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A biopsychosocial approach to understanding the contributors to chronic pain

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DECLARATIONS

This is to certify that work submitted in this thesis is the result of original research. It has been conducted substantially by myself with assistance outlined below. It has not already been accepted for any degree, diploma or other qualification. All authors and works to which reference has been made are fully acknowledged.

Data were utilised from the Survey of Health and Ageing and Retirement in Europe (SHARE). Design, data analysis and writing of the study that utilised the SHARE data were conducted by myself, under the supervision of Professor Ana M. Valdes, Associate Professor Tobias Bast, and Professor Eamonn Ferguson.

Study design, ethical application, data collection, analysis, and writing for the Cognition, Pain and Wellbeing study (Study 1) were conducted primarily by myself, under the supervision of Professor Ana M. Valdes, Associate Professor Tobias Bast, and Professor Eamonn Ferguson. Mr Anthony Kelly assisted with data collection, including QST measures and bloods.

Study design, ethical application, data collection, and general administration for the Knee Pain In the Community (KPIC) study data were conducted by the KPIC study team, including Mr Anthony Kelly, Dr Gwen S. Fernandes, Professor Ana M. Valdes, Professor Weiya Zhang, Professor David A. Walsh and Professor Michael Doherty. The study that utilised the KPIC data was designed by Professor Ana M. Valdes and myself. Data analysis and writing were done by myself.

Study design for the Investigating Musculoskeletal Wellbeing and Health (IMW&H) study was conducted by Dr Bonnie Millar, Dr Daniel McWilliams, Professor Eamonn Ferguson and Professor David Walsh. Ethical application, data collection and general application for the Investigating Musculoskeletal Wellbeing and Health study were conducted by Dr Bonnie Millar. Design of the COVID study survey that utilised the IMW&H data was conducted mainly by myself, with some input from Professor Ana M. Valdes and Dr Amrita Vijay. Data analysis and writing were conducted by myself under the supervision of Professor Ana M. Valdes.

Data were utilised from the WebEx cohort designed by Professor Ana M. Valdes and Dr Sameer Gohir and from the Omega 3 cohort designed by Professor Ana M. Valdes and Dr Amrita Vijay. Ethical application and data collection for the WebEx were conducted by Dr Sameer Gohir. Mr Anthony Kelly helped with data collection. Ethical application and data collection for the Omega 3 were conducted by Dr Amrita Vijay. The study that utilised these data was designed by Professor Ana M. Valdes and myself. Data analysis and writing were conducted by myself under the supervision of Professor Ana M. Valdes.

All data presented in this thesis were analysed by myself, and supervision of the thesis was undertaken by Professor Ana M. Valdes, Associate Professor Tobias Bast, and Professor Eamonn Ferguson.

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LIST OF PUBLICATIONS

Chapter 4 - Study 2: Kouraki, A., Bast, T., Ferguson, E., & Valdes, A. M. (2022). The association of socio-economic and psychological factors with limitations in day-to-day activity over 7 years in newly diagnosed osteoarthritis patients. Scientific reports, 12(1), 943. https://doi.org/10.1038/s41598-022-04781-3.

Press release: https://www.nottingham.ac.uk/news/osteoarthritis

Chapter 6 - Study 4: Kouraki, A., Doherty, M., Fernandes, G. S., Zhang, W., Walsh, D. A., Kelly, A., & Valdes, A. M. (2022). Different genes may be involved in distal and local sensitization: A genome-wide gene-based association study and meta-analysis. European Journal of Pain, 26, 740– 753. https://doi.org/10.1002/ejp.1902

Chapter 7 - Study 5: Kouraki A, Vijay A, Gohir S, Spector TD, Kelly A, Bast T, Ferguson E, Valdes AM. Reproducible microbiome composition associations with anxiety are not mediated by short chain fatty acids or indoles. *In preparation*

ABSTRACT

Background: The biopsychosocial model suggests that chronic pain is the result of a dynamic interplay between biological, psychological, and social factors. Psychological variables, such as anxiety, are recognised as key contributors to chronic pain. However, their role, and particularly that of cognitive ability, in chronic pain from osteoarthritis (OA) - the main cause of chronic pain worldwide - is less well understood. Social stressors, such as social deprivation may further explain the pain experience and may predict increased functional disability (limitations in activities of daily living), but their interaction with psychological factors has not been studied much, especially in the early stages of OA. Meanwhile, anxiety and social isolation extending over many months during the coronavirus pandemic may exacerbate pain in chronic pain patients, but this has not been investigated over time. Chronic pain in OA is underpinned by neuropathic and pain sensitization mechanisms that may predict resistance to therapy. Considering biological factors, such as host genetics, may enhance our understanding of these key mechanisms in OA. Lastly, little is known about the gut microbiome's role in chronic pain. Identifying the gut microbiome's link with anxiety may give us some understanding of how it might be linked to pain. Therefore, the aim of the current thesis was to explore how psychosocial factors (cognitive ability, anxiety and social deprivation/isolation), host genetics and the gut microbiome, interact in their contribution to chronic pain.

Objectives: [1] to design a study aimed at investigating the specific neuro-cognitive domains that are impaired in OA pain, [2] to explore the dynamic interplay between key socioeconomic (i.e. social deprivation, education) and psychological factors (i.e. cognitive function, anxiety) and limitations in activities of daily living in OA pain; [3] to explore the potential impact of the first coronavirus lockdown on social isolation, anxiety, pain and overall quality of life in chronic pain patients; [4] to identify genetic markers associated with pain sensitisation and neuropathic-like pain in OA; and [5] to identify bacterial taxa reproducibly associated with anxiety and the role of serum levels of microbial metabolites and neuromodulators.

Methods: [1] We recruited eight participants from the Nottinghamshire area to conduct a feasibility study. Distinct cognitive functions were measured using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Pain measures included Quantitative Sensory Testing and pain questionnaires, such as painDETECT. Confounders were measured, including depression/anxiety, sleep quality, quality of life and physical function. Additional cognitive measures were taken, along with the National Adult Reading Test that served as a proxy of general premorbid IQ. Data on prosociality were also collected. For identifying molecular biomarkers, gut microbiome measures and genetic markers, blood, urine and faecal samples were collected. We assessed the feasibility of the study based on the experiences of the patients. Descriptive statistics were reported and correlational analysis was used to investigate the relationship between cognitive function, anxiety and

pain.

[2] We used data from waves 4, 5, 6 and 7 of the Survey of Health, Ageing and Retirement in Europe (SHARE) (n = 971) and selected a subsample of respondents who initially did not report a diagnosis of OA until wave 6. We used path models to test how social deprivation, education and anxiety, before diagnosis (waves 4 and 5), affect the relationship between cognitive ability, pain and limitations in activities of daily living following diagnosis (waves 6 and 7).

[3] We analysed data from a sample of chronic pain patients (N = 305 of which 28.9% reported having OA) from Investigating Musculoskeletal Health and Wellbeing cohort thrice during the pandemic (May 2020, July 2020, September 2020). We employed repeated measures ANOVAs (and Kruskal–Wallis where appropriate) to compare measures of affect, social isolation, exercise changes due to COVID-19, quality of life and pain at the various time points.

[4] We performed a genome-wide gene-based association study using pressure pain detection thresholds (PPTs) from distal pain-free site (anterior tibia), a measure of distal sensitisation, and from proximal pain-affected site (lateral joint line), a measure of local sensitisation, in 320 knee OA participants from the Knee Pain and related health in the Community (KPIC) cohort. We next performed gene-based fixed-effects meta-analysis of PPTs and a neuropathic-like pain phenotype (painDETECT) using genome-wide association study data from KPIC and from an independent cohort of 613 OA participants, respectively.

[5] We meta-analysed data from two cohorts (WebEx: n = 37 and Omega 3/Fibre: n = 58) and published literature (n = 60) in order to identify bacterial taxa reproducibly associated with anxiety. We further investigated the role of serum levels of SCFAs and indoles on anxiety and the links between anxiety-associated operational taxonomic units (OTUs) and neuromodulators involved in anxiety. Gut microbiota was characterized using 16S rRNA sequencing in stool samples. OTU data were transformed and normalized. Serum metabolites were measured using tandem mass spectrometry.

Results: [1] The study was feasible and all practical aspects were executed as planned, and the study was well received by the patients. Our preliminary findings suggested that anxiety, pain and cognitive ability are correlated and that global cognitive impairment and impairments in certain aspects of cognitive ability might be associated with poor physical function, increased neuropathic-like pain and increased central pain processing.

[2] We showed that high social deprivation before diagnosis predicted greater limitations in activities of daily living after diagnosis, with this effect partly mediated by impaired cognitive ability. We also found that higher educational attainment before diagnosis may protect against limitations in activities of daily living after diagnosis via better cognitive ability and lower anxiety.

[3] Covid-19 lockdown increased anxiety in May and September, whereas pain levels were unaffected. The highest levels of social

isolation and depression occurred in the early stages of lockdown but declined thereafter. The at least a bit anxious group had heightened pain in May and September compared to the not at all anxious group. Doing at least an hour of exercise per day (on a typical day) was a protective factor against increased anxiety during lockdown, and quality of life improved by September when lockdown rules were eased.

[4] The most significant genes associated with distal and local sensitisation were *OR5B3* and *BRDT*, respectively. We also found previously identified neuropathic pain-associated genes – *KCNA1*, *MTOR*, *ADORA1*, and *SCN3B* – associated with PPT at the anterior tibia and an inflammatory pain gene – *PTAFR* – associated with PPT at the lateral joint line. Meta-analysis results of anterior tibia and neuropathic-like pain phenotypes revealed genes associated with bone morphogenesis, neuro-inflammation, obesity, type 2 diabetes, cardiovascular disease and cognitive function.

[5] We identified reproducible microbiome associations of anxiety with *Escherichia-Shigella* (fixed-effect beta (95% CI) = 0.28 (0.11, 0.45), False Detection Rate (FDR)-adj. $p = 3 \times 10^{-3}$), *Christensenellaceae R-7 group* (fixed-effect beta (95% CI) = -0.32 (-0.48, -0.15), FDR-adj. $p = 1 \times 10^{-3}$) and *Ruminococcaceae UCG-010* (fixed-effect beta (95% CI) = -0.24 (-0.40, -0.08), FDR- adj. $p = 6 \times 10^{-3}$). We found no significant associations between anxiety and gut-derived metabolites nor between anxiety-associated bacteria and anxiety-related markers after meta-analysis and correcting for multiple testing.

Conclusion: This thesis showed that improving cognitive ability and reducing anxiety early in the disease course might protect against the negative impact of socioeconomic factors on subsequent functional disability in painful OA. Furthermore, regular exercise during lockdown and a balanced gut microbiome may contribute to reduced anxiety levels in chronic pain. Therefore, interventions like cognitive behavioural therapy, exercise, and probiotic supplementation might alleviate the influence of pain and anxiety on poor health outcomes in older adults with OA. Future research will investigate the role of inflammation, and the gut microbiome in the link between pain sensitisation and psychosocial factors, such as cognitive ability.

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Chapter 1 General Introduction

1.1. Overview: multiple biological, psychological and social factors contributing to chronic pain

The current thesis is motivated by a biopsychosocial approach to chronic pain; it recognises the influence of emotion on chronic pain, which is integrated in its definition, and accounts for relevant contributors, such as cognitive ability, socioeconomic factors, COVID-19 stressors, genetics, and the gut microbiome, in our understanding of chronic pain. This multidisciplinary approach might help us to delineate the mechanisms underlying chronic pain, and might allow us to identify potential targets for intervention.

Chronic pain conditions have a great societal impact with regards to illness burden, suffering and cost (Apkarian, Baliki, & Geha, 2009). The related cost to the NHS is approximately £3.2 billion per year, imposing a huge economic burden (Neogi, 2013). At present, chronic pain management is almost entirely based on symptoms (Apkarian et al., 2009; Mansour, Farmer, Baliki, & Apkarian, 2014). However, there is a limitation of end-organ pathology to fully explain pathophysiology in chronic pain patients (Abeles, Solitar, Pillinger, & Abeles, 2008; Finan et al., 2013). This is reflected in the recently revised International Association for the Study of Pain (IASP) definition of pain: ‘an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage’

(Raja et al., 2020) with the inclusion of ‘resembling that associated with’.

The biopsychosocial (BPS) model proposed by Gatchel and colleagues is now widely recognised as the most holistic approach to chronic pain (Gatchel, Peng, Peters, Fuchs, & Turk, 2007). According to this model, central and peripheral mechanisms interact to shape an individual’s experience of chronic pain (Figure 1-1). The expression of chronic pain is also influenced by a person’s genetic predisposition. The BPS model proposes that central pain processing is comprised of an interplay between biological, cognitive, somatic, and affective inputs. Efferent and afferent pathways exist that link central to peripheral processes, such as an individual’s immune response, and vice versa. Afferent signals from the periphery send input to central processes, which further exacerbates an individual’s pain perception. It further recognises that social factors, such as environmental stressors, activities of daily living, and social support/isolation can contribute to an individual’s experience of chronic pain and disability. The purpose of this project is not to test the biopsychosocial model as a whole, but to add information about the different components of the model and their interactions for which we know less about, such as cognitive ability (Study 1 & Study 2), social deprivation/isolation (Study 2 & Study 3), genetics (Study 4), and the gut microbiome (Study 5). This may help us gain a more complete view and a deeper understanding of the BPS model in chronic pain.

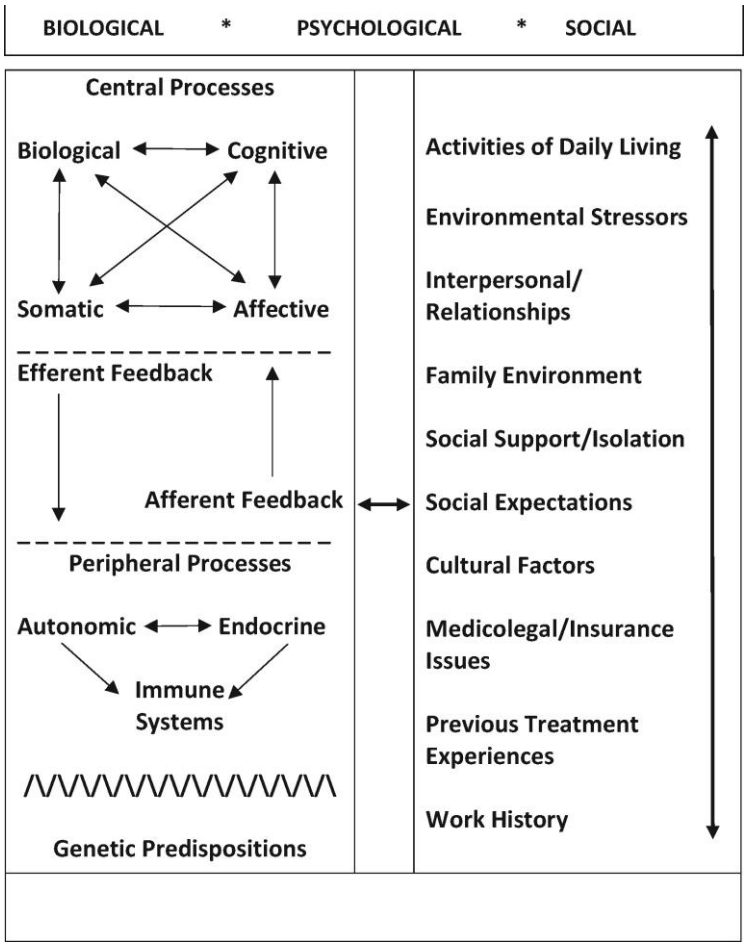


Figure 1-1. A conceptual model of the biopsychosocial approach to chronic pain.

From: Gatchel RJ. Comorbidity of chronic pain and mental health disorders: The biopsychosocial perspective. Am Psychol 2004;59(8):795–805.

Briefly, the BPS model suggests that chronic pain is the result of a dynamic interplay between biological, psychological, and social factors (Gatchel et al., 2007). First, psychosocial factors, such as anxiety, cognitive ability and social deprivation, may explain the pain experience (Burston et al., 2019; James & Ferguson, 2019; Luong, Cleveland, Nyrop, & Callahan, 2012). Second, anxiety and social isolation extending over many months during the coronavirus pandemic may

further exacerbate pain (Clauw, Häuser, Cohen, & Fitzcharles, 2020). Third, considering biological factors, such as genetic mechanisms and the gut microbiome, may further improve our understanding of chronic pain (Guo, Chen, Xing, & Liu, 2019; Mogil, 2012).

In the current thesis, we mainly focus on osteoarthritis (OA), which is the main and fastest growing cause of chronic pain worldwide, with an estimated 4 in 10 people with knee OA for which pain is not adequately controlled (Cisternas et al., 2016; Neogi, 2013). Out of those, many are resistant to therapy and report significant suffering from pain, which, in turn, limits their participation in everyday life activities, changes their behaviour and emotion and dramatically reduces their overall quality of life (Alkan, Fidan, Tosun, & Ardıçoğlu, 2014; March & Bagga, 2004; Neogi, 2013; Spencer L James, 2018).

Chronic pain conditions like OA are often co-morbid with anxiety (Barnett et al., 2018; Stubbs, Aluko, Myint, & Smith, 2016). Anxiety enhances pain in patients with OA (Burston et al., 2019) and there is a strong link between the pathways underlying pain, stress, and anxiety (Butler & Finn, 2009; Johnson & Greenwood-Van Meerveld, 2014). Despite evidence of a neural basis for the effect of emotion and cognition on chronic pain (Apkarian, Hashmi, & Baliki, 2011; Butler & Finn, 2009; Giesecke et al., 2005; Glass et al., 2011; Johnson & Greenwood-Van Meerveld, 2014), there is a need to identify the specific neuro-cognitive domains that are impaired in OA and disentangle the relationship between pain modulation and other top-down pain-regulating mechanisms, including cognitive and emotional factors

(Study 1).

Social factors may further explain the pain experience and may influence daily function, which is important for good quality of life (Craig et al., 2020; Kiadaliri, Gerhardsson de Verdier, Turkiewicz, Lohmander, & Englund, 2017). Work demonstrating interactions between psychological factors, such as cognitive ability and anxiety, and social factors, such as social deprivation, in individuals experiencing pain from OA, are scarce (Harris, Loxton, Sibbritt, & Byles, 2013; Siviero et al., 2020). Hence, the dynamic interaction between key socioeconomic and psychological factors and limitations in activities of daily living needs to be explored in individuals with OA pain (Study 2). Furthermore, there is paucity of evidence on the potential impact of the coronavirus pandemic and mitigation procedures on the levels of anxiety, pain and overall wellbeing among individuals with chronic pain. Social distancing and isolation, and closure of group activity programs such as gyms and pools may result in reduced health-related physical activities, which are a vital management strategy for chronic pain (Macfarlane et al., 2017). Social isolation and loneliness will also contribute to poor psychological health (Raina et al., 2021a). These numerous and persistent stressors may exacerbate pain, even in the absence of viral illness (Clauw et al., 2020) (Study 3).

Lastly, considering biological factors may contribute to our understanding of chronic pain. For example, little is known about the genetic mechanisms involved in pain sensitisation and neuropathic-like pain. Increased pain sensitisation and neuropathic-like pain symptoms

might be a barrier to treatment response (Kurien, Arendt-Nielsen, Petersen, Graven-Nielsen, & Scammell, 2018; Petersen, Graven-Nielsen, Simonsen, Laursen, & Arendt-Nielsen, 2016; Wylde et al., 2015), and thus, understanding the genetic mechanisms involved may promote better profiling and diagnosis of pain patients (Study 4). In addition, evidence of a potential role of gut microbiota in the pathophysiology of chronic pain is still scarce (Moloney et al., 2016). Identifying consistent microbial profiles of associations with anxiety gives us some understanding of how the gut microbiome may be linked to pain. This can then pave the way for future studies aimed at understanding the overlapping mechanisms linking anxiety and heightened pain perception with alterations in the gut microbiome (Study 5).

With the BPS model in mind, we first designed a study aimed at disentangling the relationship between pain and pain-regulating mechanisms, including cognitive and affective factors, in OA. We then wanted to explore the complex interplay between cognitive ability, anxiety and key socioeconomic factors and their associations with limitations in day-to-day activity in a large representative sample of newly diagnosed OA patients. In addition, we aimed at determining the potential impact of the pandemic on social isolation, anxiety, pain and overall quality of life in chronic pain patients of the Nottinghamshire area. Lastly, we sought to identify the underlying genetic mechanisms of pain sensitisation and neuropathic-like pain in OA and identify

reproducible gut microbial signatures of anxiety in OA and healthy individuals.

In the following sections, first, I will define chronic pain and, second, I will review the influence of psychosocial factors as contributors to chronic pain with a focus on anxiety, cognitive ability and social deprivation/isolation. Then, I will deal with the potential influence of the coronavirus pandemic and mitigation procedures for people living with chronic pain. The last part of this chapter will focus on the role of biological factors and, in particular, genetics and the gut microbiome, in chronic pain and the potential link with psychological factors, mainly anxiety.

1.2. Chronic pain

Chronic pain (CP) is defined as pain that persists past the healing phase (IASP, 2011). Most of the time, the end of the healing phase is hard to establish and for ease of diagnosis, fixed times have been agreed by clinicians to define ‘chronic’, which are practical but often arbitrary and do not associate well with underlying mechanisms (Apkarian et al., 2009). Numerous CP conditions have been characterised, which are usually identified by the area or type of injury, such as arthritic, neuropathic, diabetic, and myofascial. Frequently, multiple pain conditions give rise to clinical manifestations and vice versa, multiple distinct tissues often contribute to a single pain condition. For the majority of the individuals that experience acute pain,

the underlying injury normally alleviates or heals and they will not go on and develop CP (Apkarian et al., 2009).

1.2.1. Osteoarthritis: a CP-associated disease

Osteoarthritis, a chronic degenerative disease of the joints, is a condition commonly associated with CP, and potentially with cognitive decline (Huang et al., 2015; Neogi, 2013). It involves cartilage loss, cyst formation, subchondral bone sclerosis and osteophyte development. Chronic knee pain is defined as any pain in or around a knee on most days for more than 3 months. Knee OA, more than any other condition, hugely contributes to walking aid dependence, difficulty climbing up or down stairs and other activities that depend on the lower body (Guccione et al., 1994; March & Bagga, 2004).

In a study which assessed participants with hip and knee OA, two different types of OA pain were described: 1. intermittent pain but largely intense, and 2. persistent background pain (Hawker et al., 2008b). Approximately one-third of knee OA patients report symptoms of numbness, burning, tingling, and pins and needles. These patients are characterised by a neuropathic pain component (Hochman, French, Bermingham, & Hawker, 2010). Furthermore, different OA stages have been identified: early stages that are marked by activity-related pain, usually progressing over time to more persistent background pain interspersed by intermittent intense pain. Apart from distinct pain types, daily pain intensity is widely variable among individuals (Allen, Coffman, Golightly, Stechuchak, & Keefe, 2009).

1.3. Psychological factors that contribute to CP

IASP's definition of pain highlights the subjectivity of pain and recognises its dependence on the individual's perception and cognitive functioning aside from its dependence on sensory processing (IASP, 2011). Melzack and Wall (1965) emphasise the importance of the affective-motivational and cognitive-evaluative components of pain. The authors state that pain is a devastating experience that consumes one's attentional resources, and interferes with one's behaviour and thinking (Melzack & Wall, 1965).

Psychosocial variables are predictive of psychological impairments in OA patients, pain variance, and gait speed (Linton & Shaw, 2011; Somers et al., 2009), influence pain perception in healthy controls (Somers et al., 2009; Weissman-Fogel, Sprecher, & Pud, 2008) and may contribute to avoidance behaviour maintaining disability (Linton & Shaw, 2011; Turk & Wilson, 2010).

1.3.1. Stress and anxiety

As mentioned above, the emotional component of pain is emphasised in its definition (IASP, 2011) and Melzack's neuromatrix model of pain. The neuromatrix model of pain further highlights the importance of the stress system in pain (Melzack, 2001). According to this model, pain is perceived as a stressor that overactivates the stress response system and eventually causes muscle, bone and neural tissue degeneration. This leads to heightened pain, creating a vicious cycle. It further

suggests that cortisol is a marker of the degree of stress that may parallel CP development (Garofalo, Robinson, & Gatchel, 2006; Melzack, 2001). Another pain theory is that of 'diathesis-stress', which poses that a traumatic (actual or perceived) experience, such as a car accident, is more likely to lead to disability in individuals with predisposing characteristics (e.g., who are prone to catastrophizing or with anxiety sensitivity) (Turk, 2002).

Evidence exists of a link between the pathways underlying pain, stress and anxiety (Butler & Finn, 2009). In CP patients, imaging data have revealed enhanced activation of brain areas involved in emotional processing (e.g. amygdala, nucleus accumbens) (Parks et al., 2011b). At the same time, brain regions frequently associated with CP, the prefrontal cortex and anterior cingulate cortex, have also been involved in mood and anxiety disorders (Price & Drevets, 2012). Furthermore, there are links between anxiety and quantitative sensory testing (QST) measures of central sensitisation in knee OA pain (Burston et al., 2019; Lluch, Torres, Nijs, & Van Oosterwijck, 2014). Such evidence provides a neural basis for the effect of emotion on knee OA pain.

1.3.2. Anxiety

CP conditions like OA are often co-morbid with anxiety (Barnett et al., 2018; Stubbs et al., 2016). Individuals with OA and co-morbid anxiety or co-morbid anxiety and depression are more likely to experience chronic or excessive CP, along with other negative disease outcomes (Carmona-Terés et al., 2017; Clauw & Hassett, 2017). Indeed, anxiety-related negative OA outcomes, including heightened pain (Akin-

Akinyosoye et al., 2018; Burston et al., 2019) and impairments in activities of daily living (Nur, Sertkaya, & Tuncer, 2018; Sinikallio, Helminen, Valjakka, Väisänen-Rouvali, & Arokoski, 2014; Stamm, Pieber, Crevenna, & Dorner, 2016) are increasingly being reported in the literature. Although generally poorly studied at the early disease stages, prospective evidence from cohorts that underwent total knee arthroplasty, revealed that greater anxiety predicts persistent pain and poorer outcomes post-operatively (Alattas, Smith, Bhatti, Wilson-Nunn, & Donell, 2017; Rice et al., 2018). Recently, it was demonstrated that higher anxiety predicted an increased risk of knee pain onset in 3274 originally pain-free individuals over a 1-year period, and OA subjects with high anxiety scores, independently from depression, exhibited central and peripheral sensitisation (Burston et al., 2019).

1.3.3. Cognition

As highlighted in the previous sections, pain and emotion, and particularly anxiety, interact in several ways and may contribute to an individual's experience of pain. While emotion is the more immediate reaction to pain, cognitions attach meaning to the emotional experience and may elicit subsequent emotional reactions, thereby leading to heightened pain (Gatchel et al., 2007).

A lot of evidence exists from animal and human studies that learning, attention/salience as well as repetition are significant drivers of plastic changes in the primary somatosensory and motor cortices (Braun et al., 2002; Jenkins, Merzenich, Ochs, Allard, & Guic-Robles, 1990; Stefan, Wycislo, & Classen, 2004; Zhang et al., 2018). Cognitive and emotional

behaviours are partly controlled by prefrontal and limbic brain areas and functional and anatomical alterations in these regions have been involved in a variety of CP conditions and musculoskeletal disorders (Apkarian et al., 2011; Mansour et al., 2014). Such evidence provides a neural basis for the effect of cognitive processing in CP.

Although there are variations between the regions activated depending on the CP condition, as well as between individuals, generally a shift is observed towards regions regulating emotional and motivational states - prefrontal and mesolimbic brain areas - and away from areas encoding sensory information about pain - the so-called ‘pain matrix’, which is comprised of cortical brain areas. It is suggested that the repeated activation of prefrontal and mesolimbic brain areas should produce alterations in assessment and processing of emotional cues and eventually lead to alterations in cortical learning and memory processing (Apkarian et al., 2011; Wiech, Ploner, & Tracey, 2008). Furthermore, altered valuation may impact nociceptive processing in the spinal cord, coupled with or further supported by modifications in prefrontal cortical emotional shifts. These downward modulatory pathways contribute to central sensitisation in the spinal cord as evident in animal models (Gardell et al., 2003) and thereby possibly contribute to pain chronicity in humans. Consequently, brain physiological, morphological and metabolic reorganisation produces cognitive, sensory and motor behavioural changes in specific patterns specific to different pain conditions. The array of cognitive dysfunctions in CP is large and their independent contributions and links to specific clinical

conditions remain to be established (Apkarian et al., 2011; Moriarty, McGuire, & Finn, 2011).

Cognitive ability is crucial for everyday tasks, such as following a conversation and remembering new information, and, thus, it is essential for independence and self-care in old age (James & Ferguson, 2019). CP patients report numerous cognitive impairments (Kewman, Vaishampayan, Zald, & Han, 1991; McCracken & Iverson, 2001; Muñoz & Esteve, 2005) and numerous clinical studies have demonstrated pain-related cognitive deficits, using behavioural tests of attention, learning and memory, processing speed and executive function in a variety of common chronic pain conditions (Apkarian et al., 2004; Brown, Scholtes, Shenker, & Lee, 2020; Dick & Rashiq, 2007; Oosterman, Derksen, van Wijck, Veldhuijzen, & Kessels, 2011; Sjøgren, Christrup, Petersen, & Højsted, 2005; Veldhuijzen et al., 2006; Verdejo-García, López-Torrecillas, Calandre, Delgado-Rodríguez, & Bechara, 2009; Weiner, Rudy, Morrow, Slaboda, & Lieber, 2006). While these studies provide clear evidence of an association between cognitive impairments and CP, most of them have investigated neuropsychological performance in mixed pain populations (Tassain et al., 2003). Nevertheless, there is evidence that distinct CP conditions might differentially affect cognitive function and cause distinct neural changes. For example, in chronic back pain, pain intensity scores inversely correlated with cognitive performance, while in complex regional pain syndrome they did not (Apkarian et al., 2004). Mutso and colleagues revealed significant decreases in hippocampal volume in

chronic back pain and complex regional pain syndrome patients but not in OA patients (Mutso et al., 2012). Furthermore, the domains of cognition affected by CP and their neural correlates have not been identified, since studies at large have used tests whose neuropsychological substrates are not well-characterised. Identifying specific domains of cognitive function affected by chronic pain has a clinical utility; specific domains regulate certain aspects of activities of daily living, such as managing personal finances, grocery shopping, remembering to take medications and, thus, dealing with pain early in the disease course could prevent subsequent cognitive loss and long-term pain (James & Ferguson, 2019). Therefore, we will design a study using translational behavioural assays of distinct neuro-cognitive constructs with well-characterized neural substrates in order to identify which areas of cognition are affected by CP as well as the nature and magnitude of cognitive deficits and their underlying brain and psychological mechanisms in chronic knee OA (Study 1, Chapter 3).

1.4. Social factors that contribute to CP

Social factors are usually overlooked as important contributors to an individual's experience of CP (Baria, Pangarkar, Abrams, & Miaskowski, 2018). In the BPS model, Gatchel et al. (2007) recognise the influence of social factors as major contributors to CP. Another important aspect of this model is that it takes into account the interplay between the separate BPS components (Gatchel et al., 2007). It is important to explore the paths linking physiological, psychological and social factors

and which of these paths are significant in predicting independent ageing, such as independence in performing daily activities. This may allow us to establish appropriate interventions with the potential to reduce the costly disability caused by OA. In the following sections, I will summarise the influence of social factors, mainly social deprivation and activities of daily living, on an individual's experience of CP focusing on how these factors interact with psychosocial variables, mainly anxiety and cognition.

1.4.1. Activities of daily living

OA can lead to limitations in activities of daily living and reduced quality of life (Hochberg et al., 2012; Zhang et al., 2008). Disability in activities of daily living implies a loss of independence and increases the risk for hospitalization, nursing home admission, and death (Phelan, Williams, Penninx, LoGerfo, & Leveille, 2004). OA strongly correlates with the ability to perform activities of daily living independently (Clynes, Jameson, Edwards, Cooper, & Dennison, 2019).

Cross-sectional studies report a significant association between pain severity and activities of daily living in older adults with OA (García-López, Llopart-Carles, Castro-Domínguez, & Rejas-Gutierrez, 2021; Stamm et al., 2016). Pain status in older adults with OA is an independent predictor of limitations in activities of daily living (Nanjo et al., 2021). Activities of daily living may also depend on cognitive skills, mood and motivational factors, all of them negatively associated with pain (Bushnell, Ceko, & Low, 2013; Foster & Doty, 2021). For example,

anxiety is associated with increased limitations in activities of daily living independently of depression comorbidity (Norton et al., 2012).

