The effect of treatment with the mast cell stabilizer ketotifen fumarate on chronic widespread pain in teenagers

Isha Tusharbhai Gandhi

Master of Dental Sciences – (Thesis)

Montreal, Quebec, Canada

May 2022

A thesis submitted to McGill University in partial fulfilment of the degree of Master of Dental Sciences

Table of Content

I would like to dedicate my Thesis to all teenagers who are suffering from chronic widespread pain at their younger ages, which has been a major motivation for me to find better solutions for them throughout my master's program.

Abstract

Background: Chronic widespread pain (CWP) is an idiopathic condition that affects 6 to 20% of the pediatric population. Children who have CWP are more likely to experience depression, anxiety, social isolation, lower life quality and are less capable to attend school. Mounting evidence indicates that mast cell (MC) regulated signalling pathways are essential mediators of pain, and treatment with MC stabilizers has been reported to improve pain. We thus hypothesized that treatment with ketotifen fumarate (KF), a MC stabilizer and non-competitive histamine antagonist (H1-receptor), may be beneficial for the overall well-being of patients with CWP.

Objectives: To test the effect of KF on the global health status of teenagers with CWP after 16 weeks of treatment. Our secondary goal was to test its effect on pain-related and physical and emotional functioning measures. We also explored if a response to treatment with KF correlates with MC activity, as determined by plasma levels of histamine and tryptase.

Methods: We conducted a proof-of-concept randomized, placebo-controlled, double-blinded, parallel trial of KF (6 mg/day) to treat CWP in 44 teenagers (age 12-19 years; 11 dropouts; n=33). At baseline and follow up visits 4, 8, 12, and 16 weeks later, patients answered surveys on their global health status and their pain intensity and duration, pain-related disability, mood, and sleep quality. Blood samples were collected at baseline and histamine and tryptase plasma levels were determined using ELISA. We used the Mann-Whitney U test to test the effect of treatment on the study outcomes (α=0.05). We also used the Kruskal-Wallis test to test for repeated measure differences between groups. We used the Wilcoxon Rank Sum Test to compare baseline and week 16 outcomes within groups. We used quantile regression to determine if baseline plasma levels of histamine or tryptase predict study outcomes at week 16.

Results: After 16 weeks of treatment, the global health status (primary outcome), pain intensity (except in borderline average pain intensity ($p = 0.05$)) and duration, pain-related disability, mood, and sleep quality measures of the KF group were not different from those of the placebo group. There were no group differences when considering measures assessed at all study visits. Within-group analyses revealed that the KF group displayed improvements in their pain-related disability (*P*-value = 0.008), anxiety (*P*-value = 0.001), and anxiety and depression (*P*-value = 0.000), while the placebo group displayed improvements in their average pain index (*P*-value = 0.027) and, pain-related disability (*P*-value = 0.033). We also found a significant effect of baseline histamine levels on average pain intensity (*P*-value = 0.032) of the KF group, such that higher levels predicted lower average pain intensity after 16 weeks of treatment. Tryptase levels did not predict any of the study outcomes.

Conclusion: Treatment with KF for 16 weeks at 6 mg/day does not improve the global health status, pain intensity and duration, pain-related disability, mood, and sleep quality compared to treatment with placebo. Nonetheless, our findings that higher baseline levels of histamine predict lower average pain intensity and duration at the end of treatment in the KF group could suggest that this effect is due to the MC-stabilizing effect of KF.

Résumé

Contexte: La douleur chronique généralisée (DCG) est une douleur idiopathique qui touche 6–20% de la population pédiatrique. Les enfants atteints de DCG sont plus susceptibles de souffrir de dépression, d'anxiété, d'isolement social, d'une qualité de vie inférieure et ils sont moins capables d'aller à l'école. En outre, Il y a des évidences qui indiquent que les voies de signalisation régulées par les mastocytes sont des médiateurs essentiels de la douleur, et il a été prouvé que le traitement avec des stabilisateurs des mastocytes améliore la douleur. Nous avons donc émis l'hypothèse que le traitement par le fumarate de kétotifène (KF), qui est un stabilisateur des mastocyteset un antagoniste non compétitif de l'histamine (récepteur H1), pourrait être bénéfique sur l'états général des patients de la DCG.

Objectifs: Examiner l'effet du KF sur l'état de santé global des adolescents avec la DCG après 16 semaines du traitement. Notre objectif secondaire était de tester l'effet du KFsur les mesures liées à la douleur et au fonctionnement physique et émotionnel. Nous avons également exploré si la réponse au traitement par KF était corrélée à l'activité des mastocytes, tels que déterminé par les concentrations plasmatiques de l'histamine et de la tryptase.

Méthodes: Nous avons mené un essai de preuve de concept randomisé, contrôlé par placebo, en double aveugle et parallèle du KF (6 mg/jour) pour traiter la DCG chez 44 adolescents (âgés de 12 à 19 ans ; 11 abandons ; n = 33). Lors des visites de référence et de suivi 4, 8, 12 et 16 semaines plus tard, les patients ont répondu aux questionnaires concernant leur états de santé global, l'intensité et la durée de la douleur, l'incapacité liée à la douleur, l'humeur et la qualité du sommeil. Des échantillons de sang ont été prélevés au début de l'étude et les concentrations plasmatiques de l'histamine et de la tryptase ont été mesurées par ELISA. Nous avons utilisé le test U de Mann-

Whitney pour tester l'effet du traitement sur les résultats de l'étude ($\alpha = 0,05$). Nous avons également utilisé le test de Kruskal-Wallis pour tester les différences de mesures répétées entre les groupes. Nous avons utilisé le test Wilcoxon Rank Sum pour comparer les résultats au début de l'étude et à la semaine 16 au sein des groupes. Nous avons utilisé la régression quantile pour déterminer si les concentrations plasmatiques initialles de l'histamine ou de la tryptase prédisent les résultats de l'étude à la semaine 16.

Résultats: Après 16 semaines du traitement, l'état de santé global (résultat principal), l'intensité et la durée (sauf en cas d'intensité de douleur moyenne limite ($p = 0,05$)) de la douleur, l'incapacité liée à la douleur, l'humeur et les mesures de la qualité du sommeil du groupe KF n'étaient pas différents de ceux du groupe placebo. En plus, Il n'y avait aucune différence entre les groupes lors de l'examen des mesures évaluées à toutes les visites d'étude. Cependant, les analyses au sein des groupes ont révélé que le groupe KF présentait des améliorations de son incapacité liée à la douleur (valeur $P = 0,008$), de l'anxiété (valeur $P = 0,001$) et de l'anxiété et de la dépression (valeur $P = 0,000$), tandis que le groupe placebo présentait une amélioration de l'indice de douleur moyen (valeur $P = 0,027$) et de l'incapacité liée à la douleur (valeur $P = 0,033$). Nous avons également trouvé un effet significatif des niveaux d'histamine de base sur l'intensité moyenne de la douleur (valeur P = 0,032) du groupe KF, disant que les niveaux plus élevés de l'histamine ont prédit une intensité moyenne de la douleur et un indice après 16 semaines de traitement. Par contre, les niveaux de tryptase n'ont prédit aucun des résultats de l'étude.

Conclusions: Le traitement par KF pendant 16 semaines à 6 mg/jour n'améliore pas l'état de santé global, l'intensité et la durée de la douleur, l'incapacité liée à la douleur, l'humeur et la qualité du sommeil par rapport au traitement par placebo. Néanmoins, nos découvertes que les niveaux de

base de l’histamine plus élevés prédisent une intensité et une durée moyennes de la douleur plus faibles à la fin du traitement dans le groupe KF pourraient suggérer que cet effet est dû à l'effet MC-stabilisateur de KF.

Acknowledgments

I am very much thankful to my supervisor Dr Carolina Berlado Meloto for allowing me to work under her supervision. Her insightful advice and constructive feedback helped me a lot to grow academically, professionally, and personally. It was a valuable experience for me as a young researcher in the field of pain and neuroscience to work with her. Dr Meloto's great experience in the field of pain and human genetics help me a lot to understand my project more interestingly. Her insightful comments and suggestions always helped me to boost my scientific knowledge. Her constant support and advice on my work motivate me a lot to steer in the right direction. Her encouragement to participate in different conferences and fellowship applications has strengthened my leadership and interpersonal skills.

I would also like to thank my supervisory committee member Dr Ana velly and Dr Marc o Martel to give their valuable time and suggestions for my project. Their feedback and guidance helped me to learn about my project from a different perspective.

I would also like to thank the Alan Edward Centre for Research on Pain's staff, researchers and scientists for their guidance and for sharing the scientific knowledge. Also, I am thankful to the funding agency (Réseau de recherche en santé buccodentaire et osseuse (RSBO) and the Faculty of Dental Medicine and Oral Health Sciences at McGill University for providing funds for my project. Also, many thanks to Mrs Crystal Noronha and Despoina Moirakidou for providing all the necessary administrative support to complete my degree without any obstacles.

I would especially like to thank Dr Melotos' laboratory Research Associate Mohamad Karaky, PhD for providing molecular biology training, and scientific guidance to complete my project flourishingly. His constant support throughout my project helped me a lot. I got a chance to learn many innovative scientific things from him.

I am grateful to my late grandparents Mr Arvinad Gandhi and Mrs Devilaben Gandhi and my late father Dr Tusharbhai Gandhi for their immense blessings and guidance throughout my career. I am very much pleased with my mother Mrs Komal Gandhi for always standing by me during my academic journey. Her constant support, motivation, and encouragement lead me to reach this bigger platform. My words and feelings would not be able to express her sacrifice and contribution to my life. I have achieved my goals because of her positive approach and strong determination. I would also like to thank Mr Megh Gandhi (Brother), whose moral support throughout my Canadian journey gave me valuable direction and wonderful insights into my life.

Lastly, I would like to thank all my dear friends Maha Zidan, Sahel Jahangiri Esfahani, Shirin Sadigparavr, Mohamad Karaky, Rachel Ossagie, and Hema Devnani and great colleagues Faez Al Hamed and Goodarz Koli Farhood. Their amazing encouragement, guidance and motivation helped me a lot to enjoy my scientific journey for my master's program at McGill University. Their immense love and support always boosted my confidence. Their company and energy were incredible for me to work more enthusiastically in the laboratory.

Preface

This thesis follows a traditional monography style. As per McGill University standards, the thesis is logically-coherent and has a unified theme. This thesis discusses a novel project on the effect of treatment with ketotifen fumarate, as a mast cell stabilizer,- on chronic widespread pain in a population of pediatric patients. This thesis includes a literature review section about pain, the epidemiology and pathophysiology of chronic widespread pain, mast cells and pain, and how ketotifen fumarate has been previously used to manage pain. The following section includes thesis objectives, methods and results. Finally, the last sections present a discussion of our findings, including limitations, and a conclusion.

Multiple authors have contributed to this thesis' work; a recognizable appreciation of each author's contribution is mentioned in the following section.

Contribution of authors

Isha Gandhi, BDS, M.Sc. candidate: Contributed to handling, storing, and managing blood samples and measured histamine/tryptase levels on them, carried out the literature search, statistical analysis, and interpreted data. Wrote all parts of the thesis including study tables and figures.

Mohamad Karaky, PhD: Research associate at the Human Pain Genetics Laboratory at McGill University. He provided training on ELISA for measuring histamine/tryptase levels and guided the french translation of the thesis abstract.

Dr Eduardo V. Perez, MD, Rebecca Pitt, Victor-Hugo G.Cárdenas, and Nada Mohamed: Provided support for the patient's treatment and data collection throughout the clinical study.

Dr Pablo Ingelmo, MD: was responsible for the treatment of chronic widespread pain patients, description and reporting of clinical findings.

Dr Audrey Grant, Dr Luda Diatchenko, Goodarz Koli Farhood, Marc Parisien: Suggested analytical approach for this study.

Dr Carolina Berlado Meloto, DDS, PhD: Assistant Professor at the Faculty of Dental Medicine and Oral Health Sciences, Montreal, Quebec, Canada. She contributed to the study and method design and supervised the statistical analysis, results and thesis writing.

List of Tables and Figures

Figure 1. Flowchart of study design and procedures.

Figure 2. Correlation between baseline histamine plasma levels and average pain intensity at week 16 in the KF(A) and (B) placebo groups.

List of Abbreviations

ATP	Adenosine triphosphate
BMI	Body mass index
CGRP	Calcitonin gene-related peptide
CIM	Center for Innovative Medicine
CNS	Central nervous system
CPM	Conditioned pain modulation
CPS	Chronic Pain Service
CRH	Corticotropin-Releasing Hormone
CRPS	Complex regional pain syndrome
CWP	Chronic widespread pain
EGE	Eosinophilic gastroenteritis
ELIZA	Enzyme-linked immune assay
FDI	Functional Disability Inventory
FIQR	Fibromyalgia Impact Questionnaire-Revised
FM	Fibromyalgia
fMRI	Functional magnetic resonance
GAD	Generalized anxiety disorder
Hb	Haemoglobin
Ht	Hematocrit
IASP	International Association for the Study of Pain
IBS	Irritable bowel syndrome
ICD-11	International Classification of Diseases

IFN	Interferons
IL	Interleukin
JFS	Juvenile fibromyalgia syndrome
KF	Ketotifen fumarate
MCH	Montreal Children’s Hospital
MCP-1	Monocyte chemoattractant protein-1
MCs	Mast cells
MIP-1β	Macrophage inflammatory protein-1β
NGF	Nerve growth factor
NK	Natural killer
NMDA	N-methyl-d-aspartate
NRS	Numerical rating scale
OCD	Obsessive-compulsive disorder
PACAP-38	Pituitary adenylate cyclase-activating peptide-38
PAR-2	Protease-activated receptor 2
PBMCs	Peripheral blood mononuclear cells
PD	Panic disorder
PGIC	Patients' global impression of change
PNS	Peripheral nervous system
PPT	Pressure pain threshold
PRV	Pseudorabies virus
PSQI	Pittsburgh Sleep Quality Index
RCADS	Revised Child Anxiety and Depression Scale

RCT	Randomized controlled trial
SAD	Separation anxiety disorder
SCG	Sodium cromoglycate
SGPT	Serum Glutamic Pyruvic Transaminase
SNRIs	Serotonin-norepinephrine reuptake inhibitors
SP	Substance P
TGF	Transforming growth factor
TNF	Tumour necrosis factor
VAS	Visual Analog Scale
VIP	Vasoactive intestinal protein
VIP	Vasoactive intestinal peptide

1. Introduction

1.1. Pain

According to the International Association for the Study of Pain (IASP) 2020, pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"[1].

Based on duration, pain can be classified as acute or chronic[2]. Acute pain is a short-term pain that is due to an injury, trauma, or disease and lasts less than three months. Chronic pain lasts more than three months and often leads to disability, and is associated with various psychological comorbidities[2–4]. There are two major broad categories of chronic pain according to the International Classification of Diseases (ICD-11): chronic primary pain, in which pain cannot be explained by an underlying pathology (e.g., chronic widespread pain (CWP), complex regional pain syndrome, and chronic primary musculoskeletal pain)[5]; and chronic secondary pain, which can be attributed to an underlying cause (e.g., chronic diabetic neuropathy, chronic postsurgical, and posttraumatic pain)[5]. Three distinct patterns of anatomic distribution of chronic pain are generally recognized[6]: localized, when pain is localized to a particular body site (e.g., hip, knee, face); regional, when pain is limited to a particular body quadrant (upper, lower, right or left); and widespread, when pain is felt in all four quadrants of the body. Based on pathophysiology, pain can be additionally classified as nociceptive, nociplastic, and neuropathic. Nociceptive pain is a natural body reaction that results from damaged tissues like internal organs, muscles and bones[2]. Nociplastic pain is “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain”[1]. Chronic pain conditions like fibromyalgia (FM), complex regional pain syndrome, and irritable bowel syndrome (IBS) are

reported as nociplastic pain conditions[7]. Neuropathic pain is attributed to disease, damage, or lesion of the somatosensory nervous system. It can occur in the central or peripheral nervous system[2,8].

Untreated pain has a significant impact on quality of life, including effects on sleep, cognitive processes, brain function, mental health, cardiovascular health, and sexual function[9]. Hence, developing treatment strategies that help relieve pain is critical to better the quality of life of patients living with pain.

1.2. Chronic widespread pain in adolescents

CWP is characterized by long-lasting pain in all four quadrants of the body, which is believed to be due to central sensitization[10–13]. CWP is often accompanied by other physical symptoms such as fatigue, concentration problems, and psychological distress[10,11]. CWP is a central feature of FM, often referred to as juvenile fibromyalgia syndrome (JFS) when it affects the pediatric population. Individuals with JFS are less physically active, report reduced quality of life, and struggle with school attendance[27–30.]

Chronic pain in children is a growing clinical concern, with disturbingly high incidence rates identified in some communities[14]. Approximately 10% to 12% of the pediatric population lives with chronic pain[10–13]. The prevalence of CWP in the pediatric population seems to vary depending on the region and classification criteria adopted by the study. In one of the earliest studies to report the prevalence of FM in the pediatric population, Buskila *et al*. found a JFS prevalence of 6.2%. in Israelian children (9 and 15 years old)[15]. In a prospective study, Mikkelson *et al.* investigated the new-onset and the persistence/reoccurrence of widespread pain among school children in Finland. At the 1- and 4-year follow-ups, 31% and 30% of children who had CWP at baseline reported persistence/recurrence of symptoms, respectively. At the 1-year follow-up, 18% of

children who were free of CWP at baseline had new-onset CWP, and 3% still experienced symptoms at the 4-year follow-up. also in this study, widespread pain prevalence increased with age, being 7% in children aged 0-10 years, 9% in those aged 11-13 years, and 15% in those aged 14-16 years[16]. Another cross-sectional study by Haraldstad *et al.* with Norwegian children aged 8-18 years found a chronic widespread pain prevalence of 21%. They observed that pain prevalence increased with age and that girls reported more disrupted sleep, lack of appetite and medication usage than boys[17]. In a large cross-sectional study conducted on Norwegian adolescents aged 13-19 years, Skrove *et al.* reported the prevalence of high-impact chronic multisite pain to be 11.4% among girls and 3.8% among boys[18]. Using questionnaire-derived pain data of individuals aged 17 years old participating in a prospective population-based birth cohort in the UK, Norris *et al*. reported a CWP prevalence of 4.5%, with females being twice as likely as males to have CWP[19]. Using a validated algorithm for identifying CWP status from primary care electronic healthcare records of the pediatric population, Somayajula *et al.* reported a 3.19% five-year prevalence of CWP among UK children, with prevalence increasing by age group (8 to 11 years: 1.49%; 12 to 14 years: 3.48%; and 15 to 18 years: 4.74%)[20]. Despite variations in the above-mentioned prevalence rates, it seems clear that the risk of having CWP increases with age, a fact noted as early as 1987 by Yunus & Masi[21].

Childhood pain is not a one-time occurrence and should be appropriately cared for, as evidence suggests that it increases the risk of chronic pain in adulthood[22]. Moreover, childhood pain causes physical and emotional difficulties not only for the children themselves but also for their families. These difficulties stem from social and psychological problems such as psychological distress, psychological vulnerability, and anxiety[17]. In addition, chronic pain also affects patients and their families on a financial level[18.] The overall health care expenses to treat

teenagers with moderate-to-severe chronic pain in the United States have been estimated at approximately $19.5 billion per year[23]. As such, the development of effective pain treatment options for children is urgently needed and may not only limit the overall impact of pain on patients and their families lives but also limit patients' risk of developing chronic pain at later stages in life.

