019; Orhurhu et al., 2015; Schaffer et al., 2023). There are various hypotheses for these co-occurrences, including: self-medication leading to frequent substance use, substance use causing pain chronification, or shared risk factors. One proposed mechanism is substance use may lead to pain chronification through promotion of maladaptive brain plasticity (Petre et al., 2015). Chronic pain has been linked to deficits in brain areas related to reward (Borsook et al., 2016; Smallwood et al., 2013). However, these associations may be due to predisposing factors, such as shared genetic risk. Using genetically informed methods, such as co-twin controls and twin models, the potential for causality as well as the relative level of shared genetic and environmental risk can be assessed (Loehlin, 1996; McAdams et al., 2021).

Further examination of the relationships between chronic pain and substance use disorders is needed to disentangle which risk pathways are unique to certain substance use versus are general to substance use. While it is difficult to recruit large samples with sufficient endorsement of substance use disorders and chronic pain conditions, Genomic SEM can test model hypotheses using different samples, which allows for testing novel relationships with traits that have low endorsement in the general population, such as opioid use disorder (Grotzinger et al., 2019).

Goals and Organization of this Dissertation

By using genetically informed methods, we can better understand the nature of the relationships between chronic pain and its common correlates, such as cognitive control and substance use. In Chapter II, I test whether three separable EF factors (e.g., common EF, working memory updating, and mental set shifting) predicted chronic pain phenotypically and genetically. In Chapter III, I test whether smoking was related to chronic pain through the strength of the reward pathway and examined their relative shared genetic and environmental risk. In Chapter IV, I propose using Genomic SEM to examine the extent of genetic relatedness between a general chronic pain factor with an addiction factor and individual substance use disorders. Overall, these three studies examine the interrelatedness and potential shared genetic risk between chronic pain and its prominent correlates.

CHAPTER II: CHRONIC PAIN IS SPECIFICALLY ASSOCIATED WITH UPDATING WORKING MEMORY: A LONGITUDINAL TWIN STUDY

Introduction

Chronic pain patients often show lower cognitive performance compared to individuals not in pain. In systematic reviews, individuals experiencing chronic pain perform worse across many cognitive tasks that tap various executive functions (EFs), including working memory, response inhibition, and cognitive flexibility (Baker et al., 2016; Berryman et al., 2013, 2014; Bunk et al., 2019). Pain relates to EFs throughout the lifespan, with young adolescents with pain exhibiting lower cognitive performance compared to their peers (Jastrowski Mano et al., 2020), and older adults in pain showing steeper cognitive decline (Murata et al., 2017) and increased risk for dementia (Ezzati et al., 2019). Prospectively, worse EFs predict pain incidence 9 years later, over and above various covariates that are related to pain, including psychopathology and socioeconomic status (Ng & Hartanto, 2022). Pre-operative patients with no pain prior to surgery who performed worse on cognitive tasks were more susceptible to post-operative pain chronification 6 and 12 months post-surgery (Attal et al., 2014). Understanding the role of EFs in pain development could help clarify the pathophysiology of chronified pain.

Most of this previous research has tested associations with individual cognitive tasks. Cognitive tasks, particularly EF tasks, are susceptible to measurement error and often tap multiple cognitive processes (Miyake et al., 2000). Latent variable modeling allows for the extraction of shared variance across tasks meant to capture a specific construct (e.g., working memory updating; mental set shifting), which minimizes measurement error. One study using this approach found that pain patients had worse EF performance on a global EF factor constructed from 6 tasks (Rouch et al., 2021); however, it remains unclear what EF domain(s) (e.g., mental set shifting or updating working memory) are associated with pain. Prospective associations between EFs and pain may also differ when including multiple factors.

While poorer cognitive abilities during pain are supported in the literature, findings vary across tasks, and phenotypic studies cannot evaluate whether shared predispositions explain relationships. To our knowledge, no study has evaluated the genetic and environmental etiology of relationships between pain and EFs. Around 55% of the variance of pain appears to be due to genetic risk, and remains stable across adulthood (Burri et al., 2018). EFs in adulthood are moderately to highly heritable (42% to 100%) at the level of latent variables (Friedman et al., 2016; Friedman & Miyake, 2017; Gustavson et al., 2018, 2022), and their genetic influences also remain stable across young adulthood (Friedman et al., 2016). These genetic influences on pain and EFs might overlap. There could also be overlapping environmental risk for pain and EFs, as both are sensitive to similar risk factors such as chronic metal exposure (Rafiee et al., 2020; W. Wang et al., 2023) or stress (Abdallah & Geha, 2017; Marin et al., 2011).

In this twin study, we evaluated whether individual differences in three EF latent variables measured at ages 23 and 28 years (Common EF; working-memory Updating-specific function; and mental set Shifting-specific function) are associated with pain reported at age 28. Then we decomposed the significant associations into etiological correlations. To our knowledge, this is the first study to examine longitudinal and genetic relationships between three, separable EF factors and pain. Examining associations with multiple EF factors elucidates which facets of EF (e.g., Common EF) are specifically relating to pain in young adults, and understanding the nature of these associations clarifies whether the pain-EF relationships are due to overlapping genetic and/or environmental risks.

Method

Participants

Participants were 786 individual same-sex twins (55% female, 45% male) from the Colorado Longitudinal Twin Study (LTS) with data for pain and/or EFs. Of the twins, 416 were monozygotic (MZ) and 370 were dizygotic (DZ). The LTS is a long-standing, ongoing developmental study of twins born between 1984 and 1990. Twins whose families lived within a 3-hour driving range (~300 km) from Boulder, Colorado, and whose birth weights were at least 1000 grams (though 96% weighed 1700 grams or more) were invited to participate (more details on data ascertainment are described in (Corley et al., 2019; Rhea et al., 2013)). Twins were not selected or excluded for particular conditions (e.g., pain). The LTS began testing twins born in Colorado between 1986 and 1990 at age 14 months and followed them into adulthood.

For this study, we used data collected at 2 separate visits, when the twins were mean ages 22.84 (standard deviation [SD] = 1.29, range = 21.11-28.03; total *n* = 785) and 28.80 (SD = 0.87, range = 28.02-34.58; total *n* = 649). EF data were collected at both visits, but the pain data were obtained only at the second timepoint of these visits, when the twins completed functional magnetic resonance imaging (fMRI) and questionnaires. Exclusion criteria for this time point included standard contraindications to MRI (metal in the body, recent tattoo, claustrophobia, and pregnancy).

The LTS sample self-reported their race as being 91.66% white, 4.44% Latino or Hispanic, 2.60% bi- or multi-racial, <1% Pacific Islander, <1% American Indian, and <1% self-reporting as other. Fewer than 0.5% (*n* = 5) of the sample endorsed any usage (days 1-30) of painkillers in the last 30 days; it did not significantly predict pain. All study procedures were approved by the Institutional Review Board of the University of Colorado Boulder.

Measures

Brief Pain History Questionnaire. The Brief Pain History Questionnaire (BPHQ) is a self-report questionnaire based on the Brief Pain Inventory, which is a validated tool for assessing non-malignant pain (Tan et al., 2004). The BPHQ was completed by the participants at the age 28 assessment. The BPHQ asks participants to report whether they were experiencing a "current significant episode of pain" in the last month (0 = no; 1 = yes); identify the location(s) of the pain episode on a body map; rank the pain severity (from 0 = no pain; to 100 = worst pain imaginable); describe the quality of the pain episode (tingling, stabbing, etc.); and report the age they were when the pain began. Pain severity rating scales are commonly used to measure pain intensity and show moderate to high reliability (0.67-0.96) (Kahl & Cleland, 2005). The current age of the participants in addition to their self-report of when pain began was used to construct a pain duration measure. This self-report of a pain episode includes multiple pain sites (e.g., head pain and back pain) and is best interpretable as general chronic pain (See Table 2-1 for pain sites).

Table 2-1. Frequency of self-reported pain locations

Pain locations	
Single body location	43%
Multiple body locations	54%
Lower extremity/Knee	35%
Back	27%
Upper extremity/Shoulder	13%
Head/Neck	13%
Chest	3%
Stomach/Bowel	3%
Hip	3%
Genital	2%
Chose not to report	3%

Note. The percentages above are calculated from the 176 people who reported experiencing chronic pain. Over half of the sample was experiencing pain in multiple locations.

Chronic pain inception can begin at an individual location but often spreads to multiple locations, which appears evident in our sample where 54% of those in pain are endorsing multi-site pain. It is theorized that the spreading of pain may occur due to central sensitization, which is amplification of pain due to maladaptive plasticity of the central nervous system (Harte et al., 2018; Tanguay-Sabourin et al., 2023). While chronic pain conditions are surely diverse in causes and symptoms, there is both psychometric and genetic evidence that there is shared etiology for pain across body sites. A common component underlying pain starting at a single site and spreading across body sites has been longitudinally validated (Battaglia et al., 2020). Furthermore, both twin (Vehof et al., 2014) and molecular genetic (Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023) studies have identified a common genetic factor that underlies all measured chronic pain conditions; thus, looking at general chronic pain may be useful for understanding part of the pathophysiology.

Although the BPHQ is self-reported, we attempted to ascertain a measure of pain chronicity as closely related to clinical cut-offs as possible. For these analyses, no pain was ascertained by answering "No" for experiencing a significant episode of pain (no chronic pain = 0). Participants who were experiencing non-chronified pain that was mild (mild chronic pain; pain intensity <=29) and/or has persisted for less than 3 months were coded as 1. Participants whose pain had lasted for 3+ months and was moderate to severe in intensity (moderate-severe chronic pain; pain intensity >=30) were coded as 2. The optimal cut-offs for pain severity may vary, but generally a cut off around 34 can be used for mild pain (Boonstra et al., 2014). We chose the cut-off of 30 as this has been conceptualized as clinically-meaningful pain by a previous prospective EF and pain study (Attal et al., 2014). Creating a pain variable using a combination of pain severity and duration has been used before in relation to EF (Murata et al., 2017), and is meant to ascertain a measure of chronic, debilitating pain.

Of the 637 people who filled out the BPHQ questionnaire, one person who reported pain rated their pain severity as 0, and two people with pain were missing on their pain severity rating; hence, our final *n* for the pain measure was 634. Of the people who reported having pain in our sample, 97.2% reported pain lasting more than 3 months, making our measure interpretable as general chronic pain. We decided not to exclude the 5 participants whose pain episode was less than 3 months as their pain episodes were showing persistence (range = 1.18 to 2.37 months), and our models would then lose information about their co-twins as well. To ensure these 5 individuals were not changing results, we ran a version of the phenotypic Cholesky decomposition in which we dropped them entirely. The results were consistent. While we do not have a measure of pain history at age 23, the average pain episode duration reported at age 28 was 1.88 years (22.60 months) suggesting that most of the participants pain inception occurred after age 23.

EF tasks. Participants completed 9 computerized EF tasks at the age 23 visit (antisaccade, stop-signal, Stroop, keep-track, letter memory, spatial *n*-back, number-letter, category-switch, and color-shape). At age 28, the battery was shortened to 6 tasks (antisaccade, Stroop, keep-track, letter memory, number-letter and category-switch). The tasks are briefly described below (see (Friedman et al., 2020; Gustavson et al., 2022) for more details).

The 3 response inhibition tasks required participants to avoid prepotent responses. In the *antisaccade task*, participants had to avoid the reflex to look at a cue stimulus on one side of the screen and instead immediately look in the opposite direction to see a briefly appearing target stimulus (a number from 1-9). The timing of the task was such that it would not be possible for the participants to see the target before it was masked if they saccaded to the cue. The dependent measure was the accuracy of target identification. In the *stop-signal task*, participants had to categorize an arrow as pointing right or left as quickly and accurately as possible. On 25% of the trials, the participants had to withhold their categorization if the arrow on the screen turned red (the delay before the arrow turned red was titrated according to performance). The dependent measure was the stop-signal reaction time. The stop-signal task was not collected at age 28. In the *Stroop task*, the participants had to avoid the tendency to read color words (e.g., red, blue, green) and instead report the font color. There was a block of 42 trials of colored strings of 3-5 asterisks; then a block of 42 trials of color words printed in a congruent color; then two blocks of 42 trials of color words printed in an incongruent color. The difference in mean reaction times for correct responses for the incongruent and asterisk blocks was the dependent measure.

The 3 updating working memory tasks required participants to add and delete information in working memory. In the *keep-track task*, participants had to keep track of the last word appearing in 2-5 categories from a series of words from 6 different categories (animals, colors, countries, distances, metals, and relatives). For each trial, a category list was shown and remained at the bottom of the screen while 15-25 words appeared serially for 2 seconds each. The proportion of target words recalled across all trials was the dependent measure. In the *letter memory task* participants had to rehearse the most recent 4 letters from series that were 9, 11, or 13 letters long. On each trial, consonants appeared one at a time for 3 seconds each, during which time participants would say aloud the last 4 letters including the letter currently on the screen. The dependent measure was the proportion of sets correctly rehearsed across 12 trials (132 sets total). In the *spatial n-back task* participants saw 12 squares fixed in random location on the screen. Within 6 blocks, 24 squares flashed one at a time, and the participants had to indicate whether it was the same square that flashed either two or three trials before. The 2-back and 3-back were separate conditions, and participants completed 6 blocks for each. The dependent measure was the averaged z-scores of the arcsined proportion of correct scores for both conditions. The spatial *n*-back task was not administered at age 28.

The 3 set-shifting tasks required participants to switch between two sub-tasks based on a cue that appeared 350 ms before the stimuli in each trial. In each trial of the *number-letter* task participants saw a number-letter/letter-number pair in one of four quadrants of a square. They categorized the number as odd or even when the pair appeared in one of the two top quadrants and categorized the letter as consonant or vowel if the pair appeared in one of the two bottom quadrants. In each trial of the *color-shape* task the participants saw a colored shape and categorized the color as red or green when it appeared with a "C" cue, or categorized the shape as circle or triangle when it appeared with an "S" cue. The *color-shape* task was not administered at age 28. In each trial of the *category-switch* task participants saw a word and categorized it as either being smaller or bigger than a soccer ball or living or nonliving depending on which cue symbol appeared. In all three tasks half the trials required repeating the subtask from the prior trial and the other half required shifting sub-tasks. The dependent measure for all the switch tasks was the difference between the average reaction time for switch trials and the reaction time for repeat trials.

Study Design

The design of the current study is an observational individual twin differences twin study (i.e., no experimental manipulation). Our sample was not selected for chronic pain nor for EF performance levels. We used the longitudinal, observational data of our sample to run cross-sectional and longitudinal structural equation models (SEMs). We used the twin structure of our sample to run genetic models.

Statistical Procedures

We used Mplus Version 8.10 (B. Muthén & Muthén, 2017) to estimate both the phenotypic and genetic models. The analyses were conducted on raw data, and the models used all available data (see Table 2-2 for the *n* for each variable). The EF models consisted of continuous data and were estimated using the robust maximum likelihood (MLR) estimator. The models that included the ordinal pain measure were estimated using the diagonally weighted least squares mean and variances adjusted (WLSMV) estimator. Mplus' TYPE=COMPLEX option was used for the phenotypic models, which employs a sandwich estimator to adjust standard errors and chi-square tests to correct for the non-independence due to twin structure (Rebollo et al., 2006). A non-significant chi-square indicates that the model is perfectly reproducing the observed covariance matrix, however due to the chi-square's sensitivity to sample size, a significant chi-square does not necessarily indicate an unreasonable fit (it indicates non-perfect fit). Therefore, we used comparative fit index (CFI) > .95 and root-mean-square error approximation (RMSEA) < 0.06 to indicate good fit (Hu & Bentler, 1998). A significant parameter was assessed at $p < 0.05$ for the Wald tests of the phenotypic parameters. The significance of the variance components of the genetic models were assessed using Mplus's difftest procedure, as the standard errors in the genetic models are not invariant to model parameterization, but the chi-square difference test is (Neale et al., 1989).

Data transformation. Sex was included as a covariate in all analyses and age and sex effects were regressed out of the EF tasks at each respective time point. Age was not included as a covariate for pain as it was a non-significant predictor estimated at near 0 ($r = 0.00$ [0.01], $p =$

0.996). The cohort showed narrow age range, and the testing protocol attempted to minimize age variance. All the EF tasks were re-scaled to have a standard deviation of around 1 to help speed model estimation (Kline, 2016).

EF factors. The EF model we used is based on previously published analyses of these EF data (see Figure 2-1) (Friedman et al., 2020). The factors reflect a unity and diversity model of EFs, which has been well-supported in the literature (Friedman & Miyake, 2017). The Common EF captures variance common to all the EF tasks and is thought to reflect general goal maintenance or top-down biasing of attention (Miyake & Friedman, 2012). The Updating-specific factor captures left-over shared variance of the three updating working memory tasks after accounting for Common EF variance. Working memory is a short-term information retention memory system that maintains features of importance, filters out unnecessary sensory signals, and continuously adds and deletes information (Baddeley, 1992). Similarly, the Shifting-specific factor captures variance shared by the three shifting tasks that is not accounted for by the Common EF factor. There is no factor specific to the inhibiting tasks because the Common EF factor captures all the variance shared by the three response inhibition tasks.

This EF model was also applied to the age 28 data based on a previous study using these data (Smolker et al., 2018). Unlike at age 23, the stop-signal, spatial *n*-back, and color-shape task were not included in the age 28 battery. Since the age 28 Updating-specific and Shifting-specific factors were left with only 2 indicators each, the loadings were equated (after scaling the tasks to have similar variances) to allow for identification (Kenny & Milan, 2012). For longitudinal models, residual covariances between the same task across time were included. At age 23, a residual covariance between the antisaccade and spatial *n*-back task was included, as prior studies using these EF data have reported the model with this shared measurement error (Friedman et al., 2016).

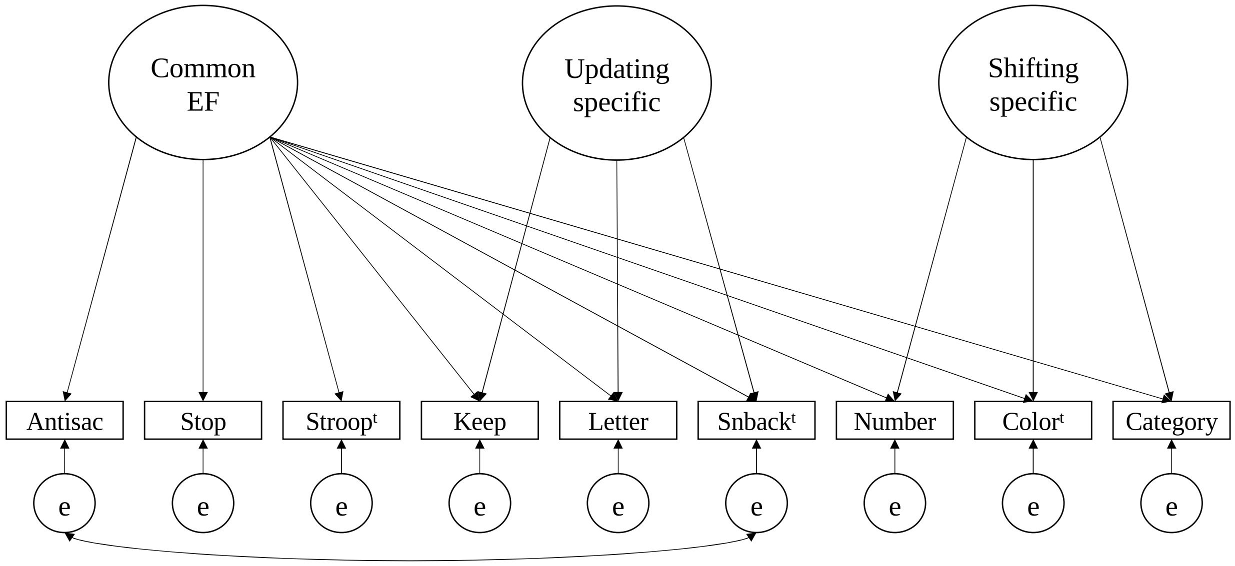


Figure 2-1. Executive function (EF) model
This model was specified based on prior publications using these EF data (Friedman et al., 2016; Reineberg et al., 2022). The Common EF factor captures shared variance across all the EF tasks. The Updating-specific factor captures shared variance specific to the working memory updating tasks and the Shifting-specific factor captures shared variance specific to mental set-shifting, both after the variance attributable to Common EF is considered. Each task is allowed an error term which includes the measurement error and variance unique to each task not explained by the three factors. The Antisaccade and Spatial n-back share a residual correlation possibly because both involve rapid eye movements to different spatial locations on the screen. This residual correlation has been included in all publications which use this data as it improves model fit and aids in convergence for the genetic models, see Friedman et al. (Friedman et al., 2016) for details. Antisac = Antisaccade; Stop = Stop-signal; Keep = Keep-track; Letter = Letter memory; Snback = Spatial n-back; Number = Number-letter; Color = Color-shape; Category = Category-switch
^{t}These three tasks were not collected at age 28 and consequently not included in the age 28 model of EF.

Phenotypic longitudinal Cholesky decomposition

We used a phenotypic Cholesky decomposition to answer whether it is the stable variance components of the EF factors (variance at age 23 and the variance it shares with age 28) or the new EF variances that appear concurrently with pain at age 28 that relate to pain at age 28. A Cholesky decomposition is a useful technique for longitudinal data as it extracts all variance from the second time point of a variable that is shared with the first time point, and partitions the residual variance of the second timepoint into a separate factor (Loehlin, 1996). Thus, it allows for examination of partial correlations with the second time point, controlling for the earlier time point.

