The effect of nonsteroidal anti-inflammatory medications on pain chronification

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DEDICATION

This work is dedicated to my family, my husband Mr. Carlos Victoria, and my son Mr. Abdulkarim Ani, for their endless love, support, and encouragement throughout my master's program.

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Abstract

Background and objective: Analgesics are among the most commonly used over-the-counter medications and NSAIDs or acetaminophen are used as the first-line treatment for chronic pain in Canada. While their pain relief efficacy has been studied, the longer-term effect of taking these medications on pain outcomes, particularly on the transition from acute to chronic pain, remains unclear. We have previously shown that the risk of chronification for acute back pain is particularly enhanced by taking NSAIDs during the acute phase, explained by the dampening of pain resolution through an inflammatory response. To determine the generalizability of the chronification effect of NSAIDs, we investigated the effect of a range of analgesics on body-site specific pain in the Canadian Longitudinal Study on Aging (CLSA) including back pain. Significant findings were tested for replication in another cohort, the UK Biobank (UKB).

Design and Methods: Based on the CLSA Comprehensive cohort of 27,765 individuals using both baseline and first follow-up (FU1) data (3 years interval), analyses were conducted on back pain, jaw pain, and knee pain. Individuals at baseline were asked about their experience of pain at each site; for back pain and jaw pain, the referral period was the prior 12 months, while for knee pain, this was during the last 4 weeks and on most of the days, (5,323/2,215/4,862) responded "Yes" respectively. Each pain type was analyzed in separate logistic regression models with site-specific pain as the outcome. Cases were defined as those with site-specific pain at baseline still reporting pain at the same site at FU1(3 years interval) (N= 2,957/946/2,517) and controls as those who had recovered (no pain) (N= 2,366/1,269/2,345). In this study, chronic pain is defined as site-specific pain present at both baseline and FU1.

We considered five analgesic classes (NSAIDs, paracetamol, opioids, anti-depressants, and gabapentinoids) as predictors in logistic regression models for each site-specific pain. We tested for association between taking medications and the development of chronic pain, adjusting for age, sex, ethnicity, intensity of pain, and BMI.

We used the nominal *p*-value threshold of 0.05 to define statistical significance in the CLSA and tested significant findings for replication in the UKB. Specifically, knee pain models were tested for replication. Cases and controls were defined for the CLSA: 7,110 UKB subjects with knee pain who answered "Yes" for having pain that interfered with their usual activities in the last month were included. Individuals who reported knee pain at any of the next visits were considered as cases (3,331), while others who did not report any pain, were considered as controls (recovered) (3,779).

Results: In a full model including all medication classes, chronic back pain showed a strong association with taking analgesics for all classes. Back pain subjects taking NSAIDs are at 1.29 times greater risk of developing chronic pain than those not taking NSAIDs (OR = 1.29; P = 0.0035). For knee pain patients, NSAIDs (and no other class) were identified as a risk factor for developing chronic knee pain (OR = 1.35; P = 0.0004). For jaw pain patients, the number of cases was very small. Opioids and antidepressants are associated with chronicity. Replication of knee pain results in the UKB showed that NSAIDs (and no other class) were identified as significant in the full model (OR = 1.15; P = 0.01).

Conclusion: Individuals taking NSAIDs for pain are at a higher risk of having chronic pain 2-3 years later, compared to individuals taking other analgesics. These results imply that the

detrimental effect of NSAIDs on pain chronicity is independent of reported pain bodily site and stage of pain. Modifications to NSAID indications are warranted.

Résumé

Contexte et objectif: Les analgésiques sont couramment pris sans ordonnance, et les médicaments anti-inflammatoires non stéroïdiens (AINS) ou l'acétaminophène sont utilisés en première ligne en traitement contre la douleur chronique au Canada. Bien que leur efficacité de soulagement de la douleur ait été étudiée, l'effet, à plus long terme, de la prise de ces médicaments sur de la douleur, en particulier sur la transition de la douleur aiguë à la douleur chronique, reste incertain. Nous avons précédemment montré que le risque de chronicité pour les lombalgies aiguës a particulièrement augmenté par la prise d'AINS pendant la phase aiguë, expliquée par l'atténuation de la douleur grâce à une réaction inflammatoire. Pour déterminer la généralisabilité de l'effet de chronicité des AINS, nous avons étudié l'effet d'une gamme d'analgésiques sur la douleur spécifique à un emplacement corporel dans l'étude longitudinale canadienne sur le vieillissement (ELCV), y compris les douleurs au bas du dos. Les résultats significatifs ainsi obtenues ont été répliques dans une autre cohorte, la UK Biobanque (UKB).

Conception et méthodes : Selon la cohorte globale de l'ÉLCV, 27,765 personnes ont donné les informations à la collecte de départ et du premier suivi (1er suivi) (intervalle de 3 ans). Les analyses ont été menées sur les douleurs lombaires, les douleurs à la mâchoire et les douleurs au genou. Les individus au départ ont été interrogés sur leur expérience de la douleur sur chaque site ; pour le bas de dos et la douleur à la mâchoire, la période de référence était les 12 mois précédents, tandis que pour la douleur au genou, il s’agissait au cours des quatre dernières semaines dans la plupart des jours (5,323/2,215/4,862) ont répondu oui respectivement). Chaque type de douleur a été analysé

dans des modèles de régression logistique séparés avec une douleur spécifique lié à l'emplacement corporel comme résultat. Les cas ont été définis comme les individus qui présentaient une douleur spécifique au même emplacement corporel au départ qui subsistait toujours au moment du 1er suivi (intervalle de 3 ans) (N = 2,957/946/2,517) et les témoins comme ceux qui avaient récupéré (pas de douleur) (N = 2,366 /1,269/2,345). Dans cette étude, la douleur chronique est définie comme une douleur localisée présente à la départ fois et au 1er suivi.

Nous avons considéré cinq classes d'analgésiques (AINS, paracétamol, opioïdes, antidépresseurs et gabapentinoids) comme prédicteurs dans les modèles de régression logistique pour chaque type de la douleur spécifique à l'emplacement corporel. Nous avons testé l'association entre la prise de médicaments et le développement de la douleur chronique, en ajustant sur l'âge, le sexe, l'origine ethnique, l'intensité de la douleur et l'indice de masse corporelle (IMC).

Nous avons utilisé le seuil nominal de valeur p de 0,05 pour définir la significativité statistique dans l'ÉLCV et testé des résultats significatifs pour la réplication dans l'UKB. Plus précisément, des modèles de douleur au genou ont été testés pour la réplication. Les cas et les témoins ont été définis comme pour l'ELCV. Ces 7,110 sujets du UKB souffrants de douleurs au genou, ayant répondu oui à la question sur les douleurs gênantes dans leurs activités habituelles au cours du dernier mois, ont été inclus. Les personnes qui ont signalé une douleur au genou lors d'une des visites suivantes ont été considérées comme des cas (3,331), tandis que les autres qui n'ont signalé aucune douleur ont été considérées comme des témoins (guéris) (3,779).

Résultats: Dans un modèle complet incluant toutes les classes de médicaments, la lombalgie chronique a montré une forte association avec la prise d'analgésiques pour toutes les classes. Les sujets lombalgiques prenants des AINS ont 1,29 fois plus de risque de développer des douleurs

chroniques que ceux ne prenants pas d'AINS (OR = 1,29 ; *P* = 0,0035). Pour les sujets souffrant de douleurs au genou, les AINS (et aucune autre classe) ont été identifiés comme un facteur de risque de développer une douleur chronique au genou (OR = 1,35 ; *P* = 0,0004).

Pour les patients souffrants de douleurs à la mâchoire, le nombre de cas a été très faible. Opioïdes et antidépresseurs ont été associés à la chronification. La réplication des résultats de la douleur au genou dans l'UKB a montré que les AINS (et aucune autre classe) ont été identifiés comme significatifs dans le modèle complet (OR = 1,15 ; *P* = 0,01).

Conclusion : Les personnes prenant des AINS contre la douleur courent un risque plus élevé de développer des douleurs chroniques 2 à 3 ans plus tard par rapport aux personnes prenant d'autres analgésiques. Ces résultats impliquent que l'effet préjudiciable des AINS sur la chronicité de la douleur est indépendant de l’emplacement corporel de la douleur signalé et du stade de la douleur. Des modifications des indications des AINS sont justifiées.

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Contribution of authors

Maha Zidan: BDS, M.Sc. Candidate: Conducted the literature search and was responsible for assembling study datasets, proceeding with all study analyses under the guidance of Dr. Diatchenko, and Dr. Grant the candidate's MSc supervisors. In addition to the data interpretation and the preparation of all study tables and figures. Ms. Zidan wrote the initial version of all sections of the present thesis. The supervisors (Dr. Diatchenko, and Dr. Grant) provided their recommendations and revisions. The findings reported in the present thesis represent original scholarship and a new contribution to knowledge.

Dr. Audrey V. Grant: Assistant Professor, Department of Anesthesia, Faculty of Medicine, McGill University: conceived this investigation, designed, and supervised this study, carried out the statistical analysis, and contributed to the thesis writing by providing her recommendations and revisions.

Dr. Luda Diatchenko, MD, Ph.D., Professor, Faculty of Dental Medicine and Oral Health Sciences, Department of Anesthesia, Faculty of Medicine, McGill University: brings her expertise as a researcher in Human Pain Genetics, supervising this study and contributed to thesis writing providing her recommendations and revisions.

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List of abbreviations

NSAIDs	Non-Steroidal Anti-inflammatory Drugs
CLSA	Canadian Longitudinal Study on Aging
UKB	United Kingdom Biobank
FU1	First follow-up
FU2	Second follow-up
IASP	International Association for the Study of Pain
WHO	World Health Organization
COX	Enzyme Cyclooxygenase
COM	Comprehensive cohort at baseline
COF1	Comprehensive cohort first follow-up
DCS	Data collection site
MCQ	Maintaining Contact Questionnaire
DPD	Canada Drug Product Database
DIN	Drug Identification Number
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
SD	Standard deviation
eQTL	Expression Quantitative Trait Loci
OR	Odds Ratio
CI	Confidence Interval
GWAS	Genome Wide Association Study
QC	Quality Control

SNP Single Nucleotide Polymorphisms

LD linkage disequilibrium

1.0 General introduction

1.1 The concept of pain

The International Association for the Study of Pain (IASP) Has defined pain as "an unpleasant sensory and emotional experience associated with or resembling that associated with, actual or potential tissue damage"[1]. It seems that the pathophysiology of acute and chronic pains differ substantially, and over the past decades, the conceptional meaning of chronic pain has changed, and now it is recognized to be a disease itself instead of being a symptom of other diseases[2].

However, pain has been one of the most common concerns in recent years[3], and studies have shown that 1 in 5 adults experience a pain disorder, while 1 in 10 adults are diagnosed with chronic pain each year[4]. Chronic pain conditions have wide-reaching impacts and result from pain, 21% of individuals suffer from depression; 13% had switched their jobs; 61% were working from home after the alteration of their physical functions[5]. Nevertheless, severe chronic pain is a risk factor for premature mortality[6], and the management process is costly, exceeding that of heart disease, cancer, or even diabetes[7]; with an estimated cost in 2010 US$ between US$ 560 to US$ 635 billion[8].

These facts point to the need for efficient medical care[9], taking into consideration individual differences that make the experience of pain different and related to multiple variables contributing to this diversity[10].

1.2 Types of pain

Pain has been classified in different ways, the most commonly used according to the World Health Organization (WHO) are anatomic, etiologic, duration, and pathophysiological

classification systems[11]. The (IASP) has classified pain types based on their duration and symptoms into “acute” and “chronic”.

Acute pain lasts less than 3 months[12]; this nociceptive pain is caused by intense promptings like injury, trauma, medical procedures, and diseases[13]. It may have different degrees of severity, but in general, its onset is fast, while prolonged long-standing alterations affect the central nervous system (CNS) leading to chronic pain conditions[14].

Chronic pain is hard to define. Most systems of classification are based on the discomfort that lasts more than the normal healing time, which is usually 3 months. It represents the transition from the acute stage induced by peripheral and central nervous system alterations[2]. It is assigned as persistent or recurrent pain lasting more than 3 months[12], and reflects real or potential tissue damage[15]. Chronic pain is affected by several biological, psychological, and sociological factors[16]. As a considerable problem in the community[3], chronic pain patients experience depression, anxiety, sleep disturbance, and fatigue[17,18], in addition to activity limitations. Managing chronic pain most often helps to achieve rehabilitation rather than recovery[5], in other words, preserving residual function and preventing secondary complications.

There are four types of chronic pain: nociceptive, inflammatory, neuropathic, and centralized/ dysfunctional[19]. Nociceptive pain is typically the result of tissue injury, presenting the activation of nociceptive neurons which deliver high threshold unpleasant impulses to the CNS[19]. The second type is inflammation pain. Substantially, inflammation protects our bodies and boosts the healing process at the acute stage; meanwhile, it works conversely at the chronic stage, damaging tissue and causing pain[20]. Neuropathic pain develops after nerve injury (lesion or disease) either in the periphery or centrally[21]. Finally, dysfunctional or centralized pain is a term used to describe chronic, often widespread pain conditions such as fibromyalgia in which there is no noxious

stimulus, no detectable inflammation, and no structural damage to the nervous system or any other tissue, and which appears to be caused by abnormal nerve function; it is inexplicable pain with negative effects on quality of life for the patients[22].

It is important to reach the right assessment of chronic pain as a critical prerequisite for treatment selection, providing information regarding the pathophysiological mechanism of pain conditions and the severity of the pain which can help guide pain management[23].

1.3 Pain conceptual models

The mechanism underlying pain perception has been a mystery for many decades. In ancient cultures, the explanations were related to religious beliefs, such that pain was considered a punishment for humanity or even a way to be redeemed from sins[24]. During the Renaissance and Enlightenment periods, many theories have been proposed. In the seventeenth century, René Descartes introduced pain as a real, not imaginary phenomenon, which comes from the brain after physical stimulation (the Dualistic Theory)[25]. He proposed that the body is more like a machine, and the pain was considered to be a disruption that traveled through the nerve fibers until it reached the brain. By this theory, the pain was turned from a spiritual experience to gain a physical, mechanical sense. Another presentation followed in the year 1811 by Charles Bell, referred to as the Specificity Theory, which identified specific pathways for different sensory receptors[24]. Scientists spent the next century and a half further developing the Specificity Theory. In 1929, another theory, the Pattern Theory by John Paul Nafe, opposite to the old Specificity hypothesis, became very popular. According to Nafe, each of the four sensory modalities (cold, pain, heat, and touch[26]) does not have its own receptor. Instead, he proposed that each feeling sends the brain a distinct pattern or sequence of impulses, and the sensation experienced is related to which pattern

the brain reads[24]. Later, in 1954, the term biopsychosocial was first introduced by Roy Grinker[27]. In the 1960s, the Gate control theory was proposed by Melzack and Wall[28]. It suggested that there is a neurological gate for pain signals in the spinal cord that blocks or transduces the signals to the brain. In other words, pain impulses are carried by small fibers that enter the dorsal horn of the spinal cord where other cells transmit the signals from the spinal cord up to the brain[24]. This theory has received considerable interest and has certainly been a major improvement on the early pain theories which explained the potential role of the nervous system and the effect of psychological factors in the complex phenomenon of pain[29]. Over the next 30 years, Melzack introduced the Neuromatrix model, based on his first Gate control theory, stating that pain can be affected not only by physical factors but also by cognitive and emotional factors. Melzack suggested that increased levels of stress will lead to a higher level of pain[30].