1.4.2. Social deprivation and education

Social factors may further affect limitations in activities of daily living, although their role in OA is not well understood. Indeed, individual and neighbourhood social factors are associated with risk for developing pain that interferes with daily activities (Jordan, Thomas, Peat, Wilkie, & Croft, 2008; Kiadaliri et al., 2017; Luong et al., 2012; Rijk et al., 2020). Furthermore, there is a cross-sectional link between higher social deprivation, assessed using 9-digit ZIP codes, and greater limitations in activities of daily living in musculoskeletal conditions (Rijk et al., 2020). In OA, low educational attainment is associated with greater reported pain and poorer health status outcomes independently of socioeconomic factors, such as income (Callahan et al., 2011; Cleveland et al., 2013; Hannan, Anderson, Pincus, & Felson, 1992; Kiadaliri et al., 2017; Knight et al., 2011; Luong et al., 2012; Thumboo, Chew, & Lewin-Koh, 2002).

Based on the above, limitations in activities of daily living among older people are the result not only of health problems, but also of their interactions with psychological and socioeconomic factors (Kostanjsek, 2011; Organization, 2007). At present, these interactions have not been studied in detail, especially at the early stages of OA, and hence our Study 2 (Chapter 4) will explore the interplay between socioeconomic, psychological factors and limitations in day-to-day activities in newly diagnosed OA patients. We will use data from the SHARE, a large,

European, longitudinal cohort of community dwelling adults aged 50 years and older, which includes measures of anxiety, cognitive ability and pain as well as detailed information about general health, demographics, social networks and socioeconomic factors (Börsch-Supan et al., 2013).

1.5. Emotional and social stressors contributing to CP during COVID

The COVID-19 pandemic has influenced the lives and health of people across the world. Throughout the pandemic, daily living has been disrupted across all sectors, including in individuals living with CP. The impact of this pandemic goes beyond physical illness, with key psychosocial stressors that include extended periods of restrained social contact, isolation, loneliness, COVID-related anxiety, employment instability, and financial strain (Clauw et al., 2020). In this setting, we will examine the potential impact of the pandemic and mitigation procedures in people with CP of the Nottinghamshire area from the Investigating Musculoskeletal Health and Wellbeing cohort (IMH&W) (Study 3, Chapter 5). It is hypothesised that for some CP patients their symptoms may be aggravated due to public health or personal implications attributed to COVID-19. Factors that require consideration are: limited health-related physical activities due to closure of gyms and pools, limitations in activities of daily living, reduced social support that adds to poor psychological health, and COVID-related anxiety that may contribute to heightened pain perception in the absence of infection (Clauw et al., 2020).

1.6. Biological factors that contribute to CP

According to the BPS model, the biological mechanisms of pain (e.g., cartilage loss in OA pain) are important contributors to the pathophysiology of CP and can interact with psychological variables to influence pain perception (Fillingim, 2017; Gatchel et al., 2007). Every biological function depends on genetic expression and over- or under-expression of a gene produces functional consequences, such as over- or under- production of a protein or peptide. Genome-wide gene-based association studies (GWGAS) are designed to map the polygenic architecture of complex diseases by identifying the genes significantly associated with a disease trait/phenotype. GWGAS may have higher power than conventional genome-wide association studies (GWAS) as it considers the joint effect of several genetic variations within a single gene (Chung, Jun, Dupuis, & Farrer, 2019; Kang, Jiang, & Cui, 2013; Neale & Sham, 2004). Therefore, GWGAS may enhance our understanding of the genetic and molecular mechanisms involved in response to noxious stimuli (Gatchel et al., 2007; Neale & Sham, 2004). A noxious stimulus is actually, or potentially, damaging to tissue and liable to cause pain, but does not invariably do so (Cervero & Merskey, 1996). Nociceptive neurons are responsible for encoding noxious stimuli (IASP, 2011). In the following sections, I will summarise the influence of host genetics and the gut microbiome in CP and their connection with psychological factors, and especially anxiety.

1.6.1. Genetics of CP and shared genetic risk with anxiety

Genetic variations can predispose individuals to develop CP (Foulkes & Wood, 2008) and OA (Valdes et al., 2011b). Candidate gene studies have revealed that TRPV1 gene variants are associated with increased risk for developing painful knee arthritis (Valdes et al., 2011b). Moreover, recent research has revealed that pain perception is also influenced by SNPs (Davies et al., 2016; Lee et al., 2020). Over the years, pain mutations, twin studies and association studies in humans have identified significant molecular factors and pathways involved in altered pain behaviour offering new insights of the pain mechanisms and of potential novel treatment targets (Davies et al., 2016). However, there is a gap in our knowledge regarding the genes and molecular pathways influencing central versus peripheral sensitisation in knee OA. Central sensitisation is defined as an amplification of neural signalling within the central nervous system (CNS) that elicits pain hypersensitivity (i.e. increased responsiveness and reduced threshold of nociceptive neurons) (Nijs et al., 2021; Woolf, 2011). Instead, sensitization of nociceptive neurons in the periphery, known as peripheral sensitization, usually indicates peripheral tissue injury and inflammation (Vardeh & Naranjo, 2017). Both peripheral sensitisation (e.g., peripheral tissue injury, inflammation) and central sensitisation mechanisms are implicated in OA pain (Nijs et al., 2021; Sofat, Ejindu, & Kiely, 2011). In knee OA and persistent postsurgical pain, neuropathic pain symptoms and persistent mechanical hyperalgesia appear to be driven largely by central pain mechanisms, respectively (Fernandes, Valdes, Walsh,

Zhang, & Doherty, 2018; Wheeler et al., 2019). Evidence indicates that signs of increased central sensitisation as well as neuropathic pain symptoms identified using the painDETECT questionnaire might be a barrier to treatment response (Kurien et al., 2018; Petersen et al., 2016; Wylde et al., 2015). More work is needed to discern between peripheral and/or central mechanisms that drive nociceptive and neuropathic-like pain in OA. Using data from the Knee Pain and related health In the Community (KPIC) cohort (Fernandes et al., 2017) and an independent Nottingham cohort study of neuropathic-like pain (Warner, Walsh, et al., 2017), we performed a GWGAS and gene-based meta-analysis of pain sensitisation and neuropathic-like pain phenotypes to identify genes involved in these key processes (see Chapter 6 for more details).

Risk for developing central pain augmentation and neuropathic-like pain may be determined by a person's genetic risk to chronic pain along with shared genetic risk factors with psychological traits, such as anxiety (Descalzi et al., 2017; Meng et al., 2020). Several genes have been implicated in pain sensitivity that have arisen from candidate gene studies. Nevertheless, there is an imperfect overlap between these genes and chronic pain conditions that may be due to multiple overlapping pathways between pain and psychological responses to pain. For instance, two neurotransmitter pathways have been reproducibly associated with musculoskeletal pain, the adrenergic pathway and the serotonergic pathway. A well-characterised example in the adrenergic pathway is that of *COMT*, the gene encoding the enzyme catechol O-methyltransferase, which facilitates the inactivation

of catecholamine neurotransmitters, including dopamine, adrenaline, and noradrenaline. Reduced COMT enzymatic activity seems to lead to amplified pain sensitivity and temporal summation of pain (Diatchenko et al., 2004). In the serotonergic pathway, genes, such as the 5-hydroxytryptamin receptor 2A (*HTR2A*) and 5HT transporter (*SLC6A4*), have been associated with chronic pain conditions, such as fibromyalgia and chronic widespread pain (Cohen, Buskila, Neumann, & Ebstein, 2002; Nicholl et al., 2011). Affective traits, such as anxiety have been associated with genetic variation in these neurotransmitter pathways and are associated with risk for chronic pain (Gottschalk & Domschke, 2017) and increased central and peripheral sensitisation measures (Burston et al., 2019). Therefore, our Study 4 will determine to what extend the genes found to be involved in neuropathic-like pain and pain sensitization phenotypes are known to be associated with psychological traits, such as anxiety.

1.6.2. Gut microbiome in CP: a role of inflammation

The hidden kingdom that resides in our gut plays an important role in metabolic (Le Chatelier et al., 2013) and immune (Hooper, Littman, & Macpherson, 2012) functioning and consumption of pro- and prebiotics has been found beneficial for the host's health. For example, consumption of pro- and prebiotics may help prevent obesity caused by high fat diet (Poutahidis et al., 2013) and control cardiovascular function and blood pressure (Marques, Mackay, & Kaye, 2018).

A relationship between CP and the gut microbiome is becoming increasingly clear. For instance, in humans changes in gut microbiome

diversity and abundance have been observed in a variety of CP conditions, such as OA-related knee pain, fibromyalgia, visceral pain, and chronic pelvic pain (Boer et al., 2019; Braundmeier-Fleming et al., 2016; Minerbi et al., 2019a). In animal studies, restoring the microflora after dysbiosis ameliorates pain in neuropathic (Ramakrishna et al., 2019; Shen et al., 2017), inflammatory (Amaral et al., 2008) and visceral pain models (Luczynski et al., 2017; O'Mahony et al., 2014). For example, an interesting mouse study demonstrated that obese mice were lacking beneficial Bifidobacteria, while they were abundant of proinflammatory bacterial species. Oligofructose supplementation was associated with reestablishment of lean gut microflora, reversal of inflammation in the colon, preservation of articular cartilage, and protection against OA (Schott et al., 2018). In a case-control study in women with fibromyalgia, significant differences were found in bacterial taxa composition and microbiome variation was highly correlated with clinical indices of fibromyalgia (Minerbi et al., 2019a).

Recent evidence has revealed that brain cells, namely endothelial, pericytes, astrocytes, microglia and inflammatory T cells and monocytes/macrophages, are able to receive signals from the gastrointestinal tract through the blood brain barrier and vagal nerve (Duan et al., 2018; Rafalski, Merlini, & Akassoglou, 2018; Tang et al., 2017). Activation of these cells may lead to neuroinflammation - astrogliosis and microgliosis- and subsequent activation of pro-inflammatory factors such as, TNF-α, IL-1β, and CXCL1. These immune mediators contribute to increased glutamatergic synaptic

neurotransmission and/or decreased GABAergic synaptic neurotransmission, both involved in central sensitisation and hypersensitivity to pain (Ji, Xu, & Gao, 2014; Latremoliere & Woolf, 2009a). Importantly, host microflora continuously modulate maturation and immunological function of microglia in the central nervous system via the gut-brain axis (Abdel-Haq, Schlachetzki, Glass, & Mazmanian, 2018; Erny et al., 2015) and may be involved in chronic pain (Dworsky-Fried, Kerr, & Taylor, 2020). Therefore, it remains to be elucidated whether neuroinflammation-induced central sensitisation underlying CP can be directly or indirectly controlled by the gut microbiome.

1.6.3. Gut microbiome in anxiety

The influence of gut bacteria on the brain, through the gut-brain axis, is a new area of interest. Despite methodological difficulties, studies have demonstrated promising findings of a role of the gut microbiome on the nervous system, and especially in emotion and stress (Sarkar et al., 2018). In addition, preclinical studies have provided extensive evidence of a role of microbial metabolites and neuromodulators in gut-brain communication (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015; Stilling et al., 2016; Strandwitz et al., 2019). The impact of anxiety on the gut-brain axis has been mostly demonstrated in animal models (Molina-Torres, Rodriguez-Arrastia, Roman, Sanchez-Labraca, & Cardona, 2019). Although animal studies are valuable for investigating fundamental cellular processes, it is crucial to translate their findings in humans. To date, mixed evidence has derived from human studies investigating the anxiety-microbiome link (Chen, Bai, et al., 2019;

Jackson et al., 2018; Jiang et al., 2018; Kleiman et al., 2017; Mason et al., 2020; Naudé et al., 2020), which has not allowed the establishment of microbial composition signatures. Furthermore, direct evidence of a link between microbial metabolites and changes in the gut microbiota and anxiety levels is lacking in humans. Identifying consistent microbial profiles of associations with anxiety might enable us to identify critical targets for interventions to prevent and/or alleviate anxiety symptoms and promote brain health (see Chapter 7 for more details).

Based on the above evidence, Study 5 will first sought to identify consistent microbial signatures of anxiety by conducting a meta-analysis of associations of anxiety levels with microbiome composition using data from two independent cohorts (an OA and a healthy cohort) and published literature. Then, we aim to find reproducible associations of anxiety levels with gut-derived metabolites, and identify consistent correlations among anxiety-related bacteria (as identified from the meta-analysis) and anxiety-related markers across the two cohorts. Identifying the gut microbiome's role in anxiety might provide us with some understanding of its role in CP and may be pave the way for designing future studies (see section 1.6.4).

1.6.4. Gut microbiome in CP: a link with anxiety and cognition

Stress and anxiety can cause increased pain perception mainly via three different mechanisms or pathways: (1) HPA axis activation, (2) sensitized spinal afferents, and (3) altered descending pain pathways (Moloney et al., 2016). There may be a bidirectional interaction between anxiety and the gut microbiota, with anxiety enhancing the production of

pathological species and reducing the production of beneficial bacteria (also known intestinal dysbiosis), which, in turn, may lead to HPA axis dysregulation (Moloney et al., 2016; O'Mahony et al., 2015; Stilling et al., 2016; Strandwitz et al., 2019). As described above, various systems and mediators may play a role in this complex interplay including altered production of neuromodulators, altered immune system functioning, and altered production of gut-derived metabolites and mediators. At the same time, cognitive processing is involved in pain, as described above, and may also be linked to alterations in gut microbiome, although evidence from human studies is mixed (Allen et al., 2016; Benton, Williams, & Brown, 2007; Carlson et al., 2018; Chung et al., 2014; Fernandez-Real et al., 2015). The overlapping mechanisms linking anxiety and pain sensitisation with alterations in the gut microbiome and the exact pathways by which the microbiota can exert direct effects on pain processes and vice versa remain to be investigated. Potential targets may include altered inflammatory and cognitive processes (Bakshi et al., 2021). Therefore, as part of our Study 1 protocol and future studies, the complex interplay between anxiety, pain and bacterial taxa in knee OA patients will be explored taking into account the potential influence of cognitive domains and inflammatory markers (see Chapter 3 and section 8.3, Chapter 8, for more details).

1.7. Summary

From the above, it emerges that individual and combined influences of biological and psychosocial contributors to pain form a unique map of factors that shapes an individual's pain experience. Despite recent advances in our understanding, we still know little about several BPS components and their interactions that contribute to CP (Figure 1-2). For example, we know little about the neuro-cognitive domains that are involved in OA pain (Study 1), the influence of social deprivation on the cognition-pain link in OA (Study 2), and the influence of the coronavirus lockdown on the levels of social isolation, pain and anxiety in CP (Study 3). In addition, we know little about the genes involved in central versus peripheral pain mechanisms in OA (Study 4) and even less about the gut microbiome's role in pain and anxiety (Study 1 and 5). Therefore, by performing the studies included in this thesis, we attempt to enhance our understanding of some of these key components and their interactions thereby gaining a more complete view of the BPS map in CP, which may then pave the way towards a more informed and personalized pain care.

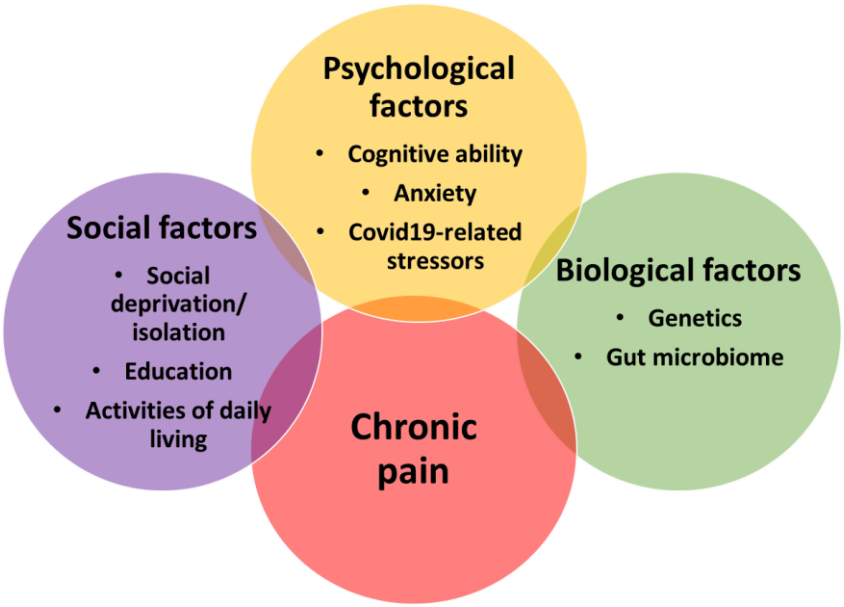


Figure 1-2. The biopsychosocial contributors to chronic pain and their interactions explored in the current thesis.

1.8. Aims and Objectives

In order to address important gaps in our current knowledge and understanding, the aim of the current study is explore of the role of key biopsychosocial contributors to CP using five separate studies:

1) Study 1, Chapter 3 – To design an observational study aimed at investigating the specific neuro-cognitive domains that are impaired in OA pain and the links between cognitive domains, anxiety, pain and bacterial taxa. The objectives of this study will be:

a. To correlate pain sensitivity measures (e.g. QST) and pain questionnaire measures (e.g. WOMAC, PD-Q) with objective behavioural measures of cognitive function (CANTAB®) in knee OA participants.

b. To compare cognitive measures between no, low and high pain participants if a sufficient enough sample size can be achieved (i.e., at least 100 participants overall).

c. To explore the correlation between inflammatory biomarkers and gut microbiome measures with pain sensitivity measures and anxiety as well as measures of cognitive ability and assess.

We hypothesise that pain severity and sensitivity will strongly correlate with cognitive impairment and anxiety, and increased peripheral inflammation and altered intestinal microbiota might mediate this relationship.

2) Study 2, Chapter 4 – To explore the dynamic interplay between key socioeconomic (i.e. social deprivation, education) and psychological factors (i.e. cognitive ability, anxiety) and limitations in instrumental activities of daily living (IADL) in OA pain with a longitudinal study. Specifically, we will test the following hypotheses: (a) higher levels of pain will be related to impaired cognitive ability and higher anxiety after controlling for pain before diagnosis and confounders, (b) better cognitive ability will be related to reduced pain levels and lower social deprivation after controlling for cognitive ability before diagnosis and confounders, (c) greater limitations in IADL will be associated with higher anxiety and poorer cognitive ability after controlling for limitations in IADL before diagnosis and confounders and (d) pain, cognitive ability and anxiety will act as the mechanisms indirectly linking socio-economic

factors (social deprivation and educational attainment) to limitations in IADL.

3) Study 3, Chapter 5 – To explore the potential impact of the pandemic and mitigation procedures, including perceived social isolation on anxiety, pain and quality of life in chronic OA patients. To do this, we will compare measures of anxiety, quality of life and pain, which were collected at three different times; when lockdown rules were first applied (May), later eased (July), and when the second wave began (September) in chronic pain patients from the Nottinghamshire area.

4) Study 4, Chapter 6 – To identify genetic markers associated with pain sensitisation and neuropathic-like pain and to what extend these markers are known to be involved in anxiety. To achieve this GWAS and GWGAS of pain pressure thresholds traits and neuropathic-like pain will be conducted to identify related SNPs and genes, as well as gene-set analysis to identify related genetic pathways.

5) Study 5, Chapter 7 – To identify bacterial taxa reproducibly associated with anxiety and the role of serum levels of microbial metabolites and neuromodulators. To achieve this we will meta-analyse data from two cohorts (OA and healthy controls) and published literature and investigate the role of serum levels of SCFAs and indoles on anxiety and the links between anxiety-associated operational taxonomic units (OTUs) as identified from the meta-analysis and neuromodulators involved in anxiety.

Chapter 2: Methodological Approaches

This Chapter will provide more detail on some of the main cohorts that contributed data to the results chapters of the current thesis, such as the KPIC and IMH&W cohorts. It will also provide further detail on the quantitative methods employed throughout the thesis. Table 2-1 below summarises the results chapters of the thesis, the cohorts that contributed data and the quantitative analysis used in order to address the aims of each study.

Table 2-1. Details of studies and quantitative analysis employed across thesis result chapters.

Results	Study sample	Aims	Time points	Quantitative analysis
Study 1 (Chapter 3)	KPIC and IMH&W	a. Specific cognitive domains impaired in OA pain b. Links between cognitive domains, anxiety, pain and bacteria taxa	Baseline & follow-ups	Spearman's correlations
Study 2 (Chapter 4[a])	SHARE	The association of socio-economic and psychological factors with limitations in day-to-day activity in OA patients	Waves 4, 5, 6 & 7	Path analysis
Study 3 (Chapter 5[a])	IMH&W	The impact of the COVID-19 pandemic on the mental health and quality of life of chronic pain patients	Baseline & follow-ups	Repeated measures ANOVA
Study 4 (Chapter 6[a])	KPIC & Nottingham post-TJR cohort	Genetics of pain sensitivity and shared genetic risk with psychological traits in OA patients	Baseline	Meta-analysis (gene-based)
Study 5 (Chapter 7[a])	WebEx (KPIC) & Omega 3 (TwinsUK)	Gut microbiome signatures of anxiety in OA patients and healthy controls	Baseline	Meta-analysis

[a]Chapters describe secondary analyses of previously existing dataset

2.1. Knee Pain and Related Health in the Community (KPIC) cohort

The Knee Pain and Related Health in the Community (KPIC) study cohort (Fernandes et al., 2017) provided us with a large sample of individuals with knee pain (n = 2152), some of whom underwent detailed clinical assessments, including knee radiographs; quantitative sensory testing; and biomarker sampling (n = 320). Baseline and year 1 recruitment began in 2014 and 2015, and ended in 2015 and 2016, respectively (Akin-Akinyosoye, 2020).

For the purpose of the current thesis, data from the larger sample of KPIC were used for participant recruitment for Study 1 (Chapter 3). As part of our Study 4 (Chapter 6), data from the 320 participants that completed detailed clinical assessments at baseline were used for secondary analyses.

2.1.1. Ethics

The KPIC study protocol was approved by the Nottingham Research Ethics Committee 1 (NREC Ref: 14/EM/0015) and registered (clinicaltrials.gov portal: NCT02098070) (Fernandes et al., 2017).

2.1.2. Study design, participants and recruitment

The KPIC study is a prospective community-based cohort of men and women aged 40 years or over within the Nottinghamshire and Derbyshire community. The KPIC study team used a cluster sampling procedure with General Practice (GP) serving as the cluster. Twelve regional GPs were approached and a healthcare professional in each GP decided eligibility using the GP register. Individuals on the GP

register were eligible to take part, irrespective of knee pain status. Individuals were not eligible to take part if they suffered from any known terminal illnesses, severe psychiatric illness and dementia, or any other conditions or circumstances that would make them unsuitable to receive a questionnaire. Postal questionnaires were sent to all eligible individuals along with a covering letter introducing the aims and objectives of the study. At the end of the questionnaire, participants were asked whether or not they wanted to receive: (i) further information about a single visit to Academic Rheumatology to undergo knee radiographs and other assessments; (ii) a similar postal questionnaires in one year's time; and (iii) information about future studies related to knee pain and knee OA (Fernandes et al., 2017).

2.1.3. KPIC survey design

The baseline postal survey acquired detailed information about the individual, including their medical history and any known risk factors for developing knee pain and knee OA (Fernandes et al., 2017). Participants were asked about presence of current knee pain with a validated question: "Have you had knee pain for most days of the past one month?" (Yes/No) (O'Reilly, Muir, & Doherty, 1996). The Intermittent and Constant Osteoarthritis Pain (ICOAP) questionnaire was used to record a participant's knee pain patterns (Hawker et al., 2008a). Current knee pain was captured with a numerical rating scale (NRS) ranging from 0–10, where '0' is no pain at all and '10' is worst imaginable pain. Information on various psychological and physiological factors were collected, such as anxiety and depression with the

Hospital and Anxiety Depression Score (HADS) (Zigmond & Snaith, 1983), and physical and mental health with the 12-item Short Form Survey (SF-12) (Ware, Kosinski, & Keller, 1996). The follow-up survey contained similar questions to the baseline survey (Akin-Akinyosoye, 2020).

2.1.4. KPIC clinical assessment

A subsample of the questionnaire responders who indicated willingness to take part in a single clinical visit in Academic Rheumatology to undergo knee radiographs and other assessments were recruited in three distinct groups (early knee pain, established knee pain and no knee pain) based on their responses to explore how knee pain and associated factors change over time.

The inclusion criteria for clinical assessment at baseline were:

i. Participants with recent-onset knee pain were defined as mild/moderate and/or intermittent knee pain occurring for the first time in the past 3 years for most days of at least one month, unrelated to obvious major trauma;

ii. Participants with established persistent knee pain: defined as knee pain for over 3 years which has been moderate or severe (NRS >6) and/or persistent for most days of the past 3 months, unrelated to obvious major trauma;

iii. Participants with no knee pain: defined as no knee pain within the past 5 years (Fernandes et al., 2017).

PPT is defined as the minimum force required to induce pain (Maquet, Croisier, Demoulin, & Crielaard, 2004). PPTs can serve as mechanism-based classification indices, such as for comparing responses within participants (e.g. affected vs. unaffected sides) (Arendt-Nielsen et al., 2010). PPTs were assessed for each participant using a standardised protocol performed by trained researchers (Fernandes et al., 2017). This has be described in detail in Chapter 6 (Study 4). Intra-rater and inter-rater agreements for PPT scores used in KPIC have been previously published (Akin-Akinyosoye et al., 2018).

Tibiofemoral (TF) and patellofemoral (PF) radiographs were taken using a standardised protocol (standing posterior–anterior and skyline views) and scored by experienced observers with intrarater and interrater reliability tests performed. Grading of radiographs for changes of OA included the summated Kellgren–Lawrence grade (KL) score (Kellgren & Lawrence, 1957) and the Nottingham logically devised line drawing atlas for individual scoring of osteophyte (0-5) and joint space width (−1 to +5) for each medial TF, lateral TF, and PF compartment. Individuals were classified as having bilateral OA if they had a KL ≥2 at either compartment in both knees (Burston et al., 2019).

2.1.5. KPIC Data management

A unique study identifier was allocated to each participant at baseline linked to follow-up responses by the same participant if available. Data were entered into a Microsoft Access 2007 database constructed by the database manager for the KPIC study. The database was constructed such that the possible range for each variable was set to the minimum

required to limit erroneous scoring and any missing observations were coded as ‘999’. The questionnaires and clinical assessment data were appropriately stored within Academic Rheumatology (Akin-Akinyosoye, 2020).

2.2. Investigating musculoskeletal health and wellbeing (IMH&W) cohort

The Investigating musculoskeletal health and wellbeing (IMH&W) study cohort consists of participants with or at risk of developing musculoskeletal-related problems (i.e. pain, frailty and disability), aged 18 years or over and able to provide informed consent. Participants were excluded if: (i) they are unable to understand verbal information in written English, or who have special communication needs, and (ii) they have any major non-musculoskeletal conditions likely to preclude follow up or eligibility for nested research studies. Participants were recruited from GP surgeries, or from databases of participants in previous studies undertaken by the University of Nottingham who had consented to be contacted with information about future research (Millar et al., 2020).

2.2.1. Ethics

The study was approved by the Central London Research Ethics Committee (REC ref 18/LO/0870) and was performed in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) and the UK Policy Framework for Health and Social Care Research, 2018 (Millar et al., 2020).

2.2.2. IMH&W participants: a comparison with KPIC

The IMH&W questionnaire was designed to capture self-reported pain and frailty as well as detailed information about the individual and their medical history (demographics, comorbidities, medications) (Millar et al., 2020).

The baseline IMH&W data included 2744 adults with a mean age of 71.38 years and 58.13% were female. The IMH&W cohort are on average ten years older compared to participants from the KPIC, perhaps due to the enrichment of the IMH&W study with individuals who were already flagged as frail by their GP. The KPIC study participants reported a lower NRS pain score compared to IMH&W. This is expected, as pain is associated with frailty, by recruiting more frail individuals they are likely to have higher pain scores (W Chaplin 2022, personal communication, 9 February).

For the purpose of the current thesis, data from the larger sample of IMH&W were used for participant recruitment for Study 1 (Chapter 3) and for the survey in Study 3 (Chapter 5).

2.3. Other cohorts

Within the current thesis, data from the Survey of Health, Ageing and Retirement in Europe (SHARE) and from a Nottingham post-TJR cohort as well as baseline data from the WebEx study and the Omega 3 study cohorts were employed. Briefly, SHARE is a community dwelling cohort of Europeans aged 50 years and over. Nottingham post-TJR cohort

includes individuals with severe OA who had undergone a joint replacement and pain where present refers to persistent joint pain after one or more years of surgery. WebEx includes knee pain participants aged 45 years or older with a clinical diagnosis of knee osteoarthritis, as well as radiographically established knee osteoarthritis that were mainly recruited from KPIC and Omega 3 is a healthy cohort of individuals aged 18 years or over and enrolled from the TwinsUK registry. More details about these cohorts are provided in the corresponding thesis Chapters.

2.4. Statistical analysis

2.4.1. Descriptive analysis and basic statistics

All statistical analyses were carried out using Stata or R statistical packages, and P values less than 0.05 were considered statistically significant. Descriptive statistics were used to analyse demographic and clinical characteristics of the samples. Continuous variables were summarised as mean or median (SD, interquartile range) and categorical variables as percentages (%) (Armitage, Berry, & Matthews, 2008).

Correlation analysis was done to investigate relationships between variables (Pearson's if normally distributed data or Spearman's (Rho) if not) or to determine which variables were entered in the multivariate models. Chi square tests were used to compare categorical variables, while a two sample t-test for independent groups compared normally distributed continuous data obtained from two independent groups. A

paired t-test was used to compare normally distributed continuous data from a dependent group (e.g. test score compared between baseline and follow-up) (Armitage et al., 2008).

Analysis of variance (ANOVA) was conducted to compare data between groups (if more than two), provided that the assumptions of normality and equality of variance in the population underlying its utilization were verified. Otherwise, Kruskal-Wallis test was used. In the case of subjects measured on three occasions over time as in Study 3, repeated measures ANOVA was employed (Armitage et al., 2008).

Multivariate linear and logistic regression models were used to investigate intercorrelations between variables and to adjust for confounders. Confounding variables were chosen due to their clinical relevance based on findings from previous studies (Hosmer Jr, Lemeshow, & Sturdivant, 2013).

2.4.2. Path analysis

Path analysis is an extension of multiple regression analysis whereby equations representing the effect of one or more variables on others can be solved to estimate their relationships. Path analysis, thus, represents the hypothesized ‘causal’ relationships among variables to be tested (Beran & Violato, 2010; Streiner, 2005). However, it cannot establish causality but can only determine whether the data are consistent with the model (Streiner, 2005). The degree of correlation among variables that determines the importance of a given path of influence from causes (or factors that influence) to effect are calculated

as path coefficients (Streiner, 2005; Wright, 1920). Path coefficients are measured simultaneously for all endogenous variables (i.e. variables that can be both a predictor with respect to a variable and an outcome with regards to another variable) rather than sequentially as in regular multiple regression models (Beran & Violato, 2010).

There are several steps in path analysis (Beran & Violato, 2010):

1. *Identifying the research problem.* The researcher based on previous research, empirical findings or theory, first, develops hypotheses about the relationships between the variables. Then, the nature of these relationships is determined (e.g. direct or indirect whereby intervening variables may mediate the effect of one variable on another, unidirectional or bidirectional) which is also guided by previous research and theoretical predictions. Lastly, the researcher outlines the model by determining the number and relationships of variables.

2. *Identifying the model*. For each parameter in the model to be estimated, there must be at least as many values (i.e., variance and covariance values) as model parameters (e.g., path coefficients). This step will determine if a model can be feasibly estimated. If a model is impossible to solve mathematically is known as underidentified. This can occur for various reasons, such as when variables are highly intercorrelated (multicollinearity).

3. *Estimating the model*. The default estimation procedure in most software packages is maximum likelihood (ML); an iterative process that estimates the extent to which the model predicts the values of the

sample covariance matrix, with values closer to zero indicating better fit. The name maximum likelihood is based on its calculation. The estimate maximizes the likelihood that the data were drawn from its population. The estimates require large sample sizes, but do not usually depend on the measurement units of the measured variables. It is also robust to non-normal data distributions (Muthén, 1993).