Previous research using an earlier wave at age 17 and the age 23 wave of the current sample has found that EF factors are highly stable across time (Friedman et al., 2016). Thus, we decided to use a Cholesky decomposition of the age 23 and 28 waves: The first factor predicts all the variance in age 23, and the variance at age 28 that is shared with age 23; the second factor captures age 28 variance that is unique (see Figure 2-2). We correlated both the age-23 and the age 28-specific variance components of EFs with pain, for a total of 6 covariances with pain. We did so to assess whether pain is relating to the stable components of EF, that which existed 6 years prior to pain report, or whether it is relating to EF variance specifically at age 28, concurrent with time of pain report.

Genetic models. We employed a series of genetic models to decompose the variances and covariances of pain and EFs into genetic and environmental influences (Rijsdijk & Sham, 2002). The classical twin model partitions phenotypic variance into additive genetic (A), shared environmental (C), and non-shared environmental (E) variance. Since MZ twins share 100%, but DZ twins share on average 50%, of their segregating genes, the A components correlates at 1.00

and 0.50, respectively. Twins who are reared in the same environment are assumed to correlate 1.00 on the C component (e.g., socioeconomic status, nutrition). The E component is uncorrelated for both zygosities as it represents unique influences (e.g. injury of one twin) and includes measurement error for non-latent factors.

Cholesky decompositions are also often applied to twin models with longitudinal data to partition the genetic (A) and environmental (C, E) variances into variance in the second time point that is shared with the first time point and unique variance that has newly emerged at the second timepoint (Loehlin, 1996). We first estimated a genetic and environmental Cholesky decomposition of just the EF factors to assess whether there were any new, significant A, C, or E influences for the EF factors that emerged at age 28. We estimated all possible cross-paths at both the factor level (e.g., shared genetic variance of the Common EF factor at age 28 with age 23) and at the task-specific level (e.g., shared genetic variance of Antisaccade task at age 28 with age 28 not accounted for by the Common EF factor).

Based on the genetic EF model, we included the non-zero, significant variance components in our model with pain. As our pain variable is ordinal, it requires a diagonally weighted least squares estimator which sometimes has difficulty converging in large models with many parameters estimated at 0. In our genetic Cholesky of pain and EFs, we decomposed the phenotypically significant associations between pain and the EF factors and assessed whether they were genetic or environmental in nature. The estimates from this twin Cholesky decomposition were used to derive genetic (*r*A) and non-shared environmental (*r*E) correlations between EFs and pain (Loehlin, 1996). For example, the *r*A for Common EF and pain quantifies the overlap of genetic influences (Rijsdijk & Sham, 2002). We conducted power analyses for these models which can be found in the Appendix A.

Results

Descriptive Statistics

Descriptive statistics for the EF tasks are presented in Table 2-2, and zero-order correlations are presented in Table 3-2. Pain scores were available for 634 participants: *n* of no chronic pain = 458; *n* of mild chronic pain = 102; and the *n* of moderate-severe chronic pain = 74. For the genetic models, the cross-tabulation of number of twin pairs in each zygosity and chronic pain level is shown in Table A-1.

Table 2-2. Descriptive statistics for executive function tasks

		n	Mean	SD	Min	Max	Skew	Kurtosis	Reliability
EF age 23									
	Antisaccade	748	0.62	0.16	0.20	0.96	-0.13	-0.67	0.90[a]
	Stop-Signal	735	215 ms	30	116	315	-0.23	0.25	0.63[a]
	Stroop	737	156 ms	74	-73	387	0.71	0.71	0.96[b]
	Keep track	749	0.72	0.09	0.44	0.96	-0.36	0.11	0.66[a]
	Letter memory	749	0.70	0.13	0.38	1.00	0.22	-0.64	0.92[a]
	Spatial *n*-back	749	-0.01	0.91	-2.74	2.70	-0.31	-0.03	0.75[b]
	Number-letter	748	246 ms	157	-241	735	0.91	0.92	0.91[b]
	Color-Shape	743	221 ms	182	-239	792	1.05	1.19	0.90[b]
	Category-switch	747	198 ms	161	-81	735	1.14	1.28	0.94[b]
EF age 28									
	Antisaccade[c]	613	0.00	1	-2.36	2.48	-0.04	-0.74	0.90[a]
	Stroop	632	147 ms	78	-0.90	385	0.70	0.73	0.96[b]
	Keep track	629	0.77	0.14	0.35	1.00	-0.69	0.20	0.75[a]
	Letter memory	637	0.73	0.15	0.31	1.00	-0.05	-0.87	0.93[a]
	Number-letter	616	183 ms	121	-60	566	0.89	0.82	0.92[b]
	Category-switch	636	180 ms	150	-103	662	1.19	1.45	0.97[b]

Note. Descriptive statistics of the executive function (EF) tasks. ms = milliseconds; SD = standard deviation.

[a] Internal reliability was calculated using Cronbach's alpha

[b] Internal reliability was calculated by using split-half reliability corrected with the Spearman-Brown prophecy formula

[c] The antisaccade task at age 28 was a z-score combination of two difficulty levels, so these descriptive statistics reflect the standardized score.

Phenotypic Associations

The phenotypic model of correlations between age 28 pain and the age 23 EF factors fit well, $\chi^2(26) = 31.406$, $p = 0.214$, CFI = 0.993, RMSEA = 0.016, model $n = 785$. Pain significantly correlated with the age 23 Updating-specific factor ($r = -0.20$, $p = 0.006$), but not with the Common EF ($r = -0.06$, $p = 0.349$) nor Shifting-specific ($r = -0.03$, $p = 0.733$) factors. The phenotypic model of the age 28 EF factor correlations with pain fit acceptably, $\chi^2(10) = 43.440$, $p < 0.001$, CFI = 0.923, RMSEA = 0.072, model $n = 649$. Pain significantly correlated with the age 28 Common EF factor ($r = -0.15$, $p = 0.015$), marginally with the Updating-specific factor ($r = -0.15$, $p = 0.061$), but non-significantly with the Shifting-specific factor ($r = 0.09$, $p = 0.228$).

We conducted descriptive phenotypic correlations between pain and the EF tasks to inform our interpretation of the full model (See Table 2-3). Consistent with significant and marginally significant correlations of pain with the Updating-specific factors at ages 23 and 28, respectively, across both timepoints, pain had significant associations with the keep-track and letter memory tasks, which load onto the Common EF and Updating-specific factors. At age 23, the third highest association was with the *n*-back, which is also an Updating-specific task, but this did not reach significance. At age 28, the associations of the Antisaccade and Stroop tasks with pain were approaching significance, which likely contributed to the significant association between pain and Common EF at this age.

Table 2-3. Zero-order correlations between pain and executive function tasks

		Pain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age 23	1. Antisaccade	-0.02 [0.05]	-													
	2. Stop	0.03 [0.05]	**-0.22**	-												
	3. Stroop	0.05 [0.05]	**-0.32**	**0.13**	-											
	4. Keep	**-0.14** [0.06]	**0.25**	**-0.12**	**-0.22**	-										
	5. Letter	**-0.17** [0.05]	**0.40**	**-0.10**	**-0.28**	**0.50**	-									
	6. Spatial *n*-back	-0.09 [0.05]	**0.36**	-0.04	**-0.18**	**0.32**	**0.40**	-								
	7. Number	0.03 [0.06]	**-0.27**	**0.08**	**0.18**	**-0.11**	**-0.09**	-0.04	-							
	8. Color	0.03 [0.05]	**-0.19**	0.02	**0.16**	**-0.14**	**-0.11**	**-0.10**	**0.43**	-						
	9. Category	0.05 [0.06]	**-0.34**	**0.14**	**0.29**	**-0.22**	**-0.19**	**-0.14**	**0.51**	**0.42**	-					
Age 28	10. Antisaccade	-0.10^{t} [0.05]	**0.65**	**-0.20**	**-0.30**	**0.29**	**0.41**	**0.28**	**-0.20**	**-0.14**	**-0.28**	-				
	11. Stroop	0.09^{t} [0.05]	**-0.24**	**0.14**	**0.49**	**-0.21**	**-0.27**	**-0.14**	**0.15**	**0.13**	**0.26**	**-0.25**	-			
	12. Keep	**-0.14** [0.05]	**0.29**	**-0.13**	**-0.25**	**0.53**	**0.50**	**0.32**	-0.06	**-0.17**	**-0.19**	**0.38**	**-0.25**	-		
	13. Letter	**-0.17** [0.05]	**0.37**	**-0.11**	**-0.31**	**0.51**	**0.84**	**0.37**	-0.08	**-0.14**	**-0.19**	**0.44**	**-0.30**	**0.55**	-	
	14. Number	0.02 [0.05]	**-0.25**	**0.16**	**0.15**	**-0.10**	-0.07	-0.07	**0.55**	**0.31**	**0.47**	**-0.34**	**0.22**	**-0.11**	**-0.11**	-
	15. Category	0.00 [0.05]	**-0.34**	**0.20**	**0.31**	**-0.25**	**-0.23**	**-0.13**	**0.47**	**0.38**	**0.66**	**-0.34**	**0.28**	**-0.22**	**-0.23**	**0.54**

Note. Pain was assessed at age 28. Executive function tasks were completed longitudinally at ages 23 and 28 years. Age and sex effects were regressed out of the executive function tasks. Sex was included as a covariate for pain. Age was not included as a covariate for pain since it had a non-significant association estimated at 0. The standard errors for correlations with pain are shown in brackets, corrected for the non-independence of twin pairs. Bolded font indicates $p < 0.05$, accounting for nonindependence.

$^{t}p < 0.10$

Phenotypic Cholesky decomposition

The phenotypic Cholesky decomposition is visualized in Figure 2-2. The phenotypic Cholesky decomposition of the EF factors fit well, $\chi^2(81) = 143.598$, $p < 0.001$, CFI = 0.965, RMSEA = 0.031, model $n = 786$. There was significant residual variance specific to age 28 Shifting-specific (residual = 0.31, $p < 0.001$), but the residual variance for age 28 Common EF (residual = 0.09, $p = 0.097$) and Updating-specific (residual = 0.10, $p = 0.276$) factors were not significant.

Pain significantly correlated with stable Updating-specific variance ($r = -0.21$, $p = 0.008$), but was not significantly related to stable Common EF variance ($r = -0.06$, $p = 0.350$), nor stable Shifting-specific variance ($r = -0.03$, $p = 0.709$). Pain did not significantly relate to new Common EF ($r = -0.29$, $p = 0.142$), Updating-specific ($r = 0.16$ $p = 0.506$), nor Shifting-specific variance at age 28 ($r = 0.20$, $p = 0.063$).

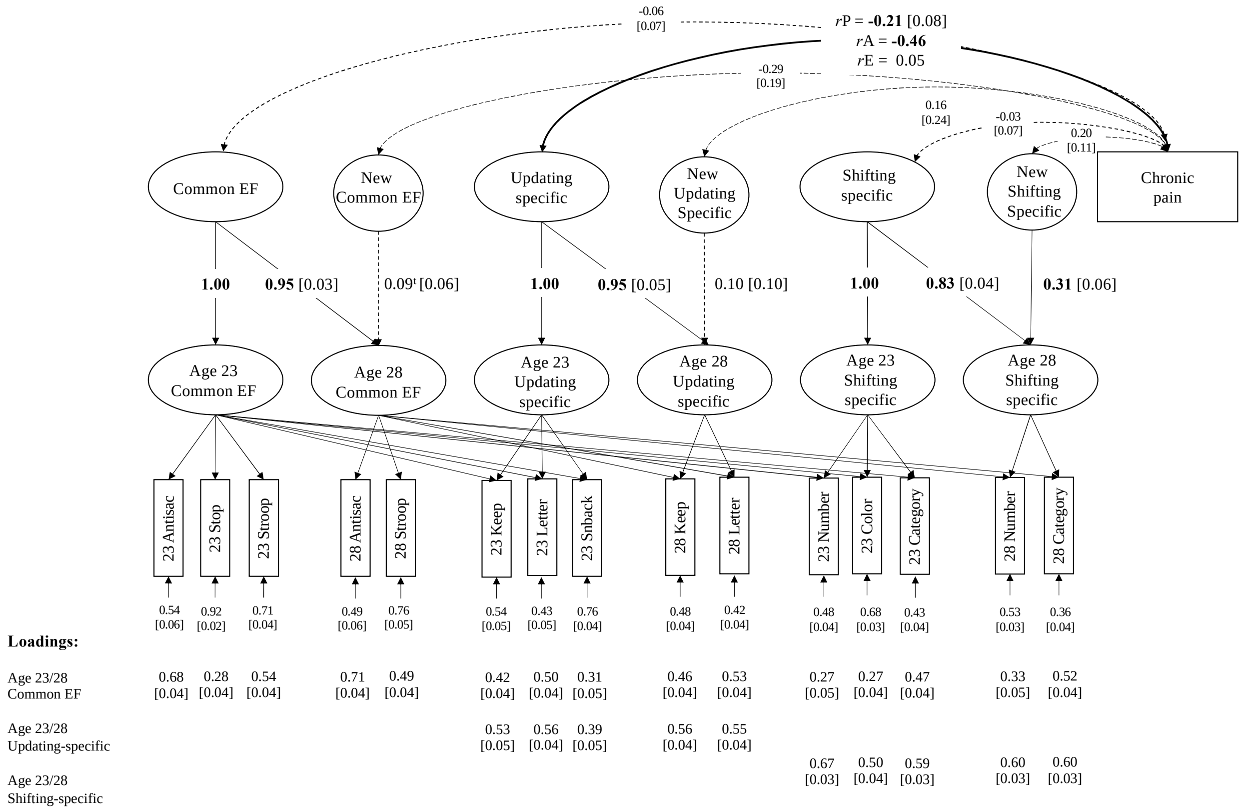


Figure 2-2. Estimates from Cholesky decomposition of executive function (EF) factors and their associations with pain

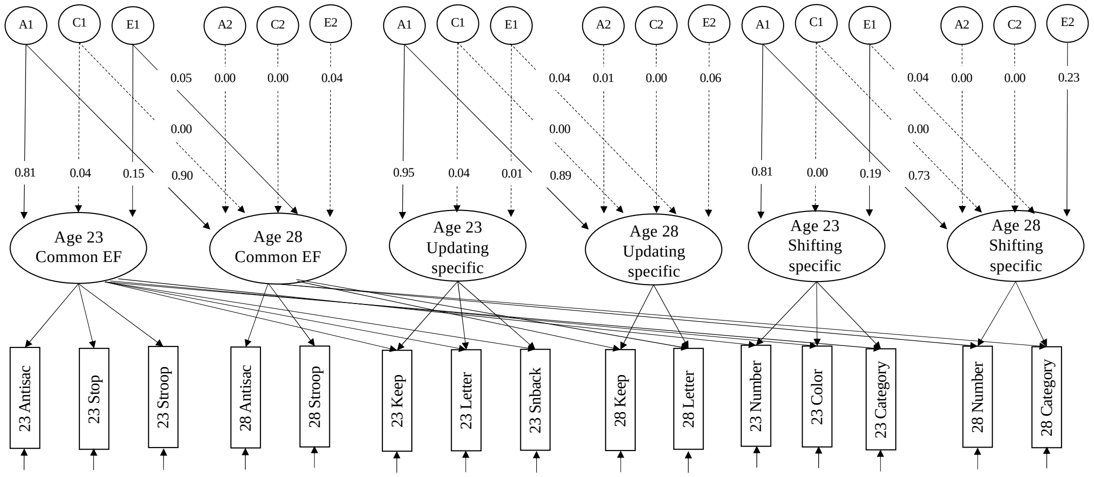
Only the Shifting-specific factor had significant residual variance at age 28; however, we phenotypically correlated pain with all three stable factors and all three residual factors. Pain significantly correlated with the stable Updating-specific variance, but not the stable Common EF nor Shifting-specific variances. Pain did not significantly associate with any of the age 28-specific EF variances. We display the genetic and environmental decomposition of the phenotypic Updating-specific and pain correlation estimated in the genetic Cholesky decomposition. The loadings of each task on their respective factors are shown. Standard errors are in brackets. rP = phenotypic correlation; rA = genetic correlation estimate from the genetic Cholesky decomposition; rE = non-shared environmental correlation estimated from the genetic Cholesky decomposition; 23 = Age 23; 28 = age 28; Antisac = Antisaccade; Stop = Stop-signal; Keep = Keep-track; Letter = Letter memory; Snback = Spatial n-back; Number = Number-letter; Color = Color-shape; Category = Category-switch. Bold font indicates p<.05, based on Wald estimates in the phenotypic model, and chi-square difference tests in the genetic model.

^{t}Indicates significance at a $p < 0.10$ level

Genetic Models

The MZ twin correlation for pain ($r = 0.46$, $p < 0.001$) was over twice as large as the DZ twin pair correlation for pain ($r = 0.12$, $p = 0.368$), which is indicative of additive genetic effects (A) but no shared environmental effects (C). A univariate AE twin model for this pain measure indicated significant A = 41%, $\chi^2(1) = 16.902$, $p < 0.001$, and E = 59%, $\chi^2(1) = 45.410$, $p < 0.001$, influences (the latter include measurement error).

Prior to adding pain into the EF model, a genetic and environmental Cholesky decomposition of EFs to assess whether there was significant stable and/or new ACE variance (See Figure 2-3). All possible cross-paths were included at the EF factor-level as well as at the task-specific level (See task-specific ACE estimates in Table A-2). We used the path estimates to calculate the genetic and environmental correlations from age 23 to age 28 EFs. The model fit well, $\chi^2(867) = 1109.173$, $p < 0.001$, RMSEA = 0.037, CFI = 0.945, model $n = 402$. The residual paths suggested there was not new significant A or C variance unique to age 28 for any of the EF factors. There was no significant unique E variance for Common EF (0.04, $\chi^2(1) = 2.104$, $p = 0.147$) nor for the Updating-specific factor (0.06, $\chi^2(1) = 0.015$, $p = 0.903$) at age 28 (see Figure 2-3). The only significant new variance that emerged at age 28 was unique E variance for the Shifting-specific factor (0.23, $\chi^2(1) = 8.521$, $p = 0.004$). Hence, genetic variance at age 28 was entirely shared with age 23 genetic variance for Common EF (rA = 1.00, $\chi^2(1) = 46.651$, $p < 0.001$), Updating-specific (rA = 1.00, $\chi^2(1) = 27.539$, $p < 0.001$), and Shifting-specific (rA = 1.00, $\chi^2(1) = 15.569$, $p < 0.001$). Shared environmental variance was non-significant for all three factors and was estimated at 0 or near 0. The Common EF factor shared significantly stable E variance across time (rE = 0.74, $\chi^2(1) = 9.671$, $p = 0.002$). Non-shared environmental variance was estimated near 0 and not significant for the Updating-specific factor at age 23, hence it could not share stable E variance with age 28 (rE = 0.64, $\chi^2(1) = 0.155$, $p = 0.694$). Non-shared environmental variance at age 28 did not share significantly stable non-shared environmental influences with the age 23 Shifting-specific factor (rE = 0.40, $\chi^2(1) = 2.533$, $p = 0.111$).



See Supplement for task-specific A, C, and E estimates

Loadings:

Age 23/28 Common EF	0.71 [0.04]	0.28 [0.04]	0.51 [0.04]	0.71 [0.04]	0.43 [0.05]	0.42 [0.04]	0.56 [0.04]	0.35 [0.05]	0.48 [0.04]	0.56 [0.04]	0.34 [0.04]	0.27 [0.05]	0.49 [0.04]	0.39 [0.04]	0.52 [0.04]
Age 23/28 Updating-specific						0.57 [0.05]	0.50 [0.04]	0.37 [0.05]	0.56 [0.04]	0.55 [0.04]					
Age 23/28 Shifting-specific											0.66 [0.04]	0.52 [0.04]	0.55 [0.04]	0.58 [0.03]	0.56 [0.03]

Figure 2-3. Estimates from the genetic Cholesky decomposition of executive function (EF) factors

The A1s have direct paths to the Age 23 factors as well as a cross-path to the Age 28 factors. Hence, A1 captures genetic variance in the age 23 factor and the variance it shares with the age 28 factors. A2 has a direct path to the Age 28 factors and is the residual genetic variance specific to the age 28 factor. This decomposition was done for additive genetic (A1, A2), shared environmental (C1, C2) and non-shared environmental (E1, E2) variances for each EF factor (i.e., Common EF, Updating-specific, and Shifting-specific). In this Cholesky decomposition, the tasks also all had their own task-specific ACE decompositions (e.g., an A1 for age 23 Antisaccade and age 28 Antisaccade and an A2 for age 28 Antisaccade). These task-specific estimates are not visualized here for space but can be found in Table A-2. The solid lines symbolize significance at $p < 0.05$; dashed lines indicate $p > .05$, determined with chi-square difference tests (the A1, C1, and E1 paths cannot be tested with single-df difference tests when the age 28 variables are included, as dropping the first path requires also dropping the cross-paths to ensure model identification; therefore, significance tests for the age 23 paths were obtained from Friedman et al., 2016, which presented the age 23 model). A = additive genetic effects; C = shared environmental effects; E = non-shared environmental effects (includes measurement error); 23 = Age 23; 28 = age 28; Antisac = Antisaccade; Stop = Stop-signal; Keep = Keep-track; Letter = Letter memory; Snback = Spatial n-back; Number = Number-letter; Color = Color-shape; Category = Category-switch.

Based on the phenotypic Cholesky decomposition of pain and EFs and the genetic and environmental EF Cholesky decomposition, we estimated a genetic and environmental Cholesky decomposition for pain and EFs including cross-paths to all phenotypically significant associations (see model fit statistics in Table 2-4). The only significant phenotypic correlation was with the Updating-specific factor; hence, this was the only association we decomposed (but for thoroughness we present decomposition fit statistics for a model that includes all cross-paths with non-significant phenotypic associations and non-significant variance components in Table A-3). The model fit well, $\chi^2(1083) = 1122.372$, $p = 0.198$, RMSEA = 0.013, CFI = 0.984, model $n = 403$. Pain genetically correlated with the stable genetic component of the Updating-specific factor (rA = -0.46, $\chi^2(1) = 7.879$, $p = 0.005$). Pain did not share significant non-shared environmental correlations with the stable Updating E component (rE = 0.05, $\chi^2(1) = 0.039$, $p = 0.844$).