A lot of work for many researchers was done to illustrate the etiology of pain through these theories and the biopsychosocial model[27]. Now, we can clearly explain the pain experience by the dynamic interaction of three contributors: biological, psychological, and sociological factors[31]. Each of these factors may have its own independent impact[10].

Biological factors

Multiple biological variables, including demographic and genetic factors, play a major role in the individual pain experience. Biological changes occurring at different levels of the nervous system may also represent an important factor affecting pain development[32], for example, alterations at the supra-spinal level, such as gray matter with a lower intensity, have been reported in chronic pain patients' brains in multiple regions[33].

Pain prevalence differs depending on sex, age, and ethnicity[34]. A lot of research studies have observed that women have a higher level of reported pain than men. Furthermore, old people (aged 65 and older) are more susceptible to developing chronic pain and have a lower recovery rate[35]. The same is true for genetic variation: it has been shown that genetic factors contribute to the development of chronic pain[36]. Many of them contribute to variation in psychological distress and sensitivity to pain[37]. Now, with rapid developments in genotyping methods and other genetic technologies besides the widespread adoption of Genome-wide association studies (GWAS), the number of specific genes associated with different pain conditions is growing fast[38]. GWASes helped to deliver remarkable discoveries in human genetics, and to detect associations between human diseases and genetic variants, revealing more about the genes, variants, and biological pathways and making it possible to create a genetic predictor for diseases, and as a result, better prevention, and treatment strategies[39].

Psychological factors

Many studies have shown that psychological factors play an important role[40] and influence both the experience and management of pain[41,42]. For example, fear, anxiety, depression, catastrophizing, and other negative factors may lower patients' pain threshold[42,43]; while positive emotions and pain beliefs produce responses exactly the opposite, as the case of patients who avoid catastrophizing and believe that they can control their pain reported lower pain intensity and better function[44]. Higher pain intensity and disability have been noticed among patients reporting low levels of self-efficacy beliefs[32], and poor coping skills[44].

Another such factor is sleep deprivation as a reason for hyperalgesia; in fact, chronic pain is frequently associated with sleep disturbance; in the meantime, deprivation or disturbance of sleep enhances pain sensitivity leading to pain[45].

The majority of patients who have depression also report chronic pain[46]. Similarly, elevated fear response manifests as a substantial feature for a considerable number of individuals with musculoskeletal pain who developed chronic conditions[42]. Overall, psychological factors play an important role in the transition to pain chronicity, contributing at least as much as other factors[47].

Social factors

The experience of pain depends also on the intervention of the social factors, with social support, social learning, and socioeconomic status associated with physical and psychological states influencing chronic pain conditions[48]. One of the most commonly studied social factors is social support[48]. Clearly, chronic pain is significantly improved when social support is received[49]. Among patients with injury, chronic pain may also appear when there is an experience of unfairness and unnecessary physical and emotional suffering[50].

Moreover, socioeconomic status showed that lower levels of education and frequency of chronic pain conditions are correlated, further demonstrating that this factor plays an important role in the development of chronicity[48] and worsening of pain[51].

1.4 Pain and inflammation

Pain is known as a manifestation of inflammation[52]; however, pain is a complex phenomenon involving psychosocial and biological mechanisms as discussed above. Studying the

pathophysiological mechanisms of pain starts with a noxious stimulus (thermal, mechanical, electrical, or chemical). Also, it may arise from injury or damaged tissue. As a result, non-neuronal cells (e.g., mesenchymal, immune, glial, and epithelial cells) release neuromodulator mediators producing pain signals by somatosensory neurons (nociceptors). These signals are carried to the dorsal horn of the spinal cord, and transmitted to the brain, transferring information about the intensity and duration of peripheral noxious stimuli[53]. In the case of inflammation, the activation of the nociceptors is direct via a wide variety of immune cells which can stimulate sensory neurons by producing pro-inflammatory mediators [53].

Many injuries trigger persistent inflammation, and in this context, pro-inflammatory mediators stimulate nociceptors causing chronic pain. In both tissue injury and inflammation, pain is a response to prevent the damage to the tissue and initiate tissue repair mechanisms[54], and in both, we can see the involvement of biochemical mediators such as cytokines, neuropeptides, and growth factors, and neurotransmitters[55]. Each pain condition has an inflammatory profile that comprises multiple inflammatory mediators leading to pain manifestations. So, we can say that the biochemical origin of the pain is inflammation[55]. These presentations diverge from one person to another and even vary in the same person at different times or different stages in the lifecycle[56]. Understanding this process will help in the first place to manage pain conditions[55].

1.5 Management of pain

The goal of studying pain disorders is to guide the management process through a better understanding of the underlying pain mechanisms[23] and identifying risk factors impacting pathophysiological processes contributing to patients' pain states[57].

Rehabilitation and improvement of patients' quality of life are the headmost concern for physicians in their practice to manage pain conditions[11]. Pain is a complex experience which affects an individual's quality of life. Thus, to manage the pain, many approaches are applied such as medications, nerve blocks, physical therapy, and lifestyle modifications. Most often, more than one treatment is needed to obtain complete pain relief.

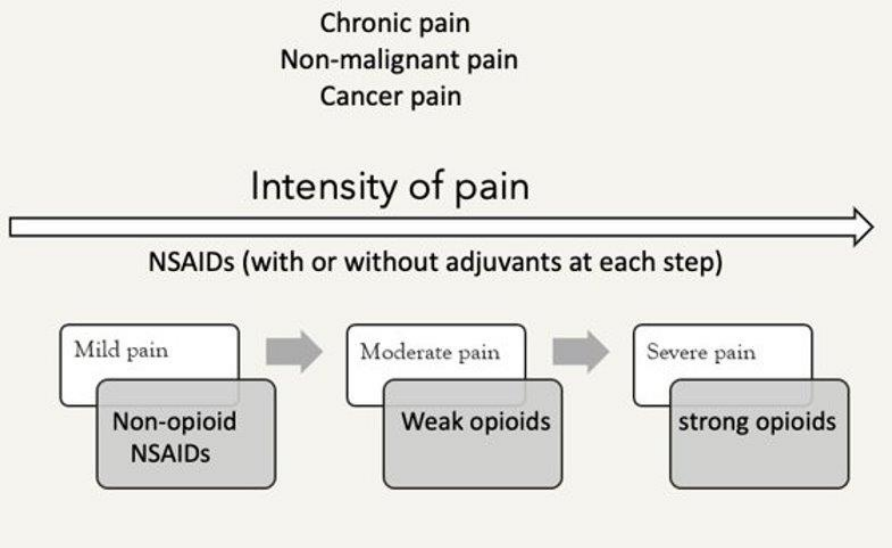
So far, the use of pharmacological agents is the first-line approach for pain management[58]. They are cheap, work fast, and are relatively safe even with possible side effects[59].

Among non-prescribed medications, analgesics are widely used. Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are highly consumed over-the-counter (OTC) analgesics used by approximately 17% to 23% of the population in the US each week, most often by the elderly[60].

For prescribed analgesics, the World Health Organization (WHO) has promoted a three-step ladder model that relies on pain intensity reported by patients as a mean criterion[15]. This guideline marked three steps for the sequential use of analgesics, where drug selection is based on the severity of pain as follows: non-narcotics, weak narcotics, and narcotics[61]. In the first step, with mild pain, treatment begins with non-opioid analgesics such as NSAIDs and acetaminophen. Moderate pain, as a second step, is considered when pain persists, treated with mild opioids (e.g., codeine, tramadol, alone or combined with tramadol), with low doses of strong opioids in some cases. If the pain persists and/or is severe, using strong opioids is recommended as the third step in the treatment (e.g., morphine, buprenorphine fentanyl, oxycodone, hydromorphone, and tapentadol), in combination or not with non-opioids[15,62]. The combination with opioids and non-opioids or the use of adjuvant analgesics can be an addition at any step of the ladder in the

pharmacological treatment to reach the desired adequate relief [63]. The drug selection order is shown in Figure 1.

Figure. 1. WHO World Health Organization (WHO) Analgesic ladder.



1.6 Effects of nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are both one of the most prescribed and heavily used over-the-counter classes of analgesics[64]. Studies remarked NSAIDs make up around 5-10% of prescribed medications each year[65]. It is a class of medicines that are widely used to relieve pain, reduce inflammation, and bring down a high temperature. The mechanism of action is mainly to inhibit the enzyme cyclooxygenase (COX) which controls the production of prostaglandins (mediators of inflammatory and anaphylactic reactions), and thromboxane (mediators of vasoconstrictions) so it affects platelet adhesion[66]. Two isoforms of COX enzymes were identified, with Cox-2 primarily seen at the site of the injury to maintain the inflammatory response. COX-1, which is expressed in most tissues, is responsible for physiological protective functions, such as maintaining the

gastrointestinal mucosal lining, kidney blood supply, and platelet aggregation[67]. Most NSAIDs are nonselective and inhibit both COX-1 and COX-2; thus, according to the roles of prostaglandins in the body, restraining these activities by taking NSAIDs can have both positive and negative effects. On the other hand, NSAIDs affect bone healing. Many human studies demonstrated an increased risk of delayed healing of union or nonunion fractures and surgical fusions[68]. NSAIDs appear to have an inhibitory effect on collagen production by tenocytes leading to reduced collagen formation, tensile strength, and delayed maturation of healing tendons[69]. Risk and complications are typically greater for people who take NSAIDs for a long period[64]. Furthermore, in some people, NSAIDs produce serious side effects, impacting cardiovascular, gastrointestinal, or renal function[66].

1.7 Pain chronicity - gaps of knowledge

Chronic pain is pain that carries on for longer than three months. It is a common, complex, and challenging condition, that originates from different types of tissue damage. Chemical mediators and pain transmission pathways are involved[56].

Effective pain management demands analgesic regimens safely suitable to various types of pain. Despite the advanced research and treatment protocols that reported great improvement in managing pain conditions, less interest was given to the results for long-term management[70]. Furthermore, the pathophysiology of the transition from acute to chronic pain is under investigation but is only vaguely understood[71,72]. Some prospective studies in this area suggested that this transition occurs across several cellular and molecular levels; so far, the immune process is considerably engaged in both neuropathic and inflammatory pain[73]. In fact, the immune process takes part in promoting and maintaining chronic pain[74,75]. Intervention in the inflammatory process

often leads to various conditions and diseases[76]; also, this may affect the healing process itself, which is complex, involving interactions among the mediators and the cells[77]. Drugs that suppress this active response, such as NSAIDs used in the management of acute injury may interfere with the recovery process[67]. Here appears the need for evidence from prospective longitudinal studies to establish and determine the effects of NSAIDs on pain chronicity.

2.0 Study objectives and hypothesis

The overall objective of this project is to better understand the risk factors of pain chronicity. We specifically tested the effect of NSAIDs as a distinct analgesic category on pain chronicity and the contribution of the genetic factors to an increased risk of chronicity of pain though the genome-wide association study (GWAS) design.

Previously, we investigated the role of NSAIDs on pain chronicity in a prospective analysis of the associations between analgesic medications taken at the acute pain stage and the development of chronic back pain was conducted in our research laboratory. The result of the analyses demonstrated that back pain chronicity at a subsequent time point was found to be enhanced by NSAIDs taken during the acute phase[75]. To determine the generalizability of the effect of developing chronic pain by NSAIDs on different muscular-skeletal conditions at different stages of pain states, we investigated the effect of taking a range of analgesic groups on multi-visit pain at different body sites as a proxy for chronic pain. We hypothesize that NSAIDs that suppress the inflammatory response among individuals with pain increase the risk for chronicity at body sites other than the back.

We also studied the genetic contribution using the GWAS as an approach to find the genes contributed to body site-specific pain. This approach had been used before in different studies

to identify the high frequency genetic variants associate with a specific disease or the severity of this disease. The large number of published pain-relevant GWASs showed the importance of this approach and helped to understand biological pathways contributing to pain states, including back pain[78], shoulder pain[79], temporomandibular disorders[80] and many other chronic musculoskeletal pain conditions[81,82].

The specific objectives are:

I. Investigate the effect of medications on the chronicity of body site-specific pain using the cohort Canadian Longitudinal Study on Aging (CLSA).
II. Study the effect of different groups of analgesics on chronicity, including NSAIDs and other categories of analgesics that don't affect the inflammatory process.
III. Test for replication of the findings from the CLSA in another cohort, the United Kingdom Biobank (UKB).
IV. Identify the genetic variants associated with a risk for body site-specific pain in CLSA by conducting a GWAS.

3.0 Methods

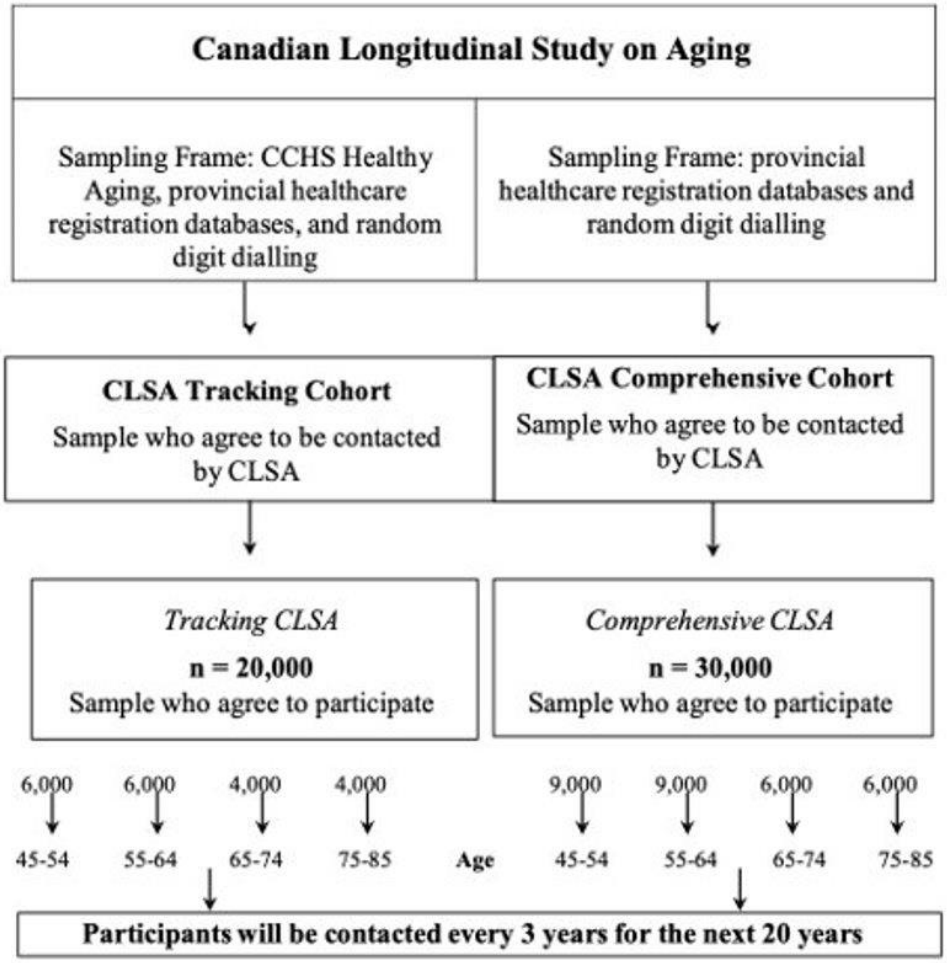
3.1 Study design and participants

Data for the analysis was extracted from the prospective cohort study, the Canadian Longitudinal Study on Aging (CLSA). The CLSA is a broad, long-term, observational study that follows approximately 50,000 Canadians who were between the ages of 45 and 85 at recruitment. These participants were grouped into two study components with different sampling designs (the CLSA Tracking and Comprehensive) and will be followed up for at least 20 years, or until death. The data collection scheme is shown in Figure 2. The main goal of the CLSA is to improve the quality of life and health conditions for Canadians[83] by understanding the dynamic process of aging that affects the quality of life.