4. *Determining the model's goodness of fit*. Fitting the variable path model involves minimizing the difference between the sample covariances and the covariances predicted by the model. Model fit can be determined by examining the model's fit indices with most ranging from 0 to 1 with a high value indicating a great degree of variance in the data accounted for by the model. Another important thing to consider is the sample size that needs to be large enough to obtain stable estimates of the parameters. Generally, a sample size of at least 100 but preferably 200 cases are needed.

5. *Respecifying the model if necessary*. To obtain improved fit results, the above sequence of steps is repeated until the most succinct model is derived for example with the addition of a path link, or inclusion of mediating or moderating variables (if theoretically supported).

Path analysis is a powerful statistical technique that allows for more complicated and realistic models than multiple regression (Streiner, 2005). Another advantage of this technique is the inclusion of autoregressive effects where a variable measured at two time points is correlated with itself, which corrects for an overestimation of the

relationship among variables (Beran & Violato, 2010). Therefore, path analysis was used within the current thesis to assess the complex relationships between socioeconomic and psychological factors and limitations in daily activities in Study 2 (Chapter 4).

2.4.3. Meta-analysis

In general, meta-analysis is a qualitative method for combining the results of independent research studies. Several methods exist for synthesising research findings from independent studies that aids in our understanding of a relationship (e.g., anxiety-microbiome). One approach is combining research findings using r and d effect size estimates, which involve the transformation of several common statistics. In this case, the overall significant effect from several studies using transformed statistics is obtained by averaging either the r or d effect size measures. Meta-analysis compares the relative importance of findings in several research studies by interpreting effect size measures, which are on a common metric. The overall d effect size measure can be weighted by sample size to compute an unbiased, average d effect size measure (Schumacker & Tomek, 2013). The meta-analysis methods employed within the thesis are described in more detail in the relative Chapters (i.e. Chapters 6 and 7).

Chapter 3: Impairments in which cognitive domains are associated with chronic pain in knee osteoarthritis? Study protocol and preliminary results of an observational cohort study[1]

3.1. Abstract

Background: Although there is substantial evidence that chronic pain patients have some cognitive impairment, limited evidence is available about the specific neuro-cognitive domains that are impaired in patients with osteoarthritis (OA) pain. To address this gap, we plan to determine the relationship between pain and behavioural measures of distinct cognitive functions (e.g. prefrontal executive function, hippocampal memory functions), as measured using translational behavioural assays of well-characterized neuro-cognitive constructs.

Methods: We anticipate recruiting participants from the Nottinghamshire area, who have identified as having knee OA and age- and sex-matched controls (n_{total} = 100). Distinct cognitive functions will be measured using the Cambridge Neuropsychological Test Automated

[1] Chapter 3 was sent out for peer review in *BMJ Open*. Despite much effort, the journal was unable to obtain the appropriate number of referees for our manuscript in a timely manner and, therefore, the manuscript was sent back to us after several months.

Battery (CANTAB). Pain measures will include Quantitative Sensory Testing and pain questionnaires, the Western Ontario and McMaster Universities Osteoarthritis Index and PainDETECT. Confounders will be measured, including depression/anxiety (Hospital Anxiety and Depression Scale), sleep quality (Pittsburgh Sleep Quality Index), quality of life (12-Item Short Form Health) and physical function (30s Sit to Stand Test and Timed Up and Go Test). Additional cognitive measures will be taken using the Cognitive Failures Questionnaire, the Cognitive Reflection Test and the Virtual Delayed-Matching-to-Place test of rapid place learning (optional), along with the National Adult Reading Test that will serve as a proxy of general premorbid IQ. Data on prosociality will be collected using the Dictator Game. For identifying molecular biomarkers, gut microbiome measures and genetic markers, blood, urine and faecal samples will be collected. We assessed the feasibility of study based on the experiences of the patients. Correlational analysis will be used to investigate the relationship between cognitive function and pain. We will compare cognitive function between pain participants and age and sex-matched controls, using independent samples t-tests. Multivariate regression models will be used to investigate intercorrelations between variables and to adjust for confounders.

Preliminary Results: Until now 8 participants have been screened, of which 7 identified as having knee pain from OA and 1 as control (no pain). The study was feasible, since most of the practical aspects were executed as planned, and the study was well received by the

participants. Our preliminary results showed that higher anxiety correlated with higher pain duration and poorer cognitive function, such as lower IQ, higher cognitive impairment and more sensorimotor deficits on a familiarisation test.

Discussion: We expect the results from this study to help us improve our understanding of which distinct cognitive domains are impaired in chronic knee OA pain, of the underlying biological mechanisms of chronic pain and cognitive function in OA, and potentially point the way for better treatment options in the future and contribute to the identification of biomarkers for this disease.

Trial registration: The study was retrospectively registered in November 2020 with trial registration number: NCT04620525.

3.2. Background

Osteoarthritis (OA) is the most common form of arthritis, and for 4 in 10 people pain from OA is not adequately controlled (Neogi, 2013). A huge economic burden is attributed to OA with an estimated cost to the NHS of approximately £3.2 billion per year (NICE, 2014).

Cognitive ability is crucial for everyday tasks, such as following a conversation and remembering new information, and, thus, it is essential for independence and self-care in old age (James & Ferguson, 2019). Chronic pain patients report numerous cognitive impairments, including in memory, attention and concentration (Kewman et al., 1991; McCracken & Iverson, 2001; Muñoz & Esteve,

2005) and numerous clinical studies have demonstrated pain-related cognitive deficits, using behavioural tests of attention, learning and memory, processing speed and executive function in a variety of common chronic pain conditions (Apkarian et al., 2004; Dick & Rashiq, 2007; Oosterman et al., 2011; Sjøgren et al., 2005; Veldhuijzen et al., 2006; Verdejo-García et al., 2009; Weiner et al., 2006). Although these studies provide clear evidence of an association between cognitive impairments and chronic pain, most of them have investigated neuropsychological performance in mixed pain populations (Tassain et al., 2003) and, thus, it is difficult to infer information about the extent and nature of cognitive impairments in specific chronic pain conditions. Studies have suggested that the type of pain condition might play a significant role in cognitive function and cause distinct neural changes (Apkarian et al., 2004; Mutso et al., 2012). Two recent epidemiological studies have revealed a bidirectional link between cognitive function and pain in newly diagnosed OA patients (James & Ferguson, 2019; Kouraki, Bast, Ferguson, & Valdes, 2021). However, information about the specific cognitive domains that are affected in OA is lacking, since previous studies have mainly used tests whose neuropsychological substrates are not well-characterised, making it difficult to identify specific cognitive domains affected by chronic pain and their neural correlates. In contrast to previous studies, our study will use objective translational assays of well-characterized neuro-cognitive constructs, including Cambridge Neuropsychological Test Automated Battery (CANTAB) (Barnett et al., 2010) and a virtual maze assay of rapid place

learning, the Virtual Delayed-Matching-to-Place (vDMP) test (Buckley & Bast, 2018). Using these assays, we will test a variety of cognitive domains in patients with knee OA (including prefrontal executive functions and hippocampal memory functions). Identifying a standard array of cognitive impairments experienced by chronic pain patients has clinical utility, since specific domains regulate certain aspects of activities of daily living, such as managing personal finances, grocery shopping, remembering to take medications (Moriarty et al., 2011). Moreover, a characterization of key cognitive impairments, using translational, i.e. cross-species, assays of distinct neuro-cognitive functions, may point the way for better treatment options in the future, and would enable pre-clinical study of these impairments and potential treatments in well-established rodent models of knee OA. In addition, using Quantitative Sensory Testing (QST), we will investigate whether cognitive deficits are associated with different dimensions of pain, such as allodynia, hyperalgesia and temporal summation (Arendt-Nielsen et al., 2010; Sterling, Treleaven, Edwards, & Jull, 2002). Studies in several musculoskeletal diseases have shown impaired therapeutic outcomes, including from surgery, in patients presenting with hyperalgesia and allodynia (Davis & Moayedi, 2013; Gwilym, Oag, Tracey, & Carr, 2011; Sterling et al., 2002).

The gut microbiome has been implicated in modulating central nervous system functions and in various neurological conditions (Cryan, O'Riordan, Sandhu, Peterson, & Dinan, 2020). However, little effort has been placed in investigating the link between gut microbiome dysbiosis

and OA, and although there is some evidence to support a potential role of gut microbiota in the pathophysiology of chronic pain (Clos-Garcia et al., 2019; Minerbi et al., 2019a; Minerbi et al., 2019b), relevant studies are still scarce. There is evidence that the gut microbiome can activate peripheral (Schott et al., 2018) and central nervous system inflammation (Duan et al., 2018; Rafalski et al., 2018; Tang et al., 2017) and, thus, might contribute to peripheral and central sensitisation underlying chronic pain (Krustev, Rioux, & McDougall, 2015). Furthermore, rodent studies have revealed a potential bacteria-cognition link mediated via the gut-brain axis, but findings from human studies are scarce and have revealed mixed results (Benton et al., 2007; Carlson et al., 2018; Davidson, Cooke, Johnson, & Quinn, 2018; Fernandez-Real et al., 2015; Sarkar et al., 2018). Increased levels of cytokines and glucocorticoids have been suggested to independently impair cognitive function (Donzis & Tronson, 2014). Given that reduced microbiome diversity and increased inflammation have been associated with cognitive impairment (Davidson et al., 2018; Donzis & Tronson, 2014) and chronic pain (Krustev et al., 2015; Minerbi et al., 2019b; Valdes et al., 2017), they are potential mediators of the pain-cognition link. Therefore, we will investigate whether gut microbiome dysbiosis correlates with inflammatory markers, pain sensitivity measures and deficits in cognitive function and whether increased peripheral inflammation and intestinal microbiota dysbiosis mediates the pain-cognitive function relationship in a cohort of knee OA patients.

3.2.1. Rationale

This study will examine the link between measures of pain and measures of distinct cognitive functions in people with knee OA. We will test a range of distinctive cognitive domains, using CANTAB and the vDMP task (optional), and will take into account the multidimensional nature of pain, using QST to assess different dimensions of pain responses. The study will also explore the link between pain sensitivity, cognitive function, biomarkers of inflammation and intestinal microbiota.

3.2.2. Primary objective

The primary objective of this study is to determine the relationship between pain severity/sensitivity, as measured by QST, and distinct cognitive functions, as measured using CANTAB, in knee OA.

3.2.3. Secondary objective(s)

The secondary objectives are to compare cognitive measures between pain participants and age and sex-matched controls, provided such a group can be recruited and a sufficiently high sample size (i.e., at least 100 participants overall) can be achieved, and to explore the correlation between inflammatory and molecular biomarkers, gut microbiome measures and knee pain, as well as cognitive measures. We will also offer participants the option to participate in testing of rapid place learning on the vDMP task, in addition to CANTAB, and if sufficient participants choose this option, will examine the link between pain sensitivity and vDMP measures and compare vDMP measures in pain and no-pain participants.

3.3. Methods/design

3.3.1. Study design

This is a cross-sectional cohort study in the primary care setting in Nottingham with participants identified as having knee OA and age and sex-matched controls, provided such a group can be recruited and a sufficiently high sample size (i.e., at least 100 participants overall) .

3.3.2. Patient and public involvement

The concept of patient involvement translated to the study design and execution phases of the research. On 12.06.2019 a meeting with an advisory group consisting of key stakeholders (clinicians and patients with various chronic musculoskeletal pain conditions) was conducted. A 15 min presentation was given about the research concepts, our aims and objectives and our research questions. Following that, the study design and research methods were discussed with patients, using lay language to facilitate common understanding and solicit their feedback. Through this process, patients contributed to aspects of the study design. More specifically, the group provided feedback that led to reconsideration of the exclusion criteria for study participation. As a result, people who suffer from gastrointestinal issues (such as, Crohn's disease, Irritable Bowel Syndrome) will be recorded and will form a different group in the analysis stage of the gut microbiome data. The group supported the aims and methods of the project and offered feedback regarding the usefulness of the study, overall aims, its

relevance to patient needs, benefits to patient care and outcomes. A consultation model of user involvement will continue to be followed throughout the project. The group were also given the patient information sheet developed for the current study to read at home and sent back to us by post in prepaid envelopes along with their feedback. The group's feedback led to a reconsideration of the patient information sheet to make it less wordy, containing more bullet points and pictures that would make it clearer to the lay person and in a larger font, for ease of reading.

3.3.3. Eligibility criteria and recruitment

Table 3-1 outlines the inclusion and exclusion criteria of the study. Participants, who have previously expressed an interest to be contacted for future research, will be recruited from existing databases (Fernandes et al., 2017; Gohir, Greenhaff, Abhishek, & Valdes, 2019) in Academic Rheumatology, City Hospital, University of Nottingham, and will be included in the study if they meet all the inclusion criteria.

Table 3-1. Inclusion/Exclusion Criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
Aged ≥ 40 years	Not adequate understanding of verbal explanations and written information in English
Able to read and write English	Special communication needs
Have English as their first language	English not first language
	Dementia
	Dialysis
	On home oxygen
	Serious mental illness
	Terminal illness

3.3.4. Sample size and justification

We aim to invite approximately 100 people to take part in the study in order to achieve sufficient statistical power (Knofczynski & Mundfrom, 2007). Previous studies with chronic pain patients, including with knee OA, reported that average pain intensity and cognitive function, as measured with the Trail-Making Test A and B, the Bourdon-Vos test and Digit-span backward test, correlated negatively with Pearson's r within 0.23-0.38 (Attal et al., 2014; Oosterman et al., 2011). An estimated minimum sample size of 83 volunteers would provide 80% statistical power to detect correlations of 0.3 at $p < 0.05$. The formula for calculation is based on two-tailed test (Draper & Smith, 1998; Stuart

& Kendall, 1963). A previous study (Suokas et al., 2012a) comparing knee pain and no knee pain groups, reported a minimum Cohen's d of −0.68 and -0.74 for pressure pain thresholds (the negative value indicates lower pressure pain thresholds in OA), which corresponds to a population SD of 2.57 and 2.03 for distal and affected site joints, respectively. To detect this effect size, the minimum sample size is 30 participants per group for a two-group comparison using two-tailed unpaired t-test and $p<0.05$, to achieve a power of 80% (Cohen, 2013). An estimated 90% of invited participants are expected to agree to participate in this study forming an effective sample size of 90 participants.

3.3.5. Research assessment

The following cognitive and behavioural measures will be taken from each participant:

Questionnaire measures (see Appendix 1.1, p. 235, for questionnaire booklet) and tests:

- Cognitive Failures Questionnaire (CFQ), 25 items, to assess everyday failures in perception, memory, and motor function (Broadbent, Cooper, FitzGerald, & Parkes, 1982).
- Cognitive Reflection Test (CRT), 3 items, to assess intuitive versus rationale cognitive style (Frederick, 2005).
- The National Adult Reading Test (NART) will be administered as a proxy of general premorbid IQ (Nelson, 1982).

- The Dictator Game (DG) to explore the role of altruism in motivating subjects' behaviour (Eckel & Grossman, 1996; Hoffman, McCabe, Shachat, & Smith, 1994).
- Tests from the Cambridge Neuropsychological Test Automated Battery, CANTAB® (Barnett et al., 2010), will include:
- Motor Screening Task (2 min) – Familiarisation test
- Intra-Extra Dimensional Set Shift (7 min) – Cognitive flexibility test comparable to the Wisconsin Card Sort Test. Aspects of cognitive flexibility measured include set-shifting (from shapes to lines or vice versa) and reversal learning (shifting from a previously rewarded visual stimulus to a previously unrewarded). Tasks of attentional set-shifting and reversal learning were found to require different portions of the prefrontal cortex in marmosets (Dias, Robbins, & Roberts, 1996), and frontal lobe damage in the corresponding areas of the human brain caused impairments on these tasks (Owen, Roberts, Polkey, Sahakian, & Robbins, 1991; Rahman, Sahakian, Hodges, Rogers, & Robbins, 1999).
- Paired Associate Learning (8 min) – Visual object-location associative learning. Participants learn to associate non-verbal patterns with locations on the screen with reminders in-between each successive attempt. Paired Associate Learning has been found to cause both bilateral hippocampal hyperactivation and hypoactivation in the same participants with mild cognitive impairment depending on memory load and task stage

(encoding/retrieval) (de Rover et al., 2011; Polkey & Robbins, 1995).

- Rapid Visual Information Processing (9 min) - Sustained visual attention test. Participants are required to monitor a stream of single digits for a sequence of three-digit numbers. Performance of the Rapid Visual Information Processing task has been found to engage frontal, parietal, occipital, thalamic, and cerebellar region activation (Lawrence, Ross, Hoffmann, Garavan, & Stein, 2003).
- Five Choice Reaction Time (4 min) – Processing and psychomotor speed. In the first trial, the participant has to observe a single location, while in the second trial, five locations are monitored. This test was developed from the well-known five-choice serial reaction time task used in rodents to measure visuospatial attention and motor impulsivity and has been found to be highly dependent on fronto-striatal mechanisms in rodents (Higgins & Silenieks, 2017; Pezze, McGarrity, Mason, Fone, & Bast, 2014; Robbins, 2002). Evidence of the translational utility of this test comes from a human study, which showed that serotonin depletion induced 'waiting impulsivity' on this test (Worbe, Savulich, Voon, Fernandez-Egea, & Robbins, 2014). 'Waiting impulsivity' has been associated with pathological behaviours, such as gambling and substance addictions, in humans (Leeman & Potenza, 2012) and with compulsive cocaine taking in rats (Belin, Mar, Dalley, Robbins, & Everitt, 2008).

- Spatial Working Memory (4 min) – Executive function, strategy use, retention and manipulation of visuospatial information (De Luca et al., 2003). Participants need to search for hidden tokens without returning to previous token locations. A human PET study found both ventral and dorsolateral areas of the frontal cortex to be activated during a Spatial Working Memory task (Owen, Doyon, Petrides, & Evans, 1996).
- Multi-Tasking Test (8 min) – Executive function and multitasking is known to involve frontal brain regions, including the anterior cingulate and immediately adjacent areas and the right dorsolateral prefrontal cortex (Burgess, 2000).

The neuropsychological substrates of the above assays are well characterised from patient and neuro-imaging studies and from rodent and non-human primate studies that have used directly equivalent tests. Computerised cognitive assessments are beneficial because they decrease measurement error variability between sites as well as participant attrition (Barnett et al., 2010).

In addition, we will also use the vDMP test (30-60 min), a new rapid place learning test in a virtual environment presented on a computer screen (Buckley & Bast, 2018), and performance on this test was shown to be associated with engagement of a brain circuit including the medial temporal lobe in human participants (Bauer, Buckley, & Bast, 2020). This test is optional and would be offered to participants at the end of the visit or as an online version to complete at home.

Additionally, the participants will complete the following assessments and questionnaires on pain sensitivity and severity, sleep quality, anxiety and depression, quality of life, and functionality:

- QST that assesses pain sensitivity such as pressure pain thresholds, temporal summation and conditioned pain modulation. Pressure pain thresholds is a valid measure of tenderness around the knee, temporal summation is a measure of central sensitization and conditioned pain modulation assesses the function of endogenous pain inhibitory pathways (Arendt-Nielsen et al., 2010).
- The Numerical Rating Scale (NRS), a subjective measure in which individuals rate their pain on an eleven-point numerical scale from 0 (no pain) to 10 (worst pain) (Farrar, 2001).
- The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), 24 items questionnaire to assess pain, stiffness and physical function (McConnell, Kolopack, & Davis, 2001).
- The PainDETECT questionnaire (PD-Q), which is used to identify neuropathic pain components (Freynhagen, Baron, Gockel, & Tölle, 2006b).
- The Pittsburgh Sleep Quality Index (PSQI), a questionnaire on sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989).
- The Hospital Anxiety and Depression Scale (HADS), a 14-item measure designed to assess anxiety and depression symptoms (Zigmond & Snaith, 1983).
- The 12-Item Short-Form Health Survey (SF-12), which assesses health-related quality of life (Ware et al., 1996).

- Objective assessments of physical function with the 30-Second Sit to Stand Test (30CST) (Jones, Rikli, & Beam, 1999) and the 'Time Up and Go' (TUG) test (Podsiadlo & Richardson, 1991) based on the recommendations of Osteoarthritis Research Society International (Dobson et al., 2013).

Participants will be asked to provide a urine and faecal (optional) sample for the purpose of identifying inflammatory biomarkers, molecular biomarkers, gut microbiome measures and genetic markers (Valdes et al., 2011b; Valdes, Meulenbelt, Chassaing, Arden, Bierma-Zeinstra, Hart, Hofman, Karsdal, Kloppenburg, & Kroon, 2014; Valdes, Meulenbelt, Chassaing, Arden, Bierma-Zeinstra, Hart, Hofman, Karsdal, Kloppenburg, Kroon, et al., 2014; Valdes et al., 2018).

3.3.6. Start and end dates

The trial has started in November 2019 and has been paused due to COVID-19.

3.3.7. Study visits

Participants from previous research in the department, who have expressed an interest in future research, will be informed of the study both orally and in writing. Those who qualify for the study will give signed informed consent. The consent will be taken by an experienced member of the research team.

The participants will have an assessment session with experienced staff, and NRS, SF-12, HADS, PD-Q, WOMAC, PSQI, 30CST, TUG, QST, CANTAB tests, CFQ, CRT, NART, DG and urine and blood samples will be taken. These samples will be assessed for circulating levels of glucose, C reactive protein, triglycerides, low-density lipoprotein, high-density lipoprotein, tumour necrosis factor-alpha, interleukin-6 and interleukin-1 (Valdes, Meulenbelt, Chassaing, Arden, Bierma-Zeinstra, Hart, Hofman, Karsdal, Kloppenburg, & Kroon, 2014; Valdes et al., 2018).

3.3.8. Data management

Data for this study will be collected during a single visit or online for vDMP. Data will be collated by trained local research staff, and data entry in a relational Microsoft Access database will be completed in a standardised fashion. The clinical research forms from the study visit will be sent to the data entry site. CANTAB provides an outcome measures report for the tests and variants used in this study, which can be downloaded directly from the application. The investigator performs and monitors data entry. This will include questionnaires (SF-12, HADS, PD-Q, WOMAC, PSQI, CFQ) and data from CANTAB, QST, 30CS, TUG, CRT, NART, and DG.

3.3.9. Data analysis

Correlational analysis will be done to investigate the relationship between cognition and pain. We will compare cognitive function between pain participants and age and sex-matched controls using independent samples t-tests. Multivariate linear and logistic regression models will be used to investigate intercorrelations between variables and to adjust for confounders. Confounding variables were chosen due to their clinical relevance with chronic pain and cognitive function based on findings from other studies (Attal et al., 2014). Correlational analysis between the study variables will be done to determine which variables will be entered in the multivariate models. The independent variables that demonstrate a significant relationship with the dependent variable (pain severity and sensitivity) will be entered in the multivariate model if they are considered to be of high clinical significance (e.g. age, sex) or were able to differentiate between pain-patients and controls in comparative analysis at $P<0.20$, a level of significance that has been recommended in logistic regression analysis (Hosmer, Lemeshow, & Sturdivant, 2013).

3.3.10. Adverse events

As all procedures are being carried out following standard methods, the occurrence of an adverse event as a result of participation in this study is not expected and no adverse event data will be collected. However, debriefing will provide an opportunity for any issues to be raised.

3.3.11. Criteria for terminating the study

The current study involves a single assessment session only and an online assessment if participants choose to complete the online version of vDMP at home. Since it is not an interventional study nor does involve investigational medicinal products or medical devices, no serious adverse events are expected to arise that will require termination of the study.

3.3.12. Ethics and dissemination

The study results will be disseminated to peer-reviewed journals, seminars and conferences and to non-academic stakeholders and the general public, if and as appropriate, e.g. through Versus Arthritis UK or other press releases. Study participants' identity will not be disclosed when publishing the results. Study participants will also be informed of the results if requested.

3.3.13. Insurance and indemnity

The University of Nottingham and the NIHR Nottingham Biomedical Research Centre indemnifies their staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

3.3.14. Study conduct

Study conduct will be subject to systems audit for inclusion of essential documents; permissions to conduct the study; Curriculum Vitaes of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria); accountability of study materials and equipment calibration logs.

3.3.15. Study data

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. This will be managed by the direct study team.

Entries on study forms will be verified by inspection against the source data. A sample of the forms (10%) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by the FMHS-REC as required.

3.3.16. Record retention and archiving

In compliance with Good Clinical Practice guidelines and in accordance with the University of Nottingham Code of Research Conduct and Research Ethics, the lead investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least seven years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The study master file held by the leading investigator on behalf of the sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all anonymised study databases and associated meta-data encryption codes.

3.3.17. Statement of confidentiality

Individual participants' medical or personal information obtained as a result of this study are considered confidential, and disclosure to third parties is prohibited. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files. Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

Data generated as a result of this study will be available for inspection

on request by the participating physicians, the University of Nottingham representatives, the FMHS-REC and the regulatory authorities.

3.4. Preliminary results

Participants provided feedback throughout their visit to the study team as well as with feedback forms provided at the end of the study visit (Appendix 1.2, p. 256). The study was feasible, since all the practical aspects were executed as planned, and the study was well received by the participants.

3.4.1. Descriptives

Preliminary analysis was conducted for eight participants that were screened so far. Table 3-2 presents their demographic characteristics; Table 3-3 summarises the WOMAC and PainDETECT scores among the chronic pain group (n=7); Table 3-4 includes raw QST data among the different pain groups: severe (n=5), moderate (n=2) and no pain (n=1); and Table 3-5 summarises the main CANTAB outcome measures for severe and moderate pain participants and controls (n=2, a test run was included as additional control).

Table 3-2. Characteristics of participants

				Education, n (%)				Smoking status, n (%)			
Characteristics	**Age, mean (±SD)**	**Female Gender, n (%)**	**BMI, median (min, max)**	**Master's degree or equivalent**	**Undergraduate degree or equivalent**	**Left formal education at age 17-18**	**Left formal education before age 16**	**Ex-smoker**	**Never smoker**	**OA diagnosis, n (%)**	**Opioid medication, n (%)**
Cases (n=7) /Controls (n=1)	69 (12.46)	37.5	32.86 (25.73, 47.78)	12.5	37.5	25	25	25	75	75	25

Table continued on next page

SD, Standard Deviation; BMI, Body Mass Index; NART, National Adult Reading Test.

Table 3-2(Cont.). Characteristics of participants

		Psychological and Quality of life factors, median (min, max):						
Characteristics	**NART-predicted IQ, mean (±SD)**	**Anxiety[1]**	**Depression[1]**	**Physical health-related quality[2]**	**Mental health-related quality[2]**	**Sleep quality[3]**	**Cognitive style[4]**	**Cognitive impairment[5]**
Cases (n=7) /Controls (n=1)	114.72 (6.74)	6.5 (0, 10)	6.5 (0, 12)	34.38 (17.89, 54.22)	50.47 (38.35, 58.84)	9 (4, 13)	0.88 (0, 3)	36.5 (17, 52)

SD, Standard Deviation; BMI, Body Mass Index; NART, National Adult Reading Test.

[1]Hospital Anxiety and Depression Scale (HADS)

[2]12-Item Short Form Survey (SF-12)

[3]Pittsburgh Sleep Quality Index (PSQI)

[4]Cognitive Reflection Test (CRT)

[5]Cognitive Failures Questionnaire (CFQ)

Table 3-3. Characteristics of knee OA and neuropathic pain symptoms among cases

Characteristic	**Case knees (n=7)**
WOMAC score, median (min, max):	
Pain (/20)	11 (7, 13)
Stiffness (/8)	5 (3, 6)
Physical function (/68)	37 (29, 55)
Total (/96)	51 (39, 73)
Neuropathic pain symptoms:	
PD-Q score, median (min, max)	11 (6, 18)

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; PD-Q, Pain Detect Questionnaire

Table 3-4. Raw QST data for most painful knees of severe and moderate pain participants and control

QST parameters	**Severe (n=5)**	**Moderate (n=2)**	**Control (n=1)**
Pressure Pain Threshold (kPa), median (min, max):			
Superolateral patella	206.8 (64.7, 912.4)	692.35 (576.2, 808.5)	605.6
Superomedial patella	197 (73.5, 691.9)	802.15 (593.9,1010.4)	445.9
Medial joint line	194 (77.4, 900.6)	656.6 (372.4, 940.8)	350.8
Tibialis anterior muscle	180.3 (102.5, 963.3)	532.1 (380.2, 684)	626.2
ECRL	217.55 (88.2, 406.7)	418.45 (342, 494.9)	394
Wind up ratio (ratio)	0.6 (0.125, 0.9125)	0.725 (0.7, 0.75)	0
Conditioned Pain Modulation score (C-U)	61.8 (-343, 240.1)	-123.45 (-159.7, -87.2)	1

ECRL, Extensor carpi radialis longus

Table 3-5. CANTAB outcome measures scores for severe and moderate pain participants and controls

CANTAB measures*	Controls (n=2)	Moderate (n=2)	Severe (n=5)
IED EDS Errors	20.5 (12.0)	32.0 (33.9)	90.6 (76.7)
IED Total Errors (Adjusted)	710.0 (158.0)	851.0 (353.0)	953.0 (290.0)
MOT Mean Latency	31.0 (75.0)	88.2 (70.4)	92.9 (72.4)
MTT Incongruency Cost (Median)	105.0 (43.1)	196.0 (76.7)	341 (148.0)
MTT Multitasking Cost (Median)	2.0 (0.0)	3.5 (0.7)	15.0 (8.5)
MTT Total Incorrect	11.5 (0.7)	10.0 (0.0)	10.0 (2.1)
PAL First Attempt Memory Score	23.0 (11.3)	33.5 (9.2)	31.6 (9.1)
PAL Total Errors (Adjusted)	206.0 (114.0)	283 (43.8)	364.0 (136.0)
RTI Median Five-Choice Movement Time	298.0 (33.9)	362.0 (110.0)	372.0 (25.1)
RTI Median Five-Choice Reaction Time	0.96 (0.003)	0.91 (0.05)	0.85 (0.07)
RVP A'	353.0 (25.1)	456.0 (131.0)	424.0 (95.0)
RVP Median Response Latency	0.005 (0.001)	0.02 (0.01)	0.006 (0.004)
RVP Probability of False Alarm	36.0 (1.4)	42.0 (5.7)	NA
SWM Between Errors 12 Boxes	10.0 (14.1)	20.0 (4.2)	25.6 (22.9)
SWM Between Errors	6.5 (2.1)	7.5 (2.1)	8.2 (3.5)

IED, Intra-Extra Dimensional Set Shift; MOT, Motor Screening Task; MTT, Multi-Tasking Task; PAL, Paired Associate Learning; RTI, Five Choice Reaction Time; RVP, Rapid Visual Information Processing; SWM, Spatial Working Memory.

**for CANTAB outcome measures description please see Appendix 1.2.4*

3.4.2. Correlational analysis

We performed correlational analysis to investigate the relationship between the variables. Higher anxiety levels on the HADS scale correlated with increased pain duration (rho = 0.86, p = 0.014), higher predicted IQ assessed with NART (rho = 0.75, p = 0.032), higher cognitive impairment i.e. higher CFQ score (rho = 0.87, p = 0.005) and higher mean latency on the motor screening familiarisation task (i.e. sensorimotor deficits or lack of comprehension) (rho = 0.75, p = 0.032). Higher depression on the HADS scale correlated with higher cognitive impairment on the CFQ (rho = 0.87, p = 0.005) and a higher median time taken for a subject to release the response button after the presentation of a target stimulus on the Five Choice Reaction Time test (i.e. lower processing and psychomotor speed) (rho = 0.74, p = 0.037). In turn, lower processing and psychomotor speed on the Five Choice Reaction Time test positively correlated with OA diagnosis (rho = 0.85, p = 0.008), higher pain severity (rho = 0.86, p = 0.014) and lower PPT score (i.e. higher pain sensitisation) on the tibialis anterior muscle during conditioning (rho = -0.72, p = 0.046). Poorer physical function (i.e. lower score on the 30CST) correlated with higher cognitive impairment (i.e. higher CFQ) (rho = -0.73, p = 0.038) and higher pain duration (rho = -0.81, p = 0.029). In addition, higher cognitive impairment (i.e. higher CFQ) correlated with higher pain severity and duration (rho = 0.83, p = 0.02 and rho = 0.79, p = 0.035, respectively). Rationale cognitive thinking instead of intuitive (i.e. higher CRT)

correlated with reduced knee pain (rho = -0.72, p = 0.044) and higher PPT scores (i.e. lower pain sensitisation) at the superolateral patella (rho = 0.84, p = 0.009), superomedial patella (rho = 0.81, p = 0.015), medial joint line (rho = 0.86, p = 0.007) and tibialis anterior muscle (rho = 0.77, p = 0.026). Poorer multitasking ability (i.e. more incorrect responses on the multi-tasking task) correlated with poorer rapid visual information processing ability (i.e. lower sensitivity to the target sequence – lower Rapid Visual information Processing A' score) (rho = -0.87, p = 0.005). In turn, poorer multitasking ability (higher score) and poorer visual information processing (lower score) correlated with higher neuropathic pain scores (i.e. higher PD-Q) (rho = 0.78, p = 0.022 and rho = -0.85, p = 0.007, respectively) as well as higher central sensitisation (i.e. higher temporal summation score) (rho = 0.73, p = 0.039 and rho = -0.76, p = 0.028, respectively). Furthermore, poorer multitasking ability and poorer visual information processing ability correlated with more time taken to complete the TUG test (i.e. poorer physical function) (rho = 0.84, p = 0.009 and rho = -0.75, p = 0.031, respectively). Lastly, higher central sensitisation (i.e. higher temporal summation) correlated with lower memory score on the paired associates learning test and a lower score on the spatial working memory test (rho = -0.76, p = 0.029 and rho = -0.77, p = 0.044, respectively).