1.3. Pathophysiology of chronic widespread pain

CWP can sometimes be attributed to other disorders, such as autoimmune disorders, sickle cell disease, or osteoarthritis[24]. In this case, it is referred to as secondary CWP and discussing its pathophysiology is beyond the scope of this thesis. Patients with primary CWP, however, report pain and other somatic symptoms that cannot be adequately explained by any other disorder or any apparent damage or inflammation in peripheral tissues, and its pathophysiology are only partly understood. Strong evidence led to a consensus that CWP is linked to a fundamental problem of augmented pain or sensory processing in the central nervous system (CNS). This evidence includes but is not limited to, the nature of pain, which affects multiple body sites and also presents itself in the form of diffuse hyperalgesia (increased pain to normally painful stimuli) and/or allodynia (pain to normally nonpainful stimuli), and the decreased threshold to other noxious (e.g., heat, cold, electrical)[25–28] and sensory (e.g., sound)[29–31] stimuli. Moreover, CWP is often comorbid with fatigue, memory difficulties, sleep and/or mood disorders, which are also CNS-mediated symptoms.

Findings from imaging studies further substantiate the involvement of CNS-mediated pain amplification in CWP. For instance, functional magnetic resonance (fMRI) studies have shown that FM patients display an increased volume in regions that process pain, including the insula and the secondary somatosensory cortices[32–34]. Furthermore, studies employing methods that quantify

the connections between different brain regions have shown an increase in the connectivity between the default mode network (i.e., interconnected brain regions that are active when a person is resting) and the insula in individuals with FM[35,36] that has been reported to correlate with pain intensity[37].

While there is inconclusive evidence that a CNS-mediated phenomenon known as temporal summation, in which the sensation of pain increases with repeated stimulation with the same painful stimulus (i.e., same location, intensity), is increased in individuals with FM compared to pain-free controls, there seems to have consistent evidence that decreased conditioned pain modulation (CPM) is considerably more common in people with FM. CPM is a centrally-mediated phenomenon that produces analgesia by recruiting the descending pain inhibition system involving the periaqueductal grey and the rostral ventromedial medulla[38]. Its analgesic effect can be measured by applying a painful stimulus to a body part and asking study participants to rate their pain intensity. A second painful stimulus is then applied (normally by submerging the person's arm in a cold water bath or applying a pressure calf to their arm), followed by a third stimulus with the same location and intensity as the first. Individuals with FM consistently display a decreased capacity to inhibit their pain, as determined by a lower reduction in their pain ratings to the third stimulus compared to healthy controls[25,39]. CPM has been reported to be attenuated in individuals living with other types of chronic pain as well[25,39–43]. Consistent with these findings, an fMRI-based study investigating the connectivity between different brain regions in individuals with and without FM has reported a reduced connection between regions involved in the descending inhibitory system[35].

Additionally, studies that quantified different neurotransmitters in people with FM have found differences compared to pain-free controls. Early in the 1990s, studies found that the excitatory

substance P (SP) was increased in the cerebral spinal fluid of people with FM[44]. More recently, glutamate – another excitatory neurotransmitter – has been found to be increased in the CNS of FM patients, including in the insula[45–51]. Unfortunately, patients do not seem to tolerate well treatment with ketamine, dextromethorphan, or memantine, which are N-methyl-d-aspartate (NMDA) glutamate receptor antagonists[52–55]. The neurotransmitters most consistently implicated in FM likely are serotonin and norepinephrine. Elevation of their levels with treatment with serotonin-norepinephrine reuptake inhibitors (SNRIs; e.g., tricyclic antidepressants, duloxetine, milnacipran, tramadol) have shown beneficial effects for FM and other chronic pain patients in many clinical studies, which is believed to be in part due to the restoration of descending analgesic activity[24,56]. Moreover, resting-state functional connectivity randomized, placebo-controlled, cross-over clinical trial of the SNRI milnacipran found that individuals with FM with lower baseline functional connectivity between pro-nociceptive (i.e., the rostral part of the anterior cingulate cortex and the insula) and antinociceptive (i.e., the periaqueductal gray and the insula) brain regions had the greatest reduction in clinical pain.

While central amplification of pain certainly partly underlies the pathophysiology of CWP, I must note that there is also evidence of a peripheral neural component in FM, with different studies that biopsied the skin of FM patients reporting they have less epidermal nerve fibres in their skin compared to controls[57–61].

It is also becoming increasingly evident that the immune system plays a pathophysiological role in CWP. Cytokines are small proteins produced and secreted mainly by immune cells that control the activity of other immune systems and are crucial for adequate immune and inflammatory responses. Imbalances in the levels of circulating cytokines have been reported in individuals with CWP compared to pain-free controls[62–66], as well as in the levels of cytokines

produced by stimulated and unstimulated cultures of peripheral blood mononuclear cells (PBMCs) of individuals with CWP[62,66–70]. Interestingly, a study has investigated the expression of the pro-inflammatory cytokines interleukin (IL)-1beta, IL-6, and tumour necrosis factor (TNF)-α in skin biopsies of 53 women with FM and 10 age- and sex-matched controls. They detected IL-1beta, IL-6m and TNF-α in the biopsies of approximately 30% of patients, while in none of the biopsies from controls[71]. When comparing different cytokine genes expression in the peripheral blood of individuals with CWP and pain-free controls, Uceyler found that those with CWP expressed significantly less IL-4 and IL-10. These findings were consistent with their protein findings, as the concentration of IL-4 and IL-10 in the serum of CWP patients was also significantly reduced compared to controls[64]. In line with this, Behm *et al*. collected PBMCs from 110 FM patients and 91 healthy controls and found that, when stimulated, PBMCs from FM cells produced significantly fewer Interferons- γ (IFN-γ), IL-5, IL-6, IL-8, IL-10, Macrophage inflammatory protein-1β (MIP-1β), Monocyte chemoattractant protein-1 (MCP-1), and MIP-1α than controls. This suggests that cell-mediated immunity may be impaired in people with CWP[70]. Another study that assessed differentially expressed genes in women with and without FM identified that the B-cell development and primary immunodeficiency signaling pathways were linked to FM. Pathways related to homeostasis were also linked to FM[72].

Imbalances in the subpopulations of immune cells in individuals with and without CWP have also been reported. In 1994, Hernanz *et al.* used flow cytometry to compare lymphocyte subpopulations in 65 patients with FM and 56 controls. They found a decrease in the number of T cells expressing the activation markers CD69 and CD25 in FM patients, suggesting a defective T cell activation system in FM[73]. Macedo *et al*. have reported an increased expression of cell adhesion molecules on neutrophils and monocytes of individuals with FM compared to controls[74].

Recently, Verma *et al*. have used multiparametric flow cytometry to unbiasedly evaluate immune cell subsets on PBMCs of individuals with and without FM. They have found that the major circulating subsets of natural killer (NK) cells were less frequent in FM patients, with a more pronounced reduction in the subset of $CD56^{bri}$ NK cells that are known for their inflammatory and immune-regulatory functions[75,76]. DNA and RNA analysis of PBMC cells also have revealed an enrichment of cell activation pathways driven by NK cells in FM patients. These findings were further substantiated by the detection of an increased expression of an NK activation ligand on subepidermal nerves and by the presence of NK cells near peripheral nerves in skin biopsies of FM patients[77].

Collectively, studies mentioned in this section highlight the complex and heterogeneous pathophysiology of CWP that likely explains the difficulty in achieving effective and sustainable pain relief for CWP patients.

1.4. Mast cells and pain

Canonically recognized as effectors in IgE-mediated immediate type I hypersensitivity and in allergic responses such as asthma[78], mast cells (MCs) are immune cells derived from multipotent hematopoietic progenitor cells in the bone marrow that express CD34/CD117[79]. They pass through the blood wall and infiltrate tissues like skin, mucous membranes, respiratory, gastrointestinal tracts, peritoneal cavity and meninges where they reside[79]. MCs are found in both the peripheral nervous system (PNS) and the CNS, especially in the spinal cord and thalamus[80]. Of particular relevance to this thesis is the fact that MCs seem to have essential roles in the regulation of physiological and pathological pain pathways[81].

MCs contain granules filled with different types of mediators that are released upon activation and can directly (i.e., by activating nerve terminals) and/or indirectly (i.e., by initiating an

inflammatory response and cascade of immune-related events) cause pain. This includes the biogenic amines histamine and serotonin, enzymes (acid hydrolases, phospholipases, chymase, tryptase and different proteases), cytokines [IL-1 to IL-6, IFN, transforming growth factor (TGF), granulocyte-macrophage colony-stimulating element, leukaemia inhibitory factor, tumour necrosis factor (TNF)], chemokines, neurotoxic chemicals, lipid metabolites (leukotrienes, prostaglandins, platelet-activating factor), adenosine triphosphate (ATP), neuropeptides (SP, vasoactive intestinal peptide), growth factors (e.g., nerve growth factor – NGF), and nitric oxide[82,83].

The contribution of MCs to pain seems to be multileveled. An interesting initial observation is a fact that the scientific literature is replete with studies reporting the co-occurrence of different chronic idiopathic pain conditions[84–88], including FM[89], and asthma – which pathophysiology is largely linked to MCs[90]. Moreover, several clinical studies have linked the activation and/or abundance of MCs to painful conditions including migraine[91,92], chronic pelvic pain syndrome[93], complex regional pain syndrome[94], vulvodynia[86,95,96], gastrointestinal inflammatory illnesses[97–99], endometriosis[100], and FM[101]. Pre-clinical studies have also shown a role for MCs in thermal and mechanical hyperalgesia[102–106], migraine[107–109], pelvic pain[110], and postoperative pain[111–113114–118].

Specifically, in one of the clinical studies, the authors have investigated migraine patients (n=18) during headache attacks. They have found elevated plasma histamine levels during the attacks and in symptom-free periods and suggested that MC-activation may play a role in the onset of migraine[59]. Done *et al.* have focused on the role of MCs and NGF in chronic pelvic pain. In this study, they found increased levels of tryptase and NGF in expressed prostatic secretions of patients with chronic pelvic pain compared to healthy controls. In parallel, they conducted a pre-clinical study using an experimental autoimmune prostatitis model and MC-deficient mice ($Kit^{W\text{-}sh}$/ $Kit^{W\text{-}}$

^{sh}). Compared to naïve animals, the prostate of mice with induced autoimmune prostatitis displayed an increase in the number of and in the activations of MCs that were linked to pelvic pain behaviour. Among mice with induced autoimmune prostatitis, MC-deficient mice showed attenuated pelvic pain behaviour and reduced concentration of intraprostatic nerve growth factor. Treatment of these mice with a mast cell stabilizer combined with a histamine 1 receptor antagonist resulted in a synergistic decrease in chronic pelvic pain. The authors have then suggested that MCs may mediate chronic pelvic and may represent potential targets for therapeutic intervention[93].

In a study aimed at characterizing early complex regional pain syndrome (CRPS)-like features in 43 patients who underwent elective hand and wrist surgery followed by several weeks of cast immobilization (a set of factors known to predispose patients to develop CRPS), Pepper *et al*. have found an increase in tryptase expression in skin biopsies collected after removal of the casts. They have suggested that MCs may play a role in CRPS, as the biochemical changes in the skin of patients after hand/wrist surgery and immobilization are similar to those observed in CRPS patients[119].

An increased number of MCs and increased MC-degranulation have been detected in vulvodynia[95,96,120]. Bornstein *et al*. have immunostained the vestibular tissue of 40 women with severe vestibulitis and 7 healthy controls for MCs and tryptase. They have found an increase in the number of MCs and tryptase in patients compared to controls. Notably, they have also reported a 10-fold increase in the total nerve fibre area in vestibulitis patients that positively correlated with the number of MCs[95]. In a subsequent study to examine the relationship between vestibular hyperinnervation and MCs function in localized vulvodynia, Bornstein *et al*. have immunostained vestibular tissue from 7 women with severe localized vulvodynia and 7 healthy controls. They have found that women's vulvodynia display an increased number of MCs, increased subepithelial

heparanase (an enzyme secreted by MCs), and increased intraepithelial hyper-innervation compared to controls. The authors have speculated that heparanase secretion by MCs may be implicated in the pathophysiology of vulvodynia, as heparanase is capable of degrading the vestibular stroma and epithelial basement membrane, thereby permitting stromal proliferation and intraepithelial extension of nerve fibers[96]. Goetsch *et al*. collected skin biopsies of tender and non-tender vestibular sites from 10 primary and 10 secondary vulvodynia patients, as well as 4 pain-free controls. They have found a greater density of MCs in tender sites of patients with vulvodynia (whether primary or secondary) compared to controls. Notably, there were no differences among groups in the nontender sites[120].

With the goal of detecting and quantifying MCs in peritoneal, ovarian, and deep infiltrating endometriosis and of studying the relationship between MCs and nerves in endometriosis, Anaf *et al.* have obtained 69 biopsies from women who underwent laparoscopic excision of endometriosis due to pain and 37 biopsies of normal uterine tissue. Using immunohistochemistry for chymase and tryptase, they have found that MCs and degranulating MCs are more abundant in endometriotic than in normal uterine tissues. Notably, they also have found a greater number of MCs and of degranulating MCs in deep infiltrating lesions, which also displayed MCs in closer proximity to nerves than peritoneal and ovarian lesions. As patients with deep infiltrating lesions also reported increased pain scores for dysmenorrhea, deep dyspareunia, and rectalgia than those with peritoneal and ovarian lesions, the authors speculated that MC-released mediators may contribute to the pain and hyperalgesia in endometriosis by directly affecting neurons, especially in deep infiltrating lesions[100].

The link between MCs and gastrointestinal inflammatory conditions has been consistently shown[97]. Of note, Henderson *et al*. have collected and immunostained gastrointestinal mucosa

biopsies of children (mean age: 11.9 ± 2.9 years) with non-inflammatory bowel disease (i.e., irritable bowel syndrome or functional abdominal pain; n=26) and inflammatory bowel disease (n=22) for IL-6, MCs, enterochromaffin cells, 5-hydroxytryptamine, and SP. Interestingly, they have found a borderline significant increase in the number of MCs among children with the non-inflammatory disease subtype and stated that their findings further substantiate the involvement of MCs in pediatric functional gastrointestinal disorders[99].

In a study that collected skin tissue sections from a cohort of 63 FM patients and 49 matched controls from the general population, Blanco *et al*. have reported a robust increase in the number of MCs in the papillary dermis of FM compared to controls and concluded that FM may be a MC-associated condition and a potential target for the development of therapeutics[101].

These observations have prompted pre-clinical researchers to develop MC-focused models of pain syndromes in rodents to better understand pain's underlying processes. With little previous evidence on the direct effect of MC degranulation on pain, Chatterjea *et al*. have investigated if inducing MC degranulation with compound 48/80 (c48/80) could produce thermal hyperalgesia, edema, and neutrophil influx in mice. They have found that plantar injection of c48/80 causing MC degranulation leads to both thermal hyperalgesia and tissue edema, and that hyperalgesia is mediated by local neutrophil infiltration. Using the histamine-1 and -3/4 receptor antagonists diphenhydramine and thioperamide, respectively, significantly reduced c48/80-induced thermal hyperalgesia and partially blocked tissue edema. Further highlighting the direct role of MCs on pain, plantar MC reconstitution restored hyperalgesia and tissue edema in MC-deficient ($Kit^{W\text{-}sh}$/ $Kit^{W\text{-}sh}$) mice[102].

Based on findings from previous studies suggesting that MC-released inflammatory molecules stimulate meningeal nociceptors to promote a prolonged migraine headache[121,122], Levy *et al*. have

investigated whether MC degranulation can promote prolonged activation of meningeal nociceptors. Using *in vivo* electrophysiological recording of meningeal nociceptors and immunocytochemical labeling of activated meningeal nociceptors, the authors have provided the first evidence that MC degranulation *per se* can cause long-lasting excitation of primary afferent nociceptors. Interestingly, the immediate induction of neuronal excitation induced by MC degranulation with c48/80 was completely blocked by depleting the dural MC content, and by pretreatment with the MC stabilizer sodium cromoglycate (SCG)[107]. In a subsequent study, the same research group has examined whether MC degranulation can also lead to the activation of pain pathways that originate in other body regions. They have found that intraperitoneal injection of the MC secretagogue c48/80 induces MC degranulation in the dura matter, hind paw skin, and bladder of rats that are blocked by pretreatment with SCG. To provide additional evidence of the MC-induced activation of pain pathways, they also have checked for the expression of c-fos, a marker of neural activation (including nociceptor activation) in spinal dorsal horn neurons. Surprisingly, c-fos expression was increased only at two rostrocaudal levels: the medullary/C2 level and the caudal lumbar/rostral sacral level (L6-S2). These findings were paralleled by a simultaneous increase in tactile pain hypersensitivity in all areas tested that was blocked by pretreatment with SCG. The authors have suggested that MC degranulation is a nociceptive stimulus that leads to the development of tactile pain hypersensitivity that is mediated by spinal processes[109]. Based on previous studies showing that the pituitary adenylate cyclase-activating peptide-38 (PACAP-38) can effectively cause headache and migraine and long-lasting skin flushing and dilatation of the superficial temporal artery[123] in humans, which are symptoms consistent with MC activation, Baun *et al.* have investigated if PACAP-38 and related peptides can induce MC degranulation in mice. They have found that PACAP-38 (most potently) and other

related peptides induce concentration-dependent MC degranulation in the dura mater and suggested that this points to MC degranulation as a major contributor to PACAP-38 triggered migraine[108].

Pre-clinical studies have also implicated MCs in pelvic pain. Using an established murine model that injects the attenuated Bartha's strain of pseudorabies virus (PRV) in mice to produce symptoms consistent with interstitial cystitis, including lamina propria MC accumulation and pelvic pain,[110,124,125] Rudick *et al*. have examined whether MCs mediate pelvic pain directly. Contrarily to wild-type mice, MC-deficient ($Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$) mice did not develop symptoms of cystitis following infection with PRV. Notably, PRV-induced pelvic pain was fully restored in MC-deficient mice following MC reconstitution by transfusion with wild-type mice bone marrow. These findings indicate a role for MCs in pelvic pain[110].

MCs have also been implicated in post-surgical pain. Oliveira *et al*. have studied the role of MCs in postoperative pain by inducing MC degranulation by plantar surgery. Degranulation was confirmed by the high levels of histamine and serotonin in the perfused tissue 1-hour post-surgery. As result, animals developed mechanical allodynia that was averted by pre-treatment with the MC stabilizer SCG. Additionally, local therapy with histamine-1, -3, or -2A receptor antagonists were able to reduce postoperative nociception in mice but completely reversed mechanical allodynia in operated animals when administered collectively. Importantly, local depletion of MCs with c48/80 before surgery significantly prevented the development of post-surgical pain and the levels of histamine and serotonin in the tissue[111]. Yasuda *et al.* have investigated the role of MCs in postoperative pain through an incision in the hind paw of BALB/c mice. The MC stabilizer SCG was administered before paw incision, and three types of postoperative pain responses (i.e., guarding behaviour, mechanical allodynia, and heat hypersensitivity) were measured at 1, 2, and

7 days after the incision. They observed that, compared to animals treated with saline, SCG-treated animals displayed reduced guarding behavior and greater withdrawal threshold to mechanical stimulation on days 1, 2, and 7. SCG does not affect withdrawal latency to heat[113].