The recruitment process took place between 2010 and 2015. Each recruited participant has been recontacted again for the first follow-up (FU1) between 2015 and 2018. During this phase, the same information was collected as for baseline along with several new measurements. In this longitudinal design, the follow-up interval is every 3 years until 2033 or death[84]. Over 51,000 participants were included in the CLSA. More than 21,000 individuals provided information through telephone interviews (Tracking cohort), while 30,000 participants were assessed through in-home interviews and data collection site visits (DCS) (Comprehensive cohort). Additional data collection involved contacting all participants by telephone 18 months after each full telephone or in-home interview to complete the Maintaining Contact Questionnaire (MCQ). Physical examinations and biological specimen collection (blood and urine) have been run at one of 11 sites across Canada: Vancouver/Surrey (two sites), Victoria, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax, and St. John's[85]. In addition, these data were linked to the health

administration database (e.g., publicly funded drug plans, medical services plans, hospitalization, continuing care/long-term care, and/or mortality) which is an important complement to data collection, making it possible to collect information on medication use, health services utilization, and to ascertain deaths and causes of death.

Figure. 2. CLSA study design



3.2 Procedures and measures

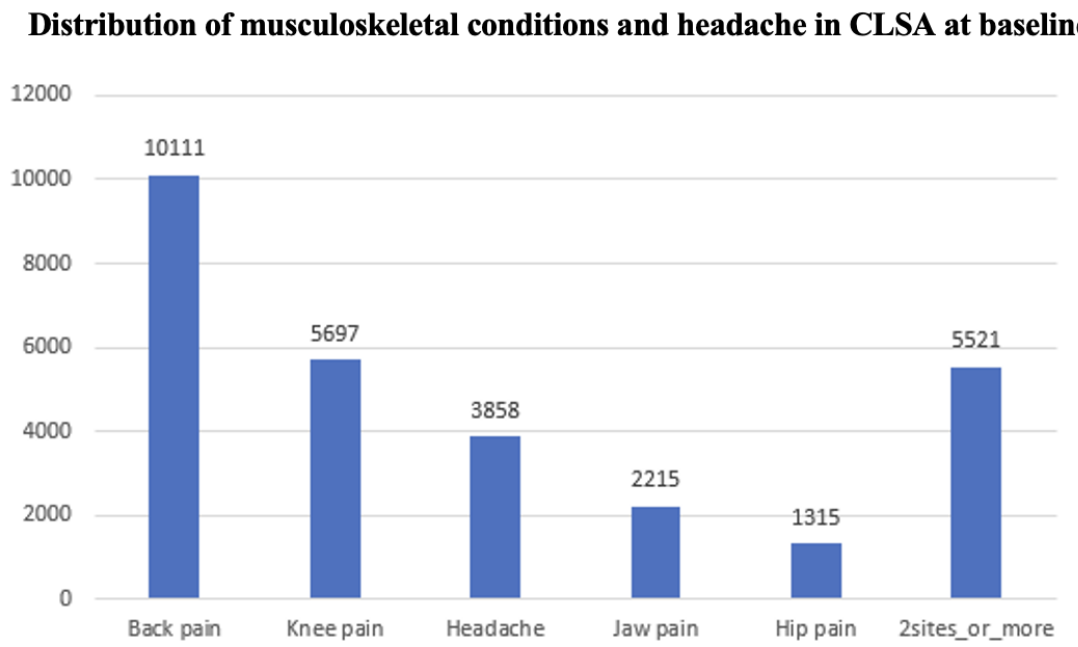
CLSA data based on two-time points have been released: baseline and FU1. Baseline data encompassed phenotypes, medications, and genetics. More detailed information was collected from Comprehensive cohort participants, including an in-person review of medications during the in-home visit and a disease symptom questionnaire during the DCS visit. Our study was limited to the Comprehensive cohort.

3.2.1 Phenotypes

The CLSA provides opportunities for interdisciplinary investigation. It has more than 8,800 variables across Biology, Genetics, Medicine, Psychology, Sociology, Demography, Economics, Epidemiology, Nutrition, and Health services. Comprehensive cohort questions related to pain are in different categories relating to conditions that cause pain at specific body sites and pain intensity[86].

The most common types of musculoskeletal pain are back pain, knee pain, jaw pain, and hip pain. The only form of headache reported in the CLSA relates to migraine. Other types of chronic pain such as neck and shoulder pain and abdominal pain were featured as questions on symptoms of other diseases (e.g., chest pain). The cohort description and clinical characteristics of all participants relating to musculoskeletal conditions and headache at baseline in the CLSA are shown in Figure 3, it also shows participants who reported two or more types at the same time.

Figure. 3. Distribution of musculoskeletal conditions and headache at baseline in the CLSA. The type of pain is shown on the *x*-axis and the number of subjects on the *y*-axis.



At each clinical visit, participants were asked to report the presence of each of these phenotypes by answering specific questions. Among the five pain conditions, two of them (hip pain and headache) were excluded. The reasons for excluding them are as follows:

In the case of the hip pain, the subjects were asked the question "Do you experience hip, leg, or calf pain during a 4-meter walk test?" A physical assessment was performed[87]. The outcome is pain induced by exercise rather than chronic pain. The test is designed to address physical capacity in specific clinical conditions.

For headaches, the question was "Has a doctor ever told you that you have migraine headache?" Only one type of headache (migraine) is targeted by the question. Essentially, there are 4 major types of headaches: (migraines, tension-type headaches, cluster headaches, and new daily

persistent headaches [88 89]). Since migraine is a neurobiological disorder[90], it does not fall under the category of idiopathic pain conditions. Moreover, we are unable to create a broader phenotype definition for headaches in general. We, therefore, continued our analysis on back pain, jaw pain, and knee pain.

Pain Characteristic phenotype

Additional data collection was conducted through telephone interviews for the baseline visit and a site questionnaire for FU1 to explore general characteristics of pain. The first question was "Are you free from pain most of the time?"; those who answered "No", were also asked, "How would you describe the usual intensity of your pain or discomfort?" To this question, the answer could be: "Mild", "Moderate", "Severe", or "Don't know". For the purpose of this research, participants answered "Yes" if they were free from pain on most days; they were categorized as having infrequent pain (=0), while the others were classified as mild (=1), moderate (=2), or severe (=3). Musculoskeletal pain phenotypes and pain characteristics are shown in Table 1.

Table. 1. Musculoskeletal pain phenotype and pain characteristics in CLSA at baseline and FU1.

Pain type	Variables-name	Dataset	Question
Back pain	OST_BP_COM/ OST_BP_COF1 OST_BCKPPM_COM/ OST_BCKPPM_COF1	COM/COF1	Have you ever had BP for at least 1 month? / Have you had this pain in the last 12 months?/ For how long?
Jaw pain	ORH_EXP_JJP_COM/ ORH_EXP_JJP_COF1 ORH_EXP_JWS_COM/ ORH_EXP_JWS_COF1	COM/COF1	In the past 12 months have you experienced any of the following? (Oral health problems - Jaw joints painful/ Jaw muscles soar).
Knee pain	OSK_PAIN_COM/ OSK_PAIN_COF1	COM/COF1	During the past 4 weeks, have you had knee pain on most days?
Characteristics of pain			
	HUP_FREE_COM/ HUP_FREE_COF1	COM/COF1	Are you usually free of pain or discomfort?
	HUP_INTNSTY_COM/ HUP_INTNSTY_COF1	COM/COF1	How would you describe the usual intensity of your pain or discomfort? Would you say it is mild, moderate, or severe?

3.2.2 Medications

Medications in the CLSA were administered as a part of the in-home questionnaire for the Comprehensive cohort. They were reported depending on the Health Canada Drug Product Database (DPD) using their Drug Identification Number (DIN). DIN is a computer-generated eight-digit number assigned by Health Canada to each drug product prior to being marketed in Canada, and it uniquely identifies the drug. The (DPD) system presents products approved for use by Health Canada, containing human, veterinary, disinfectant, and radiopharmaceutical products[91] classified in four groups: approved, marketed (active), canceled (inactivated), and dormant products.

Each one of these groups is linked to another 11 tables with information about companies, drug product, form, active ingredients, packaging, pharmaceutical standard, route of administration, schedule, product status, therapeutic class, and veterinary species.

In this work, to be able to study the effect of the drugs, we aimed to classify individual participant-level data according to the Anatomical Therapeutic Chemical (ATC) classification system assigned by the World Health Organization (WHO)[92]. In this system, medicinal products are classified in groups at five different levels according to the main therapeutic use of the main active ingredients and the organ or organ system on which they act. Pharmacological groups are assigned at the 2nd, 3^{rd,} and 4th levels allowing for drugs containing the same active ingredient to have more than one therapeutic uses[93]. The ATC codes proposed by WHO is the reference to define the therapeutic indication for CLSA medications by matching the DIN to ATC codes.

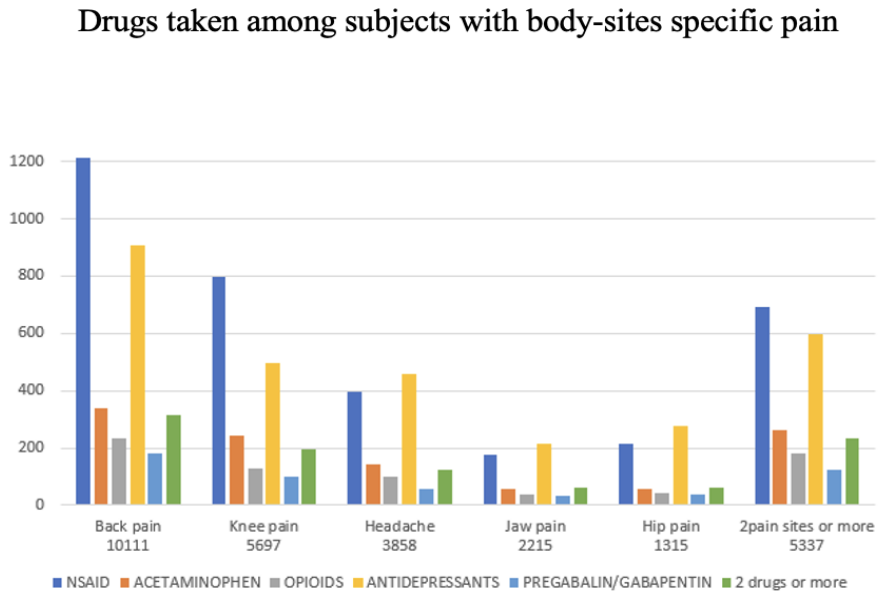
Matching across the DIN and ATC system was done as follows: first, using the CLSA datasets, we extracted the medication data including the DIN, drug name, dose, frequency, duration, the start date of medication, the reason for use, and whether the medication was prescribed by a

physician or was non-prescription. Next, we classified each drug depending on Canada Drug Product Database (DPD) using the two files: “Drug.type” contains (drug code, DIN, drug type, class, brand name) and “Ther.type” contains (drug code, ATC, drug name, category). From the first file, we had the (drug code-DIN), and from the second file, we had (drug code-ATC). Finally, by matching these two files, we had the relation (DIN-ATC) which made it possible to correspond with the ATC-coding system for WHO classification.

We took into consideration 5 types of analgesics in our analysis: NSAIDs, acetaminophen, opioids, antidepressants, and gabapentinoids which correspond to classification at the third level of the ATC classification system. classification system. Figure 4 presents these groups of analgesics across individuals with pain at specific body sites at baseline.

Figure. 4. Consumption of analgesics for pain across specific body-sites in the CLSA at baseline. Each individual may contribute to more than one category. (A) bar chart for analgesics groups taken for each body-site specific pain. It also shows the consumption in participants with two or more pain sites. (B) shows analgesics taken by numbers.

A.



B.

	NSAID	ACETAMINOPHEN	OPIOIDS	ANTIDEPRESSANTS	PREGABALIN/GABAPENTIN	2 analgesic or more	No analgesic	Total
Back pain	1212	339	233	908	180	316	7591	10111
Knee pain	796	241	130	497	101	195	4146	5697
Headache	396	145	102	459	57	126	2838	3858
Jaw pain	176	57	38	214	32	60	1615	2215
Hip pain	175	83	51	134	38	66	900	1315
2pain sites or more	690	261	179	596	126	236	3745	5337

3.3 Data analysis

Descriptive statistics at both baseline and FU1 were generated to investigate the relationship between body site-specific pain (back pain, knee pain, jaw pain) and the effect of the drugs. Next, each pain type was analyzed in separate logistic regression models using R v.4.0.2 with what we

define as multi-visit site-specific pain as the outcome. Cases were defined as those with site-specific pain at baseline who also reported pain at the same site at FU1 (this is our definition for multi-visit body-site specific pain, a construct we used in the present study), while controls were those with site-specific pain at baseline who had recovered (no pain). Multi-visit site-specific pain is a compromise definition that attempts to capture chronic pain. The CLSA does not follow study subjects closely such that we would be able to consider continuous periods of pain experience lasting three or more months. For back pain, and only back pain, a question was asked on the number of months or years of pain experience which could be dichotomized to result in a set of individuals with more than three months of pain. In order to work with a consistent definition for classifying cases across body sites, we chose to make cases of those who reported pain at two-time points with an approximately 3-year interval. We, therefore, make the assumption that cases will be enriched for individuals with chronic pain while acknowledging that there may be some misclassification. Since misclassification might reduce effect sizes towards the null without leading to false positives, this strategy is legitimate.

