Understanding the impact of widespread pain on women's health and wellbeing



Danielle Perro

Linacre College

University of Oxford

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Abstract

Chronic pelvic pain is highly burdensome in women, yet too often, treatments targeting the periphery are ineffective at managing such pain. While advances have been made in improving our understanding of chronic pelvic pain, an underexplored area is extra-pelvic, or widespread pain. Within this thesis, I used body maps, validated questionnaires, and psychophysical pain testing (i.e. conditioned pain modulation and quantitative sensory testing) to characterise widespread pain and its associated burden in women with chronic pelvic pain.

In a small cohort of women with endometriosis and chronic pelvic pain, I used the body map to characterise extra-pelvic pain. In this cohort I investigated the relationship between widespread pain and psychological health measures, such as pain catastrophizing, depression, and anxiety. I further investigated the number of comorbid pain conditions and other comorbidities, and importantly, reproductive outcomes, with respect to widespread pain. I found a significant relationship between widespread pain and poor psychological health; however, no significant association was observed with the other factors. This chapter generated important hypotheses upon which this thesis is grounded.

The relationship between chronic pain and reproductive outcomes is not well understood. In this thesis, I explored the relationship between reproductive outcomes and chronic pain, as well as different classes of pain conditions, using data from the UK Biobank. Chronic pain was shown to be associated with an increased odds of miscarriage, and further, when stratifying chronic pain conditions by their underlying mechanisms, those with an inflammatory chronic pain diagnosis had an increased odds of being nulliparous.

Sensory perturbations have been demonstrated in individuals with widespread pain. In this thesis, I investigated sensory perception and descending pain modulation in women with chronic pelvic pain and a widespread pain phenotype. However, contrary to my hypotheses, minimal alterations in sensory processes were observed with increasing pain spread.

Finally, conditioned pain modulation is a widely used paradigm to understand descending pain inhibition. However, protocol standardisation, reliability and covariates influencing conditioned pain modulation are not well established. In this thesis, I explored the reliability and covariates which may influence a well-established conditioned pain modulation paradigm in women with chronic pelvic pain and healthy controls. Although the paradigm was reliable in this cohort, I could not identify a single covariate which explained why so few women exhibited inhibition.

Overall, the aim of this thesis was to advance our understanding of widespread pain in women with chronic pelvic pain. Adequate management of *all* their symptoms is of the utmost importance to women suffering from such pain, and novel methods of pain phenotyping, such as widespread pain, may shed light on areas of clinical importance which could ultimately improve patient outcomes.

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A DPhil thesis is the culmination of years of hard work, not only by the student, but by their community which supports them. Without mine, both in the UK and Canada, this thesis would simply not be possible. Nor would it be possible without the hundreds of women across the UK who have donated their time and shared their experiences for my research and public engagement work. The opportunity to learn from you and hear about your experiences has been one of the greatest honours of my studies.

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COVID-19 impact statement

Prior to the COVID-19 pandemic, my DPhil plan was to perform a lab-based project. As outlined in my future directions of this thesis, I intended on investigating the role of extracellular vesicles in understanding endometriosis-associated widespread pain. However, due to many delays prior to the pandemic in obtaining ethical approval, and the repeat closures of the lab space, this work was not possible. Originally, I intended on recruiting women with endometriosis and widespread pain into a small cohort study, and collecting biological fluids at three separate visits (at different phases of the menstrual cycle), which would've provided very rich phenotypic and biological insights into widespread pain in such a cohort.

I ended up switching the direction of my project to use epidemiological and pain-testing approaches to understand widespread pain. Because limited data were being collected in-person, most of the data that I used was already available, and therefore, may not have been the most ideally suited to answer the questions I set out to answer. In addition to the implications on my project, I struggled with my mental health and wellbeing during the pandemic, which meant that the PhD took longer than anticipated. Especially during the first lockdown, being far away from home, it was challenging to focus wholly on my work, whilst I instead focused on trying to achieve mental wellbeing in such uncertain circumstances.

Publications and Awards

Journal Articles

Published

Perro, D., Becker, C., "Endometriosis: Pandemic Backlog Risks Making It Even Harder for Women to Get Help." *The Conversation,* 26 April. 2022. https://theconversation.com/endometriosis-pandemic-backlog-risks-making-it-even-harder-for-women-to-get-help-181803

Perro D, Seglah H, Abrahams V, Weckesser A, Griffith VAS. Black women's menstrual and reproductive health: a critical call for action in the UK. *BMJ*. 2022;379:o3052. Published 2022 Dec 22. doi:10.1136/bmj.o3052

In press

Rahmioglu, N., Morlock, S., Ghiazi, M., Møller P.L., Stefansdottir, L., Galarneau, G., Turman, C., Danning, R., Law, M., Sapkota, Y., Christofidou, P., Skarp, S., Giri, A., Banasik, K., Krassowski, M., Lepamets, M., Marciniak, B., Nõukas, M., Perro, D., … Zondervan, K. (2021). The genetic basis of endometriosis and comorbidity with other pain and inflammatory conditions. [Manuscript submitted for publication to Nature Genetics]

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Gemmell, L*., Perro, D*, Rahmioglu, N., Harris, H., Missmer, S., Zondervan, K., Vincent, K. Chronic pain is associated with an increased risk of premature menopause, a mediator of the relationship with future morbidity: A cohort study of women in the UK Biobank [Target journal: The British Medical Journal]

Presentations

Invited

Cambridge Femtech Society, Virtual: "*Communication and public perspective: Unheard of podcast",*
April 2022

History of the gendered body Seminar, University of Oxford, Virtual: "*Hysterical women: How historical negation and psychologising of women's pain and suffering has led to modern delays in diagnosis of endometriosis",* November 2020

Oral

UK Endometriosis Research Network Meeting, University of Warwick: *"Exploring the association between a chronic pain diagnosis and reproductive outcomes in the UK-Biobank",* September 2021

World Congress on Endometriosis, Virtual: "*Exploring the association between a chronic pain diagnosis and poor reproductive outcomes in the UK-Biobank",* March 2021
World Congress on Endometriosis (Virtual)

Linacre College, University of Oxford: *"Endometriosis: More than just a chronic pelvic pain condition?"*, November 2019

International Pelvic Pain Society Conference, Toronto, Canada: *"Widespread Pain is Associated with Poorer Psychological Health in Women with Chronic Pelvic Pain Presumed Secondary to Endometriosis",* October 2019

Poster

Focus on Pain Day, The British Pain Society, London, UK: *"Phenotyping Women with Endometriosis According to Widespread Pain: A Replication Study in the UK Biobank",* February 2020

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Awards and Distinctions

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Science Communication and Outreach Work

Pain Research Forum Correspondent, North American Pain School, Montreal, Canada

NIHR Open Day Volunteer & NDWRH co-lead, Oxford

Co-host, World endometriosis society – Voices and visions podcast

Blogger, AIMDay in Women's Health, St. Anne's College, University of Oxford

Principal Investigator/Project Lead: Won't you look a little closer? Pain beyond the pelvis in endometriosis: Public engagement with research seed fund project

Co-host, *Unheard of: The hidden voices of endometriosis* podcast

Finalist, FameLab Oxford Heat
Oxford

Volunteer, Wellcome Future of Genetics Day, Cheney School

Volunteer, Oxford Hands-On Science

Glossary of terms

5-HTT: Serotonin transporter

ACR: American College of Rheumatology

ALSPAC: Avon longitudinal study of parents and children

ANOVA: Analysis of variance

ANS: Autonomic nervous system

BCE: Boston Centre for Endometriosis

BDI: Beck's depression inventory

BMI: Body mass index

BPI: Brief pain inventory

BPS: Bladder pain syndrome

CBP: Chronic back pain

CDT: Cold detection threshold

CNS: Central nervous system

COPC: Chronic overlapping pain conditions

CPM: Conditioned pain modulation

CPP: Chronic pelvic pain

CPT: Cold pain threshold

CRPS: Complex regional pain syndrome

CRF: Case report form

CS: Conditioning stimulus

CSE: Certificate of secondary education

CSI: Central sensitisation inventory

CUA: Congenital uterine abnormalities

CWP: Chronic widespread pain

Df: Degrees of freedom

DFNS: German Neuropathic Pain Network

DMA: Dynamic mechanical allodynia

EABP: Endometriosis and bladder pain symptoms

EAP: Endometriosis associated pain

EDTA: Ethylenediaminetetraacetic acid

ENDOX: Study to identify biomarkers in endometriosis in Oxford

EP: EndoPain

ESSIC: European Society for Study of Interstitial Cystitis

EV: Extracellular vesicles

FM: Fibromyalgia

GCSE: General certificate of secondary education

GWAS: Genome-wide association study

HADS: Hospital Anxiety and Depression Scale

HADS-A: Hospital Anxiety and Depression Scale – Anxiety

HADS-D: Hospital Anxiety and Depression Scale – Depression

HLA-DRβ1: Human leukocyte antigen complex gene

HND: Higher national diploma

HPA: Hypothalamic-pituitary-adrenal

HPT: Heat pain threshold

IBD: Inflammatory bowel disease

IBMC: Institute of Molecular and Cell Biology

IBS: Irritable bowel syndrome

IC: Interstitial cystitis

ICD: International classification of diseases

IPPS: International pelvic pain society

IQR: Interquartile range

IUS: Intrauterine system

k: Kappa

Kg: Kilograms

kPA: Kilopascals

MaPP: Multidisciplinary Approach to the Study of Pelvic Pain

MBM: Michigan body map

MCP: Multi-site chronic pain

ME: Myalgic encephalomyelitis

mmHg: Millimetres of mercury

mN: Millinewtons

MPS: Mechanical pain sensitivity

MPT: Mechanical pain threshold

MRI: Magnetic resonance imaging

N: Newtons

NICE: National Institute for Health and Care Excellence

NLR: Neutrophil lymphocyte ratio

NMDA: N-methyl-d-aspartate

NP: Neuropathies

NRS: Numerical rating scale

NVQ: Work based qualification

OA: Osteoarthritis

OC: Oral contraceptive

OR: Odds ratio

PCOS: Polycystic ovarian syndrome

PCS: Pain catastrophizing scale

PHS: Paradoxical heat sensation

PID: Pelvic inflammatory disease

PPT: Pressure pain threshold

PROMIS: Patient Reported Outcomes Measurement Information System

QST: Quantitative sensory testing

RA: Rheumatoid arthritis

rASRM: Revised American Society for Reproductive Medicine

RBC: Red blood cells

REC: Research Ethics Committee

S1/M1: Primary sensory/motor cortex

SD: Standard deviation

SE: Shared epitope

SEM: Standard error of measurement

SF-36: Short Form 36 Health Survey Questionnaire

SLC6A4: Sodium-dependent serotonin transporter and solute carrier family 6 member 4

SLE: Systemic lupus erythematosus

SNP: Single nucleotide polymorphism

SPSS: Statistical product and service solutions

SS: Symptom severity

STAI: State-trait anxiety inventory

TDI: Townsend deprivation index

TMJ: Temporomandibular joint disorder

TNF: Tumour Necrosis Factor

TRiPP: Translational Research in Pelvic Pain

TSA: Thermal Sensory Analyzer

TS: Test stimulus

TSL: Thermal sensory limen

UCPPS: Urological chronic pelvic pain syndrome

USA: United States of America

UK: United Kingdom

UKBB: UK Biobank

US(A): United States (of America)

VAS: Visual analogue scale

VC: Voluntary childlessness

VDT: Vibration detection threshold

VFF: Von frey filaments

WBC: White blood cells

WDT: Warm detection threshold

WPI: Widespread pain index

WUR: Wind up ratio

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Chapter 1 – Introduction

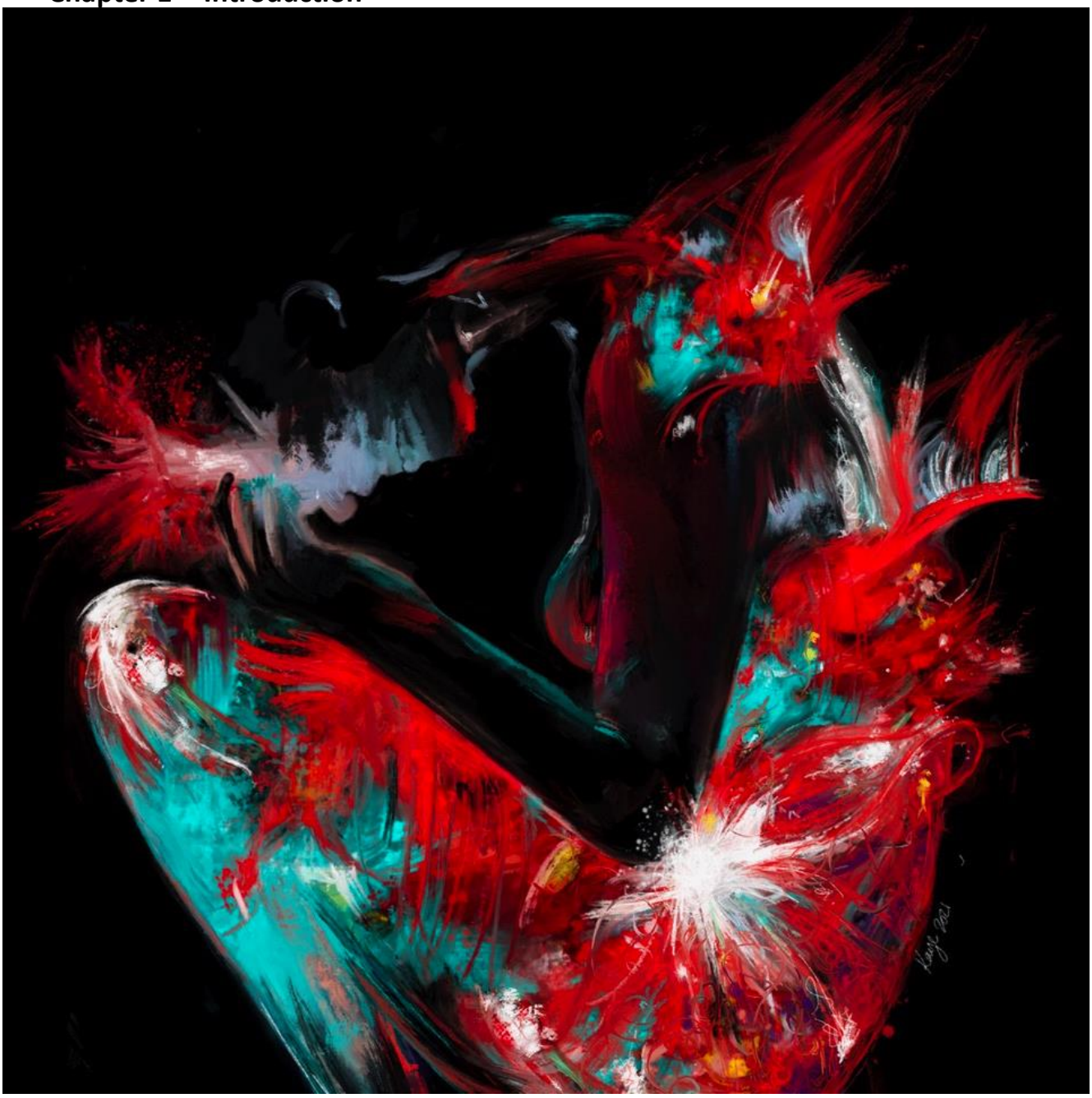


Figure 1.1. It's all in your head, Kaye Sedgwick-Jones, 2021.

It is said that a picture is worth a thousand words. For someone living with chronic pain, where sometimes those words are not taken seriously, a picture is surely worth thousands more. The image above (**Figure *1.1***) was created by artist and Endometriosis sufferer Kaye Sedgewick Jones for a public engagement project of mine. More on this project and the

evaluation of it can be found at the end of this thesis. Within this thesis, I have written more than 40,000 words to further understand the widespread pain experience of women living with chronic pelvic pain. However, I hope that before reading on, some time is spent with the image above. Whilst I have used numerical rating scales, psychophysical pain testing, and validated questionnaires to characterise widespread pain in this thesis, Kaye's portrait of her pain experience simply, beautifully, and sadly illustrates those 40,000 words in a single image. This thesis was by no means qualitative in nature, but the story of my research feels incomplete without showing even a glimpse of the lived experiences I've heard over the last four years, which are all too similar to Kaye's. Women living with chronic pelvic pain deserve better, and I hope that in some way my work will contribute positively to change in the field.

1.1. Thesis overview

Irrespective of the underlying pathology, chronic pelvic pain (CPP) is often poorly managed in women. Recent efforts have begun to better phenotype the pain experience in people with CPP, with the aim of improved patient stratification and ultimately, improved treatment for those suffering. One strategy for improving our understanding of the pain in people with CPP is by investigating the prevalence and burden of extra-pelvic pain. To contribute to this growing body of literature in women with CPP specifically, I have used epidemiological analyses and psychophysical pain testing to phenotype widespread pain in different studies of women with CPP and understand its burden. By doing so, the aim is to provide enough evidence to support the consideration of widespread pain in clinical practice, to provide more personalised approaches to pain management. Within this thesis,

I have used the body map (Described further in Chapter 2) to characterise extra-pelvic pain in women with CPP to in the first instance determine whether such a phenotyping method is useful in women, and finally, assess potential mechanisms underlying such widespread pain. In Chapter 3, I explore widespread extra-pelvic pain in women with endometriosis and chronic pelvic pain using questionnaire data, and generate important hypotheses upon which this thesis is grounded. In Chapter 4, which was driven by findings in my third Chapter, I investigate the relationship between chronic pain and reproductive outcomes using the UK Biobank. In Chapter 5, I test my hypotheses about widespread pain in women with chronic pelvic pain in a much larger cohort using questionnaire data, and psychophysical testing parameters (i.e. Quantitative sensory testing[1] and conditioned pain modulation (CPM)). In my final experimental Chapter (6), I explore the value of assessing pain modulation with a widely used CPM paradigm in pain-free women and women with chronic pelvic pain.

Below, I have presented background literature relevant to this thesis and the results presented throughout. In the first instance, I will provide an overview of chronic pain, generally and with respect to women. I will then define chronic pelvic pain and outline current evidence about the underlying mechanisms, before further discussing a highly common CPP condition, endometriosis. Thirdly, I will give an overview of widespread pain, its underlying mechanisms and burden, as well as recent research which has used widespread pain as a method for pain phenotyping and stratification. Finally, I will describe the aims of this thesis.

1.2. Chronic pain

1.2.1. What is chronic pain?

Pain is defined as an 'unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage'[2,3]. Pain is classified as acute or chronic depending on its duration: pain persisting for three or more months is considered to be chronic[4]. Chronic pain is a subjective and deeply personal experience, which makes it challenging to understand in a research or clinical setting[5]. However, knowledge of the mechanisms which generate and maintain it, can facilitate pain management and communication in such settings. It is important to note that the development of chronic pain does not occur simply because of the persistence of acute pain[6]. While acute pain can in fact become chronic, chronic pain is often driven by independent mechanisms which are shared amongst different chronic pain conditions, and distinct from the initial injury causing acute pain[7].

Chronic pain is often described in terms of its relation to disease. It may be classified as 'chronic primary pain'[8] when the pain could be considered a disease on its own, without an additional disease causing it. An example of a primary chronic pain condition is fibromyalgia[9]. 'Chronic secondary pain' on the other hand, is pain caused by an underlying pathology or disease[2]. Such an example might be dysmenorrhoea, or pain associated with menstruation, caused by underlying endometriosis[10].

1.2.2. Mechanisms of chronic pain

Pain can generally be subdivided into three subtypes according to the underlying mechanisms which generate it: nociceptive, neuropathic or nociplastic pain. Furthermore,

it can be described in terms of where the pain originates, either viscerally or somatically. The pain mechanisms, and examples of them in chronic pain, have been described in **Table *1.1***.

Table 1.1. Mechanisms of chronic pain, largely according to the Kyoto protocol of the International Association for the Study of Pain basic pain terminology[24], unless otherwise stated.

*Not all patients with osteoarthritis or rheumatoid arthritis will have completely nociceptive pain.

Term	Definition	Evidence in chronic pain
Nociceptive pain	Pain arising from actual or threatened damage to non-neural tissue which occurs due to nociceptor activation	Osteoarthritis[11] and rheumatoid arthritis[12]; pain generated in both conditions is caused by damage to non-neural tissue*
Inflammatory pain	A subtype of nociceptive pain; pain caused by ongoing inflammation, which involves the activation of nociceptors by inflammatory mediators[13]	Inflammatory bowel disease: pain arises at least in part, from inflammation in the intestines
Neuropathic pain	Pain arising from lesions or disease of the somatosensory system	Classically, diabetic neuropathy[14], but other chronic pain conditions (i.e. chronic low back pain[15] and endometriosis[16]) have evidence of a neuropathic-component to their pain
Nociplastic pain	Pain which arises from altered nociceptive signalling without evidence of actual or threatened tissue damage activating peripheral nociceptors, as would be the case with nociceptive pain, or evidence of disease or lesion of the somatosensory system, which would be the case with neuropathic pain[17] Typical presentation of nociplastic pain includes multi-site pain (beyond what's expected given the disease), somatosensory system hypersensitivity, poor sleep and fatigue[18]	Fibromyalgia is the classic example of a nociplastic pain syndrome, associated with multifocal pain, and other CNS-associated symptoms such as fatigue and poor sleep[18,19]
Central sensitisation	Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input. Characterised by allodynia, hyperalgesia, or secondary hyperalgesia[20]	A subset of patients with chronic low back pain have been shown to exhibit features of central sensitisation; experience allodynia in regions outside of the back[21]
Visceral pain	Pain originating from the internal organs	Irritable bowel syndrome; causes recurrent abdominal pain attacks[22]
Somatic pain	Pain arising from somatic tissues (i.e. skin, muscle, bones, joints)	Cancer-related chronic bone pain[23]

Of the pain mechanisms described, there is great controversy and debate amongst the scientific community with respect to the third pain type: nociplastic pain. While the mechanisms underlying such pain are not yet well understood, centrally mediated pain and perturbations in endogenous pain modulatory processes are likely culprits in the generation of nociplastic pain[25]. Nociplastic pain is explored further in my experimental chapters with respect to widespread pain.

While in some cases the pain mechanisms described above function independently, it is not uncommon for individuals with chronic pain to have multiple underlying mechanisms, with substantial overlap of nociceptive and neuropathic mechanisms suggesting a 'mixed pain' phenotype[26]. To further exemplify the connectedness of these pain mechanisms, it has been suggested that nociceptive pain is a risk factor for nociplastic pain, due to ongoing hypersensitivity caused by such pain[17].

As a result of the pain mechanisms described above, people living with chronic pain may experience altered pain processing and responses to various stimuli, driven at least in part by central sensitisation[17]. At the molecular level, a critical mechanism involved in initiating central sensitisation is the activation of the N-methyl-d-aspartate (NMDA) receptor in the dorsal horn[27,28]. Whilst typically blocked by magnesium[29], in cases of chronic pain whereby nociceptors are continuously activated, glutamate and substance P are released from the nociceptors to initiate downstream signalling cascades, and ultimately, result in activation of the NMDA receptor, which contributes to the increased excitability of nociceptive neurons [20].

Methods used to assess pain mechanisms include questionnaires and psychophysical pain testing. For example, the painDETECT questionnaire is used to assess the neuropathic component of one's pain[30,31]. Furthermore, quantitative sensory testing (QST)[1] could be used to assess nerve functioning and identify indications of allodynia or hyperalgesia[32]. These tools are further described in Chapter 2.

1.2.3. Chronic pain in women

Before delving further into chronic pain in women, it is important to briefly acknowledge the role that both sex and gender play in pain. According to the Canadian Institute of Health Research[33], sex is defined as 'a set of biological attributes in humans and animals'. It is associated with physical and physiological features including chromosomes, gene expression, hormone levels and function'. Gender on the other hand is 'the socially constructed roles, behaviours, expressions and identities of girls, women, boys, men, and gender diverse people. It influences how people perceive themselves and each other, how they act and interact, and the distribution of power and resources in society'[34]. While considering gender in its sociocultural sense was outside the scope of this thesis, I have considered sex as a biological variable such that only female participants were included in my studies.

Both sex and gender importantly contribute to the pain experience and how others perceive your pain[34]. Females have been shown to be more sensitive to experimental pain than males[35]. They are additionally more likely to experience pain in multiple body regions[36–38]. Differences in the pain experience are likely driven by a multitude of factors such as hormones[39], psychological effects (i.e. pain catastrophizing) and genetics[40].

Furthermore, previous work has shown a significant negative correlation between gender-specific stereotypes related to pain, and pain threshold, such that those who self-identified as more masculine than a 'typical' man demonstrated increased pain thresholds[41]. Additionally, the sex of the prescribing physician has been shown to influence the amount of analgesic prescribed to a patient in pain, such that male physicians offered less to female than male patients, and female doctors, vice versa[42].

Boerner et al. performed a search in the journal *PAIN* in 2017 and found that only 56% of articles reported on sex, and only 16% on gender[34]. Many rodent studies have historically included a single sex (male), by justifying that females are more variable when considering the cyclical nature of their hormones. While there is no denying such variability, meta-analyses of neuroscience studies using rodent models have shown that female rats are not more variable than males, which calls into question the outdated beliefs which contribute to the exclusion of females in animal studies[43]. All of this considered, sex and gender are still not adequately incorporated into pain research, which introduces limitations to its generalisability and inclusiveness. Despite chronic pain being more common in females[44,45], pain research which has been foundational to our understanding of pain has long excluded females, and largely negated the role that gender has in our understanding of pain.

1.2.3.1. Chronic pain conditions in women

An in-depth review of the mechanisms responsible for such sex differences in chronic pain are beyond the scope of this thesis. As mentioned previously, most chronic pain conditions

are more prevalent in females. This includes but is not limited to the following: migraine[46], fibromyalgia[47,48], temporomandibular joint disease[49], rheumatoid arthritis[50], complex regional pain syndrome[51] and bladder pain syndrome[52]. The disproportionate prevalence of chronic pain conditions in women represents an important area of both research and clinical need. A chronic pain of particular relevance to this thesis is chronic pelvic pain, both primary and secondary in nature, in females.

1.3. Chronic pelvic pain in females

Chronic pelvic pain (CPP) is defined by the IASP as pain persisting in the pelvic region for three or more months. Before this, it was defined by the American College of Obstetricians and Gynecologists (as well as the Royal College of Obstetricians and Gynaecologists[53]) as pain in the pelvic region persisting for 6 or more months[54] to account for the intermittent and often cyclical nature of pelvic pain. CPP is highly prevalent, with as many as one in six adult women in the UK affected[55], and has a detrimental impact on the quality of life of those living with it[56]. CPP is assumed to be a symptom rather than a diagnosis in its own right[53], such that further clinical investigation as to the underlying pathology is warranted when women present with CPP.

In addition to CPP, other chronic pains within the pelvis such as dysmenorrhoea[57], dyspareunia[58] (pain during or after sexual intercourse) and dyschezia (painful bowel movement)[59] amongst others, are highly prevalent in women. Like CPP, these pains can be primary in nature, and in fact when laparoscopies are performed to determine the aetiology, 35% of CPP patients are left without identifying a further cause of their pain[60].

However, some of the most common causes of chronic pelvic pain in women are pelvic inflammatory disease[61], uterine fibroids[62], bladder pain syndrome/interstitial cystitis[63], ovarian cysts, irritable bowel syndrome[64], and importantly, endometriosis[65]. Endometriosis is explored in further detail below.

1.2.4. Mechanisms of chronic pelvic pain

Generally, little is known about the mechanisms which underlie chronic pelvic pain, especially in women. Due to the common techniques used to characterise and investigate such mechanisms (i.e. neuroimaging, psychophysical pain testing), a limitation of many available studies is that they are performed on a relatively small sample of women. Furthermore, CPP is complex and often heterogeneous in presentation, and for reasons described in **Table *1.2***, it can be very challenging to understand where the pain is generated. However, there are some key peripheral and central mechanisms underlying CPP which have been proposed.

Table 1.2. Features of chronic pelvic pain in women.

Clinical pain feature	**Definition**	**Example in chronic pelvic pain**
Visceral hyperalgesia	Increased sensitivity to pain in the viscera	Lack of correlation between endometriosis-associated pelvic pain and disease extent[66]
Viscerosomatic referral[67,68]	Noxious stimulation of visceral organs initiates a referred pain response in somatic sites	Referred myofascial sensitisation in women with endometriosis and chronic pelvic pain[69]
Viscero-visceral convergence	Convergence of multiple primary afferents from different viscera at a single dorsal horn neuron	Bladder pain syndrome patients often experience hypersensitivity amongst gastrointestinal and reproductive-related organs[70]
Inflammation	Local inflammation within the pelvis can contribute to visceral hyperalgesia	Inflammation surrounding the gut can cause sensitisation of nerves, and subsequent visceral hypersensitivity[71]

1.2.4.1. Peripheral mechanisms

While some of the peripheral mechanisms underlying CPP have been described above in **Table *1.2***, there is further evidence to support peripheral generators of this pain. Whitaker et al. have demonstrated that women with CPP have a neuropathic component to their pain, by using the painDETECT questionnaire outlined in Chapter 2[72]. Furthermore, an increased number of substance P (pro-inflammatory neuropeptide) positive nerve fibres in women and rats with IC/BPS has been shown, demonstrating a mechanism for increased nociception within the bladder[73]. The release of pro-inflammatory cytokines and mediators

into the pelvic cavity by a process of neurogenic inflammation can contribute to peripheral sensitization of local nerves and contribute to the observed CPP phenotype[74].

1.2.4.2. Central mechanisms

Irrespective of the underlying pathology driving the development of CPP, women with CPP have been shown to have central mechanisms, at least in part, generating and maintaining the chronic pain[75]. According to Brawn et al., women with CPP demonstrate sensory perturbations which are distinct from healthy control participants; this includes changes to the autonomic nervous system, hypothalamic-pituitary-adrenal (HPA) axis, and brain structure [76]. As shown in **Table *1.3***, many groups have identified increased pain sensitivity in extra-pelvic regions using QST, which demonstrate an element of central sensitisation[72,77,78]. Furthermore, neuroimaging studies have identified alterations in brain volume of key pain processing regions in women with CPP, as demonstrated below by As-Sanie et al.[77] and Huang et al[79].

Table 1.3. Evidence for central mechanisms of chronic pelvic pain. NRS;Numerical rating scale. CPP; chronic pelvic pain.

Author (Year published)	**Participant information**	**Method**	**Finding**
As-Sanie et al. (2013)	Women with endometriosis & CPP (n=42) vs. women with endometriosis & dysmenorrhoea (n=15) vs. pain-free women with endometriosis (n=35) vs. CPP without endometriosis (n=22) vs. healthy controls (n=30)	Quantitative sensory testing: Pressure pain threshold (PPT) protocol applied to thumbnail	Decreased PPT in regions outside of the pelvis in all subgroups of women with CPP vs. healthy controls[77]
As-Sanie et al. (2013)	Women with endometriosis & CPP (n=17) vs. women with endometriosis & no CPP (n=15) vs. women with CPP & no endometriosis (n=6) vs. healthy controls (n=23)	Voxel-based morphometry	Decreased volume of regional grey matter in key pain processing regions in all groups of women with CPP, irrespective of endometriosis diagnosis[80]
Huang et al. (2016)	Men and women with urological chronic pelvic pain syndrome (UCPPS) (n=52; 23=women), irritable bowel syndrome (n=39;24=women), and healthy controls (n=61; 32=women)	Magnetic resonance imaging (structural and functional)	Regional white matter changes, which are proportional to UCPPS symptom severity[79]
Harte et al. (2019)	Male and female participants with urological chronic pelvic pain syndrome (UCPPS, n=153) and healthy controls (n=100)	Quantitative sensory testing (pressure pain threshold)	Participants with UCPPS had increased extra-pelvic pain sensitivity when compared to healthy controls[81]
Hellman et al. (2020)	Women with dysmenorrhea (n=147), healthy controls (n=37), and	Quantitative sensory testing	Widespread sensory sensitivity was prevalent in women with dysmenorrhoea

	women with bladder pain syndrome (n=25)		and bladder pain when compared to healthy controls[78]
Author (Year published)	**Participant information**	**Method**	**Finding**
Kmiecik et al. (2022)	Women at risk of developing CPP (n=200) i.e. low menstrual pain (<3/10 NRS) **or** moderate-severe menstrual pain (>5/10 NRS) **or** participants with bladder pain syndrome **or** non-pelvic chronic pain diagnosis) vs. healthy controls	Quantitative sensory testing & questionnaires	Multi-modal hypersensitivity (i.e. reduced thresholds toward different stimuli) predicted risk of developing pelvic pain after four year follow-up period[82]
Ryan et al. (2022)[83]	Women aged ≥18 years old presenting with chronic pelvic pain at a gynaecology outpatient clinic for the first time (n=162)	Questionnaire (Central sensitisation inventory)	Nearly 75% of women with CPP had a CSI score ≥40 (signifying questionnaire-identified central sensitisation)

1.2.5. Additional factors contributing to chronic pelvic pain maintenance

In addition to the mechanisms described above, there are further factors which play a role in its maintenance. Generally, poor sleep quality is known to predict the onset and exacerbation of chronic pain, and chronic pelvic pain is no exception[84]. While of course chronic pain also contributes to poor sleep, the association between poor sleep increasing the development and exacerbation of chronic pain is far more significant[85]. Additionally, psychological comorbidities are highly prevalent amongst women with CPP[86]. While the temporality of onset of the comorbid conditions is not certain, poor psychological health may contribute to the maintenance of CPP, and in particularly affected individuals,

appropriate management of any psychiatric comorbidity may lead to improved pain symptoms as well[87].

As mentioned previously, a CPP condition which is very common yet not much is understood about the underlying pain is endometriosis. While I investigate pain in endometriosis in later chapters (Chapter 3 & Chapter 5), I have outlined more about the condition and possible pain mechanisms underlying endometriosis-associated pain in detail below.

1.3. Endometriosis

1.3.1. What is endometriosis?

Endometriosis is a steroid-dependent chronic inflammatory condition, characterised by the growth and implantation of uterine-like tissue outside of the uterus[88,89]. While historically deemed solely a gynaecological condition, very welcomed recent efforts have been made to shift the narrative[90], to define endometriosis as more of a *systemic condition*[91,92]. In the first instance endometriotic lesions are not restricted to the pelvis[93], and the disease does not solely impact reproduction or the reproductive organs. This shift in language is critical to reducing stigma associated with the disease and women's health more generally, as ultimately endometriosis can impact every single aspect of a woman's body, relationships, and quality of life[90].

Unfortunately, the cause of endometriosis remains largely unknown. However, several aetiological theories have been proposed. Perhaps the most widely accepted is Sampson's

theory of retrograde menstruation[94]. In 1927 Sampson proposed that during menstruation, rather than the entirety of the uterine lining being shed through the vagina, some travels in reverse[95]. As such, it was stated that this tissue travelled upwards through to the fallopian tubes, where it then implanted within the peritoneal cavity. While this theory is interesting, up to 90% of women experience retrograde menstruation[96] and only 10% of women experience endometriosis. This is where other theories of endometriosis' origin begin to fill the gaps and aim to explain how some women have endometriosis deposits outside of the pelvis. Coelomic metaplasia[97–99] is the theory that the mesothelium of the peritoneal cavity transforms into endometrium, however, this doesn't explain how endometriotic lesions can implant extra-pelvically. Furthermore, the role of the lymphatic/vasculature systems as a vessel for transporting endometriosis tissue has been proposed as a potential cause of endometriosis at a distant site[100]. Finally, there is evidence to suggest that altered immunity may contribute to disease pathogenesis, such that the lesions themselves recruit immune cells and other pro-inflammatory cytokines, which are in turn elevated in the peritoneal fluid in endometriosis patients. It is still not entirely clear whether such alterations precede or occur as a result of the disease[101].

While these theories describe how tissue arrives at the site of implantation, they do not necessarily cover how the endometrial tissue implants and progresses. In brief, women with endometriosis exhibit decreased apoptosis and increased proliferation of endometrial cells, which allows for increased survival in the peritoneal cavity[102]. This is likely in part driven by gene expression of key hormonal and inflammatory regulators, such as the dehydrogenase 17βHSD-2[103], and cytokines IL-6[104] and TNF-α[105] amongst others. In order for the lesions to attach to the peritoneum, it has been suggested that damage to the

mesothelial layer is required. Only then can induction of injury via menstrual effluent take place, and the subsequent implantation of endometrial tissue. The inflammatory factors found within the peritoneal fluid, such as TGF-beta, which has been shown to induce endometrial cell invasion into the peritoneum[106], likely create a microenvironment conducive to implantation.

To sustain the lesions once they have implanted, local neuroangiogenesis is crucial. VEGF is a likely candidate which is released by peritoneal macrophages and contributes to the local angiogenesis at the lesion[107]. Over time, the peritoneal lesions will progress and their appearance will change, highlighting why early diagnosis and management of the disease are crucial. According to a prospective study of surgical observations of the disease over time, lesions will most typically begin as highly vascularised red lesions, as commonly seen in younger women, and progress until they become more fibrous and appear as white plaques, as found largely in older women[108].

1.3.2. Risk factors for endometriosis

In addition to the theories outlined above, there are further lifestyle and genetic factors which may contribute to disease pathogenesis. Characteristics of early reproductive life are associated with an increased risk of endometriosis, including early age at menarche (first period/menstrual cycle), as well as shorter menstrual cycles and heavier/longer periods[109,110].

Furthermore, the role of genetics in endometriosis has been identified[111], such that the risk of disease attributable to genetics is estimated to be 50%. However, compared to other chronic conditions with a similar prevalence and associated burden to endometriosis, our understanding of the genetic underpinnings of the disease lags far behind and requires further (and ongoing) investigation[88].

Lifestyle and environmental factors have also been shown to increase the risk of developing endometriosis. For example, while the strength of the association is variable depending on the study, an inverse relationship between smoking and endometriosis risk has been shown[112]. Furthermore, it has been shown previously that consuming high levels of long-chain omega-3 fatty acid was associated with a 22% reduction in the likelihood of being diagnosed with endometriosis when compared to women who consumed the least amount of the fatty acid[113]. In addition, given that endometriosis is an oestrogen-dependent condition, exposure to substances such as polychlorinated biphenyl (which is known to disrupt hormone balance) is suggested to influence endometriosis[114]. In the case of both lifestyle and environmental factors, further research is warranted to understand their impact on endometriosis risk. Given that lifestyle factors are modifiable, this understanding may be an important step towards disease prevention.

1.3.3. Diagnosis & associated diagnostic delay

The gold standard of diagnosis for endometriosis is laparoscopic visualisation of disease followed by histological confirmation[115]. The disease is staged according to the revised American Society for Reproductive Medicine (rASRM) staging protocol based on the

location of endometriotic lesions and how deeply they infiltrate within the pelvis, as shown in **Figure *1.2*** [116]. A major limitation of this staging system is that it is not well, if at all, correlated with predominant symptoms impacting women with endometriosis, such as pain[117–119]. A staging system which instead of disease presentation alone, further considers the symptomology of endometriosis patients (i.e. their pain) would be welcomed by members of the endometriosis community.

Due to heterogeneity in disease presentation and the highly skilled nature of appropriately diagnosing via surgery, establishing a non or minimally invasive method for diagnosing endometriosis would be beneficial. An area of research which is quickly developing for endometriosis is ultrasound[76,120,121]. However, this again has its own limitations, in that it is prone to missing superficial or peritoneal endometriosis[122].

In the United Kingdom, the average delay from symptom onset to receiving a diagnosis, otherwise referred to as the *diagnostic delay*, of endometriosis is 7.5 years[123]. Ghai et al. have described some of the primary reasons for this delay as menstrual pains in early adolescence (in that earlier symptom onset often means a later diagnosis), societal normalisation of pain in women, and the harmful attitudes of health care professionals towards women's pain experience[124]. Additionally, because the current gold standard for diagnosis is laparoscopic surgery followed by histological confirmation, a further delay is incurred on account of the time waiting for surgery[125]. As a ripple effect, further delays to beginning appropriate treatment are inflicted upon the individual[91,125]. Diagnostic delays have been shown to be harmful to the women affected, as they are associated with worse

health-related quality of life[126]. Furthermore, even with surgery, there is a high recurrence of disease and pain symptoms, which further impact quality of life[127].

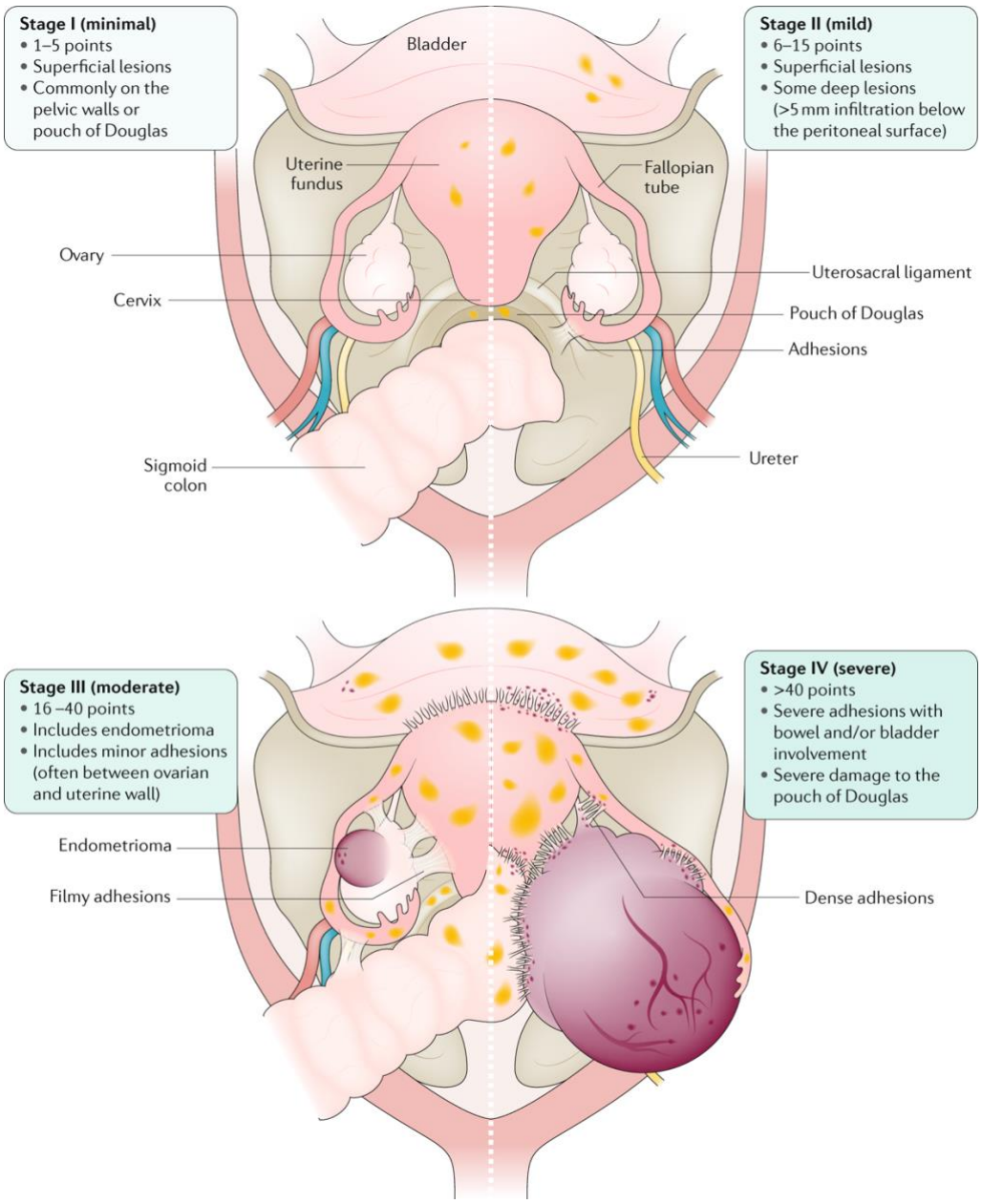


Figure 1.2. Revised American Society for Reproductive Medicine (rASRM) endometriosis staging, reused from Zondervan et al, 2018[94], with appropriate copyright permissions, Appendix 1A. Stage I: Few observable superficial lesions, often found on the peritoneum. Stage II: In addition to superficial lesions, deeper infiltrating lesions are often observed as well. Stage III: Typically presents with endometriomas, sometimes in combination with more superficial lesions or adhesions. Stage IV: In addition to characteristics of stages I-III, increased severity of adhesions, and large endometriomas found on both ovaries.

1.3.4. Symptoms of endometriosis and disease burden

Endometriosis is most prevalent in women experiencing chronic pelvic pain (CPP) or infertility[92]. As many as 40% of women with CPP will receive a diagnosis of endometriosis, and as many as 50% of women with endometriosis are infertile[65].

1.3.4.1. Reproductive outcomes

Epidemiological work (cohort study) by Prescott et al. has shown that the risk of incident infertility in women with endometriosis is around 2.12 times that of women who are endometriosis free, which is slightly less than what's been reported in cross-sectional analyses[128]. This discrepancy may in part be due to how endometriosis is defined in such epidemiological analyses, and whether it was laparoscopically confirmed. Nonetheless, infertility is an important symptom of endometriosis. It has been proposed that the underlying causes of infertility in endometriosis may be driven by the anatomical distortions caused by the disease locally in the pelvis, or immunological factors, amongst others[129]. Anatomical distortions in endometriosis may be a likely explanation for women experiencing infertility with a more severe disease stage; adhesions found within the peritoneal cavity may prevent appropriate oocyte release from the ovary, or adequate oocyte retrieval by the fallopian tubes[65]. Local inflammation within the pelvis may further prevent proper implantation from occurring. For example, with increased levels of peritoneal fluid found within the peritoneal cavity, women with endometriosis have high levels of inflammatory mediators such as Interleukin-1 and Tumour Necrosis Factor (TNF)[130].

In addition to infertility, women with endometriosis are at an increased risk of adverse reproductive outcomes, such as stillbirth[131] and miscarriage[132]. Santulli et al. have shown that women with endometriosis are at a higher risk of having a miscarriage than women without (incidence rate ratio=1.7 (95%CI=1.34-2.16))[133]. Further work by Schwartz et al. has shown that the miscarriage rate was increased in women with endometriosis when compared to women without endometriosis, and this was most pronounced in women with 'milder' forms of endometriosis (i.e. participants with stage I or stage II endometriosis (as diagnosed according to the rASRM classification))[134]. Furthermore, a recent systematic review on endometriosis and risk of adverse pregnancy outcomes found that when compared to women without the condition, those with endometriosis had an increased risk of stillbirth, in addition to hypertension, pre-eclampsia and preterm birth[135]. The mechanisms underlying the relationship between stillbirth and endometriosis currently remain unknown, but it's possible that the association is in part mediated by increased gestational hypertension amongst other factors.

1.3.4.2. Pain

Our understanding of chronic pain in women with endometriosis largely revolves around the pelvis. As such, some of the most well studied and prevalent pains in women with the condition are chronic pelvic pain (non-menstrual related pelvic pain persisting for 3+ months), dysmenorrhoea (pain with menstruation), and dyspareunia (pain during or after sexual intercourse). Although dysmenorrhoea can be primary in nature, such that it occurs without an underlying pathology, endometriosis is the most common cause of secondary dysmenorrhoea[136]. In brief, the pains affecting women with endometriosis are

mechanistically likely attributable to both peripheral[137] and central mechanisms[76], as shown in **Figure *1.3***.

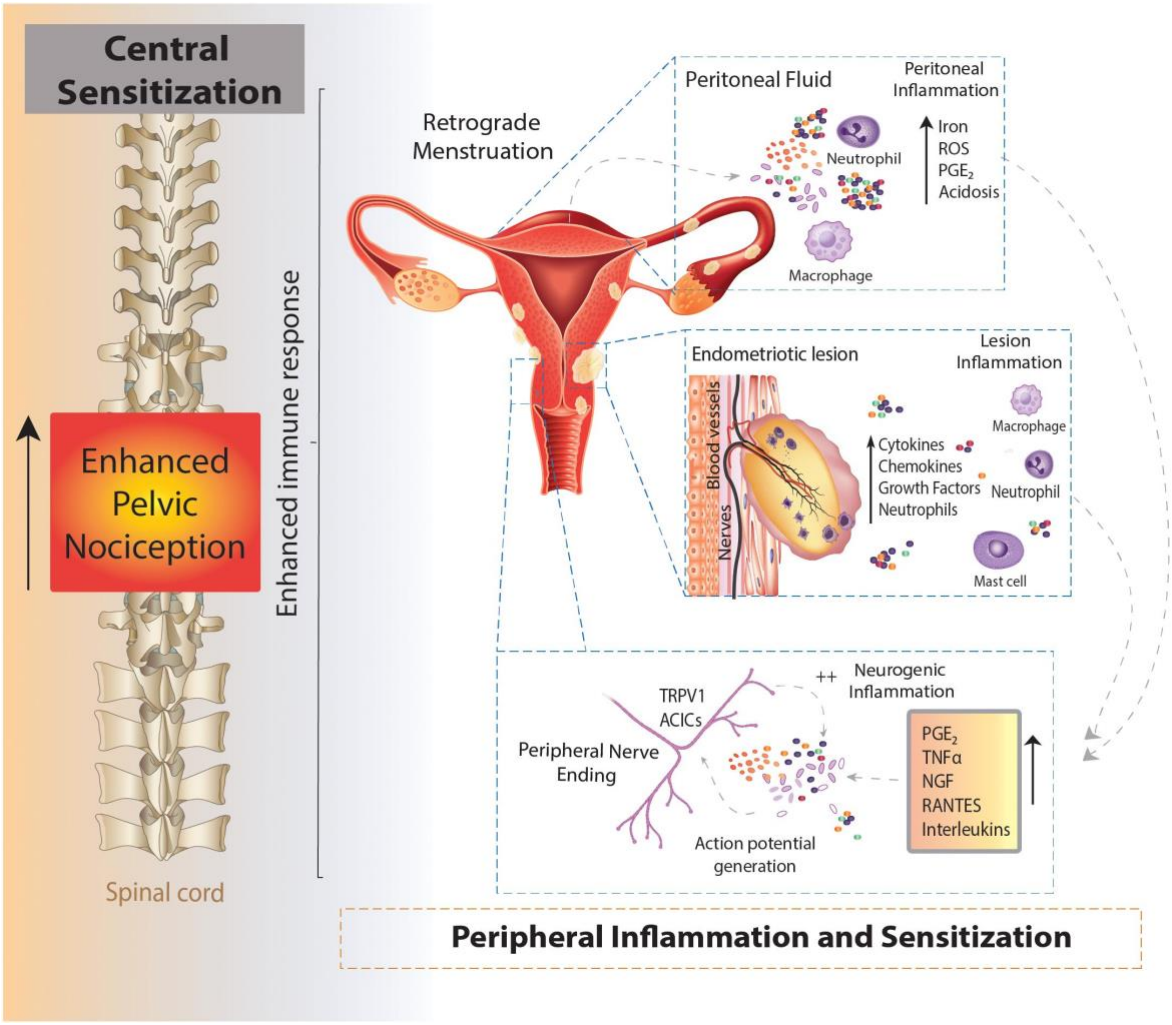


Figure 1.3. Peripheral inflammatory drivers of endometriosis-associated pain. The peritoneal fluid is full of inflammatory mediators released by fragments of endometrium within the peritoneal cavity. Furthermore, the endometriotic lesions themselves release inflammatory mediators (such as cytokines, chemokines, etc.). Inflammatory mediators then act upon the peripheral nerve endings, to elicit an action potential. Constant firing of the peripheral nerve causes peripheral sensitisation and can contribute to central sensitisation at the level of the spinal cord. This figure has been reused from Maddern et al. 2020, under the creative commons license (CC BY)[138].

Peripheral mechanisms of pain in endometriosis

In the periphery, endometriotic lesions are innervated by their own nerve supply[139]. There is however conflicting evidence as to whether differences in the pain experience of women with endometriosis are attributable to nerve fibre density[140,141]. Furthermore, the contents and inflammatory milieu of the peritoneal cavity have been shown to be important contributors to the pain experience. McKinnon et al. have shown that the endometriotic lesions themselves excrete inflammatory mediators, such as TNF-α and IL-1β, into the peritoneal fluid where they can activate nerves to elicit a painful sensation[142]. In addition, Gibson et al. have demonstrated that the abundance of macrophages in the peritoneal cavity correlates with pain severity, positively or negatively depending on the status of CD14; $CD14^{high}$ peritoneal macrophages were negatively correlated, whereas $CD14^{low}$ macrophages were positively correlated[143]. When stratifying by pelvic pain type, rather than disease stage, this association remained significant in participants with non-menstrual pain, dysmenorrhea, or dyspareunia. It is likely that inflammatory factors in the peritoneal cavity, as described above and in **Figure *1.3***, contribute to the activation of peripheral nerve endings, and ultimately results in peripheral sensitization[138].

Central mechanisms of pain in endometriosis

Furthermore, there is a growing body of evidence to suggest that central mechanisms are involved in pain associated with endometriosis[76]. Using quantitative sensory testing, Grundström et al. has shown that women with CPP and presumed endometriosis had significantly reduced pain thresholds when compared to women who were pain free, and

further, the longer the participant had chronic pelvic pain the more sensitization they experienced[144]. As-Sanie et al. have additionally shown widespread hyperalgesia in women with endometriosis and chronic pelvic pain, which did not differ from women with chronic pelvic pain and no endometriosis[77]. Furthermore, using an intramuscular saline injection at a test (pelvic) and control (hand) site, Bajaj et al. showed that women with endometriosis experience central sensitisation, as demonstrated by increased pain intensity post-injection, pain in the control site, and a hypersensitivity to stimulation via pressure[145].

Neuroimaging studies have further shown central changes within the brain in women living with endometriosis and pain[103]. As-Sanie et al. have shown that increased connectivity in the medial prefrontal cortex and anterior insula glutamatergic neurotransmission contribute to CPP in women with endometriosis, independent of the disease itself[146].

Using questionnaire measures of central sensitisation, Orr et al. have further shown a centralised component of deep dyspareunia in women with endometriosis using the central sensitisation inventory[147,148].

Comorbidities

Endometriosis seldom exists in isolation[149]. Surrey et al. have shown that women with endometriosis are at a significantly higher risk of developing comorbid conditions such as uterine fibroids, bladder pain syndrome, and irritable bowel syndrome[150]. Furthermore, endometriosis is considered a chronic pain condition that typically clusters with other chronic pain conditions, such as vulvodynia, temporomandibular joint disorder, migraine,

and fibromyalgia amongst others[151]. Together, these are coined *chronic overlapping pain conditions*[151,152]. These conditions share similar aetiological underpinnings, and in turn, the presence of one increases the risk of developing another due to increased pain sensitivity and impact on psychological health. As such, these must be considered in a clinical setting[149–151].

Women with endometriosis are further at an increased risk of developing comorbid psychiatric comorbidities, such as depression, anxiety and attention-deficit hyperactive disorder when compared to women without endometriosis[153]. Additionally, according to a systematic review and meta-analysis performed by Shigesi et al., women with the condition are at an increased risk of developing autoimmune condition, such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease[154].

Disease burden

Endometriosis hugely impacts the lives of those living with it. Olliges et al. found that, in a mixed method study, women with endometriosis experienced higher anxiety and depression scores (as assessed by the Hospital Anxiety and Depression Scale – See more in Chapter 2), as well as reduced mental and physical quality of life, higher stress ratings and worse functional wellbeing when compared to age-matched endometriosis-free controls[155]. Furthermore, women in this study reported that on account of their endometriosis and associated symptoms, they 'no longer felt like themselves'. More quantitative research investigating the burden of endometriosis has demonstrated that it

is the symptoms associated with endometriosis which dictate the poor quality of life observed in women with the disease, rather than the disease itself[156,157]. This suggests that whilst of course diagnosing and managing the underlying endometriosis is important, further emphasis must be put on managing the associated symptoms, such as pain, as they are consistently associated with a poorer quality of life.

In addition to the obvious physical and psychological burden of endometriosis, in the UK alone endometriosis is associated with an £8.2 billion cost to the economy[158], occurring because of treatment and healthcare costs, as well as a substantial loss of work. At the level of the individual, women with endometriosis lose an estimated 9,579 euros a year because of the reduced ability to work on account of their endometriosis[158].

Treatment for pain

According to the National Institute for Health and Care Excellence (NICE) guidelines for endometriosis, pain should be managed in the first instance using either analgesics or hormonal treatment[159]. Surgery may also be offered, however is done so in consultation with the patient considering the risks of such a procedure, and high likelihood of pain recurrence[127]. Häuser et al. have recommended that women with chronic overlapping pain conditions avoid repeat surgery to manage their pain, as it likely that their pain has a centralised component that may instead benefit from centrally-acting medications, such as Amitriptyline[149].

As it currently stands, hormonal treatments for endometriosis-associated pain offer poor management of their symptoms[75,139]. These are additionally not without side effects. Although hormones are used by millions around the world, some women taking the combined hormonal contraceptive, will have negative side effects on mood, that outweigh the possible benefits of taking the pill[160]. While this phenomenon is well reported amongst combined hormonal contraceptive users, similar trends are seen in other hormonal options, such as Mirena coil[161], amongst others. Surgery may also be offered for pain management; however, the rate of non-response is as high as 20% [148] , and many women will require subsequent surgeries[162]. Therefore, although standard practice, surgery is not necessarily a reliable option for pain management and requires careful consideration and shared decision making between the physician and patients.

Taking all of this into consideration, there is not a uniformly effective method for managing pain in endometriosis, and as demonstrated above, treatments that target the pelvis by way of managing the disease itself, are often ineffective. Therefore, there is a need to better phenotype the chronic pain experienced by women with endometriosis to, importantly, develop or repurpose existing treatments which can reduce the pain experienced by those living with the condition. One method of phenotyping such pain is to take a step back from a pelvis-focused approach to endometriosis-associated pain, by investigating widespread or extra-pelvic pain.

1.4. Widespread pain

Chronic widespread pain (CWP) is defined by the American College of Rheumatology (ACR) as pain lasting for 3+ months, which is present on both sides of the body, below and above the waist and in the axial skeleton[163]. Assessed using the widespread pain index as part of the fibromyalgia scale diagnostic criteria (2010)[164,165], widespread pain is necessary but not sufficient for a diagnosis of fibromyalgia. There is additionally further evidence suggesting that the two are more distinct from one another than originally thought[166]. The estimated prevalence of CWP in the general population is as high as 15%, and like most chronic pain, is more prevalent in females than males[167]. CWP is associated with a substantial burden, both at the individual and population levels. At the level of the individual, a reduced quality of life on account of CWP is reported[168–170]. This is largely driven by the physical pain itself, in addition to fatigue, somatic symptoms, and poor psychological health[163]. At the population level, and similar to endometriosis, there is a high level of work absenteeism associated with such pain contributing to economic losses[171].

1.4.1. Aetiology of CWP

CWP has a considerable heritable component, estimated at around 50%[172], however not much is known about specific genetic variation contributing to disease aetiology. In a Genome Wide Association Study (GWAS) conducted by Peters et al., the minor C-allele of the rs13361160 gene on chromosome 5p15.2 was associated with a 30% increased risk of having CWP[172]. Further work by Rahman et al. has shown that the RNF123 and ATPC1 loci, which are involved in calcium regulation, were associated with CWP[173]. Additionally, a review by Kerr et al. showed that studies investigating the relationship between CWP and

psychological diagnoses (i.e. anxiety and depression) found significant involvement of the serotoninergic pathways, thus suggesting their role in CWP[174]. Future work is warranted to replicate previously published findings, as a first step in better understanding the underlying genetic factors which may contribute to the CWP phenotype.