Preliminary data analysis was only conducted on the whole sample, since number of participants of the groups is too small for comparisons between pain participants and controls. Similarly, due to lack of power

we could not conduct multivariate regression analyses to investigate intercorrelations between variables and to adjust for confounders.

3.5. Discussion

Osteoarthritis pain has traditionally been associated with peripheral pain due to joint damage and inflammation. However, the presence of radiographic joint pathology does not always explain high pain sensitivity (Finan et al., 2013; Valdes et al., 2012). Chronic pain patients report significant suffering from pain and multiple impairments in cognitive function, which limits their participation in everyday life activities, changes their behaviour and emotion and significantly reduces their overall quality of life (Cacioppo & Hawkley, 2009; James, Walsh, & Ferguson, 2018; Moriarty et al., 2011) and treatment success (Attal et al., 2014).

Human imaging data have revealed important anatomical and functional brain alterations in chronic pain conditions, including OA (Apkarian et al., 2011; Baliki & Apkarian, 2015; Baliki et al., 2012; Iwabuchi et al., 2020; Mansour et al., 2013; Parks et al., 2011a; Reckziegel, Raschke, Cottam, & Auer, 2016). Brain reorganisation produces cognitive, sensory and motor behavioural changes in distinct patterns specific to different pain conditions (Bushnell, Čeko, & Low, 2013; Moriarty et al., 2011; Parks et al., 2011a; Wiech et al., 2008). The array of cognitive functions impaired in chronic pain is large, and their independent contributions and links to specific clinical conditions, such

as OA remain to be established (Apkarian et al., 2011; Moriarty et al., 2011). This has a clinical utility, as a better understanding of key cognitive mechanisms that are impaired may point the way for better treatment options in the future.

Recently, evidence has emerged of a bacteria-cognition link mediated via the gut-brain axis that requires further investigation (Benton et al., 2007; Carlson et al., 2018; Fernandez-Real et al., 2015). At the same time, human studies have revealed significant changes in gut microbiome diversity in chronic pain patients mainly with fibromyalgia compared to controls coupled with metabolic changes, such as in short chain fatty acids and glutamate and serine metabolism (Clos-Garcia et al., 2019; Minerbi et al., 2019b). Therefore, we designed a study aimed at investigating the specific neuro-cognitive domains that are impaired in OA pain and the links between cognitive domains, anxiety, pain and bacterial taxa. The study was feasible as all the practical aspects were executed as planned, and the study was well received. Our preliminary findings showed that anxiety, pain and cognitive ability are correlated and impairment in certain aspects of cognitive function might be associated with poorer physical function (slower TUG time), increased neuropathic-like pain (i.e. higher PD-Q) and central pain processing (i.e. higher temporal summation). These results suggest that a decrease in motor abilities is linked to poorer executive functioning in line with findings from previous studies in healthy individuals (Donoghue et al., 2012) and Parkinson's disease patients (Manenti et al., 2014). Poorer multitasking ability and poorer visual information processing were

correlated with higher neuropathic pain scores on the PD-Q. This is consistent with data from Attal et al. (2014) that found a correlation between the Rey-Osterrieth Complex Figure Test Copy and Recall and neuropathic pain symptoms on the DN4 questionnaire. We build on these findings using quantitative sensory testing to determine whether these cognitive variables may also predict more specific components of pain, such as allodynia, hyperalgesia or temporal summation. Our preliminary findings indicate that enhanced temporal summation correlated with lower memory score on the paired associates learning test, a lower score on the spatial working memory test, poorer multitasking ability and poorer visual information processing. Therefore, pain of a neuropathic nature and central pain processing seem to be most closely linked to variables assessing executive function, memory, visuospatial information processing and visual object-location associative learning. This is consistent with findings showing that morphometric abnormalities involving the prefrontal cortex are strongly related to pain characteristics, including the neuropathic nature of pain (Apkarian et al., 2009), and experimental data indicating that hippocampus-mediated behaviour, synaptic plasticity and neurogenesis are abnormal in neuropathic rodents (Mutso et al., 2012). Nevertheless, these preliminary results need to be interpreted with caution, as power was insufficient to perform comparisons between pain participants and controls and multivariate regression analyses adjusting for confounders.

Completion of this study will reveal for the first time whether increased peripheral inflammation and intestinal microbiota dysbiosis mediates

the anxiety-cognitive function-pain relationship in a cohort of knee OA patients. This will aid our understanding of the underlying biological mechanisms in the link between chronic pain and emotional factors (cognitive function and anxiety) in OA and will potentially contribute to the identification of biomarkers for this disease.

To the best of our knowledge, this study is the first to use translational behavioural assays of well-characterized neuro-cognitive constructs and to test a range of cognitive domains in patients with knee OA. Using Quantitative Sensory Testing, we explored for the first time whether cognitive deficits are associated with different dimensions of pain, such as allodynia, hyperalgesia and temporal summation. However, this is an observational study and, thus, we cannot make causal inferences about the potential link between anxiety, cognitive function and chronic pain. Furthermore, we recruited individuals with self-reported diagnosis of OA and, thus, we cannot rule out non-OA causes of knee pain. Although using OA participants allows us to identify cognitive domains specifically associated with OA pain, it limits generalization of our findings to other types of chronic pain conditions. Future results from this study may help to improve our understanding of which distinct cognitive domains are impaired in chronic knee OA pain, and of the underlying biological mechanisms of chronic pain, anxiety and cognitive function in OA, and potentially point the way for better treatment options in the future and contribute to the identification of biomarkers for this disease.

3.6. List of abbreviations

CANTAB: Cambridge Neuropsychological Test Automated Battery

CFQ: Cognitive Failures Questionnaire

CRT: Cognitive Reflection test

DG: Dictator Game

HADS: Hospital Anxiety and Depression scale

NART: National Adult Reading Test

NRS: Numerical rating scale

OA: Osteoarthritis

PD-Q: PainDETECT Questionnaire

PSQI: Pittsburgh sleep quality index

QST: Quantitative Sensory Testing

SF-12: 12-Item Short Form Health Survey

TUG: Time up and go test

vDMP: virtual Delayed Matching to Place

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

30CST: 30-second sit to stand test

3.7. Declarations

3.7.1. Ethics approval and consent to participate

The study has received approval from the University of Nottingham Research Ethics Committee (REC) (ref: 355-1907). Any modification to the approved protocol will require resubmission of modifications and

further approval from the REC. Participants from previous research in the department who meet the eligibility criteria and wish to take part in the study after they have been informed about its procedures, they will be asked to sign an informed consent form.

3.7.2. Consent for publication

Not applicable: No data from any individual person were included in this manuscript.

3.7.3. Availability of data and materials

Not applicable: The datasets that will be used or analysed during this current study are not yet available because the study is presently paused. They will be available from the corresponding author upon reasonable request once recruitment and data collection is complete.

3.7.4. Competing interests

None declared.

3.7.5. Funding

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Chapter 4: The association of socio-economic and psychological factors with limitations in day-to-day activity over 7 years in newly diagnosed osteoarthritis patients

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4.1. Abstract

Previous research has established links between chronic pain and impaired cognitive ability, as well as between chronic pain and anxiety, in osteoarthritis. Furthermore, there is evidence linking risk of osteoarthritis to lower educational attainment. However, the inter-play of these factors with key social factors (e.g., social deprivation) at the early stages of osteoarthritis are not understood. Here, we used data from waves 4, 5, 6 and 7 of the Survey of Health, Ageing and Retirement in Europe (SHARE) (n = 971) and selected a subsample of respondents who initially did not report a diagnosis of osteoarthritis until wave 6. We used path models to test how social deprivation, education and anxiety, before diagnosis (waves 4 and 5), affect the relationship between cognitive ability, pain and limitations in activities of daily living following diagnosis (waves 6 and 7). We show that high social

deprivation before diagnosis predicts greater limitations in activities of daily living after diagnosis, with this effect partly mediated by impaired cognitive ability. We also find that higher educational attainment before diagnosis may protect against limitations in activities of daily living after diagnosis via better cognitive ability and lower anxiety. Therefore, improving cognitive ability and managing anxiety may mitigate the associations of social deprivation and low educational attainment with limitations in activities of daily living.

4.2. Introduction

Osteoarthritis is the most common form of arthritis (Cross et al., 2014) and is characterized by pain, which can lead to limitations in activities of daily living and reduced quality of life (Hochberg et al., 2012; Zhang et al., 2008). Indeed, cross-sectional studies report a significant association between pain severity and activities of daily living in older adults with osteoarthritis (García-López et al., 2021; Stamm et al., 2016).

Pain status in older adults with osteoarthritis is an independent predictor of limitations in instrumental activities of daily living (IADL) (Nanjo et al., 2021). IADL reflect the ability to perform activities, such as doing housework, shopping, taking medications, preparing a hot meal, making calls, and managing money, independently (Lawton & Brody, 1969). Limitations in IADL generally occur before limitations in basic activities of daily living (Edjolo, Proust-Lima, Delva, Dartigues, & Pérès,

2016). In older adults, they progress cumulatively (Barberger-Gateau, Rainville, Letenneur, & Dartigues, 2000), and therefore interventions for preventing limitations in IADL during the early stages of osteoarthritis may limit further decline.

IADL depend on cognitive skills, mood and motivational factors, all of them negatively associated with pain (Bushnell, Ceko, et al., 2013; Foster & Doty, 2021). For example, anxiety is associated with increased limitations in IADL independently of depression comorbidity (Norton et al., 2012). In osteoarthritis, higher levels of anxiety are associated with heightened pain, as well as limitations in activities of daily living (Akin-Akinyosoye et al., 2018; Burston et al., 2019; Nur et al., 2018; Sinikallio et al., 2014; Stamm et al., 2016). Moreover, the existing literature shows that in osteoarthritis, good cognitive ability is protective against experiencing higher pain intensity, and higher pain intensity is associated with cognitive impairment (Attal et al., 2014; Berryman et al., 2013; James & Ferguson, 2019; Moriarty et al., 2011).

Social factors may affect limitations in activities of daily living, although their role in osteoarthritis is not well understood. Indeed, individual and neighbourhood social factors have been associated with risk for developing pain that interferes with daily activities (Jordan et al., 2008; Kiadaliri et al., 2017; Luong et al., 2012; Rijk et al., 2020). Furthermore, a cross-sectional link between higher social deprivation, assessed using 9-digit ZIP codes, and greater limitations in activities of daily living in musculoskeletal conditions has been reported (Rijk et al., 2020). For this study, social deprivation is defined as social isolation (i.e. feeling

left out of things), lack of social support, poor quality of the local area (i.e. cleanness, vandalism, presence of helpful people, feeling part), ease of access to local amenities (e.g., the nearest bank, pharmacy, and grocery shop), and lack of social activities (Levitas et al., 2007). Limitations in activities of daily living among older people are the result not only of health problems, but also of their interactions with psychological and socioeconomic factors (Kostanjsek, 2011; Organization, 2007). Indeed, it has also been shown that social engagement promotes better executive functioning in old age (Carlson et al., 2015; Carlson et al., 2008; Fried et al., 2013; Stern, 2012). Thus, cognitive ability is a potential mediating mechanism in the relationship between social deprivation and limitations in IADL (Foster & Doty, 2021).

In osteoarthritis, low educational attainment is associated with greater reported pain and poorer health status outcomes independently of socioeconomic factors, such as income (Callahan et al., 2011; Cleveland et al., 2013; Hannan et al., 1992; Kiadaliri et al., 2017; Knight et al., 2011; Luong et al., 2012; Thumboo et al., 2002). There is also evidence that educational attainment has positive effects on late-life cognitive ability (Lövdén, Fratiglioni, Glymour, Lindenberger, & Tucker-Drob, 2020). Therefore, higher educational attainment may protect against increased pain levels and limitations in IADL, via its association with improved cognitive ability.

It should be noted that although cognitive ability and education are correlated, they are distinct from one another, and one can act as a

moderator of the other with respect to life outcomes (Auld & Sidhu, 2005). First, there are other, especially social, determinants of whether or not people achieve certain educational outcomes (Deary, Weiss, & Batty, 2010). Second, educational attainment is more closely related to crystallized abilities (i.e. stored knowledge that has been accumulated through learning, such as grammar and academic knowledge), than to fluid abilities, such as processing speed and abstract reasoning (Baltes, Staudinger, & Lindenberger, 1999; Cattell, 1987). The measure of cognitive ability used in this study mainly reflects fluid cognition.

All of the factors discussed above will contribute to limitations in activities of daily living in general, but how they interact to contribute to limitations in IADL caused specifically by osteoarthritis pain is not yet well understood. Therefore, the present study builds on this previous work by exploring in more detail how social deprivation and educational attainment influence the dynamic relationship between IADL, pain, anxiety and cognitive ability before and after a diagnosis of osteoarthritis. As affective and cognitive factors are influenced by the experience of pain (Attal et al., 2014; Berryman et al., 2013; James & Ferguson, 2019; Moriarty et al., 2011), it is necessary to assess these factors prior to the emergence of disease associated with pain (osteoarthritis) in order to show their unconfounded effects on subsequent pain and limitations in IADL. This would allow us to identify factors that could be targeted for early interventions to reduce the disability caused by osteoarthritis (Neogi, 2013).

In the present study, we analysed data over four consecutive waves from the Survey of Health, Ageing and Retirement in Europe (SHARE), a large longitudinal survey covering 28 European countries (Börsch-Supan et al., 2013), which allowed us to follow the trajectory and development of osteoarthritis over time (i.e. before and after diagnosis). We assessed the role of social deprivation, anxiety and cognitive ability before a diagnosis of osteoarthritis in limitations in IADL, pain and cognitive ability after the onset of osteoarthritis in a selected sample of newly diagnosed osteoarthritis patients. Specifically, we tested the following hypotheses: (1) higher levels of pain will be related to impaired cognitive ability and higher anxiety after controlling for pain before diagnosis and confounders, (2) better cognitive ability will be related to reduced pain levels and lower social deprivation after controlling for cognitive ability before diagnosis and confounders, (3) greater limitations in IADL will be associated with higher anxiety and poorer cognitive ability after controlling for limitations in IADL before diagnosis and confounders and (4) pain, cognitive ability and anxiety will act as the mechanisms indirectly linking socio-economic factors (social deprivation and educational attainment) to limitations in IADL.

4.3. Results

4.3.1. Descriptives

Descriptive statistics for the sample are reported in Table 4-1. All fit indices suggested the model fits these data well [Root Mean Square

Error of Approximation (RMSEA) = 0.044, 95% CI 0.033–0.055, Standardised Root Mean Square Residual (SRMR) = 0.026, Comparative Fit Index (CFI) = 0.983, Tucker Lewis Index (TLI) = 0.957] (see Appendix 2.1, p. 272).

Table 4-1. Descriptive statistics (n = 971)

										Education, n (%):			
Characteristics	**Age (53-101)**	**BMI (16-53)**	**No. of chronic diseases (0-9)**	**Social deprivation index[1] (0-0.65)**	**Cognitive ability total score (0-107)**	**Level of pain (1-4)**	**Anxiety[1] (0-20)**	**IADL (0-7)**	**Female Gender, n (%)**	**Low – ISCED code 0,1 and 2**	**Medium – ISCED code 3 and 4**	**High – ISCED code 5 and 6**	**Other**
W4 (2011)	67.04 (9.33)	27.10 (6.90)	2.18 (1.6)	-	33.77 (10.82)	**	-	0.31 (0.83)	73.53	43.56	35.53	19.15	0.62
W5 (2013)	69.04 (9.33)	27.23 (6.77)	1.97 (1.53)	0.20 (0.15)	33.59 (11.81)	2.48 (1.09)	7.95 (2.96)	0.39 (0.96)	73.53	41	34.81	18.33	0.62
W6 (2015)	71.04 (9.33)	26.95 (7.31)	3.16 (1.72)	-	33.37 (11.46)	3.09 (0.67)	-	0.56 (1.15)	73.53	40.88	34.91	18.33	0.61
W7 (2017)	73.04 (9.33)	26.73 (7.51)	3.27 (1.78)	-	*	*	-	0.72 (1.43)	73.53	41.3	39.34	18.54	0.62

Table continued on next page

**It is not reported here due to more than 50 % missing values in this variable. Note: The missing values were not due to nonresponse but simply because some of the participants received a condensed set of questions at wave 7.*

*** Pain intensity was not assessed in wave 4 of the SHARE. However, 75.56% participants reported being bothered by pain in back, knees, hips or other joint at wave 4.*

[1]It was only measured at wave 5 of the SHARE

[2]It was not measured at wave 4 of the SHARE

Table 4-1(Cont.). Descriptive statistics (n = 971)

	Marital status, n (%)			Physical activity, n (%)		Alcohol consumption, n (%)				Smoking status, n (%)			
Characteristics	**Married and living together**	**Divorced or widowed**	**Other**	**Other**	**Never vigorous or moderate**	**Not at all in the last 3 months**	**Less than once a month**	**Once or twice a month**	**More than once or twice a month**	**Smoker**	**Non smoker**	**Affective/emotional disorder[2], n (%)**	**At risk of severe deprivation[1], n (%)**
W4 (2011)	46.76	21.52	5.35	88.67	10.5	66.22	12.56	5.97	4.42	14.21	50.77	-	-
W5 (2013)	50	45	5	86.81	13.18	78.88	9.54	4.73	3.5	12.77	82.18	6.9	12.56
W6 (2015)	45.07	49.29	11.27	84.96	15.04	80.02	6.28	4.94	4.74	*	*	9.37	-
W7 (2017)	*	*	*	*		*	*	*	*	*	*	9.47	-

**It is not reported here due to more than 50 % missing values in this variable. Note: The missing values were not due to nonresponse but simply because some of the participants received a condensed set of questions at wave 7.*

*** Pain intensity was not assessed in wave 4 of the SHARE. However, 75.56% participants reported being bothered by pain in back, knees, hips or other joint at wave 4.*

[1]It was only measured at wave 5 of the SHARE

[2]It was not measured at wave 4 of the SHARE

4.3.2. Direct Effects

The key findings are highlighted in the path model in Figure 4-1 and in Appendix 2.3, p. 275 (for all outcomes of our analysis, see Appendix 2.2, p.273). Hypothesis 1 stated that higher levels of pain will be related to reduced cognitive ability and higher anxiety after controlling for pain before diagnosis and confounders. Results from the path model show a direct association of higher anxiety before diagnosis with pain after diagnosis, such that greater anxiety is associated with more severe reports of pain. We find no significant association between cognitive ability before diagnosis and pain following diagnosis. Hypothesis 2 stated that better cognitive ability will be related to reduced pain levels and lower social deprivation after controlling for cognitive ability before diagnosis and confounders. In support of hypothesis 2, we find a direct association of higher levels of pain before diagnosis with reduced cognitive ability following diagnosis. In line with hypothesis 2, we find a direct association between social deprivation before diagnosis and cognitive ability following diagnosis, such that lower social deprivation is linked to better cognitive ability. Hypothesis 3 stated that greater limitations in activities of daily living will be associated with higher anxiety and poorer cognitive ability after controlling for limitations in activities of daily living before diagnosis and confounders. In support of hypothesis 3, results show that increased anxiety, before diagnosis, predicted greater limitations in activities of daily living (i.e., a higher IADL score) after diagnosis, whereas greater cognitive ability, before

diagnosis, predicted reduced limitations in activities of daily living after diagnosis.

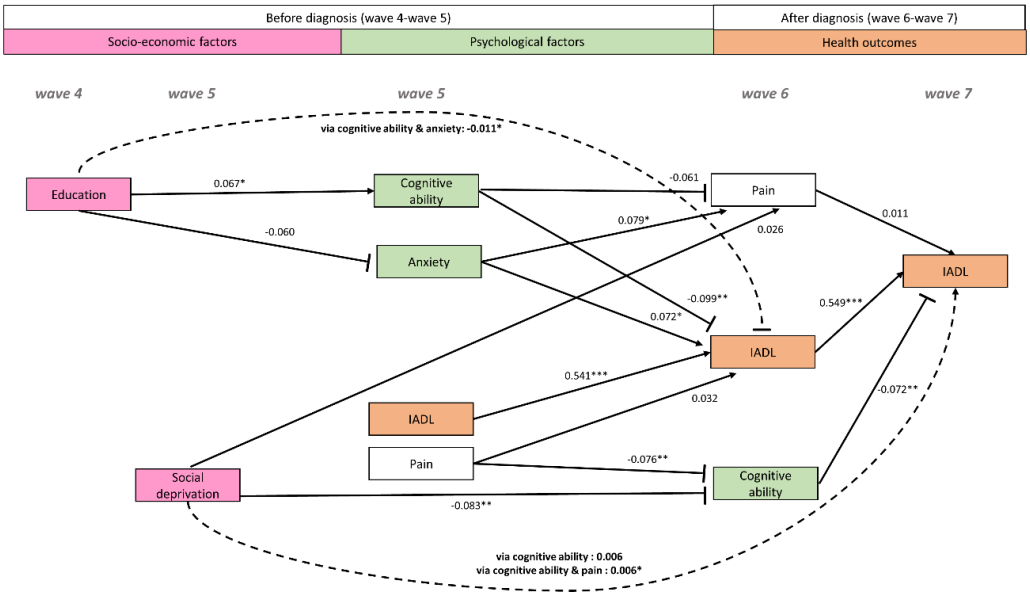


Figure 4-1. Illustration of key direct and indirect paths

*from socio-economic (education and social deprivation) and psychological factors (anxiety and cognitive ability) at waves 4 and 5 (before diagnosis) to health outcomes (pain and instrumental activities of daily living, IADL – note a higher IADL score indicates more difficulties with these activities) at wave 5, health outcomes and cognitive ability at wave 6 (following diagnosis) and IADL at wave 7 (after diagnosis). Inhibition arrows depict negative associations, whereas point arrows represent positive associations. Solid lines and dashed lines depict direct and indirect associations, respectively. Note: In path analysis, a variable can be both a predictor with respect to a variable and an outcome with regards to another variable as well as a mediator when testing for indirect effects (Streiner, 2005). For example, wave 6 cognitive ability is a predictor with regards to wave 7 IADL, an outcome with respect to wave 5 social deprivation and a mediator of the path from wave 5 social deprivation to wave 7 IADL. Standardised effects and FDR-adjusted p-values are presented. * = p < .05, ** = p < .01, *** = p < .001. n = 971.*

4.3.3. Indirect Effects

Hypothesis 4 stated that pain, cognitive ability and anxiety will act as the mechanisms indirectly linking socio-economic factors (social deprivation and educational attainment) to limitations in activities of daily living. Examining the indirect effects (Table 4-2), higher social deprivation, before diagnosis, predicted greater limitations in activities of daily living following diagnosis, through its negative effect on cognitive ability (wave 5 social deprivation -> wave 6 cognitive ability -> wave 7 IADL), suggesting that one mechanism underlying the link between social deprivation and limitations in activities of daily living is via cognitive ability. Looking at the total indirect effects, higher social deprivation before diagnosis was associated with greater limitations in activities of daily living after diagnosis (wave 7) via its association with reduced cognitive ability and higher pain levels at wave 6. In addition, higher educational attainment, prior to diagnosis was associated with lower limitations in activities of daily living after diagnosis via greater cognitive ability and lower anxiety before diagnosis (wave 5). Therefore, our results support hypothesis 4.

Table 4-2. Indirect paths predicting pain at wave 6 and IADL at waves 6 and 7 and their total indirect effects

Indirect paths	W5 Anxiety→ W6 Cognitive ability→ W7 IADL	W5 Anxiety→ W6 Pain→ W7 IADL	W4 Education→ W5 Cognitive ability→ W6 Cognitive ability	W4 Education→ W5 Cognitive ability→ W6 Pain	W4 Education→ W5 Anxiety→ W6 Pain	W4 Education → W5 Cognitive ability → W6 IADL	W4 Education → W5 Social Deprivation → W6 IADL	W4 Education → W5 Pain → W6 IADL	W4 Education → W5 Anxiety → W6 IADL	W5 Social deprivation → W6 Cognitive ability → W7 IADL	W5 Social deprivation → W6 Pain → W7 IADL
SD coefficient	0.000	0.000	0.043	-0.004	-0.005	-0.007	-0.001	-0.001	-0.004	0.006	0.000
SE	0.002	0.001	0.019	0.003	0.003	0.003	0.001	0.001	0.003	0.003	0.001
CI lower	-0.003	-0.001	0.007	-0.01	-0.011	-0.013	-0.003	-0.004	-0.01	0.001	-0.001
CI upper	0.004	0.002	0.079	0.001	0.001	0.000	0.002	0.001	0.001	0.011	0.002
P	0.778	0.622	0.020*	0.143	0.134	0.049*	0.631	0.415	0.114	0.024*	0.677
FDR-adj. P	0.821	0.717	0.043*	0.219	0.207	0.090	0.717	0.547	0.184	0.050	0.756

Table continued on next page

W, wave; SD, standardised; SE, standard error; CI, confidence interval; FDR-adj., false detection rate-adjusted; IADL, independent activities of daily living.

* = p < .05, ** = p < .01, *** = p < .001. n=971

Table 4-2(Cont.). Indirect paths predicting pain at wave 6 and IADL at waves 6 and 7 and their total indirect effects

Total indirect effects	W5 Anxiety → W6 Cognitive ability+W6 Pain → W7 IADL	W4 Education→ W5 Cognitive ability+W5 Anxiety → W6 Pain	W4 Education → W5 Pain+W5 Social deprivation → W6 IADL	W4 Education → W5 Cognitive ability+W5 Social deprivation → W6 IADL	W4 Education → W5 Anxiety+W5 Social deprivation → W6 IADL	W4 Education→ W5 Cognitive ability+W5 Pain → W6 IADL	W4 Education→ W5 Cognitive ability+W5 Anxiety → W6 IADL	W4 Education→ W5 Pain+W5 Anxiety → W6 IADL	W5 Social deprivation → W6 Cognitive ability+W6 Pain → W7 IADL
SD coefficient	0.001	-0.009	-0.002	-0.007	-0.005	-0.008	-0.011	-0.005	0.006
SE	0.002	0.004	0.002	0.004	0.003	0.004	0.004	0.003	0.003
CI lower	-0.003	-0.017	-0.005	-0.014	-0.011	-0.015	-0.019	-0.011	0.001
CI upper	0.004	-0.001	0.002	0.000	0.001	-0.001	-0.003	0.000	0.012
P	0.630	0.032*	0.346	0.040*	0.120	0.031*	0.011*	0.071	0.021*
FDR-adj. P	0.717	0.065	0.479	0.075	0.186	0.065	0.025*	0.125	0.044*

W, wave; SD, standardised; SE, standard error; CI, confidence interval; FDR-adj., false detection rate-adjusted; IADL, independent activities of daily living.

* = p < .05, ** = p < .01, *** = p < .001. n=971

4.4. Discussion

In a group of newly diagnosed osteoarthritis patients, we show that social deprivation before diagnosis predicted poorer cognitive ability after diagnosis. We also identified a link between social deprivation, prior to diagnosis and greater limitations in activities of daily living (as reflected by a higher IADL score) following diagnosis through its effect on reduced cognitive ability assessed early after diagnosis. This suggests that cognitive ability is a potential mechanism underlying the relationship between social deprivation and limitations in activities of daily living. We also demonstrated that higher levels of education before diagnosis might be protective against limitations in activities of daily living following diagnosis via better cognitive ability and lower anxiety.

To our knowledge, no previous study has assessed the interplay of social, cognitive and affective factors before a diagnosis of osteoarthritis and its impact on limitations in activities of daily living and pain severity following diagnosis. Previous longitudinal studies on the relationship between osteoarthritis pain, activities of daily living, deprivation and anxiety mostly included participants with longstanding osteoarthritis, making it hard to generalise their findings to those presenting with early disease (Hawker et al., 2011; Jordan et al., 2008; Machado, Gignac, & Badley, 2008; Peat, Thomas, Handy, & Croft, 2004; Siviero et al., 2020). For example, Hawker et al. (2011)

constructed a path model to test the interrelationships between pain, depression, fatigue and disability over time, in participants with already established osteoarthritis (Hawker et al.) and Harris *et al.* (2013) explored the impact of psychosocial risk factors (mainly perceived stress) on onset of osteoarthritis (Harris et al., 2013). We extend these findings by exploring the mechanisms linking social deprivation and educational attainment with limitations in activities of daily living over time in newly diagnosed osteoarthritis patients taking into account the role of cognitive ability and anxiety in this relationship. While many aspects of social context can be difficult or impossible to change, understanding the pathways linking such aspects to health outcomes at the early disease stages also allows us to identify areas for health interventions.

The link between higher social deprivation and greater limitations in activities of daily living and the role of lower cognitive ability as a mediating mechanism may be explained by cognitive reserve theory. This theory suggests that continued stimulation of cognitive abilities, due to social engagement inhibits atrophy in these abilities and promotes better executive functioning in old age and is supported by previous research in humans (Carlson et al., 2015; Carlson et al., 2008; Fried et al., 2013; Stern, 2012). At the same time, both higher pain levels and impaired cognitive ability might further reduce social participation and increase loneliness in seniors with and without arthritis (Cacioppo & Hawkley, 2009; James et al., 2018), creating a vicious cycle. It has been shown that impaired cognitive ability is associated

with higher pain levels in arthritis (Gale, Allerhand, & Deary, 2012; James & Ferguson, 2019). Here, we found that pain and cognitive ability may act together early after diagnosis as a mechanism indirectly linking higher social deprivation with greater limitations in activities of daily living. In line with this finding, a previous cross-sectional study found that non-cancer pain and cognitive impairment are independently associated with limitations in IADL and limitations in IADL are even greater when both pain and cognitive impairment are present (Shega et al., 2010). The mechanisms by which pain and impaired cognitive ability interact to exacerbate limitations in activities of daily living are unknown. A number of studies have demonstrated changes in brain morphology and connectivity, such as cerebral atrophy due to grey matter loss and bilateral hippocampal volume loss, in patients with chronic pain including with osteoarthritis (Mutso et al., 2012; Shi, Yuan, Dai, Ma, & Sheng, 2016). It is possible, therefore, that the synergistic effect on limitations in activities of daily living of pain and impaired cognitive ability occurs via alterations in brain morphology.

It has been previously shown that low educational attainment is associated with higher pain levels and limitations in activities of daily living in osteoarthritis (Atlas & Skinner, 2010; Callahan et al., 2011; Cleveland et al., 2013; Hannan et al., 1992; Kiadaliri et al., 2017; Knight et al., 2011; Luong et al., 2012; Thumboo et al., 2002). There is also a link between increased risk of osteoarthritis and less years of education seen in very large genetic studies (Tachmazidou et al., 2019; Zengini et al., 2018). Furthermore, education is an important determinant of

cognitive ability in old age (Olivera, Andreoli, Leist, & Chauvel, 2018). However, the pathways linking these factors in osteoarthritis are unknown. We found that education might be protective against limitations in activities of daily living via improved cognitive ability and lower anxiety. Bidirectional links between cognitive ability and affect have been demonstrated previously in arthritis (Gale et al., 2012; James & Ferguson, 2019). However, we showed that these associations were influenced by previous educational attainment and that their interactions before diagnosis predicted activities vital to independence and self-care after diagnosis. Therefore, managing anxiety and potential problems with cognitive ability early in the disease course has potential clinical benefit in terms of activities of daily living. Cognitive-based interventions, involving the education about pain processing and false beliefs about movement can result in substantial improvements in disability and performance (Koes, van Tulder, van der Windt, & Bouter, 1994; Louw, Diener, Butler, & Puentedura, 2011; Moseley, Nicholas, & Hodges, 2004; Nijs et al., 2014; Pelletier, Higgins, & Bourbonnais, 2015), and could be considered as part of the arsenal of treatment options.