Table 2-4. Fit statistics for the Cholesky decomposition of the Updating-specific association with pain

	Model Fit					Chi-square Difference	
Model	χ^2	df	*p*	RMSEA	CFI	$\Delta\chi^2(1)$	*p*
Full model	1122.372	1083	0.198	0.013	0.984		
Drop stable A Updating-specific association	**1132.240**	**1084**	**0.150**	**0.015**	**0.980**	**7.879**	**0.005**
Drop stable E Updating-specific association	1123.021	1084	0.200	0.013	0.984	0.039	0.844

Note. Presented above are the fit statistics of the genetic Cholesky decomposition between pain and EF. We only decomposed significant phenotypic associations between pain and EF, which was the association between pain and the stable Updating-specific factor. A significant worsening of model fit when dropping an association from the model suggests that the association is statistically significant. Pain shares a significant genetic association with Updating-specific factor, but not an environmental association. Sex was included as a covariate for pain; age and sex effects were regressed out of the executive function tasks. A = additive genetic effects; E = non-shared environmental effects (includes measurement error); df = degrees of freedom; RMSEA = Root mean square error approximation; CFI = confirmatory fit index. Bolded font indicates $p < 0.05$, determined with chi-square difference tests.

Discussion

To our knowledge, this is the first study to examine the phenotypic, longitudinal, and genetic/environmental relationships between chronic pain and three, separable EF latent variables (Common EF, Updating-specific, and Shifting-specific factors). We found that chronic pain in young adults is associated with lower scores on a factor specific to working memory updating, and that this relationship is entirely due to shared genetic risk.

Worsened working memory Updating-specific function predicting pain is not surprising. Working memory Updating is highly correlated with more general working memory factors based on complex working memory span tasks (Schmiedek et al., 2009). Meta-analyses and systematic reviews have found that across multiple pain syndromes and ages, chronic pain patients tend to perform worse on working memory tasks (Berryman et al., 2013; Bunk et al., 2019). Clinical pain patients also self-report pronounced working memory deficits compared to pain-free controls (Baker et al., 2016). Pre-operative pain-free patients who performed worse on processing speed tasks were at higher risk for developing chronified pain 6- and 12- months post-operatively; however, the tasks used also tapped into cognitive flexibility (Attal et al., 2014). Our findings suggest that the impairments are specific to working memory updating, as variance shared with response inhibition and set-shifting impairments were controlled for by the other latent variables.

The lack of significant correlations between pain and the Common EF and Shifting-specific factors differs from what has been reported in studies that used single EF tasks. Multiple studies have found that chronic pain patients perform worse on individual inhibition and shifting tasks (Berryman et al., 2014; Bunk et al., 2019), although our findings align with a study of Japanese older adults that found that individuals with musculoskeletal pain showed slower processing speed but no significant differences on a shifting task (Murata et al., 2017) compared to controls. A strength of our research is its large sample size and the use of latent variable modeling, which minimizes measurement error common in cognitive tasks. Including the multiple EF latent variables, which partitions the effects of different EF domains, we were able to isolate that chronic pain is relating to Updating-specific working memory over and above Common EF and Shifting-specific ability. It is possible that shared variance with working memory in the inhibition and shifting tasks has driven associations in other research. Alternatively, Updating-specific impairments may be related specifically to mechanisms of general chronic pain, such as central sensitization (Harte et al., 2018).

Updating-specific function prospectively predicting pain suggest working memory-specific deficits could play a role in pain chronification. A Midlife Development in the United States study (average age = 54.43) constructed a mean *z*-score of 5 EF tasks tapping inhibition, working memory, and set-shifting and found that baseline EF predicted the development of chronic pain 9 years later; even after adjusting for depressive symptoms, personality, age, and gender (Ng & Hartanto, 2022). A 12-year longitudinal study using the PAQUID cohort (average age = 77.46) found that pain status was related to impairment on a global latent factor of multiple EF tasks (Rouch et al., 2021). Our results expand the finding of a factor-level cognitive impairment to a young adult sample and isolates that it is Updating-specific functioning that relates to pain chronification.

We used behavior genetic methods to understand what risk factors were contributing to the co-occurrence of pain and the Updating-specific factor. When decomposing the pain-Updating-specific factor association we found that the relationship was entirely genetic. Variation in Updating-specific variance is almost 100% accounted for by additive genetic effects (Friedman et al., 2016) and general chronic pain is usually over 50% attributable to genetic effects across time (Burri et al., 2018). Shared genetic risk can be interpreted in multiple ways, but can suggest that the same or proximal risk variants are causally impacting pain and working memory, possibly through shared biological pathways (J. Martin et al., 2018). While the current study cannot elucidate biological pathways, our finding of a genetic correlation supports the conclusion that the pain-EF relationship is due to inherited factors and not directly due to environmental influences, such as to a stressful life event over which the individual has no control. However, gene-environment correlations, in which genetic predisposition drives exposure to certain environmental influences (e.g. engaging in risky behavior) or genotype-by-environment interactions, in which genetic risk genotypes may be more influential in some environmental contexts (e.g., with greater exposure to air pollution), are still plausible (Deater-Deckard & Mayr, 2005; Plomin et al., 1977).

More generally, the genetic relationship between pain and the Updating-specific factor is notable, as the only other known phenotype related to the Updating-specific factor is intelligence, and this relationship occurs in addition to a relationship with Common EF (Miyake & Friedman, 2012). Thus, the pain-Updating-specific relationship is the only known association in which the Updating-specific factor shares a relationship with a phenotype exclusive of the other EF factors. Previous research has found that the processes that contribute to both working memory and pain perception seem to share the same limited cognitive resources (Buhle & Wager, 2010), which is consistent with our finding.

There are multiple mechanisms by which working memory updating could be impaired in the pain population. Working memory enables individuals to maintain, manipulate, and update (gate) relevant information, particularly in the context of interference (Baddeley, 2003; Engle, 2002). One possibility is that the working memory system inefficiently gates ascending pain signals. Failure to filter out irrelevant sensory information could enhance pain perception in those suffering with chronic pain. Another possibility is that a dispositional hypervigilance to threat cues or fear of pain may lead working memory to be selectively monitoring for pain signals, and inappropriately updating pain-related information. Anxiousness is related to poorer working memory performance (Moran, 2016) and to pain (Lerman et al., 2015); additionally, neuroticism shares genetic overlap with pain conditions. However, EF has been shown to predict later pain over and above personality (Ng & Hartanto, 2022) and trait anxiety (Attal et al., 2014). In healthy individuals, the perception of pain depends on the attention that they allot to an experimental nociceptive stimulus, a process that may involve working memory (Legrain et al., 2011). Taxing working memory by engaging in a cognitively demanding task inhibited the perception of pain severity in experimentally induced pain across both younger and older adults (Deldar et al., 2018, 2019). Transcranial magnetic stimulation of the dorsolateral prefrontal cortex (dlPFC), the cortical hub for working memory, during a working memory task also decreased the perception of pain severity (Deldar et al., 2018, 2019). However, activation of the dlPFC without engagement in a cognitive task did not inhibit pain severity (Deldar et al., 2018). These findings suggest that, in healthy individuals, working memory may work with selective attention to help allocate limited cognitive resources and shield against nociceptive signals while maintaining attention on a current process. Genetic predisposition to deficiencies in this system could lead to improper filtering of pain and result in chronification.

Clinically, these findings support the usefulness of cognitive prioritization training to help minimize pain. Pain Reprocessing Therapy, which is a treatment focused on re-evaluating the threat value of chronic pain, has been shown to provide pain relief in those with chronic back pain (Ashar et al., 2022). Furthermore, high-frequency transcranial magnetic stimulation over the dlPFC has shown to decrease neuropathic pain for up to 3 months post-treatment (Che et al., 2021).

This study has a few limitations. At the second timepoint, three cognitive tasks were not included in the battery, making it difficult for the estimator to discriminate between Common EF and Updating-specific variance. Upon examination of the task-level correlations with pain, the only significant correlations were with the two updating tasks and including the age 23 data made it clear the association was with the Updating-specific factor. However, the phenotypic correlations of pain with the Common EF tasks were marginally significant at age 28, hinting at possible emerging issues. Furthermore, the measure of pain was self-report and the retrospective report of "when did your pain begin" is susceptible to bias (Blome & Augustin, 2015). We do not have a measure of pain history at age 23 and while most participants reported their pain beginning after age 23, they could have history of another pain episode. Finally, the results of this study may be limited to white individuals, as the LTS sample is not very diverse.

Given the novelty of these findings, there are many potential future directions to explore. First, this is a young adult sample, and it would be interesting to replicate these findings in an older adult sample, such as the UK Biobank, as an older population may have age-related cognitive declines and greater pain prevalence (Dahlhamer et al., 2018). Second, this study focused on general chronic pain. Individual pain conditions have their own unique risk which could lead to differential relationships with EF, and differences in the underlying nature of the relationships. Third, once sample sizes become large enough to conduct a genome-wide association study of working memory, biological annotation of the genetic effects could elucidate what sorts of cellular mechanisms could be involved in working memory issues, and subsequently pain chronification. Finally, while this study included a robust battery of cognitive tasks, it by no means captures all the nuanced facets of EFs. Future research could benefit from looking at other factors of EF.

In conclusion, there is a shared genetic risk between worse working memory performance and pain in young adults. Issues with working memory could be mechanistically involved in pain chronification. An impaired gating mechanism of working memory or dispositional hypervigilance to pain cues could lead to over-prioritization of pain signals.

CHAPTER III: FAMILIAL EFFECTS ACCOUNT FOR ASSOCIATION BETWEEN CHRONIC PAIN AND PAST MONTH SMOKING

Introduction

Smoking is robustly associated with pain across both adolescence and adulthood (John et al., 2006; Palmer et al., 2003; Scaini et al., 2022), and is more common among chronic pain patients compared to the general population (Orhurhu et al., 2015). Former or current heavy smoking is associated with experiencing multiple pain locations and greater pain intensity, even when controlling for risk factors for pain such as alcohol use, obesity, and biological sex (John et al., 2006).

Some studies have identified a dose-response association between smoking and pain, which supports the causal hypothesis that smoking damages peripheral tissues and exacerbates pain (Ferreira et al., 2013; Hestbaek et al., 2006). Another proposed causal mechanism is that smoking promotes maladaptive plasticity in pain processing neural pathways over time. Smoking is associated with pervasive cortical thinning (Karama et al., 2015) and decreases of subcortical volumes (Hanlon et al., 2016). In one study, the relationship between past-year smoking and later pain chronification was mediated by the strength of the resting-state connectivity between the nucleus accumbens and medial prefrontal cortex (NAc-mPFC) (Petre et al., 2015).

Smoking may be an exacerbating risk factor for chronic pain, but the relationship could arise for different reasons. Chronic pain patients may self-medicate with cigarettes, as nicotine can provide transient analgesic effects (Borsook et al., 2016). Because nicotine is highly addictive (Pontieri et al., 1996), smoking may become frequent. Or, other predisposing factors, such as genetic or shared environmental (e.g., nutrition) effects, may confound the smoking-pain association. Previous twin studies have found that pain and smoking are moderately heritable

(Junqueira et al., 2014; Maes et al., 2004), and that smoking shares genetic associations with traits that are genetically correlated with pain (Edwards et al., 2011; Khan et al., 2020).

Co-twin control analyses use monozygotic (MZ) and dizygotic (DZ) co-twins as quasi-experimental controls to estimate effect sizes when controlling for familial similarities, including genetic and familial environmental effects (McAdams et al., 2021). When accounting for familial factors (e.g., when looking at whether the twins who smoke are more likely to have pain compared to their non-smoking co-twins), the relationship between pain and smoking often becomes non-significant (Hestbaek et al., 2006; Suri et al., 2017). For example, a study of male veteran twins found that the association between chronic back pain and smoking status was entirely due to familial effects (Suri et al., 2017). However, another study found that smoking increased musculoskeletal pain risk by two-fold and that odds ratios were similar within families, (i.e., when controlling for familial confounds) (Holley et al., 2013).

We examine whether these findings extend to chronic pain in our young adult twin sample. We assess whether smoking frequency increases pain, and using the brain coordinates from a previous study (Baliki et al., 2012), whether the resting-state NAc-mPFC connectivity mediates this relationship. We use a co-twin control analysis to evaluate these associations when controlling for familial confounds. Finally, we use classical twin models to assess the degree of shared genetic and environmental risk between smoking and pain.

Method

Participants

Participants were 692 same-sex twins (54% women; 46% men) from the Colorado Longitudinal Twin Study (LTS) who had data for at least one measure used in this study. The LTS is a long-standing, ongoing developmental study of twins born between 1984 and 1990.

Twins whose families lived within a 3-hour driving range (~ 300 km) from Boulder, Colorado, and whose birth weights were at least 1000 grams (though 96% weighed 1700 grams or more) were invited to participate; twins were initially assessed at age 14 months. More information on ascertainment and history for the LTS are provided elsewhere (Corley et al., 2019; Rhea et al, 2013).

The data for this study were obtained between June 2014 and July 2019 when the twins completed functional magnetic resonance imaging (fMRI) and questionnaires (mean age=28.8 years, *SD*=0.56). Exclusion criteria included standard contraindications to MRI (metal in the body, recent tattoo, claustrophobia, and pregnancy). The pain questionnaire was only administered on the scanning day, so individuals who did not attend the scanning session did not have pain data. The smoking questions were administered as part of a phone interview prior to the scanning session and individuals who did not enroll in the scanning study were assessed as part of another study, so more data are available for smoking behavior. Those who participated in scanning did not significantly differ in smoking frequency ($p = 0.178$) compared to those who did not participate in scanning.

Demographic information and descriptive statistics are provided in Table 3-1. The sample self-reported their race as being 91.66% white, 4.44% Latino or Hispanic, 2.60% bi- or multi-racial, <1% Pacific Islander, <1% American Indian, and <1% “other.” Of the 637 individual twins who responded to the pain questionnaire, 52.12% were MZ and 47.88% were DZ. Of the 687 twins who completed the smoking questionnaire, 51.82% were MZ twins and 48.18% were DZ. A total of 632 individual twins and 298 complete twin pairs completed both the smoking and pain questionnaires.

Measures

Brief Pain History Questionnaire. Participants completed the Brief Pain History Questionnaire (BPHQ) just prior to the MRI scanning session. The BPHQ is a self-report questionnaire developed for the LTS based on the Brief Pain Inventory (BPI), which is a validated tool for assessing pain episode presence and quality (Tan et al., 2004).

The BPHQ assesses whether participants are experiencing a pain episode as well as what age their pain began, the severity, the location(s) on a body map, and the descriptive quality of the pain episode (tingling, stabbing, etc.). The participants answered (yes or no) whether they were experiencing a "current significant episode of pain." A current significant episode of pain was defined as "A 'significant episode'; is one that has an impact on your quality of life that you consider to be important. An episode could be relatively brief (days to weeks) or prolonged (months to years)." Participants who answered yes then rated the severity of their pain on a numeric pain rating scale of 0-100, with 100 being the worst pain imaginable and 0 being no pain. Numeric pain rating scales are commonly used to measure presence of pain intensity and exhibit moderate to high reliability (0.67-0.96) (Kahl & Cleland, 2005). Of the participants experiencing pain, 42% were experiencing pain in one body location, 54% were reporting pain in multiple locations, and 4% chose not to report a pain location. Of the total reported pain locations, 35% reported lower-extremity/knee pain, 27% reported back pain, 13% reported head/neck pain, 14% reported upper-extremity/shoulder pain, 3% reported chest pain, 3% reported stomach/bowel pain, 3% reported hip pain, and 2% reported genital pain.

Our measure captured multiple types of pain syndromes (e.g., back pain, head pain) and is thus interpretable as a measure of general chronic pain. Chronic pain may start at an individual location but tends to spread to multiple locations by becoming amplified by the central nervous system, otherwise known as central sensitization (Harte et al., 2018; Tanguay-Sabourin et al., 2023). While chronic pain conditions at different body sites are diverse in causes, symptoms, and consequences, there is psychometric and genetic evidence for shared etiology for pain across body sites. A longitudinal, psychometric analysis has supported the validity of a common component underlying pain across body sites (Battaglia et al., 2022). Consistent with this psychometric evidence for a general pain factor, twin and genome-wide association studies have identified a common genetic factor that contributes to many chronic pain conditions (Vehof et al., 2014; Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023). Of the people who reported pain in our sample, 97.2% reported pain lasting more than 3 months, characterizing the measure as chronic. We decided not to exclude the 5 participants whose pain episode was less than 3 months (range = 1.18 to 2.37 months), as our models would then lose information about their co-twins as well.

PhenX Toolkit - Substance Use Interview. The PhenX toolkit (Hamilton et al., 2011) is a web-based toolkit funded by the National Human Genome Research Institute and National Institutes of Health that include well-established measures of multiple phenotypes, including substance use. The phone substance use interview included multiple self-report questions about substance use behaviors (frequency, quantity, and dependence) with alcohol, tobacco, marijuana, and other substances. Smoking frequency was defined as the participants self-reported number of days they smoked in the last 30 days (0-30).

Resting-State Functional Connectivity. The LTS sample were scanned in a Siemens Tim Trio 3T or Prisma 3T scanner. Resting-state data were acquired with a 6.25-min T2*-weighted echo-planar functional scan (acquisition parameters: number of volumes = 816, TR = 460 ms, TE = 27.2 ms, matrix size = 82 × 82 × 56, voxel size = 3.02 × 3.02 × 3.00 mm, FA = 44.0°, slice thickness = 3.00 mm, field of view (FOV) = 248 mm) (see Reineberg et al., 2020). While the participants were undergoing the resting-state scan, they were instructed to relax and stare at a fixation cross.

The fMRI data were pre-processed using the FMRIB Software Library (FSL) build 509 (FSL) (Jenkinson et al., 2012). Detailed pre-processing information can be found elsewhere (see Reineberg et al., 2020). FSL tools flirt, fslmaths, and fslmeants were used to extract the resting-state functional connectivity between the NAc and mPFC using previously published, priori-defined seeds (Petre et al., 2015). The seed coordinates indicate the center of the regions of interest (ROIs: NAc; 10, 12, -8; mPFC 2, 52, -2) and were 6x6x6 mm cubed. Average time series for each ROI were created, and then correlated within individuals to produce a functional connectivity score. The functional connectivity correlation was Fisher's *z*-transformed (Silver & Dunlap, 1987). After quality assurance checks, 600 twins' imaging phenotypes were suitable for analyses.

Statistical Analysis

Data Transformation

Due to non-normality of the smoking and pain variables, we used square-root transformations (see Table 3-1). However, the distributions were still non-normal which prompted us to bin the untransformed variables to avoid biased estimates in the twin models (Verhulst & Neale, 2021). In the mediation and co-twin control models, pain (the outcome) was kept ordinal, but the smoking predictor variable was square-root transformed. Normal distribution of independent variables is not an assumption of regression, and the square-root transformed smoking variable allowed for more variability in the between- and within-family scores.

The distribution of past-month smoking frequency was non-normal, with peaks at 0 days/month and 30 days/month. Past-month smoking frequency was binned into 3 categories to reflect these peaks: past month smoking, no = 0; 1-29 days = 1; 30 days = 2. These bins correspond to daily smokers (30 days), non-smokers (0 days), and social smokers (1-29). The pain severity variable was also categorized into 3 bins: no pain, 0 = 0; mild pain, 1-29 = 1; moderate to severe pain, 30-100 = 2. To ensure that the binned variables were not changing the pattern of results, all analyses were also conducted with the square-root transformed variables (presented in the Appendix B Tables 2-4) to evaluate consistency.

Statistical Analyses

Phenotypic and twin analyses were completed in Mplus version 8.10 (L. K. Muthén & Muthén, 1998). Sex was included as covariate for all of the models and models appropriately accounted for the non-independence of twins by specifying family clustering (Rebollo et al., 2006). Additional analytic details are provided in Appendix B.

We conducted a phenotypic mediation model to evaluate whether the data are consistent with a model in which the NAc-mPFC connectivity (M) mediates the direct effect (c) of smoking (X) on pain (Y). If the effect of smoking on pain controlling for M (c’) is not significant but the indirect effect (a*b) is, complete mediation has occurred. If all paths are statistically significant, partial mediation has occurred. If the c’ path is significant but a*b is non-significant, the significance of a mediation effect is not supported (Hayes, 2009). The NAc-mPFC was regressed on X, Y, and Z plane, head movement covariates.

All significant associations from the phenotypic mediation model were tested in the co-twin control model. The co-twin control design uses one twin in a pair as a matched control for the other twin within a regression framework. We used a mixed-effects logistic regression to investigate the within-twin pair association of pain and smoking while controlling for between-family confounds (genetic and shared environment effects). Specifically, we estimated a multi-level co-twin control model with both within-family and between-family effects. The independent variable for the between-family regressor was the family mean on a trait (here, the mean of smoking frequency for each twin pair). This between-family effect reflects familial effects, which can include shared environmental and genetic effects (Carlin et al., 2005). The independent variable for the within-family regressor was the deviation of each twin from their family mean (here, the deviation of smoking from the mean of both twins). Regressing this discordance estimate on the outcome of interest shows the direct effect while controlling for familial confounding (Carlin et al., 2005). We also tested an interaction of zygosity with the within-family effect since MZ and DZ twins differ in their genetic relatedness.