The two strongest risk factors for developing CWP include being female and age[173], as the prevalence of CWP is highest in females and increases across the lifespan. Increased body mass index (BMI) is an additional well-established risk factor for the development of CWP[175]. Furthermore, polygenic risk scores for multi-site chronic pain (a metric derived in the UK Biobank by summing the total number of body regions impacted by chronic pain) have been shown to significantly predict CWP[176]. This suggests that although someone may not present with CWP at present, those with pain in multiple sites around the body are at a higher risk of developing it.

Early-life factors may also play a role in CWP development. Notably, premature birth, adverse childhood experiences, behavioural problems, and trauma have been shown to increase the risk of developing CWP[177–179]. Furthermore, a family history of CWP increases the risk of developing CWP across the lifetime[178]. Somayajula et al. have investigated widespread pain in adolescents, and their work demonstrated an allostatic load hypothesis for the development of CWP: the more risk factors a child or adolescent had, the greater the odds were of them developing CWP[180]. They found that children with three or more risk factors, ranging from poor mental health to genitourinary or gastrointestinal symptoms, amongst others, had a significantly increased odds of developing CWP when compared to children without any risk factors present (OR (95%CI)=9.17 (5.9-14.3)).

Understanding early life risk factors could facilitate the prevention or early intervention for CWP and understanding of widespread pain across the lifetime. Both of which are key to reducing the possible burden on both the individual and society.

1.4.2. Pain mechanisms of widespread pain

Central mechanisms

Efforts have been made to better understand the central mechanisms underlying widespread pain, and much research supports central sensitisation as a possible driver of widespread pain. Firstly, perturbations in sensory processing have been shown in people with widespread pain. Work by Bergman et al. has shown that self-reported widespread pain was associated with lower pressure pain thresholds (PPT) across the body, when compared to participants without chronic pain[181]. This has been further supported by work from Gerhardt et al. which shows widespread hyperalgesia in participants with CWP[182]. Furthermore, they showed that in females there was a negative correlation between PPT and increasing number of self-reported chronic pain regions. Gupta et al. have suggested that the reduced PPT occurs as a result of the widespread pain, rather than preceding it[183].

Further elements of the quantitative sensory testing (QST) paradigm (a paradigm which I have described in great depth in Chapter 2) such as temporal summation and the wind-up ratio are also useful in characterising central sensitisation. Temporal summation refers to the increased perception of pain from repetitive noxious stimuli[184]. This can be elicited experimentally using the QST protocol. Windup, or the windup ratio (which can be

calculated using QST data) is an increase in the excitability of neurons in the spinal cord, after stimulation of afferent C-fibres[185]. Experimental assessment of the windup ratio can help researchers to better understand mechanisms underlying central sensitisation[186]. Participants with fibromyalgia and widespread pain have been shown to have an increased wind-up ratio when compared to controls, suggesting that in fact after the stimulus has been repeatedly applied to the testing area, that the pain experienced is maintained over time[187].

Endogenous pain modulatory processes have also been shown to be altered in participants with widespread pain. Gerhardt et al. have shown that using a conditioned pain modulation paradigm on patients with chronic back pain (CBP), those with isolated CBP showed higher conditioned pain modulation (i.e. more inhibition) than participants with CBP & chronic widespread pain[188]. They further showed that CPM dysfunction was greater in participants with CBP & chronic widespread pain than those with CBP & fibromyalgia. Thus, highlighting a diminished ability to engage descending pain modulatory pathways in participants with widespread pain. Further supporting the notion that widespread pain and fibromyalgia, although related, may be distinct from one another mechanistically. Further neuroimaging work has been performed by Harris et al. to understand pain modulation at the level of the brainstem in people with fibromyalgia [189]. They showed reduced binding potential in the μ opioid binding receptor in brain regions known to play an important role in pain modulation (i.e. nucleus accumbens, amygdala) in fibromyalgia patients. Thus, suggesting reduced endogenous analgesic properties of the opioid system. Jensen et al. have further shown that fibromyalgia patients show reduced connectivity in regions of the brain

involved in descending pain modulation pathways during a pressure pain threshold task, when compared to healthy controls[190].

Central sensitisation is likely an important generator of widespread pain in cases where the pain has become more diffuse than would be expected after a localised injury or pain[20]. It has been suggested that neuroinflammation occurring at the spinal cord may in part be underlying central sensitisation in widespread pain by Li et al. Such that upon activation of glial cells and astrocytes in the spinal cord, cytokines such as TNF, IL-1β amongst others induce central sensitisation in surrounding synapses to cause widespread pain beyond the primary site of injury[191].

It has been previously shown that when assessing central sensitisation in chronic pain patients via the *central sensitisation inventory* (CSI) questionnaire, that widespread pain significantly contributed to the variance observed in central sensitisation[192]. Furthermore, in participants with localised pain conditions such as knee osteoarthritis[193] and shoulder pain[194], more widespread pain was associated with higher CSI scores. This therefore suggests that central sensitisation may play an important role in widespread pain, and as such, screening for it might help to provide more personalised pain management to people with localised pain conditions.

Peripheral mechanisms

There is conflicting evidence about the peripheral generation and maintenance of widespread pain. Widespread pain is often associated with myofascial tender or trigger

points, and Staud has suggested that abnormalities to peripheral tissues may contribute to the generation of chronic widespread pain[195]. Furthermore, that local ATP, lactic acid and cytokines may contribute to sensitisation of nociceptors at peripheral sites.

The role of cytokines is however not well established. While many groups have hypothesised that systemic inflammation may be an important mechanism underlying widespread pain, there is conflicting evidence to support this. Gerdle et al. first showed that systemic low-grade inflammation was associated with CWP, when compared to pain free women[196]. Upon further investigation, they found additional evidence to support systemic inflammation in women with CWP, after finding that oleoylethanolamide and palmitoylethanolamide were altered in women with CWP when compared to controls[197]. Karshikoff et al. on the other hand showed that IL-8 may be protective for widespread pain in women with chronic pelvic pain but found no evidence to support systemic inflammation in a much larger cohort than the work previously performed by Gerdle et al[198].

Further work investigating the underlying inflammation in widespread pain has shown lower levels of serum cytokines IL-4 and IL-10, which are anti-inflammatory in nature[199]. This therefore provides further support for the hypothesis that inflammation may play an important role in widespread pain.

Neuropathic pain

Using the painDETECT questionnaire to assess aspects of neuropathic pain (which has been described in more depth in Chapter 2), twin studies of participants with CWP have shown

that nearly 16% had clinical features of neuropathic pain[200]. They similarly found a shared genetic heritability between neuropathic pain and CWP. McBeth et al. have also explored neuropathic pain with respect to widespread pain, rather investigating the risk of developing CWP in those with and without neuropathic pain[201]. Using the Douleur Neuropathique 4 questionnaire to assess a neuropathic component of pain, they found that neuropathic pain only predicted a very small amount of new widespread pain cases. As such, they recommended that treatment of neuropathic pain would only prevent a small percentage of people from developing CWP.

1.4.3. Burden of widespread pain

Like any form of chronic pain, widespread pain can have a detrimental impact on an individual's quality of life and health status[169].

Quality of life

In participants with knee osteoarthritis, those with additional widespread pain demonstrated poorer quality of life than those without[202]. Similar findings in knee osteoarthritis have been shown by Berman et al., such that widespread pain was associated with worse scores on seven of the eight 36-item short form survey (SF-36) domains, with only social functioning being non-significant ($p=0.014$)[168]. Furthermore, it has been shown that families of people with CWP experience greater family dysfunction than those with more localised chronic pain, suggesting that the impacts of widespread pain are felt far beyond the physical experience of the individual. Using the SF-12 questionnaire, the MaPP

research network has shown that widespread pain in participants with urological chronic pelvic pain syndrome (UCPPS) was associated with poorer physical and mental functioning, particularly when compared to participants who suffered from UCPPS and pelvic pain alone[203].

Psychological wellbeing

The relationship between poor mental health and chronic pain are likely bidirectional[204], such that poor mental health contributes to the pain experienced and is worsened by it as well. Widespread pain is no different, such that those with more widespread pain experience higher anxiety and depression scores than those with a more localised pain phenotype[203,205]. While the directionality of this is not well understood, it does highlight the importance of considering the impact of widespread pain outside of the context of the physical pain itself.

Sleep and fatigue

Both poor sleep quality and fatigue have been shown to predict CWP onset[206]. It has been proposed that poor sleep quality can impair the endogenous pain inhibitory pathways which contribute to the body's ability to attenuate painful stimuli[207]. In a population study of adolescents using the ALSPAC cohort, the comorbidity of CWP and chronic fatigue syndrome was high (nearly 17%)[208].

(Co)Morbidity and mortality

CWP rarely exists in isolation. People living with CWP have an increased odds of experiencing additional pain-related comorbidities such as joint pain (OR(95%CI): 7.41 (6.7, 8.21)) and irritable bowel syndrome (OR(95%CI):5.17 (4.55, 5.88)), as well as chronic fatigue (OR(95%CI): 23.53 (19.67, 28.16))[209]. In addition, the diagnosis of a chronic pain condition such as rheumatoid arthritis, endometriosis or inflammatory bowel disease can increase the risk of developing chronic widespread pain[210]. This therefore suggests that central sensitisation associated with the original diseases (i.e. endometriosis, RA, IBD) may increases the likelihood of developing more generalised hyperalgesia across the body.

Unfortunately, chronic widespread pain is also associated with an increased risk of mortality[211]. Andersson has shown that compared to participants without chronic pain, people with CWP have an increased risk of death (Hazard ratio (95%CI)=1.95 (1.26, 3.03)), which is driven by factors such as poor sleep, and low physical activity[212]. Work by Tesarz et al. has similarly shown an increased risk of death amongst participants with widespread pain, largely attributable to cardiovascular causes[213]. This highlights that while CWP can have devastating impacts on the day-day lives of those living with, it can also increase the risk of mortality if associated factors are not considered in the pain management plan.

1.4.4. Widespread pain as a continuum

A clinical diagnosis of CWP is highly prevalent, however many recent studies have focused attention on pain spread rather than a diagnosis of CWP, per se. The term pain

'widespreadness' has been used to describe how much of the body, either divided into regions (as shown in Chapter 2 on the Body Maps) or percentages, pain is experienced in. The study of widespread pain as a continuum rather than a binary diagnosis has important clinical implications that I will share below, but to which I have additionally demonstrated in my own work, in Chapters 3 and 5.

Many groups have begun to look outside of the primary disease site or where pain was initiated in localised pain conditions. Barad et al. have characterised widespread pain in participants with migraine and found that with increasing pain spread outside of the head, patients report worse pain-related physical functioning, increased health-care utilization as well as more adverse life events that those with a localised pain phenotype[214].

Nickel et al. originally proposed widespread pain grouping nomenclature that subdivided participants into the number of self-reported regions impacted by pain into 'pelvic pain only' or 'pelvic pain and beyond'[215]. 'Pelvic pain and beyond' identified patients who had at least one additional body region impacted by pain. This grouping system has been used in further studies characterising widespread pain and adapted to reflect different widespread pain subgroups. A group that has been leading the way in the phenotyping of localised pain conditions such as chronic pelvic pain, is the Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MaPP) research network[216]. They further stratified the 'pelvic pain and beyond' nomenclature into 'intermediate' (experiencing pain in 1-2 additional regions outside of the pelvis) and 'widespread pain' (experiencing pain in 3-7 additional regions outside of the pelvis)[205]. Using the body map and their adapted nomenclature, they have shown that participants with urological chronic pelvic pain

syndrome can be subdivided into clinically meaningful groups based on how many regions are impacted by chronic pain outside of the pelvis. 75% of their cohort reported extra-pelvic pain and increasing pain spread was shown to be associated with greater sleep disturbances, depression, anxiety, and worse quality of life[205].

Work by Tripp et al. similarly phenotyped women with bladder pain syndrome and found that increasing pain spread was associated with greater pain severity and depression[215,217]. Hah et al. have investigated widespread pain in a cohort of (predominantly) female patients with abdominal pain and found that widespread pain was associated with increased anger[218]. Importantly, they showed that the widespread pain phenotype was relatively stable over a 12-month period, and that risk factors for having widespread pain at the one-year follow-up were having at least one chronic overlapping pain condition (COPC) and widespread pain at baseline. The MaPP group have also shown that the widespread pain phenotype is stable over a 12-month period[219]. Together, this suggests that patients presenting with widespread pain must be appropriately managed when initially presenting in clinic, and that risk factors for the progression to widespread pain, such as COPC, be considered. More recently, Phan et al. investigated myofascial dysfunction and sensitisation in women living with chronic pelvic pain and endometriosis and found that such dysfunction was widespread beyond the pelvis, suggesting an unmet clinical need in the management of CPP in this cohort[220].

1.5. Clinical importance of addressing widespread pain

All of this considered, there is an evident need to work toward patient stratification[221] based on pain symptoms, to ultimately improve pain management for women with CPP. An area of particular promise is widespread pain. As demonstrated above, assessing widespread pain can be simply and quickly done using a body map, and the results of this work signify a gap in the way pain is managed: that pain beyond the primary disease site is often not considered in clinical practice[53], but is evidently important to the overall health and wellbeing of the individual. For women with CPP who are often left with inadequate pain management, widespread pain is an underexplored area of interest which may contribute invaluable knowledge to our growing understanding of CPP. Within this thesis, I have investigated widespread pain in women with chronic pelvic pain and different underlying pathologies. I have outlined in **Appendix 1B** the key hypotheses of this thesis, the datasets used to address them, and how each chapter is linked.

1.6. Thesis aims

As such, my thesis aims to:

- Investigate the frequency and burden of extra-pelvic pain in women with endometriosis and chronic pelvic pain
- Explore the relationship between chronic pain and reproductive outcomes
- Investigate widespread pain in a larger cohort of women with chronic pelvic pain to better understand the mechanisms underlying widespread pain

- Explore the value of the conditioned pain modulation paradigm in a cohort of women with chronic pelvic pain and healthy controls

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1.8. Appendix

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Appendix 1B: Theoretical diagram reflecting hypotheses tested, and links between chapters.

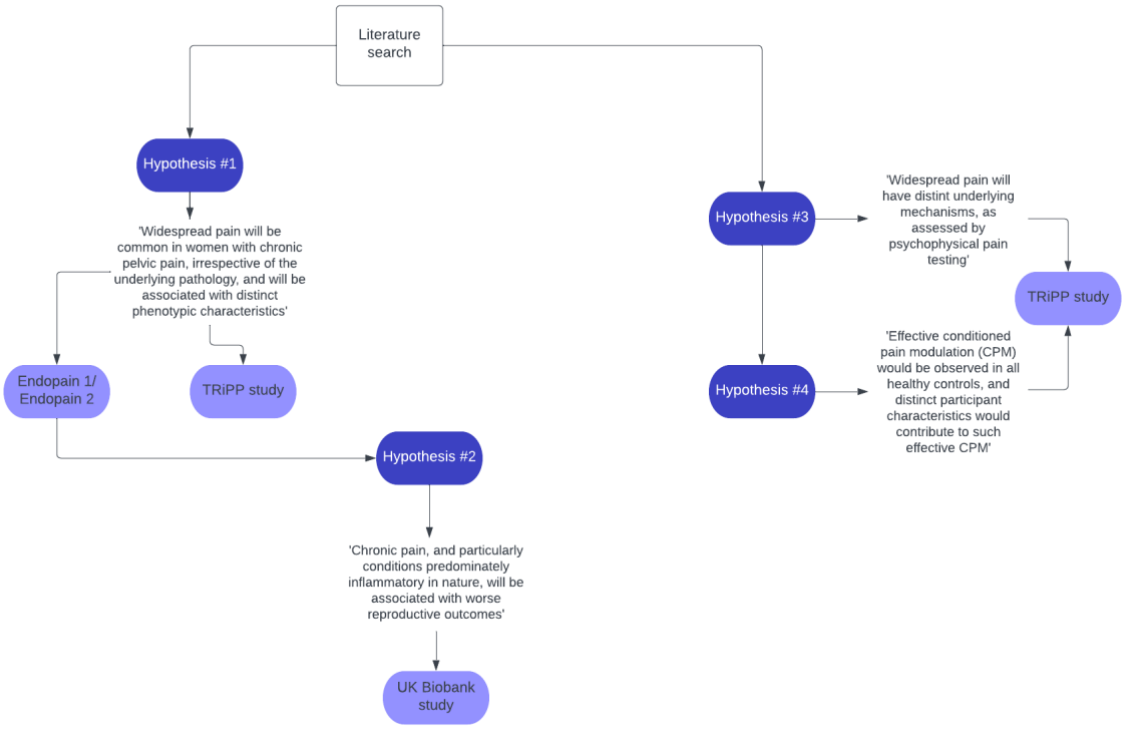


Figure 1.4. Theoretical diagram of the primary hypotheses tested in each chapter, which datasets were used to test each hypothesis, and how the chapters are linked. CPM; conditioned pain modulation, TRiPP; translational research in pelvic pain.

Chapter 2 – General Methods: How is pain studied?

Pain is subjective in nature, and one's experience of it will only ever be truly known to them. However, there are numerous tools available to study and facilitate our understanding of pain, bearing the very subjectivity of it in mind.

Within this thesis the following questionnaires and psychophysical testing paradigms have been used to better understand and characterise the widespread pain phenotype in women with chronic pain.

2.1. Questionnaires

2.1.1. The body map

The body map has been integral to characterising pain spread in this thesis. Body maps have widely been used to understand where individuals suffer from chronic pain in the body[205,214,217]. Of the body maps used in this dissertation, the one used in the first experimental chapter (Chapter 3) was developed by the International Pelvic Pain Society (**Figure *2.1***)[222]. In this free-standing body map, the individual is asked to 'share areas of pain and write a number from 1 to 10 at the site(s) of pain', where 10 is the most severe pain imaginable. This type of simplistic body map has been recommended by an expert consensus investigating pelvic floor dysfunction in women, to better characterise pain[223].

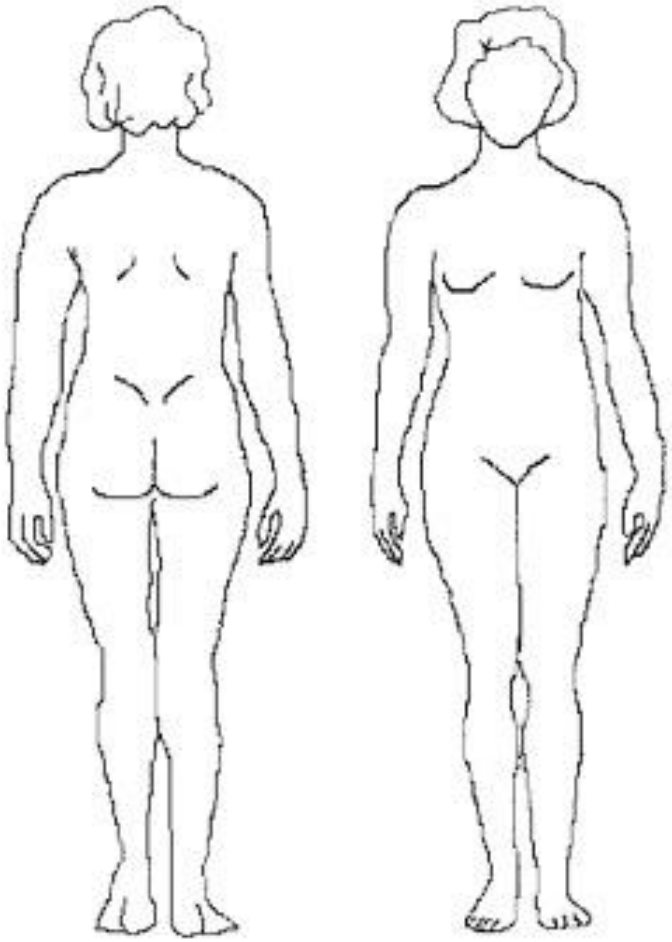


Figure 2.1. International pelvic pain society body map. This image was borrowed from the IPPS pelvic pain assessment form, which was intended to be freely reproduced and distributed while the copyright remains intact.

More recently in 2016, the Michigan Body Map (MBM)[224] was re-conceptualised and validated against other measures of pain spread, such as the Widespread Pain Index (WPI) which is used as part of the 2011 Fibromyalgia Survey Criteria[225], or the Brief Pain Inventory Body Map[226,227]. The MBM is a widely used self-reported assessment of chronic pain location, which contains 35 checkable boxes on both the front and back, as well as left and right sides of the body (**Figure *2.2***). It takes the participant less than a minute to complete and is preferred by participants over the WPI[224]. The MBM is a useful tool to assess pain

distribution in participants with chronic pain, and as a result, to help us understand the centralised components of pain[228]. Because of this, the MBM is used to characterise widespread pain within the Fibromyalgia Survey Score[225].

In my third experimental chapter (Chapter 5), whereby I more deeply phenotype the pain of women with CPP and pain-free controls, I have used the MBM.

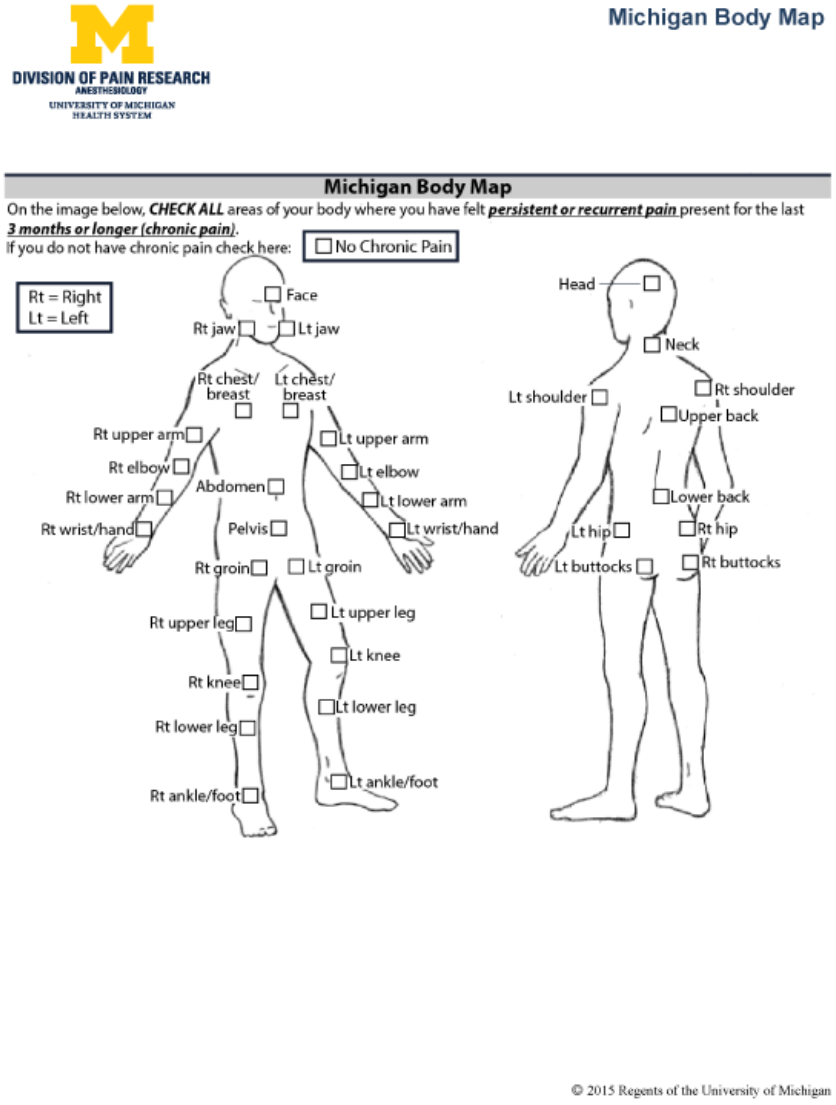


Figure 2.2. The Michigan Body Map. This was designed to be used freely in clinical and research settings.

2.1.2. Numerical Rating Scales (NRS)

Numerical rating scales (NRS) are one of the most used tools to assess someone's rating of their pain. Traditionally, NRS are an 11-point scale whereby participants select a score ranging from 0 (indicating no pain at all) to 10 (indicating the worst pain imaginable). In addition to NRS, visual analogue scales (VAS) are a commonly used subjective measure. VAS includes a straight line, typically 100mm, whereby participants are instructed to select a location on the line that aligns with their experience of pain. For example, one end represents no pain at all, and the other, worst pain imaginable. The NRS was chosen for inclusion in this thesis and associated studies, because it is well correlated with other assessments of pain intensity ratings[229] and shows the most sensitivity and stability over time when compared to distinct but similar scales (i.e. VAS) used to assess pain intensity in people with chronic pain[230].

2.1.3. Pain catastrophizing scale

Pain catastrophizing refers to the negative attitudes towards one's experience or anticipated experience of pain. It is described by three characteristics: rumination, magnification and feelings of helplessness about their pain[231]. The pain catastrophizing scale (PCS) was developed and validated in 1995 to understand the impact that catastrophic thinking has on one's pain experience[232]. The PCS is a 13-item scale, and comprises questions to assess rumination, magnification and helplessness such as: '*I worry all the time about whether the pain will end*', '*I feel I can't go on*', and '*I keep thinking about how badly I want the pain to stop'*[233]. The PCS is a widely-used scale, as it correlates highly with pain intensity, depression and anxiety[234,235]. The total PCS scale has excellent

reliability (Chronbach's α =0.93), as do the rumination(α=0.91), helplessness (α=87), and magnification (α=0.75) subscales [236].

Individuals completing this questionnaire are asked to indicate how much they agree with the 13 statements, either 0 (not at all), 1 (to a slight degree), 2 (to a moderate degree), 3 (to a great degree) and 4 (all the time). A total PCS score is calculated by summing all responses, to a total out of 52. If a participant scores greater than 30, this is considered to be clinically significant, as this corresponds to the 75th percentile of chronic pain participants completing the survey[237]. The three independent subscales within the PCS are scored out of 16 (rumination), 12 (magnification), or 24 (helplessness). Clinically significant scores in these subscales are 11, 5, and 13, respectively[238].

Of worthy consideration, is the patient perspective on the term 'pain catastrophizing'. Amongst patients with chronic pain, the term 'pain catastrophizing' is seen as derogatory and is not preferred. Crombez et al. proposed that the language should instead be person-focused and be described as 'pain-related worrying'[239]. While in this thesis catastrophizing terminology is used, it is important to recognise and continuously consider the impact on and opinions of the very people who spend time completing these questionnaires, donating their time for research or for whom the findings are relevant. For without their participation and vested interest, this thesis would not be possible.

2.1.4. painDETECT

painDETECT is a screening tool to assess a neuropathic component to pain; pain due to disease or lesion of the somatosensory system[240]. It was initially designed and tested in a smaller cohort of just over 400 participants, prior to a larger epidemiological validation in over 8,000 participants with low back pain[31]. However, since its conception, this questionnaire has been used in many other chronic pain conditions, such as fibromyalgia and endometriosis[16,241]. Further validation in these clinical cohorts is however still required. The questionnaire is widely used because patients complete it independently, and it has high sensitivity and specificity[31]. The painDETECT questionnaire is comprised of a body map where participants are instructed to mark their main area of pain, a 7-item gradation of pain measure, a 4-item pain course pattern, and a single-item question about radiating pain. The 7-item questionnaire comprises descriptives of the participant's pain such as '*Do you suffer from a burning sensation (e.g., stinging nettles) in the marked areas?*', '*Is light touching (clothing, a blanket) in this area painful?*' or '*Do you suffer from a sensation of numbness in the areas that you marked?*', amongst others. Each of the seven questions were scored out of five; 0 (never), 1 (hardly noticed), 2 (slightly), 3 (moderately), 4 (strongly), 5 (very strongly).

The 4-item pain course pattern question asks participants to select the picture that best describes their pain course. They were described and scored as follows; persistent pain with slight fluctuations (0), persistent pain with pain attacks (-1), pain attacks without pain between them (+1), or pain attacks with pain between them (+1). The single-item question asks '*Does your pain radiate to other regions of your* body?', and is a binary outcome (yes

/no). A '*yes*' response was given a score of 2, and '*no*', 0. If participants select yes, they are then instructed to draw on the body map where their pain radiates to.

A total painDETECT score is calculated by summing the three different questionnaire components, for a total score out of 38. In the initial validation study, Freynhagen et al. suggested the following cut-offs be used, to infer with confidence whether someone has a neuropathic component to their pain[31]. Scores from -1-12, are classified as nociceptive and therefore is unlikely (<15% likelihood of neuropathic component) that a neuropathic component existed. Scores from 13-18 are classified as mixed or unclear, and scores from 19-38 suggest that a neuropathic component is likely (>90% likelihood of neuropathic component). There is considerable debate amongst the scientific community as to whether in fact the painDETECT questionnaire assesses elements of nociplastic pain, rather than neuropathic pain. Nociplastic pain is a third pain type, which can occur independent of other pain types, or in combination with nociceptive and neuropathic pain components[18]. As such, it can be difficult to know whether questionnaires, such as painDETECT, are in fact assessing neuropathic or nociplastic pain. In a review of rheumatic diseases and the prevalence of neuropathic pain, it has been suggested that many patients with a label of a neuropathic pain component will have a nociplastic pain component that can be identified using this questionnaire[242]. Furthermore, it has been suggested that the 'mixed pain' phenotype includes elements of all three pain types[26]. Therefore, while in this thesis I have used the original descriptors in the painDETECT questionnaire (i.e. nociceptive, mixed, or neuropathic-like component), I recognise that there is debate over the terminology, and that participants with a neuropathic-like component to their pain may in fact have a nociplastic element to their pain.

2.1.5. Questionnaires assessing anxiety and depression

It is well-established that there is a strong relationship between different qualities and characteristics of chronic pain, and both anxiety and depression[243,244]. In this thesis, I have used three separate tools to investigate anxiety and depression, as the questionnaires prepared for each study were established *a priori* by the study Chief/Principal Investigator(s). Each questionnaire is self-administered. Most often, the Hospital Anxiety and Depression Scale (HADS) is used to assess anxiety or depression, and the State-Trait Anxiety Index (STAI) is used to assess different elements of anxiety (state or trait). Oftentimes the HADS-Anxiety and STAI-State are used interchangeably[245]. It has been shown however in a cohort of patients with an implantable cardioverter defibrillator that the prevalence of anxiety using the clinical cut-off for STAI-S (state anxiety) identified more participants with anxiety than did the HADS-A (anxiety)[246].

HADS is a 14-item questionnaire comprised of 7-item anxiety and depression subscales. Each question is scored from 0-3, a higher score indicating more anxiety/depression. Once totalled, a score of 0-7 is classified as normal, 8-10 borderline abnormal, and 11-21 abnormal (or a case of anxiety or depression). In chronic pain patients, HADS has been shown to be a reliable measure of overall emotional distress[247].

STAI is a 40-item questionnaire, subdivided into two subscales to assess state and trait anxiety. State anxiety can be described as the anxiety the participant is experiencing while completing the questionnaire and is a more transient phenotype than trait anxiety. Trait anxiety assesses someone's inherent tendency to be anxious and exhibit anxious behaviour

or thoughts. Each subscale is comprised of 20 questions, and participants are asked to select how often they feel the statement. This is scored from one to four (1=almost never, 4=almost always)[248]. STAI has high internal consistency (α=0.86-0.95).

STAI has been widely used in the study of psychological morbidity in chronic pain patients, including people with rheumatoid arthritis, fibromyalgia and chronic widespread pain[245,249].

The Beck's Depression Inventory (BDI) is a self-reported 21-item questionnaire which assesses symptoms of depression[250]. When it is used to assess depression, the STAI is often used in parallel to assess anxiety. The internal consistency, or how related questions from the same questionnaire are, is high for the BDI (α=0.73-0.92)[251]. The BDI can be self-scored, but in the case of this thesis, I scored the questionnaire for my first experimental chapter.

2.1.6. Fatigue and Sleep

In TRiPP, fatigue and sleep with respect to widespread pain were evaluated (Chapter 5). In order to assess fatigue and sleep, Patient-Reported Outcomes Measurement Information System (PROMIS) measures were used[252].

The fatigue PROMIS measure used in TRiPP was a custom short form, which assessed the participants' experience of fatigue, but also the impact that fatigue had on their daily functioning[253]. Fatigue short forms have been shown to have acceptable reliability and validity in healthy and clinical populations[254].

The fatigue-SF is comprised of 8-items, whereby participants scored how often they felt the following over the last 7 days: "*I felt exhausted, I felt that I had no energy, I was too tired to do my household chores, I felt fatigued, I was too tired to leave the house, I was frustrated by being too tired to do the things I wanted to do, I felt tired, I had to limit my social activity because I was tired".* Participants were asked to rate their experience on a 5-point Likert Scale; these were scored as follows; 1=never, 2=rarely, 3=sometimes, 4=often, 5=always.

The 8-item sleep disturbance PROMIS short-form v1.0 for adults was used to assess disturbances to sleep amongst TRiPP participants[255,256]. Participants were asked to again score how often they had the following experiences over the past 7 days, using a 5-point Likert scale, as above.

To score the fatigue and sleep questionnaire responses, data was inputted into the *HealthMeasures Scoring Service powered by Assessment Center*^{SM}*.* This user-friendly online tool involves formatting participant responses into an excel sheet as per protocol, uploading it onto the Scoring Service, and receiving an output with the raw score, t-score and standard error. Normative scores are calculated based on comparisons to a healthy population, with a population mean of 50, and standard deviation of 10. Higher scores amount to more fatigue.

Sleep scores were similarly scored. However, in the case of PROMIS sleep scale scores, higher scores equate to better sleep quality.

2.1.7. SF-36 Questionnaire

The 36-item Short Form Survey, or SF-36, is a 36-item quality of life questionnaire which measures eight different domains of health; physical functioning, physical role limitations, bodily pain, general health perceptions, energy, social functioning, emotional role limitations and mental health. It was designed to assess general quality of life but has since been adopted to study quality of life in specific diseases since its conception. It is a self-administered questionnaire and has excellent reliability (α>0.85)[257].

2.2. Psychophysical testing

In addition to questionnaires, it was important that to truly understand widespread pain in women from a mechanistic point of view, psychophysical testing methods were used. In the third experimental chapter, quantitative sensory testing and conditioned pain modulation paradigms were used to achieve this.

2.2.1. Quantitative sensory testing

Quantitative sensory testing (QST) is a psychophysical testing paradigm, which assesses somatosensory function of a participant, ultimately allowing for the determination of loss or gain of function. Previously, QST has been used to distinguish between participants with chronic low back pain and localised vs widespread pain, and fibromyalgia[182]. In Chapter 5, QST data from the Translational Research in Pelvic Pain (TRiPP) study was used to better characterise widespread pain.

In Oxford, myself and a colleague were responsible for collecting data during clinical study visits. Prior to data collection, I received standardised training and certification from the German Network of Neuropathic Pain (DFNS) on their QST paradigm, and how to analyse it. This was ultimately to ensure that data from the three TRiPP study sites could be amalgamated.

The DFNS protocol was developed using data from 180 healthy control participants[1]. In short, it involves the application of different sensory stimuli at a control site and a distant test site, in that order. In the case of TRiPP, the control site was the dorsum of the right foot. The test site was the lower abdominopelvic region, as this was relevant to the cohort of women with chronic pelvic pain. It was decided by members of the TRiPP study team, in conversation with members of the DFNS, that it was comparable to the reference dataset, which uses the trunk. After obtaining valid informed consent, a standardised set of instructions were read to the participant. They were then asked to confirm whether they understood what was said to them and were given the opportunity to ask any questions. The testing took place in a temperature-controlled room (~20°C). Participants were sat on a fully adjustable chair, as this allowed for participant comfort, and importantly, easy access to the test and control sites. QST data was collected and recorded on the DFNS case report form (CRF). All data were stored within the NDWRH before being input via double entry into an online database by members of the TRiPP team and saved on a high compliance server.

All information listed below about the specific QST tests has been acquired from the DFNS protocol, unless otherwise stated.

Thermal detection and thermal pain thresholds

Within the QST paradigm, there is a battery of thermal detection and pain thresholds, including cold (CDT) and warm (WDT) detection thresholds (as the name implies, the ability to detect warm or cold sensations) and cold (CPT) and heat (HPT) pain thresholds (the perception of cold or hot sensations as painful). Additionally, the Thermal Sensory Limen (TSL), or one's ability to detect changes in temperature was also assessed.

To perform thermal sensory tests, the Thermal Sensory Analyzer (TSA-II, Medoc, Israel) with a $9cm^3$ thermode and prerequisite software were used. The thermode was applied to the control site, followed by the test site, with a baseline temperature of 32°C. To ensure consistency in the thermal threshold measurements, the thermode temperature changed (increased or decreased) at a rate of 1°C/s. All threshold tests were completed three times, and the temperatures were recorded after each run through. Between each of the three runs, the thermode returned to the baseline temperature. There was a 6 second or 10 second interval between stimuli for the detection thresholds and pain threshold, respectively. For the Thermal Sensory Limen, participants were instructed to say when they felt a change in sensation, and label the sensation (i.e. hot, cold). For some participants, it was the case that a change in temperature was perceived oppositely to the actual change (i.e. warm to cold was perceived as cold to warm). This was classified as a paradoxical heat sensation and was recorded on the CRF.

For each thermal measure, the participant was handed a button, which they were instructed to press at different time points, depending on which paradigm was taking place.

For example, for detection thresholds, they were instructed to press the button once they detected a change in sensation. For the TSL, they were asked to report when they noticed the successive temperature change. The order of the QST paradigm was consistent across participants and TRiPP sites: CDT, WDT, TSL, CPT, HPT.

Mechanical detection threshold

Von Frey filaments (VFF) were used to assess mechanical detection threshold (MDT). These small filaments are very fine hair-like structures of different thicknesses, with a bulbous end. The thicker the VFF, the greater the stimulus intensity (measures in mN). Participants were instructed to look away from the area of skin being tested. They were told to say 'yes' as soon as a touch sensation was perceived. The MDT protocol assesses Aβ fiber function[258].

VFF from Somedic SenseLab AB (Somedic Aesthesiometer II) were used at the following intensities: 0.63mN, 1.37mN, 3.14mN, 16.67mN, 50mN, 81.4mN and 235.36mN. The MDT protocol begins by placing the 16.67mN VFF on the skin until it bends slightly. If the participant feels this VFF at this intensity, then the next lowest intensity VFF is applied until the participant can no longer feel it. If the sensation is not felt, then the next greatest intensity is applied until the participant can feel it. If the participant can still feel the lowest intensity VFF, then half of the intensity is recorded (0.315mN), and if the highest intensity VFF cannot be felt, double the intensity is recorded (470.72mn). A modified method of limits protocol is followed. As such, five sequential thresholds are determined using a series

of ascending and descending stimulus intensities. The final threshold is the geometric mean of the five series of supra- and subthreshold stimuli intensities.

Mechanical pain threshold

The mechanical pain threshold (MPT) is tested using weighted pinprick stimuli. A similar method is used as with the MDT, as ascending and descending stimulus intensities are applied to the skin. Pinpricks of 8mN, 16mN, 32mN, 64mN, 128mN, 256mN, and 512mN, from MRC Systems, were used. The stimulus was applied perpendicularly to the skin for one second. Participants were asked to identify each stimulus as 'blunt' (if the sensation was perceived as touch only) or 'sharp' (as soon as the blunt sensation became additionally painful). The paradigm started at 8mN and was sequentially increased until the participant rated the stimulus as 'sharp'. Once this occurred, stimulus intensity was sequentially decreased until the participant again rated the stimulus as 'blunt'. This was repeated five times, with the stimulus intensity being recorded each time the descriptor changed. As with the MDT, the final threshold is the geometric mean of the five series of supra- and subthreshold stimulus intensities. If the participant found the 8mN intensity to be 'sharp', then 4mN was recorded. If the highest intensity, 512mN was 'blunt', then 1,024mN was recorded. The MPT tests Aδ fibres.

Stimulus/response functions

Following from MDT and MPT, mechanical pain sensitivity (MPS) and dynamic mechanical allodynia (DMA) are assessed. MPS tests C and Aδ fibres, whereas DMA tests Aβ fibre functioning. This involves a battery of stimuli, including the pinpricks described above, in

addition to a paint brush (SENSELab Brush-05), a Q-tip and a cotton wisp (part of the pinprick set, from MRC). The order of application for both the test and control site are randomised and performed according to the CRF. After application, participants rated the pain of the stimulus from 0-100 (0=no pain and 100=worst pain imaginable). If the participant experienced any form of stinging, sharp or pricking sensation, they were instructed to rate the pain greater than 0. Pain ratings were recorded on the CRF.

Wind-up ratio

Wind-up is an increase in the excitability of neurons in the spinal cord, which occurs after rapid and repetitive stimulation of C fibres[259]. In brief, pain intensity increases after repetitive stimulation of the fibres. To better understand this, a wind-up ratio (WUR) can be calculated. To assess the WUR, a pinprick of fixed intensity (256mN) was used. First, a single stimulus was applied, and the participant was asked to rate their pain experience from 0-100 (0=no pain, 100=worst imaginable pain). After 10 seconds, this was followed by a series of ten stimulations to a nearby site (within $1cm^2$). The series was applied at a rate of 1Hz. The participant was again asked to rate the average painfulness of the series from 0-100, as stated above. This protocol was repeated 5 times.

When compared to healthy controls, the WUR has been increased, and thus exhibited characteristics of central sensitization, in patients with fibromyalgia[260,261].

Vibration detection threshold

To assess the vibration detection threshold (VDT), a Rydal Seiffer 64Hz tuning fork (8/8 scale) was applied to a bony prominence at both the control and test sites. The tuning fork was hit to induce vibration, before being placed on either the bone in the dorsum of the foot (control site) or the pubis symphysis (test site). The participant was asked to say when they could no longer feel the vibration. At that point, the VDT was determined as a disappearance threshold (out of eight) and the score was recorded. This was repeated three times. The average score of the three was then calculated before group comparisons were performed. VDT assesses the function of the Aβ fibres.

Pressure pain threshold

To assess pressure pain threshold (PPT), a pressure algometer was applied over muscles in the control and test site at a steadily increasing rate (0.5kg/s). The participant was asked to describe when the sensation of the stimulus changed from a pressure to painful (i.e. burning, aching, stinging) quality. The pressure (kPA) was recorded after each run. This procedure was repeated three times. In analysis, the arithmetic mean of the three series was calculated[1]. In TRiPP, the 10kg/100N algometer from Wagner Instruments was used. PPT assesses function of the Aδ fibres.

2.2.1.2. Data Analysis of QST data

Prior to QST analysis, scores were z-transformed to age and sex matched control reference data, as published by the German Network of Neuropathic Pain (DFNS)[1]. These transformations were performed by a colleague prior to me receiving the data. Participants

with a score greater than +1.96 (equal to one standard deviation) were considered to have a gain of function, and those with a score less than -1.96, a loss of function.

For my analyses in Chapter 5, I used data from the foot only, as perturbations in nerve functioning at this site would give a better understanding of any central sensitisation or central pain mechanisms occurring outside of the pelvis in women with chronic pelvic pain.

It has previously been shown that in participants with chronic low back pain, there was a strong correlation between Fibromyalgia 2011 criteria score and PPT at the thumbnail (control site)[262]. Further, in women, there is a negative correlation between greater number of self-reported pain sites and PPT[181,263].

2.2.2. Conditioned pain modulation

Conditioned pain modulation (CPM) is a psychophysical test which assesses functioning of the descending inhibitory pain pathways[264]. Essentially, these pain pathways should function such that 'pain inhibits pain'. In brief, the paradigm involves the application of a test stimulus (typically thermal or pressure), followed by a conditioning stimulus at a distant site, completed with the application of the test stimulus again at the original test site. If a participant has efficient or intact CPM, the pain rating of the follow-up test stimulus should be less than the original one. While there is variation in CPM paradigms used between studies, there are recommendations for CPM best practice which the TRiPP paradigm has adhered to[265].

TRiPP participants were sat in a temperature-controlled room. To determine CPM, an algometer test stimulus (TS) was applied to the right dorsal foot at 1kg/cm^2 per second, three times, and the pressure (kPa) was recorded after each application. The mean of the three pressures was calculated to determine the baseline pressure pain threshold ($PPT1_{average}$), as described by the German Research Network on Neuropathic Pain (DFNS). A pressure cuff conditioning stimulus (CS) was applied to the left arm; the cuff was pumped up at a rate of ~20mmHg/second until participants identified the stimulus as painful. The cuff was maintained at that pressure for 60 seconds. Prior to deflation, the participant was asked to rate the pain elicited from the CS out of 10 (0=not painful, 10=worst pain imaginable). The self-reported pain rating and CS pressure were recorded. Immediately after deflation, the algometer was again applied to the right dorsal foot three times, and the mean $PPT2_{average}$ was calculated.

The participant was then instructed to rest quietly for 10 minutes, prior to repeating the application of the CS and TS. Pressure, pain rating of the CS prior to deflation, and the mean $PPT3_{average}$ TS were again calculated. All testing paradigm protocols were scripted and read aloud to the participant to ensure reproducibility and consistency between sites.

CPM has been used routinely in studies of chronic pain[266], and further, to phenotype and compare chronic pain patients with localised versus widespread pain[188]. In Chapters 5 and 6, CPM was used to phenotype women with CPP[267]. In addition to comparing CPM between widespread pain groups, a robust statistical technique[268,269] was used to explore the usefulness of the CPM paradigm in the TRiPP cohort of women with CPP and healthy controls. The results from these analyses have been submitted to the journal PAIN.

2.2.2.1. CPM Data Analyses

The CPM effect in this chapter was calculated as both a percentage change, using the equation below, and absolute change ($PPT2_{average}$-$PPT1_{average}$). This was then plotted and compared between widespread pain groups.

$$\left(\frac{\mathrm{PPT2average}-\mathrm{PPT1average}}{\mathrm{PPT1average}}\ x100\%\right)$$

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Chapter 3 – Investigating the burden of widespread pain in women with endometriosis and chronic pelvic pain

3.1. Introduction

Pain is highly prevalent in those living with endometriosis: more than 60% of women with endometriosis report chronic pelvic pain (CPP)[270]. Pain is a primary motivator for patients to seek care for their endometriosis, as the symptoms can have a devastating impact on a woman's quality of life[271]. For many, this pain is poorly managed, which is in part driven by the lack of successful treatment options available[272,273]. This is likely the case, at least in part because we aren't yet effectively phenotyping and characterising pain. Without a better understanding of endometriosis-associated pain, the reality is that many will receive a one-size-fits-all treatment which isn't personalised to their pain specifically. One such way to address this is to better phenotype and characterise the pain experienced by women with endometriosis-associated pain (EAP). In order to do so, it is important that we not only better characterise pain in women with EAP, and how severe it is, but the impact that it has on the lives of those affected.

Women living with EAP are at a higher risk of developing psychiatric comorbidities[274] and experiencing poor mental health. The directionality of this relationship, however, is difficult to unpick (i.e. does pain contribute to poor mental health, or does poor mental health exacerbate pain symptoms or both), however it is clear that both pain and poor mental health can have a severe impact on overall quality of life[275].

Furthermore, infertility and poor reproductive outcomes are common in women with endometriosis. As many as 30-50% of women with the condition experience infertility[276], and have an increased risk of experiencing early pregnancy loss (miscarriage and ectopic pregnancy) and complications later in pregnancy[277]. However, there has yet to be research investigating whether a relationship exists between chronic pain and reproductive outcomes in this population.

In this chapter, I explore a possible method of phenotyping EAP and its burden: widespread extra-pelvic pain. Previous work characterising cohorts of chronic pelvic pain participants by widespread pain has highlighted an important relationship between widespread pain, comorbid conditions, and worse psychological health[205,217]. In previous CPP work, the relationship between widespread pain and poor reproductive outcomes has not been investigated. Since infertility is highly burdensome in women with endometriosis, and there is evidence that both adverse reproductive outcomes and widespread pain have a shared pathophysiological mechanism of inflammation[278,279], I believed this was an important variable to include in my analysis.

At the time of this work, phenotyping based on widespread pain was not yet performed in women with EAP. Therefore, in a cohort of women with EAP, I aimed to:

1. Subdivide the cohort based on the number of self-reported pain locations
2. Characterise and visualise the location of pain using a body map

3. Determine whether widespread pain groups represent distinct pain phenotypes with different a) psychological profiles, b) pain characteristics, c) comorbidities, and d) reproductive outcomes

As such, I hypothesised that:

1. Women with endometriosis-associated pain could be characterised according to pain spread outside of the pelvis
2. When compared to those with more localised pain, the widespread pain phenotype would be associated with worse pain characteristics, psychological health, more comorbid conditions, and worse reproductive outcomes

3.2. Methods

3.2.1. Study population

The analyses presented in this chapter use data which were collected by study members many years prior to my work. Women were initially recruited into one of two studies: EndoPain 1 (EP1) or EndoPain 2 (EP2). The primary aim of the EndoPain studies were to identify factors which predicted response to surgery in women with EAP. Women recruited to EP1 had a clinician indicate a strong suspicion of endometriosis but had not yet undergone laparoscopy to diagnose it. More specifically, after reviewing the study protocol, participants recruited into EP1 had to be female, 18 years old or older, and experience non-menstrual pelvic/lower abdominal/back pain with a mean NRS score $\geq$ 4/10 when pain is present. Additionally, a previous diagnosis of endometriosis via

laparoscopy or strong clinical suspicion by the treating clinician was required. EP1 participants were excluded if they were taking any centrally-acting medications (i.e. sleeping medications, anti-epileptic medication) at the time or any time within the month prior to the study visit, had a contraindication for participation in a magnetic resonance imaging (MRI) scan, previously received surgical management for their endometriosis or were pregnant. Women in EP2 received a diagnosis of endometriosis within the last five years and were recruited just prior to undergoing repeat surgery to manage their EAP. Participants were included if they experienced non-menstrual pelvic pain of an intensity ≥ 4/10 for more than 7 days per month, for at least 6 months total. Women were excluded from participating in this study if they were pregnant, contraindicated for a MRI scan, or were taking any centrally acting medications in the preceding three months.

3.2.2. Study design

Data collection on the EndoPain studies was completed prior to the point at which I began my DPhil. Appropriate ethical approvals were obtained for EP1 (South Central – Oxford B REC reference: 12/SC/0371) and EP2 (Oxford University Hospitals NHS Foundation Trust Research and Development REC Reference: 15/SC/0372).

Psychological questionnaires, MRI brain scans, hormone levels, and additional clinical information were collected prior to laparoscopic surgery investigating endometriosis (EP1), or 6 months post-surgery (EP2). Questionnaires included painDETECT, pain catastrophizing scale (PCS), Beck's depression inventory (BDI), and the State Trait Anxiety Inventory (STAI), all of which were described in Chapter 2.

A consort flow diagram illustrating the outcome measures analyses and how many participants were included in them can be found in **Appendix 3A**.

3.2.3. Widespread pain group allocation

As part of the questionnaires given to women in EP1 and EP2, they were instructed to complete the International Pelvic Pain Society (IPPS, Chapter 2) body map, indicating where on the body they experienced pain. All women who indicated that they experienced pelvic pain were included in this study.

I used the MaPP scoring system[205] to sum the number of widespread pain regions (out of 7) each participant was impacted by. The IPPS body map requires drawing on the body map rather than ticking boxes on affected areas of the body. Upon reviewing the body maps, I then scored them as closely as possible to the MaPP protocol[205].

After totalling the number of regions impacted by chronic pain, and for the sake of creating groups of relatively equal size, I grouped the participants into pelvic pain only (0 extra-pelvic regions), isolated (1 extra-pelvic region), intermediate (2 extra-pelvic regions) or widespread (3-7 additional regions). This differs from the MaPP widespread pain groupings used in Chapter 5, which group into isolated (pelvic pain only), intermediate (1-2 regions) or widespread (3-7 regions).

Heat maps were created for each of the pain groups using Adobe software. The colour of the region impacted by pain on the body map corresponds to the percentage of women in each group reporting pain in that region, as demonstrated by the scale.

3.2.4. Psychological & pain-related questionnaires

The BDI, and STAI questionnaires were used as measures of depression and anxiety, respectively. Furthermore, the PCS assessed the rumination, helplessness, and magnification participants felt towards their pain. Prior to retrieving the data, the questionnaires had been transferred to a computer-based database and scored by another researcher. The painDETECT questionnaire responses were similarly scored prior to receiving the data. However, all scoring of the questionnaire data followed standard protocols outlined in Chapter 2.

3.2.5. Comorbidities

While EP1 and EP2 questionnaires did ask similar questions, there were fundamental differences in the comorbidities which were enquired about. In EP1, participants were given a list of 14 comorbidities, to which they were instructed to tick a box ‘yes’ if they had ever been diagnosed with the condition, or ‘no’ if they had not. In EP2, participants were given a table with 31 possible diagnoses, to which they selected ‘yes’ or ‘no’ if they’d been diagnosed, with an ‘other’ box for free text responses. For this reason, and to increase the number of participants I could include in this analysis, I have only described the frequency of comorbidities which were a) asked about in both questionnaires and b) at least one

participant responded yes to. This included painful comorbid conditions such as irritable bowel syndrome (IBS), interstitial cystitis/painful bladder syndrome and migraine, and other comorbidities such as anxiety, depression, asthma, and chronic fatigue syndrome/myalgic encephalomyelitis (ME).

3.2.6. Reproductive outcomes

Reproductive outcomes analysed in this chapter include live births, miscarriage, multiple miscarriages (i.e. had they had more than one miscarriage–this was not necessarily recurrent or in consecutive pregnancies), and ectopic pregnancy. Participants were instructed to complete a table which asked, 'what happened in each your pregnancies?'. They were given the options listed above and were instructed to tick the appropriate reproductive outcome if they had ever been pregnant or tried to get pregnant. In addition, participants reported on whether they have had difficulties getting pregnant (didn't necessarily need to meet the criteria for infertility, that is, not conceiving after one year of consecutively trying to). This question was again asked differently in EP1 and EP2. In EP1, participants were asked 'Have you ever had problems getting pregnant?', and were instructed to say yes or no. In EP2, participants were asked about whether they had ever experienced infertility; 'Have you ever tried to get pregnant for more than 6 months in a row without succeeding?' and select 'yes' or 'no'. Although worded differently, if participants responded yes to the question in EP1 or EP2, they were listed as having self-reported difficulties getting pregnant.

3.2.7. Data analysis

The Komologrov-Smirnov test was used to assess deviation from normality. Group differences in psychological health measures were subsequently analysed using the non-parametric Kruskal-Wallis test. Comorbid conditions were analysed using a chi square test for trend (df=1), to determine whether the proportion increased as pain became more spread. Finally, a chi square test for trend (df=1) was used to assess the proportion of women self-reporting difficulties getting pregnant with increasing pain spread. Analyses were initially run on the entire dataset. However, after identifying women who were endometriosis-free post-surgical exploration, sensitivity analyses were performed on women with CPP and confirmed endometriosis, and the psychological health and pain measures analyses were rerun. Data were computed using SPSS and PRISM 9. Tables were generated using Word.

3.3. Results

The combined cohorts of women with CPP consisted of 56 women, 50 of which had endometriosis after surgical confirmation. The mean cohort age was 33.5 years and mean pelvic pain symptom duration was 11.5 years: there was no significant difference in mean age (p=0.69) or mean pelvic pain symptom duration (p=0.079) between study groups.

3.3.1. Phenotyping women with CPP and confirmed or suspected endometriosis by pain spread

Data from all 56 women were analysed, and 82% reported extra-pelvic pain. Ten women

reported having pelvic pain only (A), seventeen reported pains in one additional region (B), eleven in two additional regions (C), and eighteen in 3-7 regions outside of the pelvis (D)(**Figure *3.1***).

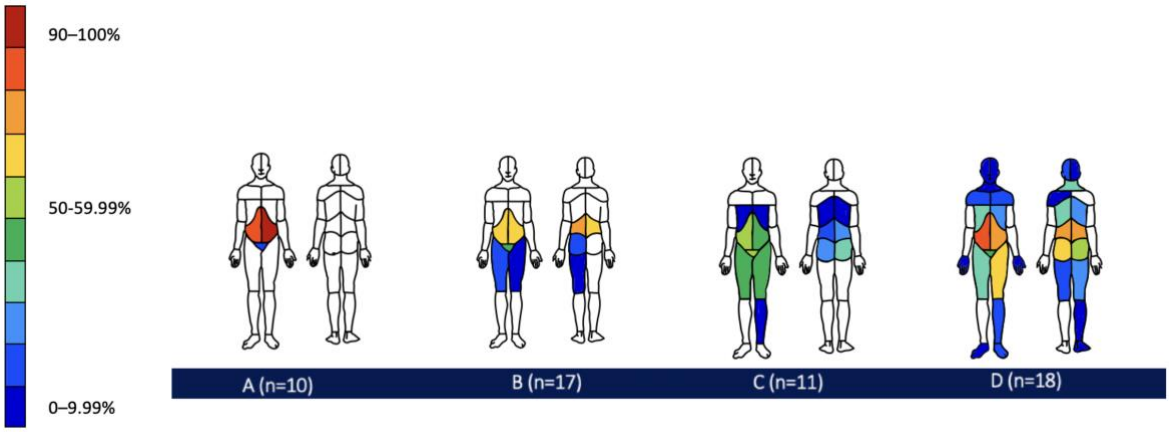


Figure 3.1. Pain spread and widespread pain grouping of Endopain 1 and Endopain 2 participants. As shown on the left-hand side, warmer colours represent a higher percentage of the group reporting pain in that area, and cooler colours indicate fewer (%) report pain in that area. A= pelvic pain only, B=isolated, C=intermediate, D=widespread pain group.

3.3.2. Measures of pain attributes, depression, and anxiety

There was a significant difference in the BDI scores between pain spread groups (p=0.024), and the highest median score was found in the most widespread pain group. However, there was no significant difference in either the state or trait anxiety scores, although it is worth noting that while not significant, anxiety scores were highest in the widespread pain group for both measures.

As shown in **Table *3.1***, there was a significant difference in the painDETECT score between pain spread groups, with higher scores reported in the intermediate and widespread pain

groups (p=0.03). Similarly, PCS scores were significantly different between pain spread groups, but the median score was highest in the most widespread pain group and was above the PCS cut-off of clinical significance of 30 (p=0.017).

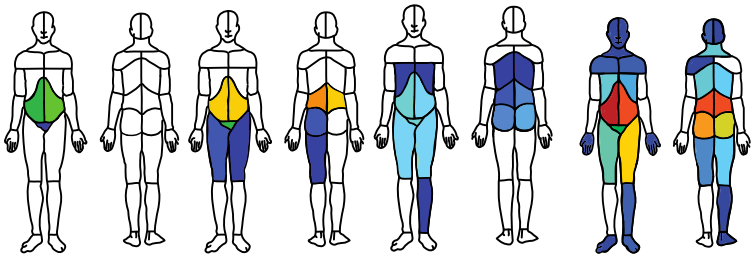
Table 3.1. Measures of pain attributes, depression, and anxiety by pain spread group. Median values are reported in each cell. A Kruskal-Wallis test has been performed to compared scores between groups. A=pelvic pain only, B=isolated, C=intermediate, D=widespread. Median scores are presented. NS; not significant.

Outcome Measure	**A**	**B**	**C**	**D**	**X^2 (p-value)**
painDETECT	10	12	16	16	8.918 (0.03)
Pain Catastrophizing	14	20	19	31	10.130 (0.017)
BDI	12	10	9	17	9.947 (0.024)
State Anxiety	28	25	21	35	5.731 (NS)
Trait Anxiety	27	26	21	34	5.305 (NS)

3.3.3. Comorbidities

As shown in **Table *3.2*** below, the percentage of participants reporting more than one comorbid pain condition was greater than 50% in all extra-pelvic pain spread groups (Groups B-D). Notably, migraine was common in the extra-pelvic pain groups (>10%). However, there was no significant trend in the proportion of women in each pain spread group self-reporting any of the diagnoses below.