It is important to acknowledge the limitations of this study. The observed limitations in activities of daily living might be due to comorbid conditions, rather than osteoarthritis. However, we controlled for number of chronic conditions in our analyses and adjusted for previous IADL at each wave. Although this study's model controlled to an extent for reverse causality between pain, IADL and cognitive ability, the

model did not include social deprivation and anxiety in waves 6 and 7. Therefore, despite having assessed social deprivation before IADL and before diagnosis, the causality could be reversed and limitations in activities could affect social deprivation. Same-source bias can arise from use of perception-based measures (social deprivation index). However, perceived measures usually serve as good proxies of actual measures, as they are often highly correlated (Curry, Latkin, & Davey-Rothwell, 2008). As we did not have information about osteoarthritis severity, despite controlling on disease duration, respondents may have had different levels of osteoarthritis pathology. We did not differentiate between the joints affected by osteoarthritis, and therefore we cannot exclude the possibility that our results may vary by joint. There is a scale by precision trade-off, which all large general cohorts have – we have a large longitudinal sample that is cross-cultural and representative and is used to address many questions, so the index of pain will, by definition, be general. It allows us to test general principles of cognitive ability, anxiety, pain, and deprivation that can be used to generate hypotheses for specific cohorts. There is also a possibility that some of the participants at the prediagnostic stage experience pain, which disrupts their ability to engage in day-to-day physical and cognitive tasks. Nevertheless, diagnosis is a critical time point, as previous studies have suggested that people often seek medical help when pain is severe enough and starts to interfere with meaningful day-to-day tasks (Ayis & Dieppe, 2009; Dieppe & Lohmander, 2005; Dominick, Ahern, Gold, & Heller, 2004; McAlindon, Cooper, Kirwan, &

Dieppe, 1993; Neogi, 2017). In order to test the potential overlap between the social deprivation index and IADL, we first examined correlations between the items in both these instruments and then conducted additional analysis using an index of material deprivation assessed in wave 5 of the SHARE study and compared it with our original results using the social deprivation index. We found no identical items between the two scales and low correlations between the items (Appendix 2.6, p. 278) and we did not observe any major differences in the results between the two indices (Appendices 2.7, p. 279 and 2.8, p. 281), suggesting that our results are not due to conceptual overlap. Although we used self-reported osteoarthritis diagnosis, March and colleagues (March, Schwarz, Carfrae, & Bagge, 1998) have found that self-reported physician diagnosed general arthritis has good congruency with clinically derived diagnoses. For the present study, two main pieces of evidence support that the measure of diagnosis is reliable. First, participants reported a diagnosis consistently at both wave 6 and wave 7. Second, we found that pain medication use increased significantly from wave 5 (before diagnosis) to wave 6 and wave 7 (after diagnosis), but did not differ significantly between the post-diagnostic waves, 6 and 7 (Appendices 2.9, p. 283 and 2.10, 283).

We show that higher social deprivation before osteoarthritis diagnosis is related to greater limitations in activities of daily living, after diagnosis with this effect partly mediated by impaired cognitive ability. We also show that, higher educational attainment before diagnosis may be protective against greater limitations in activity of daily living after

diagnosis via better cognitive ability and lower anxiety before diagnosis. Therefore, improving cognitive ability and managing anxiety may mitigate the associations of social deprivation and low educational attainment with limitations in activities of daily living and may help to promote independence in patients with osteoarthritis.

4.5. Methods

4.5.1. Study population

Data were taken from waves 4 (2011) (Abduladze & Börsch-Supan, 2013; Börsch-Supan, 2020a), 5 (2013) (Börsch-Supan, 2020b; Börsch-Supan & Malter, 2015), 6 (2015) (Börsch-Supan, 2020c; Malter & Börsch-Supan, 2017), and 7 (2017) (Bergmann, Scherpenzeel, & Börsch-Supan, 2019; Börsch-Supan, 2020d) of the SHARE study, a multidisciplinary, cross-national, and longitudinal research project focusing on community-dwelling adults aged 50 or older (Börsch-Supan et al., 2013). The time between the waves was 2 years. We did not use data from waves 1 to 3, because they did not include specific information on osteoarthritis diagnosis (only on arthritis in general), and some of the key mechanistic measures, such as social deprivation and anxiety, were only assessed at wave 5. Detailed information about the entire SHARE project is available at www.share-project.org. SHARE respondents were included in our subsample if: (a) they had participated in wave 4 and did not report a diagnosis of dementia, Alzheimer's disease, senility or Parkinson's disease at waves 4 and 5

(i.e., before the diagnosis of osteoarthritis, see next inclusion criterion); (b) they reported a diagnosis of osteoarthritis at wave 6; (d) they did not report a diagnosis of osteoarthritis at wave 5; (e) they participated in wave 7; (f) continued to report a diagnosis of osteoarthritis at wave 7; and (g) they reported pain at both waves 6 and 7 (i.e., after the diagnosis of osteoarthritis) (Figure 4-2). Of the 971 participants included in our analysis, none had missing data on measurements of pain and cognitive ability. Osteoarthritis diagnosis was assessed at all waves with the following question: “Has a doctor ever told you that you had/Do you currently have: Osteoarthritis? (With this we mean that a doctor has told you that you have this condition, and that you are either currently being treated for or bothered by this condition.)”(Börsch-Supan & Malter, 2015). The participants could have been diagnosed at any time in the period of 2 years after the wave 5 (2013) interview and before the wave 6 (2015) interview. Following diagnosis (wave 6), 479 (49.33%), 243 (25.03%), 424 (43.67%), 400 (41.19%), 56 (5.77%) participants reported to have back pain, hip pain, knee pain, pain in other joints, pain all over, respectively and 178 (18.33%), 524 (53.96%) and 269 (27.70%) reported mild, moderate and severe pain intensity levels, respectively.

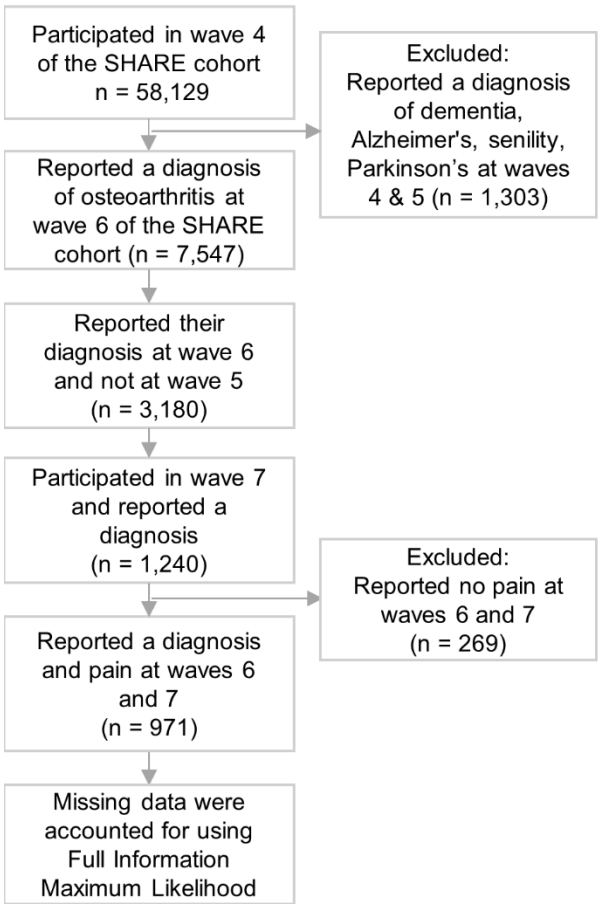


Figure 4-2. Flow chart of the assignment of respondents to the subsample analysed in this study.

4.5.2. Ethics approval and consent to participate

The SHARE data collection procedures are subject to continuous ethics review by responsible ethics committees (University of Mannheim and Max Planck Society, Germany) as well as national ethics committees in participating countries. The reviews refer to all aspects of the project, from study design to informed consent. The reviews confirm the project agrees with international ethical standards, such as the Respect Code of Practice for Socio-Economic Research and the 'Declaration of Helsinki'.

For the purpose of the present study, appropriate permission was obtained for use of the SHARE data after successful application. The applicant is required to agree that use of the SHARE data will be purely for scientific purposes, provide appropriate information regarding their scientific alignment and agree to the SHARE Conditions of Use [http://www.share-project.org/data-access/share-conditions-of-use.html?L=]. Following successful application, the data can be accessed for free upon registration. The secondary analysis performed here required no additional ethical approvals.

4.5.3. Variables

4.5.3.1. Cognitive ability

Cognitive ability was assessed at all waves and was based on multiple items: (1) immediate recall (participants were presented a list of 10 words and asked to repeat the words immediately; range = 0-10), (2) delayed recall (participants were asked for the list of 10 words after a delay; range = 0-10), (3) subtraction (participants were asked to mentally solve a subtraction task; range = 0-5), and (4) verbal fluency (participants were asked to produce as many animal names as possible within a given period of time; range = 0-100). We created a joint scale based on all items with a total score range of 0 to 125 (Cronbach's alpha = 0.80, for wave 5 and Cronbach's alpha = 0.76, for wave 6). The higher the score, the higher the participant's cognitive ability (Portellano-Ortiz & Conde-Sala, 2018). These cognitive measures are validated and are widely used in many large longitudinal surveys (Portellano-Ortiz & Conde-Sala, 2018; Rogers, Banks, Nazroo, &

Steptoe, 2015). Our calculated Cronbach's alphas demonstrated good internal consistency reliability of the cognitive ability scale. We decided not to use a standardised score of cognitive ability due to the risks associated with their use in the analysis of longitudinal data (Little, 2013; Moeller, 2015).

4.5.3.2. Pain

We constructed a pain score from two questions asked at all waves of the survey. Participants were asked whether they had been troubled by pain (yes/no). Those who replied positively were then asked to rate how bad their pain was most of the time (either mild, moderate or severe). The two variables were added to create a single score ranging from 1 (not troubled by pain) to 4 (troubled by severe pain), representing whether respondents were troubled by pain and how severe it was. This verbal rating scale used in the SHARE and other large longitudinal studies has been used widely in the pain literature (Breivik et al., 2008). Comparative and clinical trial studies (Averbuch & Katzper, 2004; Bashir et al., 2013; Bellamy, Campbell, & Syrotuik, 1999; Bolognese, Schnitzer, & Ehrich, 2003) have assessed the validity and reliability of this pain measure. Measurements using this verbal rating scale (0-4) are highly correlated with measurements using visual analogue or a numeric rating scale, and has similar precision for discriminating between treatments in osteoarthritis patients (Averbuch & Katzper, 2004; Bolognese et al., 2003).

4.5.3.3. Anxiety

In SHARE wave 5, five items were used to measure the severity of anxiety that was taken from the Beck Anxiety Inventory (Beck & Steer, 1988). The respondents were asked about anxiety symptoms ("I had fear of the worst happening", "I was nervous", and "I had a fear of dying", "I felt my hands trembling", "I felt faint") they experienced in the last 7 days and answer on a four point Likert scale ("never", "hardly ever", "some of the time", "most of the time"). We created a single anxiety scale by summing the scores of all five items to obtain an overall score, with higher scores indicating higher anxiety (Cronbach's alpha = 0.69). These items have extensively been used in large longitudinal studies like the Health and Retirement Study and to be valid for use in older populations (Smith et al., 2013). Our calculated Cronbach's alpha was .69.

4.5.3.4. Social deprivation index

A social deprivation index was provided in wave 5 of SHARE that was generated and validated by Michał *et al.* for the purpose of the SHARE study (Michał, Mateusz, & Monika, 2015). Briefly, this index was constructed using a battery of 15 questions related to participation in everyday life, social activities, and the quality of the neighbourhood following Chakravarty and D'Ambrosio (2006) and Levitas et al. (2007). In order to combine different social deprivation items into a single index, Michał *et al.* computed the weight of each item based on a regression of the chosen items on the reported values of life satisfaction (Michał et al., 2015). The most important elements of the index, those with the

highest weight are: feeling left out of things, not feeling part of the neighbourhood, having no helpful people in the local area and waiting too long to see a doctor (Michał et al., 2015).

4.5.3.5. Instrumental activities of daily living (IADL)

A modified version of IADL was used in SHARE (Lawton & Brody, 1969; Mehrbrodt, Gruber, & Wagner, 2017). IADL included seven activities in wave 5: “using a map to get around in a strange place”, “preparing a hot meal”, “shopping for groceries”, “making telephone calls”, “taking medications”, “doing work around the house or garden” and “managing money” with a total score ranging from 0 to 7. Two more items were added in waves 6 and 7: “leaving the house independently and accessing transportation services”, and “doing personal laundry”, resulting in nine items in total (score: 0-9). To account for the change in questions between waves 5, 6 and 7, we excluded from analysis the two extra items added at waves 6 and 7. A higher score indicates more difficulty with these activities (Cronbach’s alpha = 0.71, 0.80 and 0.86, for waves 5, 6 and 7, respectively).

4.5.3.6. Additional variables

Other variables included age (>50 y.o.), gender, education measured with the International Standard Classification of Education (ISCED-97) (United Nations Educational & Organization., 2006), body mass index (BMI), smoking status (Currently smoking, Ex-smoker, Never smoked, and No response), alcohol consumption (How many drinks in 3 months), physical inactivity (Never moderate or vigorous activity and

Other), number of chronic diseases (0-9), and marital status (Married and living together, Divorced, Widowed, and Other).

4.5.4. Statistical analysis

Modelling was performed using R version 4.0.1. For path analysis the 'lavaan' package was used (Rosseel, 2012). The missing mechanism of the SHARE data is assumed to be missing at random, and the level of dropout in this subsample is small (17.1%), thus, missing data were handled using full information maximum likelihood (FIML). We conducted sensitivity analyses using multiple imputations (m = 40). No significant differences between the two methods for handling missing data were found (Appendices 2.12, p. 285 and 2.13, p. 287).

Path analysis was run using the pain and cognitive ability measurements at waves 5 and 6 and IADL at waves 5, 6 and 7. The path model was constructed such that the variance in IADL at wave x controlled for the variance in IADL at wave x-1, that is: the model looks at the variance in IADL once variance due to prior IADL is controlled. The same applies to pain and cognitive ability. Social deprivation and anxiety were added as predictor variables in the model at wave 5. The path model was adjusted for age, sex, education level, number of chronic diseases, BMI and alcohol consumption at baseline. Confounding variables were chosen due to their clinical relevance to chronic pain and cognitive ability based on findings from other studies (Attal et al., 2014). Correlational analysis between the study variables was done to determine which variables were entered in the final model (see Appendix 2.5, p. 277, for correlational analyses). The standard

errors were computed using the Delta method (Greene, 2003). Our results were adjusted for multiple testing using false discovery rate (FDR<0.05).

Model fit was assessed using the CFI, the TLI, the RMSEA and the SRMR. To determine acceptable fit we used the cut-off criteria proposed by Hu and Bentler (Hu & Bentler, 1999), who recommended that an RMSEA lower than .06 and CFI and TLI greater than .95.

4.6. Acknowledgements

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4.7. Competing interests

The authors declare that they have no competing interests.

4.7. Availability of data and materials

This paper uses data from SHARE Waves 4, 5, 6 and 7 (DOIs: 10.6103/SHARE.w4.710, 10.6103/SHARE.w5.710, 10.6103/SHARE.w6.710, 10.6103/SHARE.w7.710), see Börsch-Supan, et al. [40] for methodological details.

4.8. Funding

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Chapter 5: A longitudinal analysis of the impact of the COVID-19 pandemic on pain, anxiety and quality of life of chronic pain patients

5.1. Abstract

Background: Although chronic pain patients may be more susceptible to the potential downstream consequences of COVID-19, evidence is scarce. Factors, such as anxiety, social isolation and reduced physical activity, which may be enhanced during lockdown, may exacerbate pain and contribute to poor quality of life.

Methods: We analysed data from a sample of chronic pain patients (N = 305) from the Investigating Musculoskeletal Health and Wellbeing cohort thrice during the pandemic (May 2020, July 2020, September 2020). Using repeated measures ANOVAs and Kruskal-Wallis test tests, we explored changes in the levels of pain, affect, social isolation, physical activity and quality of life at the various time points.

Results: Anxiety levels were increased in May and September, whereas pain levels were unaffected. The highest levels of social isolation and depression occurred in the early stages of lockdown but declined thereafter. Quality of life improved by September when lockdown rules were eased. The at least a bit anxious group had heightened pain in May and September compared to the not at all anxious group. Doing at least an hour of exercise per day (on a typical day) was a protective factor against increased anxiety during lockdown.

Conclusion: Overall, mental health and quality of life was worse, especially at the early stages of lockdown. Being anxious contributed to heightened pain in May and September, whereas regular exercise was a protective factor against anxiety during lockdown.

5.2. Introduction

The worldwide spread of the new zoonotic virus SARS-COV2 (COVID-19) quickly became a public health crisis that affected the physical health of the general population with more than 5 million deaths globally (Organization—WHO, 2019). This led to the first lockdown in the UK to be announced on 23rd of March, with people being urged to stay at home for months. Specific concerns have been raised for chronic pain patients, who may be more susceptible to the potential downstream consequences of COVID-19 (Clauw et al., 2020).

Apart from people surviving infection, the wider community experienced psychological, social, and economic effects that can be triggers of pain (Clauw et al., 2020; Kouraki, Bast, Ferguson, & Valdes, 2022). For example, anxiety can facilitate pain (Burston et al., 2019). Although some studies showed mental health to deteriorate during the early weeks of the pandemic (Pierce et al., 2020; Shanahan et al., 2020), other evidence suggests a decrease in anxiety and depressive symptoms during lockdown (Fancourt, Steptoe, & Bu, 2021). Furthermore, social isolation and loneliness, which may have been increased due to lockdown, may exacerbate pain (Kouraki et al., 2022;

Smith, 2017; Smith, Dainty, Williamson, & Martin, 2019). Similarly, it is well established that lack of physical activity or exercise can have negative health-related effects in chronic musculoskeletal pain patients (Booth et al., 2017).

Most studies examining the impact of the COVID-19 pandemic in chronic pain patients have been cross-sectional in design. To the best of our knowledge, no previous study compared the levels of pain, affect, social isolation and quality of life measures during the pandemic in chronic musculoskeletal pain patients. Therefore, we will explore how pain, affect, social isolation and quality of life measures changed from May to September 2020 in a cohort of chronic pain patients.

5.3. Methods

5.3.1. Study population

Participants were recruited from those consenting to further contact in the Investigating Musculoskeletal Health and Wellbeing (IMH&W) study (clinicaltrials.gov NCT03696134) (Millar et al., 2020). IMH&W participants were recruited from GP surgeries, or a database of participants in previous studies undertaken by the University of Nottingham who had consented to be contacted with information about future research. The survey was conducted online and all participants provided informed consent.

Of the 305 participants included in the final analysis, 28.9% reported having arthritis/osteoarthritis after they were asked: “In general, do you have any health problems that require you to limit your activities?”

5.3.2. Variables

Pain. Pain severity was measured at all waves of the survey. Participants were asked how much pain or discomfort they had experienced over the past week from a scale of 0 (no pain) to 10 (worst imaginable pain). This numeric rating scale has been used widely in the pain literature (Williamson & Hoggart, 2005). Comparative and clinical trial studies have demonstrated validity and reliability of this pain measure (Ferreira-Valente, Pais-Ribeiro, & Jensen, 2011) (Hjermstad et al., 2011) (Karcioglu, Topacoglu, Dikme, & Dikme, 2018).

COVID-related anxiety, depression and general anxiety. Anxiety was assessed at all study waves with two questions. The respondents were asked how anxious or worried they are feeling today and answer on a three point Likert scale (“not anxious or worried”, “a bit anxious or worried”, and “a lot anxious or worried”). In addition, participants were asked whether they suffer from anxiety and how much has this condition been affected by the COVID-19 crisis in the last 7 days from a scale of 0 (not at all) to 10 (extremely anxious). They could skip to the next question if this question did not apply to them. Depression was assessed with the following question: “If you suffer from depression, how much has this condition been affected by the COVID-19 crisis in the last 7 days”. Participants answered on a scale from 0 (not at all) to

10 (extremely depressed) or skipped to the next question if this question did not apply to them.

Social isolation and loneliness. Social isolation was assessed at all study waves with the question: “How socially isolated have you felt in the last 7 days”. Participants answered on a scale from 0 (not socially isolated) to 10 (extremely socially isolated). Loneliness was also assessed at all study waves with the question: “How often do you feel lonely”. Participants answered on a five point Likert scale (“often/always”, “some of the time”, “occasionally”, “hardly ever”, “never”).

Physical activity and exercise changed due to COVID. Physical activity was assessed at all waves with the following question: “How many hours per day of moderately intense exercise you do”. Participants answered on a Likert scale (“Less than 1 hour”, “1-2 hours”, “more than 2 hours”, “NA”). Participants were then asked whether the COVID-19 crisis has affected their ability to exercise (Yes/No).

Quality of life and limitations in activities of daily living. At all three time points, participants were asked to rate their quality of life over the last 7 days from a scale of 0 (worst imaginable) to 10 (best imaginable). They were also asked at all waves whether they had problems with doing usual activities (for example, work, study, housework, family or leisure activities) today and answered on a three point Likert scale (“no problems”, “some problems”, “a lot of problems”).

5.3.3. Statistical analysis

Participants with no missing data on anxiety and pain at all three waves were included in the analysis. Descriptive statistics were calculated for each variable at all time-points. Paired t-tests were used to compare study variables between dropouts and engaged participants in a sensitivity check and a Spearman's test was conducted for correlations between variables for all three waves. One-way and two-way repeated measures ANOVA tests (or Kruskal-Wallis where appropriate) and post-hoc tests (Tukey's and Šídák's multiple comparisons tests) were used to test differences between psychosocial variables, pain severity, physical activity/exercise and quality of life measures across study waves. Analyses were conducted in R and graphs produced using GraphPad Prism version 9.3.1.

5.4. Results

5.4.1. Descriptives and correlations

In total, 842 chronic pain patients participated in the survey and, of those, 305 (36.22 %) participated at all three time points (see Appendix 3.2, p. 301, for study flow chart). We compared the baseline demographic characteristics and baseline measures of mental health and pain of the engaged compared to dropouts (Table 5-1, Table 5-2 and Table 5-3). Despite the engaged tended to be older and less likely to have a job, compared with the dropouts, no other major differences

were observed between the two groups. Correlations between some of the main variables are shown in Appendix 3.3, p. 302.

Table 5-1. Demographic characteristics of engaged (n=305) compared to dropouts (n=537).

	Engaged (n = 305)	**Dropouts (n = 537)**
Age categories, %		
40-49	3.93	8.94
50-59	10.16	17.13
60-69	31.15	22.35
70+	51.80	46.00
n	305	537
Gender (women/men) %	56.39/43.61	59.78/40.22
n	305	537
Paid Employment	20.94/79.06	36.36/63.64
n	234	440

Table 5-2. Comparisons of baseline measures of mental health and pain between engaged and dropouts with unpaired T-tests

	Engaged (n = 305)	**Dropouts (n = 537)**	
	Mean (SD)	**Mean (SD)**	**P**
COVID Concerned (0-10)	4.49 (2.89)	4.36 (2.89)	0.51
n	303	528	
QoL (0-10)	6.46 (1.88)	6.53 (1.84)	0.60
n	296	533	
Social isolation (0-10)	4.75 (3.29)	4.67 (3.16)	0.72
n	300	533	
Depression (0-10)	1.96 (2.84)	1.66 (2.58)	0.25
n	156	282	
Anxiety (0-10)	2.25 (2.82)	2.07 (2.70)	0.49
n	169	298	
Pain (0-10)	3.06 (2.60)	2.83 (2.66)	0.22
n	301	527	

Table 5-3. Comparison of mental health, general health and behavioural measures between engaged and dropouts

	Engaged (n = 305)	Dropouts (n = 537)
Feeling anxious, % (n)		
Yes	4.97 (15)	4.49 (24)
A bit anxious or worried	45.03 (136)	46.92 (251)
No	50.00 (151)	48.60 (260)
n	302	535
Feeling depressed, % (n)		
Yes	3.30 (10)	2.80 (15)
A bit sad or unhappy	31.02 (94)	30.84 (165)
No	65.68 (199)	66.36 (355)
n	303	535
How often feeling lonely, % (n)		
Never	31.35 (95)	27.85 (149)
Hardly ever	36.96 (112)	33.64 (180)
Some of the time	12.87 (39)	16.45 (88)
Occasionally	15.18 (46)	18.50 (99)
Often/always	3.63 (11)	3.55 (19)
n	303	535
Mental Health, % (n)		
Excellent	24.34 (74)	27.72 (148)
Very good	37.50 (114)	33.52 (179)
Good	26.64 (81)	24.91 (133)
Fair	10.86 (33)	11.99 (64)
Poor	0.66 (2)	1.87 (10)
n	304	534
Health limits activities (Yes/No), %	51.18/48.82	40.04/59.96
n	297	527
Walking, %		
No problem walking	54.64 (165)	64.23 (343)
Some problem walking	36.75 (111)	25.46 (136)
A lot problems walking	8.61 (26)	10.30 (55)
n	302	534
Overall Diet, %		
Excellent	10.20 (31)	12.52 (67)
Very good	37.17 (113)	36.82 (197)
Good	42.76 (130)	36.26 (194)
Fair	8.22 (25)	12.90 (69)
Poor	1.64 (5)	1.50 (8)
n	304	535
Exercise, %		
1-2 hours	37.17 (113)	42.54 (228)
less than an hour	41.78 (127)	36.94 (198)
more than 2 hours	11.51 (35)	13.43 (72)
n	304	536
Exercise COVID (Yes/No), %	40.46/59.54	36.21/63.79
n	304	533

5.4.2. Differences in mental health and pain over time

A one-way repeated measures ANOVA was run on a sample of 305 participants to determine if there were differences in mental health levels at three time points. The results showed that mental health had changed over this period, with particularly poor mental health in May, when lockdown was implemented. More specifically, anxiety was worst in May and September, whereas depression and isolation were worst in May and improved afterwards: COVID-related anxiety, $F (1.956, 436.2) = 7.345$, $p = 0.0008$; COVID-related depression, $F (1.924, 406.0) = 10.37$, $p < 0.01$; social isolation, $F (1.982, 611.5) = 51.54$, $p < 0.001$ (Figure 5-1). We did not observe differences in loneliness during this period.

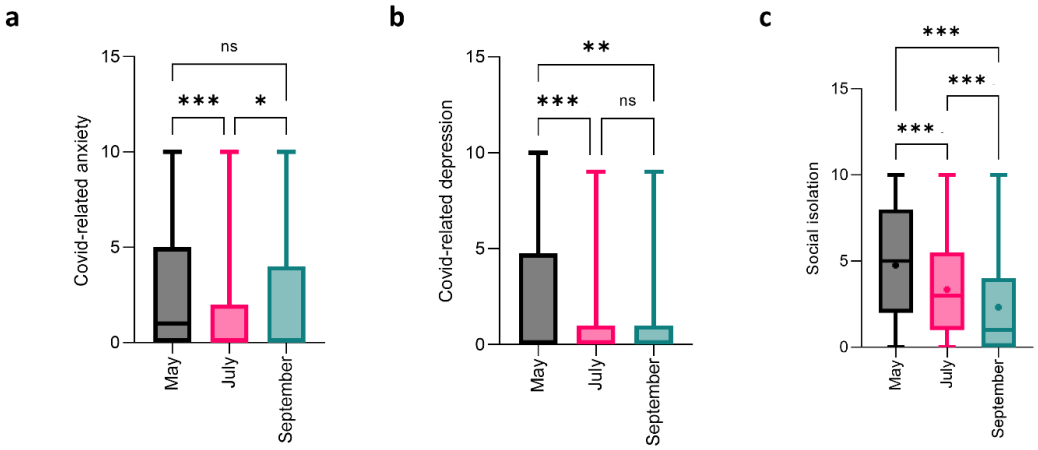


Figure 5-1. Boxplots of mental health variables during the different time points; May, July and September: a. COVID-related anxiety, b. COVID-related depression, and c. social isolation.

** = p < .05, ** = p < .01, *** = p < .0001.*

We did not find a statistically significant difference in pain levels between May, July and September (F (1.959, 607.2) < 1). We showed that pain was higher for the anxious group in May and September by performing a repeated measures two-way ANOVA and Sídák's multiple comparisons test with adjustments for multiple testing (F (2, 618) = 138.2, p < .0001) (Figure 5-2).

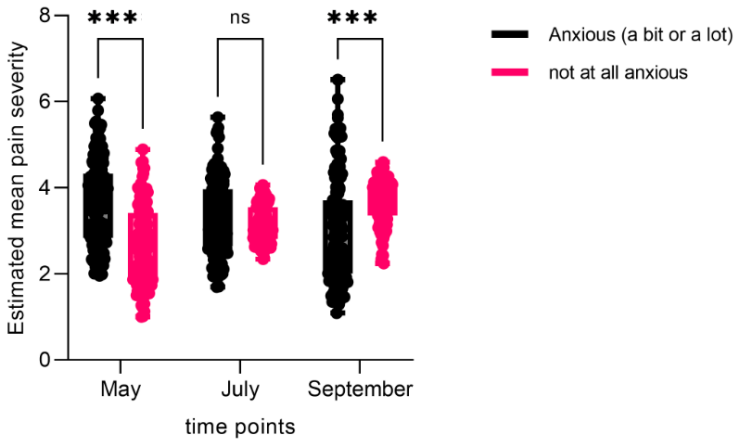


Figure 5-2. Boxplots of mean pain severity by anxiety categories: at least a bit anxious (n = 156) versus not at all anxious (n = 155) during the different time points. All data points presented.

We observed differences in physical activity from May to September (Figure 5-3 and Appendix 3.4, p. 303). Participants reported being more physically active in July and September compared to May when lockdown rules were still in place (F (1.986, 590.8) = 46.76, p < 0.001). Participants reported that COVID-19 significantly affected their exercise compared to pre-pandemic with more participants reporting being affected in May compared to September (F (1.971, 613.9) = 5.621, p = 0.004).

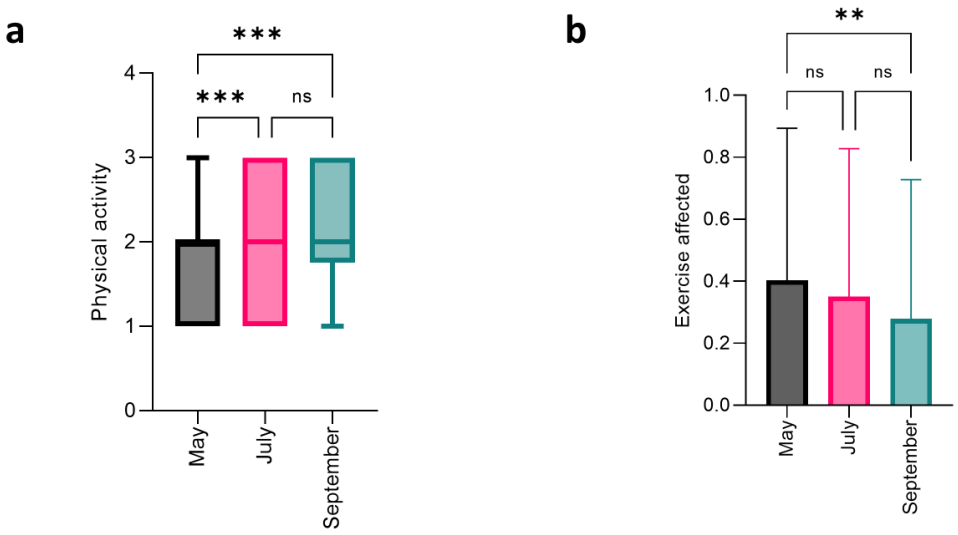


Figure 5-3. Boxplots of physical activity and exercise being affected due to COVID during the different time points; May, July and September.

** = p < .05, ** = p < .01, *** = p < .0001.*

We found that pain was higher for the least active group (less than an hour per day of exercise), Q(2) = 25.68, p < 0.001 (Figure 5-4). Exercise for more than 2 hours per day was a protective factor against anxiety at all time points, Q(2) = 13.30, p < 0.01 (Figure 5-5).