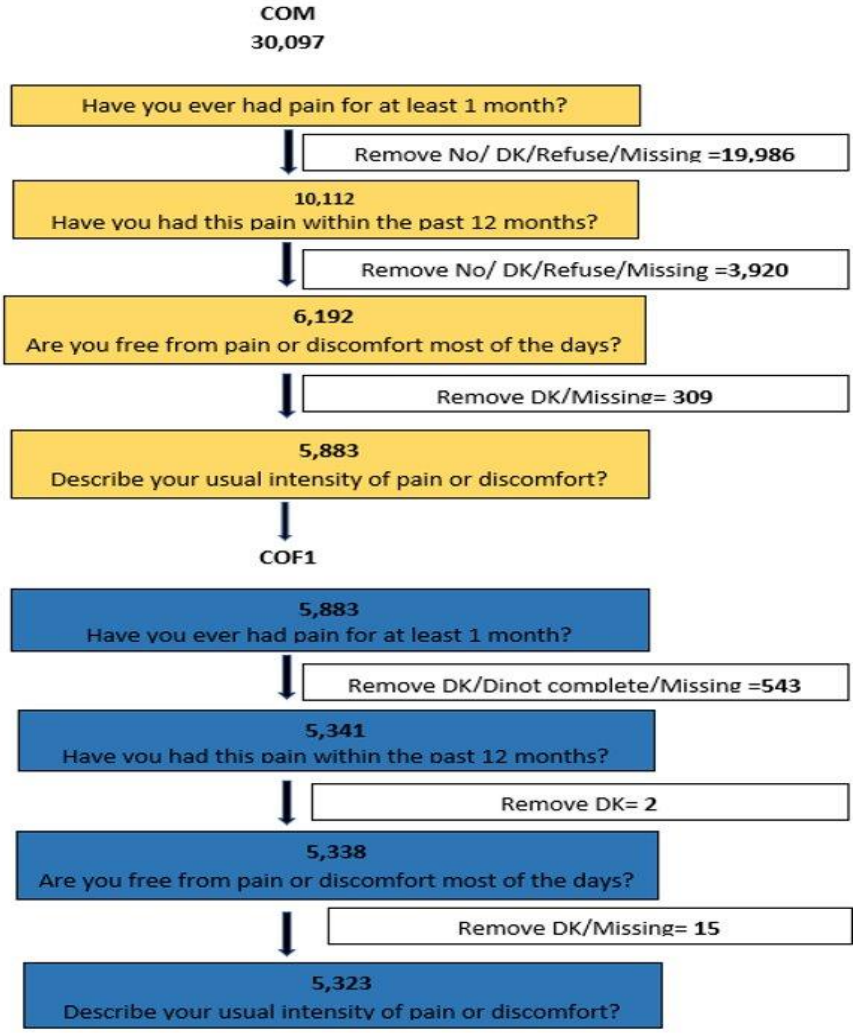
The five groups of analgesics (NSAIDs, paracetamol, opioids, anti-depressants, and gabapentinoids) were assessed as predictors in logistic regression models for each body site. We tested for association between taking medications and multi-visit pain as a proxy for chronic pain, adjusting for age, sex, ethnicity, intensity of pain, and BMI. We used the nominal *p*-value threshold of 0.05 to define statistical significance in the CLSA.

3.3.1 Data analysis back pain

The 30,097 participants included at baseline in the Comprehensive cohort were asked the question “Have you ever had back pain for at least 1 month?” Individuals who answered “Yes”

(10,112) were asked the second question:" Have you had this pain within the past 12 months?" Only 6,192 answered "Yes". They also answered another two questions related to pain intensity: "Are you free from pain or discomfort most of the days? If "No", "Describe your usual intensity of pain or discomfort?" We removed participants who answered "Don't know" or "Missing" (309); finally, we were left with 5,883 individuals who were followed to FU1; using the same algorithm to classify subjects, we were left with 5,323 individuals with non-missing data (Figure 5). They were classified at this second-time point as cases were those who reported multi-visit back pain (baseline and FU1) (2,957), and controls were those who had back pain at baseline but did not report back pain at FU1 (recovered) (2,366).

Figure. 5. Back pain analytic study flowchart for the CLSA Comprehensive cohort participants. COM: Comprehensive cohort at baseline. COF1: FU1 after 3 years.



The distribution of analgesic groups for back pain subjects at baseline is shown in Table 2.

Table. 2. Distribution of medications taken for back pain among 5,323 CLSA subjects at baseline.

Drug name	Yes	No
NSAIDS	676	4647
Acetaminophen	223	5100
Opioid	157	5166
Anti-depressants	538	4785
Pregabalin/gabapentin	110	5213

The pain conditions captured by these questions include both episodic (acute) and (chronic) pain that was experienced at least for a certain period within the last 12 months.

3.3.2 Data analysis jaw pain

Jaw pain encompasses both sore jaw muscles and jaw joint pain. All participants at baseline in the Comprehensive cohort of 30,097 were asked in the oral health section to answer the questions "In the last 12 months have you experienced any of the following?" Participants had to choose one or more of the following answers: (toothache, cannot chew adequately, dentures uncomfortable, dentures lose, dentures broken, dentures missing, swelling in your mouth, dry mouth, burning mouth, jaw muscles sore, jaw joint pain, natural tooth decayed, a natural tooth broken, natural tooth loose, sore gum around natural teeth, bleeding gum around natural teeth, denture-related sore, teeth or dentures dirty, bad breath, none of above, and other problems).

Individuals who answered "Yes" to jaw muscles sore and/or jaw joint painful questions (2,434) were considered to have jaw pain. Those cases described their pain intensity by answering the two questions: "Are you free from pain or discomfort most of the days? If "No", "Describe your usual intensity of pain or discomfort?" We removed all who answered "Do not know/ Refused to answer" (27). 2,407 individuals were followed up to the next visit at FU1 using the same criteria. Finally, we had 2,215 participants who presented jaw pain at baseline, and they were classified as cases those who reported jaw pain for multi-visit (baseline and FU1) (946), or controls (recovered) (1,269) were those who did not report any jaw pain at FU1 (Figure 6). Table 3 presents the distribution of analgesic groups for jaw pain subjects at baseline.

For this type of pain, as for back pain, the reference period was the previous 12 months.

Figure. 6. Jaw pain study overview flowchart in the CLSA Comprehensive cohort. COM: Comprehensive cohort at baseline. COF1: first follow-up (FU1)

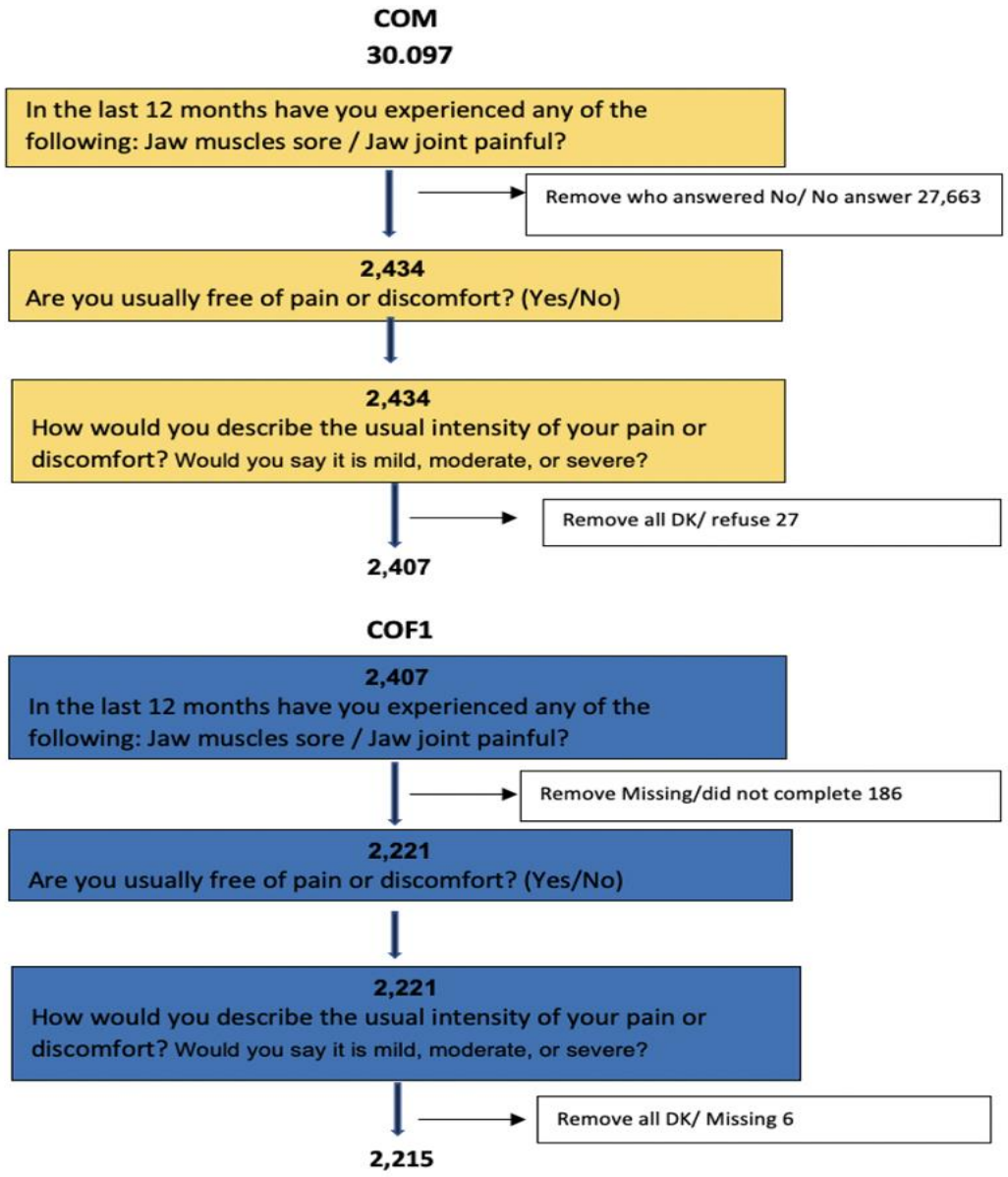


Table. 3. Distribution of analgesic groups for jaw pain subjects 2,215 at baseline in the CLSA.

Drug name	Yes	No
NSAIDS	216	1999
Acetaminophen	59	2156
Opioid	41	2174
Anti-depressants	275	1940
Pregabalin/gabapentin	37	2178

3.3.3 Data analysis knee pain

In CLSA Comprehensive cohort at baseline (N = 30,097), only individuals who answered "Yes" (5,697) to the question "During the past 4 weeks, have you had knee pain on most days?" were considered in this analysis. On the other hand, the subjects who answered "No" to the question "Are you free from pain or discomfort most of the days?" also reported on pain intensity by answering the question "Describe your usual intensity of pain or discomfort?" We removed all subjects who answered "Don't know" or "Missing". We followed 5,386 individuals to FU1, using the same criteria relating to answers to questions. Of those 4,862 individuals who reported knee pain at baseline, those who also reported knee pain at FU1and were defined as cases (multi-visit knee pain) (2,517), while controls (recovered) (2,345) were those who did not report knee pain at FU1. Figure 7 shows the knee pain classification algorithm. The distribution of medications taken for knee pain in the CLSA is presented in Table 4.

Figure. 7. Flowchart for knee pain study in the CLSA Comprehensive cohort. COM: Comprehensive cohort at baseline. COF1:first follow-up (FU1)

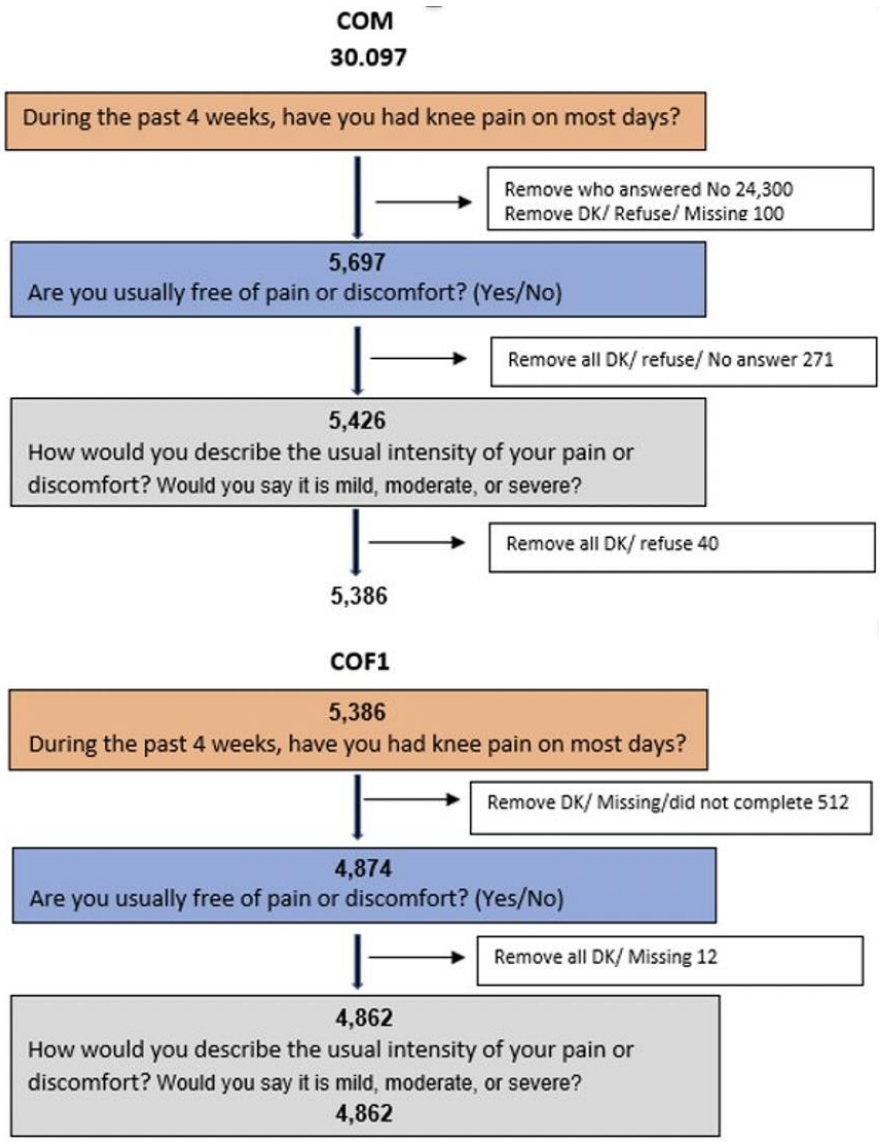


Table. 4. Analgesic distribution among 4,862 knee pain subjects at baseline CLSA.

Drug name	Yes	No
NSAIDS	686	4176
Acetaminophen	198	4664
Opioid	107	4755
Anti-depressants	415	4447
Pregabalin/gabapentin	80	4782

The knee pain definition was different from other groups as it was identified as falling within a more restricted period (in the last 4 weeks). Descriptive statistics for participant characteristics are shown in Table 5. At baseline, the sample included 57 % of women. The average age of the subjects was 63.7 yrs (SD = 9.84 yrs).

Table. 5. Demographic table for knee pain subjects in the CLSA at baseline

Variable, *Mean(SD) or N(%)*	Overall (N=4862)
Age (years)	63.7 (9.84)
Sex	
Female	2788 (57.3%)
Male	2074 (42.7%)
Ethnicity	
White	4578 (94.2%)
Black	50 (1.0%)
South Asia	42 (0.9%)
East Asia	55 (1.1%)
Others	137 (2.8%)
BMI	30.0 (6.32)

Footnotes: BMI: Body mass index; SD: standard deviations

The findings from these analyses were tested for replication in the UK Biobank (UKB).

3.3.4 Replication of knee pain in the UKB.

Across all pain types considered in the CLSA, the strongest contribution of NSAIDs towards a site-specific pain type was reported for knee pain. We, therefore, tested for replication of the knee pain findings in the United Kingdom Biobank (UKB).