Table 3.2. Frequency (n, %) of pain comorbidities between pain spread groups. A chi square test for trend (df=1) has been used to compare the proportion self-reporting a diagnosis of each condition. IBS; irritable bowel syndrome. Df; degrees of freedom. A=pelvic pain only, B=isolated, C=intermediate, D=widespread.



Pain comorbidities (n, %)	Pain Phenotype				
	A (n=10)	B (n=17)	C (n=11)	D (n=18)	X^2 (p-value)
Women Reporting 1+ Comorbid *Pain* Conditions	1 (10)	15 (88%)	6 (55%)	10 (56%)	0.886 (0.347)
IBS	1 (10%)	2 (12%)	2 (18%)	2 (11%)	0.0188 (0.891)
Interstitial cystitis/ Painful bladder	0%	1 (6%)	1 (9%)	3 (17%)	2.451 (0.117)
Migraine	1 (10%)	6 (35%)	6 (55%)	3 (17%)	0.0131 (0.910)

Table 3.3. Frequency (n, %) of additional comorbidities amongst pain spread groups. A chi square test for trend (df=1) has been used to compare the proportion self-reporting a diagnosis of each condition. Df; degrees of freedom. A=pelvic pain only, B=isolated, C=intermediate, D=widespread.

Additional comorbidities (n,%)	Pain Phenotype				
	A (n=10)	B (n=17)	C (n=11)	D (n=18)	X^2 (p-value)
Anxiety	1 (10%)	3 (18%)	3 (27%)	1 (6%)	0.197 (0.657)
Depression	2 (20%)	3 (18%)	3 (27%)	1 (6%)	0.939 (0.333)
Asthma	1 (10%)	2 (12%)	2 (18%)	1 (6%)	0.142 (0.707)
Chronic Fatigue Syndrome / Myalgic encephalomyelitis (ME)	0%	1 (6%)	1 (9%)	0%	0.0438 (0.834)

3.3.4. Reproductive outcomes

Having at least one live birth was most common amongst participants with pelvic pain only and was least common in the widespread pain group (40% vs 11%). Thirty six percent of participants in the intermediate pain spread group had at least one miscarriage. Multiple miscarriages and ectopic pregnancy were relatively uncommon amongst all pain-spread groups, as shown in **Table *3.4***.

Furthermore, whilst it could not be tested for significance (**Figure *3.2***), the percentage of women reporting difficulties getting pregnant appeared to increase with increasing pain spread. Four participants in total were missing, two from the intermediate (group C) and two from the widespread (group D) pain group.

Table 3.4. Number (%) of participants reporting each reproductive outcome by widespread pain group. A=pelvic pain only, B=isolated, C=intermediate, D=widespread.

Reproductive outcome (n,%)	**A (n=10)**	**B (n=17)**	**C (n=11)**	**D (n=18)**
At least one Live Birth	4 (40%)	4 (24%)	3 (27%)	2 (11%)
Miscarriage	1 (10%)	3 (18%)	4 (36%)	2 (11%)
Multiple Miscarriages	0%	1 (6%)	1 (9%)	1 (6%)
Ectopic pregnancy	0%	0%	1 (9%)	0%

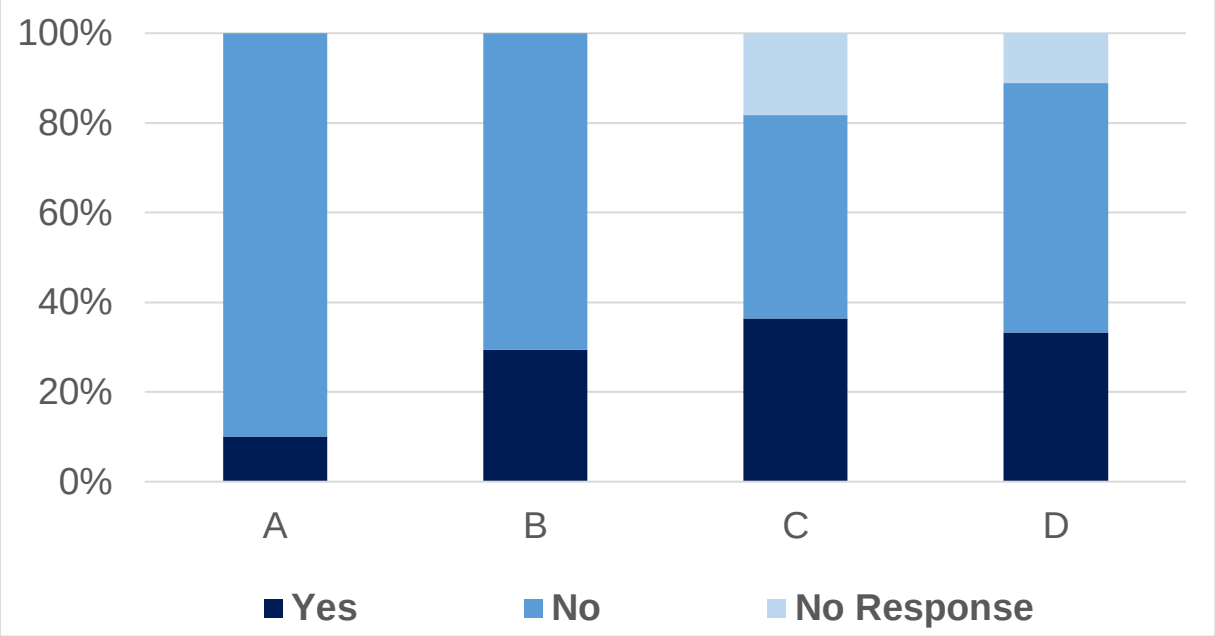


Figure 3.2. The percentage of women in each pain spread group self-reporting difficulties getting pregnant. A=pelvic pain only, B=isolated, C=intermediate, D=widespread.

Sensitivity analysis

As highlighted in **Figure *3.3***, after further review of the records six women had no endometriosis found during their surgery. This excluded one participant with pelvic pain only, two with one additional pelvic pain region, and three participants with widespread pain (3-7 additional regions). While my analyses are first and foremost interested in the pain experienced rather than the disease itself, I performed a sensitivity analysis on the psychological health and pain questionnaires to see if excluding these participants from my initial analyses would influence the results.

I found that after the sample size decreased, the association between pain spread and BDI became more significant (p=0.010). However, the painDETECT (p=0.05) and pain catastrophizing (p=0.051) scale scores were no longer significant.

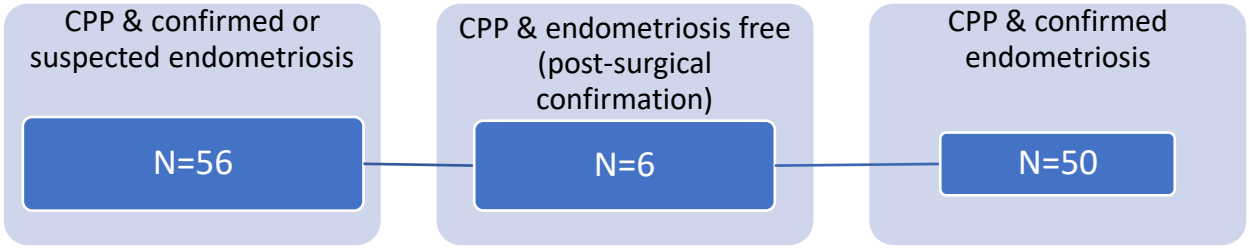


Figure 3.3. Flow chart indicating the number of participants who had confirmed endometriosis post-surgery, and the number which were excluded (n=6) for the sensitivity analysis. CPP; chronic pelvic pain.

3.4. Discussion

For the first time, I showed that women with endometriosis (confirmed or suspected) and chronic pelvic pain could be characterised according to extra-pelvic pain spread. Using a modified version of a well-defined method for grouping, I found that extra-pelvic pain was common in this population of women, as 82% experienced pain in at least one extra-pelvic region. As hypothesised, women with increasing pain spread exhibited higher depression, and pain catastrophizing scores. Additionally, more widespread pain was associated with higher painDETECT scores. Interestingly, there was no significant difference in either state or trait anxiety between groups. After performing a sensitivity analysis on these measures, the association between pain spread and depression remained significant, however likely due to a decrease in sample size, the painDETECT and pain catastrophizing were no longer significant.

Whilst there was no significant trend across the pain spread groups with respect to any of the comorbid conditions, it was interesting to observe how common an additional comorbid pain condition was in women with at least one extra-pelvic region impacted by

pain (>50%). Migraine was also very frequently reported (≥10%) across all groups. From the list of available conditions however, it wasn't necessarily the case that an additional chronic pain diagnosis corresponded to pain in that body region on the body map (i.e. although migraine was common in all pain groups, only participants in the widespread pain group identified pain in the head on the body map). This therefore suggests that there is a more generalised hypersensitivity or pain spread beyond what would be expected given the chronic pain conditions presenting in this group. It is worth noting, however, that the list of comorbidities was not exhaustive for reasons previously outlined.

I investigated reproductive outcomes with respect to pain spread, and whilst I couldn't statistically test the relationship, the frequency of women reporting at least one live birth and self-reported difficulties getting pregnant shed light on a possibly area for further investigations, in a larger and better suited cohort.

3.4.1. Psychological questionnaires

Depression scores (as assessed by the BDI) were significantly different between widespread pain groups. A similar trend is becoming widely acknowledged in research investigating the burden of widespread pain in pathophysiologically 'localised' pain conditions, such as urological chronic pelvic pain syndrome[205], IC/BPS[217], abdominal pain[218], and migraine[214]. The analyses I've presented did not allow me to unpick the temporality of the association between widespread pain and depression. While aetiologically this is important to explore further, my work is an important first step in acknowledging that the association exists, as it sheds light on the basic phenotypic

presentation of widespread pain in women with CPP and endometriosis. In Chapter 5, I have explored this finding in a larger cohort.

Interestingly, there was no association between state or trait anxiety and pain spread. This result was at first surprising because it was contradictory to my hypothesis, but also, because women taking the questionnaire completed it just prior to surgery. I would have anticipated that from surgery alone many participants would have had clinically significant state anxiety at least, but this was not the case. Since all participants underwent surgery, however, it is unlikely that this would drive any between group differences. MaPP's work phenotyping based on widespread pain was better powered to detect differences in anxiety scores between pain spread groups, and relative to other measures of psychological health, they found smaller effect sizes in anxiety scores, thus suggesting that other metrics may be more characteristic of the widespread pain phenotype[205].

Pain catastrophizing scores were significantly different between pain spread groups, and unsurprisingly, were greatest in the widespread pain group. In fact, the median PCS score in the widespread pain group was above the cut-off which indicates clinically significant catastrophizing (PCS score≥30). This relationship is typically found in widespread pain phenotyping of localised pain conditions[205,217,280], and has been similarly demonstrated in participants with fibromyalgia [281].

3.4.2. painDETECT

painDETECT scores were significantly different between the pain spread groups. In all the extra-pelvic pain groups, the median painDETECT score was ≥12, which indicates that by definition, at least 50% of participants have a mixed or possible neuropathic component to their pain[30,31]. Mechanistically, this suggests that the pelvic pain only group may be distinct from participants with extra-pelvic pain. Given recent controversies about the painDETECT questionnaire, it is also possible that the most widespread pain groups are experiencing nociplastic pain[18,242]. However, I couldn't draw conclusions about nociplastic pain based on the data included in this study. While outside the scope of this thesis, there is fMRI data available for EP1 and EP2 participants which could be used to explore the neural underpinnings of a nociplastic pain component in this cohort, as has been done previously in patients with fibromyalgia[282]. I have further explored nociplastic pain as a potential mechanism underlying widespread pain in women with CPP in Chapter 5, using psychophysical testing and more comprehensive questionnaire data.

3.4.3. Comorbidities

Although not significant, a greater proportion of women in the extra-pelvic pain groups (B D) experienced >1 comorbid pain condition when compared to the pelvic pain only group (A). In interpreting this, it is worth noting that the list of included pain conditions was by no means exhaustive, and therefore this result likely does not fully capture the true prevalence of multimorbidity. Women with endometriosis do however commonly have chronic overlapping pain conditions (COPC)[151], so it is not surprising that the frequency is so high, however it would be important to explore in a larger cohort the relationship

between widespread pain and COPC.

Furthermore, comorbidity of psychiatric comorbidities (ie. depression, anxiety) has been established in other chronic pain conditions, such as fibromyalgia[283]. While the association between psychiatric comorbidities and increasing pain spread was not significant in my analyses, evidence from participants with fibromyalgia (of which widespread pain is a signature symptom) supports the possibility that widespread pain is associated with an increased prevalence of psychiatric comorbidity.

Previously, in fibromyalgia, it has been shown that treatment of visceral comorbidities reduces fibromyalgia-associated pain[284]. This further emphasises that, with this preliminary data suggesting a high burden of widespread pain in women with EAP, that appropriate treatment of one pain may help with the symptoms of another. As such, more work is needed to fully understand the pain experience of women with EAP to offer more robust and personalised treatment options.

3.4.4. Reproductive outcomes

There was no significant association between widespread pain and any of the reproductive outcomes investigated in this chapter, as the number of events was too small to statistically compare this. Additionally, it is worth considering that due to the inflammatory milieu of the pelvis and structural barriers caused by the disease, poor reproductive outcomes are relatively common in women with endometriosis[277] and so any potential relationship between widespread pain and worse reproductive outcomes could have been masked. Furthermore, the age of the study group was on average 33.5 years old, which considering

the reproductive lifespan (menarche-menopause) is still quite young. Although there were no differences in the age of participants between pain spread groups, it is possible that I was unable to unpick the relationship between reproductive outcomes and widespread pain because not many women had been trying to conceive for long or haven't tried at all. A much larger, longitudinal study following women during their reproductive years, and recording information on widespread pain and reproductive outcomes is needed to better understand this relationship.

3.4.5. Strengths & limitations

This study, for the first time, showed that women with endometriosis could be characterised according to widespread pain, and that this pain was associated with worse psychological health. It additionally gave insight into the possible mechanisms underlying it. Ultimately though, my work on Endopain 1 and Endopain 2 was sufficient to generate important hypotheses which have been explored further in Chapters 4 and 5.

This study by nature, however, was not without limitations. Firstly, I used data from two related but distinct studies to characterise widespread pain in women with endometriosis and CPP. While this allowed an increase in sample size, this hindered the amount of information I could analyse, as I chose to only look at questions and variables which were asked in a comparable way between studies to reduce bias.

Furthermore, there is a possibility that bias was introduced into the widespread pain scoring method, as the appropriate number of regions impacted by pain relied on my

judgement of where the participant identified their pain. The resulting pain spread count was double checked after initially performed, however, to ensure accuracy in my scoring.

Additionally, the aims of the original studies were to better understand the role of surgery in pain management. Although the pain experience was assessed using questionnaires, the studies were run in 2012 and 2015. They were not intended to deeply phenotype pain, and so more advanced phenotyping methods (i.e. psychophysical testing such as conditioned pain modulation and quantitative sensory testing) were not used. I have addressed this limitation in Chapter 5.

Furthermore, I chose not to investigate the rASRM staging[285] for endometriosis across the pain groups. The rationale being that I was interested in the pain experience of these women not endometriosis itself, and it is well known that the disease staging and pain only mildly if at all correlate with one another[286,287]. However, I understand that since reproductive outcomes were investigated in my analyses that the different stages of disease could have influenced difficulties getting pregnant, for example. Upon further investigation, and although data on disease staging were only available for the EndoPain 1 study, it was not the case that participants with severe disease clustered in the widespread pain group. Rather, this group was comprised of participants with only mild or minimal endometriosis.

In the first year of my DPhil while I was awaiting ethical approval for my study *EVEP: Exploring the role of extracellular vesicles in mediating endometriosis-associated pain* (**Appendix 3B**, REC Reference: 20/SW/0032), I was given access to these datasets to

generate some preliminary data which would go on to inform the rest of my project. As such, no power calculation was performed for my analyses prior to, and this work was intended to be hypothesis generating in nature.

All in all, findings from my analyses in this chapter have made me question the role of systemic inflammation in mediating the relationships I've observed. Major depression is associated with a chronic inflammatory state[288], reproductive outcomes are influenced by the inflammatory milieu of the pelvis[278], and many comorbid pain conditions, including fibromyalgia[279], have an inflammatory component underlying them. Therefore, it is an intriguing hypothesis to explore with respect to widespread pain, but also, between reproductive outcomes and pain. Future work is warranted to explore this, as markers of inflammation were not available in this dataset.

3.4.6. Future directions & conclusions

This hypothesis-generating work produced some interesting results and the following important hypotheses which warranted further investigation:

1. Extra-pelvic widespread pain would be highly prevalent in a cohort of women with chronic pelvic pain
2. Widespread pain would be associated with worse psychological health, and general health (fatigue and sleep, higher pain catastrophizing scores, and worse depression and anxiety)

3. Comorbid conditions (pain and non-pain related) would be highly prevalent in this cohort, and more so in the widespread pain groups
4. Adverse reproductive outcomes would be associated with chronic pain, and would be driven by systemic inflammation

The aim of this chapter was ultimately to generate hypotheses about widespread pain in women with endometriosis and chronic pelvic pain. With availability of additional datasets, I've gone on to test these hypotheses, which have generated some intriguing results. As such, the first three hypotheses were tested in Chapter 5 using a larger dataset which was deeply phenotyped and included comprehensive questionnaire data and psychophysical pain testing. The final hypothesis was tested in Chapter 4 using the UK Biobank, a large cohort study design. Within the UK Biobank I explored reproductive outcomes with respect to chronic pain, but also had the opportunity to investigate markers of inflammation which were not available in EP1/EP2.

Furthermore, whilst in this thesis chapter I have only investigated questionnaire data, I am currently collaborating with a colleague trained in analysing neuroimaging data to investigate differences in brain structure amongst the widespread pain groups I have analysed in this chapter. In participants with urological chronic pelvic pain syndrome, alterations in grey matter volume have been shown in those with a widespread pain phenotype[203], when compared to those with the localised pain phenotype, which resemble work previously published in fibromyalgia. Using available data in the EP1/EP2 datasets, this is an interesting element of widespread pain to explore, to facilitate our understanding of central mechanisms underlying widespread EAP.

In conclusion, this chapter showed preliminary evidence that women with endometriosis and CPP can be characterised into meaningful groups by extra-pelvic pain spread. Further work is required to better understand the clinical significance of these findings, but ultimately, it sets the stage for the remainder of my thesis and contributes to a growing body of evidence calling for the use of body maps to phenotype in research and clinical practice.

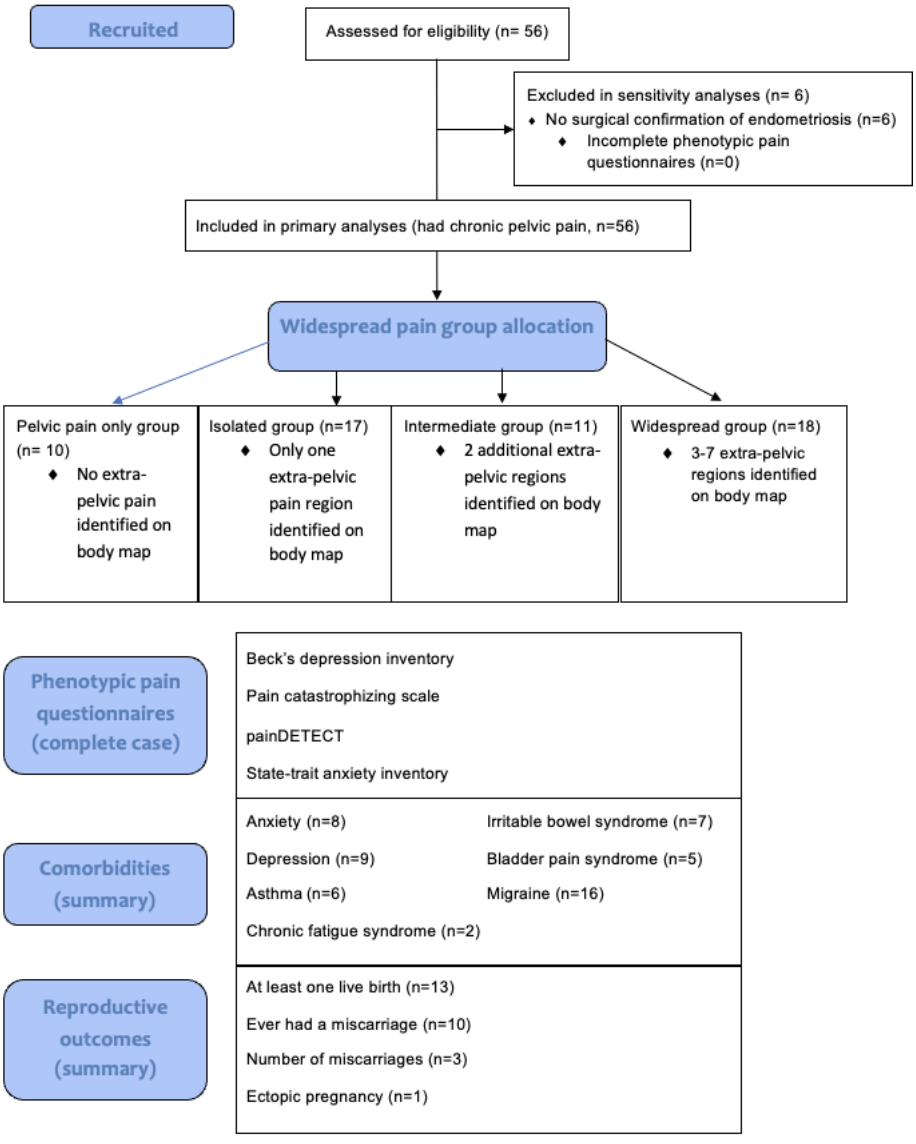
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3.6. Appendix

Appendix 3A: Consort flow diagram of analyses performed in this chapter, and the number of participants included in each analysis.



Appendix 3B: Evidence for favourable ethical opinion for EVEP study

NHS
Health Research Authority

South West - Frenchay Research Ethics Committee
Ground Floor
Temple Quay House
2 The Square
Bristol
Email: frenchay.rec@hra.nhs.uk
BS1 6PN

28 March 2022

Miss Danielle Perro
Principal Investigator, DPhil Student
University of Oxford
Level 3, Women's Centre
John Radcliffe Hospital
OX3 9DU

Dear Miss Perro

Study title:	**Investigating the role of extracellular vesicles in endometriosis-associated pain: An observational cohort study**
REC reference:	**20/SW/0032**
Protocol number:	**000000**
Amendment number:	**Substantial Amendment 1**
Amendment date:	**02 December 2021**
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The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a **favourable ethical opinion** of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Completed Amendment Tool [Completed Amendment Tool]	1.0	02 December 2021
Copies of materials calling attention of potential participants to the research [Email outline for healthy controls]	1.0	15 February 2022
Copies of materials calling attention of potential participants to the research [Leaflet advertising for additional healthy control participants]	1.0	15 February 2022
Letters of invitation to participant [Invitation Letter]	2.1	15 February 2022
Letters of invitation to participant [Tracked invitation letter]	2.1	15 February 2022
Other [Other Symptoms Questionnaire]	1.0	15 February 2022

Chapter 4 – Investigating the relationship between chronic pain and reproductive outcomes using the UK Biobank

4.1. Introduction

In my previous chapter, I summarised the frequency adverse reproductive outcomes amongst participants with increasing extra-pelvic pain. Although the sample size was too small to perform formal statistical analyses, the idea that chronic pain and poor reproductive outcomes (both of which have a significant impact on the lives of women affected) may be linked, is both intriguing and biologically plausible, and required further investigation.

My analyses using the EndoPAIN studies had many limitations, but perhaps most importantly to our understanding of this relationship was the insufficient statistical power to test my hypotheses relating to pain and reproductive outcomes. Furthermore, while I was able to evaluate the relationship between pain spread and questionnaire measures, there wasn't data available which would have allowed me to better understand the mechanisms underlying my initial findings. To explore these further, I utilised data available in the UK Biobank (UKBB).

The UKBB is a large-scale database of comprehensive biomedical data from over 500,000 individuals across the United Kingdom (UK) [289]. Recruitment took place between 2006-2010, across twenty-two centres in Scotland, England and Wales. At the time of recruitment, participants were between the age of 40 and 69 years old. Participants completed a five-part UKBB assessment process[290]. This included 1) written informed

consent, 2) a touch screen questionnaire about different lifestyle factors (i.e. diet) and health-related information (i.e. age at menopause), 3) a face-to-face interview with a study nurse, 4) anthropometric and additional physical measurements (i.e. BMI, hand grip), and 5) biofluid collection (i.e. blood, saliva). A further subset of UKBB participants (100,000) wore a 24-hour activity monitor to track and record different physiological measurements for a week, 20,000 of which have undertaken repeat measurements. Additionally, genotyping of the original cohort, and follow-up genetic data is available in the UKBB. Finally, nearly 50,000 now have undergone neuroimaging. While a follow-up experience of pain questionnaire was administered, due to the temporality of when it was administered and the relatively few participants who had completed it that were of interest to my work, I chose not to analyse this data. However, future work should consider this. A high-level overview of the UKBB data structure and study design can be found in **Figure *4.1***.

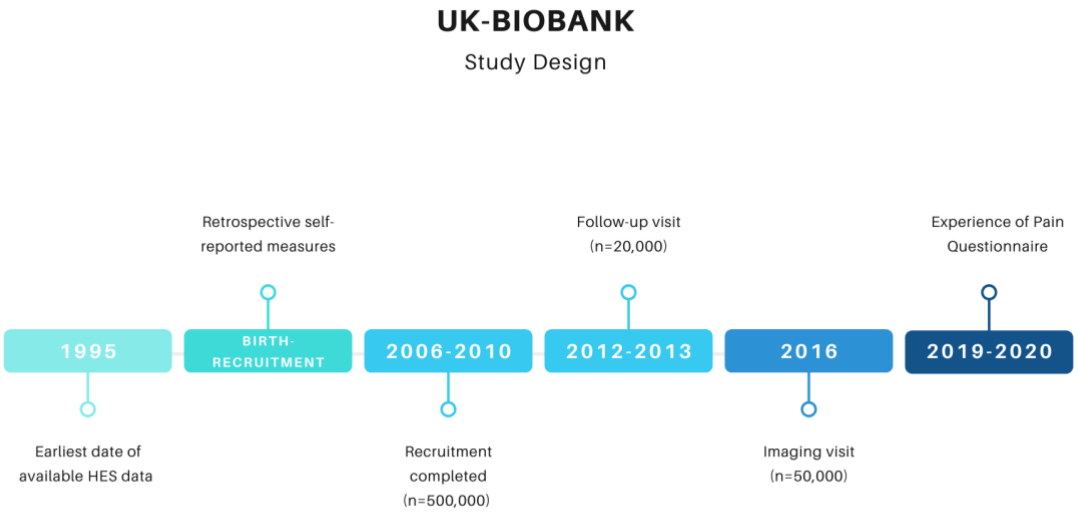


Figure 4.1. UK Biobank Study Design. HES=Hospital episode statistics.

While the UKBB is prospective in nature, a benefit of the resource is that participant ID's are linked to their hospital episode data and hospital records. For the exploration of disease prevalence and incidence, having retrospective diagnoses is an invaluable addition to the questionnaire and other physiological measurements available in the UKBB. However, something to be both mindful and cautious of when considering the presented results, is that the available hospital records only date back to 1995. Therefore, any diagnosis occurring before then would not have been captured in the hospital records, rather if it did exist, it would be found in the self-reported report of disease. This is of course prone to things like recall bias, which must be considered.

Using questionnaire, retrospective hospital episode data/health records and blood cell data available in the UKBB, I aimed to:

a) Investigate whether the odds of having poor reproductive outcomes is influenced by the presence of a chronic pain condition
b) Determine whether reproductive outcomes are influenced by the type of chronic pain diagnosis (i.e. inflammatory vs non-inflammatory in nature)
c) Assess the relationship between pain characteristics (i.e. chronic pain condition type, specific condition, and pain spread) and cellular markers of inflammation

As such, I hypothesised that:

a) The presence of a chronic pain condition would be associated with nulliparity and poor reproductive outcomes (miscarriage, and stillbirth)

b) Disparities in reproductive outcomes would be most pronounced in those with a chronic inflammatory pain condition
c) There would be an increase in cellular markers of inflammation in those with a chronic pain diagnosis when compared to controls
d) There would be an increase in cellular markers of inflammation in those with an inflammatory pain condition diagnosis, when compared to those with a non-inflammatory condition
e) Cellular markers of inflammation would be associated with inflammatory chronic pain conditions, and pain spread

4.2. Methods

4.2.1. Data structure

Since data in the UKBB has been taken at multiple time points, the data structure and coding has been labelled in such a way that allows the user to understand when the data was collected. All variables are structured as follows: X####.#.#. The characters in red indicate the field ID (i.e. what the variable is called), the green character indicates the visit the data was collected from (0=initial assessment visit, 1= first repeat assessment visit, 2= imaging visit, 3= first imaging visit), and the purple indicates the number of responses a person has to that question (i.e. for ICD10 diagnoses, a participant may have 13 diagnoses. If the 13th diagnosis is hypertensive disease, then at X41202.0.13 we would find the code I10).

Each participant in the UKBB has a unique participant ID that remains constant between study visits. This allowed me to merge different datasets (i.e. blood metrics and general questionnaire data) whilst ensuring that the responses truly belonged to each participant.

4.2.2. Study design and case ascertainment

For my UKBB work, I designed a cross-sectional study, performing tiered analyses. To address my first aim, my case group included women with a diagnosis of at least one predefined chronic pain condition, and my control group included women who were free of any of these conditions, as defined below. To address my second aim, I stratified the chronic pain group into those with a diagnosis of an inflammatory chronic pain condition versus those with a non-inflammatory chronic pain condition. Inflammatory vs non-inflammatory conditions were classified based on having a primarily inflammatory mechanism driving the pathophysiology[88,291,292], versus not.

The following chronic pain conditions used to ascertain cases in the first tier of my analysis were selected because they are highly prevalent in women: endometriosis, neuropathies (NP), complex regional pain syndrome (CRPS), temporomandibular joint disorder (TMJ), rheumatoid arthritis (RA), bladder pain syndrome/interstitial cystitis (BPS)[293], fibromyalgia (FM), migraine[291], irritable bowel syndrome (IBS) [294], lower back pain/back pain, and inflammatory bowel disease (IBD)[291].

Furthermore, the inflammatory chronic pain diagnoses which were selected for inclusion in tier two were endometriosis[88], IBD[291], and RA[292]. To be included in the non-inflammatory

pain group, participants had a diagnosis of at least one of the following conditions, and were free from endometriosis, IBD and RA: IBS, FM, osteoarthritis, migraine, BPS, and TMJ.

To ascertain chronic pain cases and controls, I utilised self-report, ICD9 and ICD10 diagnoses. Participants were classified as having a chronic pain condition if there was record in at least one of the three data sources that they had the diagnosis. The codes used to ascertain cases can be found in **Appendix 4A**.

4.2.3. Participant characteristics

In the UKBB baseline questionnaire, a comprehensive set of questions were administered to participants to characterise the cohort. In my cohort, I have summarised the following variables, as they were of importance to my subsequent analyses. *Age at baseline (X21003.0.0)* is a derived variable (performed by UKBB) which was determined by finding the difference between the date of birth and the date when the participant attended the assessment centre for their visit. This was rounded to whole year (i.e. 79 years old, rather than 79.2 years).

Ethnicity (X1001.0.0) was self-reported. Participants were asked to respond to the question 'What is your ethnic group?'. They were given twenty ethnic backgrounds to choose from, in addition to the options 'do not know' and 'prefer not to answer'. For my analyses and a summary of ethnic background, I grouped the ethnicities into the following categories: White, Asian, Black or African/Caribbean, Mixed, Other. This method of grouping is

commonly used to derive potential meaningful group differences, and to ensure that each ethnic group includes at least 100 participants.

Smoking status (X20116.0.0) and alcohol drinker status (X20117.0.0) were also self-reported variables. Participants were asked to identify whether they had never smoked, had previously smoked or were current smokers. Similarly, participants were asked to identify whether they never consumed alcohol, previously consumed alcohol, or currently consumed alcohol.

Body mass index (BMI, X21001.0.0) was determined by the UKBB using anthropometric measurements. BMI (kg/m^2) was calculated by dividing weight (kilograms, X50.0.0) by standing height (metres, squared, X21002.0.0).

Measures of socioeconomic status (SES) were largely self-reported variables. Individuals were asked to select their highest educational achievement (X6138.0.0) amongst the following: College or University degree, A levels/AS levels or equivalent, O levels/GCSE's or equivalent, CSE's or equivalent, NVQ or HND or equivalent, other professional qualifications (i.e. nursing, teaching), or none of the above. Additionally, participants were instructed to self-report (within bands) their average household income (X738.0.0), and age they completed full time education (only for those who selected a response to educational attainment other than college or university, X845.0.0).

Finally, a derived Townsend deprivation index (TDI, X189.0.0) score was assigned to each participant at recruitment based on the most recent (at the time) national census output area, and which geographical area their post code at the time of recruitment fell into.

In addition to demographic information, I summarised data pertaining to a woman's reproductive life. These self-reported variables included: 'Had menopause?' (X2724.0.0), age at first (X2754.0.0), and age at last live birth (X2764.0.0).

4.2.4. Selection and preparation of variables for analysis

Reproductive outcomes

The UKBB was not originally designed to investigate reproductive outcomes, rather diseases of aging and morbidity across the lifespan, and risk factors which predispose you to such. Because linked hospital data only goes back to 1995, there is very limited data available to understand reproductive outcomes in this respect. Therefore, for the purpose of my analyses and data completeness, I opted to use self-reported questionnaire measures of reproductive outcomes. While this question was asked at multiple time points, I chose to only use baseline questionnaire data to maximise the number of participants who were included in my analyses.

Participants were asked to identify the number of live births they've had (X2734.0.0), and this was a continuous variable. In continuity with my previous analyses in chapter 3, I converted this into a binary variable of parous (at least one child) ==1, and nulliparous (no

children) ==0. Participants were further asked if they 'Ever had a stillbirth, spontaneous miscarriage, or termination?' (X2774.0.0). If they selected yes, they were then presented with the three options to select which they had experienced. In my analyses, I chose to focus on stillbirth and miscarriage, as these were continuous with my previous work, and would more directly indicate a biological mechanism. The indication for termination was unknown, and for this reason the variable was excluded from my analyses. When determining the proportion of women in each group who had a miscarriage or stillbirth, the numerator was the number of women with at least one outcome and the denominator was the number of women who responded to the individual question about miscarriage or stillbirth, from the parent question 'Ever had stillbirth, spontaneous miscarriage, or termination?'.

Pain spread and comorbidities

In the baseline questionnaire, participants were asked about whether they had ever experienced chronic pain in different parts of their body. While for many the chronic pain may have been caused by one of the chronic pain conditions outlined above, this relationship could not be determined with the data available. Participants were asked to identify whether they experienced general pain (X2956.0.0), pain in the neck/shoulder pain (X3404.0.0), hip (X3414.0.0), back (X3571.0.0), knee (X373.0.0), stomach/abdominal region (X3741.0.0), facial regions (X4067.0.0). If they selected general pain, they were unable to select any of the other chronic pain regions. General pain in the UK Biobank has been used previously as a proxy measure for chronic widespread pain. Since participants may have

experienced chronic pain in many body regions, I calculated a total number of areas impacted by summing the total number of regions they had identified.

Many participants in the UKBB living with one of the chronic pain conditions listed above were impacted by more than one chronic pain condition. To determine the number of comorbid conditions each person had; I assigned a binary 1/0 (yes/no) to each chronic pain diagnosis. I then summed the total number of chronic pain conditions per person.

Blood Measures

At baseline, whole blood was collected from UKBB participants in 4mL ethylenediaminetetraacetic acid (EDTA) vacutainers. All samples were analysed using a Beckman Coulter LH750 Haematology Analyser, which performs automated reticulocyte analyses, and enumeration of both red blood cells (RBC) and white blood cells (WBC). Samples were typically processed and analysed within 24 hours of data collection. The parameters measured directly by the Coulter technology included the following: RBC, WBC and platelet count, lymphocyte % monocyte %, neutrophil %, eosinophil %, and basophil %. Calculated variables included the number of lymphocytes, monocytes, neutrophils, eosinophils and basophils, and plateletcrit (volume of blood occupied by platelets, %). The differential absolute quantities of the leukocyte subpopulations were calculated as followed:

$$\frac{\%\ \text{proportion of specific leukocyte}}{100}\ x\ (WBC\ count)$$

Finally, platelet distribution width was derived by the UKBB. I further derived a neutrophil: lymphocyte ratio (NLR), as this has been implicated in systemic inflammation, and calculated it as follows:

$$NLR = \frac{\text{neutrophil count}}{\text{lymphocyte count}}$$

Prior to analysis, I then performed a transformation[295] of the NLR according to Nøst et al., which was as follows:

$$Transformed\ NLR = \frac{\log(\text{NLR}) - \text{mean}(\log\ (\text{NLR}))}{\text{sd}(\log(\text{NLR}))}$$

The blood sample collection and processing protocol has been published and can be found here[296]. The typical ranges[297–301] for blood counts and the units used in the UKBB can be found in **Table *4.1***. Unfortunately, traditional measures of systemic inflammation, such as C-reactive protein[302], were not available in the UK Biobank at baseline. As such, I performed more of an exploratory analysis looking at blood cell counts, but specifically, neutrophils and lymphocytes, as well as the derived neutrophil:lymphocyte ratio, between groups to determine if there were any differences which could signal a role of inflammation.

Table 4.1. Typical/healthy ranges of blood metrics, and units used in the UK Biobank. **Continued on the next page.**

Blood Metric	Typical/ healthy ranges	Units in the UK-Biobank
Neutrophil (Count)	1.8 – 7.5	$x10^9$ cells/L
Lymphocyte (Count)	1.0 – 4.0	$x10^9$ cells/L

4.3. Data analysis

4.3.1. Participant characteristics

All participant demographic and reproductive characteristics were summarised and compared between study groups using a Mann Whitney test, as the data were not normally distributed. Data were tested against the normality assumption using the Kolmogorov-Smirnov test. Medians and interquartile range (IQR) were reported.

4.3.2. Reproductive outcome analyses

In the first instance, I performed a one-tailed chi-square test to test whether the proportion of nulliparous women in the pain group was greater than the pain-free control group. Furthermore, I performed a two-tailed chi-square test to assess whether the proportion of nulliparous women differed between those with an inflammatory chronic pain diagnosis when compared to those with a non-inflammatory chronic pain diagnosis, using a Bonferroni post-hoc test.

I then performed multiple logistic regression models for my tier I and tier II analyses to further explore the relationship between chronic pain and parity. In these logistic regression models, I defined chronic pain status as my exposure and nulliparity as my outcome. In the first set of models, I simply ran an unadjusted model, exploring the relationship between exposure and outcome. In the second set, I adjusted for age at baseline. In my final set of models, I adjusted for covariates which were selected a priori, as they are known to influence the outcome of interest, as outlined in **Table *4.2***. Similarly,

I performed the above analyses for stillbirth and miscarriage whereby pain status was the exposure, and stillbirth or miscarriage were the outcome.

Table 4.2. Risk factors for adverse reproductive outcomes available in the UKBB.

Risk Factors for adverse reproductive outcomes with Information available in UKBB	**Relevant variables in UKBB with cut-offs for categorizations**
Ethnicity	White, Mixed ethnic background, asian/asian british, black/african/caribbean/black british, other ethnic group
Body Mass Index	<18.5, 18.5-24.9, 25.0-29.9, >30.0
Age	Continuous
Smoking Status	Current, past or never smoker
Alcohol consumption	Current, past or never consumer
Uterine Fibroids	Yes/no
Pelvic Inflammatory Disease	Yes/no
Systemic lupus erythematosus (SLE)	Yes/no
Congenital uterine abnormalities (CUA)	Yes/no
Polycystic ovarian syndrome (PCOS)	Yes/no

4.3.3. Blood metric analyses

Blood metrics were tested for normality, however they all failed to meet the normality assumption. To explore this data further, I performed a sensitivity analysis by excluding values >2SD or <2-SD and re-testing the normality assumption, to determine whether outliers were skewing the distribution. After re-testing, the data were still not normally distributed, so I therefore decided to transform the blood metrics (counts and percentages)

accordingly. Blood count metrics were square root transformed, and the blood percentages were subject to an arc sine transformation[303].

To analyse this data, I firstly compared the median (IQR) of each blood count and percentage between the Tier 1 groups (chronic pain group vs controls) and the Tier 2 groups (inflammatory pain diagnosis vs non-inflammatory pain diagnosis) using a Mann-Whitney test. I then performed a stepwise multivariable logistic regression model to compare the counts, and linear regression model to compare percentages between tier 1 and tier 2 groups separately.

In the first model, I performed an unadjusted logistic regression model, where chronic pain (or inflammatory pain diagnosis in tier 2) was the exposure, and the blood count was the outcome of interest. In the second model, I adjusted for age at baseline. In my final multivariable logistic regression model, I adjusted for age, BMI, asthma (yes/no), smoking and alcohol consumption. Asthma was an important variable to consider in this model because pathophysiologically, the cell populations I've investigated in this chapter are implicated in the disease[304]. Furthermore, a diagnosis of asthma is known to be comorbid amongst participants with some of the chronic conditions under investigation[305–307]. Therefore, asthma could very well have confounded the relationship between a chronic pain diagnosis and the selected blood metrics. I controlled for the same covariates in my linear regression models.

A consort flow diagram illustrating the outcome measures analyses and how many participants were included in them can be found in **Appendix 4D**.

4.3.4. Statistical software and packages

All data was accessed using the Wellcome Centre for Human Genetics Server. Scripts were generated and saved using R Studio, however analyses were performed on my computer terminal using R software version 4.1.2[308].

The following R packages were used for data extraction and manipulation: dplyr[309], plyr[310], tidyr[311] and data.table[312]. The following packages were additionally used for descriptive analyses and regression models: oddsratio[313], psych [314], rcompanion[315].

Tables were generated on Microsoft word, and all figures were generated using GraphPad PRISM version 9.3.1.

4.4. Results

4.4.1. Participant characteristics

At the time of my original data download, N=273,404 women had available baseline data in the UKBB. Of these, n=44, 695 were diagnosed with at least one chronic pain condition, and n=228,709 were free from one of the chronic pain conditions I'd originally selected for inclusion into the tier 1 pain group. Furthermore, n=16,045 participants had a diagnosis of at least one inflammatory chronic pain condition (endometriosis, RA, and/or IBD), and n=14,071 had a diagnosis of at least one non-inflammatory chronic pain condition.

As shown in **Table *4.3***, the chronic pain group was significantly older than the pain-free controls ($p<0.0001$), a greater proportion were previous or current smokers ($p<0.0001$) and fewer currently consumed alcohol ($p<0.0001$). The chronic pain group had a higher BMI ($p<0.0001$), a lower educational achievement, and a higher TDI than controls ($p<0.0001$).

In the second tier, there was a significant difference in the age at baseline between the inflammatory and non-inflammatory pain groups ($p<0.0001$), **Error! Reference source not found.**. A greater proportion of the non-inflammatory pain group were white ($p<0.0001$), and they had a lower BMI than the inflammatory pain group. There was no significant difference in the smoking status, alcohol drinker status or other measures of SES between the two pain groups after using the Bonferroni correction for multiple comparisons.

With respect to reproductive-related measures between the tier 1 groups, more of the control group was post-menopausal, and had their first and last live births older than the pain group ($p<0.0001$, **Table 4.4**). In the tier 2 analyses, a greater proportion of the non-inflammatory pain group had undergone menopause ($p<0.0001$), however there was no significant difference between the groups with respect to age at first or last live birth (**Table *4.6***).

Table 4.3. Descriptive statistics for tier 1 groups; chronic pain group and control group. A non-parametric Mann-Whitney test was used to compare values between groups. IQR; interquartile range, AS level; advanced subsidiary level, GCSE; general certificate of secondary education, CSE; certificate of secondary education, NVQ; national vocational qualification, HND; higher national diploma. **Continued on next page**.

	Pain (N=44, 695)	**Pain-free Control (N=228, 709)**	**p-value**
Age at assessment (Median, IQR)	58 (13)	57 (13)	6.263×10^{-15}
Ethnicity (n,%)			
White	42007 (94.4)	215449 (94.2)	0.070
Asian	929 (2.1)	4649 (2.0)	0.460
Black African/Caribbean	781 (1.8)	3873 (1.7)	0.365
Mixed	320 (0.72)	1,533 (0.67)	0.263
Other	442 (0.99)	2,155 (0.94)	0.321
Smoking Status (%)			
Never	24,762 (55.4)	137,304 (60.03)	<0.0001
Previous	14,824 (33.17)	70,634 (30.77)	<0.0001
Current	4,808 (10.76)	19,559 (8.55)	<0.0001
Alcohol Drinker Status (%)			
Never	3,263 (7.35)	12,718 (5.56)	<0.0001
Previous	2,552 (5.74)	7,431 (3.27)	<0.0001
Current	38,718 (87.21)	207,892 (91.38)	<0.0001
Body Mass Index (Median, IQR)			
Body Measurements	27.1 (6.99)	26 (6.12)	$<2.2 \times 10^{-16}$
Measures of SES Qualifications (Education, n (%))			
A levels/AS levels or equivalent	6,723 (10.4)	27,167	
O levels/GCSEs or equivalent	14,946 (23.2)	52,770	
CSEs or equivalent	3,652 (5.7)	11,815	
NVQ or HND or equivalent	3,423 (5.3)	9,752	

	Pain (N=44, 695)	**Pain-free Control (N=228, 709)**	**p-value**
Other professional qualifications (i.e. nursing, teaching)	4,120 (6.4)	12,946	
None of the above	14,421 (22.4)	36,564	
Prefer not to say	816 (1.3)	2,292	
NA's	623 (0.97)	2,078	
Proportion Obtained a Post-Secondary Degree (College or University Degree)	0.245	0.325	<0.0001
Average Total Household Income, before tax (%)			<0.0001
[1] Less than 18,000	16,616 (26.1)	44,316	
[2] 18,000 – 30,999	14,531 (22.8)	49,000	
[3] 31,000 – 51,999	11,687 (18.4)	48,304	
[4] 52,000 – 100,000	6,872 (10.8)	36,449	
[5] Greater than 100,000	1,374 (2.2)	9,651	
[6] Do not know	3,454 (5.4)	12,636	
Prefer not to answer	8,088 (12.7)	25,811	
NA's	890	2,542	
Average Total Household Income (μ, SD)	2 (2)	2 (2)	$<2.2x10^{-16}$
Age Completed Full Time Education (Median, IQR)	16 (2)	16 (2)	$<2.2x10^{-16}$
NA's (n,%)	16,499 (25.6)	75,550	
Townsend Deprivation Index at recruitment (μ, SD) (Lower score means more deprived)	-1.87 (4.46)	-2.19 (4.05)	$<2.2x10^{-16}$

Table 4.4. Additional tier 1 demographic information relevant to reproductive outcomes. A non-parametric Mann-Whitney test was used to compare values between groups. IQR; interquartile range.

Pain Condition (n, %)	**Pain (N=44,695)**	**Control (N=228,709)**	**p-value**
Neuropathies	11, 575 (25.90)	0	
Complex Regional Pain Syndrome	152 (0.34)	0	
TMJ	238 (0.53)	0	
Endometriosis	8,220 (18.39)	0	
Bladder Pain Syndrome	100 (0.22)	0	
Fibromyalgia	1488 (3.33)	0	
IBS	12,084 (27.04)	0	
RA	5,716 (12.79)	0	
IBD	2,470 (5.53)	0	
Low Back Pain	7,382 (16.52)	0	
Migraine	4,132 (9.24)	0	
OA	3,964 (8.87)	0	
Conditions known to impact reproductive outcomes			
Uterine Fibroids	5,330 (11.93)	14,457 (6.32)	$<2.2x10^{-16}$
PID	1,724 (3.86)	2,129 (0.93)	$<2.2x10^{-16}$
PCOS	252 (0.56)	563 (0.25)	$<2.2x10^{-16}$
Systemic Lupus Erythematosus	296 (0.66)	409 (0.18)	$<2.2x10^{-16}$
Congenital Uterine Abnormalities	56 (0.13)	177 (0.08)	0.002
Had menopause?			
Yes	25,432 (56.90)	139,979 (61.20)	$<2.2x10^{-16}$
Unsure-hysterectomy	8,789 (19.66)	22,382 (9.79)	
Unsure-Other	1,950 (4.36)	9,782 (4.28)	
Prefer not to say	83 (0.19)	452 (0.20)	
NA's	89 (0.20)	386 (0.17)	
Age of first live birth (median, IQR)	24 (6)	25 (6)	$<2.2x10^{-16}$
NA's	14831	73585	
Age of last live birth (median, IQR)	29 (7)	30 (7)	$<2.2x10^{-16}$
NA's	14831	73585	

Table 4.5. Descriptive statistics for tier 2 groups; inflammatory pain group and non-inflammatory pain group. A non-parametric Mann-Whitney test was used to compare values between groups. IQR; interquartile range, AS level; advanced subsidiary level, GCSE; general certificate of secondary education, CSE; certificate of secondary education, NVQ; national vocational qualification, HND; higher national diploma. **Continued on next page**.

	Inflammatory Pain (N=16,045)	**Non-Inflammatory Pain (N=14,071)**	**p-value**
Age at assessment (Median, IQR)	57 (13)	57 (13)	8.167×10^{-8}
Ethnicity (n, %)			
White	15,069 (94.0)	13,479 (96.2)	2.568×10^{-13}
Asian	360 (2.3)	178 (1.3)	1.991×10^{-10}
Black African/Caribbean	276 (1.7)	171 (1.2)	0.00035
Mixed	129 (0.8)	83 (0.6)	0.030
Other	137 (0.9)	105 (0.75)	0.325
Smoking Status (n, %)			
Never	9,008 (56.53)	7,889 (56.36)	0.8939
Previous	5,273 (33.1)	4,639 (33.14)	0.847
Current	1,654 (10.38)	1,469 (10.50)	0.7444
Alcohol Drinker Status (n, %)			
Never	1120 (7.03)	927 (6.62)	0.1653
Previous	894 (5.6)	861 (6.15)	0.0468
Current	13977 (87.71)	12246 (87.5)	0.5601
Body Mass Index (Median, IQR)			
Body Measurements (1,513 NA's total)	26.8 (6.81)	26.6 (6.80)	1.313×10^{-5}
Measures of SES			
Qualifications (Education), (n, %)			
College of University degree	4,206 (28.38)	3,716 (26.94)	
A levels/AS levels or equivalent	1853 (12.5)	1,556 (11.28)	
O levels/GCSEs or equivalent	3,783 (25.5)	3,248 (23.55)	
CSEs or equivalent	100 (0.67)	898 (6.5)	

	Inflammatory Pain (N=16,045)	Non-Inflammatory Pain (N=14,071)	p-value
NVQ or HND or equivalent	822 (5.5)	762 (5.5)	
Other professional qualifications (i.e. nursing, teaching)	911 (6.1)	798 (5.8)	
None of the above	3147 (21.2)	2,814 (20.4)	
Proportion Obtained a Post-Secondary Degree (College or University Degree)	0.2838	0.2694	
Average Total Household Income, before tax (n,%)			
[1] Less than 18,000	3,788	3,442	
[2] 18,000 – 30,999	3,494	3,127	
[3] 31,000 – 51,999	3,128	2,761	
[4] 52,000 – 100,000	2,118	1,614	
[5] Greater than 100,000	442	331	
[6] Do not know	1,004	939	
Prefer not to answer	1,885	1,663	
NA's	186	194	
Average Total Household Income (Median, IQR)	2 (2)	2 (2)	0.000225
Age Completed Full Time Education (Median, IQR)	16 (2)	16 (2)	0.001678
NA's (%)	4,384	3,868	
Townsend Deprivation Index at recruitment (Median, IQR) (Lower score means more deprived)	-1.88 (4.37)	-1.96 (4.51)	0.310

Table 4.6. Additional tier 2 demographic information relevant to reproductive outcomes. A non-parametric Mann-Whitney test was used to compare values between groups. IQR; interquartile range.

Pain Condition (n, %)	**Inflammatory (N=16,045)**	**Non-inflammatory (N=14,071)**	**p-value**
Neuropathies	1,122 (6.99)	1,041 (7.4)	
Complex Regional Pain Syndrome	0	1 (0.007)	
TMJ	43 (0.27)	195 (1.39)	
Endometriosis	8,220 (51.23)	0	
Bladder Pain Syndrome	21 (0.13)	80 (0.57)	
Fibromyalgia	284 (1.77)	1,204 (8.56)	
IBS	1,233 (7.68)	10,851 (77.12)	
RA	5,716 (35.62)	0	
IBD	2,470 (15.39)	0	
Low Back Pain	785 (4.89)	875 (6.22)	
Migraine	1,781 (11.10)	2,351 (16.71)	
OA	0	3,964 (28.17)	
Conditions known to impact reproductive outcomes			
Uterine Fibroids	3,008 (18.75)	1175 (8.35)	$<2.2x10^{-16}$
PID	1,201 (7.49)	287 (2.04)	$<2.2x10^{-16}$
PCOS	152 (0.95)	64 (0.45)	$6.201x10^{-7}$
Systemic Lupus Erythematosus	153 (0.95)	78 (0.55)	$9.782x10^{-5}$
Congenital Uterine Abnormalities	26 (0.16)	19 (0.14)	0.6484
Had menopause?			
Yes	8162 (50.87)	8,119 (57.7)	$<2.2x10^{-16}$
Unsure-hysterectomy	4085 (25.46)	2,348 (16.69)	
Unsure-Other	681 (4.24)	644 (4.58)	
Prefer not to say	28 (0.17)	24 (0.17)	
NA's	29 (0.18)	21 (0.15)	
Age of first live birth (Median, IQR)	24 (6)	24 (6)	0.814
NA's	6163	4404	
Age of last live birth (Median, IQR)	29 (7)	29 (6)	0.122
NA's	6163	4404	

4.4.2. Reproductive outcomes

Nulliparity

There was no significant difference in the proportion of nulliparous women between the tier 1 groups (controls=18.78% vs pain=18.62%, p=0.206, **Figure *4.2***), however the proportion was significantly greater in my stratified analysis, when comparing those with an inflammatory chronic pain diagnosis to those with a non-inflammatory chronic pain diagnosis (inflammatory=22.55% vs non-inflammatory=16.94%, X^2*:* 176.3, p<0.0001, **Figure *4.3***).

Further analyses using a fully adjusted logistic regression model showed that the odds (OR(95%CI)) of being nulliparous was 1.41 (1.32, 1.51) greater in those with an inflammatory chronic pain condition than those with a non-inflammatory chronic pain condition. None of the models for the tier one analyses were significant.

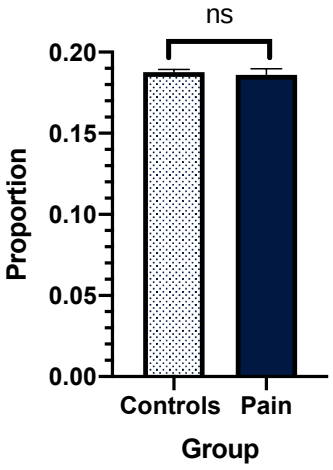


Figure 4.2. Tier 1 proportion of nulliparous women by pain group. X^2=0.671, p=0.206, ns=not significant.

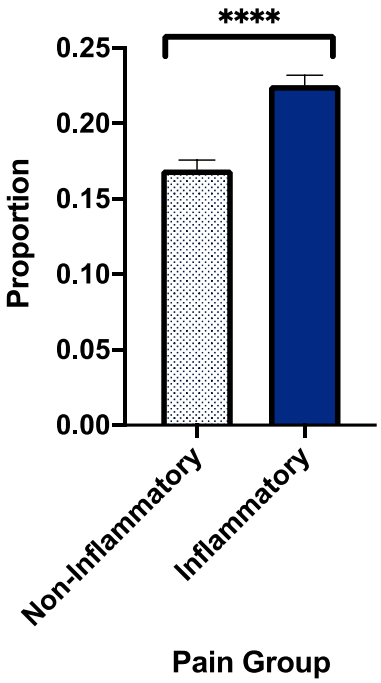


Figure 4.3. Proportion of nulliparous women by pain group, in Tier 2 analysis. X^2=176.3, ****=p<0.0001.

Miscarriage

Controls (n=72,234) and chronic pain (n=15,011) participants answered the question of whether they had ever had a spontaneous miscarriage, stillbirth, or termination. Participants with a non-inflammatory chronic pain diagnosis (n=4,846) and participants with an inflammatory chronic pain diagnosis (n=5,159) responded to the question about miscarriage.

There was a significant difference in the proportion reporting at least one miscarriage (controls=36.36% vs pain=33.52%, X^2=43.46, p<0.0001, **Figure *4.4***) when comparing

controls and participants with a chronic pain condition. Furthermore, after stratifying, the proportion of women with at least one miscarriage was significantly greater in the inflammatory pain group when compared to the non-inflammatory group (inflammatory=68.39% vs. non-inflammatory=67.15%, X^2=58.08, p<0.0001, **Figure *4.5***).

Further analyses using a fully adjusted logistic regression model showed that the odds (OR (95%CI)) of having at least one miscarriage in participants with a chronic pain diagnosis was 1.15 (1.10, 1.2) that of controls. However, after controlling for pre-defined variables, the odds of having at least one miscarriage were not significantly different between those with an inflammatory and non-inflammatory chronic pain diagnosis.

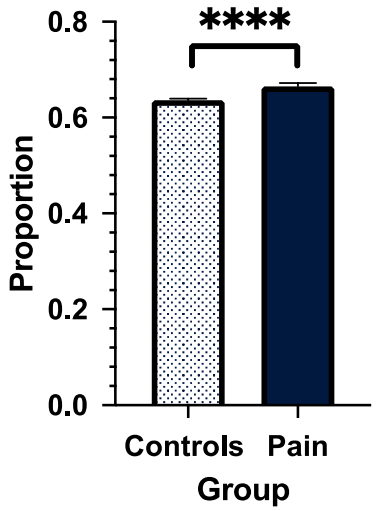


Figure 4.4. Tier 1 proportion of women reporting at least one miscarriage by pain group.

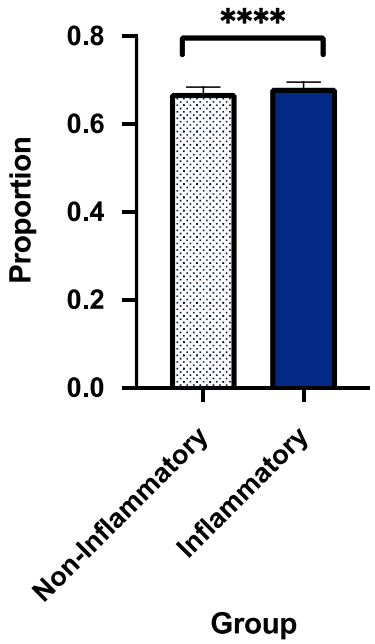


Figure 4.5. Proportion of women reporting at least one miscarriage by pain group, in Tier 2 analysis. X^2=58.08, ****=p<0.0001

Stillbirth

Controls (n=72,234) and chronic pain participants (n=15,011) answered the question of whether they had ever had a spontaneous miscarriage, stillbirth, or termination. Participants with a non-inflammatory chronic pain diagnosis (n=4,861) and participants with an inflammatory chronic pain diagnosis (n=5,175) responded to the question on stillbirth.

There was a significant difference in the proportion reporting at least one stillbirth (controls=7.92% vs pain=8.82%, X^2=13.61, p=0.0001, **Figure *4.6***) between controls and

participants with a chronic pain condition. However, after stratifying, there was no significant difference in the proportion of women with at least one stillbirth between chronic pain subgroups (inflammatory=8.46% vs. non-inflammatory=8.6%, X^2=0.059, p=0.808, **Figure *4.7***).