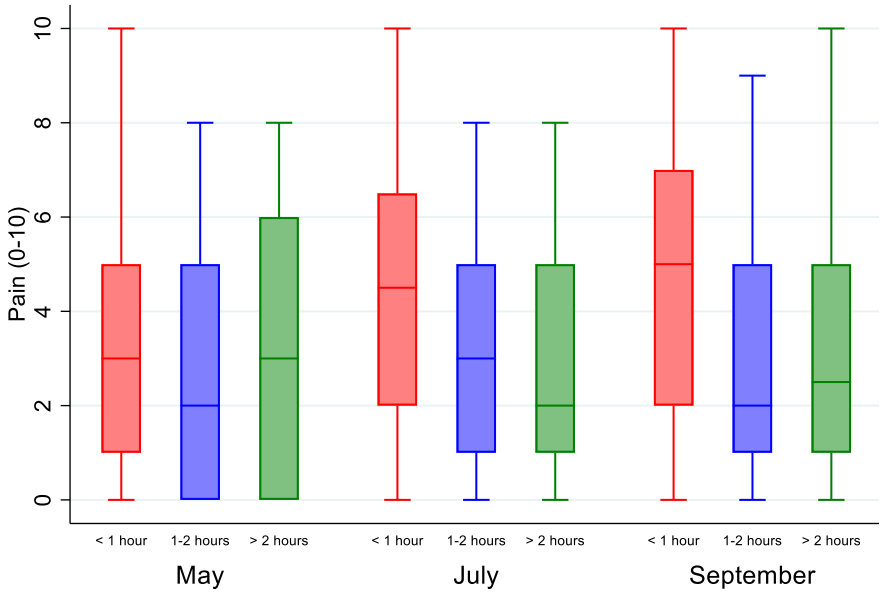


Figure 5-4. Pain by physical activity levels at the various time points: May, July and September 2020

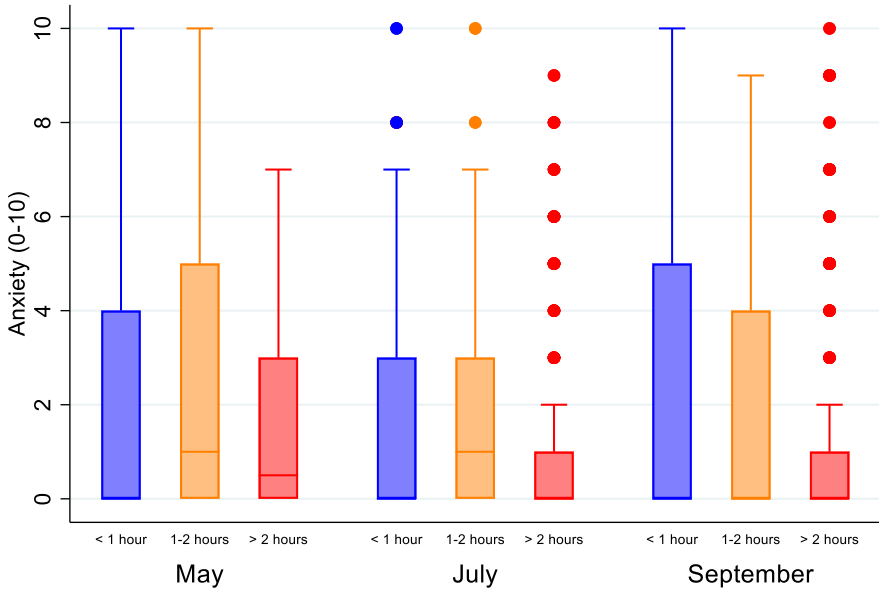


Figure 5-5. Anxiety by physical activity levels at the various time points: May, July and September 2020

Participants reported better quality of life (QoL) in September compared to May ($F(1.975, 606.3) = 4.783$, $p = 0.009$) (Figure 5-6). We did not observe significant differences in limitations in activities of daily living.

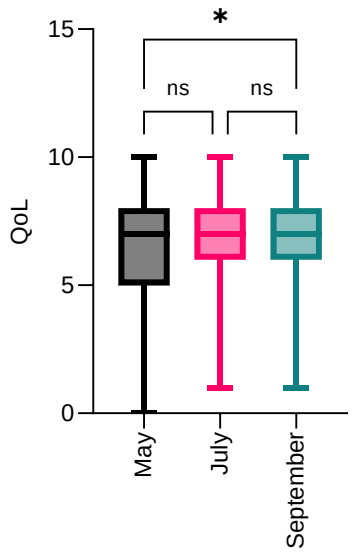


Figure 5-6. Boxplots of Quality of Life (QoL) during the different time points; May, July and September.

** = p < .05, ** = p < .01, *** = p < .0001.*

5.5. Discussion

In this study, we explored changes in the levels of affect and pain during the COVID-19 pandemic in a group of chronic pain patients. We showed that the highest levels of anxiety occurred in the early stages of lockdown and in September, while social isolation and depression were worst in May but declined thereafter. Although we did not find significant changes in pain levels, higher anxiety contributed to worse pain in May and September. Pain and anxiety levels were higher for the least active group (less than an hour per day of exercise), while QoL

improved in September when lockdown rules were eased as compared to May.

To understand these results, it is important to track how coronavirus mitigation procedures in the UK differed across our study waves. We found anxiety symptoms to be worst during a time of high COVID-19 restrictions (May 2020), while mental health recovered to some extent with lockdown restrictions easing in July and September. These results echoed findings from the UCL COVID-19 Social Study (Fancourt et al., 2021). It is possible that the improvements in mental health seen over this period reflect a process of adaptation similar to findings from studies of incarceration (Porter & DeMarco, 2019). Furthermore, the increase in online and home-based leisure activities and the widespread use of virtual and digital communication during the pandemic might have helped to ease the burden of lockdown (Brooks et al., 2020). We observed an increase in COVID-related anxiety in September, which could be attributed to the fact that the second wave of the pandemic had begun with cases starting to go up.

In contrast, for pain symptoms no changes were observed from May to September. Evidence from previous studies is mixed and there are no conclusive data on the impact of the pandemic and lockdown measures on chronic pain patients. Cross-sectional studies in chronic pain patients found an overall increase in pain severity, compared with recalled pain severity before the pandemic (Hruschak et al., 2021; Nieto, Pardo, Sora, Feliu-Soler, & Luciano, 2020; Pagé et al., 2021). A preliminary prospective study in chronic temporomandibular patients

found increased chronic facial pain severity compared to pre-pandemic levels (Asquini, Bianchi, Borromeo, Locatelli, & Falla, 2021). In contrast, a study in chronic migraine patients reported a clinical improvement for 47.1% of patients (Schiano di Cola et al., 2021). A longitudinal study in chronic pain patients found an increase in self-perceived pain during lockdown but not in measured pain intensity compared to pre-pandemic levels (Fallon et al., 2020). It is reasonable to think that a global stressful event such as the COVID-19 pandemic may lead to exacerbation of chronic pain. Nevertheless, a community-based study in the United States that surveyed people before and after the 9/11 terrorist attacks did not find any changes in pain complains (Raphael, Natelson, Janal, & Nayak, 2002), which is in line with our findings. It is more likely that individual stressors (Pillow, Zautra, & Sandler, 1996) or a reduction of daily hassles and challenges (e.g., with working from home) (Schiano di Cola et al., 2021) may have contributed to an increase or a decrease in pain severity, mainly self-reported, observed in previous studies. Indeed, we find that the group of patients that reported higher baseline anxiety (May) experienced increased pain severity in both May and September.

Despite the lockdown rules being eased in July and September, some people, such as the older groups (about 83% of our cohort is over 60), may be more susceptible to continued social isolation, loneliness and reduced levels of physical activity. Indeed, a recent longitudinal analysis of the impact of the pandemic on the mental health of middle-aged and older adults found loneliness as a predictor of worsening

depressive symptom trajectories (Raina et al., 2021b). We did not observe differences in loneliness over the study waves, but we found differences in social isolation during this period. Participants reported being more physically active in July and September compared to May when lockdown rules were still in place. In addition, they reported that COVID-19 significantly affected their ability to exercise with more participants reporting being affected in May compared to September. This perhaps reflects the easing of restrictions and reopening of exercise facilities from July to September. As we would expect, pain and anxiety was worse for the group that reported exercising for less than an hour per day.

It appears that QoL was worse in May but significantly improved in September compared to May. We did not find differences in limitations in activities of daily living during this period. This could be because although there is pre-pandemic data for the IMH&W cohort, at present we have no access to the pre-pandemic measures for comparison. The short intervals between the waves is another explanation, as limitations may take longer to develop.

This study has some limitations. Although our analysis was longitudinal, at present we have no access to the pre-pandemic data for the cohort, which makes it challenging to examine changes in mental health and pain and can obscure different patterns of change in mental health and pain over time. Furthermore, the final sample analysed is potentially biased towards older people (>49) and people without a job (including pensioners).

Overall, our findings emphasise the importance of exercise as a protective factor against anxiety during lockdowns, and highlight that groups already at risk for worse mental health before the pandemic have remained at risk, especially at the early stages of lockdown.

Chapter 6: Different genes may be involved in distal and local sensitisation: a genome-wide gene-based association study and meta-analysis

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6.1. Abstract

Background: Neuropathic pain symptoms and signs of increased pain sensitisation in osteoarthritis (OA) patients may explain persistent pain after total joint replacement (TJR). Therefore, identifying genetic markers associated with pain sensitisation and neuropathic-like pain phenotypes could be clinically important in identifying targets for early intervention.

Methods: We performed a genome-wide gene-based association study (GWGAS) using pressure pain detection thresholds (PPTs) from distal pain-free site (anterior tibia), a measure of distal sensitisation, and from proximal pain-affected site (lateral joint line), a measure of local sensitisation, in 320 knee OA participants from the Knee Pain and related health in the Community (KPIC) cohort. We next performed

gene-based fixed-effects meta-analysis of PPTs and a neuropathic-like pain phenotype using genome-wide association study (GWAS) data from KPIC and from an independent cohort of 613 post-TJR participants, respectively.

Results: The most significant genes associated with distal and local sensitisation were OR5B3 and BRDT, respectively. We also found previously identified neuropathic pain-associated genes – KCNA1, MTOR, ADORA1, and SCN3B – associated with PPT at the anterior tibia and an inflammatory pain gene – PTAFR – associated with PPT at the lateral joint line. Meta-analysis results of anterior tibia and neuropathic-like pain phenotypes revealed genes associated with bone morphogenesis, neuro-inflammation, obesity, type 2 diabetes, cardiovascular disease and cognitive function.

Conclusions: Overall, our results suggest that different biological processes might be involved in distal and local sensitisation, and common genetic mechanisms might be implicated in distal sensitisation and neuropathic-like pain. Future studies are needed to replicate these findings.

6.2. Introduction

It is estimated that pain is not adequately controlled in 4 of 10 people that suffer from knee osteoarthritis (OA) (Neogi, 2013). Knee pain hugely contributes to disability (Guccione et al., 1994; March & Bagga, 2004). Although OA pain has traditionally been associated with

peripheral pain mechanisms due to joint damage and inflammation, the presence of radiographic joint pathology does not always explain the severity of pain (Finan et al., 2013; Valdes et al., 2012). Many people with OA suffer from pain that they describe as numbness, electric shocks, or a burning, itching sensation that can initiate spontaneously and in the absence of a painful stimulus, suggestive of a neuropathic source (Cavalli, Mammana, Nicoletti, Bramanti, & Mazzon, 2019; den Boer et al., 2019). A role of the central nervous system (CNS) in augmenting nociceptive processing has been described in OA, which is known as pain sensitisation and displays similar characteristics to neuropathic pain (Chappell et al., 2009; Petersen, Arendt-Nielsen, Simonsen, Wilder-Smith, & Laursen, 2015; Soni et al., 2019).

Quantitative sensory testing (QST) can be used to quantify alterations in pain sensitivity. Reduced pressure pain detection thresholds (PPTs) at the affected site may indicate the presence of peripheral sensitisation, while reduced PPTs at a distal pain-free side is suggestive of altered pain processing in the CNS and central pain augmentation (Croft, Jordan, & Jinks, 2005; Graven-Nielsen & Arendt-Nielsen, 2002; Suokas et al., 2012b). Magnetic resonance imaging-detected inflammation in knee OA, and not severity of radiographic features, is associated with the development and worsening of local pressure pain sensitivity in the knee (Neogi et al., 2016), suggesting that inflammation is a potential mechanism underlying local sensitisation. Neuropathic pain symptoms on the painDETECT questionnaire have been associated with signs of central sensitisation

on QST, such as associations with PPTs at sites distant from the affected joints, suggesting that painDETECT may reflect central pain processing in patients with knee OA (Hochman, Davis, Elkayam, Gagliese, & Hawker, 2013; Moreton et al., 2015; Moss, Benson, Will, & Wright, 2017).

Genetic variants implicated in pain sensitivity have arisen from candidate gene studies. These included amino acid change variants in the catechol-O-methyltransferase (COMT) (van Meurs et al., 2009), the voltage gated sodium channel Nav1.7 (SCN9A) (Reimann et al., 2010) and the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) (Valdes et al., 2011a). However, there is a gap in our knowledge regarding the genes and molecular pathways influencing pain sensitisation in knee OA. Evidence indicates that signs of increased pain sensitisation might be a barrier to treatment response. Indeed, widespread hyperalgesia assessed by PPTs predicts poor outcomes to arthroplasty (Lundblad, Kreicbergs, & Jansson, 2008; Petersen et al., 2016; Rakel et al., 2012; Wylde et al., 2015). Furthermore, knee OA patients with neuropathic pain symptoms identified using the PainDETECT questionnaire are most at risk of developing chronic postoperative pain after total knee replacement (Kurien et al., 2018). Therefore, understanding the underlying mechanisms involved in pain sensitisation and neuropathic-like pain may promote better profiling and diagnosis of pain patients and development of new regimes for mechanism-based therapy. Thus, we completed two GWASs with PPTs from distal and affected sites to

identify genetic variants for distal and local sensitisation, respectively, using data from the Knee Pain and related health In the Community (KPIC) cohort (Fernandes et al., 2017). We then completed a genome-wide gene-based association analysis (GWGAS) and gene-set analysis on both PPT phenotypes separately to explore the genes and underlying genetic mechanisms of distal and local sensitisation in OA pain. Using existing GWAS data from an independent Nottingham cohort study of neuropathic-like pain (Warner, Walsh, et al., 2017), we performed a GWGAS to identify genes related to the neuropathic-like pain phenotype. GWGAS may have higher power to identify the causal variants of complex diseases compared to GWAS, because it considers the joint effect of several single nucleotide polymorphisms within a single gene (Chung et al., 2019; Kang et al., 2013). As pain sensitisation is suggested to display similar characteristics to neuropathic-like pain, we performed two separate gene-based meta-analyses by combining our neuropathic-like pain GWGAS findings with our findings from distal and local sensitisation GWGAS to identify genes common in distal sensitisation and neuropathic-like pain, as well as local sensitisation and neuropathic-like pain, respectively.

6.3. Methods

The study design is outlined in Figure 6-1. We used data from two independent study cohorts. Participants from both cohorts had similar demographic characteristics (age, sex, and BMI). We used a three-stage design for identification of any potential associations between

genetic variants and two pain sensitivity phenotypes (distal sensitisation and local sensitisation), as well as genes common in pain sensitivity and neuropathic-like pain phenotypes. First, two GWASs with PPTs from both distal and affected sites were run to identify genetic variants for distal and local sensitisation, respectively, using data from the KPIC cohort. Second, three GWGASs were run to identify the genes associated with the two pain sensitivity phenotypes and a neuropathic pain-like phenotype using the GWASs outputs from KPIC and existing GWAS data from a Nottingham cohort. Third, we conducted two meta-analyses of our GWGAS findings to identify genes common in pain sensitisation (distal and local) and neuropathic-like pain.

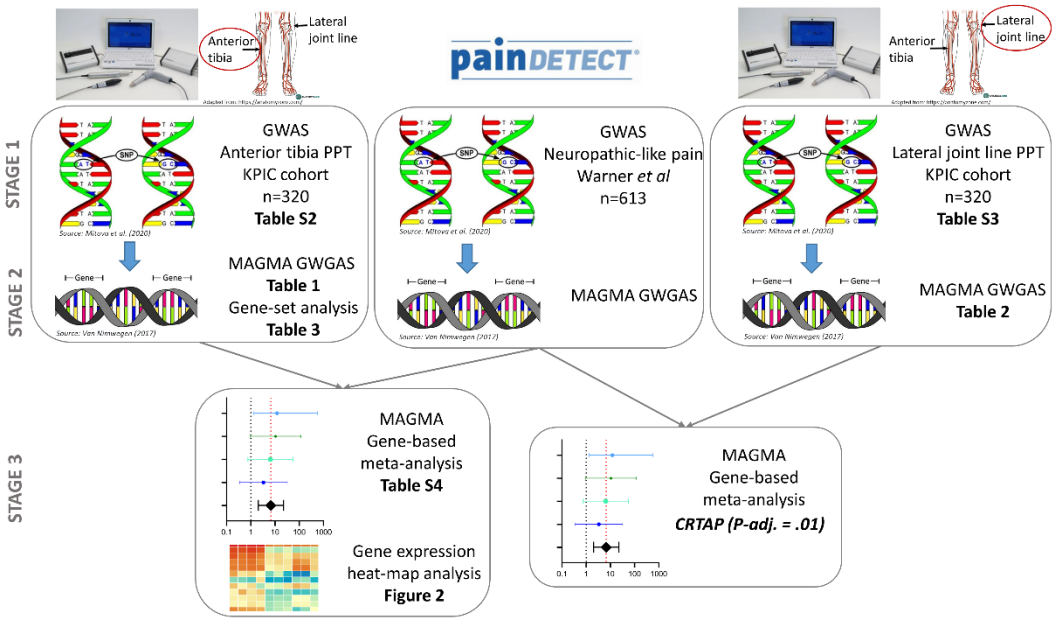


Figure 6-1. Study design.

A three-stage design for identification of associations between genetic variants and two pain sensitivity phenotypes: distal sensitisation [anterior tibia pressure pain threshold (PPT)] and local sensitisation (lateral joint line PPT), as well as genes common in pain sensitivity and neuropathic-like pain (painDETECT questionnaire) phenotypes. First,

two GWASs with PPTs from both distal and affected sites were run to identify genetic variants for distal and local sensitisation, respectively, using data from the KPIC cohort (Appendices 4.4 and 4.5). Second, three GWGASs and gene-set analyses were run to identify the genes associated with the two pain sensitivity phenotypes and a neuropathic pain-like phenotype using the GWASs outputs from KPIC and existing GWAS data from a Nottingham cohort (Tables 6-1, 6-2, and 6-3). Third, two meta-analyses of our GWGAS findings were conducted to identify genes common in pain sensitisation (distal and local) and neuropathic-like pain (Appendix 4.6) and a heat map analysis of the common genes identified from the distal sensitisation/neuropathic-like pain meta-analysis was performed (Figure 6-2).

6.3.1. Participants

6.3.1.1. KPIC cohort

Baseline data from a subgroup of the KPIC (n=320) that undertook clinical assessments including knee radiographs, knee ultrasound, quantitative sensory testing, muscle strength, balance, gait analysis and biomarker sampling and for which we had genetic information and no missing data on anterior tibia and lateral joint line PPTs was used (Fernandes et al., 2017) (Appendix 4.3, p. 306). KPIC is a prospective community-based cohort of men and women aged 40 years or over within the East Midlands region (UK).

6.3.1.2. Nottingham post-total joint replacement (TJR) cohort

Participants were recruited post-total joint replacement (TJR) for OA (n=613) from secondary care in the Nottinghamshire area (Warner, Walsh, et al., 2017). Demographic characteristics of this cohort are presented in Appendix 4.3, p. 306.

6.3.2. Phenotypes

6.3.2.1. Neuropathic-like pain

This phenotype has been described extensively elsewhere (Warner, van Meurs, et al., 2017). Briefly, neuropathic-like pain was measured with the painDETECT (Freynhagen, Baron, Gockel, & Tölle, 2006c), a self-report seven-item questionnaire with scores ranging from 0 to 39 developed to discriminate between nociceptive and neuropathic pain. The questionnaire asks about the intensity, pattern and quality of their knee pain, e.g. persistent with slight fluctuations, burning, tingling or sudden, with Likert scale and tick-box questions. Individuals were classified as having possible or probable neuropathic-like pain if they scored > 12 according to the validated cut-offs for diagnosis by Freynhagen, Baron, Gockel, and Tölle (2006a) making up 109 possible or probable neuropathic pain cases versus 504 controls (Warner, van Meurs, et al., 2017). The dichotomised value for the classification of participants to neuropathic-like pain versus controls was used for the GWAS and the results from this analysis can be found elsewhere (Warner, van Meurs, et al., 2017).

6.3.2.2. Pressure pain detection thresholds (PPT)

PPTs (in kPa) were measured in triplicate with an algometer (Somedic AB, Sweden) that is connected to a computer (HP ProBook 4520 s). Pressure was administered manually at a progressively increasing rate (standardised rate set at 30 kPa/s) by a trained researcher through the algometer, a circular rubber coated pressure probe (1 cm^2). Subjects

were instructed to push a button when the sensation changed from pressure to pain and the algometer was immediately taken off the skin. Among other areas, PPT was applied to the lateral tibiofemoral joint line adjacent to the patellar ligament of the index knee and the anterior tibia 5 cm distal to the tibial tuberosity of the contralateral knee (Fernandes et al., 2017). PPT has been shown to be a reproducible measure of nerve sensitivity throughout localized, distal and remote sites and it is part of QST, which is used to quantify pain perception. PPT at sites away from the affected joint has been interpreted as an index of central sensitisation (i.e. increased pain perception in areas away from the knee) (Arendt-Nielsen et al., 2010; Pavlaković & Petzke, 2010). PPT at an affected joint may indicate a combination of peripheral and central sensitisation. Results from a systematic review and meta-analysis revealed that people with OA have lower PPTs both at the affected joint and at areas away from the joint when compared to controls (Suokas et al., 2012b). The mean of PPT values for each site were computed from all three PPT rounds for analysis. Log transformation resulted in resulted in more symmetrical and less skewed distributions of the PPT values. Therefore, the log-transformed variables of PPT values were used for all further analyses.

6.3.3. GWAS

For all cohort study participants, genomic DNA was extracted from peripheral blood leukocytes. Genotype data were analysed using the Illumina Global screening array Inc Basic BioIT (Illumina, San Diego, USA). Quality Control (QC) checks and genotyping were done both at

the sample and single nucleotide polymorphism (SNP) level and have been described in detail elsewhere (Warner, Walsh, et al., 2017; Zeggini et al., 2012). PLINK software (version 1.07) was used to analyse GWAS data from this array (Purcell et al., 2007). Two GWASs were conducted to identify associated SNPs and genes with the two distal and local sensitisation phenotypes, respectively. The GWAS output contains information about the SNPs' location in the genome, a regression coefficient beta (β) and, a test statistic that indicate the level of association of the genetic variants with the phenotype along with a P value to determine significance.

6.3.4. Functional annotation

To gain insights into the functions of the identified genes, we next tested the probability of these genes to map into specific biological pathways as defined by the Kyoto Encyclopaedia of Genes and Genomes (KEGG) or Reactome databases using the Database for Annotation, Visualisation and Integrated Discovery (DAVID) (Dennis et al., 2003) and Reactome online platforms, respectively. The gene list was comprised of genes corresponding to all SNPs with a P-value of $P<0.0001$ in the GWAS analysis.

6.3.5. Gene-based association analysis

Subsequently, our GWAS results were used for GWGAS using Multi-marker Analysis of GenoMic Annotation (MAGMA) (de Leeuw, Mooij, Heskes, & Posthuma, 2015). MAGMA takes as input the P-values derived from the GWAS and annotates SNPs to known protein-coding genes to estimate aggregate associations based on all SNPs in a gene

(linkage disequilibrium) accounting for multi-marker effects, by applying multiple regression analyses. MAGMA then uses Fisher's test to compute P-values to test association between a gene and the phenotype and does not assign a positive or negative coefficient to a gene-based association. It differs from functional annotation as it provides a statistical gene-based test, whereas functional annotation methods map individually significant SNPs to genes. The 1,000 Genomes Project (phase 1, release 3) was used as a reference panel to calculate linkage disequilibrium between genomic variants (Auton et al., 2015). A gene-based analysis was performed for each phenotype using the results from our GWAS for local and distal sensitisation and existing GWAS data for neuropathic-like pain. The NCBI 37.3 build was used to obtain the SNPs that were attributed to each gene (de Leeuw et al., 2015). A total of 14,428, 14,428 and 14,722 protein-coding genes were assessed for an association with the distal sensitisation phenotype, the local sensitisation phenotype and the neuropathic-like pain phenotype, respectively. Benjamin – Hochberg (B–H) correction was used to determine significance.

6.3.6. Gene-based meta-analysis

Our GWGAS resulted in different genes for anterior tibia PPT and lateral joint line PPT. For anterior tibia PPT, we identified genes that have been previously related to central mechanisms of pain, whereas one of the lateral joint line PPT genes found, has been previously associated with peripheral pain mechanisms. Accordingly, we conducted two separate fixed-effects gene meta-analyses combining

our GWGAS neuropathic-like pain results with GWGAS results from anterior tibia PPT and lateral joint line PPT to identify genes common in neuropathic-like pain and distal sensitisation as well as neuropathic-like pain and local sensitisation, respectively. The Z-scores for each gene across the two cohorts, KPIC and Nottingham post-TJR, were combined using the weighted-Z method. According to this method, the weights are computed as the inverse of the squared standard error of the effect size estimate for each cohort, resulting in different weights for each cohort according to their power (Lipták, 1958; Whitlock, 2005). B–H correction was used to determine significance.

6.3.7. Pathway over-representation analysis

Significant results from the GWGAS were entered in FUMA for gene-set/GWAS catalogue over-representation analysis with MAGMA (de Leeuw et al., 2015; Watanabe, Taskesen, van Bochoven, & Posthuma, 2017). We identified statistically significant overrepresented pathways for the anterior tibia PPT phenotype after adjusting for false detection rate (FDR) with B–H correction method.

6.4. Results

6.4.1. Stage 1: GWAS

The 10 ‘top-hits’ (i.e. genes with highest p-values that survived correction for multiple testing) of the adjusted GWAS on anterior tibia and lateral joint line PPT phenotypes are shown in Appendices 4.4, p. 307 and 4.5, p. 308, respectively. The total genotyping rate was 0.99

with 700,078 variants passing filtering and QC and each tested for association with both phenotypes. The genomic inflation factor lambda (based on median chi-square) was low for both phenotypes (λ = 1.07, for anterior tibia PPT and λ = 1.00, for lateral joint line PPT). After adjusting for multiple testing, 554,759 SNPs remained in the analysis. The results of GWAS on neuropathic-like pain are described elsewhere (Warner, van Meurs, et al., 2017).

6.4.2. Stage 2: Gene-based association analysis

As knee OA is a complex, polygenic disease, we next decided to perform gene-based association analysis, which has been suggested to be more powerful at unravelling associations compared to GWAS, as it accounts for the correlations among SNPs within a single gene (Chung et al., 2019). The 'top' results, in terms of statistical significance (i.e. genes with highest p-values that survived correction for multiple testing) as well as biological relevance, of the gene-association analyses on anterior tibia and lateral tibiofemoral joint line PPT can be seen in Table 6-1 and Table 6-2, respectively. Several of the genes identified for anterior tibia PPT phenotype are traditional pain-related genes and members of the "sensory perception" gene set according to the Gene Ontology definition (GO: 0007600) (i.e. *KCNA1*, *MTOR*, *ADORA1*, *SCN3B*). For lateral joint line PPT, we found a single gene, *PTAFR*, member of the "sensory perception" gene set.

We then performed a gene-set analysis to identify pathways related to PPT phenotypes using FDR-adjusted significant genes as identified from the GWGAS. We found significant overrepresented pathways for

anterior tibia PPT after B-H correction (Table 6-3). Enrichment was seen in several metabolic related pathways.

Table 6-1. The results of interest from the MAGMA gene-based association analysis of anterior tibia PPT phenotype

Gene symbol	Chr.	Start position	Stop position	SNPs	Z statistic	Gene P-value	Adjusted P*
OR5B3	11	58169938	58170882	1	9.48	1.22E-21	1.75E-17
WNT9A	1	228109165	228135676	1	8.59	4.36E-18	3.14E-14
DNAH7	6	29364416	29365448	2	7.04	9.47E-13	2.48E-09
IFNGR1	6	139456249	139501946	2	7.01	1.04E-12	2.48E-09
HECA	11	64863587	64879332	2	7.06	1.20E-12	2.48E-09
AIM2	1	159028790	159046685	3	6.98	1.48E-12	2.68E-09
POLR3E	16	22308696	22346424	3	5.69	6.41E-09	4.87E-06
IL10RA	11	117857106	117872198	3	5.33	4.95E-08	2.98E-05
HRH2	5	175084847	175136239	6	5.04	2.37E-07	9.23E-05
ROPN1	3	123687862	123711017	1	4.96	3.46E-07	1.28E-04
KCTD11	18	13218729	13652753	93	4.78	8.70E-07	2.68E-04
CCDC14	3	123616152	123680255	4	4.78	8.96E-07	2.69E-04
KCNA1	12	5019073	5027422	1	4.32	7.98E-06	1.52E-03
MTOR	1	11166588	11322614	6	3.98	3.40E-05	4.42E-03
HRH1	10	38383264	38412280	1	3.90	4.84E-05	5.82E-03
CACNB2	10	18429373	18830688	85	3.89	5.10E-05	6.08E-03
TNFAIP3	6	138663930	138790381	11	3.71	1.05E-04	1.02E-02
ADORA1	1	203096833	203136533	9	3.39	3.56E-04	2.17E-02
SCN3B	11	123499895	123525315	6	3.20	6.87E-04	3.43E-02
TAOK3	12	118587606	118810750	8	3.08	1.04E-03	4.51E-02
CACNA2D3	3	54156620	55108584	156	3.03	1.23E-03	4.97E-02

**adjusted P values after applying B–H correction to control for multiple testing FDR*

Table 6-2. The results of interest from the MAGMA gene-based association analysis of lateral joint line PPT phenotype

Gene symbol	Chr.	Start position	Stop position	SNPs	Z statistic	Gene P-value	Adjusted P*
BRDT	1	92414928	92479985	4	6.92	2.24E-12	3.23E-08
RFX6	6	117198376	117253326	7	6.59	2.19E-11	1.05E-07
CRTAP	3	33155450	33189265	8	6.11	4.84E-10	1.74E-06
SUOX	12	56391043	56399309	2	6.06	6.73E-10	1.94E-06
CHD3	17	7788096	7816075	2	5.48	2.15E-08	5.15E-05
CRAT	9	131857073	131873070	2	5.18	1.10E-07	1.97E-04
IKZF4	12	56401268	56432219	5	4.92	4.31E-07	6.90E-04
TMEM26	10	63166401	63213208	5	4.36	6.53E-06	8.95E-03
IARS	9	94972489	95056038	1	4.35	6.83E-06	8.95E-03
PTAFR	1	28473677	28520447	1	4.02	2.89E-05	2.78E-02
C12orf10	12	53693132	53700965	1	3.94	4.09E-05	3.10E-02
DAPK1	9	90112601	90323566	4	3.85	6.01E-05	4.33E-02

**adjusted P values after applying B–H correction to control for multiple testing FDR*

Table 6-3. Gene-set analysis for anterior tibia PPT MAGMA FDR-adjusted significant genes. The results of pathway analysis showing overrepresented pathways, before and after B-H correction

GeneSet	Systolic blood pressure	Obesity-related traits	Night sleep phenotypes	Amyotrophic lateral sclerosis (sporadic)	Coronary artery calcified atherosclerotic plaque score in type 2 diabetes	Systemic juvenile idiopathic arthritis	Body mass index	Hand grip strength
N	793	756	538	164	26	34	1365	156
n	26	25	20	11	5	5	31	9
P-value	1.88E-07	2.77E-07	7.48E-07	9.94E-07	5.65E-06	2.24E-05	2.65E-05	3.21E-05
adjusted P	2.52E-04	2.52E-04	4.51E-04	4.51E-04	2.05E-03	6.77E-03	6.88E-03	7.28E-03
Genes	CPSF3L, NME7, ADORA1, WNT9A, GPR137B, CACNB2, SYNPO2L, TCF7L2, NOX4, SYT1, MYCBP2, HOXB7, INSR, RGL3, SLC8A1, COBLL1, TNS1, COL4A4, JPH2, ITPR1, HRH1, FGD5, CTNNB1, MITF, ADRB2, TBXAS1	CSF1, AIM2, CACNB2, SLC29A3, NAV2, NOX4, IL10RA, ANO2, SYT1, FAM155A, SAMD4A, MAX, FAM189A1, CEP152, SLC8A1, MACROD2, ITPR1, NCEH1, TLL1, LRFN2, GRIK2, NXPH1, AUTS2, TBXAS1, HR	SPTA1, WNT9A, LRIG3, SAMD4A, AEN, PMP22, ZNF830, RTTN, ZNF486, LRP1B, UBOX5, TASP1, DTD1, CACNA2D3, LRTM1, GALNTL6, NADK2, RANBP3L, EPB41L4A, HRH2	FANK1, RYR3, ANKRD29, RNF165, MACROD2, MYO18B, TAPT1, TLL1, LRFN2, AUTS2, TBXAS1	LRP1B, MAGI1, ZBTB49, OSBPL3, MTSS1	KLF17, WWOX, ZNF521, TAPT1, COL12A1	MTOR, FAM63A, CACNB2, TCF7L2, PNLIPRP3, STK33, SFSWAP, POLR2M, CHTF18, CYLD, MYO19, CDC27, POP4, LRP1B, COBLL1, COL4A4, MACROD2, ENTPD6, TAF4, CACNA2D3, COL25A1, FGF2, TLL1, LRFN2, COL19A1, IFNGR1, RGS17, SBDS, AUTS2, TSGA13, DMD	TCF7L2, SYT1, SAMD4A, SLC8A1, DNER, ITPR1, CADPS, LRFN2, AUTS2

6.4.3. Stage 3: Gene-based meta-analysis

We then conducted a fixed-effects gene meta-analysis of anterior tibia PPT and neuropathic-like pain in both cohorts (KPIC and Nottingham post-TJR) and performed heat map analysis (Appendix 4.6, p. 309 and Figure 6-2, respectively). Results from the meta-analysis revealed 'top-hits' (i.e. genes with highest p-values that survived correction for multiple testing) in various genes related to bone morphogenesis and neuro-inflammation, including *WNT9A*, *POLR3E*, *AIM2*, *HECA*, and *IFNGR1* (Appendix 4.6, p. 309). We visualised tissue specific expression patterns based on GTEx v6 RNA-seq data (Consortium, 2015) for each significant gene as an interactive heat map plot (Figure 6-2).