We used twin structural equation models to decompose the pain and smoking variances and their covariance into genetic and environmental components (Rijsdijk & Sham, 2002). The twin model leverages the fact that MZ twins share 100% and DZ twins share on average 50% of their segregating genetic variants. Common environmental factors (e.g. childhood home environment) are assumed to be shared 100% across MZ and DZ twins (Derks et al., 2006). Thus, when a measure correlates more strongly in MZ twins (rMZ) compared to DZ twins (rDZ) it suggests genetic influences on that trait. Moreover, when rMZ<2*rDZ, shared environmental influences are suggested, whereas when rMZ>2*rDZ, dominance genetic influences are suggested. Using these assumptions, structural equation models can be estimated to decompose the variances and covariances of a phenotype into three latent variables: (1) additive genetic effects (A), which represent the sum of all alleles that add up to the heritability of a trait; (2) genetic dominance factors (D), which represent deviance from additive effects (i.e., when one allele overrides the effect of the other allele at the same locus), *or* shared environmental factors (C), which are environmental influences that lead siblings to correlate, such as socioeconomic status or parenting styles; and (3) nonshared environmental factors (E), which are environmental influences that lead siblings to not correlate, such as an injury (Rijsdijk & Sham, 2002). The E factor also includes measurement error, as random error as error will lead twins to not correlate. rMZ<1 suggests E. In the classical twin model, C and D cannot be estimated simultaneously and the choice of which parameter to include depends on the MZ and DZ correlation patterns. If rMZ≤2*rDZ an ACE model is estimated and if rMZ>2*DZ an ADE model is estimated.

We first estimated univariate models for pain and smoking. A univariate AC/DE model estimates the A, C or D, and E parameters for a single phenotype. Nested models in which A and/or C/D are fixed to 0 and compared to the full model can be used to ascertain whether exclusion of a parameter significantly worsens model fit, using a chi-square difference ($\Delta\chi^2$) test (Kline, 2016). To estimate the genetic and environmental correlations between pain and smoking, we estimated a multivariate twin model with both phenotypes which gave us the ACE estimates for each measure and the genetic (rA) and non-shared environmental correlations (rE) between them (Loehlin, 1996). These correlations provide an estimate of the degree to which genetic (rA) and environmental (rE) influences overlap across the traits. Similar to the univariate models, nested models can be fit where rA or rE are dropped to assess their statistical significance.

Results

Descriptive Statistics

Descriptive statistics are presented in Table 3-1. The crosstabs of pain presence and past-month smoking are displayed in Table 3-2. There were 30 MZ and 44 DZ twin pairs discordant for smoking.

We tested whether smoking's effect on pain was distinct from other PhenX substance use frequency measures (alcohol, cannabis, and a composite measure of any other drug; see Table S1). Smoking frequency was the only phenotype that significantly positively predicted pain, over and above the other substance use phenotypes. Less than 0.5% (*n* = 5) of the sample endorsed any usage (1-30) of painkillers in the last 30 days, which is why it was included in the composite measure; it did not significantly predict pain.

Table 3-1. Descriptive statistics of the sample

Measure	***N***	**Mean (SD) or %**	**Min-Max**	**Skewness**	**Kurtosis**
Age	692	28.83 (0.90)	28.02-34.58	2.45	7.84
Sex	692	46% male; 54% female	-	-	-
Body mass index	646[a]	25.89 (5.65)	15.02-54.02	1.32	2.14
Pain					
Pain episode presence	637	28.1% have pain	-	-	-
Chronic pain	637	27.2% have chronic pain	-	-	-
Pain duration (months)	637	22.56 (50.58)	0-314.3	2.60	6.66
Pain severity	637	8.52 (17.11)	0-100	2.41	6.26
Binned pain outcome		72.37% no pain 15.54% mild-mod pain 12.09% mod-high pain	-	-	-
Pain impact (composite)	637	7.09 (16.58)	0-100	2.84	8.37
Pain location	637	42% single-site pain 54% multisite pain 4% declined to answer	-	-	-
Smoking habits					
Age of initiation[b]	212	18.12 (3.15)	10-28	0.58	0.35
Cigarettes per day[c]	132	7.22 (5.81)	1-30	1.30	1.51
Smoking frequency	687	4.24 (9.82)	0-30	2.08	2.54
Binned smoking exposure		80.79% no smoking 8.15% non-daily smoker 11.06% daily smoker	-	-	-
NAc-mPFC connection[d]	600	0.28 (0.30)	-0.87-1.54	-0.25	1.96

Note. Of the 692 individuals who have either pain or smoking data, 632 have both smoking and pain data. In total, 637 participants of the sample filled out the Brief History Pain Questionnaire and 687 participants filled out the PhenX Substance Use Interview. Chronic pain is defined as a pain episode that has lasted more than 3 months. The pain impact composite is a composite score of participants self-report on how pain has affected their work life, social life, and well-being. The bold face type indicates the variables used in the models; other variables are included to characterize the sample. SD = Standard deviation. NAc-mPFC = resting-state functional connectivity of the nucleus accumbens to medial prefrontal cortex. Dashes indicate not applicable for percentage statistics.

[a] Only 646 out of 692 individuals filled out the height and weight survey

[b] These descriptive statistics are of individuals who are former, occasional, or daily smokers

[c] These descriptive statistics are of individuals who are occasional or daily smokers

[d] The resting-state connectivity measure presented is z-transformed.

Table 3-2. Cross-tabulation of pain presence and past month smoking

	Non-smoker	Smoker
No pain	379/632 (58.54%)	75/632 (11.87%)
Yes pain	131/632 (20.73%)	47/632 (7.44%)

Note. The percentages show the crosstabs of pain presence and smoking presence in the past month of the 632 participants who responded to both the Brief Pain History Questionnaire and the PhenX substance use interview.

Phenotypic Mediation Model

Controlling for sex effects, pain and smoking were phenotypically associated (polychoric $r = 0.22$, $p = 0.002$). However, the NAc-mPFC connectivity did not significantly correlate with pain ($r = 0.01$, $p = 0.404$) nor past month smoking ($r = 0.02$, $p = 0.294$).

The mediation model is presented in Figure 3-1. Within the framework of the structural mediation model, the a (a = 0.01 [95% confidence intervals (CI) -0.02, 0.04], $p = 0.441$) and b (b = 0.21 [95% CI -0.48, 0.91], $p = 0.539$) paths were not significant. Consequently, the indirect path was not significant (a*b = 0.002 [95% CI -0.01, 0.02], $p = 0.736$). The only significant path was the direct path from smoking to pain (c' = 0.24 [95% CI 0.08, 0.40], $p = 0.004$). Thus, we do not have evidence that the NAc-mPFC resting state connectivity mediates the smoking-pain association in this sample. See Appendix B for additional, post-hoc analyses of the NAc-mPFC relationships to pain and smoking.

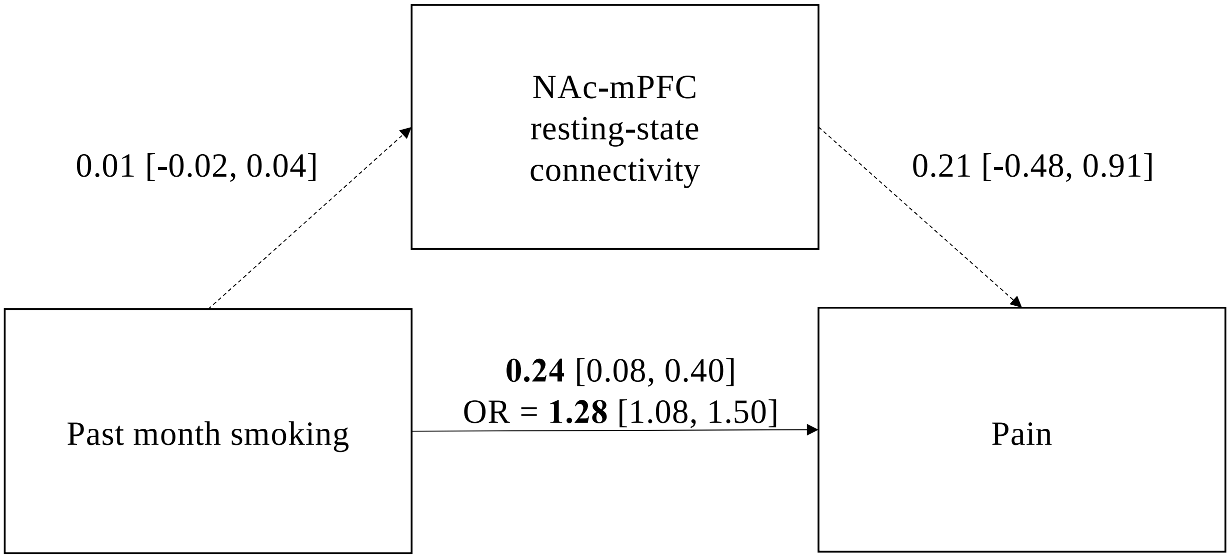


Figure 3-1. Phenotypic mediation of NAc-mPFC resting-state connectivity of the smoking-pain association

Log odds estimates of the mediation model are displayed with 95% boot-strapped (1000 times) confidence intervals. Past month smoking was measured as days smoked in the last 30 days (0-30) and was square-root transformed due to non-normality. Smoking and the brain mediator were z-scored. Hence, the estimates are the the log odds of increasing one pain level with a standard deviation increase in smoking and NAc-mPFC connectivity. The direct path from smoking to pain is significant; however, the indirect path through the brain mediator is not significant. Smoking does not significantly predict NAc-mPFC resting-state connectivity, nor does the NAc-mPFC significantly predict pain. Sex and 3 head-motion covariates were included (estimates not shown). NAc-mPFC = nucleus accumbens to medial prefrontal cortex connection strength from resting state magnetic resonance imaging. OR = odds ratio.

Co-twin Control Analyses

The co-twin control results do not provide evidence for a causal effect of smoking on pain, but they do suggest familial effects (see Table 3-3). Since the direct path between pain and smoking was the only significant association from the mediation model, this was the only association included in the co-twin control model. As seen in Table 3-3, the between-family effect of smoking on pain was significant (Odds Ratio (OR) = 1.31, $p < 0.001$), but the within-family effect was not significant and was estimated as negative (OR = 0.86, $p = 0.386$). Although the within-family effect was not significantly different from zero, it was significantly lower than the between-family effect, as a model that constrained them to be equal fit significantly worse ($\Delta\chi^2(1) = 9.892$, $p = 0.002$). The significantly lower within-family effect compared to the between-family effect suggests familial confounding (Carlin et al., 2005). The interaction of the within-family effect with zygosity was also not significant (OR = 0.90, $p = 0.730$), suggesting that the simple effects for the within-family association (reported in Table 3-3) do not differ across zygosity groups.

Table 3-3. Results of regression and co-twin control models of pain regressed on smoking

Model	Independent Variable	Effect on Pain
Model 1 Individual-level model	Phenotypic Smoking	**0.24** [0.09] **OR = 1.28**
Model 2 Co-twin Control model	Between-family Smoking	**0.27** [0.08] **OR = 1.31**
	Within-family Smoking	-0.15 [0.17] OR = 0.86
	Within MZ	-0.10 [0.21] OR = 0.90
	Within DZ	-0.20 [0.20] OR = 0.82
	Zygosity	0.09 [0.23] OR = 1.09
	Zygosity*Within	-0.10 [0.28] OR = 0.90

Note. Regression estimates [standard errors] for logistic regression models. Pain was ordinal and Smoking was the z-score of square root-transformed smoking frequency. Estimates are thus the log odds of increasing one pain level with a standard deviation increase in smoking frequency. The individual-level model regressed pain on smoking and sex without controlling for familial confounds. The co-twin control models separate the individual-level effects into between-family (suggestive of familial effects) and within-family (suggestive of direct effects) components. The models used a logit link function and maximum likelihood estimator robust to non-normality and non-independence. Sex was included as a covariate (estimates not shown). Zygosity was contrast coded (MZ = -0.5; DZ = 0.5) to obtain the within-family estimates across zygosity, whereas it was dummy coded to obtain the simple effects within each zygosity (Within MZ and Within DZ). The zygosity interaction coefficient is the difference of the within-family effect between MZ and DZ twins. MZ = Monozygotic twins. DZ = Dizygotic twins. OR = Odds Ratio. Bolded font indicates $p < 0.05$.

Twin Models

Univariate Analyses

The univariate twin models suggested that both pain and smoking are due to a mix of genetic and nonshared environmental effects. The observation that the MZ twin correlations were over double the DZ correlations for pain severity (*r*MZ = 0.46; *r*DZ = 0.10) implies dominance genetic effects, which are represented by inclusion of the D parameter. The MZ twin correlation for smoking frequency was just about double the DZ correlation (*r*MZ = 0.70; *r*DZ = 0.36), suggesting an AE model would fit well.

The variance components and model fit statistics can be found in Table 3-4. We did not have the power to detect significant A and D components when both were in the model (dominance genetic influences are difficult to detect in even very large samples; Martin et al., 1978), and the pain variance component model did not significantly deteriorate when dropping the D parameter. Pain variance was 43% [95% CI 0.18, 0.63] attributable to additive genetic effects and 57% [95% CI 0.37, 0.81] to non-shared environmental effects. For smoking the AE also fit well. For smoking frequency, 71% [95% CI 0.50, 0.85] of the variance was attributable to additive genetic effects and 29% [95% CI 0.15, 0.50] to non-shared environmental effects.

Table 3-4. Model fit statistics of the ADE pain, ACE smoking, and nested models

		Parameter Estimates			Model Fit					Chi-square Difference		
Measure	Model	A	C/D	E	χ^2	df	p	RMSEA	CFI	$\Delta\chi^2$	Δdf	p
Pain	ADE	0.00 [0.00, 0.57]	0.46 [0.00, 0.64]	0.54 [0.34, 0.79]	12.297	10	0.266	0.036	0.898			
	AE	**0.43** [0.18, 0.63]	**-**	**0.57** [0.37, 0.81]	**13.142**	**11**	**0.284**	**0.033**	**0.905**	**0.837**	**1**	**0.360**
	E	-	-	1.00 [1.00, 1.00]	31.061	12	0.002	0.094	0.154	19.158	2	<0.001
Smoking	ACE	0.70 [0.10, 0.83]	0.01 [0.00, 0.51]	0.30 [0.16, 0.51]	6.446	10	0.777	0.000	1.000			
	AE	**0.71** [0.50, 0.85]	-	**0.29** [0.15, 0.50]	**6.606**	**11**	**0.830**	**0.000**	**1.000**	**0.001**	**1**	**0.981**
	CE	-	0.61 [0.44, 0.74]	0.39 [0.25, 0.56]	10.459	11	0.490	0.000	1.000	5.000	1	0.025
	E	-	-	1.00 [1.00, 1.00]	72.516	12	<0.001	0.165	0.268	84.012	2	<0.001

Note. Model fit statistics for the full ADE pain (rMZ = 0.46; rDZ = 0.10) and ACE smoking (rMZ = 0.71; rDZ = 0.36) models and chi-square difference ($\Delta\chi^2$) tests for the nested models compared to these full models. The A (additive genetic effects), C/D (shared environmental/dominance genetic effects), and E (non-shared environmental effects) estimates presented represent the proportion of variance explained by each of the parameters. Pain and smoking frequency were ordinal and the mean- and variance-adjusted weighted least squares (WLSMV) estimator was used to account for non-normality. The model fit results show whether each model has good fit, indicated by comparative fit index (CFI) > 0.95 and root mean squared error of approximation (RMSEA) < 0.06. Chi-square difference ($\Delta\chi^2$) tests, appropriately scaled for the WLSMV estimator, were conducted to test whether dropping a parameter significantly deteriorated fit, indicated by $\Delta\chi^2$ $p < 0.05$. Dashes indicate the parameter was not estimated. df = degrees of freedom. Boldface type indicate the best models (AE) for both pain and smoking.

Bivariate Analyses

The bivariate twin model suggested that pain and smoking share a significant genetic correlation, but not a nonshared environmental correlation. The bivariate model fit statistics are presented below, and the parameter estimates are presented in Figure 3-2. The cross-twin, cross-trait correlations for MZ twins were about equal to those for the DZ twins (*r*MZ: 0.23; *r*DZ: 0.24). This pattern of correlations would suggest presence of an *r*C. We tried fitting an ACE version of pain, however, the C component in pain was estimated at 0% and in smoking at 1% (see Table 3-4). A bivariate model including *r*A and *r*E between AE pain and AE smoking fit the data well ($\chi^2(28) = 30.584$, $p = 0.336$, CFI = 0.979, RMSEA = 0.022). The *r*A correlation was significant (*r*A = 0.51, $\Delta\chi^2(1) = 11.525$, $p < 0.001$) but the *r*E correlation was not significant (*r*E = -0.18, $\Delta\chi^2(1) = 0.913$, $p = 0.339$).

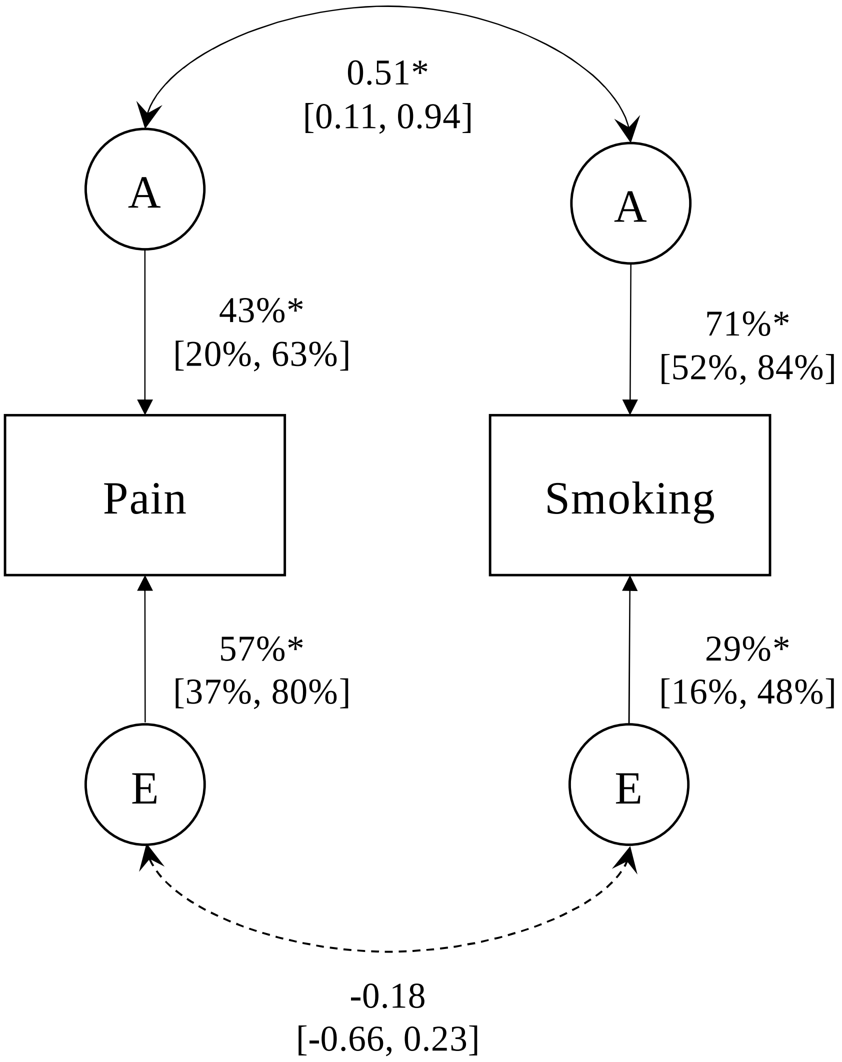


Figure 3-2. Bivariate twin model of pain and smoking
Standardized variance explained [bootstrapped 1000x 95% confidence intervals] and their correlations from the AE correlated factors model. The amount of variance explained by additive genetic effects (A) is 43% for pain and 71% for smoking. The genetic variance components of pain and smoking share a significant genetic correlation (r_g=0.51). The amount of variance explained by non-shared environmental effects (E) is 57% for pain and 29% for smoking. Pain and smoking do not show a significant non-shared environmental correlation (r_e= -0.18). Solid lines and asterisks indicate $p<.05$ and dashed lines indicate $p>.05$, based on chi-square difference tests.

Discussion

We found that chronic pain and past-month smoking are related. However, our findings do not provide evidence that smoking directly impacts pain; rather our co-twin control and twin model results suggest that there may be familial, particularly genetic, risk factors that explain their association. The mediation paths through NAc-mPFC connectivity were non-significant.

Significant shared genetic influences on smoking and pain

To our knowledge, this is the first twin study employed to estimate the genetic correlation between smoking frequency and chronic pain. Although the genetic correlation we detected may be due to a mixture of genetic and correlated common environmental effects that we are unable to discriminate, our estimate (r_g=0.51) is similar to a recent estimate from a genome-based method. Lundberg et al. (2020) found a genetic correlation (r_g=0.42) between smoking and wide-spread chronic pain in a genome-wide association study. These results suggest shared risk alleles underlie the pain-smoking association. Another study found that back pain shared a significant genetic correlation (r_g=0.35) with smoking status; interestingly, the correlation became non-significant when controlling for other related traits (e.g., body mass index [BMI], depression), suggesting that those traits might be explanatory variables (Freidin et al., 2019). Many transdiagnostic biopsychosocial factors have been detected for both smoking and pain, such as depression (LaRowe & Ditre, 2020). There may be gene sets that underlie smoking, pain, and other correlated biopsychosocial risk factors.

There are also multiple possibilities for familial environmental risk factors, which may also have gene-environment correlations with pain and smoking (in which case they might be captured by the genetic correlation for pain and smoking). Lifestyle habits such as nutrition, sleep, physical activity, and obesity, have been linked to chronic pain and smoking, both phenotypically and genetically (Lundberg et al., 2020; S. E. E. Mills et al., 2019).

A gene-environment interaction is also plausible, where genetic risk depends on an individual's environment (Purcell, 2002). Parenting styles have a gene-environment interaction with smoking and BMI, where negative parenting styles during development increased risk of genetic influences expressing for smoking and BMI (Ji & An, 2022). Chronic pain has been conceptualized as an example of gene-environment interaction where a person's genetic variants may make them at higher risk of developing pain when exposed to environmental risk (Mogil, 2012), although this hypothesis has not been directly tested within a gene-environment twin framework (Purcell, 2002). Shared environmental risk factors during development, such as poor nutrition, could contribute to increased genetic expression of pain and smoking risk alleles.