After performing logistic regression models with complete adjustments for covariates, the odds of having at least one still birth was not significantly different in tier 1, comparing those with a chronic pain diagnosis to controls (1.02 (0.95,1.10)) or in tier 2, comparing those with an inflammatory chronic pain condition to those with a non-inflammatory chronic pain condition (0.94 (0.90, 1.11)).

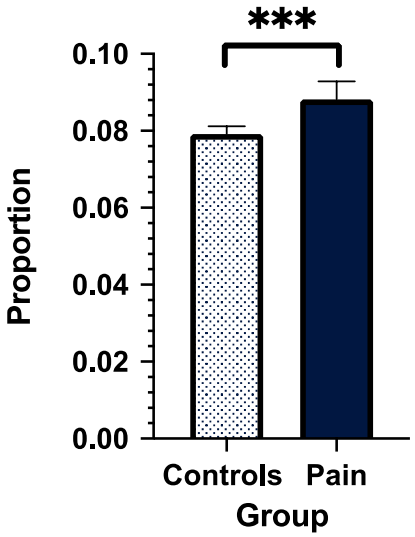


Figure 4.6. Tier 1 proportion of women reporting at least one stillbirth by pain group. X^2=13.61, p=0.0001, ***=p<0.001.

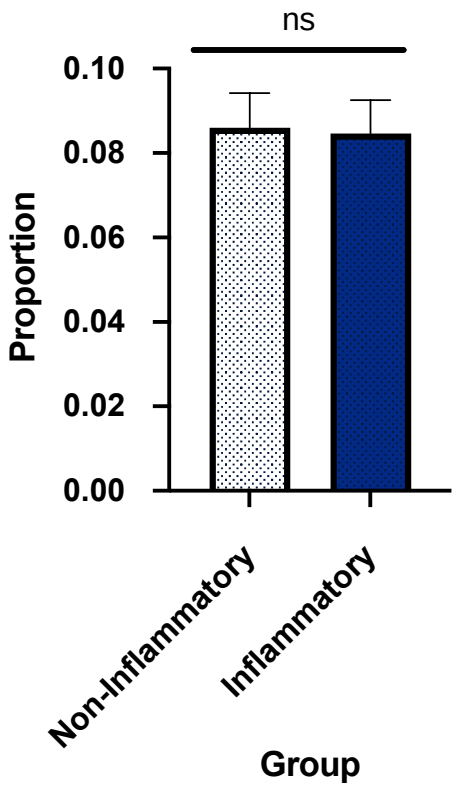


Figure 4.7. Proportion of women reporting at least one stillbirth by pain group, in Tier 2 analysis. X^2=0.059, p=0.808, ns=not significant.

4.4.3. Blood cell counts by pain group

The results presented below have been transformed using the appropriate statistical transformation. However, the clinical utility of a transformed blood metric is scant: drawing clinically meaningful conclusions from this data is challenging. Therefore, while throughout this chapter I have performed my primary analyses on transformed data, I have presented some untransformed results in **Appendices 4B/C**.

While most participants who were recruited at baseline had their blood taken, not all participants had data available for each of the blood metrics. As such, in the tables presented below I have reported the number of participants included in each analysis by study group.

Blood cell counts

In tier 1, there was a significant difference in all blood count metrics; all were increased in the pain group when compared to controls, as shown in **Table *4.7***. After stratification, and accounting for multiple comparisons, there was only a significant difference in the neutrophil count ($p=5.384x10^{-9}$) between the tier 2 groups, **Table *4.8***.

As shown in **Table *4.9***, after performing a fully adjusted logistic regression model, platelet, total WBC, and neutrophil count were increased in chronic pain participants when compared to controls, whereas eosinophils were decreased. Interestingly, in the tier 2 analyses, only neutrophils were significantly increased, and lymphocytes were significantly decreased in those with an inflammatory chronic pain condition when compared to those with a non-inflammatory chronic pain condition, **Table *4.10***

Table 4.7. Tier 1 descriptive statistics and analysed square root transformed blood count data. Threshold for significance after accounting for multiple comparisons was 0.025 (0.05/2).

Measurement	Control (Median, IQR)	Control (N)	Pain (Median, IQR)	Pain (N)	P-value
Neutrophil Count	2.0 (0.422)	216248	2.02 (0.442)	42196	$<2.2x10^{-16}$
Lymphocyte Count	1.38 (0.269)	216248	1.40 (0.288)	42196	$3.967x10^{-15}$

Table 4.8. Tier 2 descriptive statistics and analyses square root transformed blood count data. Threshold for significance after accounting for multiple comparisons was 0.025 (0.05/2). Ns; not significant.

Measurement	Inflammatory (Median, IQR)	Inflammatory (N)	Non-inflammatory (Median, IQR)	Non-inflammatory (N)	P-value
Neutrophil	2.04 (0.443)	15172	2.02 (0.419)	13302	$5.384x10^{-9}$
Lymphocyte	1.38 (0.301)	15172	1.40 (0.284)	13302	ns

Table 4.9. Odds ratio (95% CI) of tier 1 transformed blood count data (logistic regression), age adjusted and adjusted for age, BMI, asthma, smoking and alcohol consumption.

BLOOD COUNT	OR (95%CI)	p-value
Neutrophil	1.395 (1.352-1.439)	$<2.2x10^{-16}$
Lymphocyte	1.169 (1.12-1.22)	$1.15x10^{-12}$
Neutrophil	1.177 (1.139-1.216)	$<2.2x10^{-16}$
Lymphocyte	0.968 (0.924-1.015)	0.186

Table 4.10. Odds ratio (95% CI) of tier 2 transformed blood count data (logistic regression), age adjusted and adjusted for age, BMI, asthma, smoking and alcohol consumption.

BLOOD COUNT	OR (95% CI)	p-value
Neutrophil	1.25 (1.17-1.34)	$1.42x10^{-10}$
Lymphocyte	0.66 (0.60-0.73)	$3.92x10^{-16}$
Neutrophil	1.26 (1.17-1.35)	$1.78x10^{-10}$
Lymphocyte	0.62 (0.56-0.69)	<2.0x10-16

4.4.4. Comorbidities and pain spread

As shown in **Figure 4.8**, it was most common for participants in each of the pain groups to have only one chronic pain condition. However, having more than one chronic pain condition was most common in the inflammatory chronic pain group. Furthermore, it is important to consider the inclusion and exclusion criteria for my pain groups, as this is likely responsible for the trends seen in multimorbidity. As such, I did not statistically compare these proportions. In **Figure *4.9***, I've shown that generally chronic pain is highly prevalent in the UKBB, but most prevalent in participants with a diagnosis of a chronic pain condition ($p<2.2x10^{-16}$). After stratifying, it was clear that abdominal pain ($p<2.2x10^{-16}$), neck pain ($p=5.9x10^{-12}$), and back pain ($p=4.3x10^{-13}$) were highly prevalent in the non-inflammatory pain group.

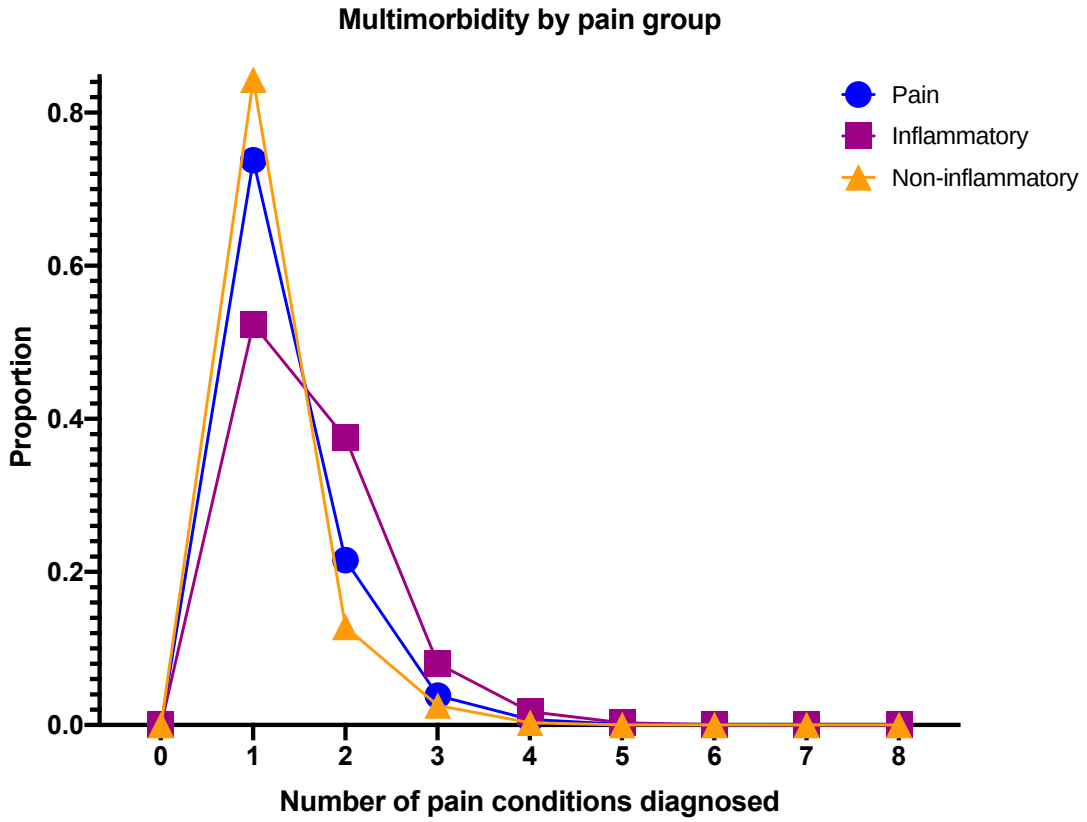


Figure 4.8. Multimorbidity of chronic pain conditions in each of the chronic pain groups.

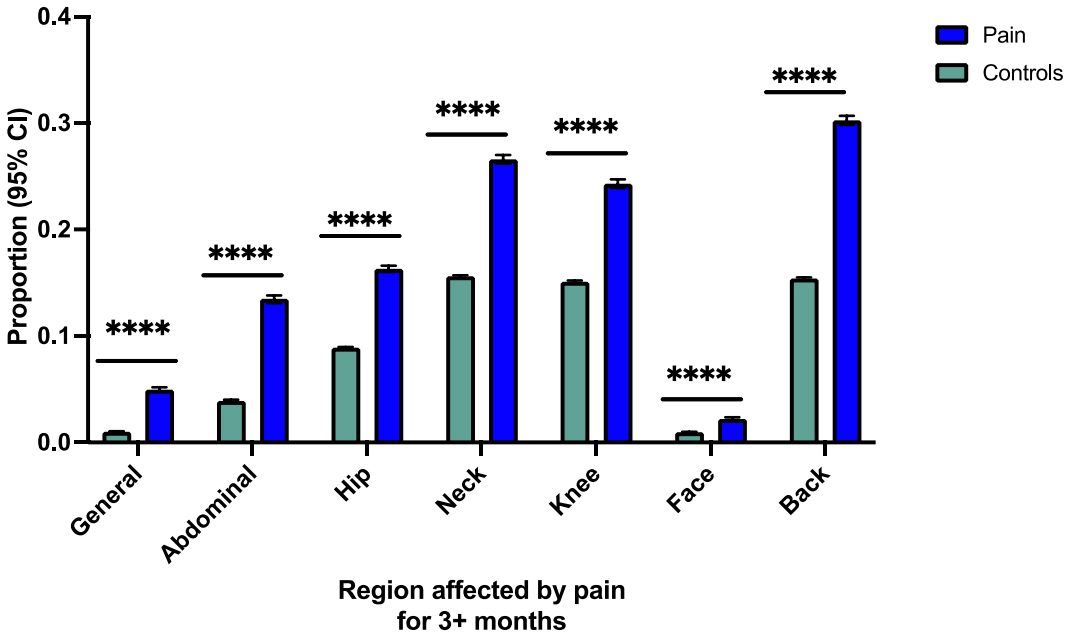


Figure 4.9. Body regions impacted by chronic pain (3+ months) by tier 1 study group. 95% confidence intervals are indicated on the bar. Levels of significance indicated by stars above the bars; ****p<0.0001.

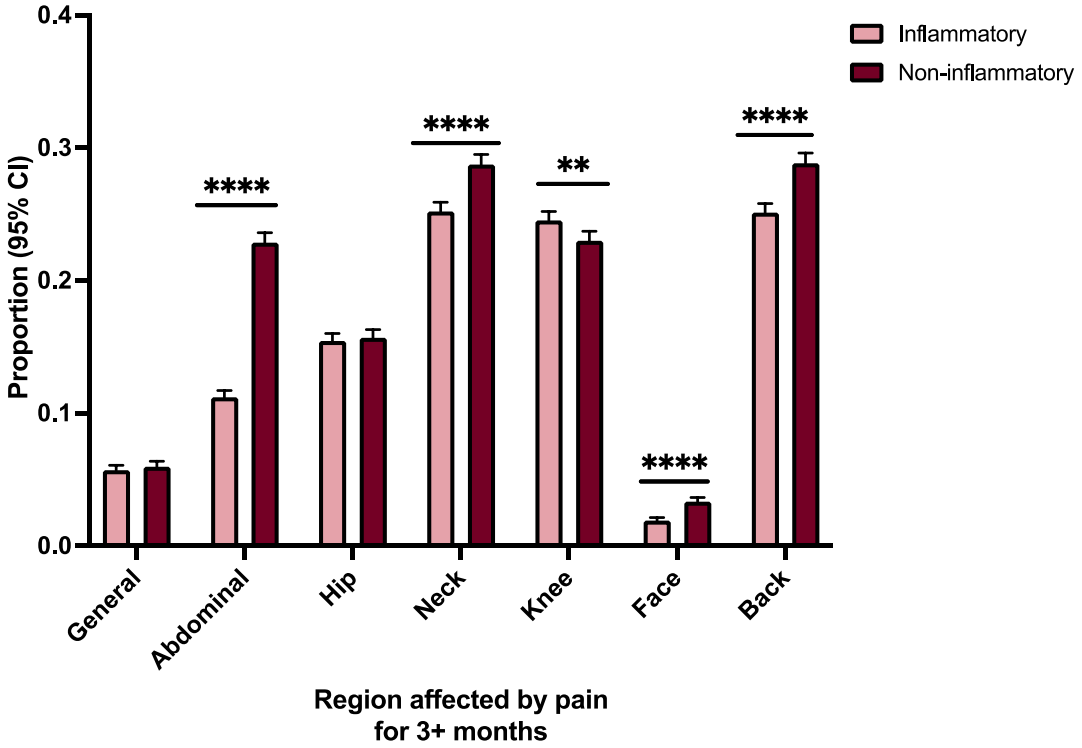


Figure 4.10. Body regions impacted by chronic pain (3+ months) by tier 2 study group. 95% confidence intervals are indicated on the bar. Levels of significance indicated by stars above the bars; ****p<0.0001, ***p<0.001, **p<0.01.

4.4.5. Sensitivity analyses & further exploratory analyses

In my tier 2 analyses, investigating both reproductive outcomes and blood measures, I observed significant differences between those with an inflammatory versus non-inflammatory chronic pain condition. However, these results warranted further investigation. In the first instance, I was keen to understand if the associations that I had observed were in fact influenced by a comorbid diagnosis of an autoimmune condition, which pathologically are often associated with underlying inflammation. In my first set of sensitivity analyses, I excluded participants in the non-inflammatory pain group who had a diagnosis of at least one of the following autoimmune conditions: systemic lupus erythematosus, Sjögren's syndrome, Coeliac disease, Multiple sclerosis or Addison's disease. I then re-ran my reproductive outcome and blood cell models, in my tier 2 groups. The prevalence of each autoimmune condition in the tier 2 pain groups can be found in **Table *4.11***.

Table 4.11. Prevalence of autoimmune conditions in tier 2 study groups.

Autoimmune condition	**Inflammatory pain (n=16,045)**	**Non-inflammatory pain (n=14,071)**
Systemic lupus erythematosus	153 (0.95%)	78 (0.55%)
Sjögren's syndrome	223 (1.39%)	94 (0.67%)
Coeliac disease	154 (0.96%)	126 (0.90%)
Multiple sclerosis	101 (0.63%)	102 (0.72%)
Addison's disease	14 (0.087%)	12 (0.085%)

After removing the autoimmune cases, the stillbirth and miscarriage model remained not significant between groups. The nulliparity model remained significant, and neither the effect size nor confidence interval exhibited considerable change after making this

exclusion (**Figure *4.11***). Therefore, considering that the prevalence of autoimmune conditions was low in these groups, it is unlikely in my data that they significantly impacted the relationships I've observed.

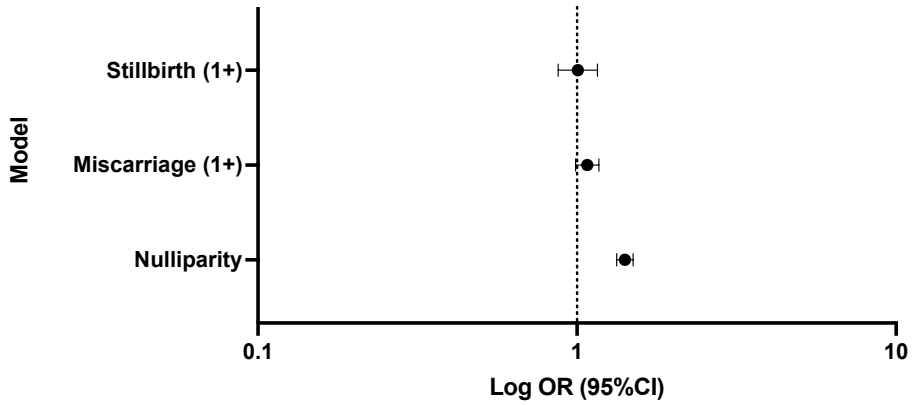


Figure 4.11. Age-adjusted reproductive outcome models after excluding participants with an autoimmune condition from the non-inflammatory pain group. OR; odds ratio, CI; confidence interval.

Furthermore, I was interested in stratifying the tier 2 model into the individual inflammatory chronic pain conditions to determine if one (RA, IBD, or endometriosis) was driving the associations previously observed. When comparing those with endometriosis to participants with a non-inflammatory chronic pain condition, the odds of being nulliparous and having at least one miscarriage were significantly increased. In the fully adjusted models (**Table *4.12***), the odds of being nulliparous were also significantly increased when comparing the IBD group vs non-inflammatory pain group, and the RA group vs non-inflammatory pain group.

Table 4.12. Inflammatory condition stratified tier 2 models ((A) unadjusted and age-adjusted and (B) fully adjusted) investigating reproductive outcomes. RA; rheumatoid arthritis, IBD; inflammatory bowel disease. Fully-adjusted models were adjusted for covariates outlined in **Table 2**.

(A)

	Endometriosis		Rheumatoid Arthritis		Inflammatory Bowel Disease	
	Unadjusted	**Age-adjusted**	**Unadjusted**	**Age-adjusted**	**Unadjusted**	**Age-adjusted**
Nulliparity	1.89 (1.77, 2.02)	1.67 (1.56, 1.781)	1.01 (0.93, 1.09)	1.14 (1.05, 1.24)	1.15 (1.03, 1.28)	1.19 (1.07, 1.33)
Miscarriage	1.10 (0.99, 1.22)	1.16 (1.05, 1.29)	1.06 (0.95, 1.20)	1.11 (0.99, 1.25)	0.93 (0.79, 1.09)	0.91 (0.78, 1.06)
Stillbirth	0.77 (0.64, 0.92)	0.89 (0.74, 1.07)	1.23 (1.02, 1.47)	1.10 (0.92, 1.32)	1.10 (0.85, 1.42)	1.05 (0.80, 1.35)

(B)

	Fully adjusted		
	Endometriosis	**RA**	**IBD**
Nulliparity	1.63 (1.50, 1.77)	1.21 (1.10, 1.34)	1.22 (1.07, 1.39)
Miscarriage	1.13 (1.003, 1.28)	1.06 (0.93, 1.22)	1.06 (0.93, 1.22)
Stillbirth	0.98 (0.79, 1.22)	0.93 (0.74, 1.16)	0.94 (0.68, 1.27)

Additionally, I reran the blood count and blood percentage models after excluding participants with an autoimmune condition from the non-inflammatory pain group. The values marked in red in **Table *4.13*** indicate significance. Neutrophil counts were significantly increased, and lymphocyte count was decreased in those with an inflammatory pain condition versus a non-inflammatory pain condition.

Table 4.13. Tier 2 blood cell count models after excluding participants with an autoimmune condition from the non-inflammatory pain group. Logistic regression model was performed comparing the neutrophil and lymphocyte cell counts between those with an inflammatory vs non-inflammatory pain condition. The results presented below are unadjusted, age-adjusted, or fully adjusted for age, BMI, asthma, smoking and alcohol consumption.

	OR (95%CI)		
Blood Count	**Unadjusted**	**Age-adjusted**	**Fully adjusted**
Neutrophils	1.26 (1.18, 1.35)	1.25 (1.17, 1.34)	1.26 (1.17,1.35)
Lymphocytes	0.65 (0.59, 0.72)	0.65 (0.59, 0.72)	0.611 (0.55, 0.68)

I further investigated blood cell counts in the stratified tier 2 pain models. I only analysed cell counts which were significant in the previously presented models excluding participants in the non-inflammatory pain group with an autoimmune condition diagnosis. This included neutrophil and lymphocyte counts. As shown in **Table *4.14***, the models remained significant for RA and IBD, however the endometriosis model was no longer significant, even prior to any adjustments.

Table 4.14. Stratified tier 2 models investigating significant blood cell counts between individual chronic pain conditions. Fully adjusted models were adjusted for age, BMI, asthma, smoking and alcohol consumption. (A) Endometriosis, (B) Rheumatoid arthritis, and (C) Inflammatory Bowel Disease vs non-inflammatory pain group, after participants with an autoimmune condition diagnosis were removed. Unadjusted, age-adjusted, and fully adjusted models are presented. Endo; endometriosis, RA; rheumatoid arthritis, IBD; inflammatory bowel disease.

(A)

	Endometriosis (n=8,220 endo vs n=13, 685 non-inflammatory)		
	Unadjusted	Age-adjusted	Fully-adjusted
Neutrophils	1.03 (0.94, 1.12)	0.95 (0.87, 1.04)	0.98 (0.89, 1.07)
Lymphocytes	0.93 (0.82, 1.05)	0.96 (0.85, 1.09)	0.99 (0.87, 1.13)

(B)

	Rheumatoid Arthritis (n=5, 716 RA vs n=13, 685 non-inflammatory)		
	Unadjusted	Age-adjusted	Fully-adjusted
Neutrophils	1.64 (1.50, 1.79)	1.70 (1.55, 1.87)	1.55 (1.40, 1.70)
Lymphocytes	0.41 (0.35, 0.47)	0.41 (0.36, 0.48)	0.31 (0.27, 0.36)

(C)

	Inflammatory Bowel Disease (n=2,470 IBD vs n=13, 685 non-inflammatory)		
	Unadjusted	Age-adjusted	Fully-adjusted
Neutrophils	1.43 (1.26, 1.63)	1.46 (1.28, 1.66)	1.66 (1.45, 1.90)
Lymphocytes	0.49 (0.40, 0.60)	0.49 (0.40, 0.60)	0.50 (0.42, 0.62)

Neutrophil:lymphocyte ratio

The blood cell counts available in the UKBB didn't include the neutrophil: lymphocyte ratio (NLR). However, this is an important variable in assessing systemic inflammation[316,317]. Additionally, I routinely found significant differences in neutrophils and lymphocytes in my earlier analyses. After excluding participants in the non-inflammatory chronic pain group with a diagnosis of an autoimmune condition, I calculated the NLR and transformed NLR as described in the methods section.

As shown in **Table *4.15***, the NLR was increased in those with an inflammatory chronic pain diagnosis when compared to those with a non-inflammatory chronic pain diagnosis. After full-adjustments, the endometriosis model was not significant (OR(95%CI)= 0.999 (0.29, 1.07)), whereas the RA (OR(95%CI)=2.058 (1.91, 2.23)) and IBD (OR(95%CI)=1.82 (1.64, 2.03)) models were.

Table 4.15. Logistic regression models for neutrophil:lymphocyte ratio. Unadjusted, age-adjusted, and fully adjusted models (age, BMI, asthma, smoking and alcohol consumption) are presented.

	Unadjusted	Age-adjusted	Fully adjusted
Tier 2 (after removing autoimmune cases from non-inflammatory group)	1.40 (1.33, 1.48)	1.40 (1.32, 1.48)	1.41 (1.34, 1.15)
Stratified endometriosis tier 2 model	1.06 (0.99, 1.13)	0.99 (0.93, 1.07)	1.0 (0.29, 1.07)
Stratified RA tier 2 model	2.01 (1.87, 2.17)	2.04 (1.89, 2.20)	2.06 (1.91, 2.23)
Stratified IBD tier 2 model	1.75 (1.57, 1.95)	1.77 (1.59, 1.97)	1.82 (1.64, 2.03)

Finally, of interest to the widespread pain aspect of my thesis, I performed a sensitivity analysis on the stratified NLR models above in those with general pain and those without general pain. As shown below in **Figure *4.12***, the NLR effect size was greatest in those with RA & general pain, and IBD with general pain, although due to the relatively small number of participants with general pain (**Table *4.16***), the confidence intervals are wider than the models without general pain.

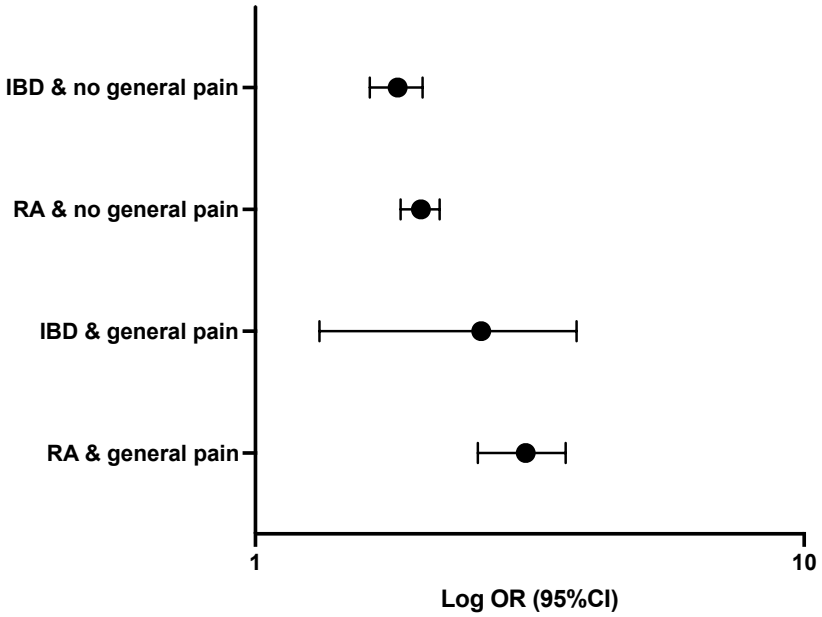


Figure 4.12. Fully adjusted log odds ratio (OR) of increased NLR in RA and IBD participants with and without general pain, compared to participants with a non-inflammatory chronic pain condition. Adjusted for age, BMI, asthma, smoking and alcohol consumption. IBD; inflammatory bowel disease, RA; rheumatoid arthritis. OR; odds ratio, CI; confidence interval.

Table 4.16.Contingency table of general pain prevalence in RA (rheumatoid arthritis) and IBD (inflammatory bowel disease) groups.

	General pain	**No general pain**
RA	677	5039
IBD	79	2391

4.5. Discussion

4.5.1. Overview

In line with my original hypothesis, the presence of a chronic pain condition in the UK Biobank was associated with a significantly increased odds of having at least one miscarriage when compared to controls. However, the odds of having at least one stillbirth or being nulliparous was not significantly different between the two groups.

After stratifying the chronic pain group into those with a classically inflammatory chronic pain condition and those with a classically non-inflammatory chronic pain condition, I found that the odds of being nulliparous were significantly increased in the inflammatory pain group when compared to the non-inflammatory group. However, the odds of having at least one stillbirth or at least one miscarriage did not differ between the two groups.

Upon performing sensitivity analyses, whereby I further stratified the inflammatory pain group into its three constituent diseases (endometriosis, rheumatoid arthritis and inflammatory bowel disease) to determine the odds of being nulliparous, the effect size was largest in the endometriosis group (OR=1.628) but both the RA (OR=1.21) and IBD (OR=1.221) fully adjusted models remained significant.

In my first experimental chapter, I was unable to assess inflammation physiologically. Therefore, in this chapter, I investigated blood cells (both as counts and percentages) to better understand inflammation which may underlie the association between pain and

reproductive outcomes. In my investigations of blood cell counts between the chronic pain and control group, most blood cell counts were significantly increased in the chronic pain group when compared to controls. Interestingly, in my tier 2 analyses, neutrophils were significantly increased, and lymphocytes decreased in the inflammatory group vs non-inflammatory group. After excluding participants with an autoimmune condition from the non-inflammatory chronic pain group, this remained true. Since the NLR has been implicated in systemic inflammation in chronic inflammatory conditions[318,319], I calculated the NLR and found that this was significantly increased in the inflammatory group vs non-inflammatory group.

To understand whether the underlying inflammation was common in each of the inflammatory pain groups, I stratified the NLR models into endometriosis, RA and IBD vs non-inflammatory. I found that the NLR was significantly increased in both the RA and IBD groups when compared to the non-inflammatory pain group, however the endometriosis model was not significant. Furthermore, the effect size of the RA and IBD models were increased in those with general pain (widespread pain proxy).

Finally, I investigated pain spread and comorbid pain diagnoses in participants with a chronic pain condition. Interestingly, chronic pain was highly prevalent in all study groups. All body regions impacted by chronic pain were however more prevalent in the chronic pain group than controls. After stratifying, the prevalence of general and hip pain was comparable between the tier 2 groups. Abdominal, neck and back pain were more prevalent in the non-inflammatory group, whereas facial pain and knee pain were more prevalent in the inflammatory pain group. When investigating number of chronic pain

diagnoses, it was more common for participants in the inflammatory chronic pain group to have more than one chronic pain condition than it was for participants in the non-inflammatory pain group.

4.5.2. Reproductive outcomes

Nulliparity

Despite being sufficiently powered to detect such differences, the odds of being nulliparous did not differ between participants with chronic pain and controls. Given the diversity of underlying pain generating mechanisms, disease pattern and temporality of disease diagnosis, it is possible that any relationship between chronic pain generally and reproductive outcomes could not be elucidated from the chronic pain population I have selected, and the results masked.

In my tier 2 analyses, however, the odds of being nulliparous were significantly greater in the inflammatory chronic pain group in comparison to those with a non-inflammatory pain condition. After stratifying my tier 2 model into the three separate conditions (RA, IBD, endometriosis), each model remained significant. This suggests that it was not a single condition driving the initial association observed, and perhaps there is a shared inflammatory mechanism driving this association. While it is possible that ongoing disease-related inflammation is a shared mediator, it is likely that there are important factors which are disease specific, driving these associations.

Observing more prevalent nulliparity in the endometriosis group is unsurprising and well supported by the literature. As many as 50% with the disease experience infertility[65]. The pelvis of someone with endometriosis often contains peritoneal fluid comprised of an inflammatory milieu. The inflammatory environment can hinder endometrial receptivity for proper implantation of an embryo[65]. Since the disease is most commonly found around the pelvic organs, anatomical disruptions caused by endometriosis can further hinder conception[129]. It is also possible that dyspareunia, or pain with sexual intercourse, prevents women from engaging in frequent sex. Unfortunately, the level of granularity required to answer questions about pain with sex, especially during the reproductive lifetime was not available in the UK Biobank. Something to consider as well is that not every woman with endometriosis experiences pain with the disease, and therefore the pelvic inflammation and anatomical limitations previously described may be predominant mechanisms in this cohort of women, as opposed to pain-related causes of nulliparity.

Contrary to endometriosis, in which the disease is known to cause challenges when conceiving, there is conflicting evidence about the relationship between parity and rheumatoid arthritis. On the one hand, parity has been hypothesised to be a protective factor against rheumatoid arthritis. It has been previously shown that the risk of RA was lower in women who were parous when compared to nulliparous women[320]. This association depended on a woman's HLA genotype for the 'shared epitope' or 'SE' (alleles in the HLA-DRβ1 gene): women with SE had a greater reduction in risk of RA than women who were SE-negative. Furthermore, women with RA are at an increased risk of developing pre-eclampsia during their pregnancies[321], which is known to increase the risk of fetal death[322], as well as long-term adverse outcomes[323] (such as hypertension, and

development of cardiovascular diseases) for mother and baby. Although further investigation would be required to determine the incidence of pre-eclampsia in this cohort, it is plausible that this may have contributed to the observed association.

Parity has also been shown to be a predictor of a more severe RA phenotype in women who are negative for anti-citrullinated protein antibodies and who are younger. Further evidence suggests that because of disease severity and some medications taken to manage RA, women with the conditions take longer to conceive after trying than women without RA[324–326]. Since the odds of being nulliparous was higher in the RA group when compared to those with a non-inflammatory pain condition, this data would be consistent with the idea that parity may have been protective against the development of RA. However, in the UK Biobank data it would have been difficult to test this hypothesis for three reasons. Firstly, the reproductive outcome data available didn't have the level of granularity to investigate risk of RA after a live birth, compared to those who were nulliparous to ultimately test this hypothesis. Additionally, I combined all RA cases for the sake of statistical power and robustness, however this is a heterogeneous group with different RA pathologies. Finally, I didn't investigate medications taken at the time of live birth which could have possibly influenced one's parity, and one's decision about trying to conceive whilst managing their RA[327]. If analyses were to continue on this research question of parity in RA, it would be important to consider whether subtypes of RA (i.e. seropositive or negative) have different disease trajectories after parity. Additionally, investigating medication use at the time when women were trying to conceive would help understand the mechanism of nulliparity in women with the disease. Qualitative data on the

participants' decision-making process around having children and managing their disease would further facilitate this understanding.

In women with inflammatory bowel disease, the association with nulliparity may in fact be driven by voluntary childlessness (VC). VC in women with IBD is likely driven by many factors related to disease burden, such as employment status, a diagnosis of Crohn's disease (rather than ulcerative colitis), and importantly, lack of education on pregnancy after a diagnosis of IBD[328]. There is a fear which is not uncommon amongst women with IBD that certain classes of treatment medications (i.e. biological therapies) may hinder the ability to have a child, or that the disease itself can be passed on to the baby[329]. While it is true that some medications taken at different stages during pregnancy (i.e. infliximab during the third trimester) can be detrimental to a healthy pregnancy, proper counselling of disease management during pregnancy would limit the risk of poor outcomes[330]. Existing evidence suggests however that patient education in a health care setting on pregnancy and disease is poor and may have been even more so when most of these women were trying to conceive[331]. It is possible that these reasons could in part explain why fewer women with IBD were parous than women with a diagnosis of a non-inflammatory chronic pain condition. Further work is needed to in the first instance understand what medications were taken at the time of trying to conceive if they were trying to conceive at all. Additional analyses could focus on generational effects, to see if parity has increased in women more recently, who would presumably have better access to education on pregnancy with IBD.

Voluntary childlessness in women with RA is less understood, however many studies have reported that women with RA have voluntarily reduced the number of children conceived on account of disease severity or progression[332,333].

Miscarriage and Stillbirth

Interestingly, the odds of having at least one miscarriage and stillbirth were significantly increased in those with a chronic pain diagnosis when compared to controls. However, the odds didn't differ between those with an inflammatory chronic pain condition when compared to those with a non-inflammatory pain condition. This suggests that an inflammatory underpinning of miscarriage or stillbirth in the chronic pain group may be less likely than other causes. Although age and additional demographic factors which could have influenced the odds of having a miscarriage were controlled for in the tier 1 analysis, it is possible that additional factors may underlie the associations which were not accounted for.

One possible explanation could be metabolic syndrome or metabolic diseases. At the group level, the mean BMI score was higher in the chronic pain group than controls, and generally, risk factors for cardiovascular disease and metabolic syndrome have been shown to be more highly prevalent in people with chronic pain than pain-free controls[334]. Furthermore, metabolic diseases in women are known to increase the risk of miscarriage and stillbirth[335], and this is most pronounced in women with poorly managed disease[51].

While I controlled for BMI in my analyses, further exploration of a previous diagnosis of metabolic syndromes and related diseases (ie. Type II diabetes, gestational diabetes, pre-eclampsia) may help to better understand the mechanisms underlying these results.

However, a significant limitation of this is that data on age at diagnosis is only available from 1995 on, and so diagnoses made prior to a miscarriage or stillbirth would be extremely limited in numbers, and would require far more advanced epidemiological techniques and study design (i.e. cox models) to answer such questions. It is also important to consider that although age at diagnosis was not explored in these analyses, the typical age of symptom onset may differ in a disease-dependent fashion (see **Table 4.17**), which could in part explain the associations observed (or lack thereof), and further necessitate the exploration of individual diseases, as I had done in the nulliparity models. Ultimately, a study designed to address questions about reproduction would likely be better suited for this.

Table 4.17. Typical age of symptom onset for chronic pain conditions in tier II analyses.
Continued on next page.
*Note the diagnostic delay: although many women with endometriosis experience symptoms from a young age, most won't receive a diagnosis until many years later

Chronic pain condition	**Typical age of symptom onset**
Inflammatory	
Endometriosis	Adolescence[52] *
Rheumatoid arthritis	Median age at symptom onset is 45 for women, depending on type[40]
Inflammatory bowel disease	15-40 years old[53]
Non-inflammatory	
Osteoarthritis	Median age at symptom onset is 50[54]
Irritable bowel syndrome	50% of patients report symptom onset before 35 years old[55]

Migraine	Incidence peaks around 35 years old for women[56]
Fibromyalgia	Between ages 30-50[57]
Bladder pain syndrome	Likely before age 30[58]
Temporomandibular joint disorder	20-40 years old[59]

4.5.3. Blood cells

As I had hypothesised, blood cell counts were altered in the chronic pain group when compared to controls. Of note were the increased neutrophils and decreased lymphocytes. After stratifying, neutrophils, and lymphocytes counts, as did the NLR, remained significantly greater in the inflammatory chronic pain group when compared to the non-inflammatory chronic pain group. This suggests that relatively speaking, there may be more active systemic inflammation[60] in the inflammatory pain group at the time the samples were collected. After further investigation of the NLR in my stratified tier 2 models, only the RA and IBD models remained significant. This may in part be due to differences in the disease trajectory of RA, IBD, and endometriosis. RA is typically diagnosed later in life relative to endometriosis, and commonly close to the time of menopause[40]. Since UK Biobank participants were older at the time of recruitment (40-69 years old) and at least 50% of participants in the inflammatory chronic pain group had already undergone menopause, it is possible that participants with RA have a more active disease state at the time of baseline. NLR has previously been used to infer the inflammatory state of RA[61], and to predict RA remission[32].

IBD on the other hand typically presents and is diagnosed in early adulthood[62]. However, considering the disease trajectory, women don't experience an improvement in symptoms

post-menopause[63]. Therefore, there is no reason to believe that at the time these blood samples were taken, women were experiencing less pain or inflammation than earlier on in their disease journey. Furthermore, NLR has been shown in some studies to be elevated with increasing disease severity and may show promise for future use in clinical practice[31,64].

Endometriosis on the other hand is as an oestrogen-dependent disease[5]. As such it is typically thought of as a disease primarily affecting pre-menopausal women of reproductive age. For many with endometriosis, related symptoms subside as oestrogen levels taper off post-menopausally, although some women do experience symptoms after menopause[65]. There is conflicting evidence about the role of NLR in endometriosis and its association with disease severity and infertility [66,67]. In both studies, women who were of reproductive age were investigated. All of this considered, it is likely that given the age of participants in the UK Biobank, most women with endometriosis were peri- or post-menopausal and may not be experiencing disease-related symptoms or systemic inflammation observed in the RA or IBD groups at the time of data collection.

General pain and NLR

Further sensitivity analyses showed that the effect size of the NLR models in RA and IBD were greatest in those with either of these diseases and general pain. This very interestingly sheds light on the possibility that systemic inflammation may be an important mechanism in widespread pain, and its association with outcomes investigated in Chapters 3 and 5.

4.5.4. Pain spread and comorbidity

In tier 1, all chronic pain areas of the body available in the UK Biobank were more prevalent in the chronic pain group than controls. Given the age of the UK Biobank participants and the trajectory of chronic pain across the lifetime[68], it is unsurprising that some control participants also experienced chronic pain. This confirmed though that a majority of the chronic pain group had in fact lived with chronic pain, which was a limitation of making group allocations based on disease diagnoses alone. In tier 2, general pain (a UK Biobank proxy for widespread pain) and hip pain were similarly prevalent between pain groups. Abdominal, neck and back pain were more prevalent in the non-inflammatory pain group than the inflammatory pain group. These pains may be more prevalent because of the selection criteria for each study group, and which diagnoses were selected. For example, abdominal/stomach pain is a hallmark symptom of IBS[69] and therefore likely contributes to this increased prevalence in the non-inflammatory pain group. Albeit the time and exact cause of such chronic pain is unknown, this provides evidence that the participants I've included in these chronic pain groups have in fact lived with chronic pain at some point in their lives.

4.5.5. Sensitivity analyses

Sensitivity analyse were performed to strengthen the conclusions I drew from my results. In the first instance, participants with autoimmune conditions were excluded from the non-inflammatory chronic pain group, as they are often inflammatory in nature and may contribute to challenges conceiving[70]. The prevalence of these conditions in the non-

inflammatory chronic pain group was not high, and therefore excluding participants from analyses did not make any significant difference to my model results for reproductive outcomes. Similarly, after stratifying the modified (no autoimmune diagnoses in non-inflammatory group) tier 2 inflammatory chronic pain conditions, the relationship with neutrophils, and lymphocytes did not change. Therefore, these results suggest that in this cohort, autoimmune conditions did not significantly impact or confound my results.

4.5.6. Strengths and limitations

This study was designed upon the foundation of work presented in Chapter 3. One of the primary limitations of that preliminary work was a small sample size, which was addressed using the UK Biobank, an invaluable resource of deep phenotypic and biological data on over 500,000 individuals across the UK. In addition, my previous work was limited in its ability to understand inflammation. While I was restricted to the use of blood cell measures with baseline data in the UK Biobank, I was able to characterise an important marker of systemic inflammation (NLR) across my pain groups.

From a professional development point of view, working in the UK Biobank hugely progressed my knowledge of epidemiology, study design and statistical concepts. I became relatively proficient in R and became acquainted and comfortable using big data. This work was performed early on in my R / big data journey, and since then I have developed my epidemiological skillset, and in other collaborative projects have employed more advanced statistical analyses using this dataset.

While this work had improved upon limitations from my hypothesis-generating chapter, working with big data brings about its own limitations that future work should aim to address.

In the first instance are inherent limitations of the UK Biobank. It was wholly convenient to use UK Biobank data during the pandemic because I had access, and it was a great learning tool. However, the database was designed to answer questions about health and disease in an older population, with hospital data spanning back only to 1995. As such, the ability to design an 'ideal' study, in which chronic pain precedes reproductive outcomes, was very challenging. Furthermore, Fry et al. have shown evidence of healthy volunteer and selection bias[71] in the UK Biobank, and lack of generalisability to the UK population[72]. In addition to biases inherent of the UK Biobank itself, it is likely that an element of recall bias was present in the variables I investigated. While ICD9 and ICD10 data was used, many measures I used relied on self-reported data, which is prone to such bias[73].

Secondly, it is important to acknowledge the limitations in my study design. Given that this was a cross-sectional study, I was able to determine associations however was restricted from drawing conclusions about causality. This was primarily because this design did not allow for the temporality of these associations to be assessed. To fully understand the relationships that I've observed in this chapter, future work should include a cohort design[74], in which exposure definitively precedes outcome, and more pain-related measures are assessed at the time of reproduction. While this study design is not without its own limitations, it is an important step in further elucidating the relationship between pain and reproductive outcomes.

A further limitation of studying chronic pain conditions in the UK Biobank data is that very limited information on the disease-associated pain experience is available. As such, we don't know whether a participant experienced pain with their disease, how severe or impactful it was on their quality of life. I consider women with endometriosis, for example, who may not have pain at all[33] and may have received a diagnosis because they had troubles conceiving rather than experiencing debilitating pain. All of this considered, future work is necessary to establish whether there is a relationship between the pain symptoms occurring as a result of disease, and reproductive outcomes.

4.6. Future directions and conclusions

Considering the limitations stated above, future work is necessary to derive appropriate causal conclusions about the relationship between chronic pain and reproductive outcomes, if they exist. To do so, a prospective cohort design on participants during their reproductive years would be ideal. This would reduce recall bias, address the issue of temporality, and participants would be more appropriately suited to study questions about reproduction. A possible database which could be used is ALSPAC, the Avon Longitudinal Study of Parents and Children[75–77]. Simply, this study follows children through adolescence into adulthood, and collects comprehensive phenotypic data about pain, and reproductive outcomes. Data collection for this is ongoing, and so it would be ideally set up to answer questions about pain and reproductive outcomes across the life course.

In this chapter, I've shown that nulliparity was more prevalent in those with an inflammatory chronic pain diagnosis than those with a non-inflammatory chronic pain

condition. However, the effect size was largest in participants with endometriosis, where a relationship with infertility is well-established[33]. The RA and IBD models were significant as well. Upon investigation of blood cells, the NLR was increased in those with an inflammatory chronic pain condition, and specifically, those with RA and IBD. This suggests that in the UKBB, RA and IBD may be associated with more of an ongoing systemic inflammation at the time of biological sample collection. In the case of endometriosis, disease-related inflammation may occur earlier on in the reproductive life and be more pertinent to the association between the disease and poor reproductive outcomes.

Chronic pain was highly prevalent, but again, I could not determine when this pain was experienced in relation to reproductive outcomes. To conclude, this work has shown that nulliparity, not miscarriage or stillbirth, may be associated with chronic pain-related inflammation in women with RA and IBD. Future work is crucial to better understand this phenomenon.

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4.8. Appendices

Appendix 4A: ICD9, ICD10 and self-reported codes for chronic pain conditions.

	Code Source		
Disease	**Self-report**	**ICD9**	**ICD10**
Endometriosis	1402	"6170", "6171", "6172", "6173", "6174, "6175", "6176", "6178", "6179"	"N800", "N801", "N802, "N803", "N804", "N805", "N806", "N808", "N809"
Neuropathies	“1468”, "1255”, "1256"	3579,"3538","3569","3568","3562","3560","356","3371","3370","3540","3541","3542","3543","355","354","3550","3551","3555","3556","3558","3562","3564","3568","3569","3575"	G609,"G603","G602","G600","G608","G60","G580","G990","G130","G56","G560","G561","G562","G563","G564","G568","G569","G57","G570","G571","G572","G573","G574","G575","G576","G578","G579","G58","G587","G588","G589","G61","G610","G611","G618","G619","G62","G620","G621","G622","G628","G629","G63","G631","G632","G633","G636","G638","G64","G590"
Complex regional pain syndrome	N/A	“33721, “3544, “35571, “33729, “33722, “33720, “3559, “73376	“G9050", “G90511", “G90512", “G90513", “G90519", “G90521", “G90522", “G90523", “G90529", “G9059", “G5640", “G5641", “G5642", “G5643", “G5770", “G564", “M890", “M8900", “M8901", “M8902", “M8903", “M8904", “M8905", “M8906", “M8907", “M8908", “M8909"
Temporomandibular Joint Disorder	N/A	5246	K076
Rheumatoid Arthritis	1464	“714”,"7140","71400","71401","71402","71403","71404","71405","71406","71407","71408","71409","71423","71424"	“M05”,"M053","M0530","M0531","M0532","M0533","M0534","M0535","M0536","M0537","M0538","M0539","M058","M0580","M0581","M0582","M0583","M0584","M0585","M0586","M0587","M0588","M0589","M059","M0590","M0591","M0592","M0593","M0594","M0595","M0596","M0597","

			M0598","M0599","M060","M0600","M0601","M0602","M0603","M0604","M0605","M0606","M0607","M0608","M0609","M069", "M0690", "M0691", "M0692", "M0693", "M0694", "M0695", "M0696", "M0697", "M0698", "M0699","M068","M0681","M0682","M0685","M0686","M0687","M0688","M0689","M064","M0640","M0641","M0643","M0644","M0645","M0646","M067","M069"
Bladder pain syndrome/interstitial cystitis	N/A	“595","5950","5951", "5952","5953","5958","5959"	“R3982”, “N301”, “N3010”
Fibromyalgia	1542	N/A	“M797”, “M7970”, “M7971”, “M7974”, “M7975”, “M7976”, “M7977”, “M7978”, “M7979”
Migraine	1265	“346”, “3460”, “3461”, “3462”, “3468”, “3469”	“G43”, “G430”, “G431”, “G432”, “G433”, “G438”, “G439”
Irritable bowel syndrome	1154	5641	“K58”, “K580”, “K589”
Lower back pain/Back pain	1534	7242	“M545”, “M5450:M5459”
Inflammatory bowel disease	1461	“555”, “5550”, “5551”, “5552”, “5559”	“K50”, “K500”, “K501”, “K508”, “K509”, “K51”, “K510”, “K511”, K512”, “K513”, “K514”, “K515”, “K518”, “K519”
Osteoarthritis	1465	“715”, “7151”, “71514”, “71515”, “71516”, “7152”, “71526”, “71527”, “7153”, “71531”, “71533”, “71534”, “71535”, “71536”, “71537”, “7158”, “7159”	“M199”, “M1990”, “M1991”, “M1992”, “M1993”, “M1994”, “M1995”, “M1996”, “M1997”, “M1997”, “M1998”, “M1999”

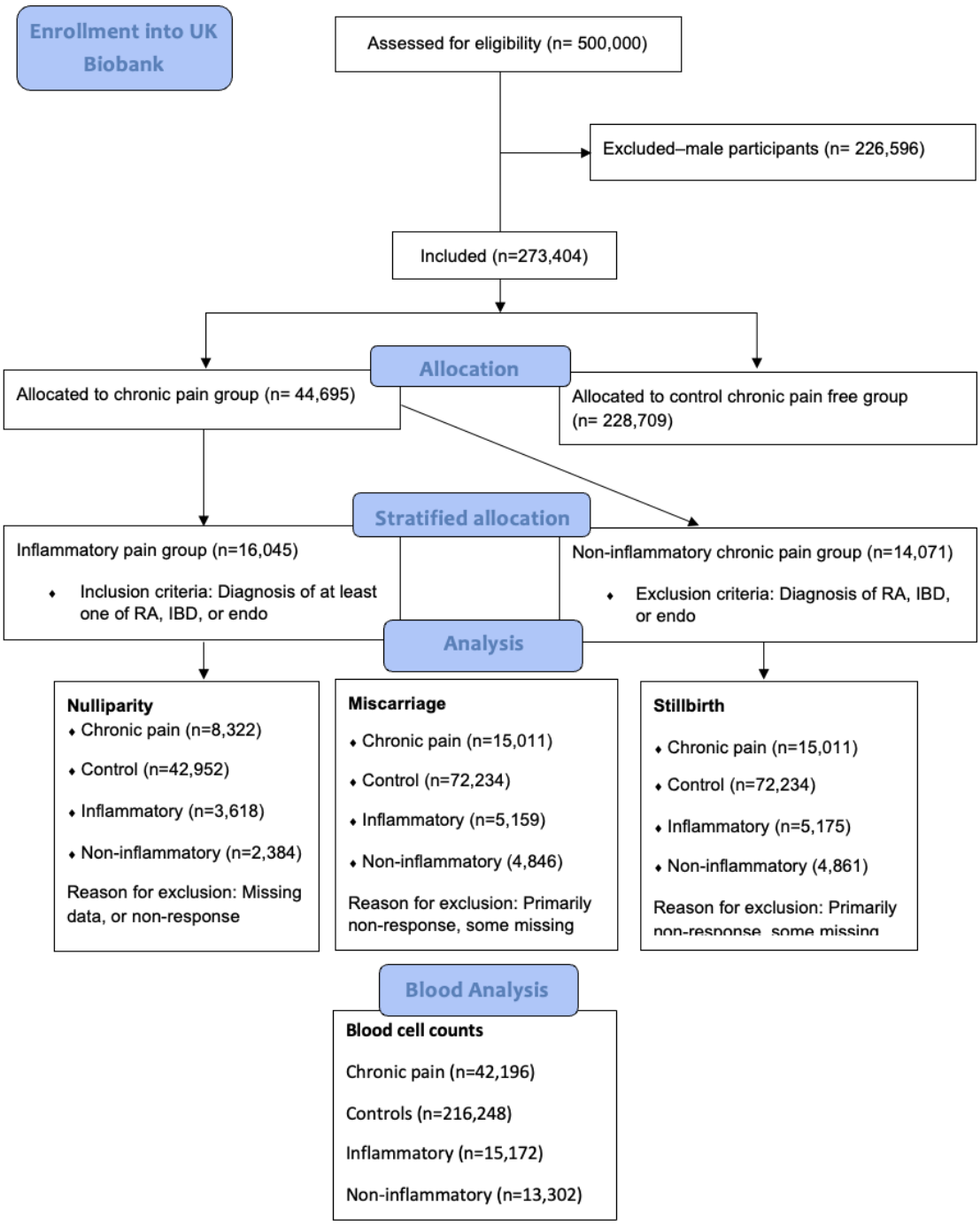
Appendix 4B: Untransformed blood cell count and percentages, tier 1 analysis.

Measurement	Control (Median, IQR)	Control (N)	Pain (Median, IQR)	Pain (N)	P-value
Neutrophil Count	3.99 (1.69)	216248	4.1 (1.8)	42196	$<2.2x10^{-16}$
Lymphocyte Count	1.91 (0.75)	216248	1.95 (0.810)	42196	$3.97x10^{-15}$

Appendix 4C. Untransformed blood cell count and percentages, tier 2 analysis.

Measurement	Inflammatory (Median, IQR)	Inflammatory (N)	Non-inflammatory (Median, IQR)	Non-inflammatory (N)	P-value
Neutrophil Count	4.15 (1.82)	15172	4.1 (1.7)	13302	$5.384x10^{-9}$
Lymphocyte Count	1.90 (0.832)	15172	1.97 (0.8)	13302	1

Appendix 4D. Consort flow diagram of analyses performed in this chapter, and the number of participants included in each analysis. Bold and italicised fonts indicate the number of participants available for a minority of blood count measures. RA; rheumatoid arthritis, IBD; inflammatory bowel disease, endo; endometriosis.



Chapter 5 – Deeply phenotyping widespread pain in a larger cohort of women with chronic pelvic pain

5.1. Introduction

Endometriosis and other pelvic pain pathologies such as bladder pain syndrome are highly burdensome in women, yet seldom exist in isolation from one another, and other more widespread pathologies[1–3]. Furthermore, treatments for these conditions targeting the pelvis are often ineffective at managing pain symptoms[4–6]. Therefore, understanding the distinct and shared pain mechanisms underlying pelvic pain pathologies is of the utmost importance. Crucially, in efforts to shift toward more personalised medicine, we must better phenotype these women. One such way to do this is to shift focus away from the pelvis, and phenotype on more systemic characteristics and impacts of pain.

The Translational Research in Pelvic Pain (Tripp) study, led by Professor Katy Vincent, aimed to deeply phenotype women with endometriosis-associated pain, bladder pain syndrome, comorbid endometriosis-associated pain and bladder pain syndrome, and chronic pelvic pain[7]. Women in this multisite study were required to complete comprehensive questionnaires about their mood, health, and pain, undergo psychophysical testing (quantitative sensory testing, conditioned pain modulation, autonomic nervous system testing, and bladder sensitivity testing), and functional magnetic resonance imaging (fMRI). A full study flowchart[7] can be found in **Figure 5.1**.

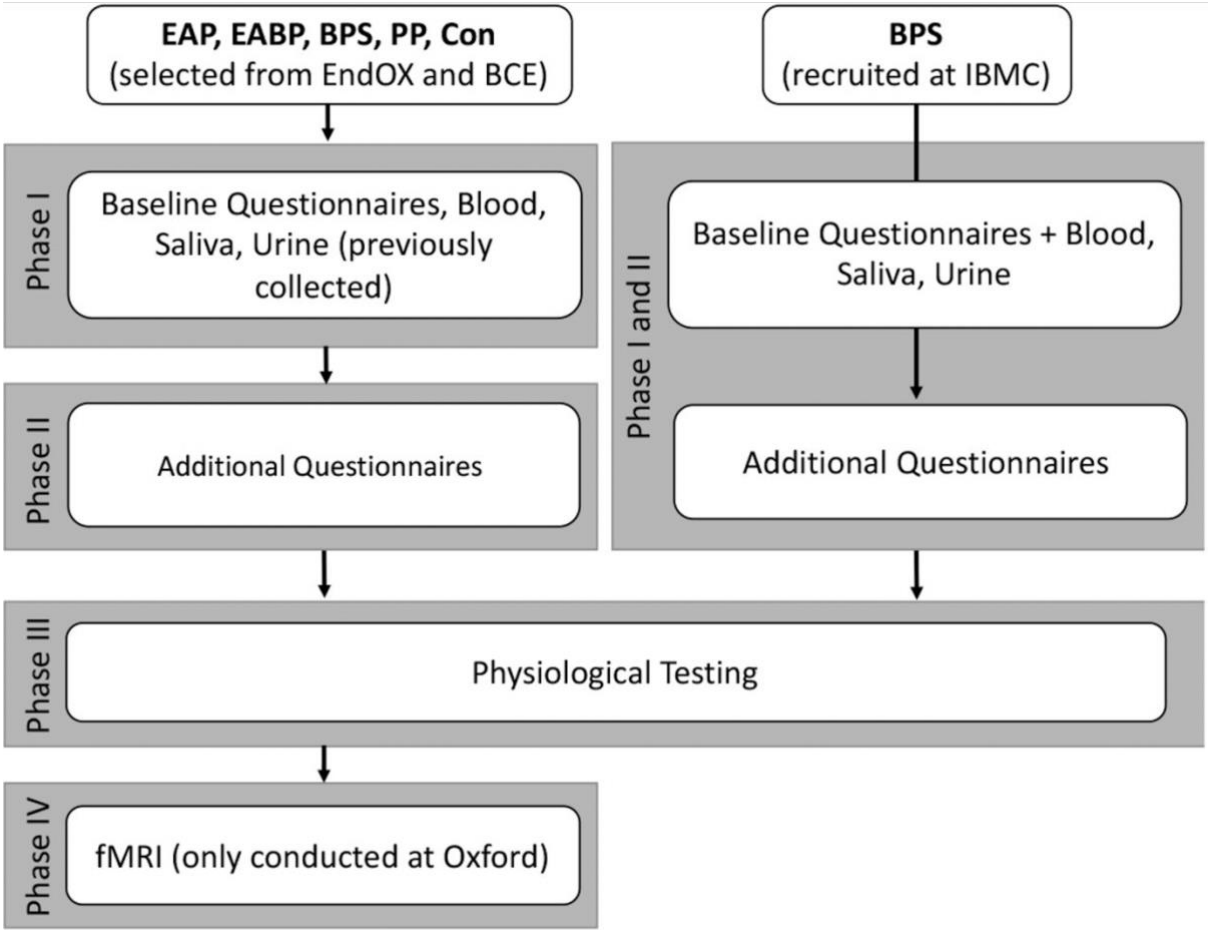


Figure 5.1. TRiPP study design flow chart, taken with permission from the TRiPP baseline characteristics paper. Phase I; completed before TRIPP started (Oxford and Boston) or at the time of TRiPP study commencement (Porto). Phase II; Additional questionnaires (either online or on paper) at each study site. Phase III/IV; Carried out by trained researchers at the respective site(s). EAP; endometriosis-associated pain, EABP; endometriosis-associated pain and bladder pain symptoms, BPS; bladder pain syndrome, PP; pelvic pain, Con; control, EndOx; study in Oxford to identify biomarkers for endometriosis, BCE; Boston Centre for Endometriosis, IBMC; Instituto de Biologia Molecular e Celular, fMRI; functional magnetic resonance imaging.