3.3.4.1 Study design and participants

Data for replication analysis was obtained from the UKB. The UKB is a large prospective cohort study with more than 500,000 participants between the ages of 40-69[94]. A subset was followed for three study visits (2 years intervals) at 35 centers in 22 cities in England, Scotland, and Wales. A wide range of variables was included in this study, including lifestyle, environment, genotype, and other exposures[94,95]. The baseline visit was carried out between 2006 and 2010; socio-demographic and medical information was assessed through different types of questionnaires[96]. An initial 5-year follow-up assessment took place between 2012 and 2013 (FU1), and the second follow-up assessment (FU2) started in 2014 and has been completed too.

3.3.4.2 Measures and procedures

Phenotype

We tried to match the CLSA definition in the UKB, so the subjects were selected for this study based on the question "In the last month have you experienced any of the following that interfered with your usual activities? (You can select more than one answer)" if the answer was "Yes" for knee pain at baseline (visit 0) (v0) (7,110 individuals). Those who also reported knee pain at any of the subsequent visits (visit 1 (v1) or visit 2 (v2)) were considered as cases (multi-visit knee pain) (3,331), while others who did not report any pain at the follow-up visits were considered as controls (recovered) (3,779).

Medication

Medications in the UKB were categorized into 6,745 groups, of which 1,809 were reported by 10 or more people[97]. Of these 1,752 (97%) were classified using the ATC Classification System at the first three ATC levels[98]. Each drug in the UKB[99] was specified by a code that refers to the

trade name or the generic category. The classification of medications was done by matching this code assigned for each drug in the UKB database and with the WHO ATC code for each drug. Following the same criteria as for the CLSA, we examined the 5 groups of analgesics: NSAIDs, paracetamol, opioids, anti-depressants, and gabapentinoids.

3.3.4.3 Data analysis of knee pain in the UKB.

7,110 subjects were included in this analysis; the mean age of participants was 56.7 yrs (SD=7.16 yrs). The sample included 47 % of women (see Table 6). The distribution of medications taken by knee pain subjects in the UKB is presented in Table 7. Logistic regression analysis was performed to test for association between each analgesic category and multi-visit knee pain to indicate chronic pain, adjusting for age, sex, ethnicity, the intensity of pain, and BMI. We used the nominal *p*-value threshold of 0.05 to define statistical significance.

Table 6. Demography of knee pain subjects in UKB at baseline.

Variable, *Mean(SD) or N(%)*	Overall (N=7110)
Age (years)	56.7 (7.32)
Sex	
Female	3399 (47.8%)
Male	3598 (50.6%)
Ethnicity	
White	6904 (97.1%)
Black	42 (0.6%)
Asian	79 (1.1%)
Mixed	28 (0.4%)
Others	57 (0.8%)
BMI	28.2 (4.95)

Footnotes: BMI: Body mass index; SD: standard deviations

Table. 7. Distribution of medication taken for 7,110 knee pain subjects in the UKB at baseline.

Drug name	Yes	No
NSAIDS	2,295	4,815
Paracetamol	1,401	5,709
Opioids	131	6,979
Anti-depressants	509	6,601
Pregabalin/gabapentin	129	6,981

3.3.5 GWAS for knee pain in CLSA.

3.3.5.1 Genotyping in CLSA

The genome-wide genotyping in CLSA was performed on DNA samples collected from the blood of 26,622 individuals from the CLSA Comprehensive cohort of men and women with 93% of European ancestry. DNA extraction and genotyping were performed at the McGill and Genome Quebec Innovation Centre, Montreal, Canada. Participants were genotyped using the Affymetrix UK Biobank Axiom array[100]. Affymetrix Axiom array genotypes for 794,409 genetic variants, of which 95% are high quality. The genotypes were imputed to the TOPMed reference panel which imputed genotypes for ~308 million genetic variants. Quality assessment includes both marker- and sample-based tests, as well as an analysis of population structure and familial relatedness. Genomic positions of the array genotyped and imputed genotype data are reported about human genome build GRCh37/hg19 and GRCh38/hg38, respectively [100]. QC filtering of imputed SNPs was done based on Hardy-Weinberg equilibrium (HWE < 1×10^{-6}), minor allele frequency (MAF < 0.01), and genotyping missing rate (INFO score less than 0.3) after running genome scan analysis. These genomic data were linked to physical, lifestyle, medical, economic, environmental, and psychosocial factors collected longitudinally in CLSA[101].

3.3.5.2 Measures

We carried out a Genome-Wide Association Study (GWAS) to identify the genetic variants associated with knee pain in 26,622 participants with genotype data from the CLSA at both baseline and FU1.

Phenotype definition was based on the question "During the past 4 weeks, have you had knee pain on most days?". Subjects who answered "Yes" for any of the time points were considered cases, all others were controls. After removing non-European samples, and those that failed quality control (QC) measures, 25,262 participants with European ancestry were retained, consisting of 7,004 cases who reported knee pain at any of the two visits (3,109 males and 3,895 females) and 18,239 controls who had no knee pain at any time point (8,571 males and 7,928 females) for the GWAS association analysis. 6,480,790 single nucleotide polymorphisms (SNPs) were used for the genome scan after post GWAS QC. We used the SAIGE software for testing the association between phenotype and genotype, with adjustment for sex, age, ethnicity, and first 50 PCs. We investigated the genomic variants that occur more frequently using FUMA, a web-based platform, which serves to annotate, prioritize, visualize and interpret GWAS results [102,103]. Positional, expression quantitative trait loci (eQTL) and chromatin interaction mapping are delivered by FUMA, in addition to analyses such as pathway analysis, gene-based associations, and tissue enrichment analysis.

4.0 Results

4.1 Back pain

5,323 individuals out of the 30,097 individuals in the CLSA Comprehensive cohort had back pain at baseline. They were defined at FU1 as 2,957 cases, and 2,366 controls (recovered). Logistic regression analysis was performed to test the association between each analgesic category and multi-visit back pain (Table 8), adjusting for age, sex, ethnicity, the intensity of pain, and BMI. A Venn diagram (Figure 8) shows the number of individuals with back pain and the distribution of analgesic groups.

Figure. 8. A Venn diagram of 5 groups of analgesics for back pain subjects in the CLSA shows the number of drugs consumed alone/ shared with other types of analgesics.

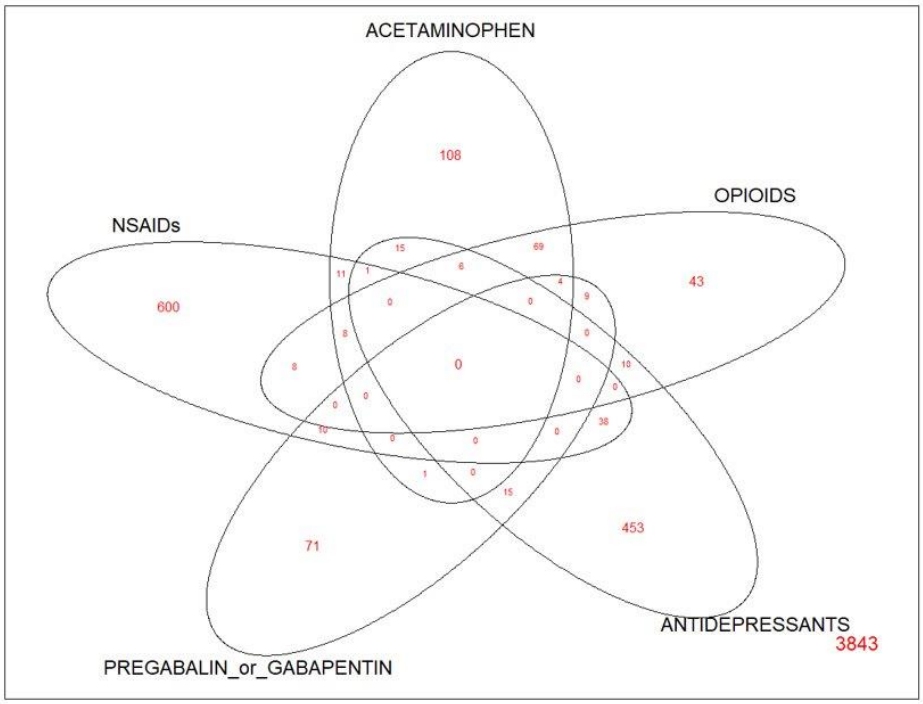


Table. 8. Logistic regression results for back pain subjects in the CLSA. The outcome is multi-visit back pain adjusted for age, sex, ethnicity, intensity, and BMI.

	Model1		Model2		Model3	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs	1.24 (1.05-1.47)	0.0108				
Acetaminophen			2.38 (1.76-3.28)	4.35E-08		
Opioids					4.5 (3.97-7.20)	1.85E-11
Anti-depressants						
Pregabalin/Gabapentin						
Age	1 (0.99-1.001)	0.677	1 (0.99-1.01)	0.66	1 (0.99-1.01)	0.429
Sex	0.85 (0.76-0.95)	0.004	0.86 (0.77-0.96)	0.009	0.85 (0.77-0.96)	0.006
Black	0.74 (0.34-1.20)	0.457	0.75 (0.34-1.65)	0.471	0.72 (0.32-1.61)	0.425
South.Asia	0.67 (0.38-1.20)	0.179	0.64 (0.36-1.15)	0.138	0.67 (0.37-1.20)	0.179
East.Asian	0.64 (0.35-1.14)	0.134	0.65 (0.36-1.16)	0.148	0.65 (0.36-1.17)	0.521
Other	1.13 (0.81-1.59)	0.482	1.12 (0.80-1.58)	0.502	1.12 (0.80-1.57)	0.47
INTENS	0.84 (0.80-0.89)	4.10E-10	0.84 (0.80-0.89)	3.26E-10	0.84 (0.80-0.89)	2.78E-10
BMI	1.03 (1.02-1.03)	3.24E-07	1.02 (1.02-1.03)	5.03E-07	1.02 (1.02-1.03)	3.92E-07

	Model4		Model5		Model6	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs					1.29 (1.09-1.52)	0.0035
Acetaminophen					1.66 (1.19-2.34)	0.0034
Opioids					3.42 (2.17-5.58)	2.94E-07
Anti-depressants	1.32 (1.1-1.59)	0.0036			1.34 (1.11-1.61)	0.0026
Pregabalin/Gabapentin			2.44 (1.58-3.88)	9.63E-05	2.26 (1.46-3.62)	0.0004
Age	1 (0.99-1.01)	0.418	1 (0.99-1.006)	0.584	1 (0.99-1.01)	0.506
Sex	0.86 (0.77-0.96)	0.0165	0.87 (0.78-0.97)	0.008	0.87 (0.78-0.97)	0.013
Black	0.79 (0.36-1.73)	0.512	0.87 (0.40-1.88)	0.544	0.7 (0.31-1.57)	0.383
South.Asia	0.68 (0.38-1.21)	0.196	0.67 (0.38-1.19)	0.188	0.69 (0.38-1.23)	0.21
East.Asian	0.63 (0.35-1.13)	0.137	0.58 (0.32-1.03)	0.122	0.67 (0.37-1.21)	0.188
Other	1.14 (0.82-1.60)	0.447	1.12 (0.80-1.57)	0.523	1.11 (0.79-1.57)	0.532
INTENS	0.84 (0.80-0.89)	5.15E-10	0.84 (0.80-0.89)	6.70E-10	0.84 (0.80-0.89)	3.22E-10
BMI	1.03 (1.02-1.04)	2.53E-07	1.03 (1.01-1.04)	2.35E-07	1.02 (1.01-1.03)	5.03E-06

Our analyses demonstrated that each analgesic class is strongly associated with multi-visit back pain in both the single medication models (Model 1- Model 5) and in the full model (Table 8). Upon further examination of the results, it can be noticed, however, going from the single model to the full model that the OR decreased for opioids (from 4.5 to 3.42), gabapentinoids (from 2.44 to 2.26), and acetaminophen (from 2.38 to 1.66), but increased for NSAIDs (from 1.24 to 1.29) and less so for anti-depressants (from 1.32 to 1.34). Therefore, subjects taking NSAIDs are at 1.29 times greater risk of multi-visit back pain than those not taking NSAIDs.

4.2 Jaw pain

Jaw pain analysis was performed on 2,215 individuals out of the 30,097 subjects from the CLSA Comprehensive cohort who had jaw pain at baseline, including 946 cases and 1,269 controls (recovered) at FU1. Logistic regression analysis was performed to test the association between each analgesic category and multi-visit jaw pain. We adjusted for age, sex, ethnicity, and intensity of pain. Results are displayed in Table 9. The breakdown of the number of individuals with jaw pain by medication consumption by analgesic class is shown in Figure 9.

Figure. 9. A Venn diagram of 5 groups of analgesics for jaw pain subjects in the CLSA shows the number of drugs consumed alone/ shared with other types of analgesics.

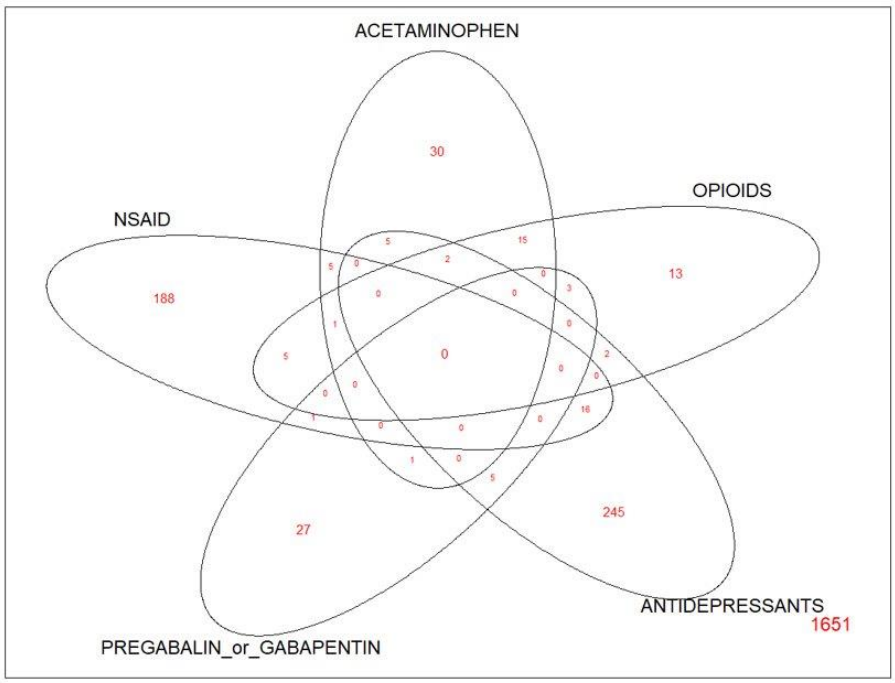


Table. 9. Logistic regression analysis results for jaw pain in the CLSA. Outcome: multi-visit jaw pain, adjusted for: age, sex, ethnicity, and intensity of pain.