Small, non-significant direct influence of smoking on pain

We did not find a significant non-shared environmental correlation between pain and smoking, which is inconsistent with the hypothesis that smoking directly causes pain. This result is consistent with our co-twin control analysis (McGue et al., 2010), in which we did not find a significant direct effect. Our co-twin control findings did show a significant family association, also consistent with the significant genetic correlation we found in the twin model. These results suggest that familial effects account for the relationship between smoking and pain, rather than there being a direct causal effect (McAdams et al., 2021).

The support for predisposition underlying the pain-smoking association corroborates multiple past co-twin control findings, which examined smoking behaviors with low back pain (Hestbaek et al., 2006; Junqueira et al., 2014; Suri et al., 2017). In one study, individual-level analyses showed that being a former or current smoker increased back pain, but when partitioning out familial confounding, the risk was not significant (Suri et al., 2017). In another study, quantity of cigarettes per day showed evidence for dose-response relationships, but within a discordant MZ twin framework, smoking status did not significantly increase concurrent or prospective risk for low back pain (Hestbaek et al., 2006). Our findings extend these conclusions to chronic pain, which includes multiple pain types in addition to low back pain.

These findings conflict with some studies of causal support found in the literature (LaRowe & Ditre, 2020). Using genomic approaches, Mendelian randomization studies have found contradicting evidence on whether smoking causally increased pain risk or was due to genetic confounding (Freidin et al., 2019; Lundberg et al., 2020). This inconsistency may be due to differential mechanisms for specific pain conditions. For example, orofacial pain severity may have a more direct association with smoking frequency, in comparison to general pain (Alamir & Quadri, 2020).

An argument in support of the causal hypothesis, regardless of mechanism, is that the cessation of smoking leads to at least partial reversibility of pain severity. The literature on this phenomenon has mixed results, and these studies are most often observational (Behrend et al., 2012; Leboeuf-Yde, 1999; Shi et al., 2011). The quasi-experimental nature of the co-twin control design is unique in its ability to control for unmeasured genetic and socio-demographic factors within families (McAdams et al., 2021). Within a co-twin framework, being a former or current smoker did not have a significant effect on pain (Suri et al., 2017).

Small, non-significant NAc-mPFC mediation of the smoking-pain association

We did not find associations with pain or smoking and maladaptive cortico-striatal connectivity. It is surprising that we did not observe a relationship between the NAc-mPFC connectivity and concurrent smoking frequency, as circulating nicotine has been well-supported in activating acetylcholine receptors in dopamine systems (Wittenberg et al., 2020). One possibility is that smoking frequency does not reflect tobacco quantity, which may be a more direct measure of this mechanism; however, smoking quantity and smoking frequency were highly colinear in this sample. The prior study that found the significant mediation had differences in comparison to the current study; it was longitudinal and assessed smoking status within a subacute back pain sample (Petre et al., 2015). Another possibility is that the imaging data may have been noisy, as the nucleus accumbens is small and near many ventricles, making it susceptible to poor data quality.

The lack of relationship between the NAc-mPFC and pain was also unanticipated. The affective dimensions of pain have been shown to correlate with this circuit's functional connectivity (Baliki et al., 2012; Petre et al., 2015). Furthermore, the circuit has been shown to mediate the impact of self-regulation and acute pain intensity (Woo et al., 2015). The pain measure in our study may fail to distinguish between sensory and affective dimensions of pain, which would reduce power.

Limitations and future directions

This study was not without its limitations. First, the pain measure was self-reported, which can be biased (Robinson et al., 1997). Substance use-related self-report measures are especially vulnerable to under-reporting, which may have created artificial discordance in our co-twin design (Johnson & Fendrich, 2005).

Second, the cross-twin cross-trait correlations were suggestive of common (familial) environmental (C) risk factors underlying the covariation of pain and smoking; however, C estimates for pain and smoking were negligible, so our models focused on genetic associations. We did not detect significant C variance in our pain and smoking measures, consistent with the literature, but that does not mean they are zero. A larger twin study may be needed to definitively reject the presence of a small C influence (N. G. Martin et al., 1978).

Although this is a relatively large study for one that includes imaging data, power for a within-family effect depends on the within-family variance (discordance). A power analysis based on the variances we observed in this sample (see Appendix B) suggested that we had good power to detect phenotypic and between-family effects similar to those that we observed, but low power to detect a within-family effect of the same magnitude. However, the estimate for the within-family effect was nowhere near that for the phenotypic effect (it was actually negative), which may somewhat reduce concerns that our effects were consistent with a causal model but did not reach significance. While the within-family effect was not significantly different from 0, it was significantly smaller than the between-family effect. Given that we found a significant between-family effect, a non-significant negative within-family effect that was significantly smaller than the between-family estimate, and a significant genetic correlation, the data are suggestive of a familial explanation for the pain-smoking association. However, we cannot rule out the possibility of a small, direct effect of smoking on pain.

This study focused on general chronic pain, but future research would benefit from observing these associations in specific pain conditions. There may be differential mechanisms and shared gene sets for differential pain conditions, such as migraine or low back pain. Future research could examine additional biopsychosocial risk factors and explanatory variables for the pain-smoking relationship, such as inflammation. Furthermore, conducting gene-environment interactions between pain and common environmental risk factors, such as stressful life events, may be informative.

Conclusions

The association between smoking and pain appears to be due to familial effects, likely genetic risk. While cessation of smoking is beneficial to overall health, it may not directly reduce pain. There are likely underlying risk factors that are driving both pain and smoking risk. Identification of these underlying risk factors may be informative in prevention of both pain and smoking exacerbation.

CHAPTER IV: EXAMINING GENETIC RELATIONSHIPS BETWEEN CHRONIC PAIN AND SUBSTANCE USE DISORDERS

Introduction

Chronic pain and substance use disorders are highly co-prevalent (Orhurhu et al., 2015; Rogers et al., 2020; Witkiewitz & Vowles, 2018). Among chronic pain patients, 10% meet the diagnosis for opioid use disorder (Martel et al., 2018), 12% meet diagnosis for cannabis use disorder (Degenhardt et al., 2015), around 20% use tobacco (LaRowe & Ditre, 2020; Orhurhu et al., 2015), and 20% report moderate-to-heavy alcohol consumption (Macfarlane & Beasley, 2015). Co-use of substances is also commonly reported among pain patients (Degenhardt et al., 2015; McDermott et al., 2018; Rogers et al., 2019). Bidirectional associations between chronic pain and substance use disorders have been observed (Koller et al., 2024; Rubenstein et al., 2022) and there is evidence of shared genetic risk between pain and substance use behaviors (Koller et al., 2024), which may partially explain the bidirectionality. While genetic correlations between pain and substance use disorders have been identified, it is unclear whether the relationship acts solely through a common substance use disorder liability or if there are unique pathways to particular substance use disorders and consumption.

Substance use disorders may cause pain chronification. Generally, substance use has been linked to maladaptive changes in central systems that incite aberrant pain processing (Borsook et al., 2016). Nicotine, opioid, and alcohol use have been linked to a deficiency in central opioid systems, which has been linked to chronic pain processing (Borsook et al., 2016; Egli et al., 2012; Shi et al., 2010). Smoking in particular has a robust literature hypothesizing tobacco's causal effect on pain, potentially through promoting maladaptive neural plasticity in overlapping reward and pain processing regions of the brain (Petre et al., 2015).

Most research on pain and substance use investigates relationships with one or two substances, as it is difficult to collect a sample with sufficient endorsement of comprehensive pain data and of multiple substance use disorders. Tobacco use is considered a prominent risk factor for chronic low back pain (LaRowe & Ditre, 2020); however, many studies have looked at smoking in isolation and did not additionally control for alcohol, cannabis, and opioid use. A multivariate classifier based on the biopsychosocial model indicated that alcohol use and tobacco use traits were a large contributor in predicting chronic pain development (Tanguay-Sabourin et al., 2023), but non-prescribed opioid and cannabis use traits were not included (Rogers et al., 2019). Thus, there remain unanswered questions about the degree that pain shares associations with substance use disorders in general or with specific substances. Understanding the shared and unique relationships may elucidate the pathophysiology of pain. For example, aberrant reward processing may be common between multiple substance use disorders and pain, whereas expression and desensitization of nicotinic acetylcholine receptors may be unique to smoking and pain (Borsook et al., 2016; Shi et al., 2010).

Latent variable modeling would allow for the extraction of a common factor capturing shared variance among substance use disorders, while also separating out each disorder's residual variance (Bollen, 2002). The common substance use disorder variance and unique substance use disorder variances can then be related to a chronic pain factor. However, due to low substance use disorder endorsement in the general population, ascertaining a sample well-powered for this type of model is unlikely at the phenotypic level (Bollen, 2002). Genomic structural equation modeling (Genomic SEM) allows for modeling relationships between phenotypes collected in separate samples, enabling latent variable modeling to be conducted on infrequently endorsed traits that would typically lead to a sparse correlation matrix in a phenotypic setting (Grotzinger et al., 2019). Hence, hypotheses about the degree of relatedness between multiple chronic pain conditions and substance use traits can be tested.

Chronic pain is characterized by both somatic and brain-based (e.g., cognitive and affective) attributes (Melzack & Casey, 1968). Chronic pain conditions certainly have their own pathologies and etiologies; however, phenotypic (Battaglia et al., 2022) and genetic studies (Scaini et al., 2022; Vehof et al., 2014; Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023) have identified a common factor among various pain conditions, irrespective of body site. A general pain factor constructed from 24 pain conditions is thought to tap into central, affective-pain mechanisms, as it shows greater genetic expression in the central nervous system and has been related to traits such as anxiety, depression, and neuroticism (Zorina-Lichtenwalter, Bango, Čeko, et al., 2023; Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023). Another general pain factor has been phenotypically linked to substance use in adolescents (Scaini et al., 2022). Genetic correlations have been identified between chronic pain and individual substance use disorders, suggesting that there is shared pleiotropy between these traits (Hatoum et al., 2022; Koller et al., 2024; Toikumo et al., 2023); however, the degree to which the central component of chronic pain relates to general (i.e., a Genomic SEM Addiction factor that captures shared genetic variance of 4 substance use disorders involving alcohol, tobacco, cannabis, and opioids; (Hatoum et al., 2022) and specific substance use disorder risk is unclear.

This study will use Genomic SEM to assess the degree of genetic relatedness of associations between the General Pain factor with the Addiction factor; with substance use disorders over and above common addiction liability; and with substance use consumption. We will examine the degree of functional enrichment of brain cell types of significant genetic associations to help inform potential biological pathways. We expect to find shared genetic risk between General Pain and Addiction and likely with smoking; however, the relationships with other substance use disorders and consumption traits and the functional enrichments tests are largely exploratory. This approach will inform the level of common and unique biological pathways shared between a chronic pain factor and substance use traits.

Method

GWAS Summary Statistics

We obtained GWAS summary statistics from previously published GWASs. GWAS summary statistics are output data files from GWASs, in which regressions are conducted between single nucleotide polymorphisms (SNPs) across the genome predicting a trait of interest. The GWAS output file contains columns including SNP IDs, chromosome locations, effect alleles, effect sizes, minor allele frequencies, sample size (or effective sample size), standard errors, and *p*-values. The summary statistics were limited to European ancestry as there were not GWASs available for many non-European ancestries due to low sample sizes.

Chronic pain phenotypes

The GWAS summary statistics for the 24 pain conditions used the United Kingdom Biobank (UK Biobank). Chronic pain was defined as pain lasting longer than 3 months or conditions with persistent pain as an important symptom, such as osteoarthritis. The conditions included were headache, migraine, neck shoulder pain, back pain, chest pain/discomfort, chest pain during physical activity, irritable bowel syndrome, gastritis, oesophagitis, stomach pain, carpal tunnel, cystitis, hip pain, knee pain, leg pain, gout, enthesopathies of lower limb, hip arthrosis, knee arthrosis, enthesopathies, rheumatoid arthritis, non-specific arthropathies including osteoarthritis, pain in joint, and chronic widespread pain. The descriptive statistics for the GWAS summary statistics can be found elsewhere (Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023). The prevalence of the conditions was 0.014-0.473.

Substance use phenotypes

GWAS summary statistics for Opioid Use Disorder (OUD) (Deak et al., 2022), Problematic Alcohol Use (PAU) (Zhou et al., 2023), Drinks per Week (DPW) (Saunders et al., 2022), Tobacco Use Disorder (TUD) (Toikumo et al., 2024), Cigarettes per Day (CPD) (Saunders et al., 2022), and Cannabis Use Disorder (CanUD) (Levey et al., 2023) were included.

The OUD GWAS (Deak et al., 2022) examined OUD case/control status. The GWAS was released in 2022 and includes data from 7 cohorts: Million Veterans Program (MVP) releases 1 and 2, Psychiatric Genetics Consortium (PGC), Yale-Penn release 3, iPSYCH, FinnGen, Partners Biobank, and Vanderbilt University Medical Center's biobank (BioVU). The cases were defined as those meeting ICD-9/ICD-10 or DSM-IV/V OUD diagnosis status. The controls included both unscreened and screened participants to maximize sample size. The European ancestry total N was 554,186 (N_{case} = 15,251; $N_{\text{effective}}$ = 56,994).

The PAU GWAS (Zhou et al., 2023) is a meta-analysis of a continuous phenotype that combines across Alcohol Use Disorder (case status determined by ICD-9/10) and Alcohol Dependence (case status determined by DSM-IV/V) and Alcohol-related Problems (identified using questions 4-10 from the Alcohol Use Disorders Identification Test–Problem [AUDIT–P]). The GWAS was released in 2023 and includes AUD, AD, or AUDIT-P data from the following cohorts: MVP release 4, UK Biobank, FinnGen, iPSYCH, QIMR Berghofer cohorts, PGC, and Yale-Penn release 3. The European ancestry total N was 903,147.

The TUD GWAS (Toikumo et al., 2024) is a meta-analysis examining case/control status across 8 cohorts: UK Biobank, BioVU, Mass General Brigham Biobank (MGBB), Penn

Medicine BioBank (PMBB), and MVP. Cases were those who met the ICD-9/ICD-10 TUD code on 2 or more occasions. Controls were screened for absence of TUD code. The total *N* for European ancestry of TUD was 739,895 (N_{case} = 174,021, $N_{\text{effective}}$ = 495,005).

The CanUD GWAS (Levey et al., 2023) was released in 2023 and includes data from the PGC, deCODE, MVP-4, iPSYCH2, MGB, Yale-Penn 3 cohorts. Cases met an ICD code for either cannabis dependence or cannabis abuse. Controls were defined as individuals without ICD codes for cannabis dependence, abuse, or use. The total *N* for the European ancestry group was 886,025 (N_{case} = 42,281; $N_{\text{effective}}$ = 161,053).

The Drinks per Week (DPW) and Cigarettes per Day (CPD) GWASs (Saunders et al., 2022) were derived from the GWAS and Sequencing Consortium of Alcohol and Nicotine Use (GSCAN). The phenotypes were continuous measures of alcohol and cigarette consumption, respectively. The European ancestry total *N* for DPW was 2,428,851 and the total *N* for CPD was 618,489.

Procedure

Linkage Disequilibrium Score Regression (LDSC) is a method that estimates SNP-based heritabilities and genetic covariances using GWAS summary statistics. First, we used the GenomicSEM package in R to run GWAS summary statistics through the “munge” function, which outputted Z-statistics that were aligned to the same reference allele. The genetic covariance and sampling covariance matrix was then estimated using the “ldsc” function. The genetic covariance matrix contains the SNP-based heritabilities on the diagonal and the genetic covariances on the off-diagonal. SNP heritability is estimated by regressing the GWAS χ^2 (test statistic) against the LD scores for every SNP. The LD scores used here reflect the 1000 Genomes Phase 3 European subsample. The estimated per-SNP heritability is the strength of association between LD and the SNP effect size (i.e., the slope), which was converted to the common variant heritability by multiplying by the number of SNPs (m) in the LD-score reference panel. Genetic covariances were estimated in a similar fashion, with the product of the GWAS Z-statistics across the two traits as the predicted outcome in the LDSC regression equation. The genetic covariance matrix was standardized into a genetic correlation by using the "cov2cor" function. Genetic correlation plots were estimated using the "corrplot" function in R. The sampling covariance matrix contains the squared standard errors on the diagonal and the sampling dependencies on the off-diagonal. These sampling dependences are estimated directly from the data and control for varying and unknown degrees of participant sample overlap across the included GWAS.

The GenomicSEM package allows for the fitting and estimation of structural equation models using the output from LDSC. The goodness of fit of the model was assessed by multiple fit indices: in general, a Comparative Fit Index (CFI) > 0.90 and an SRMR of <0.08 are considered acceptable model fits (Bollen & Long, 1993). Chi-square difference tests were conducted to assess whether dropping a parameter leads to a deterioration of fit ($p < 0.05$) for nested models.

Stratified GenomicSEM can use a stratified LDSC matrix to assess functional enrichment of model parameters (e.g., factor correlation between General Pain and Addiction) that were estimated in GenomicSEM. Stratified LDSC is similar to LDSC, but functional information is used to partition SNP heritabilities across biological categories (Finucane et al., 2015). Functional enrichment can be examined across SNPs and then grouped together into biological categories, such as specific neuronal subtypes. A functional annotation is considered enriched for the parameter of interest (e.g., factor variance) when the genetic variance of that annotation is greater than the proportional size of the annotation (Grotzinger et al., 2022). An annotation's proportional size is the number of SNPs within that annotation over the total number of SNPs analyzed. In other words, a parameter will have functional enrichment of a particular annotation when the genetic variance of that annotation is greater than would be expected for the size of the annotation. We ran Stratified Genomic SEM to identify enrichment for functional annotations (i.e., genetic expression in brain regions) using the "enrich" function. We included a total of 113 annotations for the analysis including 1) baseline annotations which includes all SNPs (e.g., Conserved Primate, Minor Allele Frequency bin, Genomic Evolutionary Rate Profiling) and 2) neuronal subtype annotations (e.g., GABAergic neurons, Astrocytic Transporter, Excitatory CA1 Hippocampal Neurons). The full list of annotations is linked in Appendix C. We applied the enrich function to test the significant paths of interest. Since the annotations are not independent, FDR correction was applied to each set of annotations tested for a model.

We prepared the summary statistics to have all the necessary columns for GenomicSEM (e.g., SNP rsID, the effect allele, the non-effect allele, the coefficient, and *p*-value). For the binary traits, we calculated the effective *N* where needed. We used cross-trait LDSC to examine the genetic correlations between the pain conditions and substance use disorders.

Models

Using GenomicSEM, we fit a series of models based on prior literature (Hatoum et al., 2022; Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023) to test our hypotheses. First, we ascertained the amount of shared genetic variance between General Chronic Pain and Addiction. We fit the measurement models for the Chronic Pain and Addiction factor models. The Chronic Pain factor model is a bi-factor-like model with a Musculoskeletal Pain-specific factor accounting for variance above and beyond the General Pain factor. The Addiction factor has loadings for all four substance use disorder traits (i.e., OUD, PAU, TUD, and CanUD). A latent genetic correlation was estimated between the General Pain factor and the Addiction factor to ascertain the degree of shared genetic variance.

Second, we conducted a Q_{trait} analysis that allowed the General Pain factor to predict all the Addiction factor indicators directly instead of correlating with the factor. A chi-square difference test was conducted between the nested model and the unconstrained model. If the nested model fits significantly worse than the unconstrained model, this is indicative that General Pain may share additional variance with the SUD indicators. We also tested the degree and significance that each Addiction indicator related to the General Pain factor over and above the Addiction-General Pain correlation. If the pathway directly to the indicator(s) is significant, this is suggestive of pain sharing additional genetic risk over and above shared genetic risk with general addiction risk.

Third, for alcohol and tobacco, we assessed the shared genetic variance specific to pain with substance consumption, while controlling for problematic substance use for that substance. The Alcohol Cholesky Decomposition included three factors: 1) Factor 1, the problematic alcohol use factor, which captured all variance of PAU and all variance of AUDIT-C and DPW shared with PAU; 2) Factor 2, the alcohol consumption factor, which captured all the variance of AUDIT-C not shared with PAU and all the variance of DPW shared with AUDIT-C; and 3) Factor 3, the alcohol frequency residual factor, which captured all leftover residual variance of DPW that did not load on either the problematic alcohol use factor nor the alcohol consumption factor. Genetic correlations were estimated between the General Chronic Pain factor and the three Alcohol factors.

The Tobacco Cholesky Decomposition included two factors: 1) Factor 1, the tobacco use disorder factor, which captured all variance of TUD and all variance of CPD shared with TUD; and 2) Factor 2, the tobacco frequency residual factor, which captured all leftover residual variance of CPD not shared with TUD. Genetic correlations were estimated between the General Chronic Pain factor and the two Tobacco factors. Because this is a Cholesky decomposition, all the substance use indicators' residual variance were fixed to 0 so that the factors fully captured the variances. Significance, valence, and effect size of the correlations were informative of how chronic pain risk relates to problematic use compared to consumption of these two substances.

Fourth, we conducted Stratified Genomic SEM to assess enrichments for functional annotations for the significant associations identified through Genomic SEM. A significant enrichment of an annotation for a pleiotropic signal can suggest a potential shared mechanism or pathway between the two traits.