With such a rich dataset, not only could I better understand the underlying mechanisms of widespread pain in these women, but importantly, the impact that different aspects of their pain have on their quality of life. I was fortunate to have received training from the German Neuropathic Pain Network (DFNS) to conduct psychophysical testing in this cohort myself at the Oxford site[8]. This training was integral to the success of the project, as all

researchers responsible for data collection were taught the same methodologies, and were instructed on the order of the paradigm, and script which was to be followed to ensure reproducibility, and the appropriate combination of data from different study sites.

In the first experimental chapter of my thesis, I showed that widespread pain in women with endometriosis-associated pain was associated with poor psychological health (depression, pain catastrophizing) and worse reproductive health outcomes. However, this study had several limitations. Firstly, and most importantly, it was a readily available dataset that was limited in sample size. Additionally, the EndoPAIN studies were not designed to answer questions about pain spread per se, nor were they designed to answer questions about pain mechanisms underlying it. In summary, the questionnaires available for the EndoPAIN analyses were limited and not comprehensive enough to get the full picture or impact of widespread pain.

Results from the first experimental chapter were used to inform the aims and hypotheses of these widespread pain analyses in TRiPP, in a more appropriate dataset designed to deeply phenotype pain in women with chronic pelvic pain.

5.1.1. Aims

1. Characterise extra-pelvic pain according to previously published protocols
2. Understand the relationship between factors impacting quality of life and widespread pain
3. Determine the prevalence of comorbidities between widespread pain groups

4. Investigate psychophysical parameters with respect to widespread pain to understand underlying pain mechanisms

5.1.2. Hypotheses

1. Extra-pelvic widespread pain is highly prevalent in the TRiPP cohort of women with chronic pelvic pain
2. Widespread pain is associated with worse psychological health, and general health (fatigue and sleep, higher pain catastrophizing scores, and worse depression and anxiety)
3. Comorbid conditions (pain and non-pain related) are highly prevalent in this cohort, and more so in the widespread pain groups
4. Psychophysical testing will reveal perturbations in pain processing mechanisms unique to those with widespread pain

5.2. Methods

5.2.1. Study population

This TRiPP cohort comprises 785 women, either with chronic pelvic pain syndrome (PP), endometriosis-associated pain (EAP), bladder pain syndrome (BPS), endometriosis & comorbid bladder pain symptoms (EABP), or pain-free controls (CON). The following inclusion criteria were met by included participants; EAP–surgical diagnosis of endometriosis, at least one pelvic pain rated >=4/10 on the numerical rating scale (NRS); EABP–surgical diagnosis of endometriosis, at least one pelvic pain >=4/10, pain perceived

as arising from the bladder and other area(s) of the pelvis; at least one urinary symptom (i.e. frequency, urge); BPS–fulfil the European Society for Study of Interstitial Cystitis (ESSIC) criteria; CPP– Surgical confirmation of no endometriosis, at least one pelvic pain >=4/10, no urinary symptoms, and pelvic pain not perceived as arising from the bladder; CON–no previous diagnosis of endometriosis; no pelvic pain or dysmenorrhoea (All NRS<3/10), no urinary symptoms.

All participants were aged 18-50 and were not currently pregnant or lactating. Women in the EAP/EABP/CON groups were previously enrolled in EndOX: A study to identify possible biomarkers in women with endometriosis at Oxford University (REC Reference 09/H0604/58, Oxford, UK) or a study at the Boston Centre for Endometriosis (IRB-P00004267, Boston, USA), and had agreed to be contacted again for participation in future research studies. Participants in the BPS cohort were newly recruited from patient support groups and BPS clinics in Oxford and Porto respectively. All participants were subject to telephone screening before allocation to the appropriate study subgroup. Recruitment and data collection occurred across three sites by appropriately trained researchers: Oxford University Hospitals Foundation Trust/University of Oxford, UK; Boston Children's Hospital, USA; and IBMC, Portugal. TRiPP received favourable ethical approval from Yorkshire & The Humber – South Yorkshire Research Ethics Committee (19/YH/0030). For further information on the TRIPP study, the full study protocol can be found on medRXiv[7].

5.2.2. Study Design

A subset of women participated in a follow-up portion of this study. Follow-up study participants completed a pack of comprehensive questionnaires, which included validated questionnaires about their pain experience, fatigue, sleep, general health (SF-36) mood (pain catastrophizing scale, Hospital Anxiety and Depression Scale), self-reported comorbidities, and importantly, the Michigan body map (MBM).

A further subset of the TRiPP cohort underwent psychophysical testing, which included quantitative sensory testing (QST), conditioned pain modulation (CPM), recordings of autonomic nervous system (ANS) activity and non-invasive bladder testing. Prior to any psychophysical testing, valid informed consent was obtained, and all participants completed a 'How are you today?' questionnaire. This short questionnaire included questions about pain (Numerical rating scale (NRS) of participants' pain rating at the start of the visit, before the CPM paradigm), the IPPS body map (where participants were instructed to circle where on the body they experience pain), mood (pain catastrophizing scale (PCS) and the State-Trait Anxiety Inventory (STAI)-State questionnaire), questions about their menstrual cycle (current hormone use, or if they did have periods, information about their last menstrual period (LMP)), and medications used at the time of the visit. Prior to the visit, participants were instructed to refrain from taking analgesic medications, and reduce caffeine consumption, as these are known to impact testing parameters.

Many participants completed all three components of the testing paradigm in a single visit. When this was the case, the experimental paradigm was always completed in the same

order (QST, CPM & ANS, bladder paradigm). Some participants chose to attend three separate visits, at which only a single test was administered in each visit. Participants completed the 'How are you today?' questionnaire before each experimental paradigm, irrespective of whether participants attended a single or three separate visits.

For the following analyses, the follow-up questionnaire and psychophysical data from TRiPP were utilised.

5.2.3. Summarising pain spread in the TRiPP cohort

Self-reported regions impacted by pain were collected using the MBM. Participants were asked to identify where on the body map they experience pain for 3+ months. This was in the first instance summarised to understand the pain spread across the TRiPP study groups. Since all chronic pain participants recruited to the TRiPP study experienced at least one type of pelvic pain rated >4/10, it was assumed that all participants had at least pelvic pain. The number of extra-pelvic regions was summarised and compared between study groups and visualised using heat maps. The dot over each region on the body represents the proportion of women reporting pain in that region; the darker and bigger the dot is, the more prevalent pain reported in that area was. The body heat maps were created by following an online tutorial[9].

5.2.4. Characterising pain spread according to the MaPP protocol

Since there was no existing literature investigating and comparing widespread pain between endometriosis, bladder and pelvic pain groups to suggest that there would be

differences in the widespread pain profiles, all chronic pain participants were amalgamated into a single group before categorising them according to the MaPP protocol[10].

MaPP's method for categorising pain spread involves grouping the 35 MBM regions into 7 categories; back, head, right leg, left leg, right arm, left arm, and trunk, as shown in **Figure *5.2***. Since all chronic pain participants in TRiPP were recruited based on having chronic pelvic pain, any woman who didn't identify extra-pelvic regions impacted by pain on the MBM, was allocated to the 'isolated' group. Participants who identified one or more extra-pelvic regions were further stratified into 'intermediate' (1-2 regions) or 'widespread pain' (3-7 regions).

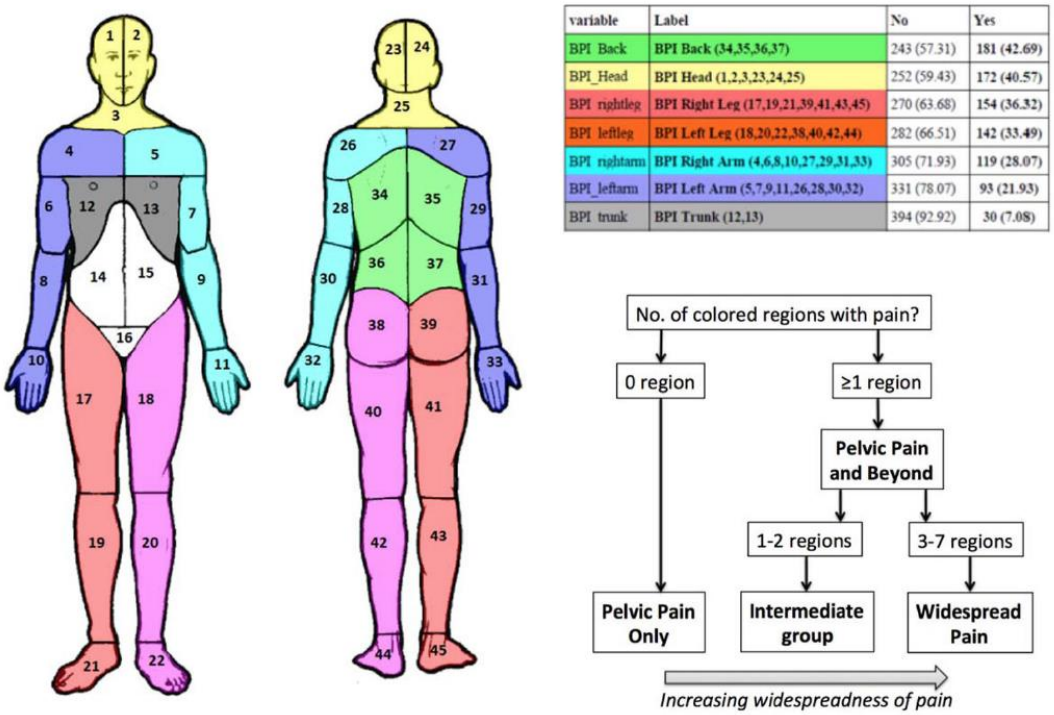


Figure 5.2. MaPP widespread pain coding, according to the number of extra-pelvic regions identified on the Michigan Body Map. This figure was originally published by Lai et al[10], but an appropriate copyright/license has been obtained from the publisher and can be found in **Appendix 5A**.

The prevalence of the seven MaPP regions was summarised in the TRiPP cohort. Furthermore, the distribution of the TRiPP study groups allocated to the different MaPP widespread pain groups was described.

5.2.5. Psychological and general health measures

The PCS, painDETECT and HADS were earlier described in the general methods of studying pain chapter (Chapter 2). As such, only a brief outline is described below.

5.2.5.1. Fibromyalgia Scale

The fibromyalgia scale screens for fibromyalgia using the 2010 fibromyalgia diagnostic criteria[11]. The newer scale itself is comprised of the widespread pain index (WPI), which includes 19 body regions, and a symptom severity (SS) scale. The rationale for excluding the tender points from this classification system is that SS is better correlated to the WPI than the tender points. The WPI regions can be totalled using the MBM. The SS scale is scored out of twelve. This scale includes the following parameters: fatigue, waking unrefreshed and cognitive symptoms. Participants can either have a score of zero (no problem), one (slight or mild problems), two (moderate or considerable problems), or three (severe or life-disturbing problems) for each question. Someone with fibromyalgia would be classified as such using this questionnaire if the following three conditions are met:

1. WPI ≥7 and SS score ≥ 5 **or** WPI 3-6 and SS score ≥ 9
2. Symptoms have been present at a similar level for 3+ months
3. The patient does not have a disorder that would otherwise explain the pain

Further to investigating widespread pain using the MBM, the fibromyalgia scale was used to determine how many CPP participants additionally met the diagnostic criteria for fibromyalgia. While widespread pain is a criterion that must be met for a diagnosis of fibromyalgia, it is not the case that everyone with widespread pain has fibromyalgia.

5.2.5.2. Pain catastrophizing scale

The PCS was administered to all follow-up TRiPP participants. Total scores and PCS subscales (rumination, magnification, and helplessness) were compared between widespread pain groups.

5.2.5.3. Hospital Anxiety and Depression Scale

The HADS anxiety and depression subscales were scored according to an online published scoring sheet[12] and were compared between widespread pain groups. Additionally, a linear test for trend was performed to assess the linear relationship between the number of extra-pelvic regions impacted by pain and the HADS anxiety and depression subscales. Finally, the proportion of women in each widespread chronic pain group meeting the clinical cut-off for the subscales was investigated.

5.2.5.4. PROMIS Fatigue and sleep

Fatigue and sleep measures were previously described in the methods chapter (Chapter 2)[13]. An ANOVA and post-hoc linear test for trend was performed to compare fatigue and sleep scores between widespread pain groups, and as an ordinal variable.

5.2.5.5. SF-36 questionnaire

Prior to performing the analyses on the SF-36 questionnaire, participant responses were scored by a colleague. Scored questionnaire responses were compared between widespread pain groups and controls appropriately.

5.2.6. Measures of pain quality

Questionnaires administered to better understand pain were given only to chronic pain participants and not healthy controls, as based on inclusion criteria it was assumed that healthy controls were not living with CPP.

5.2.6.1. painDETECT

The painDETECT questionnaire was used to assess the underlying neuropathic component of participants' pain. The characteristics of this questionnaire were previously outlined in the general methods chapter (Chapter 2). The median painDETECT scores between widespread pain groups were compared, and the percentage of participants in each widespread pain group meeting the clinical cut-off in each of the painDETECT subscales

(i.e. burning, tingling, light touch, pain attacks, cold/heat, numbness, pressure) were further summarised.

5.2.6.2. Pelvic pain severity

In the follow-up questionnaire, participants were asked to complete questions about their pelvic pain duration and intensity. They were asked to rate their pain on an NRS scale from 0 (no pain) to 10 (worst imaginable pain). The following questions were included in this analysis:

i) Please rate how severe your period pain was at its worst in the last 12 months
ii) Please rate how severe your pain was at its worst during the last time you had vaginal intercourse/penetration
iii) Please rate how severe your pain was at its worst in the 24 hours after the last time you had vaginal intercourse/penetration
iv) Please rate how severe your pelvic pain was at its worst in the last three months
v) Please rate how severe your pelvic pain was on average in the last three months
vi) Please rate your worst bladder pain in the last 7 days

5.2.6.3 Comorbid conditions

Participants were asked to self-report any comorbidities they had. This included, but was by no means limited to, diagnoses such as asthma, anxiety, depression, polycystic ovarian syndrome, irritable bowel syndrome, rheumatoid arthritis, etc. For the analyses, the prevalence of each self-reported condition amongst the widespread pain groups was

described, and multi-morbidity (at least one additional condition on top of the diagnoses used to classify TRiPP participants) between groups was summarised. The frequency of each condition, and the proportion experiencing multimorbidity, were analysed using the chi square test for trend across the pain spread groups.

5.2.7. Psychophysical testing

5.2.7.1 Quantitative sensory testing

The full methodology for the quantitative sensory testing (QST) paradigm was previously described in the general methods chapter (Chapter 2). For the analyses in this chapter, QST measures at the control site between groups were plotted and described between groups.

5.2.7.2 Conditioned pain modulation

The CPM paradigm used in TRiPP, and the statistics used to determine the CPM effect were described earlier in the general methods chapter (Chapter 2).

5.2.8. Data analysis

Data were analysed with GraphPad PRISM 9. The Shapiro-Wilk test was used to test the normality assumption. Normally distributed continuous variables were compared using an ANOVA, with a post-hoc linear test for trend. Data which were not normally distributed were compared between groups using the non-parametric Jonckheere Terpstra test for linear trend between the widespread pain groups or the non-parametric Kruskal-Wallis

test[13,14]. The chi-square test for trend was used to compare proportions between widespread pain groups. The appropriate parametric or non-parametric correlation test was used to compare continuous variables against the number of extra-pelvic pain regions. Post-hoc tests with a Bonferroni correction were performed. Values of $p<0.05$ were considered to be significant, however the appropriate p-values considering multiple comparisons were listed in each of the individual figures.

As part of the TRiPP data analysis group, I designed analysis plans for both this widespread pain analysis and the CPM work presented in Chapter 6. This work was presented to the working group, comprised of leaders in psychophysical pain testing, clinical aspects of pain, women's health and epidemiology. It was then discussed, modified after group feedback, and approved before commencing the analyses.

5.3. Results

5.3.1. Participant characteristics

One hundred and fifty-eight women took part in the follow-up part of the TRiPP study involving comprehensive questionnaires. This was comprised of n=108 chronic pelvic pain participants and n=50 healthy controls. The TRiPP study group allocation amongst the 108 chronic pain participants was as follows: n=43 EAP, n=26 BPS, n=26 EABP, and n=13 PP. The median (IQR) age of the entire TRiPP cohort was 32 (12) years old. The chronic pain participants were slightly older than healthy controls (controls, 29.5 (10) vs chronic pain, 33 (13.75), p=0.049). The total number of participants included in each of the analyses included in this chapter can be found in **Appendix 5C**.

5.3.2. Pain spread in TRiPP

As shown in **Table *5.1***, some of the most prevalent regions impacted by pain amongst the entire TRiPP cohort were the abdomen, pelvis, lower back, neck, and head. There were significant differences in the number of pain regions impacted across TRiPP study groups (X^2=32.03, p<0.0001, **Figure *5.3***). After performing a Dunn's post hoc test for multiple comparisons, the significant differences were between healthy controls and EAP (p=0.0001) and EABP (p<0.0001). There were no significant differences between the other groups. The body maps illustrating the percentage of women with pain in each body region, by TRiPP study group, can be found in **Appendix 5B**.

Table 5.1. Number of participants (%) reporting pain in that region of the body by study group (L-indicates left side of the body, R-indicates right side of the body). **Continued on next page.**

Body Region (n, %)	Healthy Controls (n=50)	EAP (n= 43)	EABP (n=26)	BPS (n=26)	PP (n=13)	Entire Pain Cohort (n=108)
Head	4 (8)	11 (25.6)	7 (26.9)	3 (11.5)	1 (7.7)	22 (20.4)
face	1 (2)	5 (11.6)	3 (11.5)	2 (7.7)	0	10 (9.3)
Rjaw	3 (6)	2 (4.7)	4 (15.4)	1 (3.8)	0	7 (6.5)
Ljaw	3 (6)	4 (9.3)	3 (11.5)	1 (3.8)	0	8 (7.4)
neck	5 (10)	13 (30.2)	7 (26.9)	9 (34.6)	1 (7.7)	30 (27.8)
Rchest	1 (2)	2 (4.7)	3 (11.5)	0	0	5 (4.6)
Lchest	0	6 (14)	4 (15.4)	1 (3.8)	0	11 (10.2)
upperback	4 (8)	9 (20.9)	7 (26.9)	6 (23.1)	2 (15.4)	24 (22.2)
lowerback	4 (8)	17 (39.5)	16 (61.5)	13 (50)	3 (23.1)	49 (45.4)
Rshoulder	2 (4)	9 (20.9)	10 (38.5)	5 (19.2)	0	24 (22.2)
Lshoulder	0	9 (20.9)	7 (26.9)	4 (15.4)	1 (7.7)	21 (19.4)
Rupperarm	0	2 (4.7)	3 (11.5)	1 (3.8)	1 (7.7)	7 (6.5)
Lupperarm	0	1 (2.3)	1 (3.8)	0	0	2 (1.9)
Relbow	0	3 (7)	2 (7.7)	1 (3.8)	1 (7.7)	7 (6.5)
Lelbow	0	3 (7)	2 (7.7)	0	0	5 (4.6)
Rlowerarm	0	2 (4.7)	1 (3.8)	2 (7.7)	0	5 (4.6)

Body Region (n, %)	Healthy Controls (n=50)	EAP (n= 43)	EABP (n=26)	BPS (n=26)	PP (n=13)	Entire Pain Cohort (n=108)
Llowerarm	0	2 (4.7)	1 (3.8)	0	0	3 (2.8)
Rwrist	1 (2)	5 (11.6)	2 (7.7)	3 (11.5)	0	10 (9.3)
Lwrist	1 (2)	4 (9.3)	2 (7.7)	2 (7.7)	0	8 (7.4)
abdomen	4 (8)	19 (44.2)	18 (69.2)	10 (38.5)	1 (7.7)	48 (44.4)
pelvis	3 (6)	26 (60.5)	18 (69.2)	17 (65.4)	4 (30.8)	65 (60.2)
Rhip	1 (2)	6 (14)	9 (34.6)	5 (19.2)	1 (7.7)	21 (19.4)
Lhip	3 (6)	5 (11.6)	11 (42.3)	5 (19.2)	1 (7.7)	22 (20.4)
Rgroin	2 (4)	8 (18.6)	8 (30.8)	2 (7.7)	2 (15.4)	20 (18.5)
Lgroin	2 (4)	10 (23.3)	10 (38.5)	2 (7.7)	1 (7.7)	23 (21.3)
Rbuttock	1 (2)	4 (9.3)	6 (23.1)	2 (7.7)	0	12 (11.1)
Lbuttock	2 (4)	1 (2.3)	8 (30.8)	1 (3.8)	0	10 (9.3)
Rupperleg	1 (2)	4 (9.3)	8 (30.8)	0	0	12 (11.1)
Lupperleg	1 (2)	3 (7)	8 (30.8)	1 (3.8)	0	12 (11.1)
Rknee	4 (8)	8 (18.6)	5 (19.2)	1 (3.8)	1 (7.7)	15 (13.9)
Lknee	4 (8)	5 (11.6)	5 (19.2)	2 (7.7)	1 (7.7)	13 (12.0)
Rlowerleg	2 (4)	3 (7)	6 (23.1)	1 (3.8)	0	10 (9.3)
Llowerleg	2 (4)	3 (7)	5 (19.2)	2 (7.7)	0	10 (9.3)
Rankle	1 (2)	4 (9.3)	3 (11.5)	4 (15.4)	1 (7.7)	12 (11.1)
Lankle	0	7 (16.3)	3 (11.5)	2 (7.7)	0	12 (11.1)

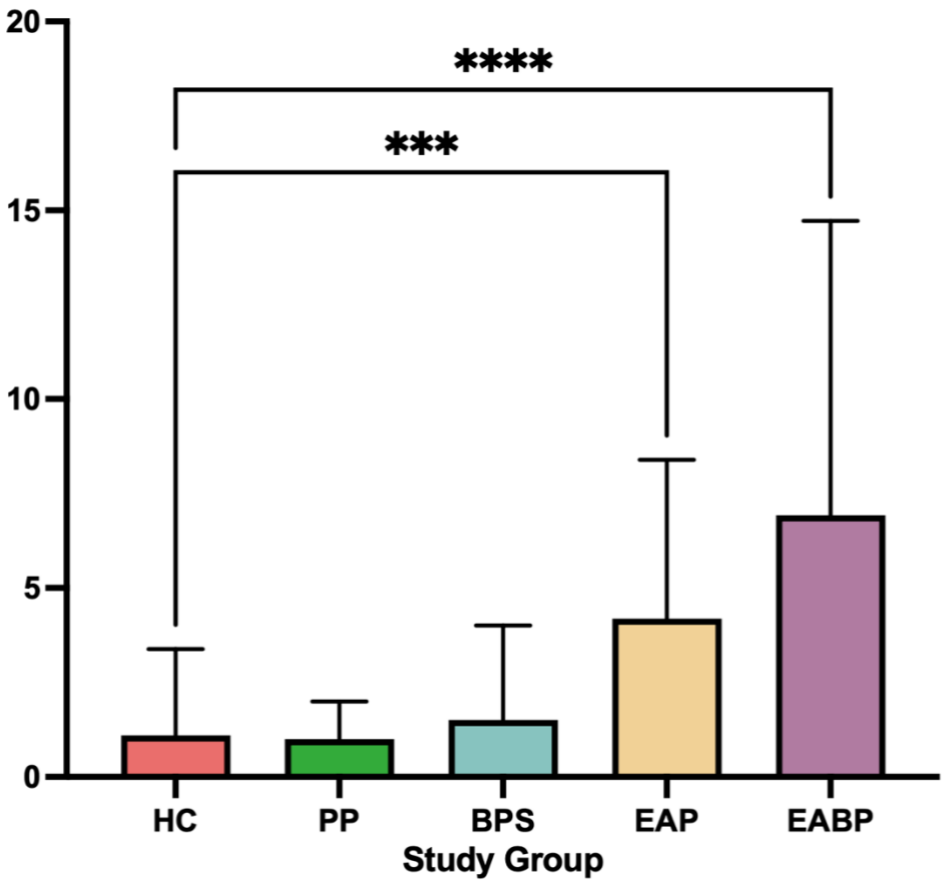


Figure 5.3. Number (median, IQR) of extra-pelvic regions affected by pain by TRiPP study group. Kruskal-Wallis test was performed to compare between groups. HC; healthy controls, PP; pelvic pain, BPS; bladder pain syndrome, EAP; endometriosis-associated pain, EABP; endometriosis-associated pain and bladder symptoms. ***p<0.001, ****p<0.0001.

5.3.3. Characterising pain spread according to the MaPP protocol

N=33 (30.6%) of TRiPP pain participants had pain only in their pelvis ('isolated'), n=31 (28.7%) had 1-2 additional regions ('intermediate'), and n=44 (40.7%) had 3-7 regions ('widespread'). As shown in **Table *5.2***, the most common MaPP reported regions impacting chronic pain participants (n=108) were back (50.9%), left leg (40.7%), right leg (38.0%), and

head (38.0%). All four TRiPP pain groups were represented in each of the MaPP widespread pain groups (**Table *5.3***, **Figure *5.4***). N=15 (34%) of participants with the widespread pain phenotype also met the clinical cut-off for a fibromyalgia diagnosis. There was a significant difference in participant age between the widespread pain groups and controls (p=0.045, **Table *5.4***. Participant age by widespread pain group).

Table 5.2. Number of participants reporting pain in the MaPP-coded pain regions in entire pain group of TRiPP (n=108), no=number (%) of participants who did not select that region, yes=number (%) of participants who selected that region as painful. MBM; Michigan body map. R indicates right side, L indicates left side of the body.

Variable	MBM regions included	No (n,%)	Yes (n,%)
MBM_Back	upperback, lowerback	53 (49.1)	55 (50.9)
MBM_Head	rjaw, ljaw, head, neck, face	67 (62.0)	41 (38.0)
MBM_Rightleg	rhip, rgroin, rbuttock, rupperleg, rknee, rlowerleg, rankle	67 (62.0)	41 (38.0)
MBM_Leftleg	lhip, lgroin, lbuttock, lupperleg, lknee, llowerleg, lankle	64 (59.3)	44 (6.7)
MBM_Rightarm	rlowerarm, relbow, rupperarm, rwrist	94 (87.0)	14 (13.0)
MBM_Leftarm	llowerarm, lelbow, lupperarm, lwrist	99 (91.67)	9 (8.33)
MBM_Trunk	rchest, lchest	97 (89.81)	11 (10.19)

Table 5.3. Number of participants per MaPP pain group, by TRiPP study group. Isolated; 0 extra-pelvic regions, Intermediate; 1-2 additional extra-pelvic regions, Widespread; 3-7 additional regions. PP; Pelvic pain only, BPS; bladder pain syndrome, EAP; endometriosis-associated pain, EABP; endometriosis and bladder pain symptoms.

Study Group	**Isolated (n, %)**	**Intermediate (n, %)**	**Widespread (n, %)**
PP (n=13)	6 (17.14)	6 (18.18)	1 (2.5)
BPS (n=26)	10 (28.57)	9 (27.27)	7 (17.5)
EAP (n=43)	13 (37.14)	11 (33.33)	19 (47.5)
EABP (n=26)	6 (17.14)	7 (21.21)	13 (32.5)
Entire pain cohort (n=108)	33 (30.6)	31 (28.7)	44 (40.7)

Table 5.4. Participant age by widespread pain group. Kruskal-Wallis test was used to compare between groups.

	Controls	Isolated	Intermediate	Widespread	p-value
Age (median, IQR)	29.5 (10)	29 (9)	33 (17)	36 (11)	0.045

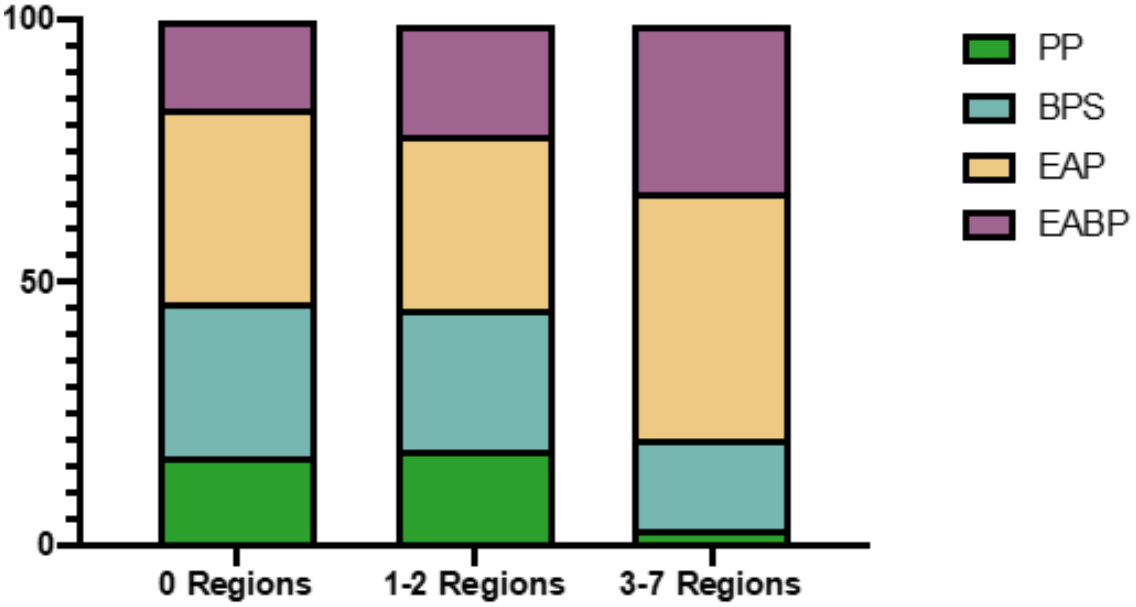


Figure 5.4. Percentage of MaPP-coded widespread pain group occupied by TRiPP pain subgroups. A greater percentage of bar chart occupied by each colour/study group indicates a higher percentage of participants from that study group in each widespread pain group. Only chronic pain participants were included in this figure. PP; pelvic pain, BPD; bladder pain syndrome, EAP; endometriosis-associated pain, EABP; endometriosis-associated pain and bladder symptoms.

In some of the analyses presented in the remainder of this chapter, the number of extra-pelvic regions rather than MaPP coded regions have been used, as this is a continuous variable. To ensure that this is representative of the MaPP coded regions, a correlation

between a participants MaPP coded regions and number of extra-pelvic regions from the MBM was performed. There was a very strong positive correlation between the two, providing assurance that using the two interchangeably is valid (rho=0.960, p<0.0001, **Figure *5.5***).

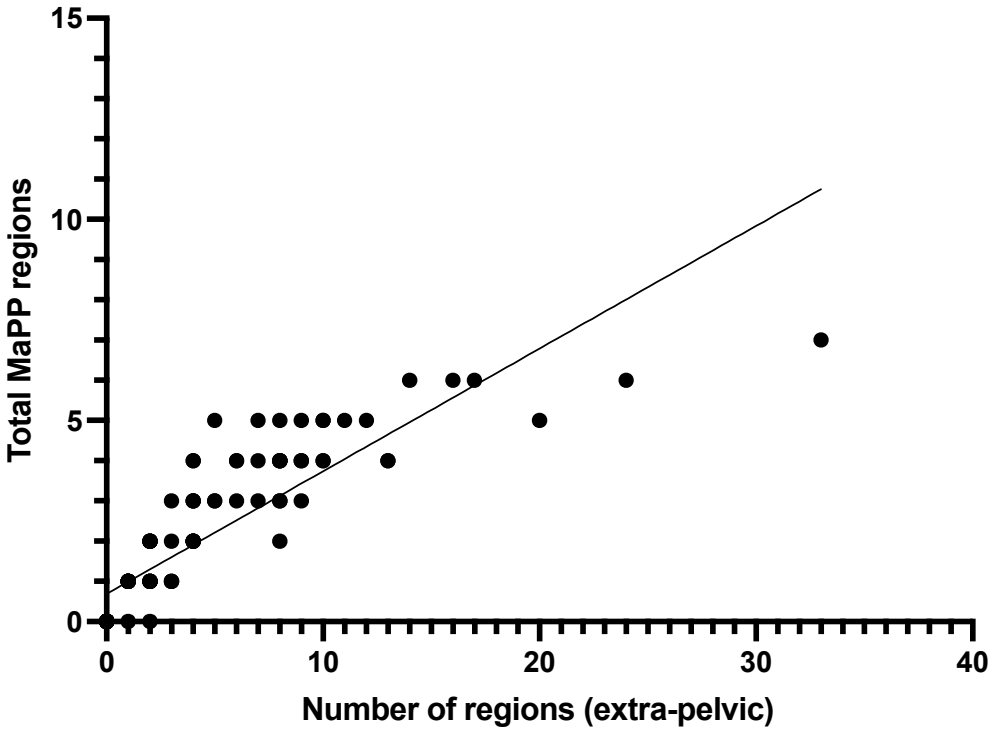


Figure 5.5. Spearman correlation between number of extra-pelvic pain regions identified on the Michigan body map, and total number of MaPP regions. Only chronic pain participants were included in this correlation analysis. Spearman rho=0.960, p<0.0001.

5.3.4. Psychological and general health measures

5.3.4.1. Pain catastrophizing scale

There was a significant difference between the widespread pain groups with respect to median pain catastrophizing scale (PCS) scores (X^2=32.40, p<0.0001), as seen in **Figure *5.6*a**. After performing a Dunn's post-hoc test for multiple comparisons, a significant association

was observed between healthy controls and the isolated ($p=0.031$), intermediate ($p=0.0073$), and widespread pain groups ($p<0.0001$).

In addition, there were significant differences in the rumination ($X^2=16.58$, $p=0.0009$), magnification ($X^2=27.03$, $p<0.0001$) and helplessness ($X^2=42.24$, $p<0.0001$) subscales. In all three cases, the most significant difference was between healthy controls and the widespread pain group ($p<0.0001$, **Figure *5.6*b-d**). There was only a weak positive correlation between the total number of extra-pelvic pain regions identified by all chronic pain participants, and the PCS score ($rho=0.296$, $p=0.01$).

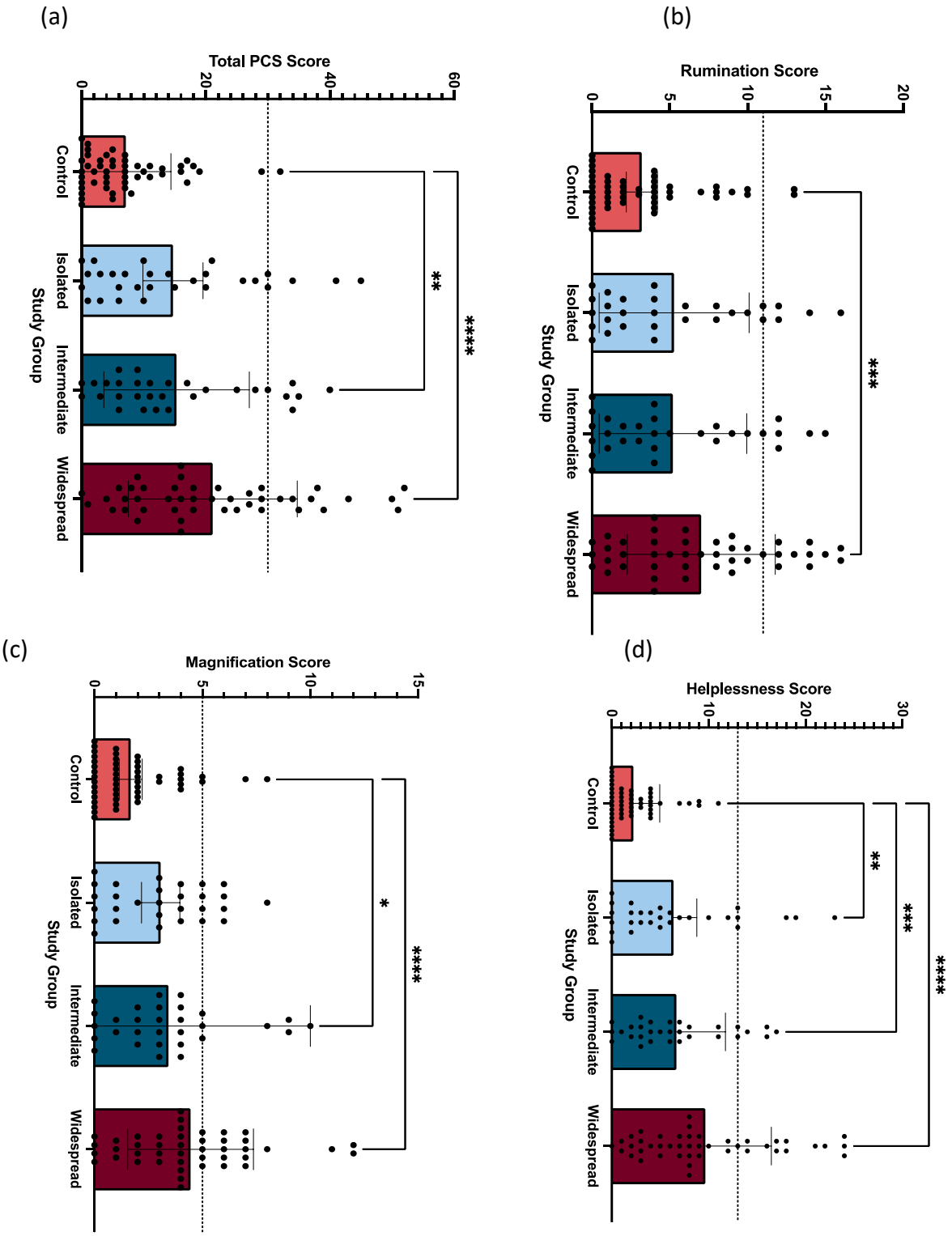
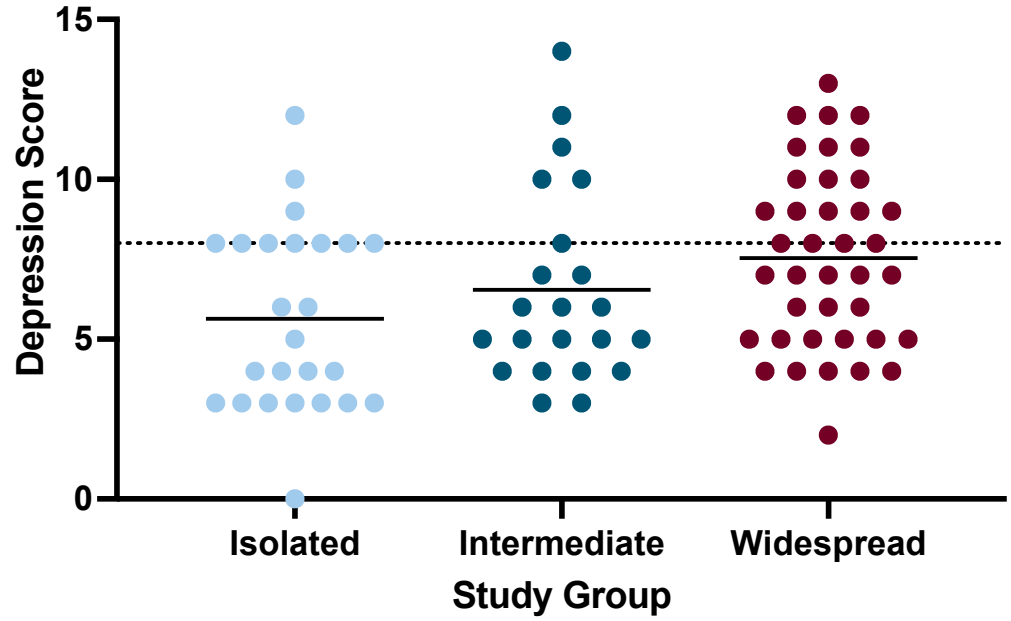


Figure 5.6. Pain catastrophizing scale (PCS) (total and subscales) by widespread pain groups and healthy controls. (a) Total PCS score compared between widespread pain groups and healthy controls. Y=30 indicates a PCS score of clinical significance. PCS subscales by widespread pain group (b) PCS-Rumination. (c) PCS-Magnification. (d) PCS-Helplessness. [Lines between two groups indicate a significant association, the number of asterisks corresponds to the level of significance; *p<0.05, **p<0.01, ***p<0.001,****p<0.0001]. Bars represent the mean and 95% confidence interval. An ANOVA was performed to compare values between groups.

5.3.4.2. Hospital Anxiety and Depression Scale

As shown in **Figure *5.7*A**, there were significant differences in the HADS-D (F=3.351, p=0.040) and HADS-A (F=7.106, p=0.0014, **Figure *5.7*B**) scores between widespread pain groups. There was additionally a significant linear trend between HADS-D and number of extra-pelvic regions impacted by pain (R^2=0.075, p=0.011), and an even stronger association between HADS-A and number of extra-pelvic regions (R^2=0.129, p=0.0007). Interestingly, many participants in the widespread pain group had a HADS-D score that was above the point of clinical significance, and the mean HADS-A score of both the intermediate and widespread pain groups exceeded the point of clinical significance.

(A)



(B)

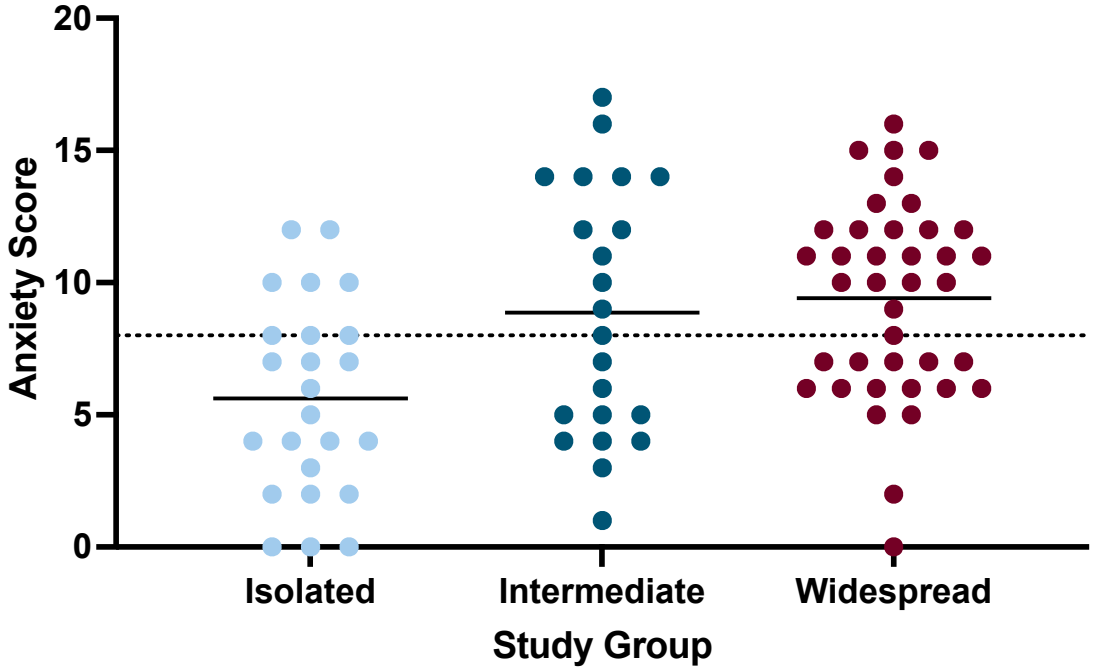


Figure 5.7. Hospital Anxiety and Depression Scale (HADS) by widespread pain groups. (A) HADS-D depression scores by widespread pain group, and (B) HADS-A anxiety scores by widespread pain group. The dotted line at y=8 on both figures represents a score of clinical significance. The bold line for each respective widespread pain group on each figure shows the group mean. Relationship between HADS-D and widespread pain; R^2=0.075, p=0.011, and between HADS-A and widespread pain; R^2=0.129, p=0.0007. An ANOVA was used to compare scores between groups, and a post-hoc linear test for trend.

5.3.4.3 Fatigue and sleep

There was a significant difference in the PROMIS fatigue scores between widespread pain groups (F=20.09, p<0.0001). After performing a post-hoc linear test for trend, there was a significant positive linear trend in the fatigue scores across ordinal widespread pain groups (y=4.292x, R^2=0.283, p<0.0001, **Figure *5.8***).

Similarly, there was a significant relationship between the PROMIS sleep scores and widespread pain (F=13.72, p<0.0001). There was a significant negative linear trend in the sleep scores across the widespread pain groups (y=-3.480x, R^2=0.223, p<0.0001, **Figure *5.9***).

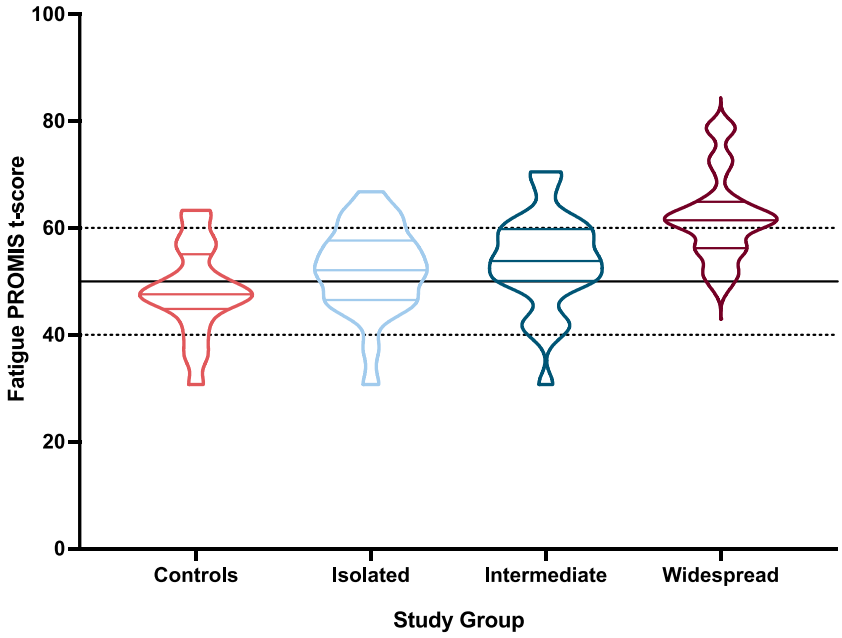


Figure 5.8. PROMIS fatigue score by widespread pain group (isolated, intermediate, and widespread pain groups) and controls. Y=50 is a US-standardised population mean, as indicated by the bold line, and +/-1 standard deviation is denoted by the dotted lines at y=40 and y=60. Higher scores indicate worse fatigue. Violin plots used above demonstrate the distribution of the data, as well as the median and interquartile range of the data, indicated by the bold lines with the individual plots. ANOVA F=20.09, p=$7.274x10^{-11}$.

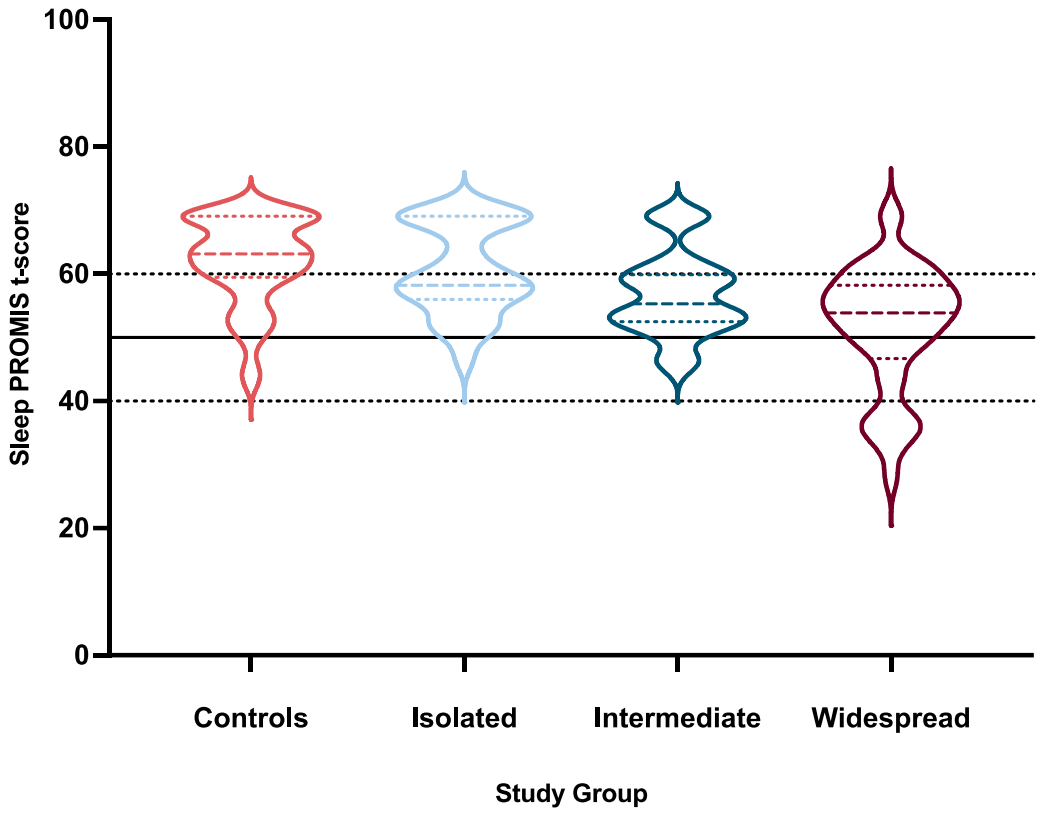


Figure 5.9. PROMIS sleep score by widespread pain groups (isolated, intermediate, and widespread pain group) and controls. Y=50 is a US-standardised population mean, as indicated by the bold line, and +/-1 standard deviation is denoted by the dotted lines at y=40 and y=60. Lower scores indicate worse sleep. Violin plots used above demonstrate the distribution of the data, as well as the median and interquartile range of the data, indicated by the bold lines with the individual plots. ANOVA F=13.72, p=7.217x10^{-008}.

Given how pronounced worse fatigue and poorer sleep were in the widespread pain group the relationship between sleep and fatigue in the widespread (3-7 regions) pain group was further investigated. There was only a weak-moderate negative correlation between sleep and fatigue measures (rho=-0.380, p=0.012, **Figure *5.10***).

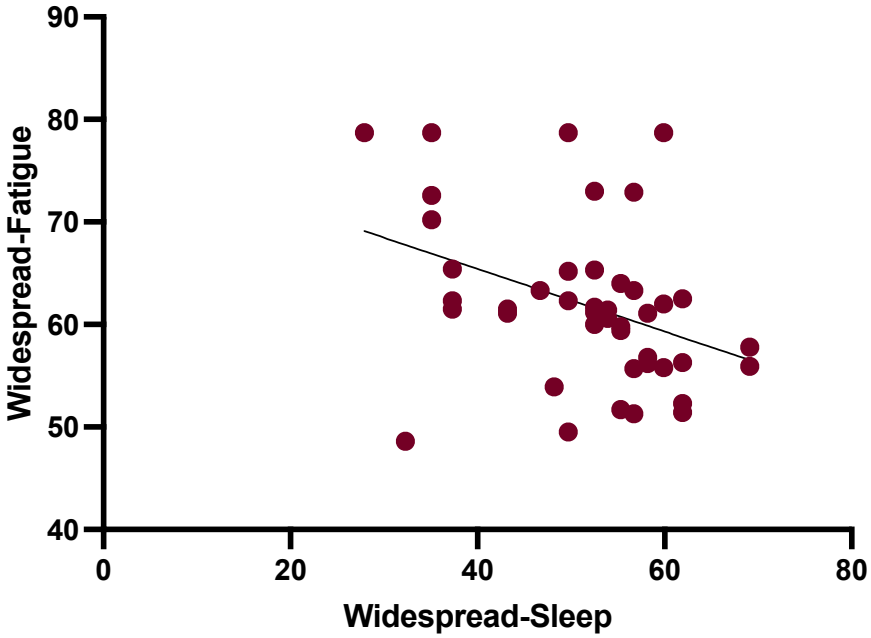


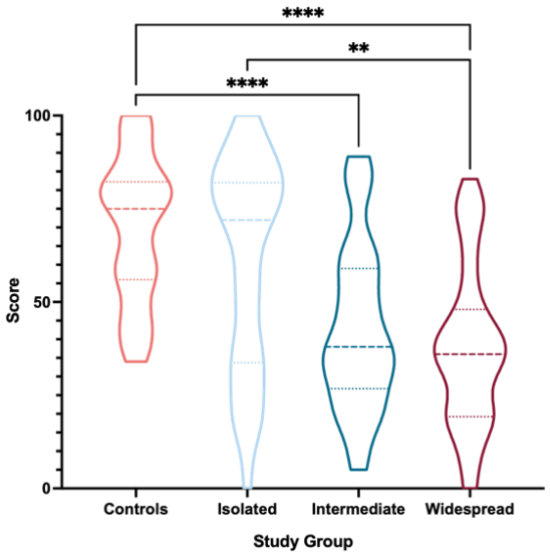
Figure 5.10. Spearman correlation between PROMIS fatigue and PROMIS sleep measures in the widespread pain group. *Rho*=-0.380, p=0.012.

5.3.4.4. SF-36

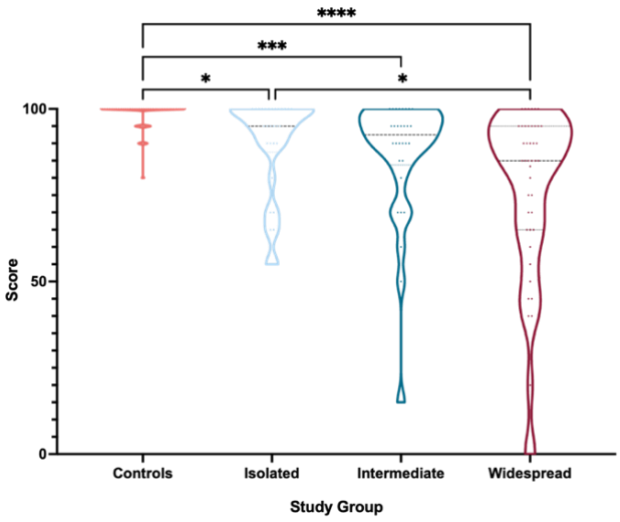
Widespread pain was associated with worse quality of life. There were significant differences in all eight measures of the SF-36 between the widespread pain groups, as shown in **Figure *5.11*a-9h**. In all cases, after post-hoc testing, there were significant differences between those with widespread pain and healthy controls. However, in general health, physical functioning, physical health limitations, fatigue, social functioning, and pain there were also significant differences between the widespread pain groups, as well as between the isolated/intermediate pain group and controls.

Figure 5.11. SF-36 questionnaire responses by widespread pain group and controls. Each figure depicts the following SF-36 subscales; (a) general health, (b) physical functioning, (c) physical health limitations, (d) emotional limitations, (e) energy/fatigue, (f) emotional well-being, (g) social functioning, (h) pain. Kruskal-Wallis test was used to compare between groups. [*p<0.05, **p<0.01, ***p<0.0001, ****p<0.0001]. **Continued on next page.**

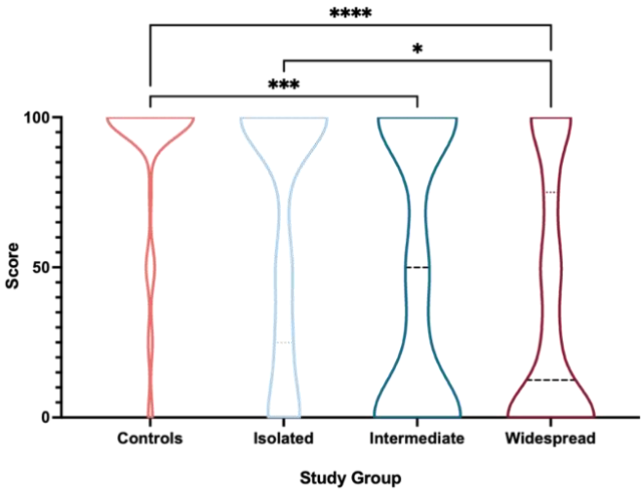
(a) General health



(b) Physical functioning



(c) Physical health limitations



(d) Emotional limitations

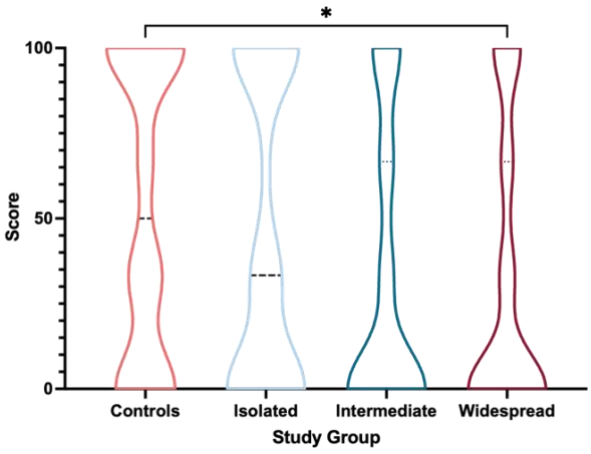
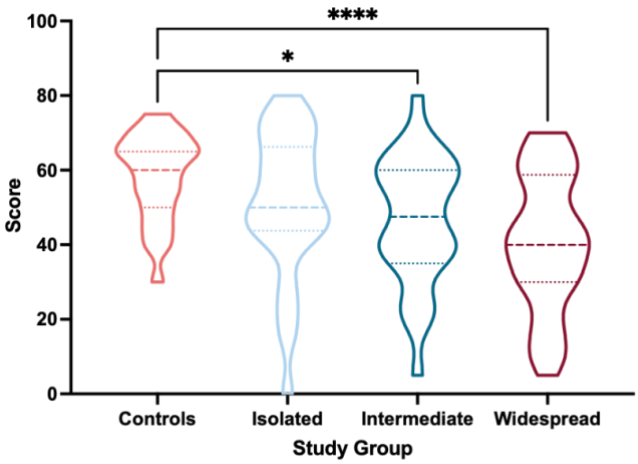
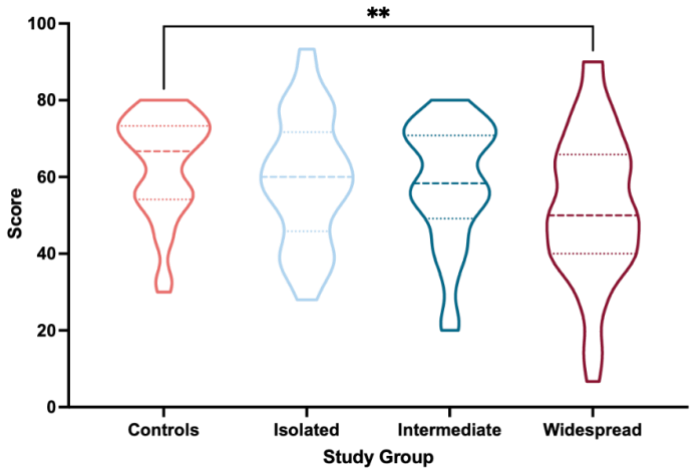


Figure 11 (continued)

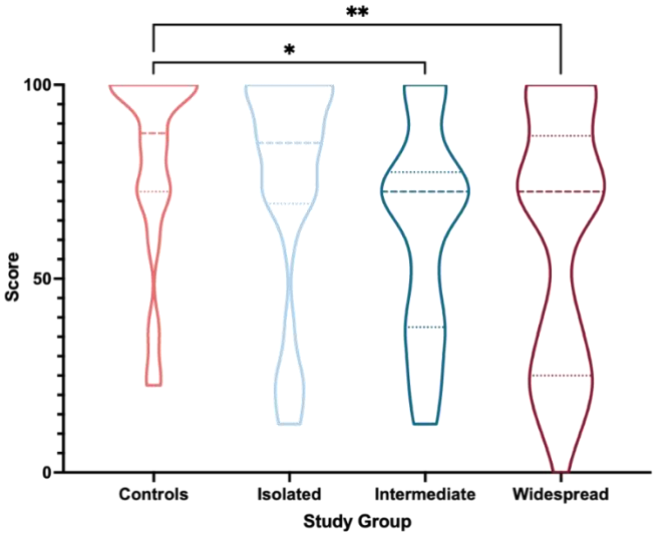
(e) Energy/fatigue



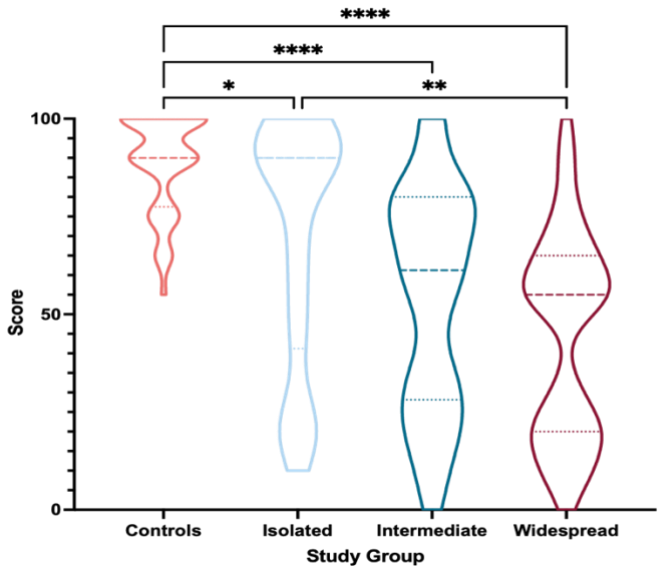
(f) Emotional wellbeing



(g) Social functioning



(h) Pain



5.3.5. Measurement of pain attributes and quality

5.3.5.1. painDETECT

There was a significant difference in the painDETECT scores between widespread pain groups; particularly between the widespread pain and isolated (p<0.0001), and widespread pain and intermediate groups (p=0.0005), as shown in **Figure *5.12***. There was no significant difference between the isolated and intermediate groups. Furthermore, there was a significant positive correlation between the painDETECT score and number of extra-pelvic regions impacted by pain in those with extra-pelvic pain (p<0.0001, **Figure *5.13***).