	Model1		Model2		Model3	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs	1.01 (0.75-1.36)	0.943				
Acetaminophen			1.52 (0.89-2.59)	0.124		
Opioids					3.04 (1.58-6.16)	0.0012
Anti-depressants						
Pregabalin/Gabapentin						
Age	0.97 (0.96-0.98)	6.44E-13	0.97 (0.96-0.98)	3.89E-13	0.97 (0.96-0.98)	7.61E-13
Sex	0.49 (0.40-0.59)	1.41E-13	0.49 (0.41-0.59)	1.55E-13	0.49 (0.40-0.59)	9.12E-14
Black	0.66 (0.20-2.01)	0.469	0.66 (0.20-2.02)	0.478	0.67 (0.20-2.05)	0.49
South.Asia	0.98 (0.35-2.62)	0.963	0.96 (0.34-2.58)	0.939	0.10 (0.35-2.68)	0.995
East.Asian	0.45 (0.14-1.18)	0.128	0.45 (0.14-1.19)	0.133	0.46 (0.15-1.21)	0.139
Other	1.03 (0.65-1.62)	0.909	1.03 (0.65-1.62)	0.912	1.01 (0.63-1.59)	0.974
INTENS	0.96 (0.87-1.05)	0.389	0.96 (0.87-1.05)	0.387	0.96 (0.87-1.05)	0.364

	Model4		Model5		Model6	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs					1.02 (0.76-1.37)	0.897
Acetaminophen					1.11 (0.62-1.98)	0.718
Opioids					2.89 (1.44-6.13)	0.004
Anti-depressants	1.37 (1.06-1.78)	0.017			1.38 (1.07-1.80)	0.015
Pregabalin/Gabapentin			1.42 (0.73-2.78)	0.306	1.33 (0.68-2.64)	0.402
Age	0.97 (0.96-0.98)	2.07E-12	0.97 (0.96-0.98)	7.44E-13	0.97 (0.96-0.98)	2.80E-12
Sex	0.50 (0.41-0.60)	5.07E-13	0.50 (0.41-0.59)	1.68E-13	0.49 (0.41-0.60)	4.92E-13
Black	0.69 (0.20-2.10)	0.517	0.66 (0.20-2.02)	0.476	0.71 (0.21-2.16)	0.549
South.Asia	0.98 (0.34-2.62)	0.962	0.98 (0.35-2.64)	0.972	1 (0.35-2.68)	0.994
East.Asian	0.46 (0.15-1.21)	0.14	0.44 (0.14-1.17)	0.122	0.47 (0.15-1.23)	0.147
Other	1.03 (0.65-1.63)	0.887	1.03 (0.65-1.62)	0.914	1.01 (0.64-1.60)	0.956
INTENS	0.96 (0.87-1.05)	0.363	0.96 (0.87-1.06)	0.394	0.95 (0.87-1.05)	0.339

Our analyses demonstrated that opioids and anti-depressants are associated with multi-visit jaw pain in both the single medication models (opioids $p = 0.0012$, anti-depressants $p = 0.017$) and in the full model (opioids $p = 0.004$, anti-depressants $p = 0.015$) as it is presented in Table 9. Upon further examination of the results, it can be noticed that the OR is decreased in the full model. However, the OR for opioids decreased in the full model from 3.04 to 2.89, and for antidepressants, it decreased from 1.37 to 1.38. It is important to note that the sample size was smaller for jaw pain analysis in comparison with back pain (n = 2,215 compared to n = 5,323).

4.3 Knee pain

4,862 individuals out of the subjects from the 30,097 CLSA Comprehensive cohort reported knee pain at baseline. Almost 48.2% at FU1 recovered (2,345) and were considered controls, while 51.8% of individuals were cases (2,517). Logistic regression analysis was performed to test the association between each analgesic category and multi-visit knee pain, adjusting for age, sex, ethnicity, the intensity of pain, and BMI (Table 10). The medication consumption of individuals with knee pain by analgesic class is shown in Figure 10.

Figure. 10. A Venn diagram of 5 groups of analgesics for knee pain subjects in the CLSA shows the number of drugs consumed alone/ shared with other types of analgesics

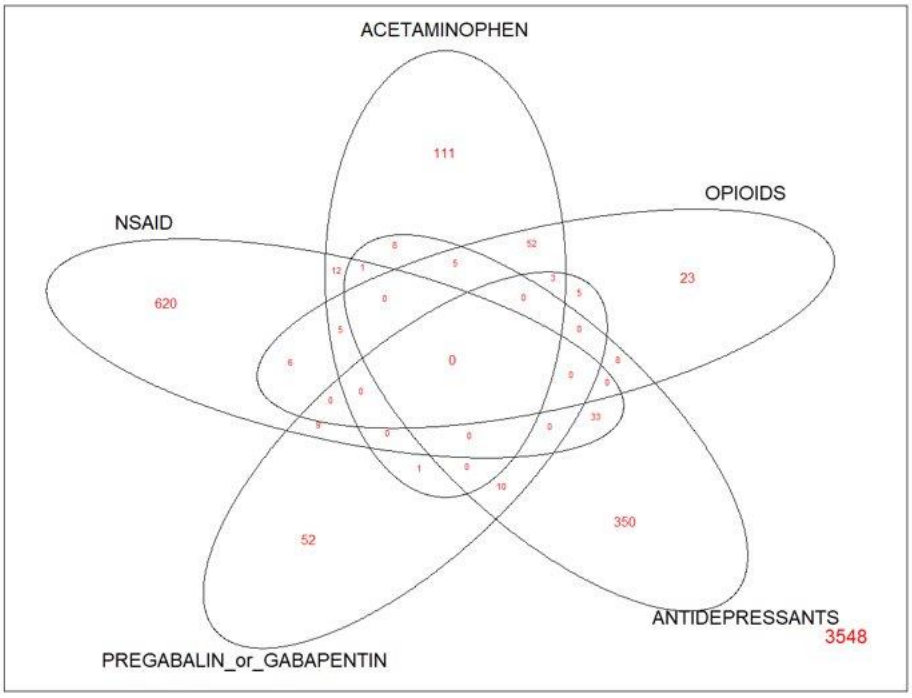


Table. 10. Logistic regression analysis results for knee pain in the CLSA. Outcome: multi-visit knee pain, adjusted for: age, sex, ethnicity, the intensity of pain, and BMI.

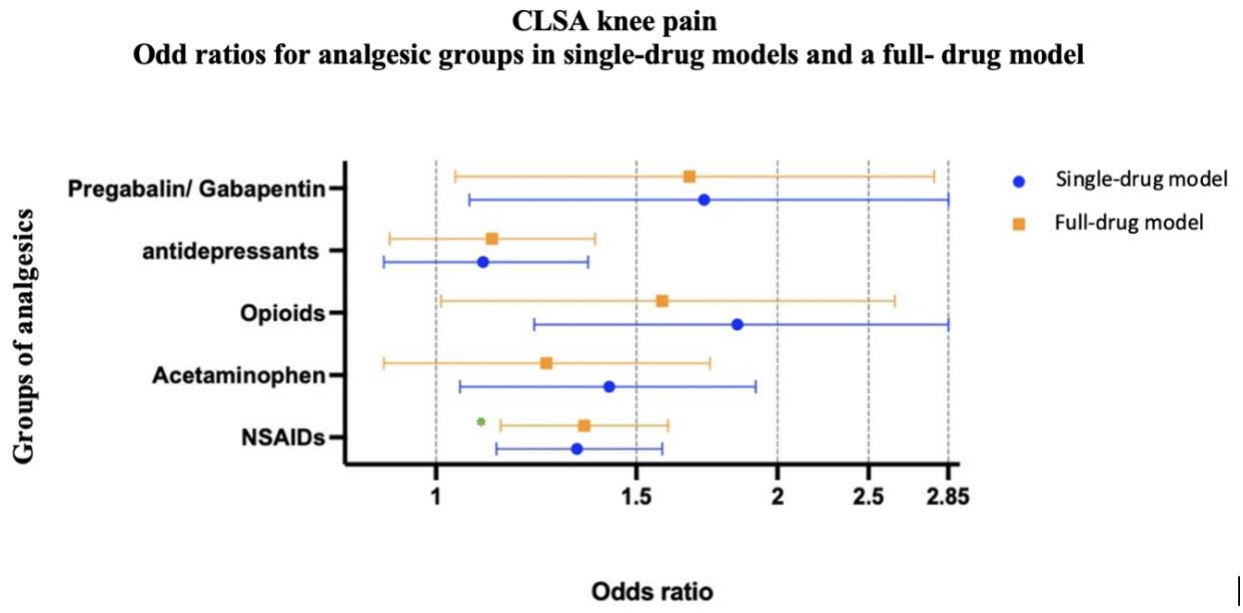
	Model1		Model2		Model3	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAID	1.33 (1.13-1.58)	0.000779				
Acetaminophen			1.42 (1.05-1.91)	0.0223		
Opioid					1.84 (1.22-2.82)	0.00403
Anti-depressants						
Pregabalin/Gabapentin						
Age	1.01 (1.001-1.013)	0.014	1.01 (1.001-1.013)	0.0098	1.01 (1.0-1.014)	0.0067
Sex	0.98 (0.87-1.10)	0.687	0.99 (0.88-1.11)	0.828	0.98 (0.88-1.10)	0.783
Black	1.23 (0.69-2.22)	0.485	1.22 (0.68-2.20)	0.511	1.23 (0.69-2.23)	0.481
South.Asia	1.16 (0.62-2.17)	0.647	1.15 (0.62-2.16)	0.663	1.17 (0.63-2.20)	0.616
East.Asian	0.81 (0.46-1.39)	0.44	0.8 (0.46-1.37)	0.413	0.8 (0.46-1.37)	0.417
Other	0.99 (0.70-1.41)	0.956	0.99 (0.70-1.40)	0.943	0.98 (0.69-1.39)	0.912
Intens	0.83 (0.78-0.88)	4.28E-11	0.83 (0.78-0.88)	5.21E-11	0.83 (0.78-0.88)	7.09E-11
BMI	1.05 (1.04-1.06)	<2e-16	1.05 (1.04-1.06)	<2e-16	1.05 (1.04-1.06)	<2e-16

	Model4		Model5		Model6	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAID					1.35 (1.14-1.60)	0.000438
Acetaminophen					1.25 (0.90-1.38)	0.186
Opioid					1.58 (1.01-2.53)	0.049
Anti-depressants	1.1 (0.90-1.36)	0.359			1.12 (0.91-1.38)	0.303
Pregabalin/Gabapentin			1.72 (1.07-2.82)	0.028	1.67 (1.04-2.74)	0.039
Age	1.01 (1.002-1.014)	0.007	1.01 (1.002-1.014)	0.0093	1.01 (1.002-1.014)	0.0117
Sex	0.99 (0.88-1.11)	0.875	0.99 (0.88-1.11)	0.846	0.98 (0.88-1.11)	0.795
Black	1.24 (0.70-2.24)	0.468	1.24 (0.70-2.24)	0.465	1.24 (0.70-2.25)	0.464
South.Asia	1.16 (0.63-2.18)	0.638	1.17 (0.63-2.19)	0.626	1.17 (0.63-2.20)	0.615
East.Asian	0.79 (0.45-1.36)	0.4	0.79 (0.45-1.35)	0.384	0.82 (0.47-1.42)	0.487
Other	0.98 (0.69-1.40)	0.929	0.98 (0.69-1.40)	0.929	0.99 (0.70-1.41)	0.959
Intens	0.83 (0.78-0.88)	5.41E-11	0.83 (0.78-0.88)	5.87E-11	0.83 (0.78-0.88)	5.56E-11
BMI	1.05 (1.04-1.06)	<2e-16	1.05 (1.04-1.06)	<2e-16	1.05 (1.04-1.06)	<2e-16

In model 1, individuals with knee pain were at 1.33 times greater risk of multi-visit knee pain if they reported taking NSAIDs (p = 0.00078) compared to those not taking NSAIDs. When adjusting for the usage of other analgesics in model 6, NSAIDs were significantly associated with multi-visit knee pain (OR = 1.35; p = 0.0004). Acetaminophen appeared as a statistically significant predictor in the single model (p = 0.022) but when adjusted for the usage of other analgesics, it did not show significance (p = 0.186). Opioids and gabapentinoids also presented significant p-values

in the single model (p = 0.004, p = 0.028 respectively), while in the full model, after adjustment for all other medications, the OR decreased for both (from 1.84 to 1.58; 1.78 to 1.67 respectively). The ORs by analgesic class are displayed in Figure 11.

Figure. 11. Odd ratios for 5 analgesic categories in both single-drug models and a full-drug model for CLSA subjects with knee pain. The analgesic category is given on the *y*-axis, and the odds ratio (log scale) is given on the *x*-axis. * Asterisks refer to significant effects.



4.4 Replication of knee pain results in UKB.

7,110 individuals from the UKB cohort answered "Yes" for knee pain at baseline; 46,8 % were thus considered cases (3,779) with knee pain at baseline and any of v1 or v2; while 53.2% of controls recovered and reported no knee pain (3,779) at any of v1 or v2. Logistic regression analysis was performed to test the association between each analgesic category and multi-visit

knee pain. We adjusted for age, sex, ethnicity, number of pain sites, and BMI (Table 11). A number of pain sites are the number of knee pain sites counted from the UKB dataset, which has been used as a proxy for pain intensity.

The breakdown of the number of individuals with knee pain by medication consumption by analgesic class in UKB at baseline is displayed in Figure 12.

Figure. 12. A Venn diagram of 5 groups of analgesics for knee pain subjects in the UKB shows the number of drugs consumed alone/ shared with other types of analgesics.

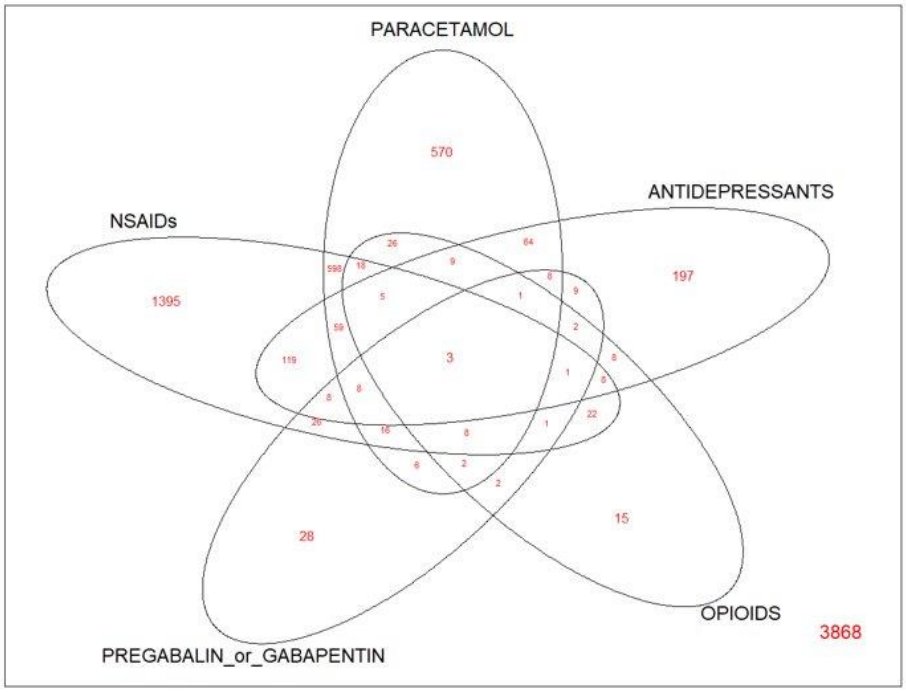


Table. 11. Logistic regression analysis results for knee pain in the UKB. The outcome is multi-visit knee pain, adjusted for: age, sex, ethnicity, number of pain sites, and BMI.