We also performed a fixed-effects gene meta-analysis of lateral joint line PPT and neuropathic-like pain in both cohorts, which yielded a single significant gene, *CRTAP* at chromosome 3 (Z=4.83, p-adjusted=0.01, after applying B–H correction).

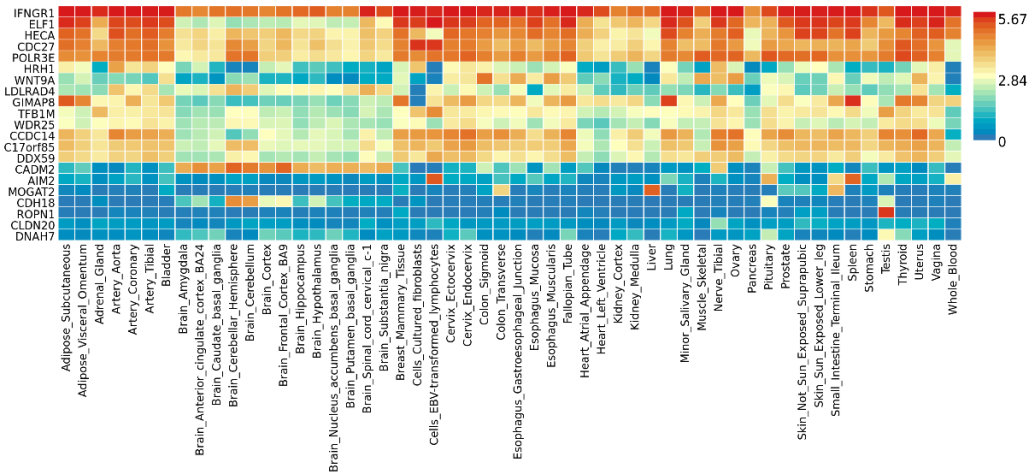


Figure 6-2. Gene expression heat-map plot of significant genes from the meta-analysis of anterior tibia PPT and neuropathic-like pain in both cohorts.

6.5. Discussion

In this study, we used a GWGAS approach to identify novel genes for pain sensitisation in knee OA. The most significant genes associated with distal and local sensitisation were *OR5B3* and *BRDT,* respectively. Additionally, we identified several traditional pain-related genes, *KCNA1*, *MTOR*, *ADORA1*, and *SCN3B*, for anterior tibia PPT phenotype and *PTAFR* for lateral joint line PPT phenotype. Gene-set analysis for anterior tibia PPT revealed enrichment of metabolic-related pathways. We found genes involved in bone morphogenesis (*CRTAP, WNT9A*), inflammation and neuro-inflammation (*POLR3E*, *AIM2*, *IFNGR1*), metabolic disease and obesity (*AIM2*, *DNAH7*, *CADM2*), and cognitive function (*CRTAP*, *CCDC14*) from meta-analysis of distal sensitisation and neuropathic-like pain traits in the two cohorts. A single gene, *CRTAP*, was significant after meta-analysis of local sensitisation and neuropathic-like pain traits.

The most significant gene associated with distal sensitisation was *OR5B3. OR5B3* is an olfactory receptor gene involved in the production of G-protein coupled transmembrane receptors, which enable the detection and transmission of olfactory stimuli. A recent study revealed that knockdown of cathepsin S (CTSS) upregulates almost all olfactory receptor family genes, and vice versa, in cell overexpressing CTSS and an increase of the expression level of olfactory receptor 5B3 protein was observed when cells were treated with an CTSS inhibitor (Chen et al., 2021). It has been shown that release of CTSS from microglial cells causes neuropathic pain (Clark & Malcangio, 2012) that could be reversed by intraspinal injection of CTSS inhibitor. Consistent with our findings, these results suggest a potential mechanistic role of *OR5B3* in neuropathic pain. *BRDT* was the most significant gene associated with local sensitisation and is a member of the bromodomain and extra-terminal (BET) family of proteins. BET proteins bind to acetylated lysine residues in the histones of nucleosomal chromatin and function either as co-activators or co-repressors of gene expression (Filippakopoulos & Knapp, 2014), where they regulate the expression of key oncogenes, anti-apoptotic proteins and many immunity-associated genes and pathways (Wang, Wu, Tang, & Kang, 2021).

Genes previously associated with neuropathic pain and signs of central sensitisation – *KCNA1*, *MTOR*, *ADORA1*, and *SCN3B* – were identified from GWGAS of anterior tibia PPT, while an inflammatory pain gene – *PTAFR* – was found from GWGAS of lateral joint line. These findings are consistent with a role of joint damage and inflammation in local

sensitisation, as well as altered pain processing in the CNS in distal sensitisation, and thus are biologically plausible. Specifically, *KCNA1* encodes for the potassium voltage-gated channel subfamily A member 1, which is a key player in the perception of mechanical stimuli (Hao et al., 2013). *ADORA1* that codes for adenosine A_1 receptor is suggested to have anti-nociceptive properties, following surgery (Gan & Habib, 2007), and to reduce thermal hyperalgesia and mechanical allodynia in animal models of neuropathic pain (Gong et al., 2010; Wu et al., 2005). *MTOR* that codes for the mammalian target of rapamycin has a role in inflammatory- and opioid- induced hyperalgesia (Xu et al., 2014; Xu et al., 2011) and acts as a regulator of neuroplasticity in the CNS (Hoeffer & Klann, 2010; Jaworski & Sheng, 2006). *SCN3B* encodes for the b3 subunit of voltage-gated sodium channel (Na_v) (Morgan et al., 2000), and is implicated in neuropathic pain (Casula et al., 2004; Lopez-Santiago et al., 2006; Pertin et al., 2005). Finally, *PTAFR* encodes for a member of the G-protein coupled receptor 1 family of proteins for platelet-activating factor, and is involved in the perception and maintenance of neuropathic pain by regulating the production of pro-inflammatory cytokines in the dorsal root ganglion (Okubo et al., 2012; Shindou et al., 2017; Tsuda, Tozaki-Saitoh, & Inoue, 2011).

The results of pathway analysis for anterior tibia PPT revealed overrepresented pathways associated with systolic blood pressure, obesity-related traits, night sleep phenotypes, amyotrophic lateral sclerosis, type 2 diabetes, systemic juvenile idiopathic arthritis, body mass index and hand grip strength. These traits are often comorbid with

OA and have been previously associated with pain from OA (Swain, Sarmanova, Coupland, Doherty, & Zhang, 2020). For instance, cardiovascular disease and OA share similar underlying disease mechanisms (Fernandes & Valdes, 2015). Obesity is a major risk factor for OA and shares common genetic variations with OA (Panoutsopoulou et al., 2014). Disturbed sleep has been associated with increased pain in OA (Doherty & Smith, 1993; Smith, Quartana, Okonkwo, & Nasir, 2009), while pain has been suggested to increase the risk of developing frailty (low hand grip strength) in OA (Valdes & Stocks, 2018; Veronese et al., 2017).

Meta-analysis of distal sensitisation and neuropathic-like pain revealed 'top-hits' in genes involved in bone morphogenesis, *CRTAP* (Morello et al., 2006) and *WNT9A* (Regard, Zhong, Williams, & Yang, 2012). A recent GWAS identified *WNT9A* as a robust novel genetic marker for hand OA (Boer et al., 2021). We identified several inflammation-related genes, *POLR3E* (Chiu, Macmillan, & Chen, 2009), *AIM2* (Hornung et al., 2009), *IFNGR1* (van de Wetering, de Paus, van Dissel, & van de Vosse, 2010), *LDLRAD4* (Nakano et al., 2014) and *HRH1* (Dong et al., 2014). Increased accumulation of pro-inflammatory factors in the joint accompanying synovitis, is highly correlated to OA pain (Berenbaum, 2013). Despite a role of *POLR3E*, *AIM2*, *IFNGR1* and *HRH1* in inflammation, evidence suggests that these genes are also involved in neuroinflammation, neurodegeneration and neuroplasticity, processes related to central pain facilitation and neuropathic pain (Ji, Nackley, Huh, Terrando, & Maixner, 2018a; Latremoliere & Woolf, 2009b; Myers,

Campana, & Shubayev, 2006). For example, *POLR3E* participates in pre-mRNA splicing and transcription and aged mice showed reduced expression of *POLR3E* (Kohman et al., 2011). Improper RNA splicing can result in abnormal translation of RNA and is associated with many age-related diseases including Alzheimer's disease (Meshorer & Soreq, 2002). Furthermore, *AIM2* is involved in neuroinflammation and neurodegeneration (Cox et al., 2015; Wu, Hung, Liu, & Hsueh, 2017), as well as in neuronal plasticity and memory (Chen, Shu, et al., 2019; Wu, Liu, Huang, & Hsueh, 2016). Moreover, deletion of *IFNGR1* results in complete abrogation of neuroinflammation and nigrostriatal degeneration, suggesting a role of this gene in neuroinflammation and neurodegeneration (Strickland et al., 2018). We also identified genes involved in obesity and adiposity, *AIM2* (Gong et al., 2019), *IFNGR1* (Locke et al., 2015), *DNAH7* (Söhle et al., 2012), and *CADM2* (Graff et al., 2017), as well as type 2 diabetes and cardiovascular disease, *DNAH7* (Vujkovic et al., 2020), *DDX59* (van der Harst & Verweij, 2018), *WDR25* (Perry et al., 2014), and *CLDN20* (Heid et al., 2010). Additionally, we found genes associated with inteligence, educational attainment, cognitive performance and psychological traits, *CRTAP* (Hall et al., 2018), *CCDC14* (Hill et al., 2019), *TFB1M, ROPN1* (Lee et al., 2018), *HRH1* (Shan, Bao, & Swaab, 2015), and *CADM2* (Okbay et al., 2016). There is strong epidimiological evidence of a link between cognitive function, depression/anxiety and pain in people with arthritis (James & Ferguson, 2019).

We do not find significant associations with some of the candidate genes previously reported to be associated with a pain phenotype, such as *COMT*, *SCN9A* and *TRPV1* in our GWGAS. This can be partly explained due to different analysis methods. In candidate gene studies, genes are selected *a priori* and only very small regions of the genome are investigated at a time, meaning that important genes may be overlooked using this method (Warner & Valdes, 2017). Furthermore, a lack of reproducibility of SNPs in candidate genes in GWAS meta-analyses has been previously shown. For example, candidate *COMT* SNPs were not reproduced in a GWAS meta-analysis of chronic widespread pain (Peters et al., 2013). In addition, although *COMT* has been extensively studied in relation to pain, results from candidate gene studies are not consistent (Hagen, Pettersen, Stovner, Skorpen, & Zwart, 2006; Hocking et al., 2010; Nicholl et al., 2010). Rare and drastic mutations in the *SCN9A* gene that explain different types of congenital insensitivity to pain have been identified (Cox et al., 2006). Nevertheless, in addition to these rare-causing mutations, it is known that the genetic risk for chronic pain is due to common variations with small effect size (Mogil, 2012). *TRPV1* has been associated with heat pain sensitivity, and thus it is not necessarily implicated in mechanical pain transduction. Indeed, it was shown that TrpV1 neurons are selectively tuned nociceptors that mediate responses to thermal but not mechanical pain (Mishra & Hoon, 2010).

Limitations of the current study include the small sample sizes of the individual cohorts used that are not sufficient to power genome-wide

significant results. We tried to overcome this by running gene-based meta-analysis of the two cohorts. Findings from GWAS are generally prone to type 1 errors (Bacanu, Devlin, & Roeder, 2000; Kozlitina, 2016). This was addressed by adjusting results for FDR with B–H (Benjamini, 2010). Another limitation with the use of GWAS is the possibility of inflated effect sizes (Ioannidis, 2008). We performed GWGAS and gene-set analysis that may have higher power to identify the causal variants of complex diseases, as it takes into consideration the correlations among SNPs within a single gene (Kang et al., 2013). Gene-based tests are designed to identify genes containing multiple risk variants that individually are weakly associated with a univariate trait (Chung et al., 2019). There is a possibility that distal PPTs are reflective of central sensitisation mechanisms but can also reflect features of peripheral (inflammatory) mechanisms and while neuropathic pain is presumed to result from abnormal neuronal activity of the somatosensory nervous system, glial cell dysfunction may also contribute (Ji, Berta, & Nedergaard, 2013). Indeed, distal and local PPTs were correlated in our cohort ($r = 0.72$, $p < .001$) and from our meta-analysis of indices of central sensitisation and neuropathic-like pain, we found some inflammation-related genes. However, these genes are also associated with neuroinflammation, neurodegeneration and neuroplasticity, processes related to central pain processing and neuropathic-like pain (Ji et al., 2018a; Latremoliere & Woolf, 2009b; Myers et al., 2006). In addition, painDETECT scores can also reflect nerve damage (Sumitani et al., 2016) but are also associated with signs

of central sensitisation (e.g. reduced PPTs) (Hochman et al., 2013). Although the majority of the patients in the KPIC cohort had unilateral OA (75.19%), 33 patients had bilateral OA and thus, for these patients the contralateral tibia could be an OA affected area.

To conclude, our results suggest that different biological processes might be involved in distal and local sensitisation, while common genetic mechanisms might be implicated in distal sensitisation and neuropathic-like pain. Further research is needed to confirm these findings and to explore whether other measures of altered pain processing (temporal summation and conditioned pain modulation) demonstrate similar results.

6.6. Acknowledgements

We are grateful to the study participants that took part in these studies.

Chapter 7: Reproducible microbiome composition associations with anxiety are not mediated by short chain fatty acids or indoles

7.1. Abstract

Gut bacteria may influence the brain via microbial metabolites (i.e. short-chain fatty acids (SCFAs) and indoles) or altering levels of neuromodulators (i.e. serotonin, GABA, cortisol and kynurenine). Meta-analysing data from two cohorts (WebEx: n = 37 and Omega 3/Fibre: n = 58) and published literature (n = 60), we aimed to identify bacterial taxa reproducibly associated with anxiety. We further investigated the role of serum levels of SCFAs and indoles on anxiety and the links between anxiety-associated operational taxonomic units (OTUs) and neuromodulators involved in anxiety. Gut microbiota was characterized using 16S rRNA sequencing in stool samples. OTU data were transformed and normalized. Serum metabolites were measured using tandem mass spectrometry. We identified reproducible microbiome associations of anxiety with *Escherichia-Shigella* (fixed-effect beta (95% CI) = 0.28 (0.11, 0.45), False Detection Rate (FDR)-adj. $p = 3 \times 10^{-3}$), *Christensenellaceae R-7 group* (fixed-effect beta (95% CI) = -0.32 (-0.48, -0.15), FDR-adj. $p = 1 \times 10^{-3}$) and *Ruminococcaceae UCG-010* (fixed-effect beta (95% CI) = -0.24 (-0.40, -0.08), FDR- adj. $p = 6 \times 10^{-3}$). We found no significant associations between anxiety and gut-derived metabolites nor between anxiety-associated bacteria and anxiety-related markers after meta-analysis and correcting for multiple testing. Overall, several reproducible anxiety-microbiome associations

were identified that were not mediated by SCFAs, indoles or anxiety-related markers, suggesting other mechanisms are at play. Given the known associations with health for the *Christensenellaceae* genus and the pathogenic nature of *E. Shigella,* our findings suggest that these taxa may be considered as biomarkers of anxiety in humans.

7.2. Introduction

The diverse community of microorganisms that reside in our gut called the microbiota has emerged as an important player in human health and disease (Codagnone et al., 2019). Indeed, knowledge is accumulating of a role of the gut microbiome composition in modulating the gut-brain axis, affecting mood and behaviour (Dinan & Cryan, 2015;2017; Rieder, Wisniewski, Alderman, & Campbell, 2017). Furthermore, microbial metabolites, such as short-chain fatty acids (SCFAs) and indoles, products of the gut microbiome, are emerging as important mediators of the gut-brain communication (McCarville, Chen, Cuevas, Troha, & Ayres, 2020; Roager & Licht, 2018). In addition, gut microorganisms can affect brain and behaviour by producing neurotransmitters and neuromodulators, such as serotonin, γ-aminobutyric acid (GABA), and kynurenine (O'Mahony et al., 2015; Strandwitz et al., 2019). Dysfunction of GABAergic and serotonergic systems and the kynurenine pathway has been implicated in psychiatric disorders like depression and anxiety (Gordon & Hen, 2004; Nemeroff, 2003; Réus et al., 2015). The gut microbiota can also increase cortisol levels (Kamada, Seo, Chen, & Núñez, 2013), which, in turn, can

contribute to hypothalamic–pituitary–adrenal axis dysregulation and promote low-grade chronic central nervous system inflammation, processes linked to the pathophysiology of affective diseases (Keller et al., 2017; Winter, Hart, Charlesworth, & Sharpley, 2018).

The impact of anxiety on the gut-brain axis has been mostly demonstrated in animal models (Molina-Torres et al., 2019). Although animal studies are valuable for investigating fundamental cellular processes, it is crucial to translate their findings in humans. To date, mixed evidence has derived from human studies investigating the anxiety-microbiome link (Chen, Bai, et al., 2019; Jackson et al., 2018; Jiang et al., 2018; Kleiman et al., 2017; Mason et al., 2020; Naudé et al., 2020), which has not allowed the establishment of microbial composition signatures. Identifying consistent microbial profiles of associations with anxiety might enable us to identify critical targets for interventions to prevent and/or alleviate anxiety symptoms and promote brain health.

Preclinical studies have provided extensive evidence of a role of microbial metabolites and neuromodulators in gut-brain communication (O'Mahony et al., 2015; Stilling et al., 2016; Strandwitz et al., 2019). However, direct evidence of a link between these metabolites and changes in the gut microbiota and anxiety levels is lacking in humans, and thus, it is unclear whether specific gut-derived and brain-related metabolites mediate the anxiety-microbiome link. Here, we focused on SCFAs, indoles, and neuromodulators (serotonin, GABA, kynurenine, cortisol), due to their well-studied effects on the gut-brain axis (Kamada

et al., 2013; O'Mahony et al., 2015; Strandwitz et al., 2019). First, we sought to identify consistent microbial signatures of anxiety by conducting a meta-analysis of associations of anxiety levels with microbiome composition using data from two independent cohorts and published literature. Then, we aimed to find reproducible associations of anxiety levels with gut-derived metabolites (SCFAs and indoles), and identify consistent correlations among anxiety-related bacteria (as identified from the meta-analysis) and anxiety-related markers (serotonin, GABA, kynurenine and cortisol) across the two cohorts.

7.3. Methods

The study design is outlined in Appendix 5.1, p. 310. We used data from two study populations with anxiety measures and 16S sequencing carried out with the same pipeline and assessed for association between anxiety level and gut microbiome composition. We further investigated published reports between the taxa identified and anxiety in the literature.

7.3.1. Study population

Data were taken from baseline visits of two independent cohorts, the WebEx and Omega 3/Fibre. WebEx is a knee osteoarthritis cohort of community dwelling individuals (Age: >45 y) recruited from the Nottinghamshire area, for a subset of whom gut microbiome composition was available (Gohir et al., 2019). Omega 3/Fibre is a healthy cohort of participants aged >18 y that were enrolled from the

TwinsUK registry (Vijay, Astbury, Le Roy, Spector, & Valdes, 2021). We only included data from baseline visits in our analysis, as these studies were randomized intervention trials. Participants were included in the final analysis if they had no missing data on anxiety scores and OTUs (n = 37, for WebEx and n = 58, for Omega 3/Fibre). Data were combined with previously published results in meta-analysis (n = 60) (Chen, Bai, et al., 2019).

All participants provided written informed consent. For WebEx, ethical approval was obtained from the Research Ethics Committee (ref:18/EM/0154) and the Health Research Authority (protocol no: 18021) and the trial is registered under the clinicaltrials.gov database (NCT03545048). The Omega 3/Fibre trial was approved by the West Midlands Black Country Research Ethics Committee (18/WM/0066) and is registered under the clinicaltrials.gov database (NCT03442348).

7.3.2. Anxiety measures

In Omega 3/Fibre cohort study, the Hospital Anxiety and Depression Scale Anxiety (HADS-A) subscale (Zigmond & Snaith, 1983) was used to measure severity of anxiety. Participants were scored based on this subscale from 0-21 with higher scores indicating higher anxiety. In the WebEx cohort study, anxiety was measured using a validated item originating from the HADS-A (Akin-Akinyosoye et al., 2021; Akin-Akinyosoye et al., 2020). The item was scored from 1-4 with a higher score indicating higher anxiety. In the study by Chen *et al*., the Hamilton Anxiety Rating Scale and Self-rating Anxiety Scale scores were used to

define the severity of anxiety symptoms, with higher scores indicating higher anxiety levels (Chen, Bai, et al., 2019).

7.3.3. Sample collection

Baseline blood, stool, and anthropometric measures (such as, height and weight) were collected in both cohort studies. Blood samples were collected from participants between 8:30am and 10am during each visit. Participants were instructed to come in a fasted state at least since 9 pm the night before (i.e. minimum fasting time was 11.5 hours). Blood samples were collected using serum separator tubes and were processed within 2–3 hours of collection for separating serum and aliquoted for storage at −80 C until the end of the intervention period.

7.3.4. Gut Microbiome Composition

Faecal sample collection and processing methods were the same in both cohort studies. Faecal samples were collected by the participant at home the night before or on the day of the study visit. Samples were brought to the study visit by the participant or were sent by post using previously provided collection kits and were frozen immediately at −80°C until further processing. Stool DNA extraction was carried out according to Goodrich *et al.* using 100 mg of the stool sample (Goodrich et al., 2014). There was no homogenization prior to this step. Gut microbiome composition was determined by 16S rRNA gene sequencing carried out as previously described (Goodrich et al., 2016; Jackson et al., 2016). Briefly, the V4 region of the 16S rRNA gene was amplified using universal primers 355F (CCAGACTCCTACGGGAGGCAGC) and 806R

(GGACTACHVGGGTWTCTAAT). Amplified DNA was sequenced on the MiSeq platform (Illumina, 300bp paired-end reads). Read filtering and clustering was carried out using the MYcrobiota pipeline. Briefly, chimeric sequences were filtered using the VSEARCH algorithm within Mothur, and reads were clustered into OTUs using closed-reference clustering against the SILVA database v132 based on a 97% similarity. Diversity metrics (Shannon index observed OTUs and Unweighted UniFrac) were calculated by rarefying the OTU table down to 7000 sequences per sample 50 times and taking the average. These analyses were carried out in QIIME2 (v2018.11).

7.3.5. Metabolite analysis

7.3.5.1. Serum short chain fatty acids

Serum SCFAs (i.e. n-butyrate, acetate and propionate) in the WebEx study cohort were measured by Metabolon Inc., Durham, USA using the standardized procedures described previously (Evans et al., 2014; Shin et al., 2014). Serum SCFAs in the Omega 3/Fibre study cohort were measured by the Mass Spectrometry Department, King's College London using in-situ pentafluorobenzylation of the free acid species, followed by gas chromatography-negative-chemical-ionisation mass spectrometry (GC-NCI-MS) determination of the resulting derivatives as described previously (Vijay, Astbury, et al., 2021).

7.3.5.2. Additional metabolites

Indoles, like indole acetic acid (IAA), indole propionic acid (IPA), and indoxyl sulphate (IndSO4) and anxiety-related metabolites (GABA, serotonin, cortisol and kynurenine) were all measured using tandem

mass spectrometry with the Biocrates MxP Qaunt 500 kit (Biocrates Life Science AG, Innsbruck, Austria) (Roberts et al., 2020).

7.3.6. Statistical analysis

All statistical analyses were carried out in R v4.0.3. OTUs with a relative abundance of <0.1% in every sample were removed, and zero inflated relative OTU abundances were inverse normal transformed and normalized before further analyses. Results from each participating cohort were standardized to allow for the different scales. All analyses were adjusted for age, gender and body mass index (BMI) (apart from when combining the WebEx and Omega-3/Fibre with the Chen *et al*. data), and multiple testing. Correction for multiple testing was performed based on the FDR or Bonferroni correction. The $p < .05$ and FDR <0.05 were considered statistically significant in all statistical analyses. Correlations were assessed by Spearman rank correlation analysis. Linear regressions were first carried out independently in both cohorts to identify significant associations between anxiety, gut microbiota composition and serum metabolites along with adjusting for covariates such as age, sex, BMI and multiple testing. The standardized estimates were meta-analysed in WebEx and Omega-3 to produce a combined effect after adjusting for covariates and multiple testing. When combining the WebEx and Omega-3 with the Chen *et al*. data, we did not adjust for control variables to ensure comparability with the paper by Chen *et al*., which reports uncontrolled coefficients (Chen, Bai, et al., 2019). Meta-analysis takes the effect size, standard error and sample size into account to give an overall effect from the different

groups studied. We used fixed-effects inverse-variance models since our cohorts were homogenous having adjusted for age, sex and BMI and having used only baseline observations. Heterogeneity, as measured with the Q statistic, was not significant between the cohorts in all meta-analyses (P>0.05) further supporting the use of fixed-effects models.

7.4. Results

The descriptive characteristics of the studied cohorts are shown in Table 7-1.

Table 7-1. Descriptive characteristics of the two cohorts at baseline

	Omega 3/Fibre **(n = 58)**	**WebEx** **(n = 37)**
	Mean (± SD)	**Mean (± SD)**
Age (y)	65.55 (9.32)	69.51 (8.77)
Men/Women (%)	8/50 (14/86)	12/25 (32/68)
BMI (kg/m^2)	26.57 (4.51)	30.31 (7.26)
Shannon mean	5.83 (0.86)	5.17 (0.91)
Anxiety	5.91 (3.94)[1]	1.44 (0.81)[2]

Abbreviations: BMI: body mass index; SD, standard deviation.

1. HADS-A: 0-7 = Normal, 8-10 = Borderline abnormal (borderline case), 11-21 = Abnormal (case)

2. A validated item from HADS-A subscale: 1-4, with 1 = Never to 4 = Always

We found that several OTUs were significantly associated in one cohort or the other with the same directions of these associations in both cohorts (Appendix 5.2, p. 311).

We conducted a systematic search of the literature to explore if any of these OTUs had been previously associated with anxiety in published cohorts (Appendix 5.1, p. 310). Of these *Escherichia-Shigella, Christensenellaceae R-7 group, Prevotellaceae NK3B31 group, Ruminococcaceae UCG-002* and *Ruminococcus 2* were found to be significantly associated with anxiety severity in Chen, Bai, et al. (2019) (Table 7-2).

Table 7-2. Linear regressions with anxiety and meta-analysis

Bacteria	**Omega 3 /Fibre (n = 58)**		**WebEx (n = 37)**		**Chen et.al, 2019[2] (n = 60)**		**Meta-analysis**		
	Beta	**SE**	**Beta**	**SE**	**ES**	**SE**	**Over all**	**95% CIs**	**FDR-P**
Shannon index[1]	-0.08	0.14	-0.53**	0.15	-	-	-0.29	-0.49, -0.09	0.033
Escherichia-Shigella	0.047	0.17	0.45*	0.15	0.4**	0.12	0.28	0.11, 0.45	0.003
Christensenellaceae R-7 group	-0.36*	0.15	-0.28	0.17	-0.3*	0.12	-0.32	-0.48, -0.15	0.001
Ruminococcaceae UCG-010	-0.38*	0.13	-0.26	0.18	-0.1	0.13	-0.24	-0.40, -0.08	0.006
Ruminococcaceae UCG-002	-0.28*	0.14	-0.088	0.17	-0.23*	0.13	-0.22	-0.38, -0.05	0.009
Prevotellaceae NK3B31 group	-0.45*	0.20	-0.011	0.17	-0.23*	0.13	-0.26	-0.45, -0.07	0.010
Ruminococcus 2	-0.29*	0.14	-0.094	0.17	-0.23*	0.13	-0.22	-0.38, -0.06	0.012

Abbreviations: SE, standard error. FDR, false detection rate. CIs, confidence intervals

1. Adjusted for age, sex and body mass index (BMI)

2. Effect sizes were estimated from a heat-map plot

*p < 0.05, **p < 0.01

We then meta-analysed the estimates of associations with anxiety in both study cohorts and in Chen, Bai, et al. (2019) (Table 7-2). Shannon diversity was negatively associated with anxiety in WebEx and Omega

3/Fibre study cohorts and reached statistical significance after meta-analysis of both cohorts giving a standardized estimate of -0.29 (95% CI: -0.49, -0.09, adj. p = 0.0329) (Figure 7-1.A). Furthermore, we found that the only OTU associated with higher anxiety was *Escherichia-Shigella* (Figure 7-1.B), which resulted in an increase of 0.28 on the standardized measure of anxiety (95% CI: 0.11, 0.45, adj. $p = 3 \times 10^{-3}$). The rest of the OTUs were associated with lower anxiety scores. *Christensenellaceae R-7 group* (Figure 7-1.C) contributed to a decrease of 0.32 on the standardized measure of anxiety (95% CI: -0.48, -0.15, adj. $p = 1 \times 10^{-3}$). *Ruminococcaceae UCG-010* (Figure 7-1.D) was associated with lower anxiety with standardized beta -0.24 (95% CI: -0.40, -0.08, adj. $p = 6 \times 10^{-3}$). *Ruminococcaceae UCG-002* (Figure 7-1.E) was associated with lower anxiety with standardized beta -0.22 (95% CI: -0.38, -0.05, adj. $p = 9 \times 10^{-3}$). *Prevotellaceae NK3B31 group* (Figure 7-1.F) was negatively associated with anxiety with standardized beta of -0.26 (95% CI: -0.45, -0.07, adj. $p = 1 \times 10^{-2}$). *Ruminococcus 2* (Figure 7-1.G) was associated with lower anxiety with standardized beta -0.22 (95% CI: -0.38, -0.06, adj. $p = 1.2 \times 10^{-2}$).