Taken altogether, the clinical and pre-clinical evidence indicate that MCs-regulated pathways as significant mediators of pain and that therapies targeting MCs may relieve pain in individuals living with pain.

1.5. Ketotifen fumarate: a MC stabilizer

The MC stabilizer ketotifen fumarate (KF) is a benzocycloheptathiophene derivative[126] and a non-competitive histamine antagonist (H1-receptor antagonist)[127]. It is commonly used orally for the prophylactic management of asthma in geriatric and paediatric patients. It is also used in allergic conditions like rhinitis and conjunctivitis[127], reactive airway diseases[126,128], bronchial asthma[126], and MC-mediated disorders like urticaria[129–132].

A few clinical studies have been conducted to examine the effect of treatment with KF on pain-related conditions. Riccardi has conducted an open-label (n=25) and a double-blind trial (n=27) of KF (2-4 mg/day) for the treatment of neurofibroma-associated itching and pain. Symptoms were assessed using a 1-10 scale at baseline, during and after treatment, which length varied among patients. Regardless of the study design, Riccardi has reported a decrease in the score of itching and pain during treatment that were increased after treatment and suggested that interference with MC function is a realistic approach to the treatment of itching and pain associated with neurofibromas[133].

Rosas *et al.* have reported cases of two individuals with eosinophilic colitis with symptoms of diarrhea, abdominal pain, and gastrointestinal bleeding that were treated with KF and displayed an improvement in symptoms, including pain[134]. Bolukbas *et al.* also have reported a case of

eosinophilic gastroenteritis mimicking abdominal emergency that was treated with KF and achieved dramatic improvements[135]. Additionally, Freeman has also reported a case of eosinophilic gastroenteritis with abdominal pain that was treated with KF and followed for over 20 years. Notably, treatment with KF provided symptom relief that promptly relapsed if the drug was discontinued[136].

Klooker *et al.* conducted a randomized controlled, double-blind, placebo-controlled trial (n=60) study to investigate the effect of 8-week treatment with KF (up to 6 mg/twice daily) on rectal sensitivity of patients with IBS. Other IBS-related symptoms and quality of life were also assessed before and after treatment. They observed that the group treated with KF reported a post-treatment increase in the threshold of rectal discomfort in patients with IBS with visceral hypersensitivity (and not in normosensitive patients with IBS), ameliorated abdominal pain, bloating, gas, diarrhoea, and incomplete evacuation, and improved quality of life. Despite these beneficial effects, the MC number and spontaneous release of tryptase in rectal biopsies of IBS patients were lower than in healthy volunteers, and the spontaneous release of histamine was mostly undetectable. Moreover, the spontaneous release of histamine and tryptase levels were not affected by treatment with KF. The authors suggested mechanisms of action independent of tryptase and histamine, such as KF-induced histamine-1 receptor blockade, may explain the beneficial effects seem with KF treatment[137].

Based on studies showing that FM patients have increased MCs in their skin compared to controls, Ang *et al.* have conducted a 10-week randomized, double-blind, placebo-controlled trial of KF (4 mg/day) for the treatment of FM symptoms (n_{KF}=24; $n_{PLACEBO}$=27). Contrarily to what they expected, individuals treated with KF did not exhibit a larger reduction in pressure hyperalgesia, average weekly pain intensity, or greater improvement in the severity of FM

symptoms compared to those who received a placebo. The authors argued that their results might be explained by a lack of sufficient MC stabilization due to the short treatment period (10 weeks) and/or low KF dose employed, or by their reduced sample size. Of note, no skin or blood samples were collected in this study to investigate the direct effects of KF on peripheral MCs[138].

Preclinical studies have shown that pre-treatment with KF can reverse pain in inflammatory[139] and postoperative pain models[112]. Anoush *et al*. have treated Sprague-Dawley male rats intraperitoneally with KF, saline, or diclofenac, followed by the injection of their hind paw with formalin or carrageenan to induce inflammatory pain. Paw edema was assessed hourly for 4 hours and pain behavior was recorded every 15 seconds for 60 minutes. KF significantly reduced paw edema starting at 2-hours post-injection like diclofenac. No treatment effectively reduced pain behavior immediately following injection, but KF overall significantly reduced pain throughout the 60-minutes assessed compared to saline, again in a manner similar to diclofenac[139]. Oliveira *et al.* have sought to investigate the role of the MC-released protease tryptase, a ligand of the protease-activated receptor-2 (PAR-2), in postoperative pain in mice. Surgical pain was induced with an incision in the plantar surface of the animal's hind paw and effectively induced mechanical pain that was prevented by MC depletion prior to surgery with c48/80. Pre-treatment with KF or SCG reduced mechanical pain and spontaneous pain behavior, with the analgesic effect of KF lasting longer than that of SCG. Pre-treatment with a tryptase inhibitor or a PAR-2 antagonist also prevented the development of post-surgical mechanical pain and of spontaneous pain behavior. Their findings reinforce that MCs have a critical role in postoperative pain and indicate that the pretreatment with MC stabilizers, particularly KF, could be used clinically to prevent postoperative pain[112]. Massaad *et al.* have studied the effect of treatment with antagonists to SP, CGRP, histamine (H)-1 or H-2 (MC-released inflammatory mediators), or with the MC stabilizer KF prior

to the induction of paw inflammation with capsaicin on mechanical and thermal pain, as well as on the levels of different cytokines (i.e., IL-1β, IL-6, TNF-α and NGF). Pre-treatment with all antagonists and with KF inhibited pain in a nearly dose-dependent manner. Additionally, pre-treatment with the SP or H-2 antagonists or with KF prevented the upregulation of all cytokines, while pre-treatment with the CGRP or H-1 antagonists prevented only the upregulation of NGF[140].

Despite showing the pain-relieving effects of KF, these investigations did not investigate whether such effects were MC-dependent. In this regard, a recent study by Meloto *et al.* has investigated the effect of stabilizing MCs with KF on inflammatory (i.e., formalin or CFA-induced inflammation) or neuropathic (i.e., spared nerve injury)-induced nocifensive behavior or mechanical pain in wild-type and MC-deficient ($Kit^{W\text{-}sh}$/ $Kit^{W\text{-}sh}$) mice. They have found that pre-treatment with KF dose-dependently inhibits nocifensive behavior in wild-type mice following formalin injection. Treatment with KF following inflammation induced by CFA significantly reduced mechanical pain in wild-type but not in MC-deficient mice. Treatment with KF following a neuropathic injury had no effect on pain. Importantly, their findings were supported by the report of a case series of individuals with CWP who showed significant clinical improvements after treatment with KF. The authors concluded that stabilizing MC with KF before and after inflammation develops effectively reduces nocifensive behavior and mechanical pain, respectively, in a MC-dependent manner. Combined with the report of a case series of patients with CWP who achieved important clinical improvements after treatment with KF, they suggested that clinical studies need to be conducted to test KF's efficacy in reducing pain in nonneuropathic pain conditions[141].

1.6. Gap in Knowledge

CWP is a complex pain condition whose pathophysiology remains to be fully understood. Plentiful evidence supports the role of pain amplification at the CNS level[24,25,32–43,45–56,142]. More recently, a peripheral component in the form of peripheral neuropathies has also been suggested[57–61]. Simultaneously, a growing body of evidence highlights the importance of the immune system in the mechanisms underlying CWP[63–69,72–74,140,141]. In that regard, MCs emerge as potentially important mediators of pain. Firstly, many studies have demonstrated the comorbidity between allergic conditions and different chronic pain conditions, including FM[82,85–87]. MCs have also been implicated in multiple clinical pain conditions[86,91–101] and their role in pain have been confirmed in various pre-clinical studies[102–118]. Of note, Meloto *et al*. have recently demonstrated that stabilizing MCs with KF alleviates mechanical pain in mice in an MC-dependent manner and their findings were paralleled by a case series of adolescents treated with KF who reported improvements in pain and other chronic pain-associated symptoms[141]. The authors warranted the conduction of clinical trials to confirm the potential analgesic use of KF, a drug normally used to treat allergic conditions. Although Ang *et al*. has conducted such a trial, they were not able to observe improvements in pain or the functional status in adults with FM but highlighted that their negative results may have been due to a lack of sufficient MC stabilization due to the short treatment period (10 weeks) and/or to the low KF dose employed[138]. Therefore, here we have sought to examine whether treatment with KF for a longer period and with a higher dose than that used by Ang *et al.* can provide symptoms improvement to patients living with CWP.

1.7. Thesis objectives

The first objective of this project was to test the effect of treatment with KF on the overall health status of teenagers with CWP. The second objective was to explore the effect of treatment

with KF on pain-related and psychosocial measures of teenagers with CWP. Lastly, we also aimed to correlate pre-treatment MC activity with overall health status and pain-related and psychosocial measures after treatment with KF.

2. Methods

2.1. Study design and participants

This was a proof-of-concept, randomized, placebo-controlled, double-blind, parallel-group clinical trial of KF to treat CWP in teenagers (n=44). All patients were recruited from the Chronic Pain Service (CPS) at the Montreal Children's Hospital (MCH). Study participants fulfilled the following inclusion criteria: 1, Patients (male and female) aged 12 to 17 years; 2, Diffuse bodily discomfort that had lasted at least three months and was accompanied by symptoms of exhaustion, sleeping difficulty, cognitive difficulties, mood problems and at least one somatic symptom to varying degrees, such as IBS, headaches, menstrual pain, lower urinary tract symptoms, myofascial pain, and temporomandibular pain; 3, Physical examination within normal ranges except for discomfort to pressure on soft tissues; (i.e. tactile hyperalgesia which is increased pain following a painful stimulus); 4, Average whole body pain score of at least 4 out of 10; 5, Functional Disability Inventory scores greater than 12 out of 60; 6, Stable dosages of their ongoing treatments for a minimum of four weeks; 6, Not having substantial changes in their overall health status (i.e., global impression of change scores less than 6) after 8 weeks of their ongoing therapy. Participants were excluded from the study based if they met any of the following criteria: 1, Known intolerance or allergy to KF; 2, Having been previously treated with another MC stabilizer; 3, Cognitive impairment interfering with clinical examinations; 4, Diagnosed with rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and/or other connective tissue illnesses; 5, Diagnosed with eczema (atopic dermatitis) or chronic urticaria (hives); 6, Diagnosed with

schizophrenia or bipolar condition; 7, Coagulopathies or chronic thrombocytopenia; 8, If they underwent an elective surgery during the time frame of the study; 9, Seizure history or current seizure therapy; and 10, Presenting abnormal lab findings in the previous 6 months (e.g., increased serum glutamic pyruvic transaminase and/or low platelet count, haemoglobin or hematocrit).

2.2. Procedures and measures

The study protocol was approved by Health Canada (control number 187113) and the McGill University Health Centre (MUHC) Ethics Review Board (#15-207-MUHC). Eligible patients attending the Chronic Pain Service (CPS) at the Montreal Children's Hospital (MCH) were invited to participate in the study. All participants and their parents or legal guardians have obtained an explanation of the research aims and methods and the participant's parents or legal guardians have signed the study's informed consent form prior to joining the study. Participants were involved in a total of five monthly study visits. Figure 1 displays a flowchart of the study design and procedures.

2.2.1. Dose, timing and length of KF treatment

The effective dose of KF for the treatment of patients with CWP has not been established yet. In our study, the starting dose (1 mg/day) has been chosen from the standard treatment for adolescents with asthma[145]. The safety profile of KF on this dose has been largely evaluated over the years[133]. The maximum therapeutic dose (6 mg/day) significantly reduced itching, pain, and tenderness, in patients with neurofibromatosis, with minimal adverse effects[133]. As oral KF may cause drowsiness early at the beginning of therapy, participants of our study have received 0,5 mg KF BID (1 mg/day) in the first week of treatment, 1 mg BID (2 mg/day) during the second week, 2 mg BID (4 mg/day) in the third week and 3 mg BID (6 mg/day) in the fourth week and thereafter until completing 16 weeks of treatment.

As noted in a previous study[141], the therapeutic effects of KF may not be clinically evident until several weeks after the initiation of therapy[146]. Additionally, regulatory agencies generally require that the treatment of confirmatory trials last for at least 12 weeks to establish the treatment effect, and to provide a reasonable amount of time to assess safety and tolerability[146]. Hence, this study was set to last for 16 weeks.

2.2.2. Randomization and blinding

Randomization was performed by a research assistant not involved in clinical treatment or data analysis using the online software (http//:www.randomization.com). We have used the software's first generator, which randomizes each subject to a single treatment by using the method of randomly permuted blocks.

The MUHC pharmacy was responsible for blinding. All clinicians and researchers involved in the recruitment, consent, data collection, experimental procedures and data analysis have remained blinded to study group allocation for the duration of the study.

2.2.3. Study visits

At the baseline visit, participants answered the following surveys: the Patient Global Impression of Change (PGIC; defined as our primary outcome); the Visual Analog Scale (VAS); the Functional Disability Inventory (FDI); the Revised Child Anxiety and Depression Scale (RCADS); and the Pittsburgh Sleep Quality Index (PSQI). They were also asked questions about their pain duration and their current use of other pain medications. After that, participants underwent a pressure pain test followed by blood collection. The next sections will describe the surveys and procedures in detail. Following blood collection, participants were instructed to proceed to the MUHC pharmacy to collect their pills. At the pharmacy, participants received four

vials of pills (KF or placebo) for the first four weeks of treatment and were given clear instructions on how many pills to take during the following 4 weeks.

On study visits, weeks 4, 8, 12, and 16 participants underwent the same procedures, except for blood collection. At the pharmacy, they received pills for the next four weeks, except for visit 16, which marked the end of their participation in the study.

2.2.4. Study surveys

2.2.4.1. Patient's Global Impression of Change (PGIC;[147,148])

The primary end point of our study was the PGIC after 16 weeks of treatment with KF or placebo. The PGIC is a rating scale that measures the change in patient life after single or multiple interventions that offers a quick and simple method of quantifying clinical progress. The PGIC is the most commonly used anchor-based method of assessing clinically important changes after treatments and has been used extensively in studies of musculoskeletal conditions. The PGIC asks patients to describe changes in limitation, symptoms, emotions and overall quality of life-related to the treatment received. For our study, the PGIC has been displayed as in previous studies of musculoskeletal pain:[149,150]

"Since beginning treatment, how would you describe the change (if any) in ACTIVITY LIMITATIONS, SYMPTOMS, EMOTIONS and OVERALL QUALITY OF LIFE-related to your painful condition?" (CHOOSE ONE).

1- No change (or condition has got worse)

2- Almost the same, hardly any change at all

3- A little better, but not a noticeable change at all

4- Somewhat better, but the change has not made any real difference

5- Moderately better, and a slight but noticeable change

6- Better, and a definite improvement that has made a real and worthwhile difference

7- A great deal better, and a considerable improvement that has made all the difference

In the PGIC, the greater the score, the more improvement in the clinical conditions. Hence, we have opted to use this measure as a continuous variable.

2.2.4.2. Pain intensity

Both self-report and behavioural pain measures have been created, validated, and widely utilised in paediatric pain[149]. Here we have assessed pain intensity using the 0-100 numerical rating scale (NRS), in which '0' means 'no pain at all' and '100' means 'the worst pain imaginable'. Study participants were asked to use the NRS to rate the lowest, average and highest pain intensity. In addition, participants were asked to specify what percent of their awaken day they spent with the lowest, average and highest pain intensity. This allowed the generation of an index termed as 'pain index', calculated as the product of the highest, average and lowest pain intensity by their respective pain durations (in hours)[151].

2.2.4.3. Pressure pain sensitivity

Pressure pain thresholds (PPT) were assessed using a digital pressure algometer applied bilaterally at the trapezius muscle. Specifically, a trained examiner applied a gradually increasing amount of pressure and participants were instructed to say "pain" when they first felt that the pressure sensation became painful. This procedure was repeated three times on each side, and the participant's PPT (in kg) was defined as the average of all six trials (three on the right and three

on the left). PPTs have an inverse relationship with pain, meaning that the lowest the PPT, the highest the pain sensitivity.

2.2.4.4. Physical and Emotional Functioning

2.2.4.4.1. Functional Disability Inventory (FDI; [152,153])

The FDI has been used in a wide spectrum of pain disorders in children and adolescents[154–157]. The FDI has excellent internal consistency, moderate to high test-retest reliability, moderate cross-informant (parent-child) reliability, and strong predictive validity[152,155]. The FDI is a 15-item scale that assesses one's difficulty in performing a wide variety of everyday physical activities, including recreational and social functioning, such as "doing chores at home", "being at school all day", or "walking the length of a football field." Answers are provided on a 5-point scale, in which 0 means "No Trouble" and 4 means " Impossible". A 0-60 score is then generated by the sum of all items, with greater scores indicating greater functional disability.

2.2.4.4.2. Revised Child Anxiety and Depression Scale (RCADS; [158,159])

The RCADS is used to evaluate the emotional functioning domain that has high psychometric qualities and has proven to be reliable and valid in clinical and nonclinical samples[158,160–162]. It consists of a self-reported questionnaire that contains 47-items assessing children's symptoms of anxiety and depression, such as "I feel sad or empty", "I worry about being away from my parents", "I worry about what other people think of me", "I worry about things", "All of sudden I feel really scared for no reason at all", "I have to do somethings just the right way to stop bad things from happening". Answers are provided on a four-point scale ranging from 0 to 3, in which 0 means "never" and 3 means "always". A raw total anxiety score is then calculated as the sum of all items assessing a wide range of anxiety-related behaviors, and a raw anxiety and behavior score

is created as the sum of all items assessing anxiety and depression behaviors. Raw scores are then converted to T-scores, with lower T-scores indicating less anxiety and/or depression behaviors.

2.2.4.4.3. Sleep

The Pittsburgh Sleep Quality Index (PSQI)[163] is a self-reported questionnaire that contains 19 items assessing a few time-related aspects of their sleep habits, as well as aspects related to sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. In the non-time related items, respondents are asked how often each of a number of items happen to them on a 4-point Likert scale with the response options of "not during the past month", "less than once a week", "once or twice a week," or "three or more times a week." These time- and non-time-related items are grouped into seven component scores that are summed to create the global PSQI score, with higher scores indicating worse sleep quality.

2.2.4.5. Analgesic medication

Study participants were not asked to stop taking any medications to participate in this study. However, they were allowed to reduce the doses or, eventually, to stop the use of other analgesic medication(s) under medical supervision if they reported a significant reduction in pain intensity (50% reduction in their pain intensity rating compared to the previous visit). The number of analgesic medications taken at each study visit was recorded and used as a proxy measure for pain severity. The suggested interpretation of this measure would be the lower the number of analgesic medications taken, the less severe the patient's current pain-related condition.