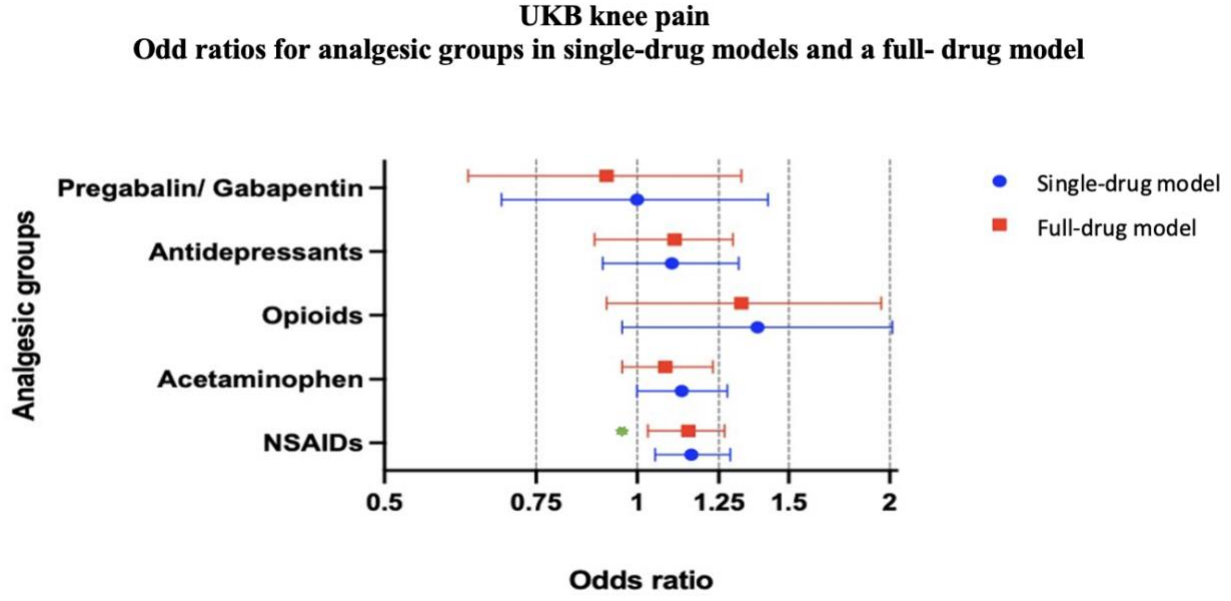
	Model1		Model2		Model3	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs	1.16 (1.05-1.29)	0.00432				
Paracetamol			1.13 (1-1.28)	0.0557		
Opioids					1.39 (0.96-2.01)	0.0822
Anti-depressants						
Pregabalin/ Gabapentin						
Age	1.01 (1.0 -1.02)	0.00262	1.01 (1.0 -1.02)	0.0007	1.01 (1.0 -1.02)	0.001
Sex	0.97 (0.88-1.06)	0.492	0.98 (0.89-1.08)	0.679	0.97 (0.88-1.07)	0.566
Mixed	0.58 (0.25-1.25)	0.172	0.58 (0.25-1.25)	0.174	0.58 (0.25-1.27)	0.183
Asian	0.91 (0.57-1.44)	0.69	0.92 (0.57-1.45)	0.717	0.92 (0.57-1.45)	0.716
Black	1.43 (0.76-2.76)	0.272	1.42 (0.75-2.74)	0.284	1.42 (0.75-2.73)	0.287
Other	0.96 (0.56-1.64)	0.882	0.95 (0.56-1.62)	0.855	0.95 (0.55-1.61)	0.835
pain_site	1.21 (1.16-1.26)	< 2e-16	1.21 (1.16-1.25)	< 2e-16	1.21 (1.17-1.26)	< 2e-16
BMI	1.05 (1.03-1.06)	< 2e-16	1.05 (1.04-1.06)	< 2e-16	1.05 (1.04-1.06)	< 2e-16

	Model4		Model5		Model6	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
NSAIDs					1.15 (1.03-1.27)	0.0113
Paracetamol					1.08 (0.96-1.23)	0.206
Opioids					1.33 (0.92-1.95)	0.129
Anti-depressants	1.1 (0.91-1.32)	0.341			1.10 (0.89-1.3)	0.447
Pregabalin/ Gabapentin			1.0 (0.69-1.43)	0.983	0.92 (0.63-1.33)	0.64
Age	1.01 (1.0 -1.02)	0.0008	1.01 (1.0 -1.02)	0.0008	1.01 (1.0 -1.02)	0.0028
Sex	0.98 (0.89-1.07)	0.616	0.97 (0.88-1.07)	0.562	0.98 (0.88-1.08)	0.615
Mixed	0.58 (0.25-1.25)	0.176	0.58 (0.25-1.26)	0.177	0.58 (0.25-1.25)	0.172
Asian	0.92 (0.58-1.46)	0.721	0.92 (0.57-1.45)	0.714	0.91 (0.60-1.45)	0.701
Black	1.44 (0.76-2.77)	0.266	1.43 (0.76-2.75)	0.275	1.43 (0.75-2.75)	0.277
Other	0.95 (0.55-1.62)	0.847	0.95 (0.55-1.62)	0.848	0.96 (0.56-1.63)	0.869
pain_site	1.21 (1.17-1.26)	< 2e-16	1.22 (1.17-1.26)	< 2e-16	1.20 (1.15-1.25)	< 2e-16
BMI	1.05 (1.04-1.06)	< 2e-16	1.05 (1.04-1.06)	< 2e-16	1.04 (1.03-1.05)	< 2e-16

From model 1, subjects with knee pain were at 1.16 times greater risk of multi-visit knee pain if they reported NSAIDs usage than if they were not taking NSAIDs $p = 0.0043$).

In the full model, NSAIDs (and no other class) still was significant ($p = 0.01$, OR =1.15). No other drugs showed statistically significant association either in the single-drug model or in the full model. These results are presented on the log scale for odd ratios in Figure 13.

Figure. 13. Odd ratios for 5 analgesic categories in both the single-drug models and a full-drug model for UKB knee subjects. The analgesic category is given on the *y*-axis, and the odds ratio (log scale) is given on the *x*-axis. * Asterisks refer to significant effects.



4.5 GWAS knee pain in CLSA

GWAS-associated analysis: The FUMA web application was used as the main annotation tool to interpret GWAS results through links made with external data sources to provide functional annotations. FUMA results showed no associations at the GWAS significance threshold of *p*-value 5 x 10^-8, so we changed this value to a less strict cutoff (1 x 10^-5) with the aim of considering suggestive signals in the context of previous findings from publicly available databases. A Manhattan plot for GWAS summary statistics was generated (Figure 14). 4 SNPs in chromosome 11 were associated with knee pain with this relaxed threshold addressing multiple testing. The SNPs showed high linkage disequilibrium (LD, r^2: 0.7-1), suggesting that these reflect a single genetic effect (Table 12). These variants were intergenic in the gene Ribonuclease/Angiogenin

Inhibitor 1 (*RNH1)* and intronic in the gene Phosphatidylserine Synthase 2 (*PTDSS2)* (Figure 15).

The *RNH1* was previously shown to be associated with BMI in multiethnic populations[104].

Table. 12. SNPs in LD. 4 SNPs in chromosome 11 with high linkage disequilibrium (r^2: 0.7-1)

Uniq ID	rsID	ch	pos	non-effect-allele	effect-allele	MAF	gwasP	Beta	SE	Genomic locus	r2	IndSigSNP	Nearest gene	dis	position
11:475257:A:G	rs370804070	11	475257	A	G	0.02485	NA	NA	NA	1	0.709608	rs139498822	PTDSS2	0	intronic
11:490196:C:T	rs139498822	11	490196	C	T	0.01789	1.81E-06	0.35	0.07	1	1	rs139498822	PTDSS2	0	intronic
11:509726:A:T	rs140921741	11	509726	T	A	0.02485	4.21E-06	0.31	0.07	1	0.709608	rs139498822	RNH1	2425	intergenic
11:513640:C:T	rs117752128	11	513640	C	T	0.04573	3.83E-06	0.21	0.04	1	1	rs117752128	RNH1	6339	intergenic

Figure. 14. Manhattan Plot (GWAS summary statistics) for knee pain in CLSA, filtering was performed only for SNPs, *p*-value ≥ 1e-5.

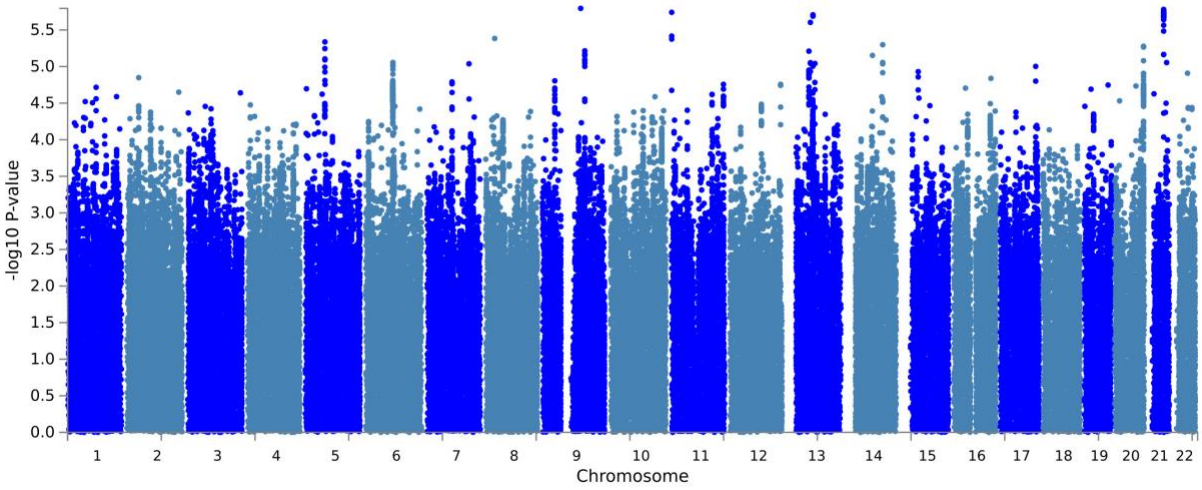
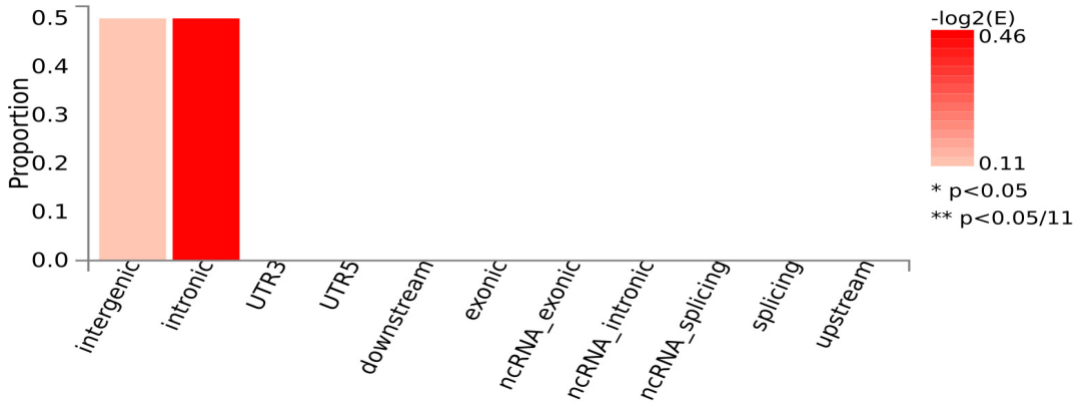
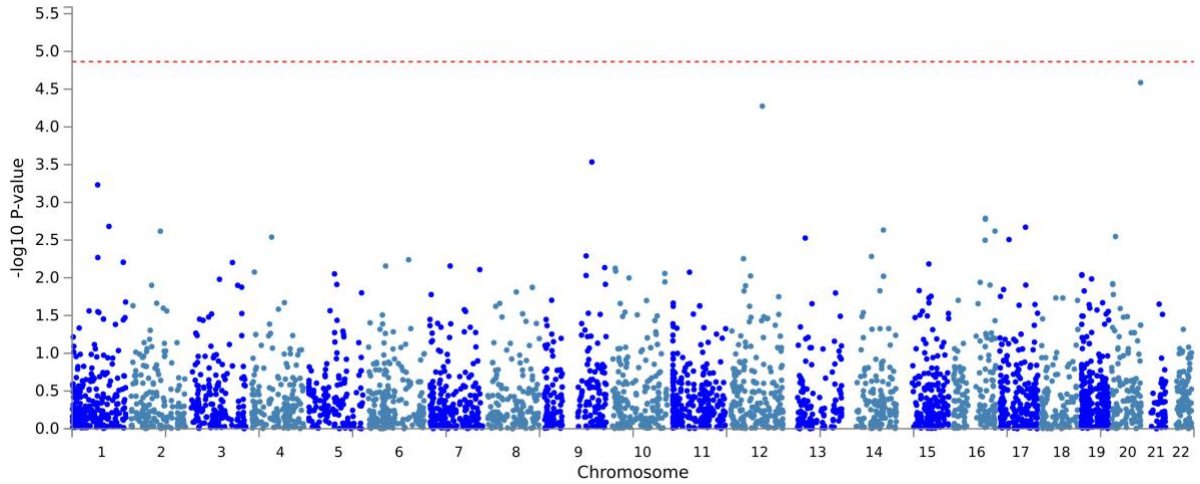


Figure. 15. Functional consequences of SNPs associated with knee pain within the gene locus. Intergenic in *RNH1* and intronic in the gene *PTDSS2*.



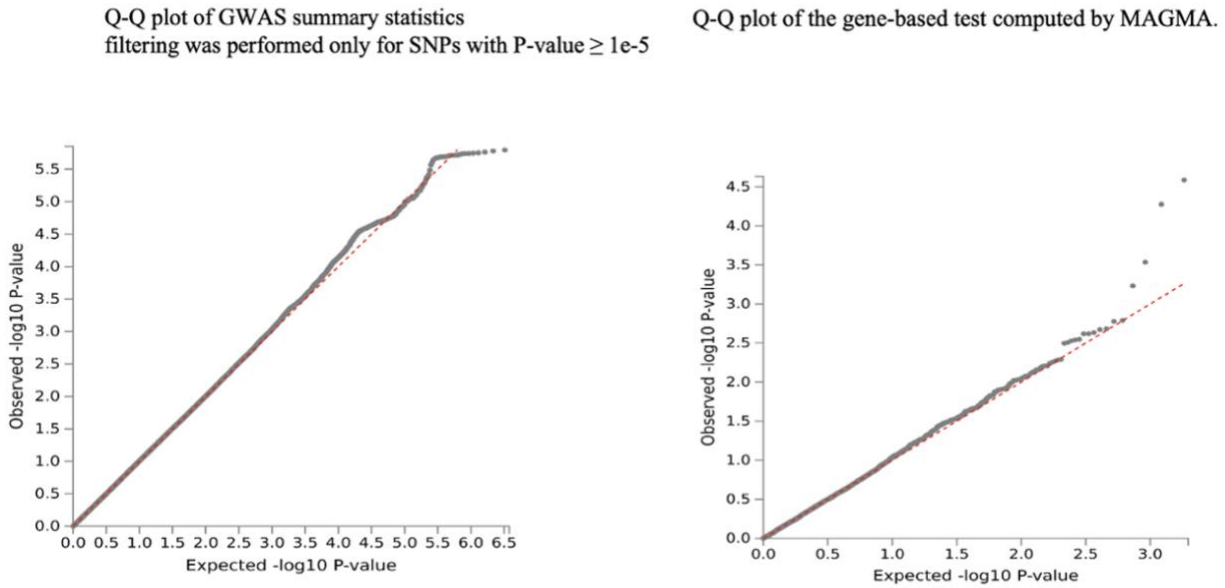
We also performed a gene-based analysis, where the *p*-values from the entire GWAS are plotted in genomic order by chromosomal position on the *x*-axis and by *p*-value on the *y*-axis (Figure 16). The value on the *y*-axis represents the $-\log 10$ of the *p*-value. This analysis produced no significant results.