Results

Shared genetic variance between pain conditions and SUDs

The genetic correlation matrix derived from LDSC of all pain condition and substance use indicators is presented in Figure 4-1. The bi-factor model of chronic pain ($\chi^2(237) = 1289.338$, CFI=0.950, SRMR=0.075) and the model of the Addiction factor both fit well ($\chi^2(2) = 6.300$, CFI=0.998, SRMR=0.033). When correlating the General Pain factor with each substance use disorder (i.e., not including the Addiction factor in the model), General Pain shared a significant genetic association with OUD ($r_g = 0.41, p < 0.001$), PAU ($r_g = 0.28, p < 0.001$), TUD ($r_g = 0.39, p < 0.001$), and CanUD ($r_g = 0.38, p < 0.001$). At the factor-level, General Chronic Pain shared a significant genetic association with Addiction, $r_g = 0.43, p < 0.001$, and the model fit well ($\chi^2(334) = 2527.876$, CFI=0.952, SRMR=0.072).

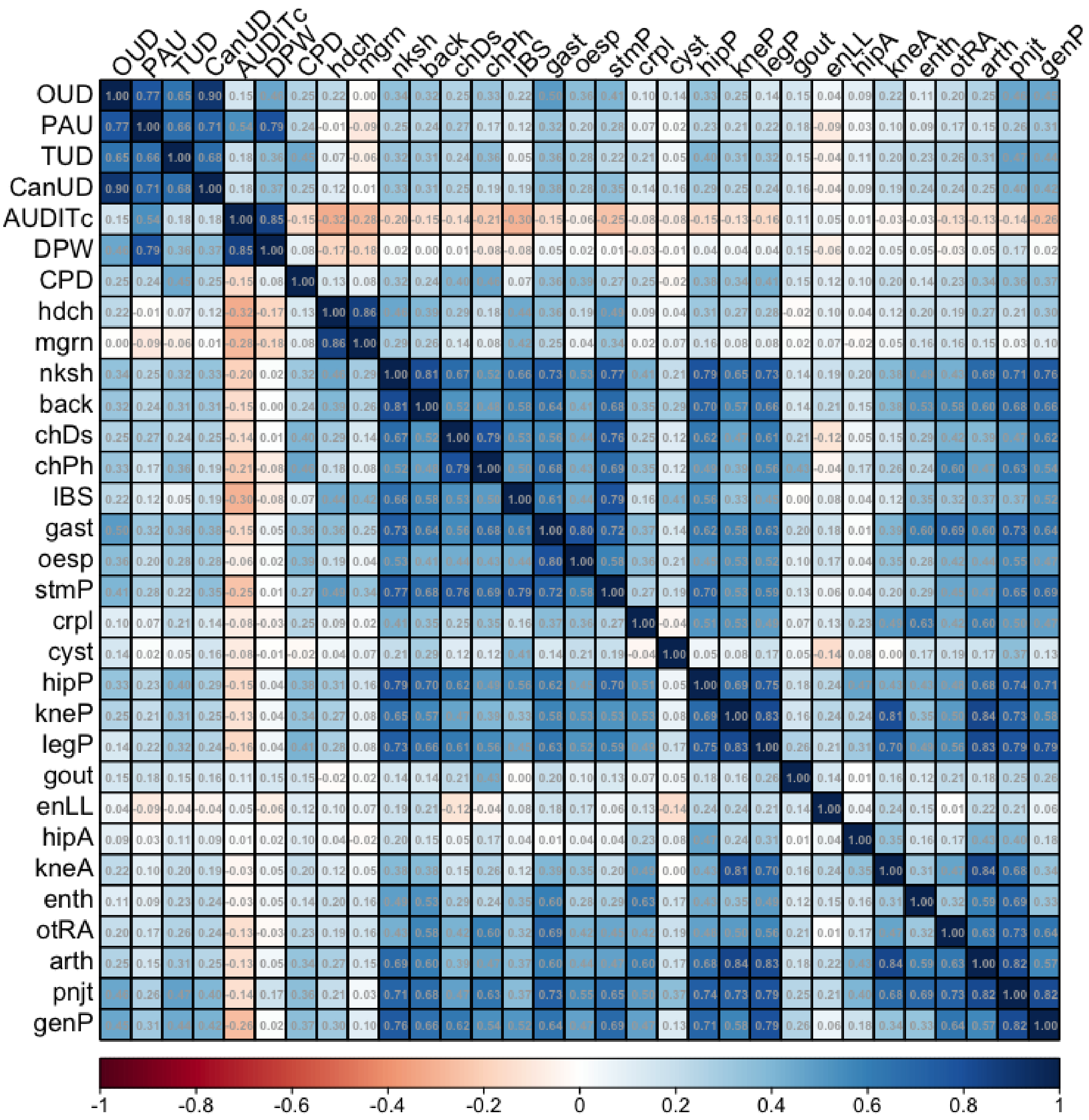


Figure 4-1. LDSC matrix of genetic correlations
The LDSC matrix of genetic correlations between all substance use and chronic pain condition traits. OUD = opioid use disorder; PAU = problematic alcohol use; TUD = tobacco use disorder; CanUD = cannabis use disorder; AUDITc = AUDIT consumption score; DPW = drinks per week; CPD = cigarettes per day; hdch = headache; mgrn = migraine; nksh = neck/shoulder; back = back; chDs = chest pain/discomfort; chPh = chest pain during physical activity; IBS = irritable bowel syndrome; gast = gastritis; oesp = oesophagitis; stmP = stomach pain; crpl = carpal tunnel; cyst = cystitis; hipP = hip pain; kneP = knee pain; legP = leg pain; gout = gout; enLL = enthesopathies of lower limb; hipA = hip arthrosis; kneA = knee arthrosis; enth = enthesopathies; otRA = rheumatoid arthritis; arth = arthropathies nonspecific including osteoarthritis; pnjt = pain in joint; genP = chronic widespread pain.

Unique genetic relationships between pain conditions and SUDs

We were unable to estimate the chi-square difference test of the nested model, where General Pain had a covariance with the Addiction factor, compared to the unconstrained model, where General Pain had direct paths to all four substance use disorder indicators. The chi-square of the unconstrained model ($\chi^2(331) = 2553.215$) is larger than the nested model ($\chi^2(334) = 2527.876$) which should statistically not happen. Models with more parameters should have a better fit (Pavlov et al., 2020). We've been troubleshooting with the creator of Genomic SEM, but have not yet figured out the solution. Prior to submission to journals, we plan to diagnose why there is this discrepancy; however, the models, the parameters, and their significance reported below should be unaffected. Controlling for the factor-level correlation with the Addiction factor, General Pain showed a significant positive genetic association with TUD ($b = 0.110$, $p = 0.004$), and a significant negative genetic association with AUD ($b = -0.138$, $p = 0.002$), but was not significantly related to OUD ($b = 0.032$, $p = 0.590$) nor CanUD ($b = -0.009$, $p = 0.831$).

Disentangling relationships with problematic use and consumption

Alcohol use. Descriptively, General Pain shared a significant relationship with the AUDIT-C indicator ($r_g = -0.231$, $p < 0.001$), but not with the DPW indicator ($r_g = 0.015$, $p = 0.513$). The Cholesky decomposition fit acceptably and is shown in Figure 4-2 ($\chi^2(306) = 576.428$, CFI=0.933, SRMR=0.075). Pain shared a significant genetic relationship with the Problematic Alcohol Use Cholesky factor ($r_g = 0.283$, $p < 0.001$). Controlling for Problematic Alcohol Use, pain shared a significantly negative, genetic relationship with the Alcohol Consumption Cholesky factor ($r_g = -0.451$, $p < 0.001$). General pain did not share a significant association with the Alcohol Frequency Residual ($r_g = 0.055$, $p < 0.540$).

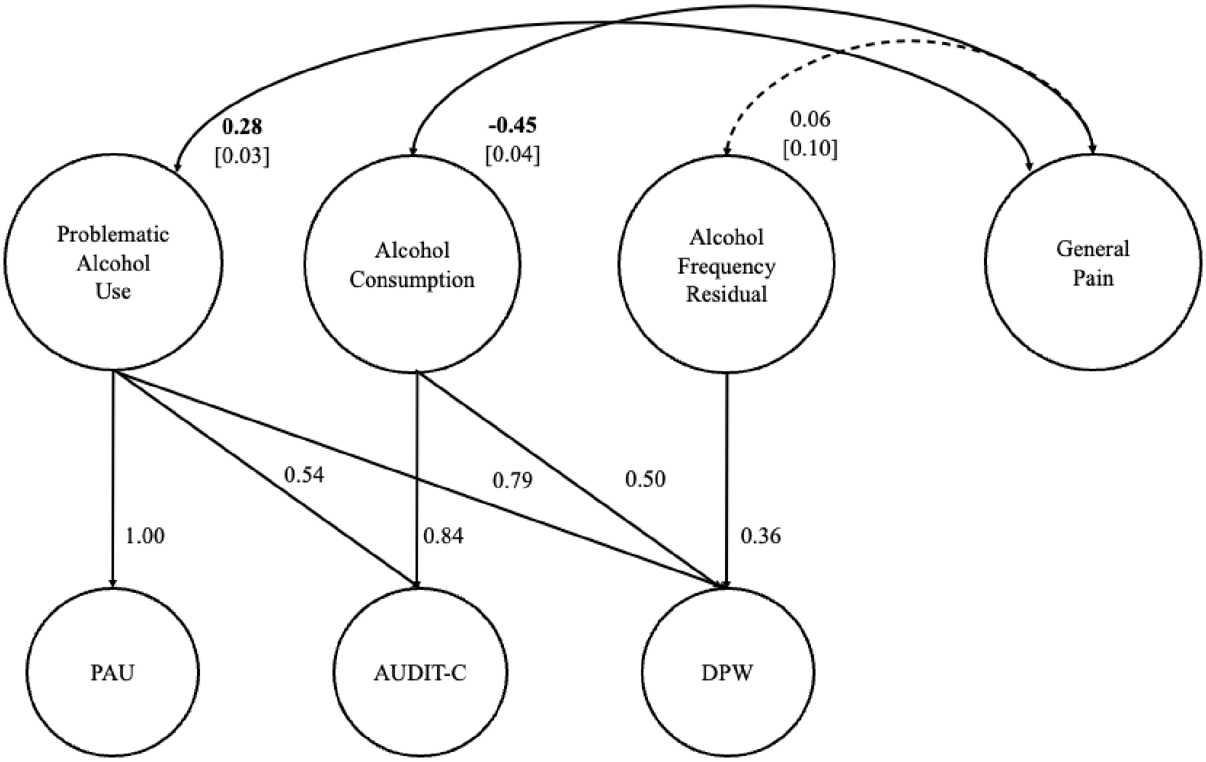


Figure 4-2. Genetic Cholesky Decomposition of Problematic Alcohol Use, Alcohol Consumption, and Relationships with General Chronic Pain

The Problematic Alcohol Use Cholesky factor captures 100% of the variance PAU indicator as well as any genetic variance that is shared with PAU from the AUDIT-C and DPW indicators. The Alcohol Consumption Cholesky factor captures all remaining genetic variance of the AUDIT-C indicator and any variance from DPW that is shared with AUDIT-C. The Alcohol Frequency Residual captures the remaining genetic variance of the DPW indicator. The double-headed arrows visualize the covariances between the General Pain factor and the Alcohol factors. The 28 pain indicators for the General Pain factor are not visualized in this diagram. PAU = problematic alcohol use; AUDIT-C = AUDIT consumption score; DPW = drinks per week.

Tobacco use. Descriptively, the General Pain factor shared a significant association with the CPD indicator ($r_g = 0.423, p < 0.001$). The Cholesky decomposition fit acceptably ($\chi^2(283) = 2187.549$, CFI=0.930, SRMR=0.075) and is shown in Figure 4-3. Pain shared a significant genetic relationship with the Tobacco Use Disorder Cholesky factor ($r_g = 0.391, p < 0.001$). Controlling for Tobacco Use Disorder, pain shared a significant genetic relationship with the Tobacco Consumption Cholesky factor ($r_g = 0.278, p < 0.001$).

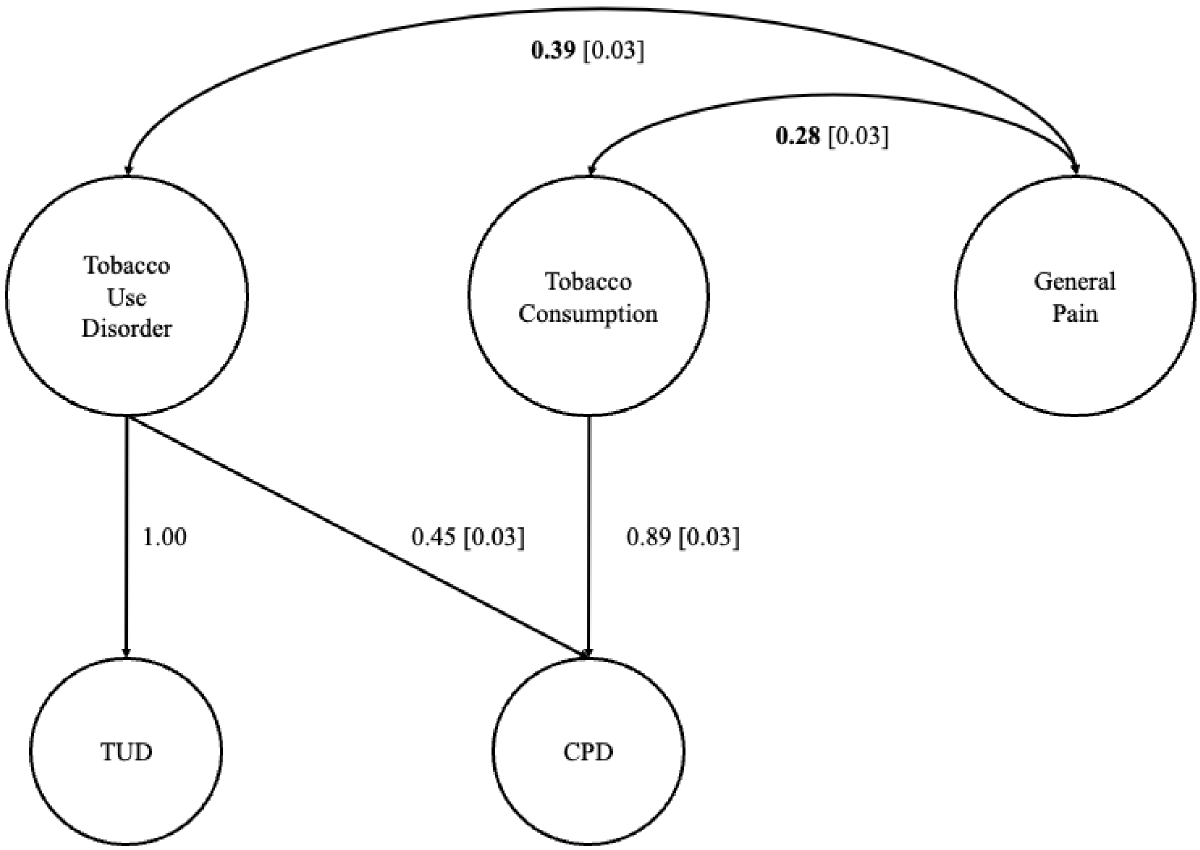


Figure 4-3. Genetic Cholesky Decomposition of Tobacco Use Disorder, Tobacco Consumption, and relationships with Chronic Pain
The Tobacco Use Disorder Cholesky factor captures 100% of the variance TUD indicator as well as any genetic variance that is shared with TUD from the CPD indicator. The Tobacco Consumption Cholesky factor captures all remaining genetic variance of the CPD indicator. The double-headed arrows visualize the covariances between the General Pain factor and the Tobacco factors. The 28 pain indicators for the General Pain factor are not visualized in this diagram. TUD = tobacco use disorder; CPD = cigarettes per day.

Functional enrichment between General Chronic Pain and Substance Use

General Chronic Pain and Addiction Association. We applied Stratified Genomic SEM to the genetic association between the Addiction factor and the General Chronic Pain factor. A total of 42 annotations met significance with the uncorrected *p*-value, and 34 annotations met significance after FDR correction (See Appendix C). The association showed significant enrichments in neuronal annotations including multiple sets of GABAergic neurons, Protein truncating variant intolerant (PI) genes, Oligodendrocyte Precursor cells, Astrocytic

Transporters, Excitatory CA1 Hippocampal Neurons, Excitatory Prefrontal Cortex Neurons, and Evolutionary annotations (all FDR-corrected $p < 0.05$) (See Figure 4-4).

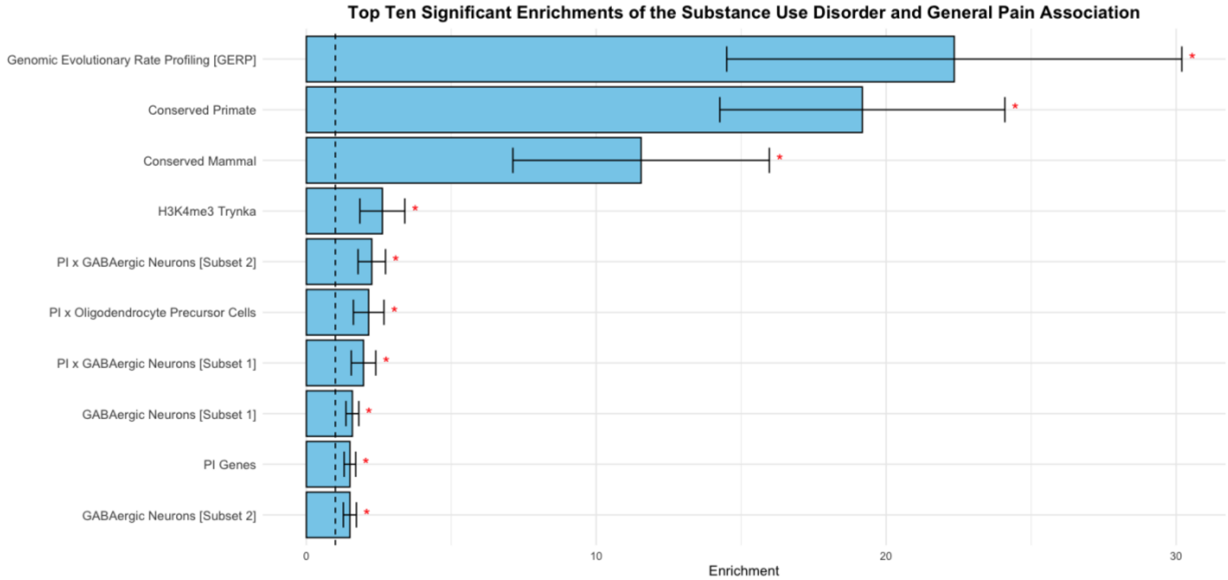


Figure 4-4. The top ten significant enrichments of the Addiction factor and General Pain factor covariance
The Addiction Factor captures variance of 4 substance use disorders. The General Pain factor captures variance of 28 pain conditions. The red asterisks indicate significance at an FDR-corrected p-value < 0.05. The error bars are the 95% Confidence Intervals.

General Chronic Pain and PAU-specific Associations. The enrichment values for all annotations did not successfully estimate. This is likely due to the low covariance between General Chronic Pain and PAU-specific variance when controlling for shared variance with the Addiction factor. To better diagnose this problem, we looked at General Chronic Pain's enrichment with PAU without including the Addiction factor in the model. The significant enrichments were the same as those with the Addiction factor reported above, confirming our suspicion that there was not much PAU-specific association.

General Chronic Pain and TUD-specific Association. The enrichment values for all annotations did not successfully estimate. This is likely due to the low covariance between General Chronic Pain and TUD-specific variance, when controlling for shared variance with the

Addiction factor. Indeed, when we looked at General Chronic Pain's enrichment with TUD without including the Addiction factor in the model, the significant enrichments were the same as those with the Addiction factor reported above.

General Chronic Pain with Problematic Alcohol Use and Alcohol Consumption. We used Stratified Genomic SEM to test the enrichments of the covariance between General Chronic Pain and two factors from the Alcohol Cholesky Decomposition (See Figure 4-2). The General Chronic Pain-Problematic Alcohol Use association had 8 un-corrected significant enrichments, but only 2 that passed FDR correction: the Conserved Primate pathway (*b* = 19.041, FDR-corrected *p* = 0.018) and the Genomic Evolutionary Rate Profiling pathway (*b* = 27.809, FDR-corrected *p* = 0.018) (See Figure 4-5).

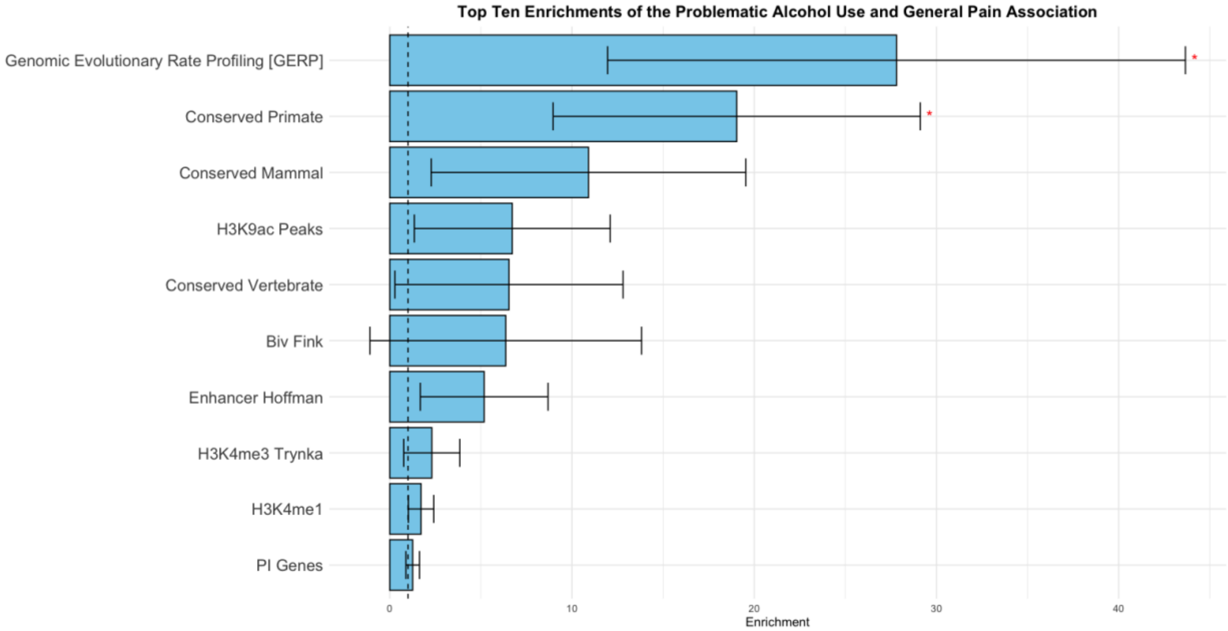


Figure 4-5. The top ten functional enrichments of the covariance between the Problematic Alcohol Use Cholesky factor and General Pain factor
These enrichments are of the association between the General Pain factor and the Problematic Alcohol Use factor from the Cholesky Decomposition. The Problematic Alcohol Use Cholesky factor captures all the variance of PAU and all its shared variance with the AUDIT-C and DPW indicators. The red asterisks indicate significance at an FDR-corrected p-value < 0.05. The error bars are the 95% Confidence Intervals.