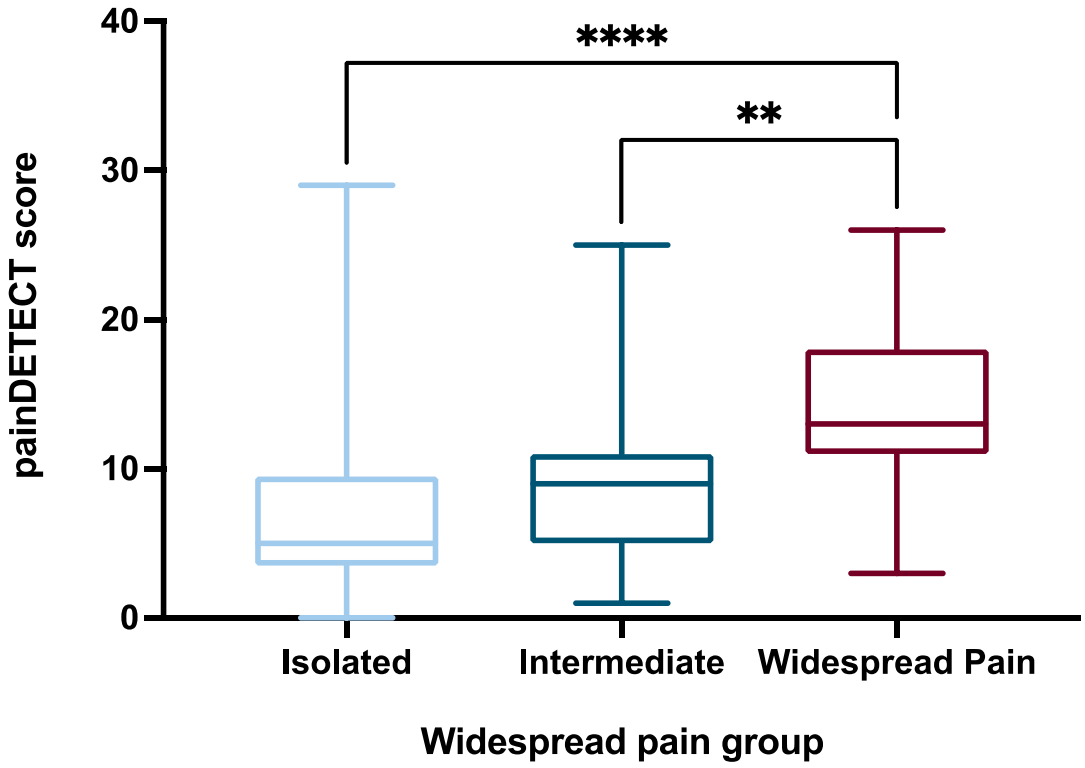


Figure 5.12. painDETECT score by widespread pain group. Kruskal-Wallis test was performed. [**p<0.01, ****p<0.0001]

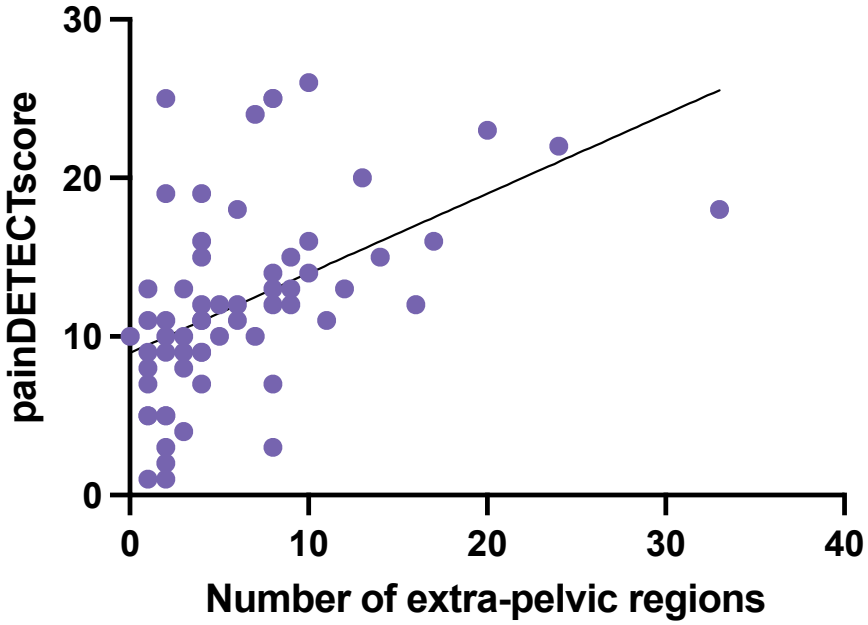


Figure 5.13. Spearman correlation between painDETECT score and number of extra-pelvic regions impacted by pain in the intermediate and widespread pain groups only. *Rho*=0.523, p<0.0001.

With respect to the painDETECT pain descriptors, a large proportion of the widespread pain group met the clinical cut-off for 'burning', 'pain attacks', and 'pressure', as shown in **Table *5.5***. These results should be interpreted with caution. There was a relatively high degree of missing data amongst all three groups (isolated; 31.4%, intermediate; 18.2%, widespread;15%). Participants with missing data have been excluded from the table below.

Table 5.5. Number (%) of participants who completed the questionnaire in each widespread pain group above the clinical cut-off for each painDETECT subscale

	painDETECT subscales						
	Burning	**Tingling**	**Light Touch**	**Pain Attacks**	**Cold/Heat**	**Numbness**	**Pressure**
Isolated (n=24)	4 (16.7)	3 (12.5)	0	8 (33.3)	0	2 (8.33)	3 (12.5)
Intermediate (n=27)	4 (14.8)	2 (7.4)	1 (3.7)	10 (37.04)	1 (3.7)	0	4 (14.8)
Widespread (n=34)	12 (35.3)	4 (11.8)	3 (8.83)	17 (50)	2 (5.9)	4 (11.8)	10 (29.4)

5.3.5.2. Pelvic pain severity

There were significant differences between the worst period pain experienced over the last twelve months (X^2=14.31, p=0.0025), but after post-hoc analysis, this was only significant between the controls and the widespread pain group (p=0.001), as described in **Table *5.6***. Similarly, post-hoc analyses revealed significant differences between only the healthy controls and widespread pain group (p=0.0194) in the self-reported worst pelvic pain in the last three months.

With respect to pain with sex, there were significant differences between groups for both (a) the worst pain the last time the participant had vaginal penetration (X^2=29.05, p<0.0001), and (b) in the 24 hours after the last time vaginal penetration was had (X^2=42.00, p<0.0001). Post-hoc analysis revealed that for (a), the significant differences

were between the widespread pain group and controls (p<0.0001), and the intermediate pain group and controls (p=0.116). For (b), the significant differences were again between the widespread pain group and controls (p=<0.0001), and the intermediate pain group and controls (p=0.0019). However, there was also a significant difference between the widespread pain group and those with isolated pain (p=0.0082).

Finally, there were significant differences in the worst bladder pain experienced in the last 7 days (X^2=35.10, p<0.0001). However, the significant differences were only between the widespread pain group and controls (p<0.0001), and the intermediate pain group and controls (p=0.0018).

Table 5.6. Pelvic pain symptom intensity scored out of 10, by widespread pain group. Median (IQR) are reported.

Pelvic Pain Symptom	**Widespread pain group**			
	Controls	**Isolated**	**Intermediate**	**Widespread**
Worst period pain experienced over last 12 months	4.5 (3)	7 (5)	6.5 (3)	8 (4)
Worst pelvic pain in the 3 three months	3 (3)	7 (7.5)	7 (2)	8 (2)
Worst pain experienced with most recent vaginal penetration	1 (2)	3.5 (6.5)	5 (5.5)	7 (4)
Worst pain experienced in the last 24 hours post-vaginal penetration	1 (0)	1.5 (4.75)	5 (6)	6 (6)
Worst bladder pain experienced in last 7 days	0 (0)	0 (4)	1 (6)	3 (7)

5.3.5.3. Comorbid conditions

Comorbid conditions were highly prevalent in the TRiPP cohort, as shown in **Figure *5.14***. The dotted line at y=10% indicates the threshold for being considered a 'common' comorbid condition. Amongst all the comorbid conditions, asthma, depression, fibromyalgia syndrome, high blood pressure, irritable bowel syndrome (IBS), migraine and spine problems were most prevalent in the widespread pain group. The prevalence of IBS significantly increased across the pain spread groups, but there was no significant difference in any of the other diagnoses (X^2=9.03, p=0.0027). 90% of all women in the widespread pain group had at least one comorbid condition, and the proportion experiencing multimorbidity significantly increased as pain became more spread (X^2=7.35, p=0.0067), as shown in **Figure *5.15***.

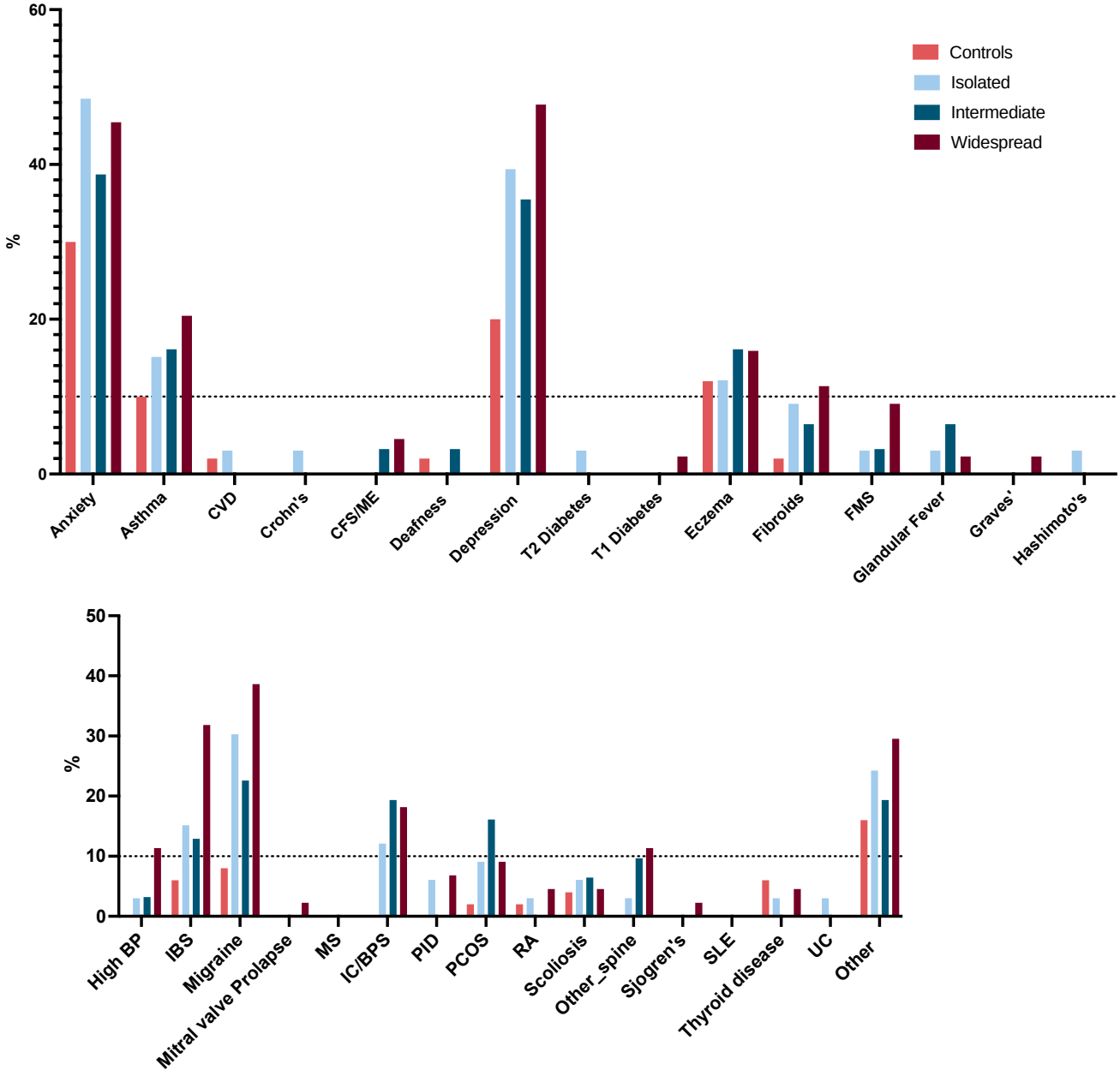


Figure 5.14. Self-reported comorbid condition prevalence (%) by widespread pain group and controls. CVD; cardiovascular disease, CFS/ME; chronic fatigue syndrome/myalgic encephalomyelitis, T1/T2 diabetes; type 1 or type 2 diabetes, FMS; fibromyalgia syndrome, High BP; high blood pressure, IBS: irritable bowel syndrome, MS; multiple sclerosis, IC/BPS; interstitial cystitis/bladder pain syndrome, PID; pelvic inflammatory disease, PCOS; polycystic ovarian syndrome, RA; rheumatoid arthritis, SLE; systemic lupus erythematosus, UC; ulcerative colitis, Other; free-text option. Dotted line at 10% indicates the percentage at which a disease could be considered 'common' in this cohort.

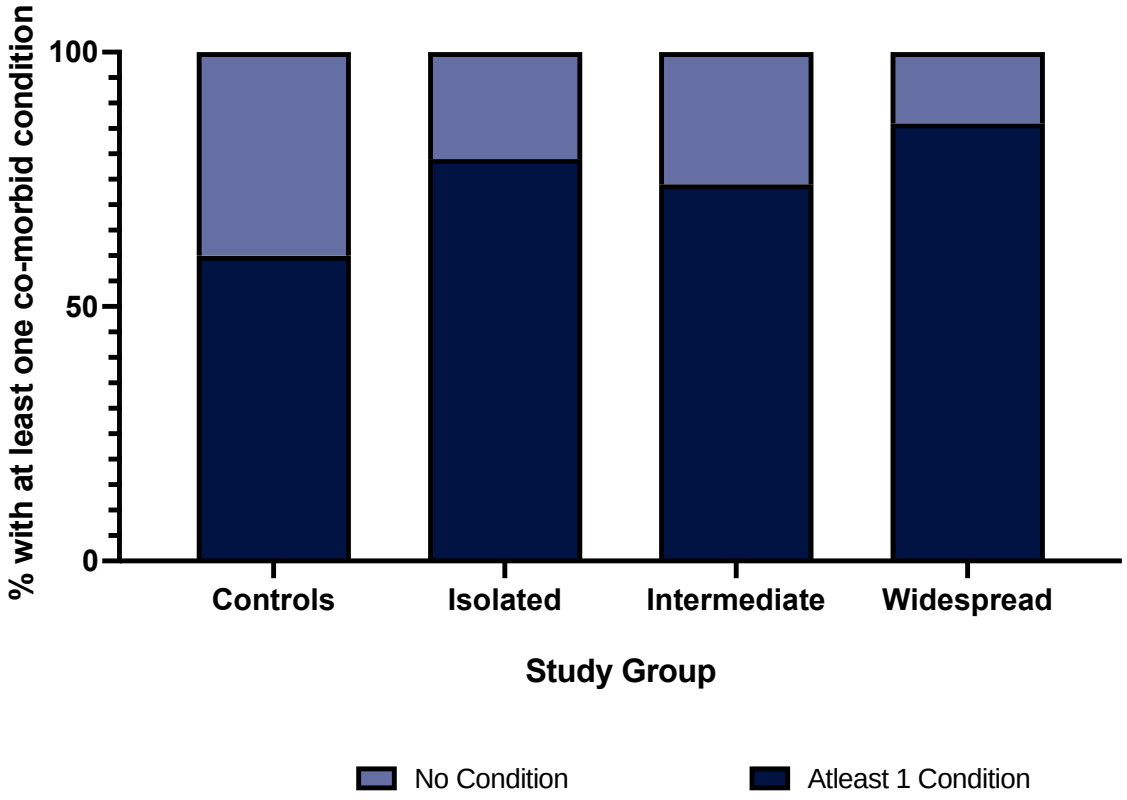


Figure 5.15. Multimorbidity in the widespread pain groups; the % of each group self-reporting at least one comorbid condition. There was a significant increasing trend in the proportion of women in each group reporting at least one condition (X^2=7.35, p=0.0067)

5.3.6. Psychophysical testing

Only a subset (n=85) of women completed the psychophysical testing paradigm; n=59 with CPP and n=26 CON. All CON participants and twenty-four CPP participants were recruited from Boston, USA. Twenty-four CPP participants were recruited from Oxford, UK, and eleven participants from Porto, Portugal. Psychophysical testing participants were comprised of the following TRiPP subgroup allocations: n=6 PP, n=25 EAP, n=14 BPS, n=14 EABP. Twenty-nine of the CPP participants fell into the widespread pain category, fifteen into the intermediate, and fourteen into the isolated pain group.

The median (IQR) age (years) at the time of the psychophysical testing visit was 29.5 (12.5) for controls and 34 (13) for CPP participants (p=0.124).

5.3.6.1. Quantitative sensory testing

Exemplified in **Figure *5.16***, which shows the group means for each of the QST parameters, there was a significant loss of function in heat pain threshold in the intermediate pain group, and a loss of function in the vibration detection threshold in the widespread pain group. Interestingly, there was a significant gain of function in the dynamic mechanical allodynia in the widespread pain group.

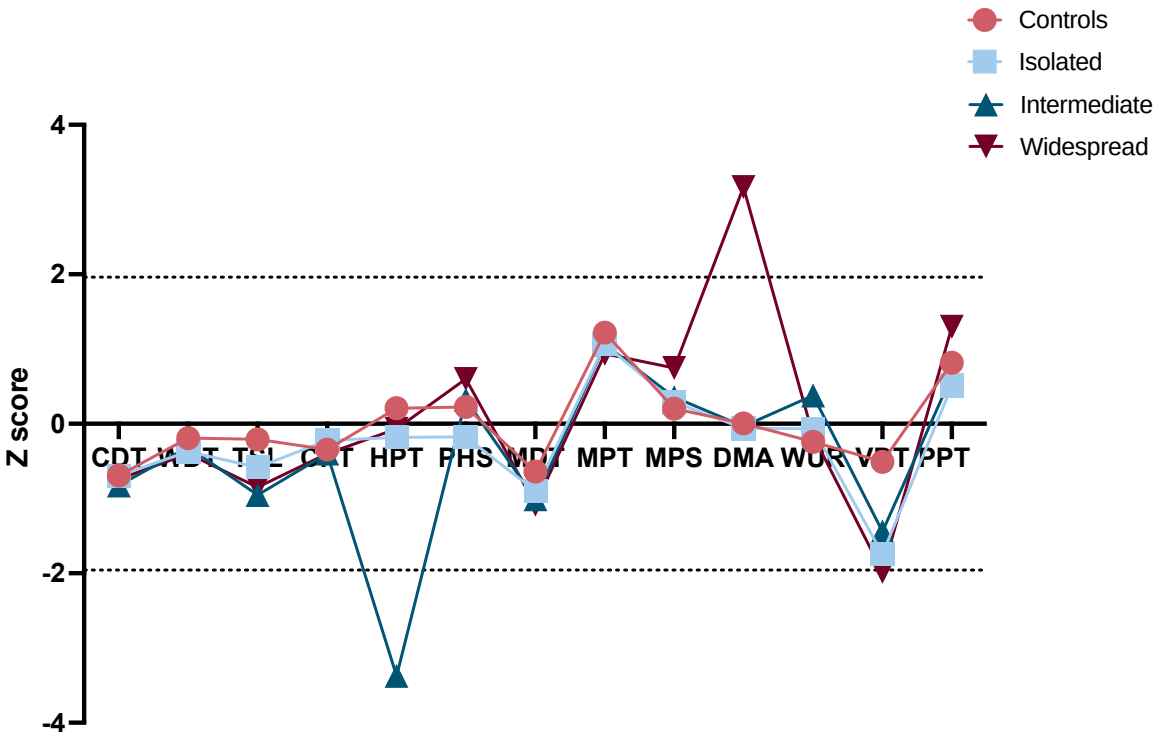


Figure 5.16. Group means for each QST parameter, by widespread pain grouping, at the control site (foot). A significant gain of function is indicated if the symbol is above the top dotted line (>+1.96), and a loss of function; a symbol below the bottom dotted line (<-1.96). CDT; cold detection threshold, WDT; warm detection threshold, TSL; thermal sensory limen, CPT; cold pain threshold, HPT; heat pain threshold, PHS; paradoxical heat sensation, MDT; mechanical detection threshold, MPT; mechanical pain threshold, MPS; mechanical pain sensitivity, DMA; dynamic mechanical allodynia, WUR; windup ratio, VDT; vibration detection threshold, PPT; pressure pain threshold.

5.3.6.2. Conditioned pain modulation

As shown in **Figure *5.17*A-D** there was variability in the CPM response amongst widespread pain groups. The range of the absolute CPM effect was determined using the difference between the average of the pressure pain threshold (PPT) after (PPT2) and before (PPT1) the conditioning stimulus. It was calculated as $PPT2_{average}$-$PPT1_{average,}$ and the mean effect in the widespread pain groups were as follows: controls (3.2), isolated (2.57), intermediate (1.43), widespread (2.93). On the plots below PPT3 was also plotted, which represents a third average pressure pain threshold which was assessed 10 minutes after the PPT2. Interestingly, there was no significance between group difference in the absolute or % change CPM response, using PPT2 and PPT1 data ($F=0.121$, $p=0.948$).

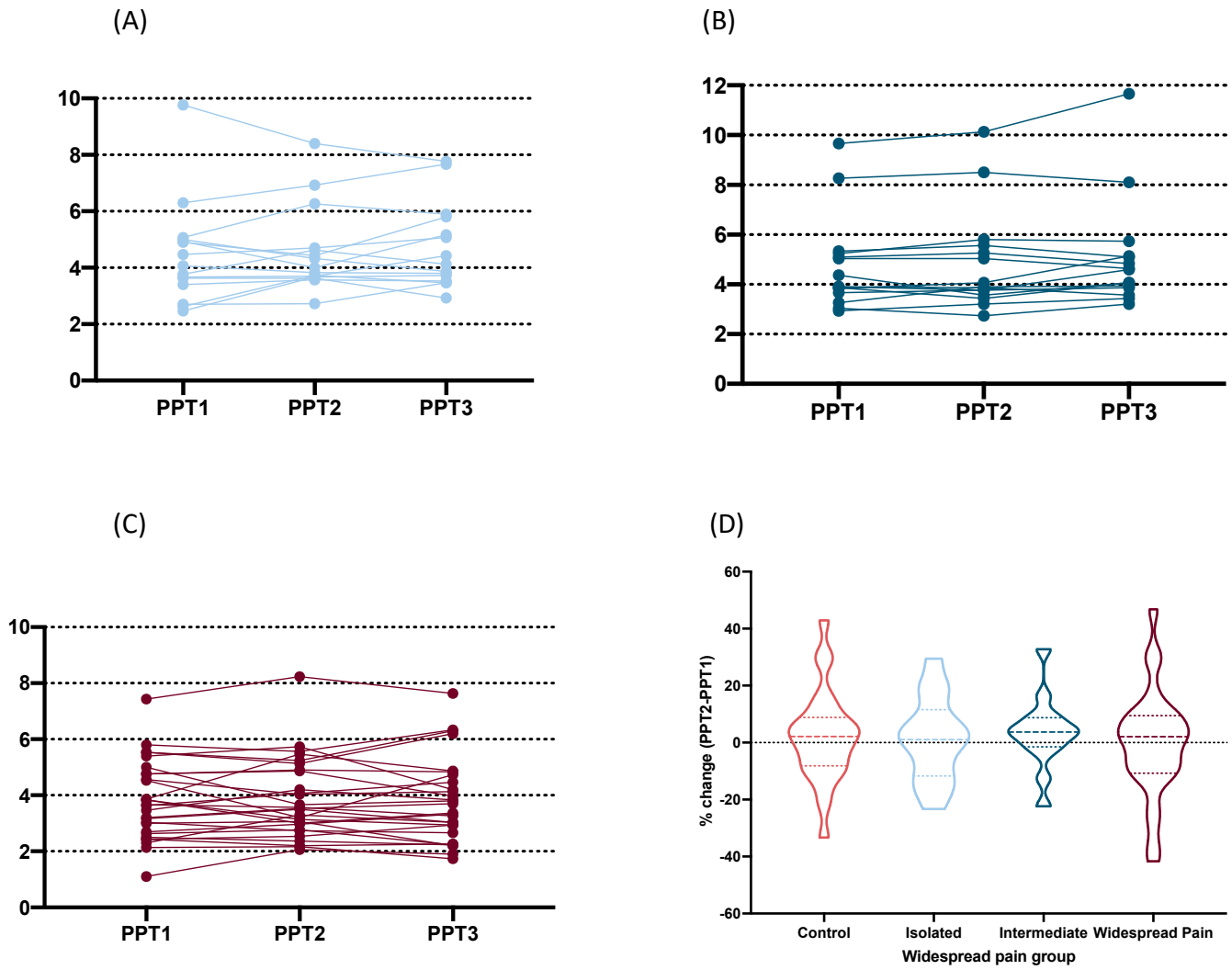


Figure 5.17. Conditioned pain modulation paradigm pressure pain thresholds before (PPT1) and after (PPT2) the conditioning stimulus, or ten minutes after PPT2 (PPT3), amongst (a) isolated (n=15), (b) intermediate (n=16) and (c) widespread pain (n=28) groups. The CPM effect, as determined by the percent change, by widespread pain group has been plotted (d).

5.4. Sensitivity & secondary analyses

While a large proportion of the CPP group was classified as having widespread pain, I wanted to investigate the number of participants in the widespread pain group falling into each of the MaPP coded regions (three, four, five, six, or seven). This was done to ensure

that while I was rating everyone with 3-7 regions as 'widespread', there was a spread of scores and everyone was not rated as a three or seven, for example. As shown below in **Figure *5.18***, there was participant representation in each of the 3-7 widespread pain regions.

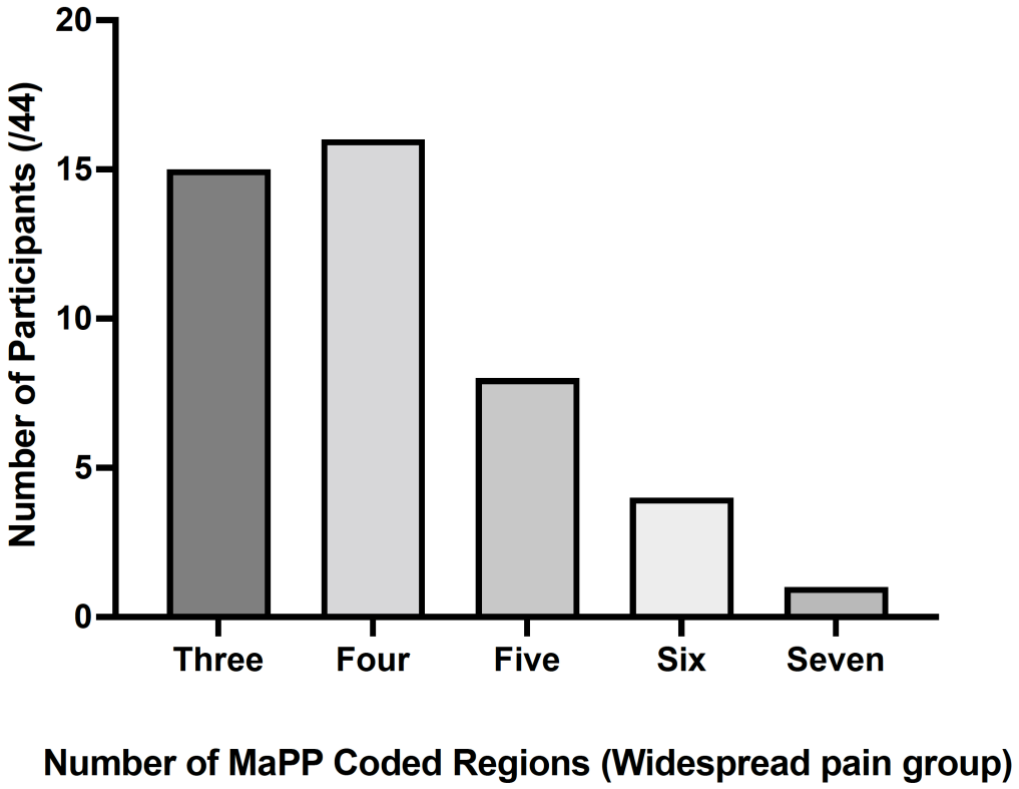


Figure 5.18. Distribution of MaPP coded pain regions in the widespread pain group; number (out of n=44 widespread pain participants) in the MaPP coded region.

5.5. Discussion

5.5.1. Overview

As hypothesised, the TRiPP cohort could be characterised according to extra-pelvic pain. While the group most impacted by widespread pain was the EABP group, it was clear that extra-pelvic pain was prevalent in all TRiPP study groups.

Widespread pain was highly burdensome in the cohort, with more than 60% experiencing pain in at least one of the seven MaPP-coded widespread pain regions. In line with previous work investigating widespread pain in localised pain conditions[15], and in further replication of the first experimental chapter, widespread pain was associated with worse psychological health in women with CPP[10,16]. Additionally, women with more widespread pain experienced higher levels of fatigue, and poorer sleep.

Interestingly, only a subset of participants with widespread pain met the criteria for fibromyalgia syndrome using the fibromyalgia scale. Thus, suggesting that while many with widespread pain have fibromyalgia, most women with widespread pain in TRiPP were living with a widespread pain phenotype, exclusive of clinical features of fibromyalgia.

Contrary to my hypothesis and previously published literature [17–21], there were few perturbations revealed in psychophysical testing which could distinguish the widespread pain group from the other pain groups and controls. With respect to QST testing on the foot, participants with a widespread pain phenotype showed a gain of function in DMA, and a loss of function in the VDT. Furthermore, participants in the intermediate pain group

showed loss of function in the HPT. With respect to CPM, there were no significant differences in the CPM effect between widespread pain groups.

5.5.2. Psychological impact of widespread pain

Widespread pain was associated with higher anxiety and depression scores, although the association with depression may be less clinically relevant in this group. In a cohort of women with abdominal pain, anxiety has been shown to be associated with widespread pain and was in fact a risk factor for its development[22]. Furthermore, depression was significantly worse in the widespread pain group, which was shown in the first experimental chapter (Chapter 3), as well as work by MaPP in participants with UCPPS[10]. Since the TRiPP study was cross-sectional, I was unable to determine directionality of these associations. However, a bi-directional relationship[23] between psychiatric comorbidity or higher anxiety/depression scores and widespread pain are plausible: anxiety may increase the risk of widespread pain but may also occur because of it.

Further, as in my first chapter, pain catastrophizing scores were highest in the widespread pain group. This phenomenon has been previously shown in patients with fibromyalgia; higher PCS scores were associated with increasing pain widespreadedness[24]. Again, pain catastrophizing is a risk factor for widespread pain[25,26], but may also increase as a result of more areas impacted by pain.

5.5.3. Impact of widespread pain on general health and quality of life

Using the SF-36 questionnaire, I showed that participants with widespread pain had the worst scores for all questionnaire subscales. Widespread pain has been previously associated with worse quality of life[10,27], and the SF-36 has previously been used as a tool to distinguish between participants with fibromyalgia, widespread pain only, and healthy controls[28].

Additionally, there was a significant trend between worse sleep, as well as more fatigue and widespread pain. Poor or unrefreshing sleep has been shown to be a risk factor for widespread pain in older adults[29]. Furthermore, widespread pain has been shown to increase the risk of insomnia onset in participants with rheumatological diseases[30], which suggests a bidirectional relationship between poor sleep and widespread pain.

Fatigue is also a hallmark symptom of fibromyalgia[31]. Only a portion of those with widespread pain met the clinical criteria for fibromyalgia, so therefore this could be the underlying cause in this subset. However, it is possible that there are distinct underlying mechanisms in participants with widespread pain which increase the risk of developing fatigue or having comorbid fatigue symptoms. This warrants further investigation.

Since there was only a moderate correlation between the two measures (sleep & fatigue) in the widespread pain group, it is likely that they are distinct from one another, and it is simply not a matter of poor sleep causing fatigue.

Finally, pelvic pain severity (namely pain with sex, bladder pain symptoms and period pain) was highest in the widespread pain group. This result is contrary to what was previously found by MaPP [10], in which there was no relationship between pelvic pain severity and increasing pain spread. Tripp et al., however, have found that those with increasing pain spread and BPS experienced worse bladder pain symptoms[16]. As-Sanie et al. have suggested that central pain amplification plays a role in the development of pelvic pain in women, as women with CPP experience increased pain sensitivity at a distant site when compared to controls[6]. Further work is needed to better understand the relationship between peripheral drivers of pelvic pain and central mechanisms of pain maintenance in the TRiPP cohort.

5.5.4. Comorbidities

Comorbidities were highly prevalent in the TRiPP cohort, and this was especially pronounced in the widespread pain group. Furthermore, chronic overlapping pain conditions were highly prevalent in this group. Comorbid conditions are generally highly prevalent in participants with widespread pain, and it's been hypothesised that this relationship is mediated by both genetics and environmental factors[32]. While the exploration of such mediating factors is outside the scope of this thesis, future work should consider factors, such as age, socioeconomic factors, or psychological disorders to name a few, in the analyses that I have already performed.

5.5.5. Mechanisms of pain

Assessed by painDETECT, participants with widespread pain had a greater neuropathic component to their pain than the other groups. Additionally, they were more likely to describe their pain as pressure, pain attacks or burning. There is growing evidence to suggest that people with widespread pain exhibit features of neuropathic pain, and interestingly, there is a shared genetic predisposition to both neuropathic and widespread pain[33]. However, others have suggested that neuropathic pain only predicts a small number of new widespread pain cases, calling into question how prominent a neuropathic feature is in people with widespread pain[34].

In addition to questionnaires, psychophysical testing was used to better understand the pain mechanisms underlying widespread pain, something that I was unable to do in my first experimental chapter. While it was only prevalent in about a third of the widespread pain group, there was a clear and distinct gain of function in PPT at the dorsum foot that was not observed in the other MaPP-coded widespread pain groups. Therefore, this suggests that these participants are experiencing widespread hyperalgesia (outside of the primary disease/pain site of the pelvis), or a heightened response to pain in a site distant to the pelvis. This reflects central nervous system (CNS) involvement in pain maintenance. Widespread central sensitisation is a hallmark of fibromyalgia, in addition to the chronic widespread pain phenotype associated with it[35].

Further, a loss of function in the VDT outside the 95% confidence interval reflects hypoesthesia, or the decreased ability to detect non-painful stimuli. This inability to detect

vibration at a distant site likely indicates disruptions to the somatosensory system. However, this requires further investigation in future studies as this is a novel finding.

The gain of function observed in DMA suggests a hypersensitivity to non-painful stimuli, or allodynia in those with widespread pain. While the cause of DMA in humans is not completely known, both peripheral and central mechanisms are likely at play. In rodent models, Piezo2 (a mechanosensitive ion channel in the skin) has been shown to mediate sensitisation to mechanical pain after nerve injury[36]. More recently in humans, a loss-of-function mutation in the channel resulted in the inability to undergo an allodynia response to touch on the skin, after inflammation of the skin via capsaicin[37]. Although I didn't investigate Piezo2 in TRiPP participants, it is possible that those with more widespread pain had an underlying mutation in the Piezo2-encoding gene which could in part explain the gain of function in DMA in this subset of participants.

The intermediate pain group showed thermal hypoalgesia to heat, as shown by a loss of function outside of the 95% confidence interval. However, the means for the other groups were all very close to zero. Interestingly this wasn't observed in the widespread pain group, which suggests that mechanistically, there may be perturbations to the somatosensory system which are unique to this intermediate pain group as well.

While outside the scope of this thesis, it would be interesting to further explore participant characteristics which could explain differences between the intermediate and widespread pain groups. It is particularly important to better understand this intermediate group, as they seem to be distinct with respect to self-reported outcomes and psychophysical testing

measures. In doing so, we may be able to understand whether assignment to this group is clinically relevant.

Interestingly, there were no between-group differences in the CPM effect. Previous work by Gerhardt et al. has shown that in patients with chronic back pain, those with localised pain have more efficient CPM than those with back and comorbid widespread pain[38]. Furthermore, there were no differences in the CPM effect between those with back pain & CWP and participants with fibromyalgia. Rice et al have similarly shown that participants with CWP have impaired CPM when compared to healthy controls[39]. On the one hand, my results could indicate that in this group of participants, descending inhibitory pain pathway functioning does not differ between widespread pain groups. However, there are methodological complexities with the CPM paradigm that could also very likely explain why there was no association.

While there are standardised paradigms for CPM, the methodologies reported in the literature are highly variable when considering the test stimulus, conditioning stimulus, stimulus application (i.e. in parallel, sequential), study design and beyond. Additionally, there is a lack of consensus on how CPM should be analysed, with recent research proposing novel statistical approaches to ensure reproducibility and reliability in the field of CPM research.

Because there were no differences between the widespread pain or TRiPP study groups, further analyses were required to better understand if this was due to a true lack of

difference, or if there were elements of the CPM paradigm itself that influenced our results, some of which have been explored in Chapter 6.

In this chapter I have evaluated the neuropathic and nociceptive elements of pain, using painDETECT. However, there is a third type of pain, 'nociplastic pain', which is likely a contributor to the mechanisms underlying widespread pain in this group.

The International Association for the Study of Pain has defined nociplastic pain as "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"[40]. Nociplastic pain is distinct from the two pain types formerly described but is not entirely understood. It is thought however that such pain is underlaid by CNS perturbations and aberrant pain modulation[41]. The hallmark of nociplastic pain, however, was highly prevalent in the TRiPP study: widespread pain which is more diffuse than one would expect given the disease or actual tissue damage. In the case of TRiPP, 40.7% of all CPP participants, who were initially recruited based on having a pelvic pain pathology, exhibited widespread pain outside of the pelvis. Nociplastic pain is also (controversially) associated with non-pain symptoms, such as fatigue, sleep, poor memory, and mood[41]. Interestingly, fatigue, sleep and psychological health measures were significantly worse in the most widespread pain group. As such, it is plausible that there is a strong nociplastic component to the pain experienced by this subgroup.

5.5.6. Strengths and limitations

In contrast to my first experimental chapter, these analyses were more appropriately powered to detect between group differences in women with CPP and widespread pain. Additionally, because TRiPP was designed to deeply phenotype pain in women, I was able to investigate the underlying mechanisms of widespread pain in this cohort using more comprehensive questionnaires and psychophysical testing parameters. Importantly, this work contributes to a growing body of evidence supporting the need to consider widespread pain clinically.

However, there are some limitations which must be considered when interpreting the results of this chapter.

Firstly, this chapter aimed to characterise widespread pain. Moving forward, however, it will be important that future analyses consider covariates which could in part or completely explain the relationships that I have observed between widespread pain and a multitude of pain-related factors. Known risk factors for widespread pain include stress, age, female sex, sleep disorders, anxiety and depression, socioeconomic status[42], increasing BMI[43] and other comorbid conditions[44]. While I didn't find any significant differences between the widespread pain groups concerning age, other factors as listed above were not accounted for in my analyses.

Furthermore, this was a cross-sectional study. As such, I was unable to unpick the temporality and directionality of the associations observed in this chapter. Having found

associations between widespread pain and anxiety, depression, and sleep, as well as a high prevalence of comorbid conditions, it is likely that these morbidities act bidirectionally[23]. As such, future work investigating these relationships should incorporate a cohort study design to better understand the temporality of these associations.

Finally, most of my analyses relied on self-reported measures. While validated questionnaires were used in this study, it is likely that this may have introduced bias into my work. Many of the pain-related questions asked participants about pains that they had experienced in the last days, months or last year. As such, it is plausible that there is a level of recall bias[50] in these responses; for people living with chronic pain, and experiencing it frequently, it may be difficult to recall their worst pain, and how they would rate it out of 10, in the last 12 months. A way to combat this in future would be to only ask questions with a shorter time between the event and the questionnaire, or, to ensure that the participant keeps a diary of their experiences which the self-reported questionnaire asks about[46]. Additionally, some of the questions that were asked (particularly with respect to pain with sex) are sensitive in nature. As such, people may have not been able to recall such a pain or respond in such a way that is inaccurate for fear of shame or stigma.

Furthermore, it is likely that for the painDETECT questionnaire, that non-response bias[47] may have contributed to the results I observed. In fact, this may have skewed my results to overestimate the prevalence of participants reporting neuropathic descriptors or neuropathic-like pain in the widespread pain groups. While it is not possible to determine from this data whether those that didn't respond experience neuropathic-like pain, it may

be important to better understand the participant characteristics of those whose data was missing.

From a disease-centred perspective, one could argue that a limitation of this study is that the widespread pain groups were comprised of women with different underlying pathologies. While it is true that there is a varied distribution of TRiPP study groups in the widespread pain group, there is a need to push away from this outdated theory that pain solely arises from the site of disease or lesion, and would thus originate in this case, from the pelvis. When we take a pain-centred approach, we are better able to holistically understand the pain experience that might otherwise be hindered if we had taken the former approach.

5.5.7. Future directions & conclusions

While outside the scope of this thesis, there are some key questions that remain unanswered. The first being the distinction between widespread pain and fibromyalgia. In the TRiPP cohort, only around one third of the widespread pain group met the clinical criteria for fibromyalgia. Although these analyses won't be sufficiently powered in the TRiPP cohort, it is important to run stratified analyses between those with widespread pain who meet the fibromyalgia criteria, and those who don't to see what distinctions there are, if any. Previous work using CPM to characterise widespread pain and fibromyalgia found differences between the two groups, and it has been proposed that fibromyalgia is in fact a more extreme phenotype of widespread pain that is associated with poor sleep and fatigue, as well as psychiatric comorbidities, like depression[38].

Previous work using psychophysical testing to phenotype widespread pain has found distinct perturbations unique to those with widespread pain[48]. However, in this cohort, I didn't find any. Perturbations in QST performed at the foot did suggest alterations to the somatosensory processing pathways and a centralised component to the widespread pain and even intermediate pain phenotype. However, there is likely a myriad of factors which contribute to the underlying pathophysiology. As such, to further enhance our understanding of widespread pain in women with CPP, it will be important to explore additional mechanistic pathways.

Notably, genetics and other omics analyses have shown distinct signatures relevant to chronic widespread pain (CWP) in the UK Biobank. A genome-wide association study (GWAS) has shown associations between the RNF123 locus and CWP, which plays a role in calcium regulation[49]. The RNF123 gene is highly expressed in musculoskeletal tissue. Additional GWAS studies[50,51] have investigated multisite chronic pain (MCP, multiple body regions self-reported in the UK Biobank as persisting for 3+ months), which likely closely resembles the widespread pain phenotyping used in this study. They found that MCP was associated with 76 independent SNP's at 39 risk loci, and genes associated with neurogenesis and nervous system development. Furthermore, a polygenic risk score for MCP successfully predicted the development of widespread pain (in the UK Biobank, this is coded as 'general pain for 3+ months')[52]. In addition, the relationship between CWP and depression, as well as CWP and fatigue have been shown to be genetically mediated[53]. Given that there is evidence of genetic underpinnings of widespread[54] and multi-site chronic pain, and associated comorbidities, it would be useful to further investigate these

associations in the TRiPP study. In collaboration with my colleague Mike, this work is ongoing and will be included in publications resulting from this work.

Additionally, neuroimaging would be a useful tool to better understand centralised mechanisms of widespread pain in this cohort. The MaPP research network has shown that in participants with UCPPS, there were distinct structural and functional brain signatures that distinguished between those with widespread pain from those with localised pain[55]. Interestingly, they were not distinct from those with fibromyalgia. Notably, there were increases in grey matter volume in the right supplementary motor area/cingulate cortex and bilateral primary sensory/motor cortex (S1/M1), and increased connectivity between the salience network and the left S1/M1 region in the widespread pain group when compared to those with localised pain[55]. Due to COVID-19, there were significant delays to the functional magnetic resonance imaging (fMRI) data collection in TRiPP, and it is only now being collected. If this was not a barrier, fMRI data would have been included in these analyses. However, there will hopefully be scope upon completion of my DPhil to investigate this further to better understand the neural underpinnings of widespread pain.

Finally, contrary to my hypothesis and previous literature[38], I did not find any differences between the widespread pain groups with respect to CPM. While not presented in this part of my thesis, I also did not find any differences in the CPM effect between those with CPP generally and controls. As published CPM results and methodologies are inconsistent and oftentimes contradictory, there is a need to better the CPM paradigm and its usefulness, especially in a cohort of women. Using the CPM data from TRiPP, I have further investigated the results and employed a novel and robust statistical approach to better understand why

we didn't observe any differences between those with CPP and controls. This work is presented in the next chapter.

To conclude, in this chapter I have shown how highly burdensome widespread pain is in a cohort of women with CPP. Mechanistically, it is likely that nociplastic pain, amongst other mechanisms, contributes largely to this phenotype. As such, future work and clinical considerations should be made to ensure that in the case of pain management, widespread pain symptoms be considered. For those with such a widespread pain phenotype, it's likely that peripherally acting medications would be ineffective, and that a more holistic approach (inclusive of psychological interventions, exercise, centrally-acting medications, etc.) would be better suited.

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5.7. Appendix

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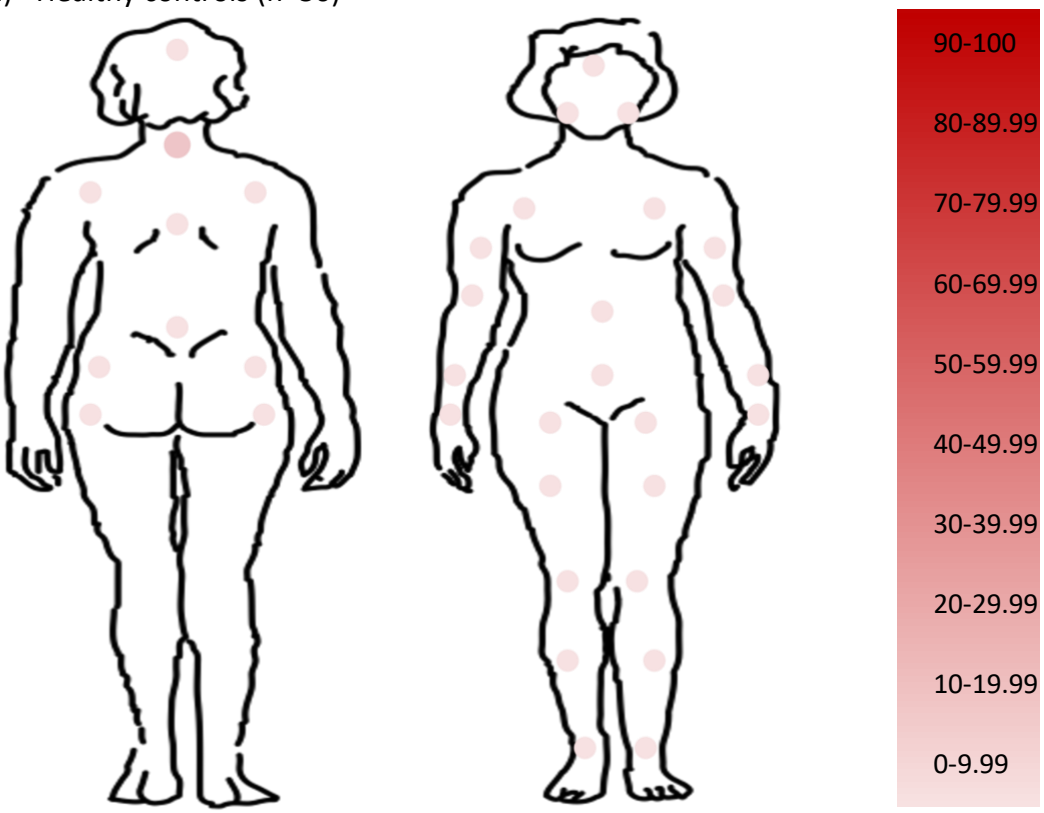
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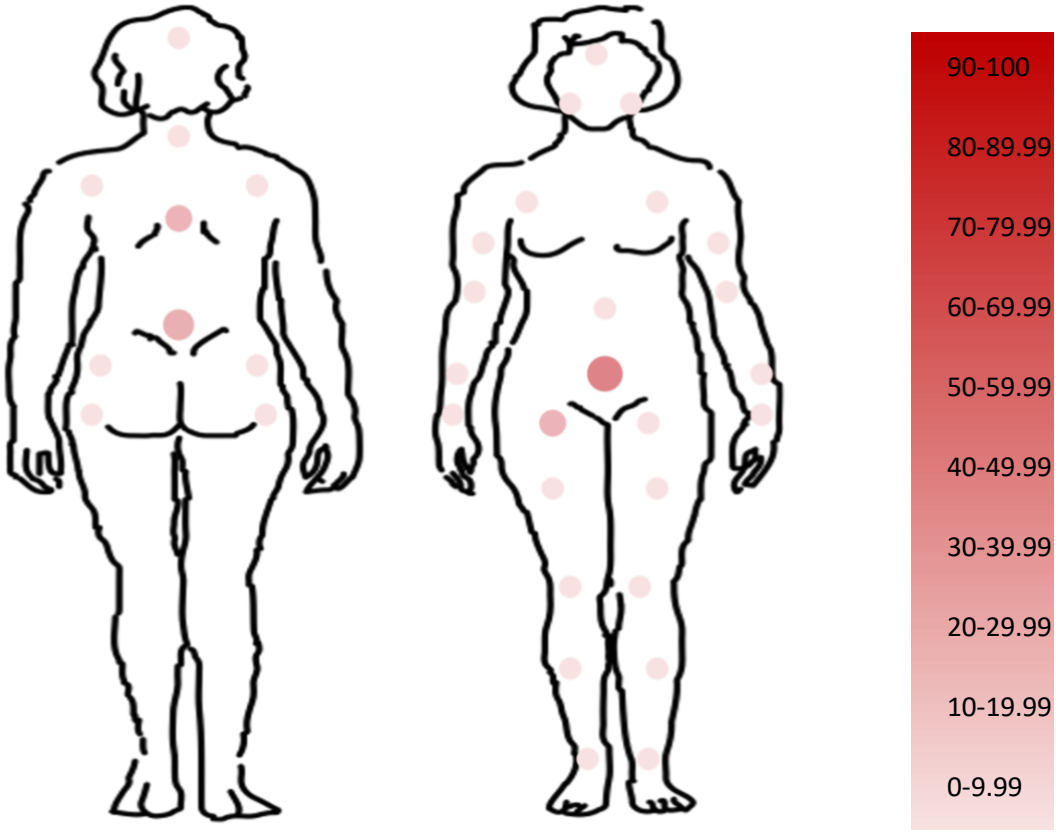
Appendix 5B: Body maps illustrating the percentage of women in each TRiPP study group with pain in the specific area.

***Note:** The size and colour of the dot correspond to the percentage of women in each group with pain in that area, such that a larger and darker red dot indicate more women in the group reported pain in that area. The scale on the right of each diagram indicates which colour corresponds to the specified range of percentages.

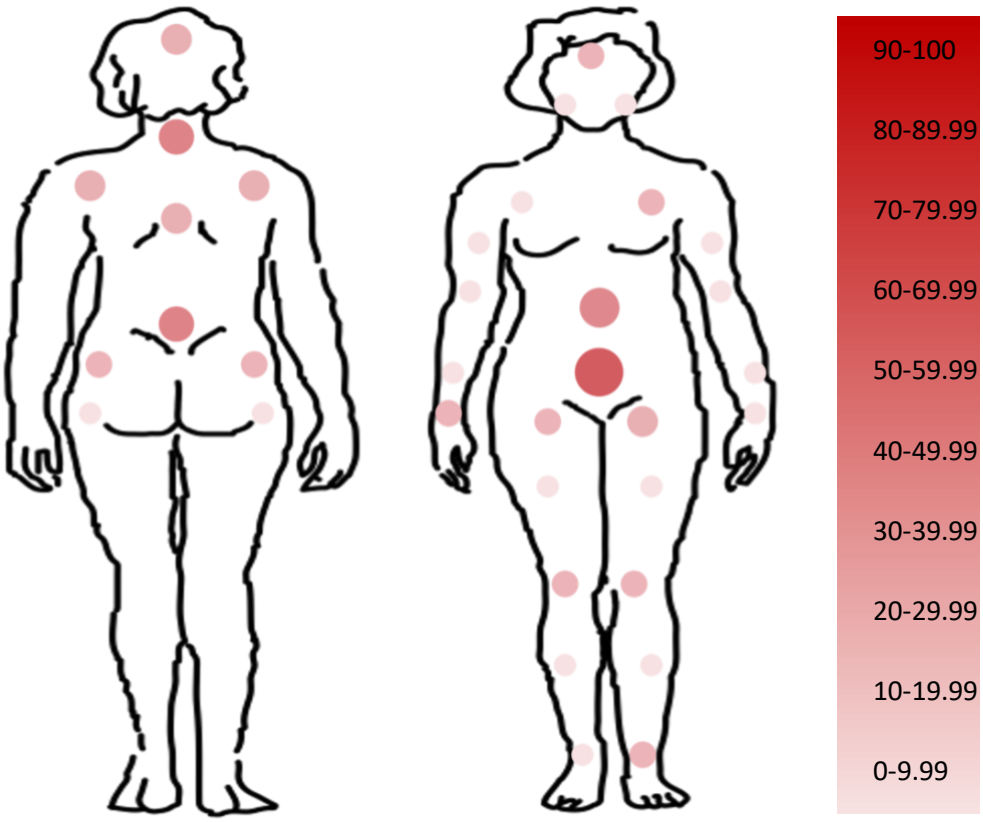
a) Healthy controls (n=50)



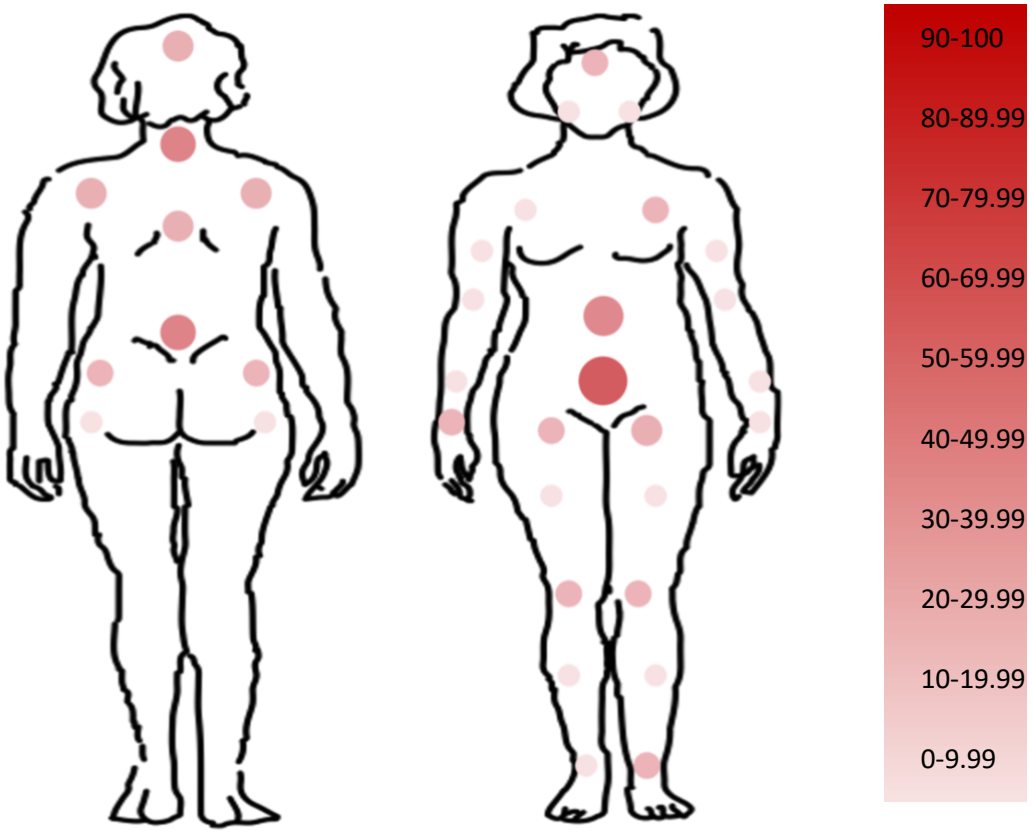
b) Pelvic pain only (n=13)



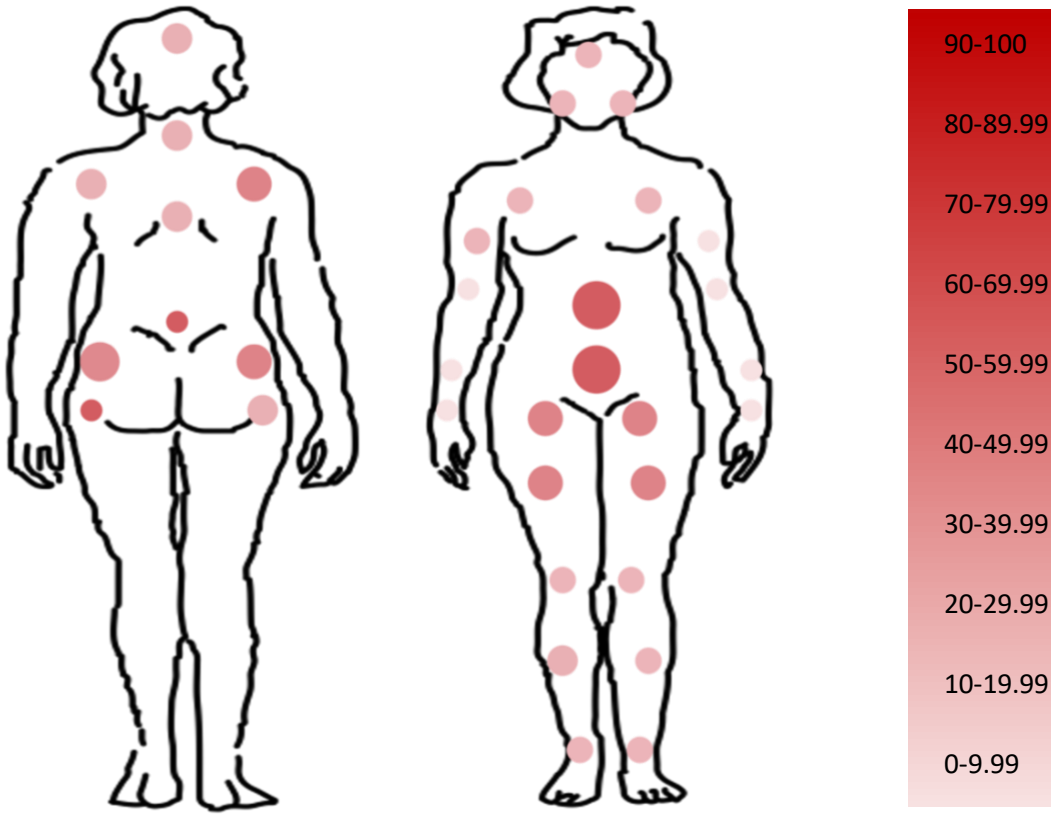
c) Bladder pain syndrome (n=26)



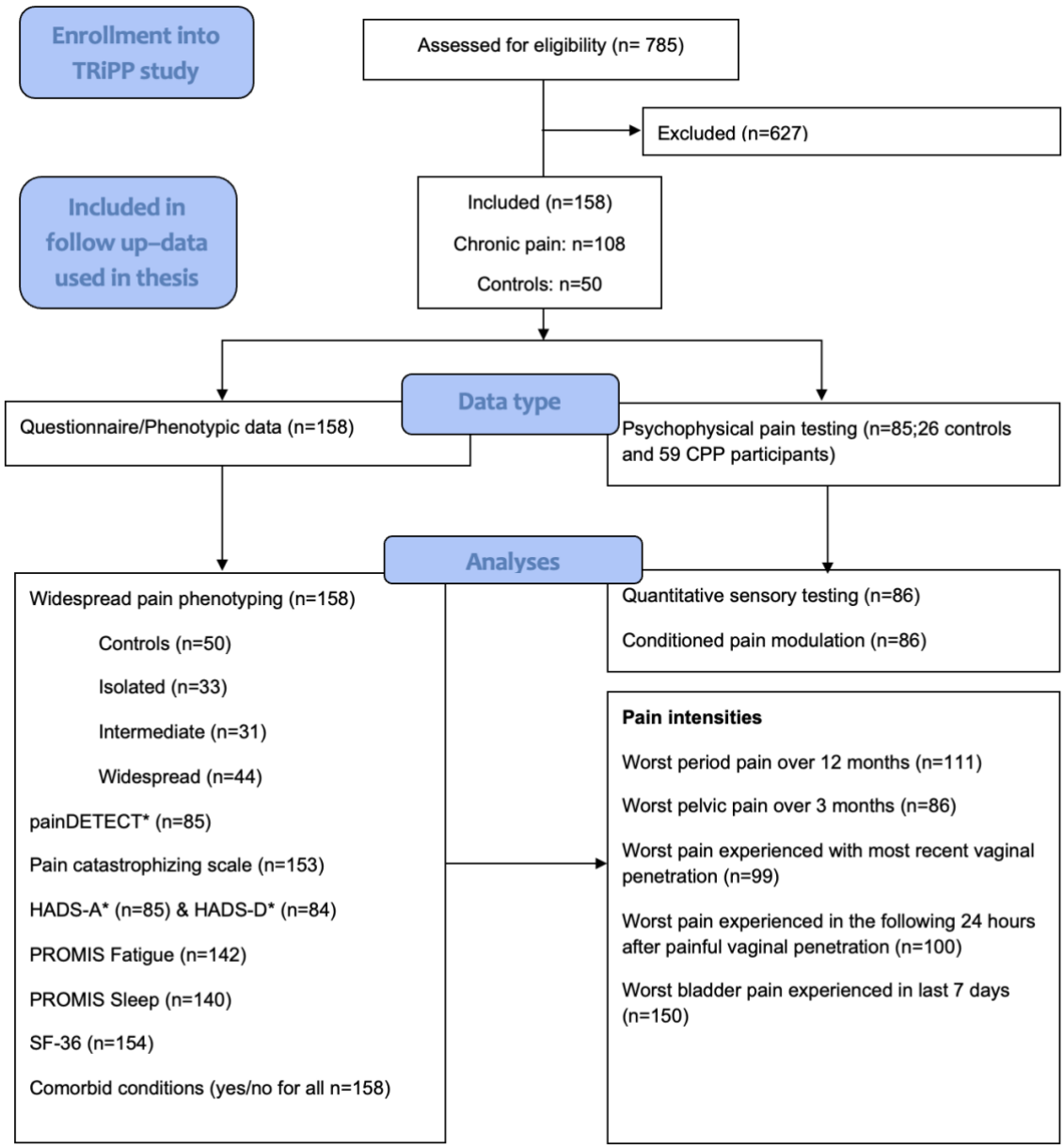
d) Endometriosis-associated pain (n=43)



e) Endometriosis-associated pain and bladder pain symptoms (n=26)



Appendix 5C: Consort flow diagram of analyses performed in this chapter, and the number of participants included in each analysis. *Indicates that this questionnaire was only administered to chronic pelvic pain (CPP) participants.