2.2.4.6. Blood collection

Twenty millilitres (20mL) of blood were drawn into two 10mL tubes from each study participant. The amount of blood was calculated based on the minimal amount of plasma needed to obtain sufficient material for the profiling of different multiple markers. In this study, we have

focused on tryptase and histamine, two markers of MC activity. To avoid adverse reactions, we have checked every patient's record for their last blood test to ensure that their haemoglobin count was normal.

After collection, blood was immediately spun down and approximately 10mL of plasma was retrieved from each participant and transferred into new coded tubes stored at -80°C until analysis.

2.2.4.6.1 Quantification of tryptase and histamine in plasma

After study completion, we used enzyme-linked immune assays (ELISA) to determine the levels of histamine (Histamine ELISA kit KA2589, Abnova) and tryptase (Human Tryptase/ TPSAB1, B2 ELISA Kit Picotine, EK0890, Boster) in the plasma obtained from study participants following the manufacturers' instructions. Briefly, plasma samples were thawed at 4°C and diluted 1:5 with sample diluent. The concentrations of histamine and tryptase were measured in triplicate (100µl each), averaged, and multiplied by the dilution factor to obtain each participant's levels of histamine and tryptase.

2.3. Data analysis

As a widely accepted statistical rule, at least 40 data points are needed to allow employing parametric tests[164]. As 11 participants dropped out of the study, our final dataset consisted of 33 participants. Hence, here we have used non-parametric tests. The type I error was set at 0.05 and data are presented as median and range, or as the number and per cent, where applicable. All statistical analyses were conducted using RStudio (Version 1.4.1717).

We have used the Mann-Whitney U test to test for differences in continuous outcomes between groups, and the Chi-Square test was used for categorical outcomes. We have used the Wilcoxon Rank Sum Test to test for differences within the group in outcomes at two different time points (i.e., baseline *versus* week 16). We have used the Kruskal-Wallis test to test for differences

within the group in outcomes at all time points (i.e., baseline, week 4, 8, 12, and 16). Lastly, we have used quantile regression to examine the relationship between baseline plasma levels of histamine or tryptase on study outcomes at week 16 by considering the 95^{th} percentile of quantile with a tau=0.5.

3. Results

We have enrolled 44 participants in the study, of which 11 dropped out (n=33; n_{KF}=19 and $n_{PLACEBO}$=14). There were no differences in the demographic characteristics between participants in the KF and placebo groups at baseline (Table 1). All study participants were females, except for one male participant in the KF group. There were also no differences between groups at baseline in all pain-related and physical and emotional functioning measures assessed in the study (Table 2).

We have not identified any statistically significant differences between the KF and placebo groups at week 16 (end of treatment), including in the participants' self-reported global health status, defined as the study's primary outcome (Table 3).

Next, we have compared baseline and end of treatment (week 16) data within groups. While there was no baseline to end-of-treatment differences in the global health status or any of the pain-related measures in the KF group, there were significant reductions in the disability [baseline = 22.0 (5.00, 47.0); week 16 = 19.0 (0, 39.0), p = 0.008], anxiety [baseline = 50.0 (28.0, 77.0); week 16 = 43.0 (29.0, 70.0), p = 0.001], and anxiety and depression [baseline = 52.0 (30.0, 80.0); week 16 = 44.0 (28.0, 76.0), p = 0.000] measures, indicating improvement of these symptoms (Table 4). In the placebo group, there were significant reductions in the average pain index [baseline = 28.8 (3.00, 49.0); week 16 = 16.8 (1.80, 27.0), p = 0.027] and disability measures [baseline = 25.0 (0,

41.0); week 16 = 16.0 (1.00, 40.0), p = 0.032], while the reductions in the anxiety measure [baseline = 43.0 (36.0, 64.0); week 16 = 37.0 (26.0, 62.0), p = 0.054] only approached significance (Table 5). When taking into account all five study visits (i.e., baseline and weeks 4, 8, 12, and 16), we have not found any statistically significant differences within the KF (Table 6) or placebo (Table 7) groups for any of the study outcomes.

To examine whether the baseline to end of treatment improvement in symptoms observed within groups was mediated by MCs, we tested if the baseline plasma levels of the MC activity markers histamine (Table 9) and tryptase (Table 10) could predict such improvements. We have first tested and found no significant differences in the plasma levels of histamine and tryptase between groups at baseline (Table 8). Notably, we have found that greater baseline plasma levels of histamine significantly predicted lower average pain intensity ratings (p-value=0.004) (Figure 2) at the end of treatment in the KF group, potentially suggesting that treatment with KF may only be beneficial for individuals with greater circulating levels of histamine. The baseline plasma level of tryptase did not predict any of the study outcomes.

4. Discussion

In response to the widely reported but understudied comorbidity between many types of allergies and several chronic pain conditions[84–87,89] and to recent findings showing both pre-clinical and clinical evidence that stabilizing MCs with KF may provide pain relief in an MC-dependent manner[141], here we have sought to prove the concept that treatment with KF may provide symptoms improvement for patients living with CWP. Ang *et al*. conducted a randomized clinical trial of KF (4 mg daily) for the treatment of FM in adults and found that it did not reduce pain sensitivity, improve clinical pain, or reduce overall FM symptom severity compared to placebo. Nonetheless, the authors warranted the conduction of clinical trials using higher doses of KF. In

addition, the length of treatment in the study of Ang *et al*. was 10 weeks[138]. In previous work of our group, we have reported that symptom improvements in teenagers treated with KF seemed to begin approximately after 10 weeks of treatment[141]. Hence, we have designed a proof-of-concept randomized clinical trial of KF for the treatment of CWP using a higher dose of the medication (6 mg daily) and a longer treatment period (16 weeks). However, we were not able to demonstrate that treatment with KF improves the global health status or pain-related (except average pain intensity) or physical and emotional functioning measures assessed in our study compared to placebo.

Despite that, our study brings to light a couple of points that should be further investigated. Firstly, within-group comparisons of variables before and after treatment revealed a robust effect of KF in improving measures of physical (i.e., disability) and emotional (i.e., anxiety and anxiety and depression) functioning. In the placebo group, this effect was specifically seen for average pain index, physical (i.e., disability) and emotional (i.e., anxiety) functioning. One logical inference is that study participants in the KF and placebo groups responded to what is known as the "placebo effect". Defined as the effect of patients' positive expectations on their state of health, placebo effects can lead to beneficial health outcomes. These effects occur in many clinical contexts, including in clinical trials[165,166]. Here, we can argue that patients' expectations of clinical improvement due to the possibility that they might be receiving a new treatment could have positively affected their ability to perform everyday physical activities, leading to a decrease in the disability scores at the end of treatment. The role of a placebo effect in our findings is further evidenced by the statistically significant before-to-after treatment reduction in the average pain index reported by participants in the placebo group. We cannot explain, however, why this putative placebo effect was not also observed for the emotional functioning measures of anxiety and

depression in the placebo group. One possibility is that these measures suffered from the lower sample size available in the placebo (n=14) compared to the KF group (n=19). Another intriguing possibility is that KF does in fact contribute to the improvement of anxiety and anxiety and depression symptoms. Although seemingly far-fetched, a few studies have demonstrated that treatment with KF can reduce or prevent the development of anxiety-[167–171] and depression-like[167,172] behaviors in rodents. It should be noted that many of these studies point to a histamine-mediated effect of KF on anxiety and/or depression. In our study, baseline plasma levels of histamine did not predict the improvement in these behaviors reported by participants in the KF group at the end of treatment, suggesting that mechanisms unrelated to histamine-mediated such improvement. In that regard, stabilization of MCs leading to a decrease in the levels of substance P and serotonin, two MC-released molecules linked to psychopathological conditions[173], could partly respond to the mood improvements reported by participants treated with KF. Substance P acts mainly by binding to neurokinin-1 receptors and it has been found to be increased in the cerebral spinal fluid of individuals with FM[44]. Substance P has been consistently implicated in anxiety-related disorders[174–177] and neurokinin-1 receptor antagonists are a promising target for the treatment of depression[178–180]. Serotonin is likewise implicated in mood-related disorders, and drugs that inhibit its neuronal reuptake at the CNS are commonly used to treat anxiety and depression, including in children[181,182]. However, KF can easily penetrate the blood-brain barrier and we cannot rule out that its beneficial effect on the symptoms of anxiety and depression may have been due to the stabilization of MCs in the CNS and/or to its postsynaptic histamine-1 antagonism[183–187].

Secondly, baseline plasma levels of histamine predicted lower average pain intensity at the end of treatment in the KF group. This suggests that treatment with KF may be particularly

beneficial for individuals whose CWP pathophysiology relates to increased circulating levels of histamine and possibly, increased MC activity, as these cells are the most relevant source of histamine in the immune system[188]. Indeed, chronic pain conditions, including CWP, are highly heterogeneous and believed to result from a complex set of biopsychosocial and environmental factors unique to each individual that interact to produce temporally dynamic symptoms and influence one's susceptibility and resiliency to pain onset and chronicity[189,190]. It is thus pointless to envisage a single cause, nor even to expect that anyone cause might be necessary or sufficient for the development and/or chronicity of pain in all individuals. From a clinical standpoint, this could mean that screening chronic pain patients for their levels of histamine could aid clinicians in the treatment decision-making process. We should highlight, though, that the report of lower average pain at the end of treatment by participants in the KF group can only be speculatively attributed to a decrease in the patients' circulating levels of histamine, as plasma was not collected from participants at the last study visit. That said, it has been previously shown that KF can decrease both the levels of histamine[191,192]. In addition, treatment with KF has also been shown to lead to a decrease in the levels of other MC-release substances, including TNF-α[193,194], macrophage-derived chemokines[195], and IL-8[196]. Although these findings originate from studies done in the context of asthma and allergic disorders, TNF-α, macrophage-derived chemokines, and IL-8 have all been shown to contribute to pain in human and animal studies[197].

Our findings contrast with a few cases reports in the literature showing the pain-relieving effects of KF[134–136], and one could argue that these reports were likely focused on individuals whose pain was linked to increased MC activity and/or histamine-1 receptor signalling. In a clinical trial of patients with neurofibroma-associated itching and pain[133], treatments with KF at doses lower (2-4 mg) than the one used in our study (6 mg) decreased both itching and pain. MCs

are present in most neurofibromas and have been shown to be critical to the origin and progression of neurofibromas in both humans[198–202] and relevant mouse models[203,204]. Moreover, histamine plays an important role in purinergic symptoms, particularly via its histamine-1 receptors[205]. It is thus possible that MCs and their mediators play a more central role in the pathophysiology of symptoms linked to neurofibromas, including pain, than in chronic pain at large. Another clinical trial, now of IBS, has found that treatment with KF (up to 12 mg) decreased rectal discomfort in patients with visceral hypersensitivity and ameliorated abdominal pain and other gastrointestinal symptoms[137]. Notably, KF doses used were up to double the dose used here. Additionally, the authors reported that rectal hypersensitivity was decreased only in patients with visceral hypersensitivity but not in normosensitive patients. This aligns with our theory that treatment with KF may be particularly useful for individuals whose symptoms are linked to increased MC activity. In fact, in addition to the well-known role of MCs in IBS[206], a recent review elaborates on MCs' particular relevance to visceral sensations[207].

The findings of our study must be cautiously considered, as our study has limitations. As a proof-of-concept trial, we aimed to recruit 44 participants, a sample size similar to that of Ang *et al.*[138]. We assumed that modifications to the study design, namely an increase in KF dose and treatment length, would be sufficient to yield statistically detectable evidence of symptoms improvement following treatment. As a result of study dropouts (n=11), we cannot rule out that we may not have been able to detect such treatment-dependent improvements due to reduced statistical power. Additionally, our finding that higher pre-treatment treatment levels of circulating histamine are linked to lower average pain at the end of treatment needs to be confirmed in future studies that assess MC markers both before and after treatment.

5. Conclusion

Daily treatment with 6 mg of KF did not improve the global health status or pain-related (except for average pain intensity) or physical and emotional functioning measures compared to treatment with placebo. Nonetheless, within-group comparisons revealed that treatment with KF may be beneficial to relieve pain in individuals with greater plasma levels of histamine. Hence, we encourage the conduction of future clinical trials that are fully powered to detect symptom improvement in response to treatment with KF. We also recommend future studies assess markers of MC activity, including but not limited to histamine and tryptase, both before and after treatment to shed light on the biological mechanisms underlying KF-mediated symptom improvement.

Table 1. Demographic characteristics at baseline of all participants in the KF and placebo groups who completed the study (n=33).

	Baseline		
Variable [data presentation]	**KF (N=19)**	**Placebo (N=14)**	***P* -value**
Age [Median (Min, Max)]	17.0 (13.0, 19.0)	16.0 (12.0, 19.0)	0.707[a]
Gender [n (%)]			
Male(%)	1 (5.3%)	0 (0%)	
Female(%)	18 (94.7%)	14 (100%)	1[b]
BMI [Median (Min, Max)]	24.2 (15.6, 36.7)	20.1 (16.9, 138)	0.113[a]

Footnotes: BMI: Body mass index; [a]Mann-Whitney test; [b]Chi-square test

Table 2. Pain-related and physical and emotional functioning measures in the KF and placebo groups at baseline.

		Baseline		
		KF (N=19)	**Placebo (N=14)**	
Variable	**N**	*Median (Min, Max)*	*Median (Min, Max)*	***P*-value[a]**
Pain intensity	33			
High pain intensity		75.0 (9.00, 90.0)	67.5 (7.00, 95.0)	0.985
Average pain intensity		50.0 (7.50, 85.0)	40.0 (5.00, 75.0)	0.673
Low pain intensity		40.0 (4.50, 70.0)	20.0 (0, 70.0)	0.280
Pain index	20	(N=14)	(N=6)	
High pain index		18.4 (0, 45.0)	22.5 (1.75, 28.5)	0.508
Average pain index		17.3 (0, 60.0)	28.8 (3.00, 49.0)	0.591
Low pain index		8.25 (0, 31.5)	5.00 (0.45, 8.00)	0.282
Pressure pain threshold	33	1.61 (0.31, 3.54)	1.30 (0.655, 3.18)	0.760
Analgesic medication	33	2.00 (0, 5.00)	2.00 (1.00, 5.00)	0.939
Disability	33	22.0 (5.00, 47.0)	25.0 (0, 41.0)	0.488
Anxiety	33	50.0 (28.0, 77.0)	43.0 (36.0, 64.0)	0.773
Anxiety & Depression	33	52.0 (30.0, 80.0)	45.0 (38.0, 68.0)	0.604
Sleep quality	33	11.0 (6.00, 17.0)	8.50 (2.00, 16.0)	0.193

Footnotes: [a]Mann-Whitney test

Table 3. Global health status, pain-related and physical and emotional functioning measures in the KF and placebo groups at Week 16.

		Week 16		
Variable	**N**	**KF (N=19)** *Median (Min, Max)*	**Placebo (N=14)** *Median (Min, Max)*	***P*-value[a]**
Global Health Status	33	2.00 (1.00, 7.00)	3.50 (1.00, 6.00)	0.423
Pain intensity	33			
High pain intensity		70.0 (20.0, 80.0)	57.5 (0, 90.0)	0.672
Average pain intensity		45.0 (10.0, 75.0)	34.0 (0, 80.0)	0.050
Low pain intensity		37.0 (5.00, 65.0)	17.5 (0, 80.0)	0.096
Pain index	20	(N=14)	(N=6)	
High pain index		14.5 (0, 74.3)	15.8 (1.75, 48.8)	0.934
Average pain index		25.8 (0.63, 42.0)	16.8 (1.80, 27.0)	0.075
Low pain index		3.88 (0, 24.0)	6.50 (0.60, 15.0)	0.508
Pressure pain threshold	33	1.45 (0.74, 5.40)	1.58 (0.623, 2.83)	0.984
Analgesic medication	33	2.00 (0, 4.00)	2.00 (0, 5.00)	0.751
Disability	33	19.0 (0, 39.0)	16.0 (1.00, 40.0)	0.898
Anxiety	33	43.0 (29.0, 70.0)	37.0 (26.0, 62.0)	0.644
Anxiety & Depression	33	44.0 (28.0, 76.0)	43.0 (26.0, 64.0)	0.686
Sleep quality	33	9.00 (2.00, 20.0)	8.00 (4.00, 17.0)	0.660

Footnotes: [a]Mann-Whitney test

Table 4. Baseline and week 16 global health status, pain-related measures and physical and emotional functioning measures of participants in the KF group.

		KF (N=19)		
		Baseline	**Week 16**	
Variable	**N**	*Median (Min, Max)*	*Median (Min, Max)*	***P*-value[a]**
Global Health Status	33	3.00 (1.00, 6.00)	2.00 (1.00, 7.00)	0.524
Pain intensity	33			
High pain intensity		75.0 (9.00, 90.0)	70.0 (20.0, 80.0)	0.367
Average pain intensity		50.0 (7.50, 85.0)	45.0 (10.0, 75.0)	0.722
Low pain intensity		40.0 (4.50, 70.0)	37.0 (5.00, 65.0)	0.812
Pain index	20		(N=14)	
High pain index		18.4 (0, 45.0)	14.5 (0, 74.3)	1
Average pain index		17.3 (0, 60.0)	25.8 (0.63, 42.0)	0.706
Low pain index		8.25 (0, 31.5)	3.88 (0, 24.0)	0.064
Pressure pain threshold	33	1.61 (0.31, 3.54)	1.45 (0.74, 5.40)	0.861
Analgesic medication	33	2.00 (0, 5.00)	2.00 (0, 4.00)	0.311
Disability	33	22.0 (5.00, 47.0)	19.0 (0, 39.0)	0.008
Anxiety	33	50.0 (28.0, 77.0)	43.0 (29.0, 70.0)	0.001
Anxiety & Depression	33	52.0 (30.0, 80.0)	44.0 (28.0, 76.0)	0.000
Sleep quality	33	11.0 (6.00, 17.0)	9.00 (2.00, 20.0)	0.134

Footnotes: [a]Wilcoxon Rank Sum Test

Table 5. Baseline and week 16 global health status, pain-related and physical and emotional functioning measures of participants in the placebo group.

		Placebo (N=14)		
		Baseline	**Week 16**	
Variable	**N**	*Median (Min, Max)*	*Median (Min, Max)*	***P*-value[a]**
Global Health Status	33	3.00 (1.00, 5.00)	3.50 (1.00, 6.00)	0.235
Pain intensity	33			
High pain intensity		67.5 (7.00, 95.0)	57.5 (0, 90.0)	0.529
Average pain intensity		40.0 (5.00, 75.0)	34.0 (0, 80.0)	0.134
Low pain intensity		20.0 (0, 70.0)	17.5 (0, 80.0)	0.573
Pain index	20		(N=6)	
High pain index		22.5 (1.75, 28.5)	15.8 (1.75, 48.8)	0.500
Average pain index		28.8 (3.00, 49.0)	16.8 (1.80, 27.0)	0.027
Low pain index		5.00 (0.45, 8.00)	6.50 (0.60, 15.0)	0.248
Pressure pain threshold	33	1.30 (0.311, 3.54)	1.58 (0.623, 2.83)	0.777
Analgesic medication	33	2.00 (1.00, 5.00)	2.00 (0, 5.00)	0.830
Disability	33	25.0 (0, 41.0)	16.0 (1.00, 40.0)	0.032
Anxiety	33	43.0 (36.0, 64.0)	37.0 (26.0, 62.0)	0.054
Anxiety & Depression	33	45.0 (38.0, 68.0)	43.0 (26.0, 64.0)	0.070
Sleep quality	33	8.50 (2.00, 16.0)	8.00 (4.00, 17.0)	0.869

Footnotes: [a]Wilcoxon Rank Sum Test

Table 6. Differences in study outcomes throughout all five study visits in the KF group.