Figure. 16. Manhattan plot of the gene-based test computed by MAGMA gene analysis, gene-set analysis, and gene-property analysis. The gene-based p-value is shown on the left side bar on the -log 10 scale. SNPs associations across chromosomes 1–22 are displayed. Input SNPs were mapped to 3660 protein-coding genes. Genome-wide significance (red dashed line in the plot) was defined at P = 0.05/3660 = 1.366e-5.



Quantile-quantile (Q_Q) plots were also generated (Figure 17). No evidence was found for inflation of the Q-Q plots which appeared without observed deviation of the observed distribution compared to expected with the genomic inflation factor λgc= l.002. So far, no cryptic relatedness was identified among the subjects that might affect our association. (i.e., kindship among the cases or controls[105]).

Figure. 17. Q-Q plots for knee pain in CLSA.



Only one distinct genomic locus was found to be associated with long-lasting knee pain in the CLSA cohort. This locus was in the *PTDSS2* gene in chromosome 11 with a *p*-value of 1.81×10^{-6} for rs139498822, containing 4 SNPs and harboring two independent risk signals. Table 13 displays these data. Genomic region[106] was presented in Figure 18.

Table. 13. Genomic Risk Loci (FUMA result), one locus in chromosome 11with 4 SNPs associated with knee pain in CLSA, and two risk signals. Risk allele C>T.

Genomic lucus	uniq ID	rsID	chr	pos	p-value	start	end	nSNPs	nGWAS SNPs	nIndSig SNPs	IndSig SNPs	nLead SNPs	LeadSNPs
1	11:490196: C:T	rs139498822	11	490196	1.8146E-06	475257	513640	4	3	2	rs139498822; rs117752128	1	rs139498822

Figure. 18. Genomic region for rs139498822. It is shown on the position 490196 on the gene *PTDSS2* extended to a 38kb.

To identify tissue specificity of the identified association results, MAGMA gene-property analysis[107] tested relationships between tissue-specific gene expression profiles and knee pain-gene associations. The gene-property analysis is based on a regression model including sex, age, ethnicity, and first

50 PCs. A heat map demonstrated that the corresponding genes were expressed in adipose-subcutaneous and visceral tissues, glands, tibial nerve, and many other tissues as shown in Figure 19. Next, we performed partitioned heritability analyses using stratified LD score regression[108], to

examine whether the observed heritability was enriched in any particular tissue using a wide range of tissue and cell types[109]. Our analysis did not identify any enrichment in any of the tested tissues at a 10% false discovery rate (FDR), however, the hist expression was found in the nerve (Figure 20)

Figure. 19. The Heatmap of differential gene expressions represents both *PTDSS2* and *RNH1* in 54 tissue types.

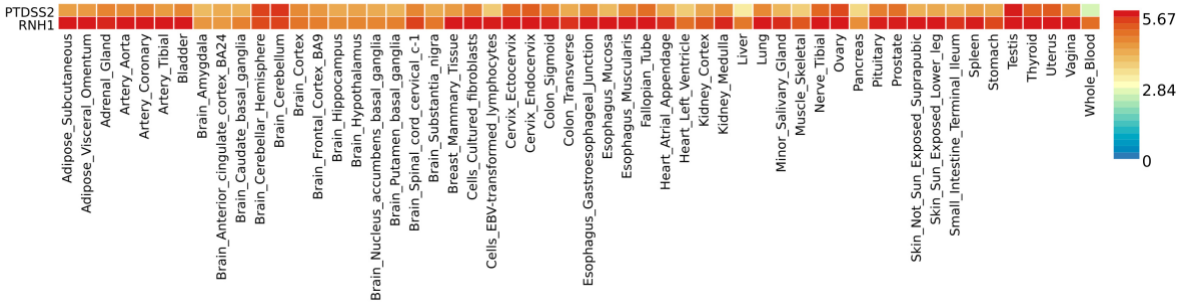
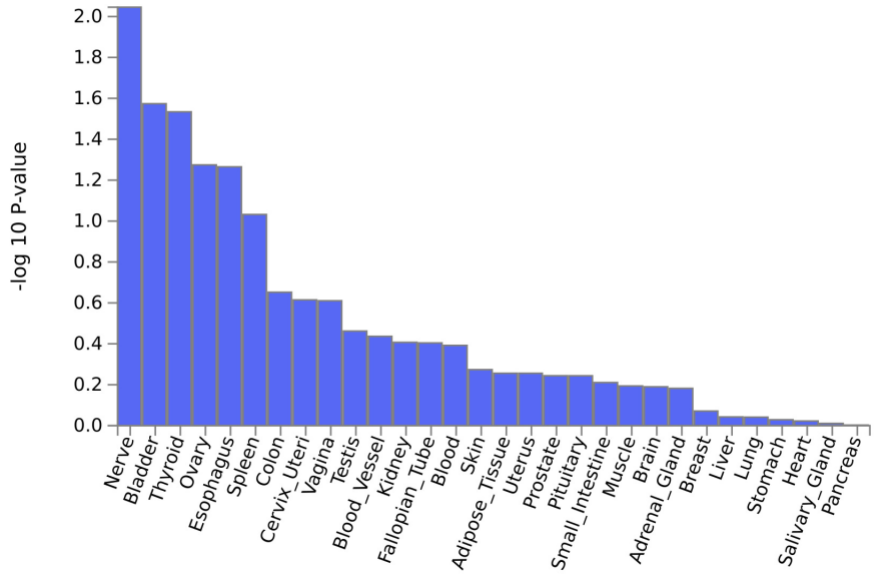


Figure. 20. Partitioned heritability for any knee pain. The statistical threshold of significance is highlighted at the FDR 10% level with a horizontal red line. Overview of the results of the MAGMA tissue enrichment analysis as implemented in FUMA using GTEx data for 54 tissue types. Nominal -log 10 *p*-value are shown on the *y*-axis. None of the investigated tissues showed a significant enrichment.



5. Discussion

In this study, we investigated the relationship between taking NSAIDs and chronicity of pain, trying to explain whether NSAIDs are a risk factor, using multi-visit site-specific pain as a proxy to indicate chronic pain. We compromised this definition because chronic pain as the pain persists for more than three months was hard to define in the CLSA. The data did not specify the duration of each body site-specific pain, except for back pain. In our work, to keep the consistency with other pain types, we considered the frequency of reporting pain as an index to chronicity, even though, it might still include some few acute cases. This misclassification was not avoidable and will not create a bias or false positives as we assumed since most of the multi-visit site-specific pain cases will be chronic.

Our first objective was to study the chronicity of pain for different pain types in groups of people who suffered from pain at baseline. This was done by focusing on reported pain at the same body site after the first visit (3 years later). We used different groups of analgesics, as a second objective, to see if this relationship was related to NSAIDs. This objective did not consider the efficacy of the medications, but rather the role of maintaining pain for multiple visits. Our third objective was to replicate our findings in another cohort the UKB. Finally, we conducted a GWAS to identify the genetic variants associated with body site-specific pain.

To achieve these objectives, we analyzed data from the CLSA, focused on older adults, by extracting different variables related to pain. The study involved a baseline and one follow-up assessment visit. Among the 5 types of musculoskeletal pain and headache conditions in the CLSA: (back pain, knee pain, hip pain, jaw pain, and headache), we excluded hip pain because this pain type was defined as pain induced by exercise, experienced at the hip, leg, or calf during a 4 m walk; also, the headache was excluded because this variable was limited to migraine, which

is a neurobiological disorder and not idiopathic pain, and no further questions allowed us to create a generic phenotype for headache.

We focused on three types of musculoskeletal pain: back pain, jaw pain, and knee pain with prevalence respectively 35%, 8%, and 20%.

First, we identified subjects who reported site-specific pain at baseline when they were first enrolled in the CLSA. Then, we questioned the prospective effects of analgesic groups on the incidence of multi-visit for this body site-specific pain.

Starting with back pain, our results showed in a full model including all medication classes, multi-visit back pain had a strong association with taking analgesics for all classes. Back pain subjects taking NSAIDs are at 1.29 times greater risk of multi-visit back pain than those not taking NSAIDs. The high *p*-value for opioids in the results displayed opioids to be associated with the outcome, but it is mostly related to the severity of pain, or in other words, it will be caused by the indication for which the opioids were used (association by indication).

For jaw pain, NSAIDs and antidepressants were in the right direction of the association, but the sample size was very small, and the statistical power was not high enough to determine the right effects. We were not able to determine whether NSAIDs are a risk factor in jaw pain model, and large sample size is required.

For knee pain subjects, NSAIDs were identified as a risk factor for multi-visit knee pain (risk factor 1.33 times more for those taking than not taking NSAIDs). This effect remained significant even after controlling for a number of relevant covariates (1.35 times more in the full model).

Among all the above results from the CLSA analyses, the strongest contribution of NSAIDs towards reporting multiple visits was in knee pain, so we replicated these significant findings related to knee pain in the UKB, and similarly, NSAIDs (and no other class) were identified as a

risk factor for multi-visit knee pain. Subjects with knee pain were at 1.15 times greater risk of multi-visit knee pain if they reported taking NSAIDs compared to not taking NSAIDs.

We can state that individuals with body site-specific pain taking NSAIDs are at a higher risk of still having pain 2-3 years later, compared to individuals taking other analgesics. The study design does not allow us to directly probe the causal pathways of the NSAIDs. The CLSA and UKB questionnaires did not help to create a clear definition for chronic pain. Nonetheless, we added intensity of pain as an indicator for pain assessment and for the selection of medications, but still could not have a concrete statement for the causality between NSAIDs and chronic pain, in addition to the insufficient sample size. However, applying Hill's causation criteria[110], this work had stated few of them: consistency, strength of association and specificity.

The association is consistent since the results were replicated in different cohort, and this causal relationship would be expected to be found consistently among different populations. In considering the strength (effect size), the small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal. Also, these results addressed the specificity of NSAIDs among other analgesic groups as a risk factor with a causal impact on developing chronic pain in contrast to other analgesic groups.

Furthermore, to identify the genetic variants associated with knee pain, we conducted a GWAS using the CLSA cohort. We defined knee pain as "During the past 4 weeks, have you had knee pain on most days" based on the information available from the study questionnaire. No evidence was found for inflation of the test statistics ($\lambda GC = 1.002$). We identified 4 SNPs associated with knee pain within one locus. By using dbSNP, we explored the highest risk allele frequency of the lead SNP rs139498822, MAF: T=0.055/33 (Northern Sweden. The frequency in other ethnicities

was T=0.0034, 0.000) for African and Asian respectively, compared to the European (T= 0.02457, we can state that European descent individuals might suffer more from knee pain than non-European descent individuals based on observations of frequency differences [106].

FUMA results showed two genes identified as *PTDSS2* and *RNH1*were implicated more in the nervous system.

PTDSS2 is a protein-coding gene. The protein encoded by this gene catalyzed the conversion of phosphatidylethanolamine to phosphatidylserine, a structural membrane phospholipid that functions in cell signaling, blood coagulation, and apoptosis. The link between phospholipid composition and altered cellular functions of obesity has been proved and *PTDSS2* was positively correlated with BMI[111].

The *RNH1* was previously shown to be associated with BMI in 3 other GWASs[112]. BMI is the most commonly used index to characterize obesity[113]. Having additional weight puts extra pressure on the knees, which can result in chronic pain. We can conclude that knee pain is influenced by obesity and by the effects of these two genes.

Limitations

There are several limitations to our study. First, the huge challenge in longitudinal studies is to motivate the participants and keep them engaged. In the CLSA, participants moved to other locations or sometimes withdrew from the study. Other reasons for the loss of participants are they might develop health-related barriers such as hearing impairment, vision loss, speech/language problems or they might experience cognitive decline. The retention rate and mortality rate, at the end of the FU1, 4.3% of participants had withdrawn from the active data collection and 2.75 died

since their baseline assessment. (4.1 % in the Tracking cohort and 1.85 in the Comprehensive cohort)[114]. In my study, these missing data affected all analyses, and the sample sizes were smaller. Second, the Comprehensive cohort was designed to recruit participants from an area of 25-50 km. The data reflected only these regions and not the 10 provinces of Canada.

Third, although the medication data were obtained by trained nurses in CLSA and UKB, the manual entry derived many varied data entry issues, as in CLSA, many entries did not match DPD. Also, the complex combination products, nondrug products, and many international products, all limited the accuracy of information. The manual classification was needed to complete the classification.

Fourth, the questionnaires in the CLSA did not clear up concrete definitions which is the main standard for the research studies. They did not provide a lot of information or details that could be helpful to develop more models or specific analyses. We were not able to define the time duration for the pain in each body site-specific pain except for back pain, and as a result, we could not classify pain types as acute or chronic as may be undertaken in the UKB. Also, the quantifying and adjusting misclassification for our definition of chronic pain was not possible, we hope it is not too much and we assume it is not going to create a bias or false positivist.

Fifth, there is no follow-up for medications-taken participants, or a protocol was mentioned, we don't know about patients' regularity taking their medications, are there breaks, for how long they were taking that drug? These questions are important in following up on the pain status. To overcome these issues, further assessments will be necessary to address more precise and indicated questions in the future.

Finally, we investigated the effect of taking NSAIDs as a risk factor to develop chronic pain in the general population from observational cohorts. Chronic pain development would be more aptly

considered within an experimental study design such as randomized controlled trials to examine cause-effect relationships, causality, randomization and reduce any bias.

Conclusion

Individuals with body site-specific pain taking NSAIDs are at a higher risk of still having chronic pain 2-3 years later, compared to individuals taking other analgesics. These results imply that the detrimental effect of NSAIDs on pain chronicity is independent of reported pain bodily site and stage of pain. Further studies are needed to investigate the timing of NSAID treatment and to understand the actual drug-related risk by moving into newer approaches or alternative strategies for pain management with more awareness of NSAID usage. Furthermore, various indications for NSAIDs still need to be investigated.

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Figure 2. CLSA study design, Canadian longitudinal study of aging protocol, https://clsa-elcv.ca/doc/511