Chapter 6 – Conditioned Pain Modulation in TRiPP

[This chapter was adapted from my first author publication (to be submitted to PAIN), entitled: Exploring the value of conditioned pain modulation in women: A TRiPP study. The analysis and presentation of the data below was performed prior to receiving feedback from the TRiPP consortium. As such, the final manuscript may differ from this work. The work presented below for this thesis, however, is original work, unless otherwise indicated.]

6.1. Introduction

Conditioned pain modulation (CPM), originally described as 'diffuse noxious inhibitory control' in rodents, is the diminution of pain after the application of a painful conditioning stimulus in a distant site, or the idea that 'pain inhibits pain'[1]. The CPM paradigm is a psychophysical test which assesses the function of the endogenous inhibitory pain pathways responsible for appropriate pain inhibition. Recent reviews have suggested that CPM may be a useful biomarker for chronic pain and have clinical utility for distinguishing between participants with pain and healthy controls, or between distinct subgroups of chronic pain participants[2–4].

In female cohorts with chronic pain, differences in CPM between participants and healthy controls are variable. In women with irritable bowel syndrome (IBS), CPM impairment distinguished between disease severity subtypes, but not IBS participants from controls[4]. Similarly, Payne et al. found no difference in CPM efficiency between women with primary dysmenorrhoea (PD) or controls, nor across the menstrual cycle[5–7]. Hellman et al. showed that women with comorbid PD and bladder pain sensitivity have decreased CPM efficiency compared to other pain groups and controls[8]. Furthermore, it's been shown that CPM is a

useful psychophysical measure to characterise those with widespread pain, compared to those with more localised pain [9], although I didn't find this in my previous TRiPP work (as shown in Chapter 5).

In healthy controls, there is heterogeneity in the reported CPM efficiency. While it is expected that relative to chronic pain participants healthy controls will exhibit greater CPM inhibition, there is evidence of variation in the individual CPM effect, due to age, sex, menstrual phase, and other known variables[10,11]. Furthermore, genomic and metabolomic analyses have been performed to better understand participant characteristics influencing the CPM response. Polymorphic variation in the 5-HTTLPR tandem repeat allele and rs25531 single nucleotide polymorphism have been shown to influence the expression of the serotonin transporter 5-HTT (encoded by the SLC6A4 gene)[10]. Reduced CPM inhibition in response to both pressure pain threshold (PPT) and heat after ischemic pain conditioning stimulus (CS) was reported for healthy participants having the genotype associated with lower 5-HTT expression[12,13]. Later studies did not find a significant 5-HTTLPR allele association with CPM response in healthy controls, using the cold pressor as the CS, and PPT [14] or heat [15] as the test stimulus (TS). Furthermore, plasma serotonin abundance was positively associated with an inhibitory CPM response in adolescents with chronic back pain, however, it was only significant in males despite the study sample including almost 4 times as many females[16].

In addition, there is growing evidence that testing parameters such as test (TS) and conditioning stimulus (CS) modality, and where they are applied to the body contribute to this variation[17,18]. While there are well-established CPM paradigms, there is no consensus

on which should be the gold standard[19]. Consequently, inconsistency in the CPM literature poses a challenge when comparing CPM results across studies.

Recent efforts have aimed to improve the standardisation and comparability of CPM testing and reporting. A statistical approach, which considers the standard error of measurement (SEM) when interpreting CPM effect, has been proposed[20]. Due to the inherent error of psychophysical testing, namely the test stimulus (TS), the % or absolute change in the TS before and after the CS are compared against the SEM. Kennedy *et al*. have suggested a cut-off point of +/-2SEM, which allows for a robust assessment of a 'true' CPM effect. This has since been adopted by many groups[21]. Additionally, there are efforts to calculate and report the reliability of the CPM effect and stimuli. The pressure pain threshold (PPT) test stimulus has shown excellent reliability, however, the degree of reliability of CPM using other stimuli is variable and may be influenced by different study characteristics[21,22]

Given the documented variability in the CPM effect between female pain participants and healthy controls, I aimed to:

1. Explore the 'true' CPM effect in women with chronic pelvic pain and healthy controls using a robust statistical approach
2. Investigate participant characteristics which may distinguish those with effective from those with ineffective CPM
3. Assess the intrasession reliability of the CPM effect in healthy controls.

I hypothesised that in the TRiPP cohort:

1. I would observe effective CPM in all healthy control participants, but only in a subset of those with chronic pelvic pain (CPP)
2. There would be distinct participant characteristics contributing to effective CPM
3. There would be good intrasession reliability of the CPM effect in healthy controls

6.2. Methods

6.2.1. Study population

As mentioned in my previous TRiPP chapter (Chapter 5), a subset of the original TRiPP study was subject to psychophysical testing. Since psychophysical pain testing occurred at three separate research centres (Oxford, Boston, Porto), all researchers responsible for data collection underwent coordinated training, performed the CPM paradigm according to a script, and used identical equipment to ensure that data was consistently across sites. I was one of two DPhil students responsible for data collection in Oxford.

6.2.2. Study Design

Prior to any psychophysical testing, valid informed consent was obtained by one of the researchers, and all participants completed a 'How are you today?' questionnaire. [22,23] Participants were instructed to refrain from taking analgesic medications and reduce caffeine consumption in the 24 hours prior to the CPM visit, as these are known to impact the CPM effect. The time of the CPM visit was recorded.

6.2.3. Conditioned Pain Modulation Paradigm

Participants sat in a temperature-controlled room, maintained around 20°C. To assess CPM, a force dial 10kg algometer with a $1cm^2$ rubber tip test stimulus (TS) was applied to the right dorsal foot at $1kg/cm^2$ per second until the participant said that the sensation had switched from a pressure to a painful sensation. This was repeated three times. The pressure (kPa) at which the participant said the stimulus was painful was recorded after each application[23]. The mean of the three pressures was calculated to determine the baseline pressure pain threshold ($PPT1_{average}$), as described by the German Research Network on Neuropathic Pain (DFNS)[23]. An pressure cuff conditioning stimulus (CS) was applied to the left arm; the cuff was pumped up at a rate of ~20mmHg/second until participants identified the stimulus as painful. The cuff was maintained at that pressure for 60 seconds; however, the participant was not told the duration of time this would last. Prior to deflation, the participant was asked to rate the pain elicited from the CS out of 10 (0=not painful, 10=worst pain imaginable). The self-reported pain rating and CS pressure were recorded. Immediately after deflation, the algometer was again applied to the right dorsal foot three times until the participant found it painful, and the mean $PPT2_{average}$ was calculated.

The participant was then instructed to rest quietly for 10 minutes, prior to repeating the application of the CS and TS as outlined above. Pressure, pain rating of the CS prior to deflation and the mean $PPT3_{average}$ TS were again calculated.

6.2.4. Data analysis

Determining CPM Effect

In line with recommended reporting requirements[19], the CPM effect was calculated and reported as both the absolute difference in pressure pain threshold ($PPT2_{average}$-$PPT1_{average}$) and percentage change ($PPT2_{average}$-$PPT1_{average}$/$PPT1_{average}$) x 100[22]. When determining the 'true' CPM effect using the SEM threshold, the absolute difference was used.

Standard error of measurement and CPM reliability

Using control PPT1 recordings, the standard error of measurement (SEM) was calculated using the formula below. Results from an analysis of variance (ANOVA) using PPT1 recordings were used to determine reliability in the SEM equation.

$$\text{Reliability baseline PPT} = \frac{\text{Residual sum of squares}}{\text{degrees of freedom}}$$

$$\text{SEM} = \text{standard deviation of baseline PPT x } \sqrt{(1 - \text{reliability baseline PPT})}$$

In line with recent research, a threshold of +/-2SEM was used to determine a 'true' CPM effect [21,24]. The absolute difference in $PPT2_{average}$ – $PPT1_{average}$ was compared against this +/-2SEM threshold. A score >+2SEM was considered an inhibitory or 'intact/efficient' CPM response, and a score <-2SEM was considered a facilitatory response. Scores between the thresholds were classified as 'non-responders'.

Intrasession reliability of the CPM effect in healthy controls was determined using the kappa statistic (k, SE)[25]. Participants were firstly classed as responders (either facilitatory or inhibitory) or non-responders, as described above, using data from $PPT2_{average}$ and $PPT1_{average}$. The CPM effect was then calculated again using $PPT3_{average}$ - $PPT2_{average}$. Participants were similarly classed according to their CPM response. A 3 x 3 table was used to generate the kappa statistic, comparing the responder group from the two separate CPM effect calculations.

Factors which may influence the CPM response

Validated questionnaires – PCS & STAI-S

Both the PCS ($\alpha = 0.92$) and STAI-S ($\alpha = 0.31 - 0.86$) are self-administered questionnaires, with poor to excellent test-retest reliability [26,27]. Although described in more depth in the general methods for studying pain chapter (Chapter 2), in brief, PCS and STAI-S scores were investigated as continuous variables. Additionally, I aimed to determine whether any participants met the clinical cut-off for PCS (y>30) or STAI-S (y>40). Questionnaires were scored according to the respective scoring protocols [27–29].

Menstrual phasing

Participants were asked to self-report whether they were taking any hormonal contraceptives, the day of their LMP, and typical length of their menstrual cycle. Those who were not currently taking any form of hormonal contraception, who indicated that they

still had menstrual cycles, were categorised by menstrual phase according to the following protocol[30]. Based on a 28-day cycle, days 1-7 were classified as menstrual phase, days 8-14 were classified as follicular/proliferative and day 15+ was luteal/secretory phase. For participants whose cycle length deviated from 28 days, 14 days were subtracted from their reported cycle length, the secretory phase being held constant, and the remaining duration was assigned to the proliferative phase. For women who reported a variable cycle length, the min, mean or max cycle length was determined, and the stage was allocated accordingly. The menstrual phase was cross-checked by another member of the research group to ensure accurate and reproducible phasing.

Pain ratings and pressure (mmHg) of the pressure cuff

The self-reported pain rating (/10) and pressure (mmHg) of the CS were recorded and compared between control CPM response groups.

Time of participant visit and medication use

The start and end time of the CPM visit was recorded by the researcher. Since the scripted paradigm took about an hour, the paradigm start time was used to code the time of participant visit into morning (7am-12pm), afternoon (12pm-4pm) or evening (4pm+).

Medication use was summarised in healthy control participants. Further investigation was done to summarise the prevalence of centrally-acting medication use amongst CPM responders and non-responders (number of participants, %).

rs25531 expression and Serotonin abundance

To determine rs25531 expression, plasma samples were genotyped using Illumina Global Screening Array (38 samples) and earlier genotyping experiments (Affymetrix Axiom - 2 samples, Affymetrix Precision - 3 samples) of biobanked samples. The genotypes were consistently imputed using TopMed-r2 imputation panel v1.0[31] using Michigan Imputation Server[32] v1.6.0 with minimac 4-1.0.2 algorithm and eagle-2.4 phasing. The rs25531 was imputed with an average estimated R^2=87.19%.

Serotonin relative abundance was quantified using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy by Metabolon Inc[33] in two batches with pooled plasma matrix used as a control. Samples with serotonin abundance below the level of detection were imputed with minimum observed values. Log_2-transformed abundance was used in the analysis. All samples were collected on average 4.4 years prior to the time that the CPM paradigm was administered.

For all omics analyses, CPP and control groups were combined to maximise power. All omics-related analyses were performed by my colleague Michal Krassowski. We collaborated on the analysis plan, and research questions, and went back and forth with iterations of the analysis. I have obtained his consent to include this shared work in my thesis.

Statistical analyses

Descriptive statistics of the 'How are you today?' questionnaire responses were calculated. Continuous variables were assessed for normality using the Shapiro-Wilks test. Mean (SD) or median (IQR) were reported, depending on whether the data were normally distributed. Questionnaire measures were compared between both pain and control groups, and between responders and non-responders in the control group. Comparisons between the pain and control groups were made using an unpaired t-test, or where appropriate, the Mann-Whitney test.

To further investigate the CPM response in the control group, differences in demographic, questionnaire measures and pain data were compared between responder groups using an ANOVA or non-parametric equivalent (Kruskal-Wallis test). A spearman correlation was used to determine the association between the CPM effect and measures of mood; STAI-S and PCS.

All participants were included in these analyses. Data from all women with CPP were amalgamated into a single group as there was no prior evidence to suggest differences in CPM between the CPP subgroups. Data were analysed and plotted using PRISM 9. A p-value<0.05 was considered to be statistically significant.

Omics Analyses

The inhibitory CPM response logistic model with serotonin predictor was estimated in R v4.1.2 using `lrm` function from `rms` v6.3 package [34]. Multiple Imputation by Chained Equations using `mice` v3.14 package was used to impute missing data for rs25531 status (15 non-genotyped samples), and antidepressants use (for 9 samples, including two missing rs25531 status) [35]. The sensitivity analyses for alternative modelling strategies were conducted using `orms::rm` function for the ordinal model and `stats::lm` for the linear model. Multiple imputation was used for all sensitivity analyses.

6.3. Results

6.3.1. Participant characteristics

CPM was performed on 85 women; n=59 with CPP and n=26 CON. All CON participants and twenty-four CPP participants were recruited from Boston, USA. Twenty-four CPP participants were recruited from Oxford, UK, and 11 participants from Porto, Portugal. CPP participants were comprised of the following TRiPP subgroup allocations (**Table *6.1***): n=6 PP, n=25 EAP, n=14 BPS, n=14 EABP.

There was no significant difference in age, the proportion of participants using exogenous hormones or the number of caffeinated beverages consumed between the controls and those with CPP. However, current pain intensity was higher for those with CPP (2(4) vs controls (0 (1.5), p=0.124), and a smaller proportion were having a menstrual cycle (20.3% vs 53.8%). N=33 (68.8%) of CPP participants and only n=12 (46.2%) of control participants

were using hormonal contraceptives at the time of the CPM visit. The median (IQR) PCS scores for CPP participants and controls were 14.5 (18.5) and 4.5 (8) respectively ($p<0.0001$). Ten CPP participants met the clinical cut-off for PCS, whereas no control participants met such cut-off. The median (IQR) STAI-S scores for pain participants and controls were 31 (10.5) and 23 (23) respectively ($p=0.0032$). Twelve CPP participants and two CON met the clinical cut-off for STAI-S.

Table 6.1. Participant Characteristics. CPP; chronic pelvic pain participants. Mann-Whitney test was used to compare values between groups. IQR; interquartile range. CPM; conditioned pain modulation.

Characteristic	**CPP (n=59)**	**Controls (n=26)**	**p-value**
Age (median, IQR)	34 (13)	29.5 (12.5)	0.124
Participants from Each Site (n, %) Boston, USA Oxford, UK Porto, Portugal	 24 (40.7) 24 (40.7) 11 (18.6)	 26 (100) 0 0	
TRiPP Pain Sub-Group Allocation (n, %)	PP: 6 (10.2) EAP: 25 (42.4) BPS: 14 (23.7) EABP: 14 (23.7)	–	–
Pain rating before CPM paradigm	2 (4)	0 (1.5)	0.0336
Number (%) of participants using a form of hormonal contraceptives	33 (55.9)	12 (46.2)	0.482
Number (%) of participants currently having menstrual cycles	12 (20.3)	14 (53.8)	0.0042
Caffeinated beverage consumption on day of CPM visit (cups, median (IQR))	0 (1)	0 (1)	0.161
PCS Score (Median, IQR) N (%) meeting clinical cut-off	14.5 (18.5) 10 (17.0)	4.5 (8) 0 (0)	<0.0001 –
STAI-S Score (Median, IQR) N (%) meeting clinical cut-off	31 (10.5) 12 (20.3)	23 (23) 2 (7.7)	0.0032 –

6.3.2. CPM effect and testing parameters

The mean absolute difference in $PPT_{average}$ before and after the CS was 0.47 (0.41) and 0.5 (0.48) for CPP participants and controls, respectively. The % change was 12.74 (15.08) and 11.88 (11.12) for CPP participants and controls, respectively (p=0.795). There was no significant difference in the CPM effect between CPP and control groups. As demonstrated in **Table *6.2***, there was a significant difference between the median (IQR) cuff pain rating (CPP= 5(5), CON= 3 (3) p=0.0070) and mean (SD) pressure (mmHg) of CS between CPP participants and controls (CPP=202.5 (63.49), CON=161.9 (40.10), p=0.0036).

Table 6.2. Conditioned pain modulation parameters. CPP; chronic pelvic pain participants. PPT; pressure pain threshold. T-test or Mann-Whitney test was used depending on whether data were normally distributed. CS; conditioning stimulus. u, SD; mu/mean, standard deviation. kPA; kilopascals, mmHg; millimetres of mercury.

Parameter	CPP (n=59)	Controls (n=26)	p-value
Change in PPT (after CS (u, SD)			
Absolute (kPA)	0.47 (0.41)	0.5 (0.48)	0.795
% Change (%)	12.74 (15.08)	11.88 (11.12)	
Cuff pain rating (Median, IQR)	5 (5)	3 (3)	0.0070
Pressure (mmHg) of CS (u, SD)	202.5 (63.49)	161.9 (40.10)	0.0036

6.3.3. +/-2 x Standard error of measurement threshold and reliability

The +/-2SEM threshold was determined to be +/-0.624. When comparing the absolute difference in PPT against this threshold, six (23.1%) controls (n=4 inhibitory, n=2 facilitatory, **Figure *6.1*A**) and nineteen (32.2%) CPP participants (n=11 inhibitory, n=8

facilitatory, **Figure *6.1*B**) had a 'true' CPM effect. There was substantial intrasession reliability of the CPM response in controls (k, SE: 0.623 (0.159).

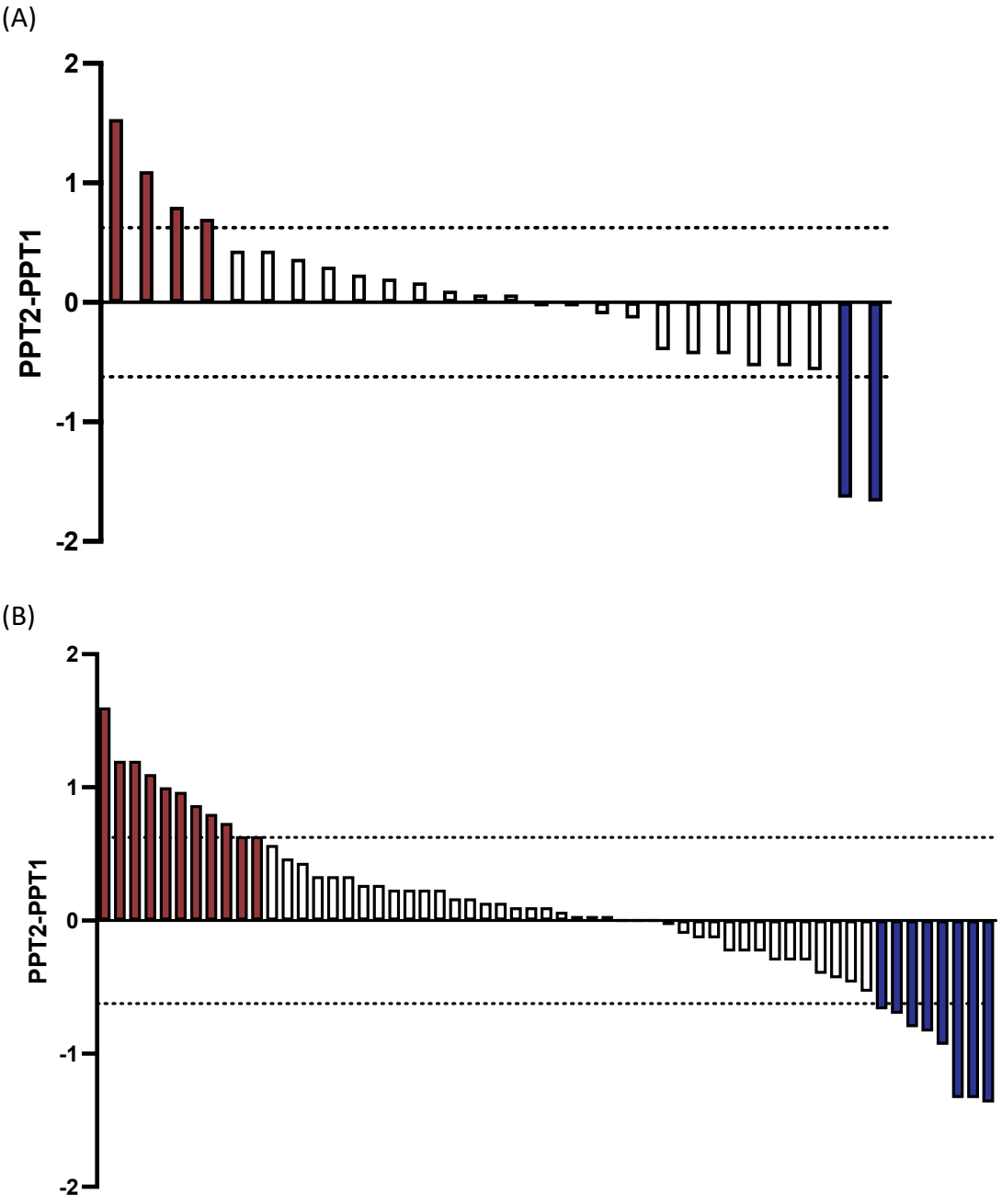


Figure 6.1. Participants with a 'true' CPM effect using the +/-2SEM threshold. There is wide variation in the CPM response amongst healthy control participants. Red bars indicate an inhibitory response, and blue, a facilitatory response. Each bar represents a single participant. A) True CPM effect for controls, n=4 exhibited an inhibitory response, and n=2 a facilitatory response. B) True CPM effect for pain participants, n=11 exhibited an inhibitory response, and 8 a facilitatory response. The dotted line in both figures represented the SEM threshold. PPT; pressure pain threshold.

6.3.4. Investigating covariates between healthy control responders and non-responders

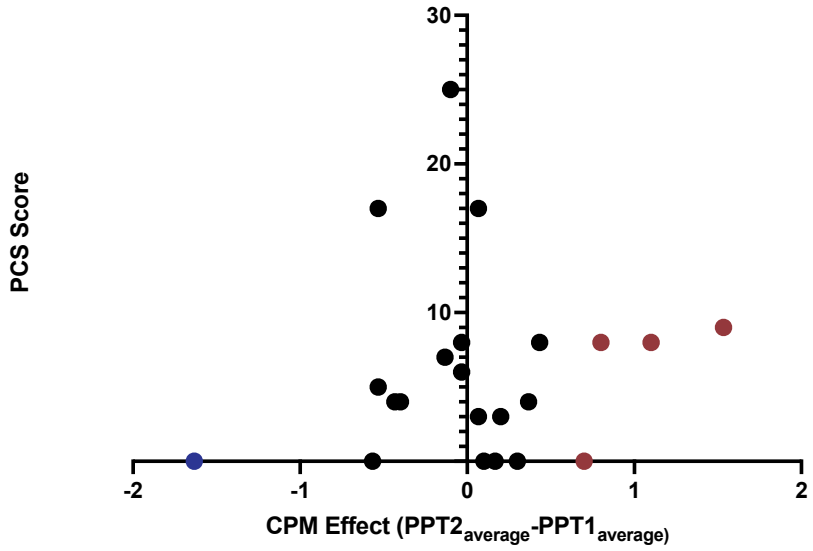
Given that only 23.1% of controls exhibited a 'true' CPM effect, and only 15.4% had exhibited 'true' inhibition, I further investigated covariates known to possibly impact the CPM response between those that responded when compared to those that did not.

6.3.4.1. Validated questionnaire measures: STAI-S and PCS

No CON participants met the clinical cut-off for PCS, indicated at y=30 in **Figure *6.2*A**. There was no significant correlation between the PCS score and the CPM effect (r=0.106, p=0.640).

Of all CON participants, only two non-responders met the clinical cut-off for STAI-S, indicated at y=40 in **Figure *6.2*B**. There was no significant correlation between STAI-S score and the CPM effect (r=-0.316, p=0.116).

(A)



(B)

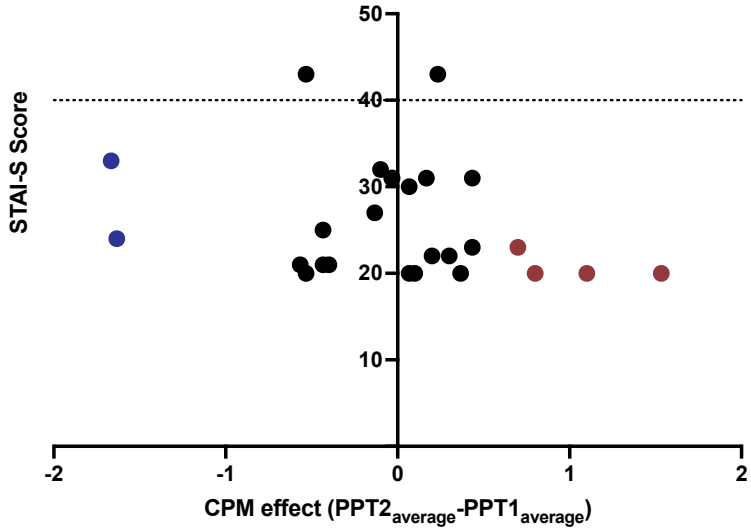


Figure 6.2. Validated questionnaire measures which assess mood showed no significant correlation with the CPM effect, in healthy control participants. In each figure, the blue dots represent participants with a facilitatory CPM response, and red dots are participants with an inhibitory response. (A) Pain catastrophizing scale (PCS) score by CPM effect. (B) State-trait anxiety inventory state (STAI-S) score by CPM effect. CPM; conditioned pain modulation.

6.3.4.2. Hormone use and menstrual phase at the time of CPM visit

Three (75%) of the inhibitory responders, twelve (60%) non-responders and one (50%) facilitatory responder were currently using hormonal contraceptives. All facilitatory responders were using unidentified oral contraceptives, whereas only 25% of non-responders and no inhibitory responders were. The use of the intrauterine system (IUS) was also prevalent in inhibitory and non-responders. Hormonal contraceptive classes by response group are further described in **Figure *6.3***. Fourteen of all CON participants had a natural menstrual cycle (i.e. were not currently on any form of hormonal contraceptives) at the time of the CPM visit, two of which did not provide menstrual cycle data. Only one (25%) inhibitory responder, four (20%) non-responders and zero facilitatory responders were currently in the follicular phase of the menstrual cycle, as shown in **Table *6.3***.

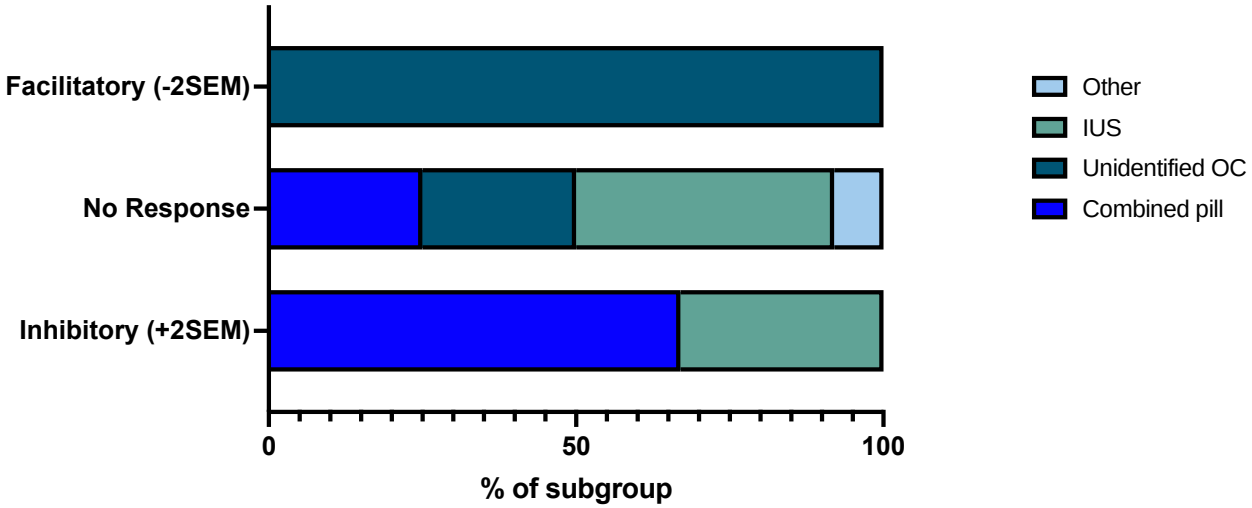


Figure 6.3. Self-reported hormonal contraceptive use by healthy control CPM responder group. IUS; intrauterine system. OC; oral contraceptive. SEM; standard error of measurement.

Table 6.3. Hormonal contraception use and menstrual phase by control CPM response group. SEM; standard error of measurement.

	Inhibitory (>+2SEM)	Non-responder	Facilitatory (<-2SEM)
Menstrual	0	4 (20%)	0
Follicular	1 (25%)	4 (20%)	0
Luteal	0	3 (15%)	1 (50%)
Number (%) of participants on hormonal contraception	3 (75%)	8 (40%)	1 (50%)
Missing menstrual phase data	0	1 (5%)	0

6.3.4.3. Pain ratings and pressure cuff measurement

As shown in **Figure *6.4*A**, there was no significant difference in either the pain rating of the CS between the three response groups (p=0.441) or the pressure (mmHg) of the CS (p=0.700). Similarly, there was no significant difference in the baseline pain rating between non-responders, those with a facilitatory response or an inhibitory response (p=0.976, **Figure *6.4*B**).

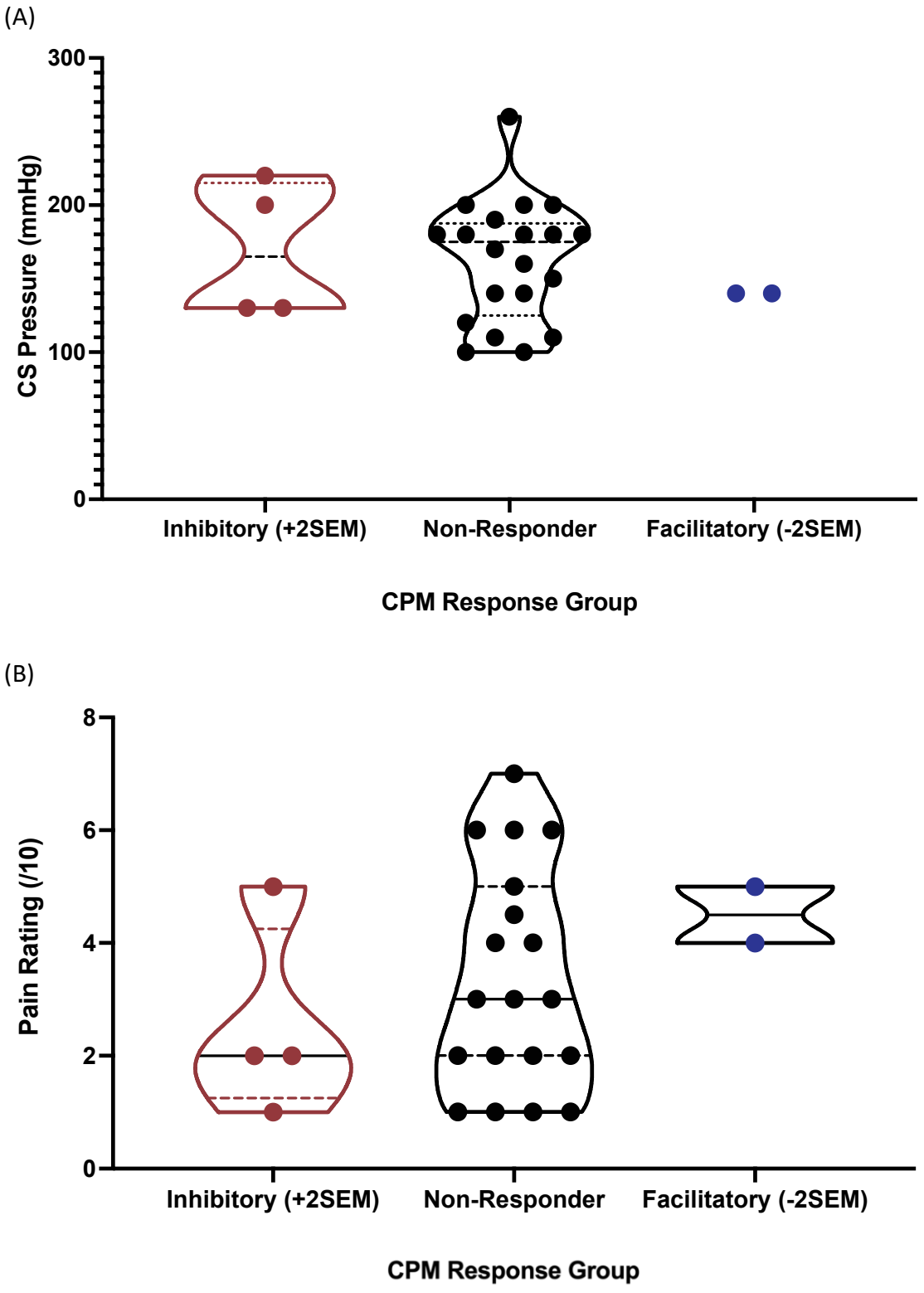


Figure 6.4. Elements of the conditioning stimulus (CS) amongst healthy control CPM response groups. (A) Pressure (mmHg) at which participant identified that the stimulus was painful, before it was maintained at that pressure for 60 seconds. (B) Self-reported pain

rating of the CS (/10), recorded prior to release of the cuff. CPM; conditioned pain modulation. SEM; standard error of measurement.

6.3.4.4. Time of CPM visit

As shown in **Figure *6.5***, morning visits were most common amongst controls; one (25%) inhibitory responder, ten (50%) non-responders and two (100%) facilitatory responders attended morning visits. Two (50%) of the inhibitory responders, and two (10%) of the non-responders undertook the CPM visit in the evening (after 4pm). One (25%) inhibitory responder and eight (40%) of the non-responders undertook the CPM visit in the afternoon.

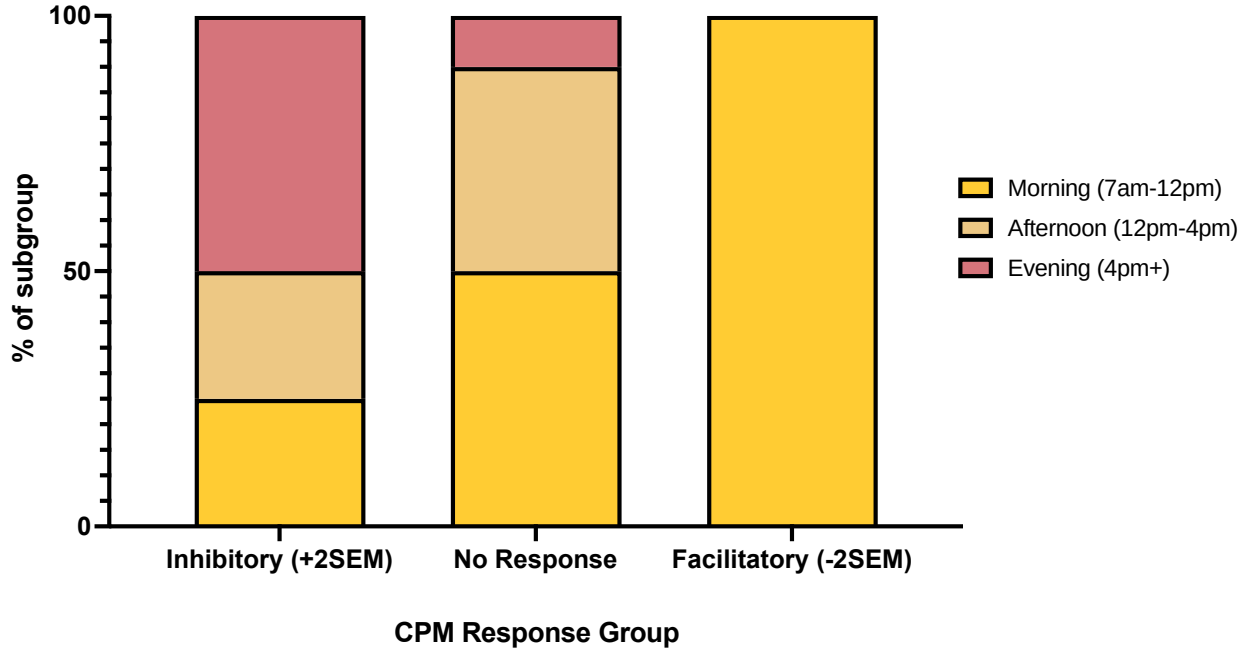


Figure 6.5. Time of day healthy control participants began the CPM visit by response groups: morning, afternoon, or evening. Inhibitory group: n=4, No response group: n=20, Facilitatory group.

6.3.4.5. Medications used

Of all CON participants, only six (23.1%) were not taking any medications at the time of the CPM visit, as shown in **Table *6.4***. The most common medications used were

multivitamins/supplements (34.6%), anti-depressants/mood stabilizers (30.8%) and antihistamines (19.2%). When further investigating centrally-acting medications between CPM responder groups, two (50%) of the inhibitory pain group and six (30%) of the non-responder group were currently taking anti-depressants. Only one participant was currently taking beta blockers, and one was taking ADHD medications, both of which were in the non-responder group (

Table *6.5*).

Table 6.4. Self-reported medications taken at the time of the CPM visit; for healthy controls only. CPM; conditioned pain modulation. SEM; standard error of measurement. **Continued on next page.**

Medication Class	**Generic Medication Name**	**N (%) participants using at least one type of medication from the medication class[a,b]**
Hormonal contraceptives	Norethindrone acetate/ethinyl estradiol tablets and ferrous fumarate, Generic oral contraceptive, Norgestimate/ethinyl oestradiol, Levonorgestrel IUS	5/26 (19.2)
Anti-depressants/Mood stabilisers	Duloxetine (SNRI), Bupropion, Escitalopram, Venlafaxine, Citalopram, Lithium, Nonbenzodiazepines	8/26 (30.8)
ADHD Medication	Adderall	1/26 (3.8)
Analgesics	Ibuprofen	1/26 (3.8)
Anti-convulsant	Lamotrigine	1/26 (3.8)
Anti-histamine	Loratadine, Hydroxyzine, Diphenhydramine, Cetirizine, Fluticasone Propionate Nasal Spray	5/26 (19.2)
Indigestion/laxative Medications	Omeprazole, Prucalopride	1/26 (3.8)
Beta Blockers	Propranolol	1/26 (3.8)
Medication Class	**Generic Medication Name**	**N (%) participants using at least one type**

		of medication from the medication class[a,b]
Multivitamins and supplements	Iron, multivitamin (Centrum), Biotin, Vitamin D3, Magnesium Complex, Probiotics, L-Proline, Melatonin	9/26 (34.6)
Antifungals or Antibiotics	Terbinafine, clindamycin benzo peroxide-gel	2/26 (7.7)
Thyroid Medication	Levothyroxine	1/26 (3.8)

[a] Some participants may be taking multiple drugs within the same medication class
[b] 6/26 (23.1%) participants were not taking any medications

Table 6.5. Self-reported medications taken at the time of the CPM visit by healthy control CPM response group. CPM; conditioned pain modulation. SEM; standard error of measurement. ADHD; attention deficit hyperactivity disorder.

	Control CPM Response Group		
Medication Class	**Inhibitory (+2SEM) N=4**	**No response N=20**	**Facilitatory (-2SEM) N=2**
Hormonal contraceptives	1/4 (25%)	3/20 (15%)	1 (50%)
Anti-depressants/Mood stabilisers	2/4 (50%)	6/20 (30%)	0
ADHD Medication	0	1/20 (5%)	0
Analgesics	0	1/20 (5%)	0
Anti-convulsant	0	1/20 (5%)	0
Anti-histamine	0	5/20 (25%)	0
Indigestion/laxative Medications	0	1/20 (5%)	0
Beta Blockers	0	1/20 (5%)	0
Multivitamins and supplements	2/4 (50%)	6/20 (30%)	1/2 (50%)
Antifungals or Antibiotics	0	2/20 (10%)	0
Thyroid Medication	0	1 (5%)	0
Multi-pharma (number of participants on >1 medications)	2/4 (50%)	10/20 (50%)	0
Number of participants not currently taking any medications	1/4 (25%)	5/20 (25%)	0

6.3.4.6. rs25531 and Serotonin abundance

Combining CPP and healthy control participants, we were able to ascertain the rs25531 single nucleotide polymorphism status in 69 participants. rs25531 was reported to be in strong linkage disequilibrium (r=0.75) with the allele associated with higher expression of 5-HTT, however, it attenuated its effect [36,37].

The observed minor allele frequency of rs25531 was significantly different (t = -6.399, df = 144.42, p-value = 2.048e-09) between chronic pain patients (8.70%) and pain-free controls (13.04%), however both frequencies fell within the range reported in databases (TopMed 11.19%, ALFA European 2.13%, gnomAD European 18.16%, 1000 Genomes European 8.95%). Interestingly rs25531 was only found in non-responders and participants with inhibitory CPM response (t = -10.114, df = 116.94, $p< 2.2x10^{-16}$). There was no significant dose-response relationship between rs25531 and the magnitude of the CPM effect.

In an exploratory analysis we found that plasma serotonin abundance in samples collected before the CPM paradigm was lower in participants with an inhibitory CPM response (Δ = -1.412 [95% CI -1.237 : -2.531] t = -5.7909, df = 85.817, p-value = 1.124e-07) (**Figure *6.6***, **Figure *6.7***).

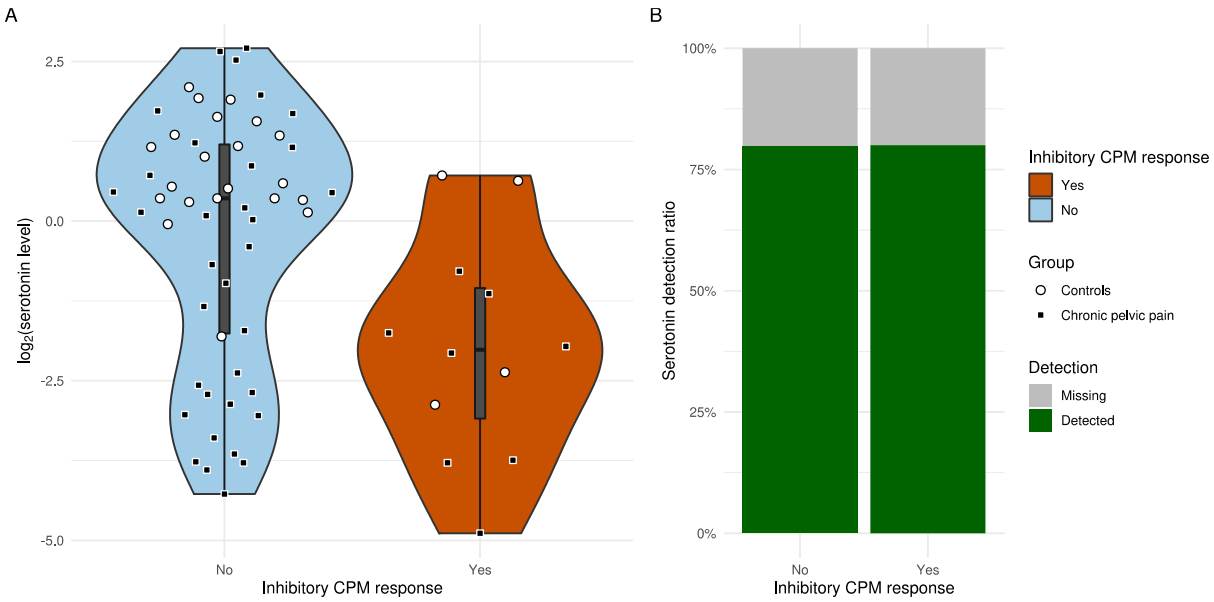


Figure 6.6. (A) Serotonin abundance and (B) detection ratio by inhibitory CPM response status

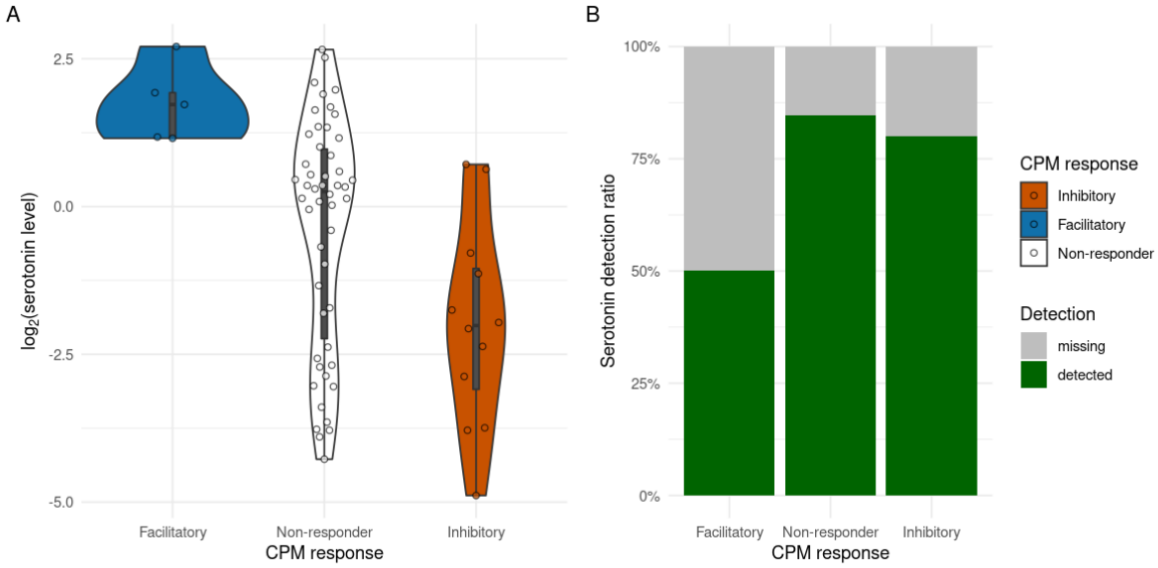


Figure 6.7. (A) Serotonin abundance and (B) detection ratio by CPM response group

To adjust for covariates known to influence the abundance of plasma metabolites (age, BMI, quantification batch) and serotonin specifically (rs25531, pelvic pain presence, use of

antidepressants) we fitted a logistic regression model. While the negative plasma serotonin association was significant in the initial complete cases (62/84 patients) analysis (OR = 0.67 [95% CI 0.46-0.97], p=0.03), the model had negative optimism-corrected coefficient of determination (R_2 = 0.19, bootstrap-corrected R_2=-0.03) suggesting poor generalizability. In subsequent analysis maximising the use of available data using multiple imputation there was no sufficient evidence to reject the null hypothesis of no effect of past plasma serotonin (OR=0.81 [95% CI 0.60-1.09], p=0.157) or rs25531 status (OR=1.51 [95% CI 0.39-5.88], p=0.549).

We performed sensitivity analyses by a) removing covariates with substantial missingness or having no obvious causal relation with the outcome (rs25531, antidepressants use, CPP status), b) removing Porto samples with missing genotype (only two out of eleven Porto samples had genotype available, potentially biasing the multiple imputation results), and c) using alternative modelling strategies with higher power (ordinal regression using CPM response status and linear regression using CPM effect size, both representing slightly different but related null hypotheses). The estimates for serotonin effect remained consistently suggestive of negative or no association with an inhibitory CPM response, and estimates for rs25531 remained suggestive of positive or no association (**Table *6.6***).

Table 6.6. Relevant estimates in sensitivity analysis models. The coefficient represents odds ratio for logistic models, unadjusted slope for linear model, and ordinal odds ratio for ordinal model. CI; confidence interval, SE; standard error, excl; excluding, CPP; chronic pelvic pain, PPT; pressure pain threshold.

Model	Parameter	Coefficient	SE	CI low	CI high	Statistic	p
linear (PPT2 - PPT1)	rs25531	0.182	0.169	-0.156	0.519	1.074374	0.28649
logistic excl. CPP status	rs25531	1.548	1.071	0.399	6.007	0.631210	0.5279
logistic excl. CPP status and antidepressants use	rs25531	1.497	1.058	0.375	5.977	0.571394	0.56773
logistic excl. Porto without genotype	rs25531	1.525	1.071	0.385	6.039	0.600606	0.5481
logistic excl. antidepressants use	rs25531	1.497	1.058	0.375	5.977	0.571394	0.56773
ordinal (inhibitory/non-responder/faciliatory)	rs25531	1.861	0.982	0.662	5.233	1.178073	0.23877
linear (PPT2 - PPT1)	serotonin	-0.004	0.035	-0.073	0.065	-0.107236	0.91491
logistic excl. CPP status	serotonin	0.837	0.109	0.648	1.082	-1.359060	0.17413
logistic excl. CPP status and antidepressants use	serotonin	0.844	0.103	0.664	1.073	-1.386619	0.16556
logistic excl. Porto without genotype	serotonin	0.807	0.126	0.594	1.096	-1.374445	0.1693
logistic excl. antidepressants use	serotonin	0.844	0.103	0.664	1.073	-1.386619	0.16556
logistic excl. rs25531 status	serotonin	0.824	0.117	0.624	1.087	-1.368247	0.17123
ordinal (inhibitory/non-responder/faciliatory)	serotonin	0.934	0.109	0.742	1.175	-0.582925	0.55994

6.4. Discussion

While my previous work aimed to understand perturbations in CPM amongst widespread pain groups, these analyses aimed to compare CPM between pain-free controls and those with CPP. Contrary to my hypothesis, I found that a greater proportion of women with CPP demonstrated effective CPM than pain-free controls. As only 23.1% of controls had a true CPM effect (only 15.4% demonstrated inhibition) according to current methods for classification, I further explored the control group to determine whether any factors underlie the CPM effect in this group. Despite having a well-phenotyped cohort I was unable to identify any single factor, which had previously been suggested to impact the CPM response, that could explain the results[21,24,38]. It was however noted by me and researchers across all three sites that the CPM paradigm itself was particularly unpleasant for participants, especially amongst a battery of other, more well-tolerated psychophysical testing paradigms (i.e. complete DFNS QST protocol and autonomic nervous system testing)[22,39].

6.4.1. Choice of conditioned pain modulation experimental paradigm

There is contradictory evidence as to whether the mode of CS impacts the CPM effect, however, there are recommendations for best practice[22,39]. It has been recommended that to reduce the element of distraction, the CS should be given sequentially rather than in parallel[19]. The CPM paradigm performed across study sites was standardised; each site researcher adhered to a pre-prepared script and used the same equipment on a distant body site, performing the paradigm sequentially[23]. As such, I am confident that our results are likely not due to deviations in the study protocol. However, stimulus-dependent

differences in the CPM response have been described, and it is possible this may have influenced our results[17].

Granovsky et al have suggested that in participants with painful diabetic neuropathy, the location of the CPM paradigm may play a large role in CPM efficiency[40]. They proposed that altered sensory function due to neuropathy at the test site could lead to enhanced CPM, as was previously shown in chronic post-stroke shoulder pain[41]. In exploratory analyses, I found some evidence of altered sensory function using quantitative sensory testing (gain of function for dynamic mechanical allodynia) at the TS in control participants with a facilitatory response (**Appendix 6A**). However, this was likely driven by a small sample size (n=2), and therefore must be interpreted with caution. Further work is necessary to explore whether standardisation of the test site in a single study is warranted or is in fact altering the observed CPM response.

El-Sayed et al. have shown that CPM inhibition is more common in healthy controls when using a pressure paradigm, rather than a heat-based one[17]. The use of the PPT paradigm only elicited an inhibitory CPM response in 15.4% of TRiPP control participants. To ensure that the PPT paradigm was in fact reliable amongst controls, I calculated a kappa coefficient which revealed substantial reliability, which is comparable to the previously reported excellent reliability of the PPT TS[21,42].

The participant's affinity for the CS has been shown to partially explain the CPM response, namely with respect to heat and cold stimuli[43]. However, there is no evidence investigating how one's affinity for an ischemic pressure conditioning stimulus may influence their

response, as it is generally well tolerated. While it was not well-tolerated in this study, I found no evidence to suggest that the CS elicited any sort of ceiling effect, which could have distracted the participants and confounded the observed CPM effect.

Furthermore, I employed the +/-2SEM threshold because it is a robust statistical technique which allows for reliable measures of a 'true' CPM effect[20,21]. This method aims to reduce the current issue of comparability in the published literature. Walker et al. previously found that in a healthy control sample of 48 participants, using the +/-2SEM threshold, 75% exhibited an inhibitory response. However, participants in this study were younger than those in TRiPP. Younger age has been shown to positively influence CPM efficiency, and therefore the older age of TRiPP participants may in part explain why fewer participants exhibited an inhibitory CPM response[44]. In a study with participants more closely age-matched to TRiPP, Kennedy et al. showed that 44-59% of healthy controls had an inhibitory CPM response, depending on the CS [21]. Neither of these studies utilised ischemic pressure as the CS, and therefore it is possible that variation in the CPM paradigms used may have contributed to the CPM effect observed after accounting for +/-2SEM.

6.4.2. CPM in controls

Participant characteristics and elements of the CPM paradigm itself have been shown to influence the CPM response in healthy controls. Notably, psychological factors such as pain catastrophizing, anxiety, and even one's perception of the CPM stimuli show conflicting evidence as to whether they enhance or attenuate CPM efficiency. Similarly, the hormonal state of the participant (i.e. menstrual phase, exogenous hormonal contraceptive use),

medications used and time of the CPM visit have been shown to influence one's ability to effectively engage descending pain pathways[11,43,45–47]. As such, I investigated these factors amongst healthy controls with respect to the CPM response.