Variable	N	*P*-value[a]
Global Health Status	19	0.670
Pain intensity	19	
High pain intensity		0.895
Average pain intensity		0.291
Low pain intensity		0.395
Pain index	14	
High pain index		0.793
Average pain index		0.829
Low pain index		0.743
Pressure pain threshold	19	0.931
Analgesic medication	19	0.966
Disability	19	0.924
Anxiety	19	0.670
Anxiety & Depression	19	0.652
Sleep quality	19	0.440

Footnotes: [a]Kruskal-Wallis Test

Table 7. Differences in study outcomes throughout all five study visits in the placebo group.

Variable	**N**	***P*-value[a]**
Global Health Status	14	0.958
Pain intensity	14	
High pain intensity		0.793
Average pain intensity		0.800
Low pain intensity		0.978
Pain index	6	
High pain index		0.963
Average pain index		0.162
Low pain index		0.941
Pressure pain threshold	14	0.997
Analgesic medication	14	0.989
Disability	14	0.495
Anxiety	14	0.675
Anxiety & Depression	14	0.638
Sleep quality	14	0.993

Footnotes: [a]Kruskal-Wallis Test

Table 8. Baseline plasma levels of histamine and tryptase in the KF and placebo groups.

		Baseline		
		KF (N=19)	**Placebo (N=14)**	
Variable	**N**	*Median (Min, Max)*	*Median (Min, Max)*	***P*-value[a]**
Histamine (ng/ml)	33	72.7 (14.6, 218)	45.3 (13.6, 114)	0.173
Tryptase (ng/ml)	33	6.77 (1.42, 47.3)	9.59 (0.64, 15.0)	0.356

Footnotes: [a]Mann Whitney U test

Table 9. Effect of treatment with KF and baseline plasma level of histamine on patient's global health status, pain-related and physical and emotional functioning measures at week 16.

Variable	Coefficient Value	*P*-value[a]
Global health status		
Treatment (Ref. Placebo)	1.000	0.278
Histamine	0.000	1.000
High pain intensity		
Treatment (Ref. Placebo)	-17.005	0.289
Histamine	-0.174	0.347
Average pain intensity		
Treatment (Ref. Placebo)	-11.440	0.410
Histamine	-0.159	0.032
Low pain intensity		
Treatment (Ref. Placebo)	-15.734	0.232
Histamine	-0.122	0.456
High pain index		
Treatment (Ref. Placebo)	-0.066	0.948
Histamine	-0.174	0.864
Average pain index		
Treatment (Ref. Placebo)	-0.779	0.447
Histamine	0.216	0.831
Low pain index		
Treatment (Ref. Placebo)	0.404	0.691
Histamine	-0.571	0.576
Pressure pain threshold		
Treatment (Ref. Placebo)	0.456	0.681
Histamine	0.003	0.763
Analgesic medication		
Treatment (Ref. Placebo)	0.033	0.964
Histamine	-0.0049	0.625
Disability		
Treatment (Ref. Placebo)	-4.078	0.548
Histamine	-0.041	0.678
Anxiety		
Treatment (Ref. Placebo)	-6.000	0.476
Histamine	0.000	1.000
Anxiety &Depression		
Treatment (Ref. Placebo)	-1.000	0.906
Histamine	0.000	1.000
Sleep quality		
Treatment (Ref. Placebo)	-1.059	0.645
Histamine	0.015	0.312

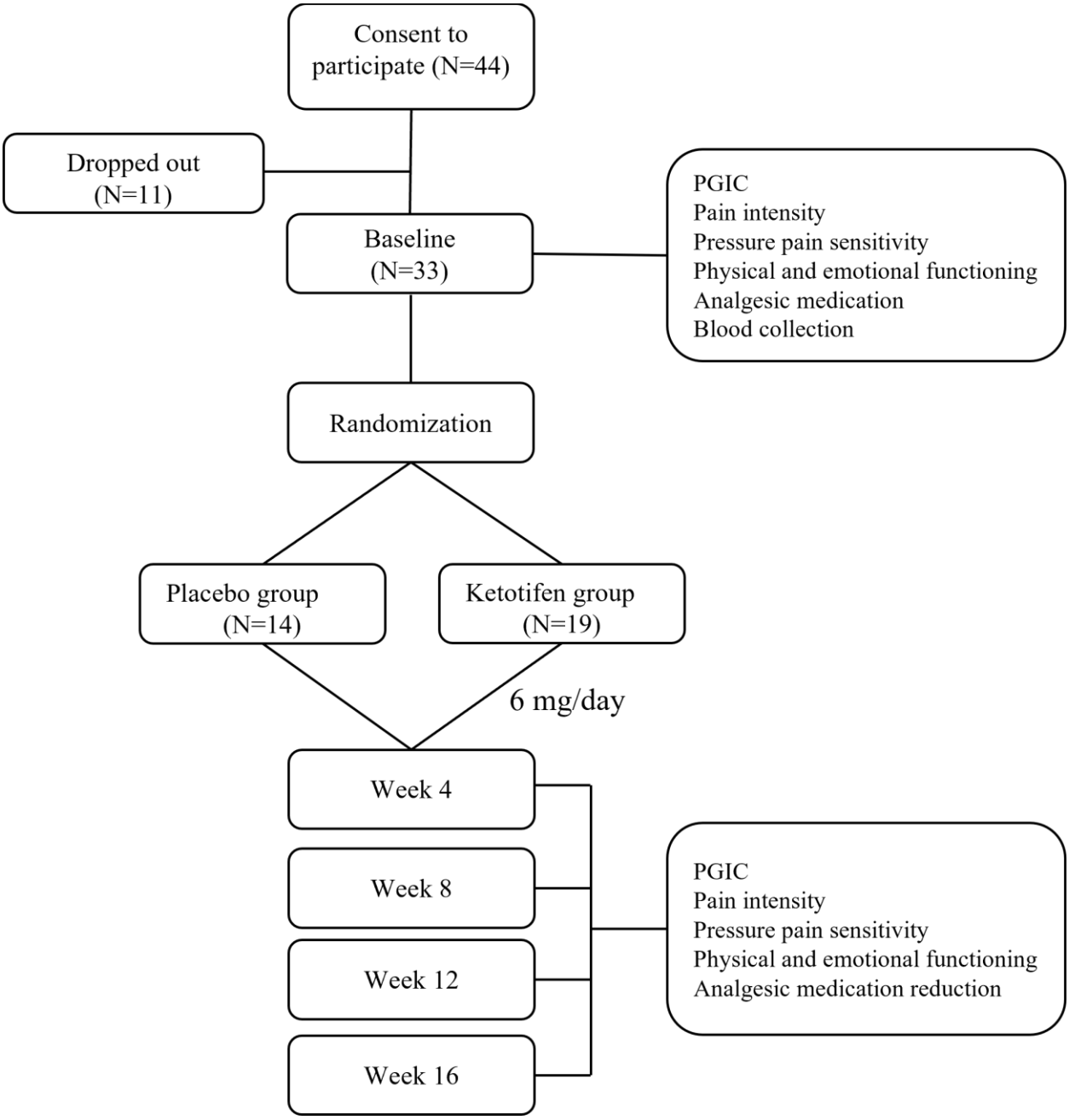
Footnotes: [a]Quantile regression

Table 10. Effect of treatment with KF and baseline plasma level of tryptase on patient's global health status, pain-related and physical and emotional functioning measures at week 16.

Variable	Coefficient Value	*P*-value[a]
Global health status		
Treatment (Ref. Placebo)	0.950	0.313
Tryptase	-0.027	0.877
High pain intensity		
Treatment (Ref. Placebo)	-1.229	0.938
Tryptase	-1.205	0.231
Average pain intensity		
Treatment (Ref. Placebo)	-11.249	0.407
Tryptase	-0.698	0.410
Low pain intensity		
Treatment (Ref. Placebo)	-8.627	0.554
Tryptase	-0.522	0.844
High pain index		
Treatment (Ref. Placebo)	-0.032	0.974
Tryptase	-0.510	0.617
Average pain index		
Treatment (Ref. Placebo)	-1.029	0.319
Tryptase	0.294	0.772
Low pain index		
Treatment (Ref. Placebo)	0.581	0.569
Tryptase	-0.077	0.938
Pressure pain threshold		
Treatment (Ref. Placebo)	0.370	0.761
Tryptase	0.013	0.949
Analgesic medication		
Treatment (Ref. Placebo)	-0.070	0.922
Tryptase	-0.023	0.349
Disability		
Treatment (Ref. Placebo)	-3.633	0.607
Tryptase	-0.165	0.441
Anxiety		
Treatment (Ref. Placebo)	-4.172	0.622
Tryptase	0.054	0.906
Anxiety &Depression		
Treatment (Ref. Placebo)	-1.014	0.900
Tryptase	0.029	0.934
Sleep quality		
Treatment (Ref. Placebo)	-2.725	0.255
Tryptase	0.033	0.564

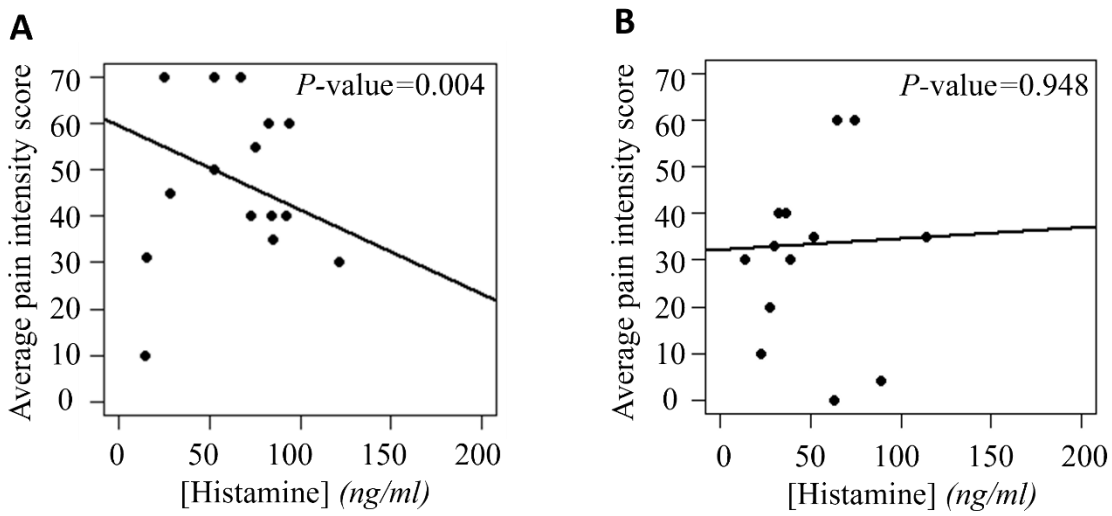
Footnotes: [a]Quantile regression

Figure 1. Flowchart of study design and procedures.



Footnotes: PGIC, Patient Global Impression of Change

Figure 2. Correlation between baseline histamine plasma levels and average pain intensity at week 16 in the KF(A) and (B) placebo groups



References

(1) Terminology | International Association for the Study of Pain. *International Association for the Study of Pain (IASP)*.

(2) Orr, P. M.; Shank, B. C.; Black, A. C. The Role of Pain Classification Systems in Pain Management. *Crit Care Nurs Clin North Am* 2017, *29* (4), 407–418. https://doi.org/10.1016/j.cnc.2017.08.002.

(3) Grichnik, K. P.; Ferrante, F. M. The Difference between Acute and Chronic Pain. *Mt Sinai J Med* 1991, *58* (3), 217–220.

(4) *ICD-11 for Mortality and Morbidity Statistics*. https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/1581976053 (accessed 2022-04-18).

(5) Treede, R.-D.; Rief, W.; Barke, A.; Aziz, Q.; Bennett, M. I.; Benoliel, R.; Cohen, M.; Evers, S.; Finnerup, N. B.; First, M. B.; Giamberardino, M. A.; Kaasa, S.; Kosek, E.; Lavand'homme, P.; Nicholas, M.; Perrot, S.; Scholz, J.; Schug, S.; Smith, B. H.; Svensson, P.; Vlaeyen, J. W. S.; Wang, S.-J. A Classification of Chronic Pain for ICD-11. *Pain* 2015, *156* (6), 1003–1007. https://doi.org/10.1097/j.pain.0000000000000160.

(6) Macfarlane, G. J. Generalized Pain, Fibromyalgia and Regional Pain: An Epidemiological View. *Best Practice & Research Clinical Rheumatology* 1999, *13* (3), 403–414. https://doi.org/10.1053/berh.1999.0029.

(7) Kosek, E.; Cohen, M.; Baron, R.; Gebhart, G. F.; Mico, J.-A.; Rice, A. S. C.; Rief, W.; Sluka, A. K. Do We Need a Third Mechanistic Descriptor for Chronic Pain States? *Pain* 2016, *157* (7), 1382–1386. https://doi.org/10.1097/j.pain.0000000000000507.

(8) Clauw, D. J.; Essex, M. N.; Pitman, V.; Jones, K. D. Reframing Chronic Pain as a Disease, Not a Symptom: Rationale and Implications for Pain Management. *null* 2019, *131* (3), 185–198. https://doi.org/10.1080/00325481.2019.1574403.

(9) Yong, R. J.; Mullins, P. M.; Bhattacharyya, N. Prevalence of Chronic Pain among Adults in the United States. *PAIN* 2022, *163* (2).

(10) Woolf, C. J. Central Sensitization: Implications for the Diagnosis and Treatment of Pain. *pain* 2011, *152* (3), S2–S15.

(11) Schmidt-Wilcke, T.; Clauw, D. J. Fibromyalgia: From Pathophysiology to Therapy. *Nature Reviews Rheumatology* 2011, *7* (9), 518–527.

(12) Gibson, S. J.; Littlejohn, G. O.; Gorman, M. M.; Helme, R. D.; Granges, G. Altered Heat Pain Thresholds and Cerebral Event-Related Potentials Following Painful CO2 Laser Stimulation in Subjects with Fibromyalgia Syndrome. *Pain* 1994, *58* (2), 185–193. https://doi.org/10.1016/0304-3959(94)90198-8.

(13) Lorenz, J.; Grasedyck, K.; Bromm, B. Middle and Long Latency Somatosensory Evoked Potentials after Painful Laser Stimulation in Patients with Fibromyalgia Syndrome. *Electroencephalogr Clin Neurophysiol* 1996, *100* (2), 165–168. https://doi.org/10.1016/0013-4694(95)00259-6.

(14) Liossi, C.; Howard, R. F. Pediatric Chronic Pain: Biopsychosocial Assessment and Formulation. *Pediatrics* 2016, *138* (5), e20160331. https://doi.org/10.1542/peds.2016-0331.

(15) Buskila, D.; Press, J.; Gedalia, A.; Klein, M.; Neumann, L.; Boehm, R.; Sukenik, S. Assessment of Nonarticular Tenderness and Prevalence of Fibromyalgia in Children. *J Rheumatol* 1993, *20* (2), 368–370.

(16) Mikkelsson, M.; El-Metwally, A.; Kautiainen, H.; Auvinen, A.; Macfarlane, G. J.; Salminen, J. J. Onset, Prognosis and Risk Factors for Widespread Pain in Schoolchildren: A Prospective 4-Year Follow-up Study. *PAIN* 2008, *138* (3).

(17) Haraldstad, K.; Sørum, R.; Eide, H.; Natvig, G. K.; Helseth, S. Pain in Children and Adolescents: Prevalence, Impact on Daily Life, and Parents' Perception, a School Survey. *Scandinavian journal of caring sciences* 2011, *25* (1), 27–36.

(18) Skrove, M.; Romundstad, P.; Indredavik, M. S. Chronic Multisite Pain in Adolescent Girls and Boys with Emotional and Behavioral Problems: The Young-HUNT Study. *European Child & Adolescent Psychiatry* 2015, *24* (5), 503–515.

(19) Norris, T.; Deere, K.; Tobias, J. H.; Crawley, E. Chronic Fatigue Syndrome and Chronic Widespread Pain in Adolescence: Population Birth Cohort Study. *J Pain* 2017, *18* (3), 285–294. https://doi.org/10.1016/j.jpain.2016.10.016.

(20) Somayajula, G. G.; Campbell, P.; Protheroe, J.; Lacey, R. J.; Dunn, K. M. Chronic Widespread Pain in Children and Adolescents Presenting in Primary Care: Prevalence and Associated Risk Factors. *PAIN* 2022, *163* (2).

(21) Yunus, M. B.; Masi, A. T. Juvenile Primary Fibromyalgia Syndrome. A Clinical Study of Thirty-Three Patients and Matched Normal Controls. *Arthritis Rheum* 1985, *28* (2), 138–145. https://doi.org/10.1002/art.1780280205.

(22) Walker, L. S.; Dengler-Crish, C. M.; Rippel, S.; Bruehl, S. Functional Abdominal Pain in Childhood and Adolescence Increases Risk for Chronic Pain in Adulthood. *Pain* 2010, *150* (3), 568–572.

(23) Groenewald, C. B.; Essner, B. S.; Wright, D.; Fesinmeyer, M. D.; Palermo, T. M. The Economic Costs of Chronic Pain among a Cohort of Treatment-Seeking Adolescents in the United States. *The Journal of Pain* 2014, *15* (9), 925–933.

(24) Clauw, D. J. Fibromyalgia: A Clinical Review. *JAMA* 2014, *311* (15), 1547–1555. https://doi.org/10.1001/jama.2014.3266.

(25) Kosek, E.; Hansson, P. Modulatory Influence on Somatosensory Perception from Vibration and Heterotopic Noxious Conditioning Stimulation (HNCS) in Fibromyalgia Patients and Healthy Subjects. *Pain* 1997, *70* (1), 41–51. https://doi.org/10.1016/s0304-3959(96)03295-2.

(26) Sörensen, J.; Graven-Nielsen, T.; Henriksson, K. G.; Bengtsson, M.; Arendt-Nielsen, L. Hyperexcitability in Fibromyalgia. *J Rheumatol* 1998, *25* (1), 152–155.

(27) Desmeules, J. A.; Cedraschi, C.; Rapiti, E.; Baumgartner, E.; Finckh, A.; Cohen, P.; Dayer, P.; Vischer, T. L. Neurophysiologic Evidence for a Central Sensitization in Patients with Fibromyalgia. *Arthritis Rheum* 2003, *48* (5), 1420–1429. https://doi.org/10.1002/art.10893.

(28) Petzke, F.; Clauw, D. J.; Ambrose, K.; Khine, A.; Gracely, R. H. Increased Pain Sensitivity in Fibromyalgia: Effects of Stimulus Type and Mode of Presentation. *Pain* 2003, *105* (3), 403–413.

(29) Gerster, J.; Hadj-Djilani, A. Hearing and Vestibular Abnormalities in Primary Fibrositis Syndrome. *The Journal of rheumatology* 1984, *11* (5), 678–680.