The association with Alcohol Consumption had 53 uncorrected significant annotations, and 51 significant enrichments that passed FDR correction. The top ten significant enrichments are visualized in Figure 4-6 and all remaining significant enrichments can be found linked in Appendix C. There were significant enrichments in multiple neuronal subtypes including Astrocytic Transporters, Endothelial Cells, Excitatory CA1 and CA3 Hippocampal neurons, Excitatory Dentate Gyrus Neurons, Excitatory Prefrontal Cortex neurons, GABAergic neurons, PI Genes, Oligodendrocytes and Oligodendrocyte Precursor Cells, and Microglia (all FDR-corrected $p < 0.05$).

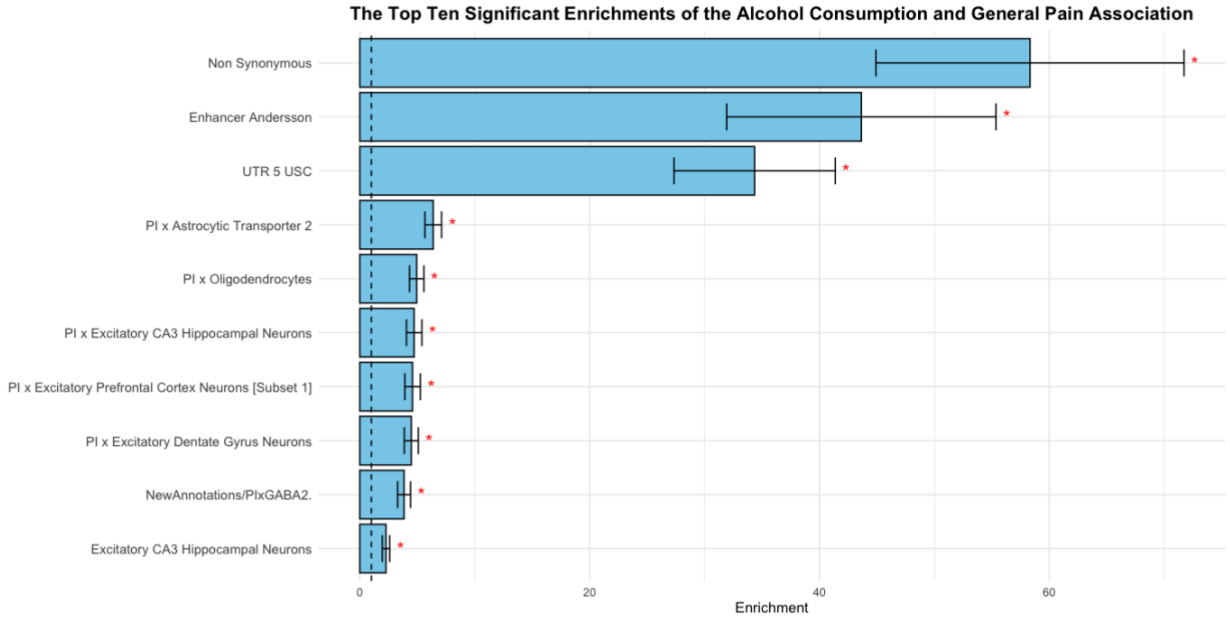


Figure 4-6. The top 10 significant enrichments of the covariance between the Alcohol Consumption factor and General Pain factor
The Alcohol Consumption factor is from a Cholesky Decomposition and captures all remaining variance of AUDIT-C and all shared variance with DPW, that is not captured by the Problematic Alcohol Use Cholesky factor. The red asterisks indicate significance at an FDR-corrected p-value < 0.05. The error bars are the 95% Confidence Intervals.

General Chronic Pain with Tobacco Use Disorder and Cigarette Consumption. We used Stratified Genomic SEM to test the enrichments of the covariance between General Chronic Pain and two factors from the Tobacco Cholesky Decomposition. The General Chronic Pain-Tobacco

Use Disorder association had 36 un-corrected significant enrichments and 18 significant enrichments that passed FDR correction (See Figure 4-7). There were significant enrichments in neuronal subtypes including Astrocytic Transporters, Excitatory CA1 Hippocampal neurons, Excitatory Prefrontal Cortex neurons, GABAergic neurons, Oligodendrocyte Precursor Cells, and PI Genes.

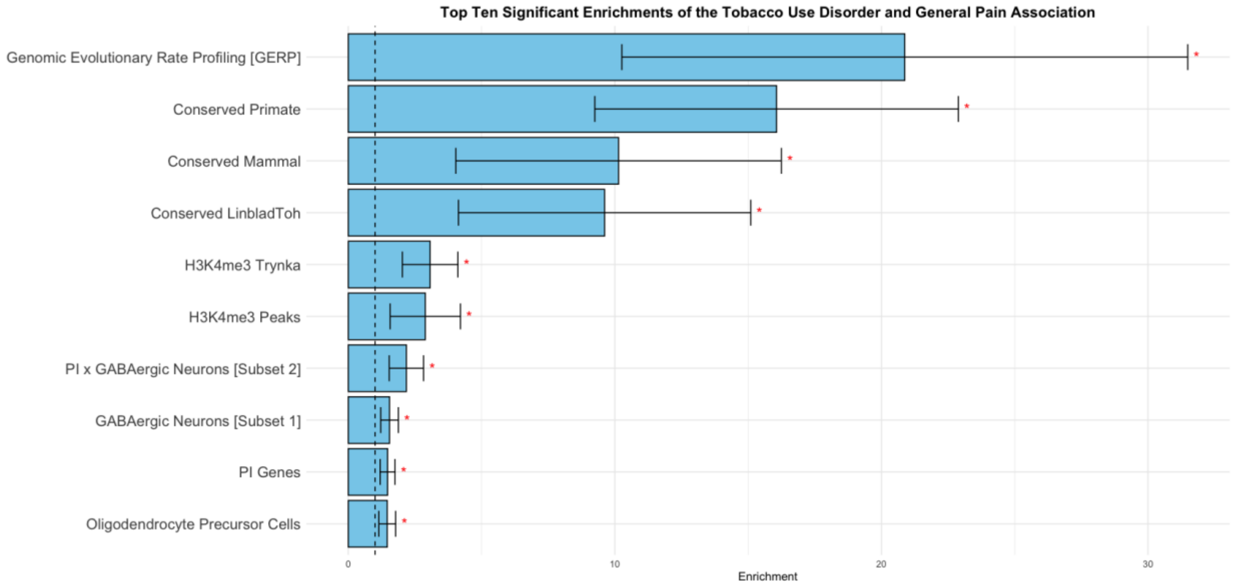


Figure 4-7. The top ten functional enrichments of the covariance between the Tobacco Use Disorder factor and General Pain factor
These enrichments are of the association between the General Pain factor and the Tobacco Use Disorder factor from the Cholesky Decomposition. The Tobacco Use Disorder Cholesky factor is all of the variance of TUD and all shared variance with TUD from the CPD indicator. The red asterisks indicate significance at an FDR-corrected p-value < 0.05. The error bars are the 95% Confidence Intervals.

The Cigarette Consumption-Pain association had 7 uncorrected significant enrichments, but 0 enrichments that passed FDR correction. We visualized the top ten enrichments to inform which were approaching significance in Figure 4-8.

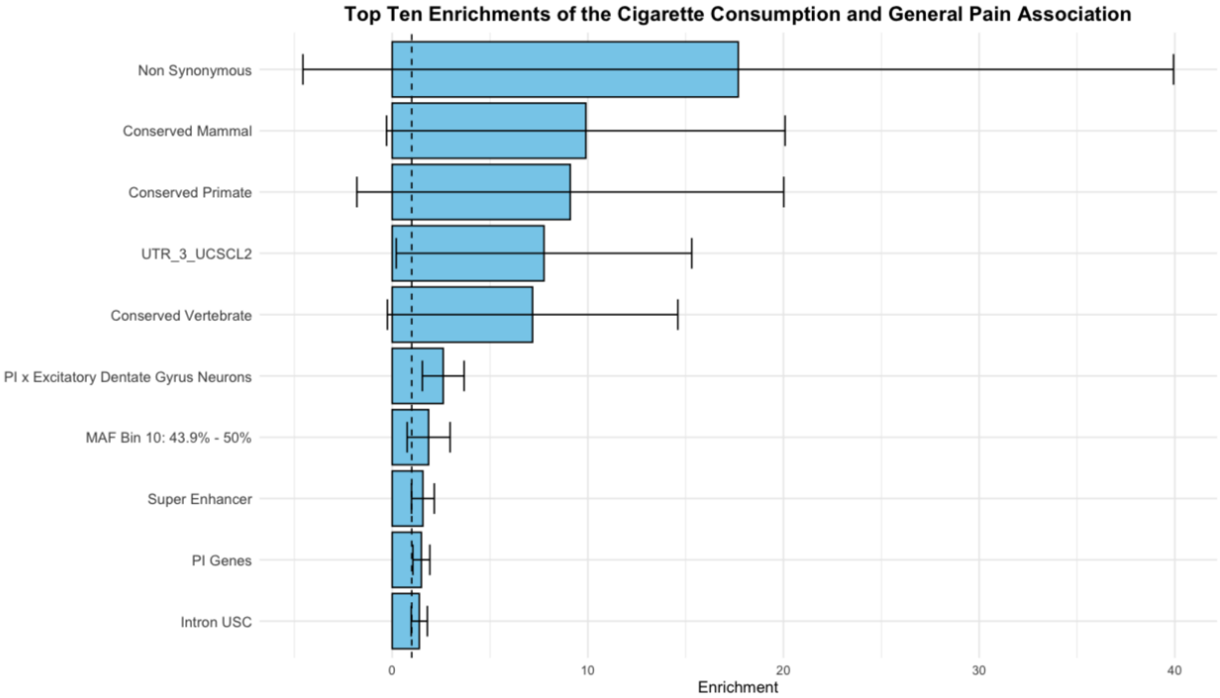


Figure 4-8. The top 10 enrichments of the covariance between the Cigarette Consumption factor and General Pain factor
The Cigarette Consumption factor is from a Cholesky Decomposition and captures all remaining variance of CPD, that is not captured by TUD. The red asterisks indicate significance at an FDR-corrected p-value < 0.05, but no enrichments survived FDR-correction in this model. The error bars are the 95% Confidence Intervals.

Discussion

We used GWAS summary statistics of 24 pain conditions, 4 substance use disorders (OUD, PAU, TUD, and CanUD), and 3 substance use consumption traits (AUDIT-C, DPW, and CPD) to disentangle genetic relationships between pain and substance use. Specifically, we used Genomic SEM to test genetic associations between a General Chronic Pain factor and a genetic Addiction risk factor, and examined whether pain shared additional, specific genetic associations with any substance use disorder. Furthermore, we used Cholesky decompositions to disentangle the genetic associations of pain with disordered substance use and substance use consumption. Finally, we used Stratified Genomic SEM to inform whether any of these pain–substance use pathways demonstrated over-represented functional expression in specific brain tissues. These models all serve to elucidate the shared genetic architecture of pain with substance use and inform potential common and specific biological pathways. We found that pain shared common genetic risk with substance use disorders but also had unique pathways with tobacco and alcohol use. These associations showed enhanced expression of multiple neuronal subtypes (e.g., GABAergic neurons, glial cells).

General Chronic Pain with Addiction risk

General Chronic Pain risk shared a moderate genetic correlation with Addiction risk. The genetic correlation could be reflective of horizontal pleiotropy, which is when genetic variants are contributing to the predisposition of two traits. However, a genetic correlation can also be indicative of vertical pleiotropy, which is when a genetic variant affects a single trait, and that trait has a causal effect on a second trait. A previous study identified pleiotropic variants associated with multisite chronic pain and opioid use disorder, alcohol use disorder, cannabis use disorder, and general addiction risk (Koller et al., 2024), supporting the existence of shared genetic predisposition. We found that this genetic association had increased expression of PI genes generally, GABAergic neurons, and specifically, expression of PI genes related to oligodendrocyte precursor cells (OPCs) and GABAergic neurons.

Damaged oligodendrocytes and the compensatory response of an increase in OPCs have been related to chronic pain, and specifically, opioid-induced hyperalgesia (Kim & Angulo, 2025). The increase of OPC expression is likely indicative of oligodendrocyte apoptosis and an attempt at re-myelination. Exogenous OPC transplantation is being explored clinically for alleviating neuropathic pain through fostering re-myelination, and could potentially be explored for patients who are experiencing additionally experiencing addiction (Kim & Angulo, 2025). Our research is unable to parse out whether endogenous OPC production is inherently heightened in people at risk of addiction and pain, or whether it is a chain reaction; either way, this mechanism may be worth further exploration in this population.

Prior work suggests that changes in GABAergic neuronal expression is related to chronic pain and substance use disorder status, compared to healthy controls. Patients with back pain exhibit increased GABA levels in the posterior cingulate gyrus in comparison to healthy controls (Peek et al., 2021). Higher GABA levels are also related to migraines and higher central sensitization (Aguila et al., 2016). Improvements in physical functioning after rehabilitation in youth with chronic pain was linked to decreased GABA in the left posterior insula (Pigott et al., 2023). Aberrant GABAergic processes are also typical in addiction, as GABA signaling modulates many addictive behaviors, including withdrawal and craving (Shyu et al., 2022). Across different substance use disorders, findings vary and GABAergic processes are both increased and decreased in certain brain areas (Shyu et al., 2022), dependent on substance and biological sex. Increased GABA may be a biomarker of centralized pain and in those vulnerable for substance use disorders.

The genetic correlation between chronic pain and addiction could also be indicative of causal or bidirectional associations. A Mendelian Randomization (MR) study used genetic instruments to find evidence of bidirectional effects between multisite chronic pain and AUD, CanUD, and PTU (Koller et al., 2024). However, another MR study that looked at 8 pain conditions found smoking had a causal effect on back, neck/shoulder, hip, and knee pain, but not on abdominal, facial, headache, or widespread pain (Farrell et al., 2023), suggesting that the directionality of associations may be more specific to individual pain conditions. Taken together, the associations are likely not strictly due to shared predisposition nor causality and it is tricky to parcel out the temporality; however, there appear to be neuronal mechanisms that are aberrant in both addiction and pain and serve as potential targets for therapeutic intervention.

Additional genetic relationships with problematic use and consumption

We further partitioned genetic variance and found that General Pain shares a negative genetic correlation with PAU and a positive genetic correlation with TUD, over and above general Addiction risk. Internalizing disorders, such as depression and anxiety, have shown this same opposing pattern of genetic associations with problematic alcohol use and alcohol consumption (Brasher et al., 2024; Colbert et al., 2021; Sanchez-Roige et al., 2019). As chronic pain shares substantial genetic risk with internalizing disorders, the negative genetic association with alcohol consumption may be due to similar pleiotropy (Zorina-Lichtenwalter, Bango, Čeko, et al., 2023). A recent study found that genetics of low positive affect partially mediated the negative genetic relationship between internalizing and alcohol use frequency (Brasher et al., 2024). Another study found that neuroticism partially mediated the relationship between chronic pain and internalizing (Zorina-Lichtenwalter, Bango, Van Oudenhove, et al., 2023). Low positive affect could potentially account for part of the negative covariance between pain and alcohol use frequency. Furthermore, the TUD and General Pain association shared significant functional enrichment of GABAergic pathway, PI genes, and OPC, suggesting that the mechanisms discussed above may be pertinent.

The genetic association we found of pain with cigarette consumption over and above the relationship with TUD emphasizes how pain may have unique pathways with different substance use traits. Even though cigarette frequency has been found to be a good genetic proxy of nicotine dependence (Sanchez-Roige et al., 2021), we found there was still unique significant genetic variance for cigarette frequency when controlling for TUD. The literature on genetic correlations and causation between pain and smoking has yielded mixed results. Evidence of unidirectional (Farrell et al., 2023), bidirectional (Koller et al., 2024), and underlying genetic predisposition (Rader, Reineberg, et al., 2024) explaining the association have been published. Our findings imply that there may be unique pathways between different smoking phenotypes and pain. However, we did not detect significant hits for the functional enrichments we tested between cigarette consumption and pain.

General Pain did not have a significant additional genetic correlation with OUD nor with CanUD. Prior to controlling for Addiction risk, the genetic correlation between General Pain and OUD was the highest of all the substance use traits; however, this association was fully captured by Addiction risk, on which OUD had the strongest loading (with non-significant residual variance). The lack of significant association with CanUD above Addiction risk suggests that shared genetic risk between General Pain and CanUD acts through more general Addiction risk.

Limitations and future directions

Well-powered GWAS summary statistics require large sample sizes, and the traits are often meta-analyzed across multiple cohorts. As a result, there was some incongruency with controls, with some controls being unscreened for substance use disorders and/or unexposed to the substances. These design decisions likely added noise to our variables. The power of latent variable modeling lies in its ability to extract common variance and leave residual variance of each indicator and its noise. Hence, the factor-level correlations may not be as impacted by this issue. However, when allowing the General Pain factor to predict each substance use disorder controlling for Addiction risk, we may have not identified genetic associations with the CanUD residual due to it being a lower-powered or noisier trait, rather there being a true lack of association. Consortiums and biobanks are collecting and releasing updated data with higher powered, less noisy traits; it may be of use to repeat these analyses at a future time.

These analyses and results were conducted using GWASs of European-like ancestry and may not be generalizable to other non-European-like ancestries (e.g., African-like, East Asian-like). GWASs stratify by ancestry to prevent false positives for trait associations which also correlate with ancestry (Carlson et al., 2013). While all humans are largely similar genetically, population migration patterns have led to differences in allele frequencies and patterns of linkage disequilibrium across ancestries. These differences often mean that though the general direction of effect of SNPs tends to remain consistent across ancestries, the effect sizes vary and are often lower and not significant in non-European-like ancestries (Carlson et al., 2013). The SNPs we examine in common-variant GWASs are just markers for the causal alleles, and the extent to which they are correlated with the causal alleles differs across ancestry (Y. Wang et al., 2020). Currently, 90% of all GWASs conducted have been on European ancestries and there is a pervasive lack of data in diverse ancestries, which prevent us from replicating our questions of interest in other ancestries (M. C. Mills & Rahal, 2020).

Conclusions

Genetic risk that is common to a multitude of chronic pain conditions shares substantial overlap with a general genetic risk towards substance use disorders. However, this general pain risk shares an additional risk with problematic alcohol use and tobacco use disorder, and differential relationships with alcohol and tobacco consumption. There may be unique biological pathways that underlie the phenotypes.

CHAPTER V: CONCLUSIONS AND GENERAL DISCUSSION

Chronic pain is a complex trait and is susceptible to both genetic and environmental risk factors. This body of work used genetically informed methods to interrogate the relationships between chronic pain and a few of its notable risk factors, including executive functioning and substance use.

Study 1: In this study, we investigated the relationships that chronic pain had with 3 executive function factors. While previous literature has found robust associations between chronic pain and EF tasks, the type of EF pain was tapping into was unclear. We found that chronic pain shared a genetic relationship with Updating working memory, controlling for variance of common EF and mental set shifting. Study 2: Smoking is an established risk factor in epidemiological literature, however many of these studies did not account for genetic effects. We found that smoking co-occurs with pain primarily through genetic pathways rather than through directly increasing risk. Study 3: Substance use disorder risk is higher in chronic pain populations. We found that chronic pain shared genetic risk with general addiction, but also with smoking, highlighting the potential for an additional biological pathway.

Interestingly, we found that substance use consumption as compared to problematic use has differential relationships with chronic pain. In Study 2, we found that alcohol frequency had a negative phenotypic relationship with chronic pain (Rader, Reineberg, et al., 2024; see Table B-1). In Study 3, we saw that alcohol consumption has a negative genetic correlation with chronic pain, over and above the positive association between problematic alcohol use and chronic pain. Potentially, alcohol consumption has a causal effect or exacerbates some pain conditions, such as migraine, which may lead to a decrease in alcohol consumption (Mostofsky et al., 2020). An MR study found evidence of reverse causality between alcohol consumption and migraine (Yuan et al., 2022), whereas alcohol use disorder was shown to have bidirectional causality with multisite chronic pain (Koller et al., 2024). The examination of substance use disorder versus consumption was exploratory; however, has yielded consistency across a community twin sample and multiple population-based, biobank samples. These findings highlight the importance of parceling out problematic substance use versus consumption in epidemiological research.

Future research may also benefit from examining how genetic predisposition to depression and anxiety relates to the association between chronic pain, executive functions, and substance use disorders. In my Master's Thesis, we found that adolescents showed significant relationships between their chronic pain and internalizing disorders (Rader et al., 2023). Furthermore, chronic pain is shown to share robust genetic risk both depression and anxiety (Zorina-Lichtenwalter, Bango, Čeko, et al., 2023).