6.4.2.1. Psychological factors

I found no correlation between the state anxiety score and the CPM response. Walker et al. showed that higher anxiety scores and PCS were correlated with pain intensity, but not the CPM effect [47]. However, in a cohort of participants with chronic low back pain, CPM efficiency was negatively associated with pain catastrophizing [48]. In my analyses, I found no correlation between the CPM effect and PCS scores.

While not investigated in this work, research has shown that in addition to one's perception of the CS, resilience, pain unpleasantness and salience of the CS contribute to the CPM effect [39,49]. Firouzian et al. have shown that in females who aren't able to effectively engage descending pain modulatory pathways, lower ratings of CS unpleasantness were associated with more CPM inhibition[39]. Although only anecdotal and not captured in our data, many of our study participants found the pain elicited by the CS cuff unpleasant. As such, it could be the case that high CS unpleasantness may have contributed to increased CPM inefficiency and may partially explain why so few participants experienced an inhibitory CPM effect [22].

There is conflicting evidence about the influence of the hormonal state, both exogenously via hormonal contraception, and endogenously across the menstrual cycle on CPM. It has

been reported that during the ovulatory phase of the menstrual cycle, when women are in a state of high estrogen and low progesterone, CPM is most effective[6,7,50]. Wilson et al. found that across four different menstrual cycles, there was no difference in CPM efficiency between the follicular and luteal phases of healthy women[51]. I did not observe notable differences in the allocation to menstrual phases across CPM response groups in TRiPP. This may be due to the fact that participants in the TRiPP study were not excluded on the basis of currently being on a form of hormonal contraception. Furthermore, there were a couple of participants who did not complete the question about menstruation. It is possible that they were amenorrhoeic and did not experience menstrual cycles at the time of the CPM visit. As such, with few participants having natural cycles if they had a cycle at all, the true effects of the menstrual cycle/endogenous hormone fluctuation may have been masked in this study population.

75% (n=3) of inhibitory responders, 40% (n=8) of non-responders and 50% (n=1) of facilitatory responders were using a form of hormonal contraception at the time of the CPM visit. The fact that more of the inhibitory response group were using hormonal contraceptives contradicts results from a systematic review, which concluded that the use of oral contraceptives may inhibit the CPM response[52]. While not everyone was using oral contraceptives, it is likely that all forms of hormonal contraceptives have some degree of systemic effects which could impact the CPM response[53].

There is great complexity in the relationship between a participant's hormonal state and a multitude of measures of pain[54]. While I aimed to summarise and compare hormone use and menstrual cycle phase between participants with and without intact CPM, the sample

size was not big enough, nor was the study designed to assess this. Finally, while I have relied on self-report measures and an algorithm for menstrual phasing, we didn't collect biological samples to sufficiently characterise the hormone phase of the participants, as Rezaii et al. have done previously[6]. Future work investigating pain modulation in women should ensure that hormone levels are acquired from participants to understand the hormonal milieu, so as to adequately characterise the cohort.

Generally, the influence of the time of CPM visit (i.e. morning, afternoon, evening) is not well understood. In a study of healthy men, rhythmic circadian changes in pain sensitivity have been observed throughout the day, with sensitivity being greatest at night and lowest in the afternoon, has been shown[55]. While that study excluded women, it is plausible that they similarly experience circadian changes in pain sensitivity. While most TRiPP CPM visits took place in the morning, afternoon and evening visits were not uncommon in each of the CPM response groups. Therefore, it is unlikely that the time of the CPM visit significantly impacted the CPM response in our study. However, there are recommendations[55] to control for this variable in the study of CPM, and therefore future studies should consider this.

I further investigated whether medication use varied between responders and non-responders, and summarised medications which have centrally-acting mechanisms. Serotonin-norepinephrine reuptake inhibitors (SNRI) and selective serotonin reuptake inhibitors (SSRI), are commonly used to treat depression, but have been proposed to facilitate pain modulation, particularly in people with neuropathic pain[56,57]. In the healthy control group, two (50%) of the inhibitory responders, and six (30%) of the non-responders

were taking a form of antidepressant. It is plausible that the 50% of controls with efficient CPM taking antidepressants may in fact have enhanced pain modulatory effects, because of this medication.

None of the inhibitory group were taking beta blockers or ADHD medications, which are also centrally acting and may have influenced pain modulation[58]. Additional investigation is warranted to understand the impact of centrally acting medications on pain modulation in female healthy controls.

6.4.3. Omics investigation of the CPM response: rs25531 and Serotonin abundance

There was a weak negative association between plasma serotonin and CPM inhibition in the entire cohort. A positive association was previously reported in males but not females with chronic back pain, suggesting potential sex differences in the molecular underpinnings of CPM inhibition[59]. The negative association (not significant in the fully adjusted model) could possibly be explained by higher platelet uptake of plasma serotonin, mediated by genetic differences in the 5-HTTLPR allele, which modulates serotonin transporter expression: the allele associated with lower expression was implicated in reduced CPM inhibition[60]. We could not attribute the lack of 'true' CPM inhibition in the healthy group to genetic differences in 5-HTTLPR allele, because only an indirect measurement of rs25531 SNP was available in our data, and there was a lack of power to detect its effect (with point estimate and frequency distribution suggestive against a genetic explanation).

Circulating serotonin abundance has been previously shown to decrease whilst on anti-depressant medication[61]. While it must be interpreted with caution, our results further support the general pain modulatory effects of such medications.

6.4.4. Limitations

The TRiPP study was carefully designed, using a recommended CPM paradigm, to address differences in CPM between those with CPP and healthy controls. However contrary to my hypothesis, only a few healthy control participants demonstrated CPM inhibition. While I investigated many known covariates which could in part explain these findings, there remain a few limitations which must be considered when drawing conclusions from these results.

Firstly, there is heterogeneity in the definition of 'healthy controls' in CPM studies[62]. Many studies exclude participants based on comorbidities, medication use and other physical characteristics because it has been proposed that such heterogeneity contributes to variability in CPM results[9,63,64]. We only excluded participants with moderate-severe CPP (>=3/10), and those with endometriosis, dysmenorrhoea, or urinary symptoms. Investigation of comorbidities showed that although they were common amongst healthy control participants, very few participants had painful conditions which would alter somatosensory functioning, as shown in **Appendix 6B**. Therefore, it is unlikely that heterogeneity with respect to comorbid diagnoses contributed to such few participants with intact CPM in this group.

While it is tempting to suggest that future CPM work should ensure that healthy controls are free from a multitude of comorbidities and factors which could influence the CPM response, one must consider the generalisability of such studies. When considering the real-world applicability of performing such exclusions, particularly in a cohort of reproductive-aged women who are at a higher risk of having pain and non-pain related comorbidities, I wonder how relevant those findings would be to our understanding of CPM in healthy controls and pain participants.

Finally, this study was initially powered to detect differences in CPM between CPP participants and healthy controls. However, this work focuses on the CPM effect in controls only (n=26), with the aim of further stratifying based on CPM response. As such, these exploratory analyses investigating covariates between CPM responders and non-responders were likely not sufficiently powered to detect relevant between group differences. Furthermore, the omics analyses were likely not powered to detect differences in the rs25531 allele or serotonin abundance with respect to the CPM response. Therefore, this should be considered when interpreting these results.

6.5. Conclusions

In conclusion, and contrary to my hypothesis, I observed efficient CPM in more CPP participants than healthy controls. Using a robust statistical approach to determine a true CPM effect, only 23.1% of healthy controls exhibited intact CPM, and further, only 15.4% exhibited CPM inhibition. After investigating covariates known to impact CPM, there was no single characteristic which explained why so few healthy controls exhibited CPM

inhibition. Additionally, many of the TRiPP healthy controls found the CPM paradigm, and in particular the CS, unpleasant. Therefore, as a result of this work, I question the general utility of the outlined CPM paradigm, and whether other methods of assessing pain modulation may be better suited for such studies aiming to better understand pain mechanisms in women. Or, if the CPM paradigm is still to be used, that location of the test site be strongly considered before moving forward with testing.

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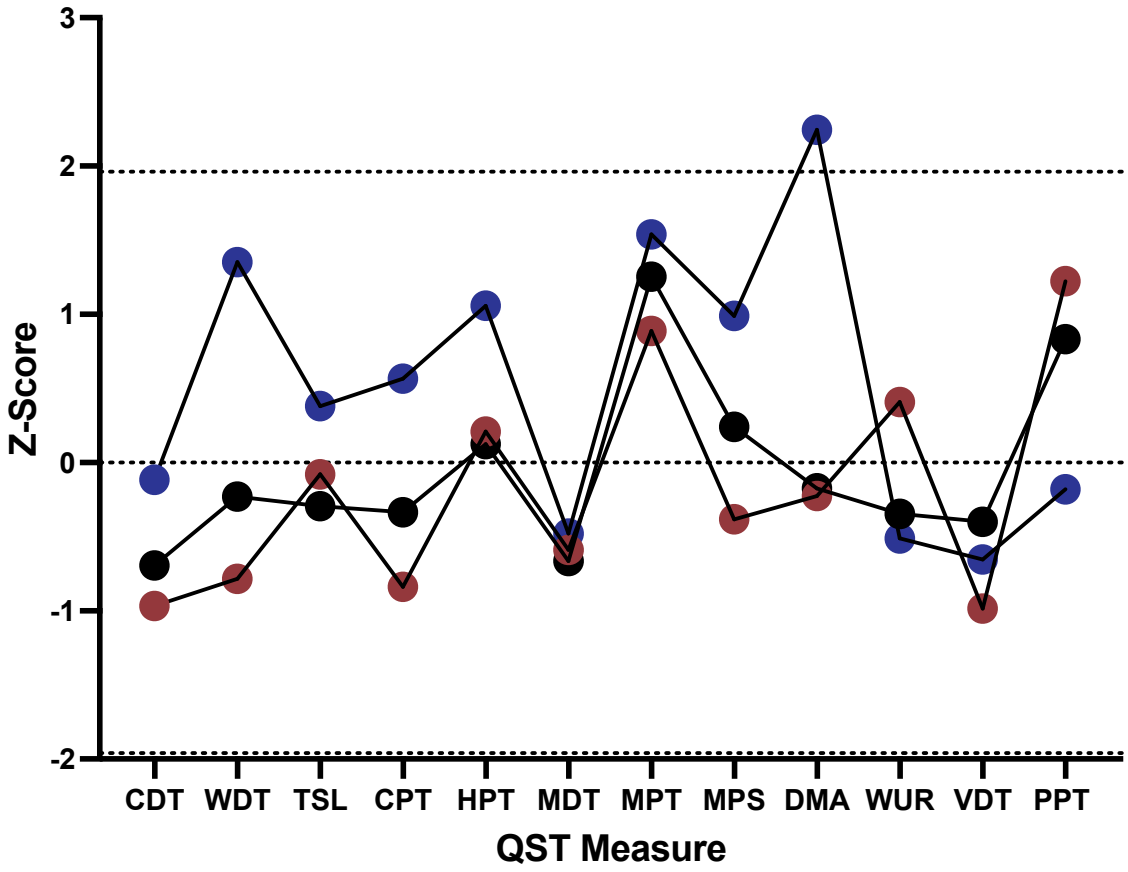
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6.7. Appendices

Appendix 6A: Quantitative sensory testing profile of healthy controls

Figure 6A-1. Quantitative sensory testing profile of control participants by CPM response group (red=facilitatory, black-non-responder, blue=inhibitory). A significant gain of function is indicated if the dot is above the top dotted line (Z>+1.96), and a loss of function; a dot below the bottom dotted line (Z<-1.96). CDT; cold detection threshold, WDT; warm detection threshold, TSL; thermal sensory limen, CPT; cold pain threshold, HPT; heat pain threshold, MDT; mechanical detection threshold, MPT; mechanical pain threshold, MPS; mechanical pain sensitivity, DMA; dynamic mechanical allodynia, WUR; windup ratio, VDT; vibration detection threshold, PPT; pressure pain threshold



Appendix 6B: Comorbid conditions by CPM response group in healthy controls

*****Participants were asked to select from a list of predefined conditions which condition they had ever received a diagnosis of. **Continued on next page.**

	Facilitatory (/2)	Non-responder (/20)	Inhibitory (/4)
Anxiety requiring medication or therapy	1	5	3
Asthma	0	2	1
Cardiovascular disease	0	0	1
Chron's disease	0	0	0
Chronic fatigue syndrome	0	0	0
Deafness/difficulty hearing	0	0	1
Depression requiring medication or therapy	0	4	1
Diabetes requiring diet control	0	0	0
Diabetes requiring insulin or tablets	0	0	0
Eczema	0	0	2
Uterine fibroids	1	0	0
Glandular fever	0	0	0
Graves' disease	0	0	0
Hashimoto's disease	0	0	0
High blood pressure	0	0	0
Irritable bowel syndrome	0	0	0
Migraine	0	1	0
Mitral valve prolapse	0	3	0
Multiple sclerosis	0	0	0
Painful bladder syndrome	0	0	0
Pelvic inflammatory disease	0	0	0
Polycystic ovarian syndrome	0	0	0
Rheumatoid arthritis	0	0	0
Scoliosis	0	0	1

	Facilitatory (/2)	**Non-responder (/20)**	**Inhibitory (/4)**
Other spine problems	0	0	0
Sjogren's syndrome	0	0	0
Systemic lupus erythematosus	0	0	0
Thyroid disease	0	1	0
Ulcerative colitis	0	0	0
Number (%) with at least one condition	2/2 (100%)	11/20 (55%)	4/4 (100%)

Chapter 7 – Discussion

7.1. Thesis summary

In this thesis, I have investigated the prevalence and burden of extra-pelvic pain in women living with chronic pelvic pain (CPP). Furthermore, I explored the relationship between reproductive outcomes and chronic pain. Finally, I've delved deeper into a commonly used psychophysical pain testing paradigm, conditioned pain modulation, in healthy women, to better understand the value it adds to our understanding of pain in women.

In Chapter 3, I characterised extra-pelvic pain in a cohort of women with chronic pelvic pain presumed secondary to endometriosis, and found that more than 80% reported pain in at least one extra-pelvic region. Women with a more widespread pain phenotype had significantly higher depression and pain catastrophizing scores, however, did not differ in their anxiety scores. Furthermore, more widespread pain was associated with a higher painDETECT score, suggesting a possible nociplastic and/or neuropathic component to this pain. Although the relationship was not significant in this dataset, this work generated important hypotheses about the relationship between chronic pain and reproductive outcomes, which warranted further investigation.

In Chapter 4, I tested the hypotheses generated in Chapter 3 about reproductive outcomes and chronic pain, and the mechanisms that may underlie it, using data from the UK Biobank. I found that women with a chronic pain condition were at an increased odds of having at least one miscarriage when compared to controls, however, the odds did not differ between the groups with respect to stillbirth or nulliparity. After stratifying the

chronic pain group into those with either an inflammatory or non-inflammatory chronic pain condition, I found the odds of being nulliparous to be increased in the inflammatory pain group when compared to those with a non-inflammatory pain diagnosis. This association remained true after stratifying into those with endometriosis, rheumatoid arthritis, and inflammatory bowel disease. This suggests a possible shared inflammatory mechanism between the chronic pain conditions and reproductive outcomes. Unlike my previous chapter, I investigated systemic inflammation (via neutrophil: lymphocyte ratio) in the chronic pain groups, to confirm that the RA and IBD group had an active inflammatory component at the time the data was collected.

In Chapter 5, I replicated my findings from Chapter 3 in a larger cohort and gained invaluable insight into the burden and pain mechanisms underlying widespread pain in women with chronic pelvic pain (CPP). In the TRiPP study, more than 60% of women with CPP experienced pain in at least one region outside of the pelvis, and this widespread pain was associated with worse psychological health and wellbeing. Furthermore, widespread pain was associated with worse fatigue and sleep. Using methods to deeply phenotype perturbations in sensory processing, quantitative sensory testing at the foot revealed a gain of function in dynamic mechanical allodynia and a loss of function in the vibration detection threshold. Contrary to expectations, there was no significant difference in conditioned pain modulation (CPM) inhibition between widespread pain groups.

In Chapter 6, I further investigated the CPM paradigm in healthy controls. Although CPM is a widely used paradigm to assess descending pain modulation in humans, only few (15.4%) healthy control participants demonstrated CPM inhibition. Within this chapter, I found no

single covariate which could distinguish between healthy control participants who exhibited CPM inhibition, and those who didn't.

All of this considered, the data presented in this thesis highlight the burden of widespread extra-pelvic pain in women with chronic pelvic pain. Further work is of course needed to better understand the mechanisms underlying the associations presented in this thesis, however, this work has demonstrated a degree of sensory disturbance and nociplastic pain as possible generators of the widespread pain phenotype. Additionally, this work has generated important questions and a preliminary understanding of the relationship between chronic pain and reproductive outcomes, which will require further investigation in a more suitable cohort. Finally, it has raised important debate about the value of the CPM paradigm presented in this thesis in women free from chronic pelvic pain: something which should be considered in future studies which aim to understand pain in women. Importantly, this thesis has used a simple method (the body map[1]) to phenotype extra-pelvic pain in women with CPP, which appears to have important clinical ramifications.

7.2. Clinical implications

Phenotyping by widespread pain in this thesis has demonstrated an important gap in our understanding and the clinical management of CPP. At present, CPP is differentially managed in part depending on the underlying pathology (i.e. endometriosis) and type of pelvic pain (i.e. dysmennorrhoea, dyspareunia, chronic pelvic pain, etc.). Irrespective of this, a recent review on international guidelines for the management of CPP found that peripherally-acting treatments to manage CPP were standard practice[2]. This typically

includes pharmaceutical interventions such as hormones (often oral contraceptives) or analgesics (both systemic, as in paracetamol, or peripheral, as in local anaesthetic). Furthermore, surgical interventions are additionally offered, particularly when trying to remove the underlying pathology, as is the case with endometriosis. However, as described earlier in this thesis, such peripheral treatments are ineffective for many[3]. My findings that extra-pelvic pain is highly prevalent in women with CPP (Chapter 3, Chapter 5) provides evidence that a more holistic approach to pain management may be necessary for patients. Whilst CPP is often managed in a gynaecological setting, perhaps patients with a more widespread pain phenotype would further benefit from a multidisciplinary pain management team, who could aim to address things like poor psychological health, fatigue and sleep, which may not be the primary focus in a standard CPP management plan, but would provide invaluable benefits to the patient's overall wellbeing. It should be acknowledged, however, that despite not being standard practice some centres (often in more academic units), are already working aligned with this model[4].

Furthermore, chronic pain and reproductive outcomes are often considered distinct entities. In the context of non-gynaecological chronic pain conditions (i.e. inflammatory bowel disease[5] or rheumatoid arthritis[6]), reproductive outcomes are seldom considered at all, except in instances of disease management during pregnancy. When considering endometriosis, chronic pain and reproductive outcomes represent two distinct pathways by which someone with the disease may seek a diagnosis[7]. However, my work provides preliminary evidence that the two may not in fact be distinct after all. Whilst further work is needed to understand the underlying mechanisms, it may represent an important area

of clinical research, which if addressed, could contribute positively to both reproductive and pain outcomes.

Finally, conditioned pain modulation has been proposed as an easy-to-use bedside tool to assess pain mechanisms and modulation[8]. However, my work draws attention to the variability in the CPM response in 'healthy' control women, using a standardised pressure pain paradigm. When considering the use of such a tool in a clinical setting, this variability, and how to control for it, must be considered in this population.

7.3. Strengths and limitations

The very nature of studying pain poses its own challenges, introduced by the subjective nature of the pain experience and the metrics we use to capture it. Whilst this challenge overarches the study of all pain, there are some limitations unique to the studies presented in this thesis which should be considered.

My work presented in Chapter 3 was the first study to phenotype widespread pain in women with endometriosis-associated chronic pelvic pain using a widely adopted protocol[9]. Although this work was likely underpowered to conclusively answer questions about the burden of widespread pain, it derived important hypotheses which were explored further throughout this thesis.

Chapter 4 addressed the issue of a small sample size, as I used data from the UK Biobank to answer questions about reproductive outcomes and chronic pain. Whilst the results of

this chapter are intriguing, it is important to consider both the demographics of the cohort and the nature of the case control study design used in this chapter, when interpreting these results. The UK Biobank participants have been shown to not be wholly representative of the UK population due to selection bias[10,11], and by design, were recruited at a time in their lives when most female participants would have been past their reproductive years or were post-menopausal[12]. Furthermore, the case control study design is limited in its ability to draw causal inferences[13]. However, this study was strengthened by the accessibility of data in the UK Biobank, robust phenotypic and biological data on thousands of participants, and a wide array of chronic pain conditions which could be studied.

The TRiPP study was not without its own challenges, largely due to the COVID-19 pandemic. TRiPP required in-person study visits, which introduced delays to recruitment and data collection as lockdowns were imposed across the world. This resulted in reduced numbers of participants recruited, and subsequently, less data collected than was originally anticipated. However, using the available data, I was able to more deeply phenotype a cohort of women with chronic pelvic pain to build upon my findings from Chapter 3.

7.4. Future directions

The work presented in this thesis provides evidence that widespread pain is burdensome to the overall health and wellbeing of women with CPP, as has been demonstrated in other chronic pain conditions[9,14–16]. However, there are many avenues which could be explored to aid in our understanding of such pain, both clinically and molecularly.

From a clinical perspective, pain in women with CPP needs to be better phenotyped. As a first step, there should be increased data availability on the subject, and to further our understanding of the mechanisms underlying widespread pain, it would be useful for the Michigan Body Map (MBM) to be routinely incorporated into clinical studies of CPP. For example, there are a standardised set of questionnaires used by endometriosis researchers in their clinical studies[17]. By including the MBM in such questionnaires, data availability on widespread pain would be robust, and the possibility to better understand its aetiology in women with endometriosis, vast. Furthermore, the utility of the tool in a clinical setting, from both the perspective of aiding the clinician's understanding of pain, and the patient's perspective of communicating their pain, should be further explored in chronic pelvic pain populations.

Clinically, the relationship between chronic pain and reproductive outcomes is an important one to explore. Given the limitations outlined in Chapter 3 and Chapter 4, a larger cohort study of women across their reproductive life, such as the ALSPAC dataset, is required to sufficiently address the causality of these associations.

Furthermore, there is important work to be done exploring the mechanisms underlying extra-pelvic pain. Improving our understanding of central mechanisms, for example, could have important implications for patient stratification in future clinical trials, investigating novel medications for the management of CPP.

From a biomolecular point of view, an interesting avenue to better understand the mechanisms underlying widespread pain, is to investigate inflammation by extracellular

vesicles (EVs)[18]. Prior to the pandemic, an original aim of this DPhil was to investigate the role of extracellular vesicles in widespread pain in women with endometriosis and chronic pelvic pain (**Appendix 3B**). EVs are small nanoparticles released by all cells in the body and can be easily isolated from peripheral blood[19], which was the plan of the EVEP study (Title: *Investigating the role of extracellular vesicles in endometriosis-associated pain*). They express surface markers of their parent cell and carry cargo (such as miRNA) which can play a role in activation or suppression of inflammation[20]. Furthermore, while they have not yet been shown to play a role in endometriosis-associated pain, they have been characterised in endometriosis[21,22], as well as other pain-related conditions, such as rheumatoid arthritis[23]. In addition, there is emerging evidence that extracellular vesicles are involved in both peripheral and central mechanisms of pain[20]. With respect to inflammatory pain, EVs have been shown to have antinociceptive properties, such that when mesenchymal stem cells derived EVs were injected into patients with osteoarthritis, improvement in pain symptoms was observed[24]. In animal models of neuropathic pain, EVs have been shown to have pronociceptive effects: EVs from a neuropathic pain model were injected into the medial prefrontal cortex of naïve rodents, and a significant reduction in thermal and mechanical pain thresholds was identified[25]. Thus, suggesting an important contribution of EVs to our understanding of pain, which should be considered in diseases like endometriosis. As a result of the COVID-19 pandemic, this project never began. However, it has received required approvals to recruit participants, and my hope is that another DPhil student will be able to undertake this project under my supervision.

Finally, further work is warranted investigating the CPM response in female healthy control participants. This will require the study of multiple paradigms and covariates known to

impact the CPM response, to more conclusively understand factors which must be controlled for in future studies employing the CPM paradigm. Only then will our understanding of CPM inhibition and reliability in chronic pain participants be aided.

7.5. Concluding remarks

The results presented in this thesis provide evidence that extra-pelvic pain is prevalent in a large proportion of women with chronic pelvic pain, irrespective of the underlying pathology. Increasing pain spread was importantly associated with worse psychological health, fatigue, and sleep, as well as demonstrated perturbations in sensory processing. Furthermore, although additional work is needed to understand the temporality of the associations, I have shown a relationship between chronic pain and reproductive outcomes, across multiple inflammatory chronic pain conditions.

Upon presentation of the data in this thesis, I hope that this work will positively contribute to our understanding of chronic pelvic pain, and the burden of pain elsewhere in the body. For too many women, management of their chronic pelvic pain is insufficient. Acknowledging and characterising the pain that exists outside of the pelvis may be an important step towards personalised medicine for women with CPP, something that is long overdue.

7.6. Bibliography

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7.7. Appendix: Public engagement with research seed fund project evaluation

This evaluation was performed by Dr Annaleise Wood, in collaboration with me and Dr Lesley Paterson. The project was generously funded by the University of Oxford's Public Engagement with Research Seed Fund.

EVALUATION REPORT

Won't you look a little closer? Pain beyond the pelvis in endometriosis

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1. Executive summary

Endometriosis is a chronic inflammatory condition affecting over 190 million women worldwide. Although highly prevalent, endometriosis remains a medical enigma that is seldom common knowledge. On Saturday 20 March 2021, an online educational event took place to explore widespread pain through visual art and highlight pain in endometriosis that exists outside of the pelvis. This online event brought together researchers, clinicians, representatives from endometriosis charities and support groups; together with artists and people living with endometriosis-associated pain (EAP), to learn about endometriosis and the unspoken impact it has on the lives of people with EAP. The event provided people with EAP an opportunity to discuss their pain, through visual art, with members of the endometriosis community who would in some capacity understand their experience.

A total of 65 people registered for the event; and 40 attended on the day. An evaluation of the online event took place to explore people's experiences of the event; what, if anything, they had learned; and any changes in people's perceptions of EAP. Audience members' feedback was gathered through an online audience interaction tool (Slido.com) – a total of 24 audience members completed the survey.



Key outcomes and impacts on respondents included:

- Increased understanding about endometriosis and EAP.
- Emotional responses to the event, for example, respondents described the event as moving and cathartic.
- Opportunity for people with EAP to express their experiences of pain visually through art.
- Raised awareness of art as a way to communicate experiences of pain.
- Feeling connected and supported by the endometriosis community.

Successes and aspects that worked well:

- Being able to learn about endometriosis from different perspectives and groups of people, including artists and researchers.
- The uplifting and inspiring nature of the event – people felt inspired, emotional and moved to learn about the lived experiences of people with endometriosis.
- Fostering a community of people with shared experiences – people valued feeling connected to the endometriosis community.

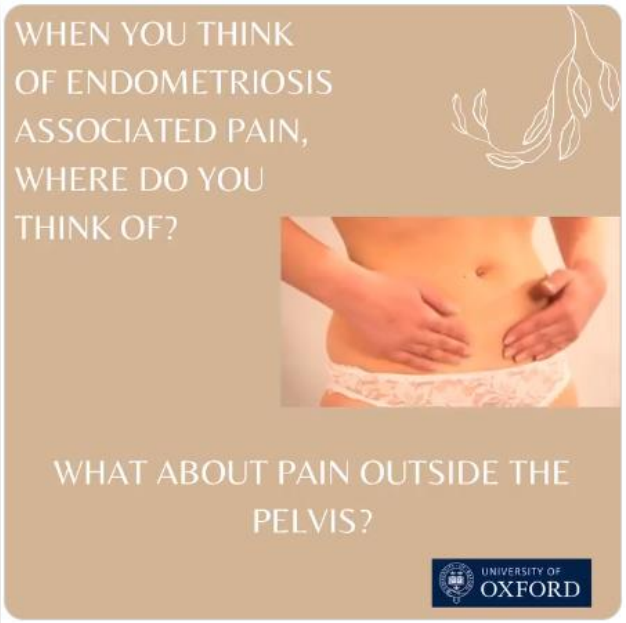
Challenges:

- The feedback from respondents was overwhelmingly positive, and only one respondent shared reflections on the wider challenges within the endometriosis community (rather than a challenge facing the online event).

2. About the online educational event

Endometriosis is a chronic inflammatory condition affecting over 190 million women and people born with a uterus worldwide. Although highly prevalent, endometriosis remains a medical enigma that is seldom common knowledge. For many endometriosis-sufferers, chronic pain predominantly in the pelvis is a reality. However, recent work done by DPhil student Danielle Perro of the Pain in Women research group at the University of Oxford suggests that many suffer from extra-pelvic pain that is inadequately addressed.

On Saturday 20 March 2021, an online educational event took place to explore widespread pain through visual art and highlight pain in endometriosis that exists outside of the pelvis. The event was part of a wider project[1] which collaborates with visual artists living with endometriosis-associated pain (EAP) and people living in Oxfordshire who have endometriosis and widespread pain to highlight, through visual art, the widespread nature of pain.



The educational event provided people with EAP an opportunity to discuss their pain, through visual art, with members of the endometriosis community who would in some capacity understand their experience. For this public audience, this was an important opportunity to learn about endometriosis and the unspoken impact it has on the lives of people with EAP. The overarching aim of the event was to increase awareness about endometriosis and EAP; and to highlight research being done in the field of pain, the Nuffield Department of Women's Reproductive Health (NDWRH; University of Oxford).

The event began with Danielle Perro presenting her DPhil work on widespread pain and endometriosis. This was followed by a virtual art gallery, where the subsequent portion invited participants who had taken part in the project to speak about their artwork and share their experiences of using art to communicate pain. Additionally, there was a facilitated discussion about research around self-management tools developed specifically for people living with endometriosis during COVID-19. Next, DPhil students from the Pain in Women and Endometriosis Care and Research groups presented their work, and the potential it has to benefit people with endometriosis. Additionally, recent research on the impact of COVID-19 on those with

[1] **Further information about the project, *Won't you look a little closer? Pain beyond the pelvis in endometriosis*, can be found here: https://sites.google.com/view/perseedfund2020/wont-you-look-a-litte-closer-pain-beyond-the-pelvis-in-endometriosis.**

endometriosis and access to relevant services were discussed. The day culminated with a summary and discussion about the event.



3. Evaluating the online event

An evaluation of the online event, '*Won't you look a little closer? Pain beyond the pelvis in endometriosis'*, was carried out to explore the outputs, outcomes and impacts resulting from the event, by engaging with the reflections and views of attendees.

Aims and objectives

The key objectives of the online event were:

1. To increase public awareness about endometriosis and endometriosis-associated pain (EAP), and highlight research being done in the field of pain, the NDWRH and Endometriosis UK.
2. To evoke conversations around EAP that challenge the heavily-focused pelvis pain narrative surrounding endometriosis.
3. To challenge current perceptions of endometriosis pain localization amongst people with endometriosis; to those who support people with endometriosis or have an interest in this this area.

Evaluation questions

1) What were the key inputs and outputs from the event?
    i. How many researchers/ DPhil students were involved in the development and delivery of the event?
    ii. How many activities or events were delivered?
    iii. How many people registered; how many people attended?
    iv. How many people with endometriosis attended?
2) What were the outcomes and impacts of the event on attendees?
    i. Did people's perceptions of EAP change as a result of the event?
    ii. Did people learn about endometriosis and EAP research being done in the field of pain, the NDWRH and Endometriosis UK?
    iii. Did people learn about pain through the means of art and science?
3) What learning and recommendations can be made for future online events?
    i. How successful was the event in its online format?
    ii. What were the challenges or barriers to having an event entirely online, inclusive of a virtual art show?

The evaluation was both summative (to explore the outcomes and impacts of the event on attendees), and formative (to gather learning and identify recommendations to enhance and improve future online events).

Evaluation methodology

Multiple evaluation tools were used to gather data and evidence for the evaluation. A summary of the different approaches is provided below:

Evaluation tool	Description	Number of responses
Data mining	• Registration and attendance data for the online event. • Demographic information was gathered through the registration form (including gender; and whether attendees have endometriosis). • The registration form also asked delegates: 'What 3 words first come to mind when you think of endometriosis-associated pain?'. This same question was repeated in the audience evaluation at the end of the event.	Number of people who registered: 65
Slido	• A Slido questionnaire was circulated to audience members at the end of the event. This was completed live during the event, and contained the following questions: 1. How would you describe your experience of today's event? 2. What, if anything, have you learned? 3. What 3 words first come to mind when you think of endometriosis-associated pain?	Number of responses: 24 Response rate: 24/28[2]

Data protection and ethical considerations

All evaluation activities were emphasised as voluntary; participants had the opportunity to not answer all the questions and could withdraw from taking part at any time.

The audience members' responses to the first two questions were hidden from other participants on Slido. Answers to this final question, 'What 3 words first come to mind when you think of endometriosis-associated pain?', were anonymised and then shared within a word cloud with attendees at the start and end of the event.

[2] By the end of the event when we asked people these questions, there were around 28 people present.

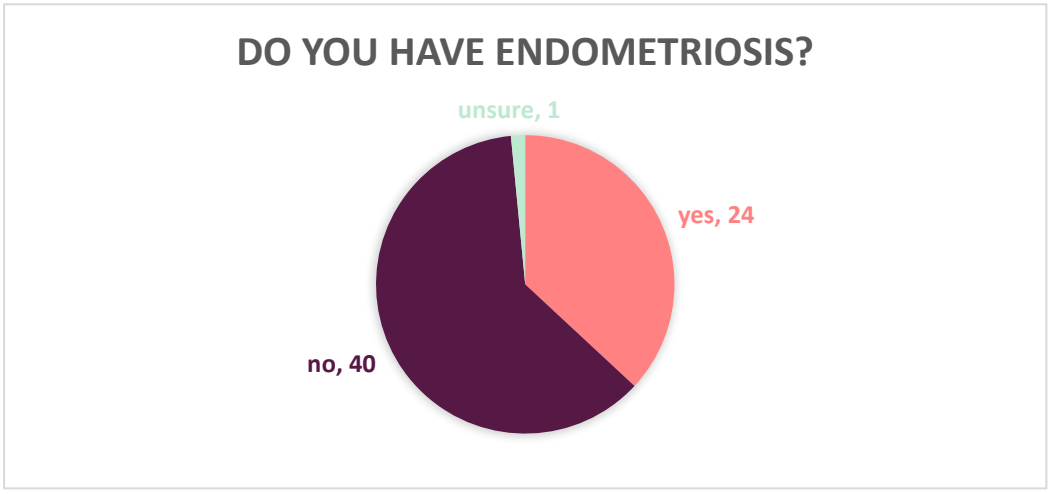
4. Evaluation findings

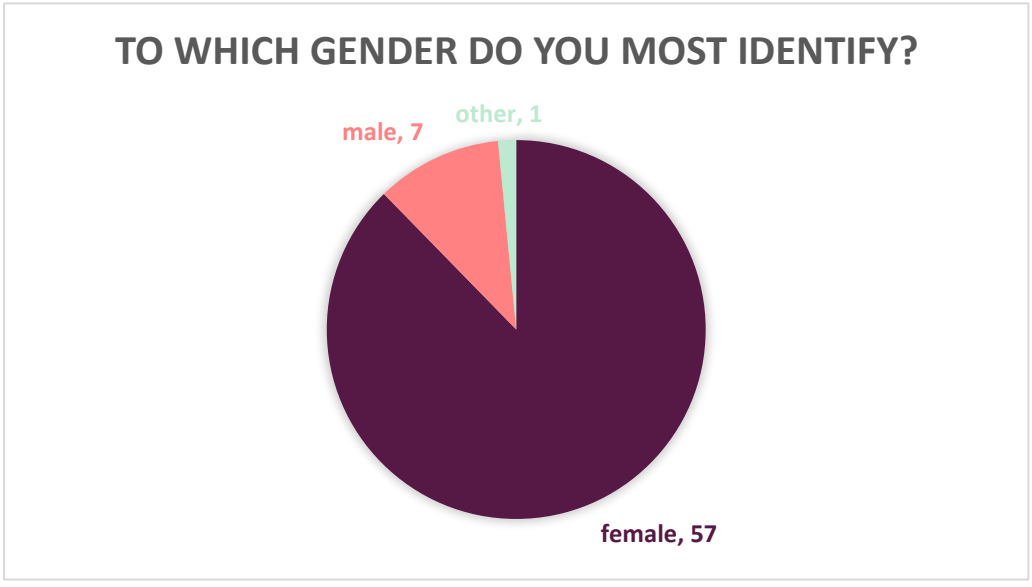
4.1 Inputs and outputs

This first section provides an overview of the key inputs and outputs from the online event.

Key inputs and outputs:

- 1 event registration page: https://medsci.zoom.us/meeting/register/tJUrc-isrDojGdRFS9V3NGJvN4etMNqBHBkw
- Event promotion via Twitter: https://twitter.com/dperro_wrh/status/1372154251674288129
- Funded by the University of Oxford's Public Engagement with Research Seed Fund in 2020/21.
- The online event was facilitated by Danielle Perro (DPhil student in the Nuffield Department of Women's & Reproductive Health, University of Oxford).
- 3 DPhil students and 1 Post-Doctoral Researcher from the Pain in Women and Endometriosis Care and Research group presented their research at the event.
- A total of 65 people registered for the event; and as many as 40 people attended the online event, which took place via Zoom (see graphs below for demographic information).






HOW DID YOU HEAR ABOUT THIS EVENT?
Word of mouth
18
Other
18
Twitter
11
I am artist involved in the project
8
Direct clinic with an event organiser
7
Instagram
3

4.2 Outcomes and impacts on attendees

This next section explores the different outcomes and impacts on individuals who attended the event.

i. An overall interesting and informative event

Overall, respondents were very positive about their experience of the online event – with people most commonly describing their experience as informative and interesting. The word cloud (figure 1) below highlights the words that respondents used to describe their experience of the event with the size of each word representing the relative frequency).

"Very interesting and informative. Great to hear about all the work being done!"

"I've absolutely loved all discussions today. All the different research projects are so interesting and it's really exciting to see that more research is going into endo and pelvic pain"



Figure 1 Word cloud illustrating the words that respondents used to describe their experience of the event

ii. Emotional response

Many respondents also experienced an emotional response to the event; and described their experience as “emotional”, “moving” and “cathartic”:

“Amazing! Informative and very emotional (I have endometriosis!)”

“Fascinating, validating, cathartic (didn't expect to get so emotional!), and uplifting.”

---

iii. Increased understanding about endometriosis and endometriosis-associated pain (EAP)

Respondents learned about a range of research taking place related to endometriosis, and that many new studies are providing hope for a greater understanding of EAP; diagnostics; and personalised treatments. Moreover, the event increased people’s understanding of how experiences of endometriosis can be similar but also differ between individuals.

“The new studies that are ongoing at present. With hope for greater understanding of endo-pain and effective, standardised diagnostics.”

“It has been fascinating to learn about the different studies and progress on the subject of Endometriosis that are currently been untaken. Hopeful.”

“Further research & work is needed in helping women with endometriosis for personalised treatment and diagnosis”

Both before and after the event, respondents commonly associated EAP with words such as “debilitating”, “stabbing”, “infertility”, “burning”, “exhausting”. These words emphasised the very challenging and difficult experiences associated with endometriosis-related pain.

At the end of the event, the word “misunderstood” frequently came to people’s minds when they thought about EAP. These ‘after’ words focused on lived experience and the terms used in presentations about ongoing research in endometriosis during the event. The word clouds below illustrate the words that people associated with EAP before the event (figure 2) and at the end of the event (figure 3). In the word clouds, the size of each word represents its relative frequency (i.e. the more frequently a word was referenced, the larger it appears in the word cloud).

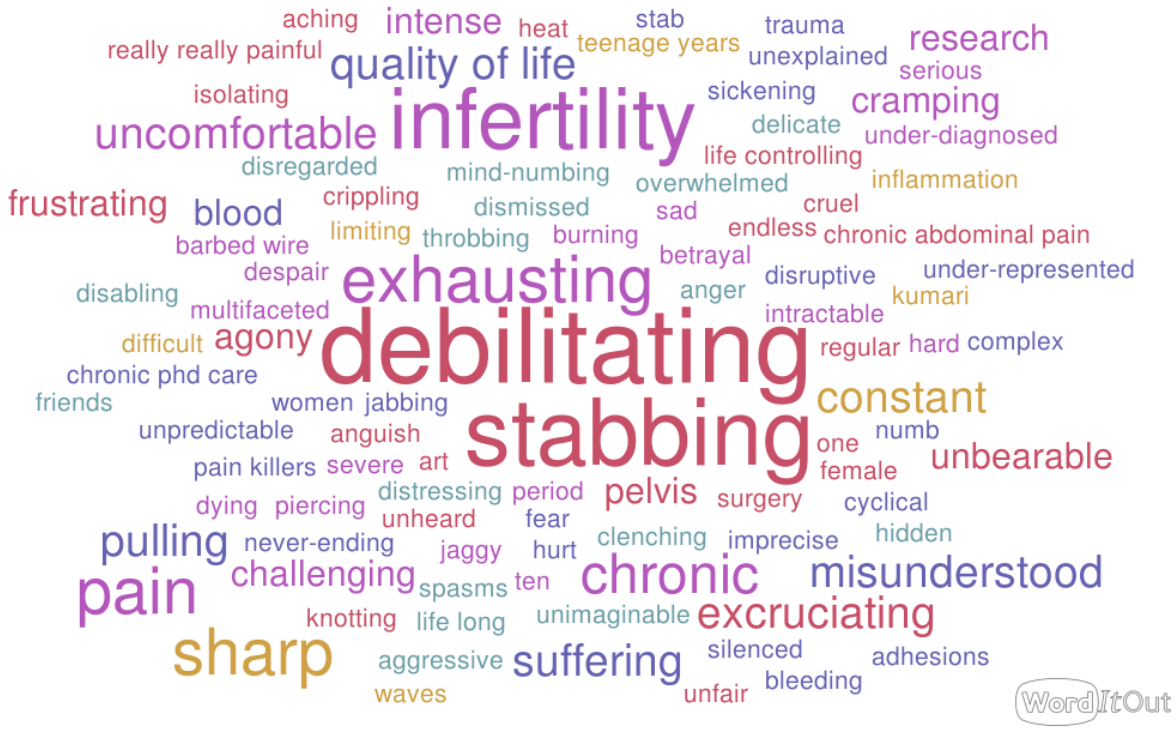


Figure 2 Words that come to mind when people think about EAP (before the event)

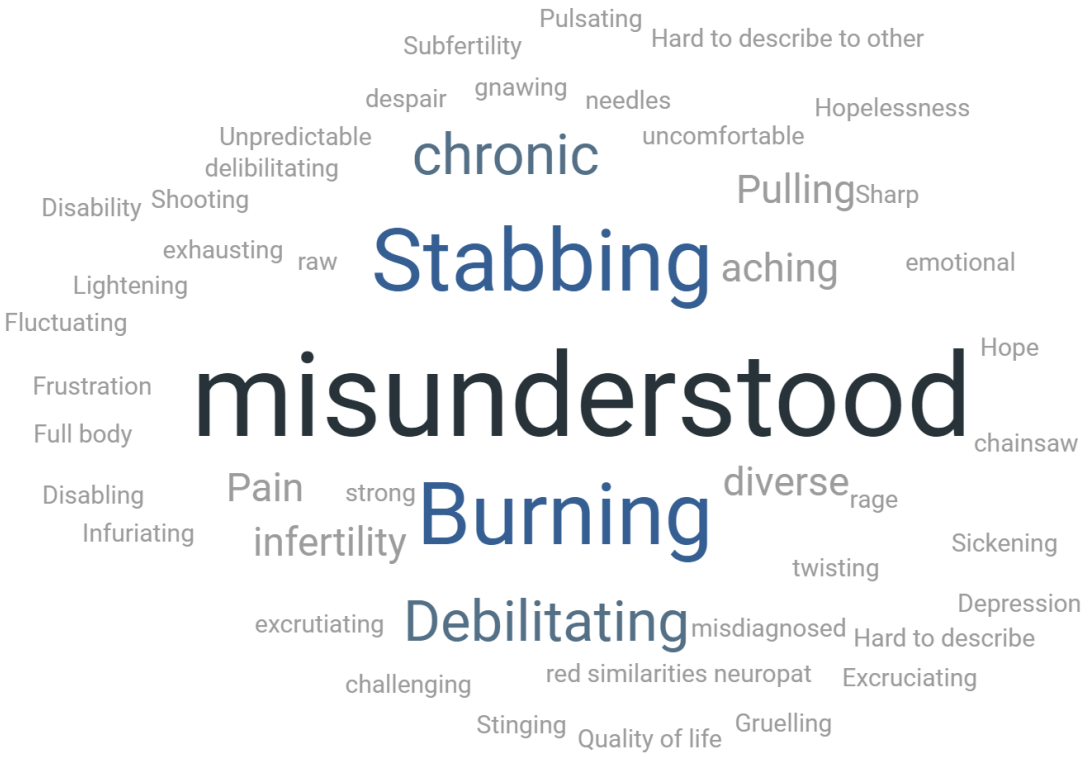


Figure 3 Words that come to mind when people think about EAP (after the event)

iv. Raised awareness of artwork as a way to communicate experiences of pain

Respondents learned about people's complex experiences of EAP through engaging with the visual art presented by project participants during the event. Attendees further emphasised that they are now more aware of artwork as an effective way for people to communicate their experiences of pain and endometriosis, which can be challenging to express verbally. The project further had a positive impact on individuals who were able to express their experiences of pain visually through art, including helping those to visually represent their pain when speaking with health professionals:

"How widely the experience of endometriosis differs from person to person, how many different research avenues are being pursued for different aspects, how cathartic using art to depict pain can be for people."

"I have learned to try and express my pain visually! I'm going to use a visual representation when I see my gynaecologist on Tuesday!"

---

v. Feeling connected and supported by the endometriosis community

Some respondents shared that they felt supported and had a stronger sense of connectedness to the endometriosis community as a result of attending the event:

"The endo community is STRONG and incredible!"

"I already knew, but this has reinforced that I am not alone. And that there is so much incredible research going on, which is very very encouraging."

"That even though you feel you are alone with endo, you are not. Those events give you strength and the feeling of not going alone through it."

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vi. Further outcomes on audience members

Respondents further reflected on the following ideas around endometriosis after attending the event:

- Increased understanding of the impact of the pandemic on people with endometriosis.
- Raised awareness of endometriosis as a full-body condition.
- Understanding the need for further research and work in helping people with endometriosis.

- Understanding the importance of collaborations between patients, researchers, and clinicians in order to understand each other's perspectives to be able to make a significant impact in improving the lives of people with endometriosis.

4.3 Successes and challenges

This final section highlights the key lessons learned through the online event; and explores the key successes and what worked well; and any challenges raised by respondents.

Successes

Respondents highlighted the following successes and aspects that worked well:

- Hearing from DPhil students about the range of research studies in this area and being able to learn about new aspects and insights into research being undertaken to help people with endometriosis.
- Being able to learn from different groups of people from both art and science backgrounds.
- Bringing together researchers, artists, clinicians and people who have endometriosis and widespread pain to illustrate the complex and individual experiences, and the widespread nature of pain.
- The uplifting and inspiring nature of the event – people felt inspired, emotional and moved to learn about the lived experiences of people with endometriosis and the range of research that is taking place to investigate EAP, diagnostics and personalised treatments.
- Fostering a community of people with shared experiences – people valued feeling connected to the endometriosis community and felt that their own experiences were validated and listened to.

Challenges and reflections

The feedback from respondents was overwhelmingly positive, and only one respondent shared reflections on the wider challenges within the endometriosis community (rather than a challenge facing the online event). This individual highlighted that while it was helpful to hear that more research was taking place related to endometriosis, some of the messages shared by medical professionals were perceived as dated. This event highlighted the need for more discussions and focus on person-first and person-guided management to ensure that each individual receives care in line with their goals.

> *"That more research is going on related to endometriosis, but some of the tropes obviously being rolled out by medics are depressingly old. The continued emphasis on maintaining fertility over quality of life seems not to have shifted at all, which is very depressing."*

5. Conclusion

In returning to the key objectives of the online event, the following conclusions can be made:

Objective 1: To increase public awareness about endometriosis and endometriosis-associated pain (EAP), and highlight research being done in the field of pain, the NDWRH and Endometriosis UK.

This online educational event brought together people with endometriosis, artists, researchers, clinicians and representatives from endometriosis charities and support groups to learn about research being done in the field of pain, and the NDWRH. Overall, many respondents highlighted that they learned about research around different aspects of EAP – for example, about people's experiences of this condition, diagnostics, personalised treatments, and the ways in which experiences of endometriosis can be similar but also differ between individuals.

Objective 2: To evoke conversations around EAP that challenge the heavily-focused pelvis pain narrative surrounding endometriosis.

The event provided a platform for DPhil researchers in the Pain in Women and EndoCaRe research groups at the University of Oxford to share findings from their research which suggests that many women suffer from extra-pelvic pain that is inadequately addressed. The educational event also provided people with EAP an opportunity to discuss their pain, through visual art, and to share their experiences of using art to communicate pain.

Through both the sharing of research findings and artwork, respondents learned about the widespread nature of pain that exists outside the pelvis and people's complex and challenging experiences of EAP. They also learned about how artwork can be an effective way for people to communicate their experiences of pain and endometriosis, which can often be difficult to express verbally. Many of the respondents emphasised they experienced an emotional response to the event, describing the event as moving and cathartic; and how it provided an important opportunity to learn about endometriosis and the unspoken impact it has on the lives of people with EAP.

Objective 3: To challenge current perceptions of endometriosis pain localization amongst people with endometriosis; to those who support people with endometriosis or have an interest in this this area.

A key message that many audience members took away from the event was that EAP is often misunderstood, and the ways in which experiences of endometriosis can be similar but also differ between individuals. The event further reinforced the importance of collaborations between patients, researchers, and clinicians in order to understand each other's perspectives to be able to make a significant impact in improving the lives of people with EAP.

6. About this evaluation

This evaluation is part of a much wider building capacity programme at the University of Oxford that aims to equip researchers with the skills, knowledge, experience and support to plan, deliver and evaluate Public Engagement with Research activities.

This evaluation will also be written up as a case study, as a practice case studies, to provide inspiration, guidance and ideas for researchers and professional services staff on the evaluation of online festivals and virtual events.

For more information about evaluating Public Engagement with Research at the University of Oxford;

- See the current training opportunities; http://www.ox.ac.uk/research/public-engagement/support-researchers
- Contact **Dr Annaleise Wood** (Engagement & Evaluation Facilitator, Public Engagement with Research, Research Services): annaleise.wood@admin.ox.ac.uk

7. Acknowledgements

Thank you to those who have shared their reflections and experiences of this online event; your time in contributing to this evaluation is very much appreciated.

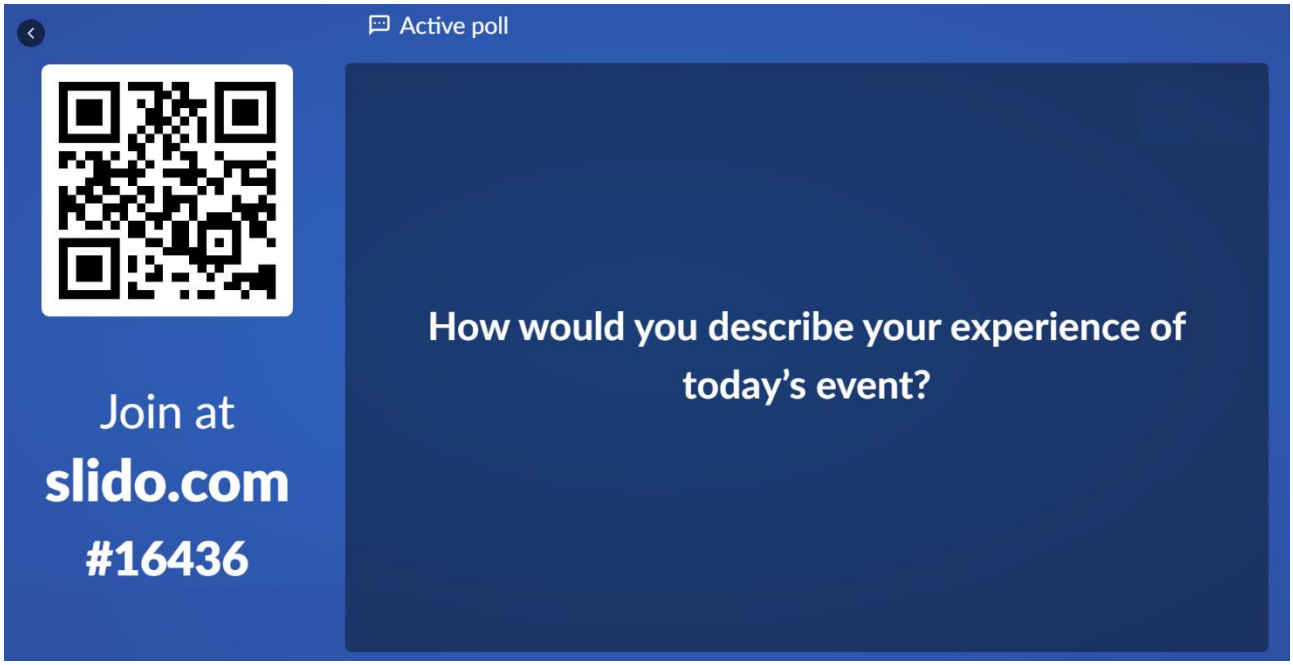
This evaluation was conducted by Dr Annaleise Wood (Engagement & Evaluation Facilitator, Public Engagement with Research, Research Services), with support from Dr Lesley Paterson (Head, Public Engagement with Research, Research Services) and Danielle Perro (Nuffield Department of Women's & Reproductive Health) at the University of Oxford.

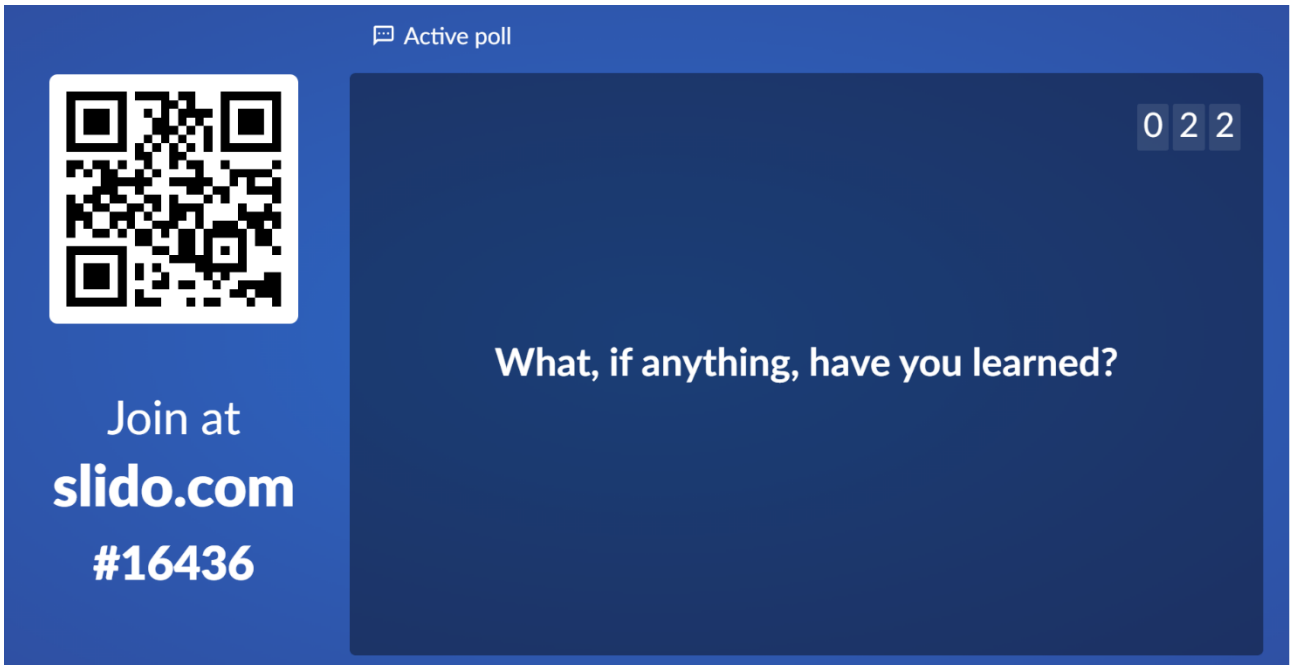
The programme to build capacity in evaluating Public Engagement with Research, which these evaluation case studies form a part of, was developed by the Public Engagement with Research Team in Research Services, and supported by the Public Engagement with Research Advisory Group (with representatives from across the Academic Divisions and the Gardens, Libraries & Museums).

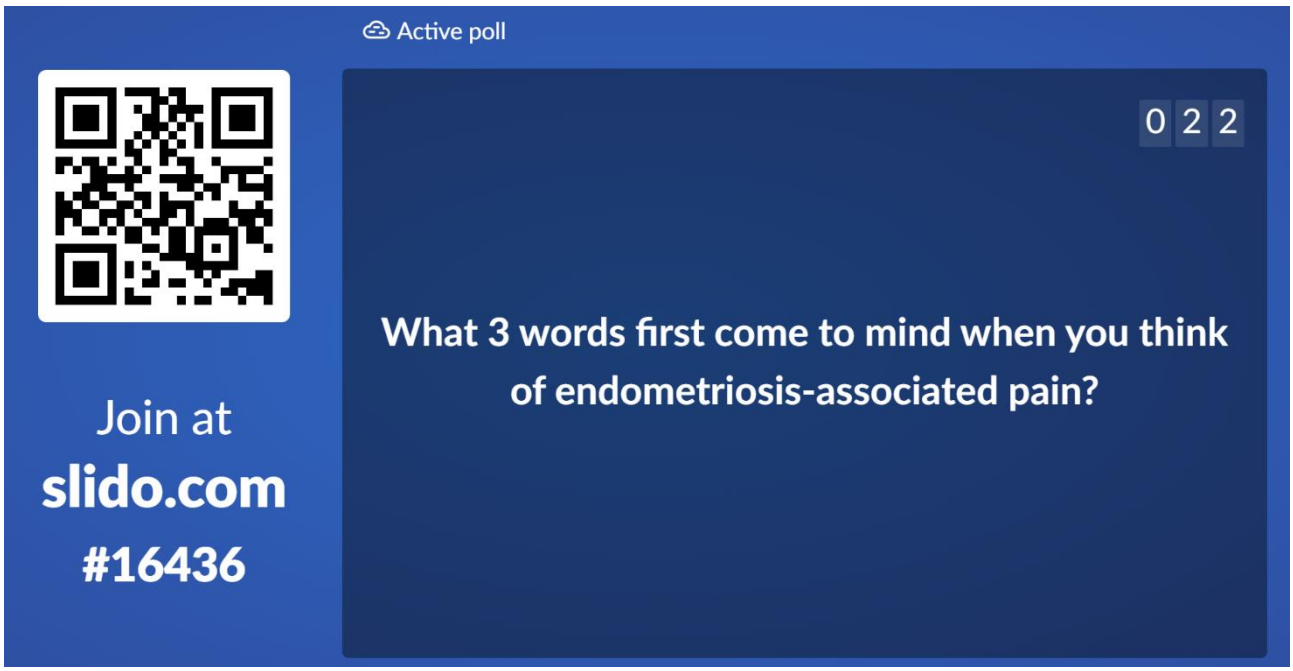
'Won't you look a little closer? Pain beyond the pelvis in endometriosis' was funded by the University of Oxford's Public Engagement with Research Seed Fund in 2020/21.

8. Appendices

Appendix 1: Slido survey questions






Active poll
0 2 2
What 3 words first come to mind when you think of endometriosis-associated pain?
Join at
slido.com
#16436