(30) Dohrenbusch, R.; Sodhi, H.; Lamprecht, J.; Genth, E. Fibromyalgia as a Disorder of Perceptual Organization? An Analysis of Acoustic Stimulus Processing in Patients with Widespread Pain. *Z Rheumatol* 1997, *56* (6), 334–341. https://doi.org/10.1007/s003930050047.

(31) Geisser, M. E.; Glass, J. M.; Rajcevska, L. D.; Clauw, D. J.; Williams, D. A.; Kileny, P. R.; Gracely, R. H. A Psychophysical Study of Auditory and Pressure Sensitivity in Patients with Fibromyalgia and Healthy Controls. *The journal of pain* 2008, *9* (5), 417–422.

(32) Coghill, R. C.; McHaffie, J. G.; Yen, Y.-F. Neural Correlates of Interindividual Differences in the Subjective Experience of Pain. *Proceedings of the National Academy of Sciences* 2003, *100* (14), 8538–8542.

(33) Gracely, R.; Geisser, M.; Giesecke, T.; Grant, M.; Petzke, F.; Williams, D.; Clauw, D. Pain Catastrophizing and Neural Responses to Pain among Persons with Fibromyalgia. *Brain* 2004, *127* (4), 835–843.

(34) Giesecke, T.; Gracely, R. H.; Grant, M. A.; Nachemson, A.; Petzke, F.; Williams, D. A.; Clauw, D. J. Evidence of Augmented Central Pain Processing in Idiopathic Chronic Low Back Pain. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 2004, *50* (2), 613–623.

(35) Napadow, V.; Kim, J.; Clauw, D. J.; Harris, R. E. Decreased Intrinsic Brain Connectivity Is Associated with Reduced Clinical Pain in Fibromyalgia. *Arthritis Rheum* 2012, *64* (7), 2398–2403. https://doi.org/10.1002/art.34412.

(36) Segerdahl, A. R.; Mezue, M.; Okell, T. W.; Farrar, J. T.; Tracey, I. The Dorsal Posterior Insula Subserves a Fundamental Role in Human Pain. *Nat Neurosci* 2015, *18* (4), 499–500. https://doi.org/10.1038/nn.3969.

(37) Napadow, V.; LaCount, L.; Park, K.; As-Sanie, S.; Clauw, D. J.; Harris, R. E. Intrinsic Brain Connectivity in Fibromyalgia Is Associated with Chronic Pain Intensity. *Arthritis & Rheumatism* 2010, *62* (8), 2545–2555.

(38) Fields, H. State-Dependent Opioid Control of Pain. *Nature Reviews Neuroscience* 2004, *5* (7), 565–575.

(39) Julien, N.; Goffaux, P.; Arsenault, P.; Marchand, S. Widespread Pain in Fibromyalgia Is Related to a Deficit of Endogenous Pain Inhibition. *Pain* 2005, *114* (1–2), 295–302. https://doi.org/10.1016/j.pain.2004.12.032.

(40) Mayer, E. A.; Gebhart, G. F. Basic and Clinical Aspects of Visceral Hyperalgesia. *Gastroenterology* 1994, *107* (1), 271–293. https://doi.org/10.1016/0016-5085(94)90086-8.

(41) Lautenbacher, S.; Rollman, G. Possible Deficiencies of Pain Modulation in Fibromyalgia. *The Clinical journal of pain* 1997, *13* (3), 189–196.

(42) Wilder-Smith, C. H.; Robert-Yap, J. Abnormal Endogenous Pain Modulation and Somatic and Visceral Hypersensitivity in Female Patients with Irritable Bowel Syndrome. *World journal of gastroenterology: WJG* 2007, *13* (27), 3699.

(43) Yarnitsky, D. Conditioned Pain Modulation (the Diffuse Noxious Inhibitory Control-like Effect): Its Relevance for Acute and Chronic Pain States. *Current Opinion in Anesthesiology* 2010, *23* (5), 611–615.

(44) Russell, I. J.; Orr, M. D.; Littman, B.; Vipraio, G. A.; Alboukrek, D.; Michalek, J. E.; Lopez, Y.; Mackillip, F. Elevated Cerebrospinal Fluid Levels of Substance P in Patients with the Fibromyalgia Syndrome. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 1994, *37* (11), 1593–1601.

(45) Sarchielli, P.; Di Filippo, M.; Nardi, K.; Calabresi, P. Sensitization, Glutamate, and the Link between Migraine and Fibromyalgia. *Curr Pain Headache Rep* 2007, *11* (5), 343–351. https://doi.org/10.1007/s11916-007-0216-2.

(46) Harris, R. E.; Sundgren, P. C.; Craig, A.; Kirshenbaum, E.; Sen, A.; Napadow, V.; Clauw, D. J. Elevated Insular Glutamate in Fibromyalgia Is Associated with Experimental Pain. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 2009, *60* (10), 3146–3152.

(47) Fayed, N.; Garcia-Campayo, J.; Magallón, R.; Andrés-Bergareche, H.; Luciano, J. V.; Andres, E.; Beltrán, J. Localized 1 H-NMR Spectroscopy in Patients with Fibromyalgia: A Controlled Study of Changes in Cerebral Glutamate/Glutamine, Inositol, Choline, and N-Acetylaspartate. *Arthritis research & therapy* 2010, *12* (4), 1–12.

(48) Harris, R. E. Elevated Excitatory Neurotransmitter Levels in the Fibromyalgia Brain. *Arthritis research & therapy* 2010, *12* (5), 1–2.

(49) Harris, R. E.; Sundgren, P. C.; Pang, Y.; Hsu, M.; Petrou, M.; Kim, S.; McLean, S. A.; Gracely, R. H.; Clauw, D. J. Dynamic Levels of Glutamate within the Insula Are Associated with Improvements in Multiple Pain Domains in Fibromyalgia. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 2008, *58* (3), 903–907.

(50) Harte, S. E.; Clauw, D. J.; Napadow, V.; Harris, R. E. Pressure Pain Sensitivity and Insular Combined Glutamate and Glutamine (Glx) Are Associated with Subsequent Clinical Response to Sham but Not Traditional Acupuncture in Patients Who Have Chronic Pain. *Medical acupuncture* 2013, *25* (2), 154–160.

(51) Foerster, B. R.; Nascimento, T. D.; DeBoer, M.; Bender, M. A.; Rice, I. C.; Truong, D. Q.; Bikson, M.; Clauw, D. J.; Zubieta, J.-K.; Harris, R. E. Excitatory and Inhibitory Brain Metabolites as Targets and Predictors of Effective Motor Cortex TDCS Therapy in Fibromyalgia. *Arthritis & Rheumatology (Hoboken, NJ)* 2015, *67* (2), 576.

(52) Graven-Nielsen, T.; Kendall, S. A.; Henriksson, K. G.; Bengtsson, M.; Sörensen, J.; Johnson, A.; Gerdle, B.; Arendt-Nielsen, L. Ketamine Reduces Muscle Pain, Temporal Summation, and Referred Pain in Fibromyalgia Patients. *Pain* 2000, *85* (3), 483–491. https://doi.org/10.1016/S0304-3959(99)00308-5.

(53) Staud, R.; Vierck, C. J.; Robinson, M. E.; Price, D. D. Effects of the N-Methyl-D-Aspartate Receptor Antagonist Dextromethorphan on Temporal Summation of Pain Are Similar in Fibromyalgia Patients and Normal Control Subjects. *J Pain* 2005, *6* (5), 323–332. https://doi.org/10.1016/j.jpain.2005.01.357.

(54) Cohen, S. P.; Verdolin, M. H.; Chang, A. S.; Kurihara, C.; Morlando, B. J.; Mao, J. The Intravenous Ketamine Test Predicts Subsequent Response to an Oral Dextromethorphan Treatment Regimen in Fibromyalgia Patients. *J Pain* 2006, *7* (6), 391–398. https://doi.org/10.1016/j.jpain.2005.12.010.

(55) Olivan-Blázquez, B.; Herrera-Mercadal, P.; Puebla-Guedea, M.; Pérez-Yus, M.-C.; Andrés, E.; Fayed, N.; López-Del-Hoyo, Y.; Magallon, R.; Roca, M.; Garcia-Campayo, J. Efficacy of Memantine in the Treatment of Fibromyalgia: A Double-Blind, Randomised, Controlled Trial with 6-Month Follow-Up. *Pain* 2014, *155* (12), 2517–2525. https://doi.org/10.1016/j.pain.2014.09.004.

(56) Bobinski, F.; Ferreira, T. A. A.; Córdova, M. M.; Dombrowski, P. A.; da Cunha, C.; Santo, C. C. do E.; Poli, A.; Pires, R. G. W.; Martins-Silva, C.; Sluka, K. A.; Santos, A. R. S. Role of Brainstem Serotonin in Analgesia Produced by Low-Intensity Exercise on Neuropathic Pain after Sciatic Nerve Injury in Mice. *Pain* 2015, *156* (12), 2595–2606. https://doi.org/10.1097/j.pain.0000000000000372.

(57) Oaklander, A. L.; Herzog, Z. D.; Downs, H. M.; Klein, M. M. Objective Evidence That Small-Fiber Polyneuropathy Underlies Some Illnesses Currently Labeled as Fibromyalgia. *PAIN*® 2013, *154* (11), 2310–2316.

(58) Albrecht, P. J.; Hou, Q.; Argoff, C. E.; Storey, J. R.; Wymer, J. P.; Rice, F. L. Excessive Peptidergic Sensory Innervation of Cutaneous Arteriole-Venule Shunts (AVS) in the Palmar Glabrous Skin of Fibromyalgia Patients: Implications for Widespread Deep Tissue Pain and Fatigue. *Pain Med* 2013, *14* (6), 895–915. https://doi.org/10.1111/pme.12139.

(59) Üçeyler, N.; Sommer, C. Objective Evidence That Small-Fiber Polyneuropathy Underlies Some Illnesses Currently Labeled as Fibromyalgia. *Pain* 2013, *154* (11), 2569.

(60) Caro, X. J.; Winter, E. F. Evidence of Abnormal Epidermal Nerve Fiber Density in Fibromyalgia: Clinical and Immunologic Implications. *Arthritis & rheumatology* 2014, *66* (7), 1945–1954.

(61) Doppler, K.; Rittner, H. L.; Deckart, M.; Sommer, C. Reduced Dermal Nerve Fiber Diameter in Skin Biopsies of Patients with Fibromyalgia. *Pain* 2015, *156* (11), 2319–2325.

(62) Gür, A.; Karakoç, M.; Nas, K.; Denli, A.; Saraç, J. Cytokines and Depression in Cases with Fibromyalgia. *The Journal of rheumatology* 2002, *29* (2), 358–361.

(63) Bazzichi, L.; Rossi, A.; Massimetti, G.; Giannaccini, G.; Giuliano, T.; De Feo, F.; Ciapparelli, A.; Dell Osso, L.; Bombardieri, S. Cytokine Patterns in Fibromyalgia and Their Correlation with Clinical Manifestations. *Clinical and experimental rheumatology* 2007, *25* (2), 225.

(64) Üçeyler, N.; Valenza, R.; Stock, M.; Schedel, R.; Sprotte, G.; Sommer, C. Reduced Levels of Antiinflammatory Cytokines in Patients with Chronic Widespread Pain. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 2006, *54* (8), 2656–2664.

(65) Maes, M.; Libbrecht, I.; Van Hunsel, F.; Lin, A.; De Clerck, L.; Stevens, W.; Kenis, G.; de Jongh, R.; Bosmans, E.; Neels, H. The Immune-Inflammatory Pathophysiology of Fibromyalgia: Increased Serum Soluble Gp130, the Common Signal Transducer Protein of Various Neurotrophic Cytokines. *Psychoneuroendocrinology* 1999, *24* (4), 371–383.

(66) Wallace, D.; Linker-Israeli, M.; Hallegua, D.; Silverman, S.; Silver, D.; Weisman, M. Cytokines Play an Aetiopathogenetic Role in Fibromyalgia: A Hypothesis and Pilot Study. *Rheumatology* 2001, *40* (7), 743–749.

(67) Amel Kashipaz, M.; Swinden, D.; Todd, I.; Powell, R. Normal Production of Inflammatory Cytokines in Chronic Fatigue and Fibromyalgia Syndromes Determined by Intracellular Cytokine Staining in Short-Term Cultured Blood Mononuclear Cells. *Clinical & Experimental Immunology* 2003, *132* (2), 360–365.

(68) Hader, N.; Rimon, D.; Kinarty, A.; Lahat, N. Altered Interleukin-2 Secretion in Patients with Primary Fibromyalgia Syndrome. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 1991, *34* (7), 866–872.

(69) Torpy, D. J.; Papanicolaou, D. A.; Lotsikas, A. J.; Wilder, R. L.; Chrousos, G. P.; Pillemer, S. R. Responses of the Sympathetic Nervous System and the Hypothalamic–Pituitary–

Adrenal Axis to Interleukin-6: A Pilot Study in Fibromyalgia. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* 2000, *43* (4), 872–880.

(70) Behm, F. G.; Gavin, I. M.; Karpenko, O.; Lindgren, V.; Gaitonde, S.; Gashkoff, P. A.; Gillis, B. S. Unique Immunologic Patterns in Fibromyalgia. *BMC Clinical Pathology* 2012, *12* (1), 1–7.

(71) Salemi, S.; Rethage, J.; Wollina, U.; Michel, B. A.; Gay, R. E.; Gay, S.; Sprott, H. Detection of Interleukin 1beta (IL-1beta), IL-6, and Tumor Necrosis Factor-Alpha in Skin of Patients with Fibromyalgia. *The Journal of rheumatology* 2003, *30* (1), 146–150.

(72) Lukkahatai, N.; Walitt, B.; Espina, A.; Wang, D.; Saligan, L. N. Comparing Genomic Profiles of Women with and without Fibromyalgia. *Biological research for nursing* 2015, *17* (4), 373–383.

(73) Hernanz, W.; Valenzuela, A.; Quijada, J.; Garcia, A.; De La Iglesia, J.; Gutierrez, A.; Povedano, J.; Moreno, I.; Sanchez, B. Lymphocyte Subpopulations in Patients with Primary Fibromyalgia. *The Journal of Rheumatology* 1994, *21* (11), 2122–2124.

(74) Macedo, J. A.; Hesse, J.; Turner, J. D.; Ammerlaan, W.; Gierens, A.; Hellhammer, D. H.; Muller, C. P. Adhesion Molecules and Cytokine Expression in Fibromyalgia Patients: Increased L-Selectin on Monocytes and Neutrophils. *Journal of neuroimmunology* 2007, *188* (1–2), 159–166.

(75) Kucuksezer, U. C.; Aktas Cetin, E.; Esen, F.; Tahrali, I.; Akdeniz, N.; Gelmez, M. Y.; Deniz, G. The Role of Natural Killer Cells in Autoimmune Diseases. *Frontiers in immunology* 2021, *12*, 79.

(76) Poli, A.; Michel, T.; Thérésine, M.; Andrès, E.; Hentges, F.; Zimmer, J. CD56bright Natural Killer (NK) Cells: An Important NK Cell Subset. *Immunology* 2009, *126* (4), 458–465.

(77) Verma, V.; Drury, G. L.; Parisien, M.; AN, Ö. A.; Al-Aubodah, T.-A.; Nijnik, A.; Wen, X.; Tugarinov, N.; Verner, M.; Klares 3rd, R. Unbiased Immune Profiling Reveals a Natural Killer Cell-Peripheral Nerve Axis in Fibromyalgia. *Pain* 2021.

(78) Méndez-Enríquez, E.; Hallgren, J. Mast Cells and Their Progenitors in Allergic Asthma. *Frontiers in Immunology* 2019, *10*.

(79) Metcalfe, D. D.; Baram, D.; Mekori, Y. A. Mast Cells. *Physiological Reviews* 1997, *77* (4), 1033–1079. https://doi.org/10.1152/physrev.1997.77.4.1033.

(80) Héron, A.; Dubayle, D. A Focus on Mast Cells and Pain. *Journal of Neuroimmunology* 2013, *264* (1–2), 1–7. https://doi.org/10.1016/j.jneuroim.2013.09.018.

(81) Woolf, C. J. What Is This Thing Called Pain? *The Journal of clinical investigation* 2010, *120* (11), 3742–3744.

(82) Johnson, D.; Krenger, W. Interactions of Mast Cells with the Nervous System--Recent Advances. *Neurochem Res* 1992, *17* (9), 939–951. https://doi.org/10.1007/BF00993271.

(83) Schwartz, L. B.; Austen, K. F. Enzymes of the Mast Cell Granule. *J Invest Dermatol* 1980, *74* (5), 349–353. https://doi.org/10.1111/1523-1747.ep12543620.

(84) Hurwitz, E. L.; Morgenstern, H. Cross-Sectional Associations of Asthma, Hay Fever, and Other Allergies with Major Depression and Low-Back Pain among Adults Aged 20-39 Years in the United States. *Am J Epidemiol* 1999, *150* (10), 1107–1116. https://doi.org/10.1093/oxfordjournals.aje.a009936.

(85) Aamodt, A. H.; Stovner, L. J.; Langhammer, A.; Hagen, K.; Zwart, J.-A. Is Headache Related to Asthma, Hay Fever, and Chronic Bronchitis? The Head-HUNT Study. *Headache* 2007, *47* (2), 204–212. https://doi.org/10.1111/j.1526-4610.2006.00597.x.

(86) Harlow, B. L.; He, W.; Nguyen, R. H. N. Allergic Reactions and Risk of Vulvodynia. *Annals of Epidemiology* 2009, *19* (11), 771–777. https://doi.org/10.1016/j.annepidem.2009.06.006.

(87) Tobin, M. C.; Moparty, B.; Farhadi, A.; DeMeo, M. T.; Bansal, P. J.; Keshavarzian, A. Atopic Irritable Bowel Syndrome: A Novel Subgroup of Irritable Bowel Syndrome with Allergic Manifestations. *Ann Allergy Asthma Immunol* 2008, *100* (1), 49–53. https://doi.org/10.1016/S1081-1206(10)60404-8.

(88) Sanders, A. E.; Maixner, W.; Nackley, A. G.; Diatchenko, L.; By, K.; Miller, V. E.; Slade, G. D. Excess Risk of Temporomandibular Disorder Associated with Cigarette Smoking in Young Adults. *J Pain* 2012, *13* (1), 21–31. https://doi.org/10.1016/j.jpain.2011.08.003.

(89) Choi, C.-J.; Knutsen, R.; Oda, K.; Fraser, G. E.; Knutsen, S. F. The Association between Incident Self-Reported Fibromyalgia and Nonpsychiatric Factors: 25-Years Follow-up of the Adventist Health Study. *J Pain* 2010, *11* (10), 994–1003. https://doi.org/10.1016/j.jpain.2010.01.267.

(90) Banafea, G. H.; Bakhashab, S.; Alshaibi, H. F.; Natesan Pushparaj, P.; Rasool, M. The Role of Human Mast Cells in Allergy and Asthma. *Bioengineered* 2022, *13* (3), 7049–7064.

(91) Heatley, R.; Denburg, J.; Bayer, N.; Bienenstock, J. Increased Plasma Histamine Levels in Migraine Patients. *Clinical & Experimental Allergy* 1982, *12* (2), 145–149.