Advantages of genetically informed methods in epidemiological chronic pain research

While a vast amount of epidemiological research has been conducted on risk factors related to chronic pain, often genetic effects are not accounted for. Twin studies have shown that about half of chronic pain risk is attributable to additive genetic effects, and that this genetic risk is shared across many various pain conditions irrespective of pain location (Vehof et al., 2014). More generally, Behavioral Genetics as a field has shown that all traits are expected to have a heritable component (Plomin et al., 2016), highlighting the importance of investigating or controlling for genetic effects.

An advantage of genetically informed methods, such as the co-twin control design, is that associations can be interrogated while accounting for genetic variance. For example, one of our studies found that smoking frequency does not necessarily have a direct effect on pain, but that the association is due to underlying familial effects (Rader, Reineberg, et al., 2024). This is not to say that an environmental risk factor, such as smoking, is entirely due to genetic predisposition. It is plausible that gene-environment interactions may exist where an environmental risk factor such as smoking may interact unfavorably with certain genotypes (Plomin et al., 1977). Gene-environment correlations are also a possibility where the frequency of certain genotypes are related to environmental exposures (Plomin et al., 1977). For example, there could be a passive gene-environment correlation, where the child may have inherited a higher genetic risk for smoking, and the parents may expose the child to an environment where smoking is commonplace and more easily available. Even risk factors that may seem purely environmental, such as engagement in a social group that smokes, may have a genetic component. It is important to account for genetic confounds to understand the nature of the relationship between a risk factor and outcome.

Advantages of latent variable modeling in epidemiological chronic pain research

Chronic pain is a complex trait and encompasses many different body sites, etiologies, and disease progressions. While each chronic pain condition certainly has its own unique risk profiles, both phenotypic and genetic research has found that there is a common component that underlies chronic pain conditions. Latent variable modeling is advantageous for extracting common variance across covarying traits, and this factor is assumed to be free of random measurement error (Bollen, 2002). Hence, there is benefit in extracting chronic pain factors and assessing how they associate with risk factors of interest. For example, in this dissertation, we extracted a genetic latent factor underlying 24 pain conditions. This genetic factor allowed us to examine how chronic pain commonly relates to substance use traits.

The use of latent variables to parcel out and control for variance using multiple factors allows for more nuanced hypotheses to be tested. In one of our studies, we further characterized the associations between executive functioning and chronic pain using latent variables. EF tasks have been associated with chronic pain in past literature, but it has been unclear whether there was a particular facet of EF that is related to chronic pain. Using latent variable modeling, we found updating working memory relates to chronic pain when controlling for Common EF and mental set Shifting (Rader, Wager, et al., 2024).

Closing remarks

In summary, this dissertation leveraged latent variable models within genetically informative designs to reveal that chronic pain shares genetic underpinnings with many of its risk factors. Exploring these genetic relationships can help clarify the pathophysiology of chronic pain and the nature of its co-occurrences with other phenotypes.

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APPENDIX A: SUPPLEMENTARY MATERIAL FOR CHRONIC PAIN IS SPECIFICALLY ASSOCIATED WITH UPDATING WORKING MEMORY: A LONGITUDINAL TWIN STUDY

Results

Power Analysis

A prior meta-analysis of the associations between pain and working memory tasks (Berryman et al., 2013) reported significant Cohen's D effect sizes ranging from -0.47 to -1.5, which correspond to Pearson correlations of -0.23 to -0.60. We were well-powered to detect latent variable associations with effect sizes on the low end of those reported as significant in that meta-analysis.

To evaluate power for our phenotypic and genetic models, we used Monte Carlo simulation (number of repetitions = 500) in Mplus. Assuming our sample size of n=634 with pain observations and our observed pain prevalence, we had 80% power to detect a phenotypic association as low as -0.18 between pain and age 23 Common Executive Function (EF) and as low as -0.19 between pain and age 23 Updating-specific or Shifting-specific abilities. For pain with age 28 EFs, we had 80% power to detect a phenotypic association as low as -0.18 with Common EF, as low as -0.22 with Updating-specific ability, and as low as 0.19 for Shifting-specific ability. For the phenotypic Cholesky decomposition, we had 80% power to detect pain associations as low as -0.18, -0.20, and -0.18 with the stable Common EF, Updating-specific, and Shifting-specific factors, respectively. The variance for age-28-unique Common EF was close to 0 and non-significant, so we had low power to detect associations (we had 80% power to detect an association as low as -0.566). Similarly, age-28-unique Updating-specific variance was nonsignificant and essentially 0, so we had low power to detect an association, even if the correlation was 1 (we only had 58% power to detect significance at this effect size). Shifting-specific was the only factor that showed significant age 28 residual variance. For age-28-unique Shifting-specific and pain, with 80% power we could detect a significant association as small as 0.324.

We used the twin model to decompose the significant and marginally significant phenotypic associations we observed. Assuming the same sample size, EF factor loadings, and the genetic and environmental variances for EFs and pain that we observed, Monte Carlo simulations suggested that we had 80% power to detect a genetic correlation of pain with stable Updating-specific variance as low as -0.398. These lower-bound estimates are similar in magnitude to the low end of the phenotypic effect sizes reported as significant in the Berryman et al. (2013) meta-analysis, suggesting that we were adequately powered to detect genetic correlations that were at least as large as these phenotypic effects. An environmental correlation of pain with stable Updating-specific variance of -1.00 was powered at 0.763 in our sample. The Updating-specific E variance was small and non-significant so it's not surprising that it would have to be a large *r*E to detect it with close to 80% power. This reflects the fact that EFs, particularly Updating-specific, don't have a lot of environmental variance so we should not really expect that phenotypic associations would be substantially attributable to an environmental correlation. We did estimate the environmental correlation to see what the point estimate was.

Table A-1. Cross-tabulation of chronic pain level by zygosity

	Twin 2 Pain Category		
Twin 1 Pain Category	None	Mid-Mod	Mod-Severe
MZ Pairs (*r*=.455, *p*<.001)			
None	87	9	11
Mild-Mod	16	6	5
Mod-Severe	5	1	6
DZ Pairs (*r*=.117, p=.368)			
None	67	18	10
Mild-Mod	15	5	2
Mod-Severe	6	1	3

Note. Displayed above is the cross-tabulation of monozygotic (MZ) and dizygotic (DZ) twin pair counts at each pain level. No chronic pain is defined as people who did not report any pain. Mild to moderate chronic pain is defined as people who have had pain for 3+ months and have rated their pain severity between 0-30. Moderate to severe chronic pain is defined as individuals who reported pain for 3+ months and rated their pain severity as between 40-100. The total n of twins who filled out the pain questionnaire, including the pain severity item, was 634, but this cross-tabulation only includes those from pairs in which both twins completed the pain measure (*n* = 546). However, pain information from twins whose co-twins' pain was not available were included in the model with EFs, as they provide information about the phenotypic and genetic associations of pain with EFs (e.g., because a co-twin with missing pain and EF data at age 28 might still have EF data at age 23).

Table A-2. Task-specific ACE parameter estimates from genetic Cholesky decomposition of executive function factors at age 23 and age 28

EF Task	A1	A cross-path	A2	C1	C cross-path	C2	E1	E cross-path	E2
Antisaccade	0.283	0.134	0.331	0.322	0.129	0.148	0.563	0.194	0.544
Stop-signal	0.349	--	--	0.262	--	--	0.855	--	--
Stroop	0.466	0.408	0.000	0.132	0.350	0.000	0.713	0.066	-0.724
Keep-track	-0.311	-0.195	0.223	0.000	0.000	0.000	0.639	-0.021	-0.628
Letter memory	0.425	0.419	0.000	0.000	0.000	0.000	0.506	0.230	0.415
Spatial *n*-back	-0.329	--	--	0.000	--	--	-0.665	--	--
Number-letter	-0.239	-0.242	0.153	0.000	0.000	0.000	0.627	0.082	0.649
Color-shape	-0.300	--	--	0.000	--	--	0.754	--	--
Category-switch	0.211	0.197	-0.186	0.000	0.000	0.000	-0.644	-0.166	-0.560

Note. In the EF genetic Cholesky Decomposition, we allowed all the tasks to have their own specific A, C, and Es which were also partitioned into stable variance and residual variance. This table displays the standardized estimates for each path in the Cholesky. These are the parameter estimates, but their significance was not tested with chi-square difference tests. A = additive genetic effects; C = shared environmental effects; E = non-shared environmental effects (includes measurement error); 1 = the first path from the shared variance factor to the age 23 tasks; cross-path = the cross-path from the shared variance factor to the age 28 tasks; 2 = the path that captures any left-over residual variance to the age 28 tasks. Dashes indicate that the path was not estimated because it was estimated at zero in an initial model and excluding it aided model convergence.

Table A-3. Cholesky decomposition fit statistics including non-significant cross-paths with pain

	Model Fit					Chi-square Difference	
Model	χ^2	df	*p*	RMSEA	CFI	$\Delta\chi^2(1)$	*p*
Full model	1117.812	1074	0.172	0.014	0.982		
Drop stable *r*A Common EF	1119.728	1075	0.167	0.014	0.981	2.297	0.130
Drop stable *r*A Updating-specific	1119.963	1075	0.166	0.014	0.981	2.705	0.100
Drop stable *r*A Shifting-specific	1118.013	1075	0.176	0.014	0.982	0.771	0.085
Drop stable *r*E Common EF	1118.667	1075	0.173	0.014	0.982	0.536	0.464
Drop stable *r*E Updating-specific	1118.608	1075	0.173	0.014	0.982	0.098	0.755
Drop stable *r*E Shifting-specific	1118.512	1075	0.174	0.014	0.982	0.003	0.956
Drop new *r*E Common EF	1118.834	1075	0.172	0.014	0.982	0.728	0.394
Drop new *r*E Updating-specific	1118.736	1075	0.172	0.014	0.982	0.277	0.598
Drop new *r*E Shifting-specific	1119.337	1075	0.169	0.014	0.982	3.084	0.079

Note. While it is typical to only decompose significant associations in a genetic model, this table displays the fit statistics of a full model where all cross-paths were estimated, including those with near 0 and non-significant variance components and non-significant phenotypic associations. Chi-square difference tests were conducted to test the significance of each cross-path from the EF factor to pain, which tests whether the genetic or environmental correlation is significant. Since there was no new A variance (estimated 0) at age 28 for any of the EF factors, the model would not converge when cross-paths with pain were included, so these paths were not estimated. *r*A = additive genetic correlation; *r*E = non-shared environmental correlation; df = degrees of freedom; RMSEA = Root mean square error approximation; CFI = comparative fit index. The chi-square differences were obtained with Mplus's DIFFTEST procedure.

APPENDIX B: SUPPLEMENTARY MATERIAL FOR FAMILIAL EFFECTS ACCOUNT FOR ASSOCIATION BETWEEN CHRONIC PAIN AND PAST MONTH SMOKING

Methods

The phenotypic, mediation, and co-twin control model used the robust maximum likelihood (MLR) estimator with a logit link function. Estimates are log odds for an increased level of pain with one standard deviation increase in smoking and/or brain connectivity. The twin models used the diagonally weighted least squares means and variances adjusted (WLSMV) estimator with a probit link function; estimates are A, C, and E variances and their correlations. Significance was assessed at $p < 0.05$ for the Wald tests of the parameters in the mediation and co-twin control models. For the twin models, the standard errors of the variance components are not invariant to model parameterization (Neale et al., 1989) so significance was assessed using chi-square difference ($\Delta\chi^2$) tests (Mplus' difftest procedure) as these tests are invariant to model parameterization. For the mediation model, we computed bootstrapped (1000x) 95% confidence intervals. We also computed bootstrapped (1000x) standard errors and asymmetric, percentile-based boot-strapped 95% confidence intervals for the twin models, since the variance components are not normally distributed (Neale & Miller, 1997).

In the co-twin control models, we assessed the interaction of zygosity with the within-family effects. We specified a random slope for the within-family effect and regressed this slope on zygosity, then fixed the slope's residual variance to zero (this procedure results in a fixed within-family effect that is allowed to vary by zygosity group). Significance of the zygosity interaction indicates that effects differ for in MZ and DZ twin pairs. Because MZ twins share all their genes and are raised together, their differences can only be attributed to non-shared environmental influences, whereas DZ twin differences can be due to both genetic and non-shared environmental influences. If the within-family estimate of the smoking-pain association is significant in both MZ and DZ twins, it is consistent with causal hypotheses because the within-family estimate controls for genetic and shared environmental effects. If the within-family estimate is only significant in DZ twins, this can suggest genetic confounding. While a larger within-family effect in DZ twins can sometimes be interpreted as evidence for genetic effects, it is not always a reliable indicator of presence or lack of genetic effects (Carlin et al., 2005). We used Mplus' type= TWOLEVEL RANDOM to account for the multi-level random intercepts. A logit link function was used since the outcome was ordinal.

For the univariate twin models, probit models with delta parameterization were used, which fixed the scaling factor to 1 and estimated all the thresholds (Prescott, 2004). The bivariate twin analyses used theta parameterization, which fixes the threshold values to their values estimated in the univariate twin models (Prescott, 2004). Good model fit was assessed using criteria of root-mean-square error approximation (RMSEA) < 0.06 and Comparative Fit Index (CFI) > 0.95 (Hu & Bentler, 1998).

Results

Post-hoc analyses

To further probe the relationship between the NAc-mPFC circuit and its relationships with pain and smoking, we conducted a few post-hoc analyses. We regressed the circuit on square-root transformed pain and the association continued to be non-significant (*b* = 0.01 [0.01], *p* = 0.404). We contrast-coded smoking to see whether there were circuit differences in non-smokers vs. smokers and non-daily smokers vs. daily smokers. There were no significant differences in smokers vs. non-smokers (*b* = 0.01 [0.01], *p* = 0.170),

Power analysis

To evaluate power for our phenotypic and co-twin control models, we used Monte Carlo Simulation in Mplus. Assuming the same sample size, pain prevalence, and between and within-family variation in smoking as we observed in our sample, we had 80% power to detect a phenotypic association as low as .27 log odds (odds ratio [OR] = 1.31), which is very similar to the significant phenotypic effect we observed (.243 log odds (odds ratio [OR] = 1.28). For the co-twin control model, we evaluated power to detect between- and within-family effects equal to our observed phenotypic effect. We had 92% power to detect a between-family effect of .243 log odds, but only 18% to detect a within-family effect of that size. We could detect a within-family effect of 0.58 log odds with 80% power.

Table B-1. Pain regressed on the PhenX Toolkit substance use frequency measures

Dependent Variable	Independent Variables	Estimate	Odds Ratio
	Tobacco Frequency	**0.24** [0.09]	**1.28** [0.11]
Pain	Marijuana Frequency	0.06 [0.10]	1.07 [0.11]
	Alcohol Frequency	**-0.22** [0.10]	**0.81** [0.08]
	Composite Frequency	0.09 [0.10]	1.10 [0.11]
	Sex effects	0.25 [0.19]	1.28 [0.25]

Note. The individual-level phenotypic model regressed pain on all other PhenX substance use frequency measures to ensure that smoking had a distinct effect separate from other substance use. A composite frequency score was created of low-endorsed substance use frequency measures including past month use of cocaine, hallucinogen, painkillers, heroine, solvents, stimulants, tranquilizers, sedatives, and any ‘other’ drug. Substance use frequency was the square-root transformed number of days used in the past month (0-30 days) which we then z-scored. The pain dependent variable was ordinal. Estimates are thus the log odds of increasing one pain level with a standard deviation increase in substance frequency. The models and used a robust maximum likelihood estimator standard errors adjusted for non-independence of twin pairs. Sex was included as a covariate (females= -0.5; males=0.5). Brackets enclose standard errors. Bolded font indicates $p < 0.05$.

Table B-2. Results of regression and co-twin control models of square-root transformed pain regressed on smoking

Model	Independent Variable	Effect on Pain
Model 1 Individual-level model	Phenotypic smoking	**0.13** [0.05]
Model 2 Co-twin Control model	Between-family Smoking	**0.11** [0.03]
	Within-family Smoking	-0.07 [0.08]
	Within MZ	-0.09 [0.11]
	Within DZ	-0.05 [0.10]
	Zygosity	0.02 [0.08]
	Zygosity*Within	0.04 [0.15]

Note. Standardized regression estimates [standard errors] for regression models. Pain and smoking were square-root transformed. The models used a robust maximum likelihood estimator. The individual-level model regressed pain on smoking and sex without controlling for familial confounds (standard error accounts for non-independence of twins). The co-twin control models separate the individual-level effects into between-family (suggestive of familial effects) and within-family (suggestive of direct effects) components. Sex was included as a covariate (estimates not shown). Zygosity was contrast coded (MZ = -0.5; DZ = 0.5) to obtain the overall within-family estimates across zygosity, whereas it was dummy coded to obtain the simple effects within each zygosity (i.e., on the Within MZ and Within DZ rows). The zygosity interaction coefficient is the difference of the within-family effect between MZ and DZ twins. MZ = Monozygotic twins. DZ = Dizygotic twins. Bolded font indicates $p < 0.05$.

Table B-3. Parameter estimates and model fit statistics from square-root transformed ADE pain, ADE tobacco, and nested models.

		Parameter Estimates			Model Fit					Chi-square Difference		
Measure	Model	A	D	E	χ^2	df	*p*	RMSEA	CFI	$\Delta\chi^2$	Δdf	*p*
Pain	ADE	0.04 [-0.89, 0.96]	0.28 [-0.70, 1.25]	0.69 [0.50, 0.88]	2.150	6	0.905	0.000	1.000			
	AE	**0.30** [0.12, 0.48]	**-**	**0.70** [0.52, 0.88]	**2.432**	**7**	**0.932**	**0.000**	**1.000**	**0.302**	**1**	**0.583**
	E	-	-	1.00 [1.00, 1.00]	14.885	8	0.061	0.069	0.574	10.232	2	0.006
Smoking	ADE	0.18 [-0.50, 0.85]	0.33 [-0.41, 1.07]	0.50 [0.32, 0.68]	4.219	6	0.647	0.000	1.000			
	AE	**0.48** [0.31, 0.65]	**-**	**0.52** [0.35, 0.69]	**4.934**	**7**	**0.668**	**0.000**	**1.000**	**0.713**	**1**	**0.398**
	E	-	-	1.00 [1.00, 1.00]	39.327	8	<0.001	0.146	0.161	27.409	2	<0.001

Note. Parameter estimates and model fit statistics for the full ADE pain and ADE smoking models, as well as the chi-square difference test for the nested models compared to these full models. The A (additive genetic effects), D (shared dominance genetic effects), and E (non-shared environmental effects) estimates presented represent the proportion of variance explained by each of the parameters. The model fit results show whether or not each model has good fit, indicated by comparative fit index (CFI) > 0.95 and root mean squared error of approximation (RMSEA) < 0.06. Chi-square difference ($\Delta\chi^2$) tests (scaled for the robust maximum-likelihood [MLR] estimator) were also conducted to test whether dropping a parameter significantly deteriorated fit, indicated by $\Delta\chi^2\ p < 0.05$. Pain and smoking frequency were square-root transformed and the MLR estimator was used to account for non-normality. The results of these continuous univariate models were similar to the categorical models presented in the main manuscript. The only difference was that the twin correlations for smoking (*r*MZ =0.50, *r*DZ=0.17) suggested an ADE model rather than an ACE model; however, the AE model was the best-fitting model for smoking using both versions of the variable. Dashes indicate the parameter was not estimated. Brackets enclose 1000x bootstrapped 95% confidence intervals. Boldface type indicate the best models (AE) for both pain and smoking. df = degrees of freedom.

Table B-4. Model fit statistics of the AE bivariate twin model with and without rA and rE correlations

	Model Fit					Chi-square Difference		
Model	χ^2	df	*p*	RMSEA	CFI	$\Delta\chi^2$	Δdf	*p*
Full model	23.269	26	0.618	0.000	1.000			
Without *r*A	**34.228**	**27**	**0.160**	**0.038**	**0.888**	**11.130**	**1**	**<0.001**
Without *r*E	24.665	27	0.593	0.000	1.000	1.372	1	0.241

Note. This table presents the model fit statistics for AE bivariate twin model as well as the chi-square difference ($\Delta\chi^2$) test for the nested models that drop *r*A or *r*E using square-root transformed pain and smoking frequency. The MLR estimator was used to account for non-normality of the variables. The results of the continuous model are consistent with results of the categorical model presented in the main manuscript. The full model included AE pain and AE smoking with shared *r*A and *r*E correlations. The univariate D components were not included for pain and smoking due to not enough power to estimate both a significant A and D component. Dropping *r*A led to a significant deterioration of fit for the model, suggesting that *r*A is significant. Satorra and Bentler (2001) corrected chi-square difference tests are displayed. df = degrees of freedom. RMSEA = root mean square error approximation. CFI = comparative fit index.

APPENDIX C: SUPPLEMENTARY MATERIAL FOR EXAMINING GENETIC RELATIONSHIPS BETWEEN CHRONIC PAIN AND SUBSTANCE USE DISORDERS USING GENOMIC SEM

Results

The supplementary tables with all Stratified Genomic SEM results can be found here: Rader_appendixC_results.xlsx. The tab ‘Annotation Names’ lists all the names of the annotations included in the Stratified Genomic SEM analyses, and their respective sources. The tab Addiction-Pain Enrich contains the enrichment results of the covariance between the General Pain factor and the Addiction factor. The tab A1-Pain Enrich contains the enrichment results of the covariance between the General Pain factor and the Problematic Alcohol Use Cholesky factor. The tab A2-Pain Enrich contains the enrichment results of the covariance between the General Pain factor and the Alcohol Consumption Cholesky factor. The tab T1-Pain Enrich contains the enrichment results of the covariance between the General Pain factor and the Tobacco Use Disorder Cholesky factor. The tab T2-Pain Enrich contains the enrichment results of the covariance between the General Pain factor and the Tobacco Consumption Cholesky factor.