(92) Theoharides, T. C.; Donelan, J.; Kandere-Grzybowska, K.; Konstantinidou, A. The Role of Mast Cells in Migraine Pathophysiology. *Brain research reviews* 2005, *49* (1), 65–76.

(93) Done, J. D.; Rudick, C. N.; Quick, M. L.; Schaeffer, A. J.; Thumbikat, P. Role of Mast Cells in Male Chronic Pelvic Pain. *The Journal of urology* 2012, *187* (4), 1473–1482.

(94) Pepper, A.; Li, W.; Kingery, W. S.; Angst, M. S.; Curtin, C. M.; Clark, J. D. Changes Resembling Complex Regional Pain Syndrome Following Surgery and Immobilization. *The journal of pain* 2013, *14* (5), 516–524.

(95) Bornstein, J.; Goldschmid, N.; Sabo, E. Hyperinnervation and Mast Cell Activation May Be Used as Histopathologic Diagnostic Criteria for Vulvar Vestibulitis. *Gynecologic and obstetric investigation* 2004, *58* (3), 171–178.

(96) Bornstein, J.; Cohen, Y.; Zarfati, D.; Sela, S.; Ophir, E. Involvement of Heparanase in the Pathogenesis of Localized Vulvodynia. *International Journal of Gynecological Pathology* 2008, *27* (1), 136–141.

(97) Walker, M. M.; Powell, N.; Talley, N. J. Atopy and the Gastrointestinal Tract–a Review of a Common Association in Unexplained Gastrointestinal Disease. *Expert review of gastroenterology & hepatology* 2014, *8* (3), 289–299.

(98) Barbara, G.; Stanghellini, V.; De Giorgio, R.; Corinaldesi, R. Functional Gastrointestinal Disorders and Mast Cells: Implications for Therapy. *Neurogastroenterology & Motility* 2006, *18* (1), 6–17.

(99) Henderson, W. A.; Shankar, R.; Taylor, T. J.; Del Valle-Pinero, A. Y.; Kleiner, D. E.; Kim, K. H.; Youssef, N. N. Inverse Relationship of Interleukin-6 and Mast Cells in Children with Inflammatory and Non-Inflammatory Abdominal Pain Phenotypes. *World journal of gastrointestinal pathophysiology* 2012, *3* (6), 102.

(100) Anaf, V.; Chapron, C.; El Nakadi, I.; De Moor, V.; Simonart, T.; Noël, J.-C. Pain, Mast Cells, and Nerves in Peritoneal, Ovarian, and Deep Infiltrating Endometriosis. *Fertility and sterility* 2006, *86* (5), 1336–1343.

(101) Blanco, I.; Béritze, N.; Argüelles, M.; Cárcaba, V.; Fernández, F.; Janciauskiene, S.; Oikonomopoulou, K.; de Serres, F. J.; Fernández-Bustillo, E.; Hollenberg, M. D. Abnormal Overexpression of Mastocytes in Skin Biopsies of Fibromyalgia Patients. *Clin Rheumatol* 2010, *29* (12), 1403–1412. https://doi.org/10.1007/s10067-010-1474-7.

(102) Chatterjea, D.; Wetzel, A.; Mack, M.; Engblom, C.; Allen, J.; Mora, C.; Paredes, L.; Balsells Hernández, E.; Martinov, T. Mast Cell Degranulation Mediates Compound 48/80-Induced Hyperalgesia in Mice. *Biochemical and biophysical research communications* 2012, *425*, 237–243. https://doi.org/10.1016/j.bbrc.2012.07.074.

(103) Lavich, T. R.; Cordeiro, R. S. B.; Calixto, J. B.; e Silva, P. M. R.; Martins, M. A. Combined Action of Vasoactive Amines and Bradykinin Mediates Allergen-Evoked Thermal Hyperalgesia in Rats. *Eur J Pharmacol* 2003, *462* (1–3), 185–192. https://doi.org/10.1016/s0014-2999(02)02947-3.

(104) Wershil, B. K.; Wang, Z. S.; Gordon, J. R.; Galli, S. J. Recruitment of Neutrophils during IgE-Dependent Cutaneous Late Phase Reactions in the Mouse Is Mast Cell-Dependent. Partial Inhibition of the Reaction with Antiserum against Tumor Necrosis Factor-Alpha. *J Clin Invest* 1991, *87* (2), 446–453. https://doi.org/10.1172/JCI115016.

(105) Cervantes-Durán, C.; Pineda-Farias, J. B.; Bravo-Hernández, M.; Quiñonez-Bastidas, G. N.; Vidal-Cantú, G. C.; Barragán-Iglesias, P.; Granados-Soto, V. Evidence for the Participation of Peripheral 5-HT_2A, 5-HT_2B, and 5-HT_2C Receptors in Formalin-Induced

Secondary Mechanical Allodynia and Hyperalgesia. *Neuroscience* 2013, *232*, 169–181. https://doi.org/10.1016/j.neuroscience.2012.11.047.

(106) Parada, C. A.; Tambeli, C. H.; Cunha, F. Q.; Ferreira, S. H. The Major Role of Peripheral Release of Histamine and 5-Hydroxytryptamine in Formalin-Induced Nociception. *Neuroscience* 2001, *102* (4), 937–944. https://doi.org/10.1016/s0306-4522(00)00523-6.

(107) Levy, D.; Burstein, R.; Kainz, V.; Jakubowski, M.; Strassman, A. M. Mast Cell Degranulation Activates a Pain Pathway Underlying Migraine Headache. *PAIN* 2007, *130* (1), 166–176. https://doi.org/10.1016/j.pain.2007.03.012.

(108) Baun, M.; Pedersen, M. H. F.; Olesen, J.; Jansen-Olesen, I. Dural Mast Cell Degranulation Is a Putative Mechanism for Headache Induced by PACAP-38. *Cephalalgia* 2012, *32* (4), 337–345. https://doi.org/10.1177/0333102412439354.

(109) Levy, D.; Kainz, V.; Burstein, R.; Strassman, A. M. Mast Cell Degranulation Distinctly Activates Trigemino-Cervical and Lumbosacral Pain Pathways and Elicits Widespread Tactile Pain Hypersensitivity. *Brain Behav Immun* 2012, *26* (2), 311–317. https://doi.org/10.1016/j.bbi.2011.09.016.

(110) Rudick, C. N.; Bryce, P. J.; Guichelaar, L. A.; Berry, R. E.; Klumpp, D. J. Mast Cell-Derived Histamine Mediates Cystitis Pain. *PLoS One* 2008, *3* (5), e2096. https://doi.org/10.1371/journal.pone.0002096.

(111) Oliveira, S. M.; Drewes, C. C.; Silva, C. R.; Trevisan, G.; Boschen, S. L.; Moreira, C. G.; de Almeida Cabrini, D.; Da Cunha, C.; Ferreira, J. Involvement of Mast Cells in a Mouse Model of Postoperative Pain. *Eur J Pharmacol* 2011, *672* (1–3), 88–95. https://doi.org/10.1016/j.ejphar.2011.10.001.

(112) Oliveira, S. M.; Silva, C. R.; Ferreira, J. Critical Role of Protease-Activated Receptor 2 Activation by Mast Cell Tryptase in the Development of Postoperative Pain. *Anesthesiology* 2013, *118* (3), 679–690. https://doi.org/10.1097/ALN.0b013e31827d415f.

(113) Yasuda, M.; Kido, K.; Ohtani, N.; Masaki, E. Mast Cell Stabilization Promotes Antinociceptive Effects in a Mouse Model of Postoperative Pain. *J Pain Res* 2013, *6*, 161–166. https://doi.org/10.2147/JPR.S41527.

(114) Chen, Y.; Yang, C.; Wang, Z. J. Proteinase-Activated Receptor 2 Sensitizes Transient Receptor Potential Vanilloid 1, Transient Receptor Potential Vanilloid 4, and Transient Receptor Potential Ankyrin 1 in Paclitaxel-Induced Neuropathic Pain. *Neuroscience* 2011, *193*, 440–451. https://doi.org/10.1016/j.neuroscience.2011.06.085.

(115) Li, W.-W.; Guo, T.-Z.; Liang, D.; Sun, Y.; Kingery, W. S.; Clark, J. D. Substance P Signaling Controls Mast Cell Activation, Degranulation, and Nociceptive Sensitization in a Rat Fracture Model of Complex Regional Pain Syndrome. *Anesthesiology* 2012, *116* (4), 882–895. https://doi.org/10.1097/ALN.0b013e31824bb303.

(116) Taiwo, O. B.; Kovács, K. J.; Sun, Y.; Larson, A. A. Unilateral Spinal Nerve Ligation Leads to an Asymmetrical Distribution of Mast Cells in the Thalamus of Female but Not Male Mice. *Pain* 2005, *114* (1–2), 131–140. https://doi.org/10.1016/j.pain.2004.12.002.

(117) Vincent, L.; Vang, D.; Nguyen, J.; Gupta, M.; Luk, K.; Ericson, M. E.; Simone, D. A.; Gupta, K. Mast Cell Activation Contributes to Sickle Cell Pathobiology and Pain in Mice. *Blood* 2013, *122* (11), 1853–1862. https://doi.org/10.1182/blood-2013-04-498105.

(118) Zuo, Y.; Perkins, N. M.; Tracey, D. J.; Geczy, C. L. Inflammation and Hyperalgesia Induced by Nerve Injury in the Rat: A Key Role of Mast Cells. *Pain* 2003, *105* (3), 467–479. https://doi.org/10.1016/S0304-3959(03)00261-6.

(119) Pepper, A.; Li, W.; Kingery, W. S.; Angst, M. S.; Curtin, C. M.; Clark, J. D. Changes Resembling Complex Regional Pain Syndrome Following Surgery and Immobilization. *The journal of pain* 2013, *14* (5), 516–524.

(120) Goetsch, M. F.; Morgan, T. K.; Korcheva, V. B.; Li, H.; Peters, D.; Leclair, C. M. Histologic and Receptor Analysis of Primary and Secondary Vestibulodynia and Controls: A Prospective Study. *American journal of obstetrics and gynecology* 2010, *202* (6), 614-e1.

(121) Ottosson, A.; Edvinsson, L. Release of Histamine from Dural Mast Cells by Substance P and Calcitonin Gene-related Peptide. *Cephalalgia* 1997, *17* (3), 166–174.

(122) Olness, K.; Hall, H.; Rozniecki, J. J.; Schmidt, W.; Theoharides, T. Mast Cell Activation in Children with Migraine before and after Training in Self-regulation. *Headache: The Journal of Head and Face Pain* 1999, *39* (2), 101–107.

(123) Schytz, H. W.; Birk, S.; Wienecke, T.; Kruuse, C.; Olesen, J.; Ashina, M. PACAP38 Induces Migraine-like Attacks in Patients with Migraine without Aura. *Brain* 2009, *132* (1), 16–25.

(124) Rudick, C. N.; Schaeffer, A. J.; Klumpp, D. J. Pharmacologic Attenuation of Pelvic Pain in a Murine Model of Interstitial Cystitis. *BMC urology* 2009, *9* (1), 1–8.

(125) Quick, M. L.; Mukherjee, S.; Rudick, C. N.; Done, J. D.; Schaeffer, A. J.; Thumbikat, P. CCL2 and CCL3 Are Essential Mediators of Pelvic Pain in Experimental Autoimmune Prostatitis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2012, *303* (6), R580–R589.

(126) Greenwood, C. The Pharmacology of Ketotifen. *Chest* 1982, *82* (1, Supplement), 45s–48s. https://doi.org/10.1378/chest.82.1.45S.

(127) Fahmy, R. H.; Badr-Eldin, S. M. Novel Delivery Approach for Ketotifen Fumarate: Dissofilms Formulation Using 3^2 Experimental Design: In Vitro/in Vivo Evaluation. *Pharm Dev Technol* 2014, *19* (5), 521–530. https://doi.org/10.3109/10837450.2013.800108.

(128) Mylona-Karayanni, C.; Hadziargurou, D.; Liapi-Adamidou, G.; Anagnostakis, I.; Sinaniotis, C.; Saxoni-Papageorgiou, F. Effect of Ketotifen on Childhood Asthma: A

Double-Blind Study. *J Asthma* 1990, *27* (2), 87–93. https://doi.org/10.3109/02770909009073302.

(129) Huston, D. P.; Bressler, R. B.; Kaliner, M.; Sowell, L. K.; Baylor, M. W. Prevention of Mast-Cell Degranulation by Ketotifen in Patients with Physical Urticarias. *Ann Intern Med* 1986, *104* (4), 507–510. https://doi.org/10.7326/0003-4819-104-4-507.

(130) Yoshida, H.; Niimura, M.; Ueda, H.; Imaura, S.; Yamamoto, S.; Kukita, A. Clinical Evaluation of Ketotifen Syrup on Atopic Dermatitis: A Comparative Multicenter Double-Blind Study of Ketotifen and Clemastine. *Ann Allergy* 1989, *62* (6), 507–512.

(131) McClean, S. P.; Arreaza, E. E.; Lett-Brown, M. A.; Grant, J. A. Refractory Cholinergic Urticaria Successfully Treated with Ketotifen. *J Allergy Clin Immunol* 1989, *83* (4), 738–741. https://doi.org/10.1016/0091-6749(89)90008-0.

(132) Snyman, J. R.; Sommers, D. K.; Gregorowski, M. D.; Boraine, H. Effect of Cetirizine, Ketotifen and Chlorpheniramine on the Dynamics of the Cutaneous Hypersensitivity Reaction: A Comparative Study. *Eur J Clin Pharmacol* 1992, *42* (4), 359–362. https://doi.org/10.1007/BF00280118.

(133) Riccardi, V. M. A Controlled Multiphase Trial of Ketotifen to Minimize Neurofibroma-Associated Pain and Itching. *Arch Dermatol* 1993, *129* (5), 577–581.

(134) Rosas Vargas, M. A.; Moncayo Coello, V.; García Cárdenas, E.; Valencia Mayoral, P.; Sienra Monge, J. J. L.; del Río Navarro, B. E. [Eosinophilic colitis. A report of two cases with non conventional treatment]. *Rev Alerg Mex* 2004, *51* (6), 231–235.

(135) Bolukbas, F. F.; Bolukbas, C.; Uzunkoy, A.; Baba, F.; Horoz, M.; Ozturk, E. A Dramatic Response to Ketotifen in a Case of Eosinophilic Gastroenteritis Mimicking Abdominal Emergency. *Dig Dis Sci* 2004, *49* (11–12), 1782–1785. https://doi.org/10.1007/s10620-004-9570-7.

(136) Freeman, H. J. Longstanding Eosinophilic Gastroenteritis of More than 20 Years. *Can J Gastroenterol* 2009, *23* (9), 632–634. https://doi.org/10.1155/2009/565293.

(137) Klooker, T. K.; Braak, B.; Koopman, K. E.; Welting, O.; Wouters, M. M.; van der Heide, S.; Schemann, M.; Bischoff, S. C.; van den Wijngaard, R. M.; Boeckxstaens, G. E. The Mast Cell Stabiliser Ketotifen Decreases Visceral Hypersensitivity and Improves Intestinal Symptoms in Patients with Irritable Bowel Syndrome. *Gut* 2010, *59* (9), 1213–1221.

(138) Ang, D. C.; Hilligoss, J.; Stump, T. Mast Cell Stabilizer (Ketotifen) in Fibromyalgia: Phase 1 Randomized Controlled Clinical Trial. *Clin J Pain* 2015, *31* (9), 836–842. https://doi.org/10.1097/AJP.0000000000000169.

(139) Anoush, M.; Mohammad Khani, M. R. Evaluating the Anti-Nociceptive and Anti-Inflammatory Effects of Ketotifen and Fexofenadine in Rats. *Adv Pharm Bull* 2015, *5* (2), 217–222. https://doi.org/10.15171/apb.2015.030.

(140) Massaad, C. A.; Safieh-Garabedian, B.; Poole, S.; Atweh, S. F.; Jabbur, S. J.; Saadé, N. E. Involvement of Substance P, CGRP and Histamine in the Hyperalgesia and Cytokine Upregulation Induced by Intraplantar Injection of Capsaicin in Rats. *Journal of Neuroimmunology* 2004, *153* (1), 171–182. https://doi.org/10.1016/j.jneuroim.2004.05.007.

(141) Meloto, C. B.; Ingelmo, P.; Perez, E. V.; Pitt, R.; González Cárdenas, V. H.; Mohamed, N.; Sotocinal, S. G.; Bourassa, V.; Lima, L. V.; Ribeiro-da-Silva, A.; Mogil, J. S.; Diatchenko, L. Mast Cell Stabilizer Ketotifen Fumarate Reverses Inflammatory but Not Neuropathic-Induced Mechanical Pain in Mice. *PAIN Reports* 2021, *6* (2).

(142) Jensen, K. B.; Loitoile, R.; Kosek, E.; Petzke, F.; Carville, S.; Fransson, P.; Marcus, H.; Williams, S. C.; Choy, E.; Mainguy, Y. Patients with Fibromyalgia Display Less Functional Connectivity in the Brain's Pain Inhibitory Network. *Molecular pain* 2012, *8*, 1744–8069.

(143) Bazzichi, L.; Giannaccini, G.; Betti, L.; Mascia, G.; Fabbrini, L.; Italiani, P.; De Feo, F.; Giuliano, T.; Giacomelli, C.; Rossi, A. Alteration of Serotonin Transporter Density and Activity in Fibromyalgia. *Arthritis Research & Therapy* 2006, *8* (4), 1–6.

(144) Gurish, M. F.; Boyce, J. A. Mast Cell Growth, Differentiation, and Death. *Clinical Reviews in Allergy and Immunology* 2002, *22* (2), 107–118. https://doi.org/10.1385/CRIAI:22:2:107.

(145) Kabra, S. K.; Pandey, R. M.; Singh, R.; Seth, V. Ketotifen for Asthma in Children Aged 5 to 15 Years: A Randomized Placebo-Controlled Trial. *Ann Allergy Asthma Immunol* 2000, *85* (1), 46–52. https://doi.org/10.1016/S1081-1206(10)62433-7.

(146) Moore, A. R.; Eccleston, C.; Derry, S.; Wiffen, P.; Bell, R. F.; Straube, S.; McQuay, H.; for the ACTINPAIN writing group of the IASP Special Interest Group (SIG) on Systematic Reviews in Pain Relief and the Cochrane Pain, P. and S. "Evidence" in Chronic Pain – Establishing Best Practice in the Reporting of Systematic Reviews. *PAIN* 2010, *150* (3).

(147) Rampakakis, E.; Ste-Marie, P. A.; Sampalis, J. S.; Karellis, A.; Shir, Y.; Fitzcharles, M.-A. Real-Life Assessment of the Validity of Patient Global Impression of Change in Fibromyalgia. *RMD Open* 2015, *1* (1), e000146. https://doi.org/10.1136/rmdopen-2015-000146.

(148) Perrot, S.; Lantéri-Minet, M. Patients' Global Impression of Change in the Management of Peripheral Neuropathic Pain: Clinical Relevance and Correlations in Daily Practice. *European Journal of Pain* 2019, *23* (6), 1117–1128. https://doi.org/10.1002/ejp.1378.

(149) McGrath, P. J.; Walco, G. A.; Turk, D. C.; Dworkin, R. H.; Brown, M. T.; Davidson, K.; Eccleston, C.; Finley, G. A.; Goldschneider, K.; Haverkos, L.; Hertz, S. H.; Ljungman, G.; Palermo, T.; Rappaport, B. A.; Rhodes, T.; Schechter, N.; Scott, J.; Sethna, N.; Svensson, O. K.; Stinson, J.; von Baeyer, C. L.; Walker, L.; Weisman, S.; White, R. E.; Zajicek, A.; Zeltzer, L. Core Outcome Domains and Measures for Pediatric Acute and Chronic/Recurrent Pain Clinical Trials: PedIMMPACT Recommendations. *J Pain* 2008, *9* (9), 771–783. https://doi.org/10.1016/j.jpain.2008.04.007.

(150) Tchivileva, I. E.; Lim, P. F.; Smith, S. B.; Slade, G. D.; Diatchenko, L.; McLean, S. A.; Maixner, W. Effect of Catechol-O-Methyltransferase Polymorphism on Response to Propranolol Therapy in Chronic Musculoskeletal Pain: A Randomized, Double-Blind, Placebo-Controlled, Crossover Pilot Study. *Pharmacogenet Genomics* 2010, *20* (4), 239–248. https://doi.org/10.1097/FPC.0b013e328337f9ab.

(151) Dampier, C.; Ely, E.; Brodecki, D.; O'Neal, P. Home Management of Pain in Sickle Cell Disease: A Daily Diary Study in Children and Adolescents. *J Pediatr Hematol Oncol* 2002, *24* (8), 643–647. https://doi.org/10.1097/00043426-200211000-00008.

(152) Walker, L. S.; Greene, J. W. The Functional Disability Inventory: Measuring a Neglected Dimension of Child Health Status. *J Pediatr Psychol* 1991, *16* (1), 39–58. https://doi.org/10.1093/jpepsy/16.1.39.

(153) Kashikar-Zuck, S.; Flowers, S. R.; Claar, R. L.; Guite, J. W.; Logan, D. E.; Lynch-Jordan, A. M.; Palermo, T. M.; Wilson, A. C. Clinical Utility and Validity of the Functional Disability Inventory among a Multicenter Sample of Youth with Chronic Pain. *Pain* 2011, *152* (7), 1600–1607. https://doi.org/10.1016/j.pain.2011.02.050.

(154) Reid, G. J.; Lang, B. A.; McGrath, P. J. Primary Juvenile Fibromyalgia: Psychological Adjustment, Family Functioning, Coping, and Functional Disability. *Arthritis Rheum* 1997, *40* (4), 752–760. https://doi.org/10.1002/art.1780400423.

(155) Claar, R. L.; Walker, L. S. Functional Assessment of Pediatric Pain Patients: Psychometric Properties of the Functional Disability Inventory. *Pain* 2006, *121* (1–2), 77–84.

(156) Kashikar-Zuck, S.; Goldschneider, K. R.; Powers, S. W.; Vaught, M. H.; Hershey, A. D. Depression and Functional Disability in Chronic Pediatric Pain. *Clin J Pain* 2001, *17* (4), 341–349. https://doi.org/10.1097/00002508-200112000-00009.

(157) Eccleston, C.; Malleson, P. N.; Clinch, J.; Connell, H.; Sourbut, C. Chronic Pain in Adolescents: Evaluation of a Programme of Interdisciplinary Cognitive Behaviour Therapy. *Arch Dis Child* 2003, *88* (10), 881–885. https://doi.org/10.1136/adc.88.10.881.

(158) Chorpita, B. F.; Yim, L.; Moffitt, C.; Umemoto, L. A.; Francis, S. E. Assessment of Symptoms of DSM-IV Anxiety and Depression in Children: A Revised Child Anxiety and Depression Scale. *Behav Res Ther* 2000, *38* (8), 835–855. https://doi.org/10.1016/s0005-7967(99)00130-8.

(159) Becker, S. P.; Schindler, D. N.; Holdaway, A. S.; Tamm, L.; Epstein, J. N.; Luebbe, A. M. The Revised Child Anxiety and Depression Scales (RCADS): Psychometric Evaluation in Children Evaluated for ADHD. *J Psychopathol Behav Assess* 2019, *41* (1), 93–106. https://doi.org/10.1007/s10862-018-9702-6.

(160) Chorpita, B. F.; Moffitt, C. E.; Gray, J. Psychometric Properties of the Revised Child Anxiety and Depression Scale in a Clinical Sample. *Behav Res Ther* 2005, *43* (3), 309–322. https://doi.org/10.1016/j.brat.2004.02.004.

(161) Esbjørn, B. H.; Sømhovd, M. J.; Turnstedt, C.; Reinholdt-Dunne, M. L. Assessing the Revised Child Anxiety and Depression Scale (RCADS) in a National Sample of Danish Youth Aged 8-16 Years. *PLoS One* 2012, *7* (5), e37339. https://doi.org/10.1371/journal.pone.0037339.

(162) Mathyssek, C. M.; Olino, T. M.; Hartman, C. A.; Ormel, J.; Verhulst, F. C.; Van Oort, F. V. A. Does the Revised Child Anxiety and Depression Scale (RCADS) Measure Anxiety Symptoms Consistently across Adolescence? The TRAILS Study. *Int J Methods Psychiatr Res* 2013, *22* (1), 27–35. https://doi.org/10.1002/mpr.1380.

(163) Buysse, D. J.; Reynolds, C. F. 3rd; Monk, T. H.; Berman, S. R.; Kupfer, D. J. The Pittsburgh Sleep Quality Index: A New Instrument for Psychiatric Practice and Research. *Psychiatry Res* 1989, *28* (2), 193–213. https://doi.org/10.1016/0165-1781(89)90047-4.

(164) Plichta, S. B.; Kelvin, E. A. *Munro's Statistical Methods for Health Care Research*; Wolters Kluwer Health/Lippincott Williams & Wilkins, 2012.

(165) Barsky, A. J.; Saintfort, R.; Rogers, M. P.; Borus, J. F. Nonspecific Medication Side Effects and the Nocebo Phenomenon. *Jama* 2002, *287* (5), 622–627.

(166) Colloca, L. The Placebo Effect in Pain Therapies. *Annual review of pharmacology and toxicology* 2019, *59*, 191–211.

(167) Cordner, Z. A.; Li, Q.; Liu, L.; Tamashiro, K. L.; Bhargava, A.; Moran, T. H.; Pasricha, P. J. Vagal Gut-Brain Signaling Mediates Amygdaloid Plasticity, Affect, and Pain in a Functional Dyspepsia Model. *JCI insight* 2021, *6* (6).

(168) Barnes, N.; Costall, B.; Kelly, M.; Onaivi, E.; Naylor, R. Ketotifen and Its Analogues Reduce Aversive Responding in the Rodent. *Pharmacology Biochemistry and Behavior* 1990, *37* (4), 785–793.

(169) Costall, B.; Kelly, M.; Onaivi, E.; Naylor, R. The Effect of Ketotifen in Rodent Models of Anxiety and on the Behavioural Consequences of Withdrawing from Treatment with Drugs of Abuse. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1990, *341* (6), 547–551.

(170) Imaizumi, M.; Miyazaki, S.; Onodera, K. Effects of Betahistine, a Histamine H1 Agonist and H3 Antagonist, in a Light/Dark Test in Mice. *Methods and findings in experimental and clinical pharmacology* 1996, *18* (1), 19–24.

(171) Kitanaka, J.; Kitanaka, N.; Tatsuta, T.; Morita, Y.; Takemura, M. Blockade of Brain Histamine Metabolism Alters Methamphetamine-Induced Expression Pattern of Stereotypy in Mice via Histamine H1 Receptors. *Neuroscience* 2007, *147* (3), 765–777.

(172) Duque-Wilckens, N.; Teis, R.; Sarno, E.; Stoelting, F.; Khalid, S.; Dairi, Z.; Douma, A.; Maradiaga, N.; Hench, S.; Dharshika, C. D. Early Life Adversity Drives Sex-Specific Anhedonia and Meningeal Immune Gene Expression through Mast Cell Activation. *Brain, behavior, and immunity* 2022, *103*, 73–84.

(173) Iftikhar, K.; Siddiq, A.; Baig, S. G.; Zehra, S. Substance P: A Neuropeptide Involved in the Psychopathology of Anxiety Disorders. *Neuropeptides* 2020, *79*, 101993.

(174) Ebner, K.; Muigg, P.; Singewald, G.; Singewald, N. Substance P in Stress and Anxiety: NK-1 Receptor Antagonism Interacts with Key Brain Areas of the Stress Circuitry. *Annals of the New York Academy of Sciences* 2008, *1144* (1), 61–73.

(175) De Araújo, J. E.; Silva, R. C.; Huston, J. P.; Brandão, M. L. Anxiogenic Effects of Substance P and Its 7–11 C Terminal, but Not the 1–7 N Terminal, Injected into the Dorsal Periaqueductal Gray☆. *Peptides* 1999, *20* (12), 1437–1443.

(176) Ebner, K.; Rupniak, N. M.; Saria, A.; Singewald, N. Substance P in the Medial Amygdala: Emotional Stress-Sensitive Release and Modulation of Anxiety-Related Behavior in Rats. *Proceedings of the National Academy of Sciences* 2004, *101* (12), 4280–4285.

(177) Zhao, Z.; Yang, Y.; Walker, D. L.; Davis, M. Effects of Substance P in the Amygdala, Ventromedial Hypothalamus, and Periaqueductal Gray on Fear-Potentiated Startle. *Neuropsychopharmacology* 2009, *34* (2), 331–340.

(178) Caberlotto, L.; Hurd, Y. L.; Murdock, P.; Wahlin, J. P.; Melotto, S.; Corsi, M.; Carletti, R. Neurokinin 1 Receptor and Relative Abundance of the Short and Long Isoforms in the Human Brain. *European Journal of Neuroscience* 2003, *17* (9), 1736–1746.

(179) Van Der Hart, M. G.; De Biurrun, G.; Czéh, B.; Rupniak, N. M.; Den Boer, J. A.; Fuchs, E. Chronic Psychosocial Stress in Tree Shrews: Effect of the Substance P (NK1 Receptor) Antagonist L-760735 and Clomipramine on Endocrine and Behavioral Parameters. *Psychopharmacology* 2005, *181* (2), 207–216.

(180) Degnan, A. P.; Tora, G. O.; Huang, H.; Conlon, D. A.; Davis, C. D.; Hanumegowda, U. M.; Hou, X.; Hsiao, Y.; Hu, J.; Krause, R. Discovery of Indazoles as Potent, Orally Active Dual Neurokinin 1 Receptor Antagonists and Serotonin Transporter Inhibitors for the Treatment of Depression. *ACS Chemical Neuroscience* 2016, *7* (12), 1635–1640.

(181) Stefánsdóttir, Í. H.; Ivarsson, T.; Skarphedinsson, G. Efficacy and Safety of Serotonin Reuptake Inhibitors (SSRI) and Serotonin Noradrenaline Reuptake Inhibitors (SNRI) for Children and Adolescents with Anxiety Disorders: A Systematic Review and Meta-Analysis. *Nordic Journal of Psychiatry* 2022, 1–10.

(182) Rink, L.; Adams, A.; Braun, C.; Bschor, T.; Kuhr, K.; Baethge, C. Dose-Response Relationship in Selective Serotonin and Norepinephrine Reuptake Inhibitors in the Treatment of Major Depressive Disorder: A Meta-Analysis and Network Meta-Analysis of Randomized Controlled Trials. *Psychotherapy and psychosomatics* 2022, *91* (2), 84–93.

(183) Yanai, K.; Ryu, J. H.; Watanabe, T.; Iwata, R.; Ido, T.; Sawai, Y.; Ito, K.; Itoh, M. Histamine H1 Receptor Occupancy in Human Brains after Single Oral Doses of Histamine H1 Antagonists Measured by Positron Emission Tomography. *British journal of pharmacology* 1995, *116* (1), 1649.

(184) Yanai, K.; Ryu, J.; Watanabe, T.; Iwata, R.; Ido, T.; Asakura, M.; Matsumura, R.; Itoh, M. Positron Emission Tomographic Study of Central Histamine H1-Receptor Occupancy in Human Subjects Treated with Epinastine, a Second-Generation Antihistamine. *Methods and findings in experimental and clinical pharmacology* 1995, *17*, 64–69.

(185) Yanai, K.; Okamura, N.; Tagawa, M.; Itoh, M.; Watanabe, T. New Findings in Pharmacological Effects Induced by Antihistamines: From PET Studies to Knock-out Mice. *Clinical & Experimental Allergy* 1999, *29*, 29–36.

(186) Okamura, N.; Yanai, K.; Higuchi, M.; Sakai, J.; Iwata, R.; Ido, T.; Sasaki, H.; Watanabe, T.; Itoh, M. Functional Neuroimaging of Cognition Impaired by a Classical Antihistamine, d-Chlorpheniramine. *British Journal of Pharmacology* 2000, *129* (1), 115.

(187) Tagawa, M.; Kano, M.; Okamura, N.; Higuchi, M.; Matsuda, M.; Mizuki, Y.; Arai, H.; Iwata, R.; Fujii, T.; Komemushi, S. Neuroimaging of Histamine H1-receptor Occupancy in Human Brain by Positron Emission Tomography (PET): A Comparative Study of Ebastine, a Second-generation Antihistamine, and (+)-chlorpheniramine, a Classical Antihistamine. *British journal of clinical pharmacology* 2001, *52* (5), 501–509.

(188) Borriello, F.; Iannone, R.; Marone, G. Histamine Release from Mast Cells and Basophils. *Handb Exp Pharmacol* 2017, *241*, 121–139. https://doi.org/10.1007/164_2017_18.

(189) Maixner, W.; Fillingim, R. B.; Williams, D. A.; Smith, S. B.; Slade, G. D. Overlapping Chronic Pain Conditions: Implications for Diagnosis and Classification. *J Pain* 2016, *17* (9 Suppl), T93–T107. https://doi.org/10.1016/j.jpain.2016.06.002.

(190) Gatchel, R. J.; Peng, Y. B.; Peters, M. L.; Fuchs, P. N.; Turk, D. C. The Biopsychosocial Approach to Chronic Pain: Scientific Advances and Future Directions. *Psychol Bull* 2007, *133* (4), 581–624. https://doi.org/10.1037/0033-2909.133.4.581.

(191) Craps, L. P. Immunologic and Therapeutic Aspects of Ketotifen. *J Allergy Clin Immunol* 1985, *76* (2 Pt 2), 389–393. https://doi.org/10.1016/0091-6749(85)90659-1.

(192) Grant, S. M.; Goa, K. L.; Fitton, A.; Sorkin, E. M. Ketotifen. A Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Use in Asthma and Allergic Disorders. *Drugs* 1990, *40* (3), 412–448. https://doi.org/10.2165/00003495-199040030-00006.

(193) Galatowicz, G.; Ajayi, Y.; Stern, M. E.; Calder, V. L. Ocular Anti-allergic Compounds Selectively Inhibit Human Mast Cell Cytokines in Vitro and Conjunctival Cell Infiltration in Vivo. *Clinical & Experimental Allergy* 2007, *37* (11), 1648–1656.

(194) Ockenga, J.; Rohde, F.; Süttmann, U.; Herbarth, L.; Ballmaier, M.; Schedel, I. Ketotifen in HIV-Infected Patients: Effects on Body Weight and Release of TNF-α. *European journal of clinical pharmacology* 1996, *50* (3), 167–170.

(195) Hung, C.-H.; Li, C.-Y.; Lai, Y.-S.; Hsu, P.-C.; Hua, Y.-M.; Yang, K. D. Discrepant Clinical Responses and Blood Chemokine Profiles between Two Non-Steroidal Anti-

Inflammatory Medications for Children with Mild Persistent Asthma. *Pediatr Allergy Immunol* 2005, *16* (4), 306–309. https://doi.org/10.1111/j.1399-3038.2005.00273.x.

(196) Ohtani, T.; Aiba, S.; Mizuashi, M.; Kawamoto, Y.; Tagami, H. Evaluation of the Efficacy of Antihistamines Using Human Monocyte-Derived Dendritic Cells Stimulated with Histamine. *J Am Acad Dermatol* 2003, *49* (2), 234–242. https://doi.org/10.1067/s0190-9622(03)01478-6.

(197) Ji, R.-R.; Nackley, A.; Huh, Y.; Terrando, N.; Maixner, W. Neuroinflammation and Central Sensitization in Chronic and Widespread Pain. *Anesthesiology* 2018, *129* (2), 343–366.

(198) Tucker, T.; Riccardi, V. M.; Sutcliffe, M.; Vielkind, J.; Wechsler, J.; Wolkenstein, P.; Friedman, J. M. Different Patterns of Mast Cells Distinguish Diffuse from Encapsulated Neurofibromas in Patients with Neurofibromatosis 1. *J Histochem Cytochem* 2011, *59* (6), 584–590. https://doi.org/10.1369/0022155411407340.

(199) Isaacson, P. Mast Cells in Benign Nerve Sheath Tumours. *The Journal of pathology* 1976, *119* (4), 193–196.

(200) Riccardi, V. Cutaneous Manifestation of Neurofibromatosis: Cellular Interaction, Pigmentation, and Mast Cells. *Birth defects original article series* 1981, *17* (2), 129–145.

(201) Riccardi, V. M.; Wald, J. S. Discounting an Adverse Maternal Effect on Severity of Neurofibromatosis. *Pediatrics* 1987, *79* (3), 386–393.

(202) Johnson, M.; Kamso-Pratt, J.; Federspiel, C.; Whetsell Jr, W. Mast Cell and Lymphoreticular Infiltrates in Neurofibromas. Comparison with Nerve Sheath Tumors. *Archives of pathology & laboratory medicine* 1989, *113* (11), 1263–1270.

(203) Zhu, Y.; Ghosh, P.; Charnay, P.; Burns, D. K.; Parada, L. F. Neurofibromas in NF1: Schwann Cell Origin and Role of Tumor Environment. *Science* 2002, *296* (5569), 920–922.

(204) Yang, F.-C.; Ingram, D. A.; Chen, S.; Zhu, Y.; Yuan, J.; Li, X.; Yang, X.; Knowles, S.; Horn, W.; Li, Y. Nf1-Dependent Tumors Require a Microenvironment Containing Nf1+/−-and c-Kit-Dependent Bone Marrow. *Cell* 2008, *135* (3), 437–448.

(205) Najafi, P.; Dufor, O.; Ben Salem, D.; Misery, L.; Carré, J. Itch Processing in the Brain. *Journal of the European Academy of Dermatology and Venereology* 2021, *35* (5), 1058–1066.

(206) Cheng, L.; Luo, Q. Q.; Chen, S. L. The Role of Intestinal Mast Cell Infiltration in Irritable Bowel Syndrome. *Journal of Digestive Diseases* 2021, *22* (3), 143–151.

(207) Hasler, W. L.; Grabauskas, G.; Singh, P.; Owyang, C. Mast Cell Mediation of Visceral Sensation and Permeability in Irritable Bowel Syndrome. *Neurogastroenterology & Motility* 2022, e14339.