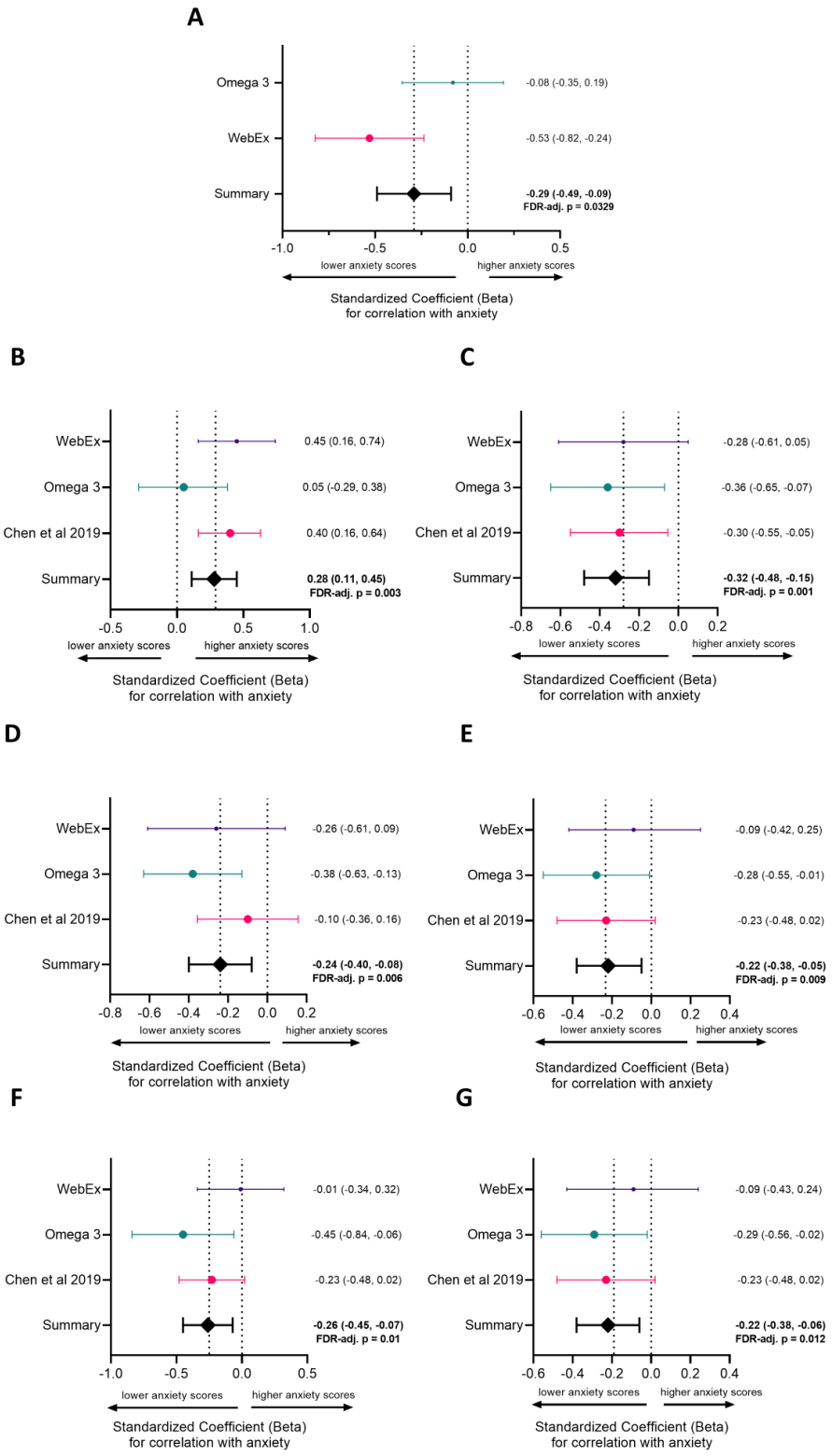


Figure 7-1. Forest plots. *A. Shannon, B. Escherichia-Shigella, C. Christensenellaceae R-7 group, D. Ruminococcaceae UCG-010, E. Ruminococcaceae UCG-002, F. Prevotellaceae NK3B31 group, G. Ruminococcus 2*

The observed associations between anxiety and alpha diversity and anxiety with specific SCFA-producing bacteria, such as *Ruminococcaceae UCG-010, Ruminococcaceae UCG-002* and *Prevotellaceae NK3B31 group,* suggest a potential role for SCFAs or other bacterial metabolites, such as indoles. We therefore investigated the association of these metabolites with anxiety as the outcome but neither of the SCFAs or the indoles were found to be statistically significant (Appendix 5.3, p. 312).

We then meta-analysed the correlation coefficients of all genera that were significantly associated with anxiety and SCFA, indoles in both cohorts and performed heat map analysis (Figure 7-2). We also tested for associations between the anxiety-associated bacteria and metabolites known in the literature to correlate positively or negatively with anxiety such as cortisol, GABA, serotonin and kynurenine. No association between any of the bacterial or anxiety-related metabolites and the anxiety-associated bacteria remained significant after correcting for multiple testing although we observed nominal associations between *Escherichia-Shigella* and IAA, *Christensenellaceae R-7 group* and kynurenine, and *Ruminococcaceae UCG-010* and IAA. The strongest association was between *Escherichia-Shigella* and IAA with a standardized correlation coefficient of -0.28 (adj. p = 0.064).

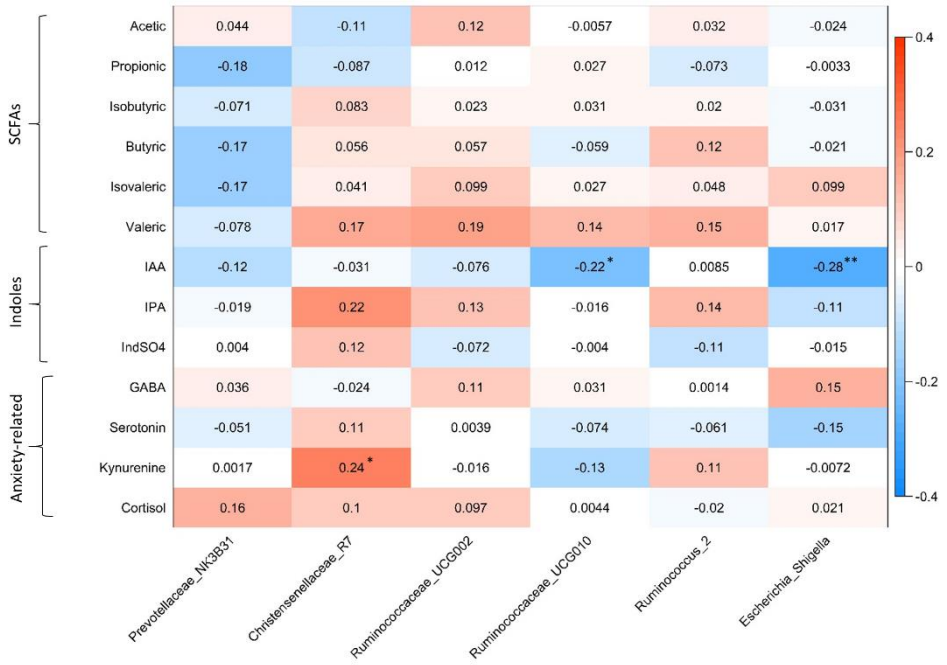


Figure 7-2. Heat map showing overall correlation coefficients of OTUs clustered at genus level with gut-related (SCFAs and indoles) and anxiety-related metabolites.

*Red and blue squares indicate positive and negative correlations, respectively, and the intensities of the colors are proportional to the degree of correlation. No association reached statistical significance after correction for multiple testing. Abbreviations: IAA: Indole-3-acetic acid, IPA: 3-indolepropionic acid, IndSO4: Indoxyl sulfate, GABA: γ-aminobutyric acid. *p < 0.05, **p < 0.01 for nominal associations.*

We then constructed a correlation network of associations between anxiety and OTUs, anxiety and gut-related metabolites, and OTUs and gut- as well as anxiety-related metabolites using our calculated fixed-effect beta estimates and correlation coefficients (Figure 7-3). Looking at the network, *Escherichia-Shigella, Christensenellaceae R-7 group, Ruminococcaceae UCG-010, Ruminococcaceae UCG-002, Prevotellaceae NK3B31 group* and *Ruminococcus 2* were significantly associated with anxiety (red and black solid line(s)). Gut-related

metabolites were not significantly associated with anxiety (grey dotted lines) and anxiety-related OTUs were not significantly correlated with gut- and anxiety-related metabolites (grey solid lines), suggesting that anxiety-related OTUs may influence anxiety directly and not through the production of bacterial metabolites or through an association with anxiety-related markers.

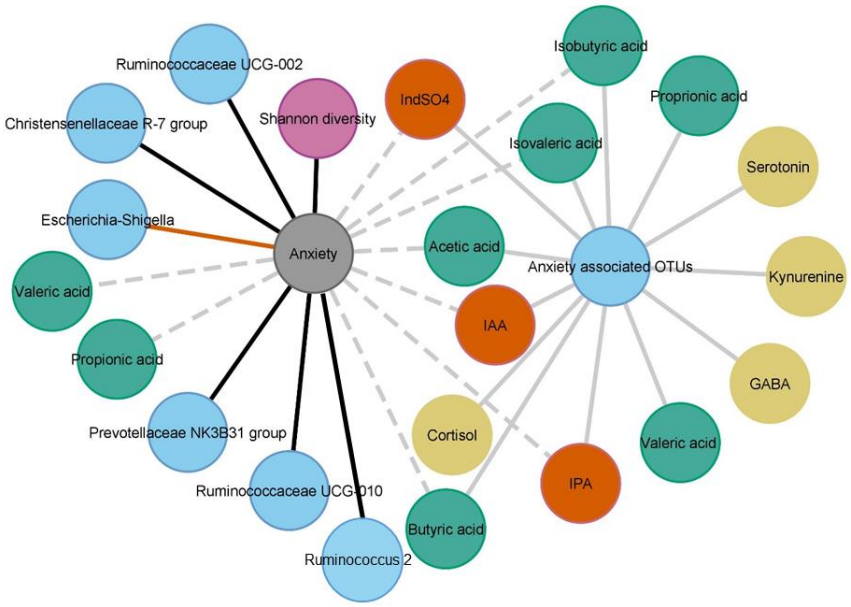


Figure 7-3. Correlation network of associations between anxiety and OTUs, anxiety and gut-related metabolites, and OTUs and gut- as well as anxiety-related metabolites.

OTUs, SCFAs, indoles and anxiety-related metabolites are color-coded in sky blue, bluish green, vermillion and sienna, respectively. Anxiety and Shannon diversity are color-coded in grey and reddish purple, respectively. Solid black and red line(s) depict significant negative and positive associations of anxiety with OTUs, respectively. Grey dotted lines show nonsignificant associations of anxiety with gut-related metabolites (SCFAs and indoles). Grey solid lines represent non-significant associations of OTUs with gut-related and anxiety-related metabolites. Abbreviations: IAA: Indole-3-acetic acid, IPA: 3-indolepropionic acid, IndSO4: Indoxyl sulfate, GABA: γ-aminobutyric acid.

7.5. Discussion

In this study, we have shown that there are several reproducible gut microbiome associations with anxiety seen in two different cohorts and in the literature. We find that one pathogenic OTU, *Escherichia-Shigella*, is strongly associated in three cohorts with higher anxiety scores, whereas the remaining OTUs and genera correlate with lower levels of anxiety.

We find that higher anxiety levels were associated with reduced Shannon diversity across two cohorts. Previous studies reported no association between the Shannon index and anxiety symptoms (Jackson et al., 2018; Kleiman et al., 2017; Mason et al., 2020; Naudé et al., 2020), possibly due to lower sample sizes.

An enrichment of *Escherichia-Shigella* was reported in generalized anxiety disorder patients (Chen, Bai, et al., 2019) and in treatment-naive generalized anxiety disorder patients (Jiang et al., 2018) and administration of a probiotic decreased the size of *Escherichia* and *Shigella* populations in subjects with stress-related symptoms (Soldi et al., 2019). Furthermore, *Escherichia-Shigella* was more abundant in Parkinson's disease patients relative to healthy subjects, positively correlated with disease severity, duration and associated with higher anxiety (Li et al., 2017). In a recent study, overgrowth of *Escherichia-Shigella* was identified in patients with irritable bowel syndrome (Liu et al., 2020). The same study also reported a positive correlation of *Escherichia–Shigella* immunoglobulin A coating index with anxiety and depression in irritable bowel syndrome participants and healthy controls

(Liu et al., 2020). *Escherichia–Shigella* was associated with increased levels of pro-inflammatory cytokines in patients with cognitive impairment and brain amyloidosis (Cattaneo et al., 2017). *Escherichia–Shigella* was also related to autism spectrum disorders (Strati et al., 2017) and was linked to poor cognition in post-traumatic stress disorder (Bajaj et al., 2019). Consistent with our results, these findings support a potential role of *Escherichia–Shigella* in central nervous system disorders. Pathogenic bacteria, like *Escherichia–Shigella,* contain lipopolysaccharides (LPS) and other toxic substances that produce an inflammatory cascade that activates the nuclear factor κB/mitogen activated protein kinase pathway, which then leads to the production of pro-inflammatory cytokines (Manco, Putignani, & Bottazzo, 2010). Increased pro-inflammatory cytokine levels can induce oxidative stress and mitochondrial dysfunction, processes that are well characterized in depression and anxiety (Hovatta, Juhila, & Donner, 2010; Scaglia, 2010). LPS may also lead to the activation of the kynurenine pathway resulting in reduced serotonin levels (O'Farrell & Harkin, 2017; Réus et al., 2015). However, this was not supported in the current study, as we did not find a significant association between kynurenine and *Escherichia–Shigella.* In rats, administration of LPS was shown to induce anxiety-like behaviours (Engeland, Kavaliers, & Ossenkopp, 2003) and in humans, acute increases in anxiety symptoms were observed when normal volunteers were injected with LPS (Reichenberg et al., 2001). Nevertheless, presence of pathogenic bacteria, such as *Citrobacter rodentium* and *Campylobacter jejuni* in the gastrointestinal

tract can induce anxiety-like behaviour regardless of a systemic immune response (Goehler, Park, Opitz, Lyte, & Gaykema, 2008; Lyte, Varcoe, & Bailey, 1998). Gut-microbiota may directly interact with the brain via the vagus nerve (Bravo et al., 2011) or via the production of neurotransmitters and neuromodulators (Lyte, 2011). For example, Escherichia species are able to produce serotonin and noradrenaline (Lyte, 2011). The link with *Escherichia-Shigella* suggests that increases in gut permeability and LPS may be responsible for increases in anxiety levels. Consumption of probiotics may strengthen the gut barrier, decrease LPS release from pathogenic bacteria like *Escherichia-Shigella* and reduce inflammation (Laugerette, Vors, Peretti, & Michalski, 2011; Soldi et al., 2019). Moreover, prebiotics and probiotics can exert anxiolytic effects and several randomized control trials have revealed that probiotics can be as effective as conventional prescription medications in alleviating anxiety and depressive symptoms (Liu, Walsh, & Sheehan, 2019; Schmidt et al., 2015).

The *Christensenellaceae* family is consistently reported to be among the most heritable gut microbial taxon (Goodrich et al., 2014; Turpin et al., 2016), typically correlated with various healthy phenotypes, including leanness (Goodrich et al., 2014) and longer colonic transit time (Roager et al., 2016). In addition, depletion of *Christensenellaceae* is identified in metabolic syndrome (He et al., 2018; Lim et al., 2017), irritable bowel syndrome and Crohn's disease (Mancabelli et al., 2017; Pascal et al., 2017). Given that gut diseases, metabolic syndrome and affective disorders are highly comorbid (de

Melo et al., 2017; Postolache et al., 2019), the link between *Christensenellaceae* and central nervous system disorders, such as Parkinson's disease (Barichella et al., 2019; Hill-Burns et al., 2017) and multiple sclerosis (Tremlett et al., 2016) comes as no surprise. At the genus level, overgrowth of the *Christensenellaceae R-7 group* was observed in controls relative to individuals affected by gut diseases, such as Crohn's (Mancabelli et al., 2017). This genus was also negatively correlated with four different stress measures in a pediatric population (Michels et al., 2019). A recent study by Stevens *et.al* identified amplicon sequence variants prevalence of *Christensenellaceae R-7 group* in the non-depressed group relative to depressed individuals with the authors suggesting a role of *Christensenellaceae R-7 group* in heritability of resistance to depression (Stevens et al., 2020). Our study is the first to report an association of *Christensenellaceae R-7 group* with reduced anxiety levels across two independent cohorts and in the literature. Taken together, the *Christensenellaceae R-7 group* may be a potential gut biomarker for several healthy phenotypes, including lower anxiety scores. Due to the robust link between the *Christensenellaceae* family and human health, it was recently suggested that genera, representative members of this family, such as *C. minuta*, could be cultured and used as therapeutic probiotics against disease (Chang et al., 2019).

The other OTUs that we find associated with lower levels of anxiety, namely*, Ruminococcaceae UCG-010, Ruminococcaceae UCG-002,*

Prevotellaceae NK3B31 group, and *Ruminococcus 2* have been reported in the literature to be associated with SCFA production (Fernández et al., 2016; Louis, Hold, & Flint, 2014). However, we were unable to find any association between SCFA levels and anxiety or anxiety-associated OTUs in our data, suggesting that the above OTU-anxiety associations might not be mediated by SCFA production. One possible explanation for this might be a direct gut-brain link through the vagus nerve (Bravo et al., 2011) or via bacterial production of neurotransmitters and neuromodulators (Lyte, 2011). Another explanation for this might be the intestinal endocannabinoid system, via which the gut microbiome has the capacity to regulate the degree of intestinal permeability (Zoppi et al., 2012). Given that increased gut permeability is implicated in anxiety (Ait-Belgnaoui et al., 2012; Foster & Neufeld, 2013), *Ruminococcaceae UCG-010, Ruminococcaceae UCG-002, Prevotellaceae NK3B31 group,* and *Ruminococcus 2* may act via the endocannabinoid system to reduce gut permeably and protect against anxiety. However, we were unable to test this hypothesis here and it needs to be explored in future studies.

The current study has its strengths and weaknesses. This is the first study to report the reproducible association of the gut microbiota with anxiety across two independent cohorts and the published literature. Although we used validated instruments to measure anxiety levels derived from a widely used scale (HADS-A), we lacked clinical measures of anxiety and thus, future studies are warranted to confirm our findings. Despite testing for several gut-related metabolites, such as

SCFAs and indoles, we could not test endocannabinoids that might have mediated the link between OTUs and anxiety levels. Moreover, we did not measure LPS or any other measure of gut permeability that might explain the link between *Escherichia-Shigella* and anxiety. We studied two independent cohorts that used the same pipeline for carrying out 16S sequencing and we could replicate our results in the literature. However, we were not able to perform metagenomics analysis, which together with metabolomics may have provided a better understanding of the underlying mechanisms mediating the gut-brain associations identified in this study.

We found strong reproducible anxiety-microbiome associations, including with *Escherichia-Shigella* and *Christensenellaceae R-7 group*. These genera have been previously associated with disease and healthy phenotypes, respectively, making our findings plausible. We did not find SCFAs, indoles or anxiety-related markers mediating these relationships, suggesting a direct link between these genera and anxiety levels. We propose these genera as potential biomarkers for anxiety. Members of the *Christensenellaceae* family should be cultured and tested in well-designed trials to identify their therapeutic potential as next-generation probiotics against anxiety.

7.6. Conflict of interest

AMV is a consultant for Zoe Global Ltd. TDS is co-founder of Zoe Global Ltd. All other authors declare no competing financial interests.

7.7. Funding

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7.8. Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Chapter 8 General Discussion

8.1. Overview

This thesis has applied a biopsychosocial (BPS) approach to chronic pain recognising the contribution of psychological (anxiety and cognitive ability), social (social deprivation/isolation) and biological factors (genetic mechanisms and the gut microbiome). The aim of the studies included in the thesis was to explore how psychosocial factors (Chapters 3, 4 and 5), host genetics (Chapter 6), and the gut microbiome (Chapter 7) interact in their contribution to chronic pain. This thesis sheds new light on some of the less well-studied components of the BPS model and the interactions between them.

Within this thesis, longitudinal, clinical-based, genetic and microbiome studies were employed. Carefully designed longitudinal studies using sophisticated statistical approaches and taking into account various outcomes, such as functional and cognitive status (Chapter 4) are important as they can unravel when is the best time and which factors to target when designing and implementing interventions to improve OA-related disability. Clinical-based studies using translational assays of distinct neuro-cognitive functions (Chapter 3) are equally important as they provide detailed information about the specific cognitive domains that are impaired in OA, thereby paving the way for better-targeted treatment options in the future. In addition, using tools, such as QST (Chapters 3 and 6) allows us to investigate different dimensions of pain, such as allodynia, hyperalgesia and temporal summation, and may potentially aid in disentangling the relationship between pain

modulation and other top-down pain regulating mechanisms. Finally, genetic and microbiome studies (Chapters 6 and 7) further our understanding of the underlying genetic and molecular mechanisms involved in chronic pain, and hence may provide useful targets for interventions or direction for future research.

8.2. Key findings and implications, and outline for future directions

8.2.1. Cognitive ability: links with pain, genetic factors, and IADL

Cognitive ability was linked to pain consistently across the different studies; global cognitive impairment was correlated with higher pain severity and duration (Chapter 3), and higher levels of pain, before diagnosis of OA, were associated with reduced cognitive ability following diagnosis (Chapter 4). Moreover, impairments in certain aspects of cognitive ability were associated with increased neuropathic-like pain and central pain processing (Chapter 3). Consistent with this finding, I found genes previously associated with cognitive ability, Alzheimer's disease and intelligence (*AIM2*, *POLR3E*, and *CCDC14*, respectively) involved in distal sensitisation and neuropathic-like pain traits from the gene-based meta-analysis of two OA cohorts (Chapter 6). These findings lend support to the idea that the risk for developing central pain augmentation and neuropathic-like pain may be partly determined by a shared genetic risk with psychological and cognitive traits (Descalzi et al., 2017; Meng et al., 2020). Some of the above-mentioned genes have also been previously involved in neuroplasticity

and neurogenesis (Kohman et al., 2011; Wu et al., 2017), which lends support to the theory that genetic differences, via their influence on physiological differences, may contribute to variation in cognition. This theory was also supported by findings from a recent combined analysis of two GWAS studies (Hill et al., 2019). As mentioned in the Introduction, altered valuation may impact nociceptive processing in the spinal cord, coupled with or further supported by modifications in prefrontal cortical emotional shifts. These downward modulatory pathways contribute to central sensitisation in the spinal cord as evident in animal models (Gardell et al., 2003). Therefore, it may be possible that a genetic variation in cognition coupled with altered cognitive processing caused by chronic pain will contribute to an increased risk for developing central sensitisation in chronic pain patients, but this will need to be tested in future studies.

Cognitive ability was also linked to IADL, such that impaired cognitive ability, before diagnosis, predicted increased limitations in activities of daily living after diagnosis (Chapter 4). Consistent with this finding, my preliminary analysis showed that poorer physical function on the timed up and go and 30 seconds sit to stand tests was linked to poorer executive functioning in knee OA (Chapter 3). Impaired ability to perform daily activities and simple performance-based tasks are early indicators of functional decline and disability in individuals with painful OA (Neogi, 2013). In addition, gait speed was associated with increased limitations in IADL independent of pain status in older adults with knee OA (Nanjo et al., 2021). Taken together, these results

suggest that simple performance-based tasks in combination with simple cognitive assessments could potentially be utilised to identify OA patients at risk for developing IADL disability, but this requires further investigation.

Cognitive ability acted as a mediating mechanism in the link between socioeconomic factors, measured before diagnosis, and health outcomes following diagnosis of OA. Specifically, higher social deprivation and lower educational attainment, before diagnosis, predicted greater limitations in activities of daily living after diagnosis, with this effect partly mediated by impaired cognitive ability (Chapter 4). This finding provides some novel insight that has potential benefit for managing chronic pain caused by OA via psychological interventions, such as cognitive behavioural therapy as will be detailed in section 8.3. Many aspects of the social context can be difficult or impossible to change. Understanding the pathways linking such aspects to health outcomes at the early disease stages allows us to identify areas for health interventions that could potentially prevent functional disability caused by chronic pain. It is equally important to understand how these social factors contribute to limitations in daily function caused specifically by OA pain for designing well-targeted and effective interventions with the potential to improve people's quality of life.

8.2.2. Anxiety: links with pain, IADL and the gut microbiome

As expected, I found that anxiety was linked to pain consistently across the studies; higher anxiety levels were correlated with increased pain duration (Chapter 3) and greater anxiety, before diagnosis of OA, was

associated with more severe reports of pain after diagnosis (Chapter 4). Anxiety was also linked to IADL, such that increased anxiety, before diagnosis, predicted greater limitations in activities of daily living (i.e., a higher IADL score) after diagnosis (Chapter 4). Furthermore, I found evidence of higher anxiety as a mediating factor in the link between lower educational attainment, before diagnosis, and greater limitations in IADL after diagnosis (Chapter 4). These findings further reinforce the idea of managing chronic pain and IADL limitations caused by chronic pain via psychological interventions.

In Chapter 5, I showed that anxiety increased during the first coronavirus (SARS-CoV-2) lockdown in 2020 amongst chronic pain patients. Pain severity was not affected by lockdown but it was higher for participants that reported increased anxiety (a lot or at least a bit) over this period. I further observed an overall protective effect of regular exercise, typically for more than an hour per day on a typical day, against increased anxiety and pain levels during the pandemic. These findings along with findings from Chapter 4, suggest that multi-domain interventions, such as a combination of psychological and exercise interventions could be better at mitigating the negative influence of external factors on health outcomes in chronic pain patients. For example, a program incorporating both cognitive-behavioural techniques and physical exercise was effective in diminishing pain intensity, pain disability and catastrophizing (Cheng et al., 2022). Despite difficulties in delivering and maintaining multidomain interventions, they were shown to be effective especially for populations

that are at higher risk, such as those with lowest levels of education (Richard et al., 2019). Therefore, these types of interventions could be designed to specifically target vulnerable groups of chronic pain patients, such as those with lowest baseline education levels or in the lead-up to future national lockdowns to try to reduce distress.

8.2.3. A complex interplay between psychological, inflammatory and gut microbiome factors in chronic pain

I found a consistent link between anxiety and gut microbiome dysbiosis, such that higher anxiety was associated with a pathogenic OTU (*Escherichia-Shigella*), whereas lower anxiety was associated with beneficial OTUs, such as *Christensenellaceae R-7 group* and SCFA-producing bacteria (Chapter 7). In Chapter 6, I also showed that genes associated with phenotypes such as depression, neuro-inflammation, and obesity (which are also correlated with microbiome composition) contribute to distal sensitisation and neuropathic-like pain in OA. The above findings together with the findings from Chapters 3, 4, and 5 that confirmed the robust link between pain and anxiety favour the idea of an overlapping mechanism linking negative affect and pain sensitisation with inflammation and alterations in the gut microbiome. Indeed, there is evidence to support that neuro-inflammation - microglia and astrocyte activation in the CNS - drives widespread chronic pain via central sensitization (Ji, Nackley, Huh, Terrando, & Maixner, 2018b). Furthermore, both genetic and clinical data have identified a potential causative role of elevated levels of circulating inflammatory markers in depression (Milaneschi et al., 2021; Ye et al., 2021). There is also

evidence of a communication between host microflora and microglia via the gut-brain axis and a connection between pain and the gut microbiome (Dworsky-Fried et al., 2020), as mentioned in the Introduction. However, there is currently no conclusive evidence of a communication between gut bacteria and neuro-inflammation driving the development and maintenance of chronic pain. In addition, the complex interplay between anxiety, pain, inflammatory markers and bacterial taxa in knee OA patients remains to be investigated. Understanding this complex interplay may help us identify molecular biomarkers of chronic pain, and thereby aid in designing effective anti-inflammatory- or microbiome-targeted interventions to promote mental and physical health. For instance, gut microbiome composition is modifiable by various therapeutic strategies, such as with dietary interventions using pro and prebiotics as well as with exercise interventions (see section 8.3 below).

The endocannabinoid (EC) system plays a role in the complex interplay between pain, gut microbiome and inflammation (Zoppi et al., 2012). Indeed, a recent study found that the anti-inflammatory effects of SCFAs are partly mediated by the EC system in chronic OA patients as well as in healthy controls (Vijay, Kouraki, et al., 2021). In addition, there is evidence of a role of inflammation in stress and anxiety (Boorman, Romano, Russell, Mondelli, & Pariante, 2015; Ye et al., 2021). Therefore, there is a possibility that the immune and EC systems act as mediating mechanisms in the link between the gut microbiome

and anxiety. This hypothesis merits investigation in future studies (see section 8.3 below).

Apart from the link between gut microbiome and pain, links also exist between the gut microbiome and cognitive ability (Dworsky-Fried et al., 2020; Sarkar et al., 2018). As central sensitisation symptoms are closely linked with psychological and cognitive traits (Akin-Akinyosoye et al., 2018; Burston et al., 2019; Kouraki, Doherty, et al., 2021), it remains to be elucidated whether the gut microbiome is a mediating mechanism in the link between emotional/cognitive processing and central sensitisation caused by chronic pain (see section 8.3 below).

8.3. Future research

My results suggest that future research is needed to reveal the overlapping mechanisms linking anxiety and pain sensitisation with alterations in the gut microbiome and the exact routes by which the microbiota can exert direct effects on pain sensitisation and vice versa. Potential targets may include altered cognitive processes and the EC and inflammatory systems, as mentioned above. I anticipate that results from Study 1 (Chapter 3) would be able to provide answers to some of these questions. In addition, I plan to conduct additional analysis using EC data and gut permeability markers (i.e. zonulin) from the WebEx cohort to test whether these might mediate the OTU-anxiety associations identified from Study 5 (Chapter 7). Future studies will also examine whether probiotic supplementation, for example with

representative members of *Christensenellaceae* family, such as *C. minuta*, exercise and cognitive behavioural therapy (CBT) or a combination of these (e.g. CBT plus exercise, exercise plus probiotics) are beneficial for patients with comorbid pain and anxiety.

CBT aligns well with the biopsychosocial approach to chronic pain, because it recognises the multifaceted and subjective nature of pain (Beck, 2012). Previous research has found that CBT utilisation is efficient in ameliorating pain and pain-related health problems in chronic pain patients, whether administered alone or in conjunction with rehabilitation treatment (Ehde, Dillworth, & Turner, 2014; Williams, Eccleston, & Morley, 2012). Essentially, what CBT does is that it identifies and changes maladaptive and pain-amplifying cognitions and behaviours, which may fuel psychological distress and lead to increased pain (Hofmann, Asnaani, Vonk, Sawyer, & Fang, 2012). Apart from reducing pain and psychological distress, CBT can increase adaptive behaviours, such as participation in day-to-day activities and exercise. In a randomised controlled trial (RCT) testing the efficacy of an exercise intervention in knee OA, obesity and anxiety/depression comorbidities predicted poorer pain and function outcomes following the exercise intervention (Legha et al., 2020). Therefore, an internet-based CBT program combined with exercise therapy may result in better treatment outcomes for OA patients with comorbid anxiety or obesity compared to CBT or exercise alone (Cuijpers, Van Straten, & Andersson, 2008; Rognsvåg et al., 2021).

Recent studies show evidence for microbiome composition being involved in OA-related knee pain (Boer et al., 2019). Other observational and interventional studies suggest that the gut microbiome is involved in pain intensity, progression and sensitivity including neuropathic pain (Ding et al., 2021) and at least in animal models these effects are mediated by the gut microbiome's role in modulating inflammation. Higher levels of dietary fibre intake have been associated with lower incidence of symptomatic, but not radiographic, knee OA in two US cohorts (Dai, Niu, Zhang, Jacques, & Felson, 2017). Although this link was mediated by BMI (which in itself is highly correlated with microbiome composition), such observations suggest that low levels of SCFAs, which are products of the gut microbiomes' fermentation of fibre, may be linked not just with increased abdominal obesity, but also with higher levels of chronic pain. There is clinical evidence that exercise interventions are effective in achieving clinically meaningful musculoskeletal pain relief for knee OA pain (Rocha, Ramos, Dias, & Martins, 2020). The EC system has emerged as a focal point to ascertain the mechanisms for how exercise benefits the body and how it reduces or controls pain. Several studies, [reviewed in (Watkins, 2018)] including Vijay, Kouraki, et al. (2021) recently, have reported increases in circulating levels of ECs after exercise, with some ECs exerting analgesic effects from exercise. Thus, physical activity is recognized as a modifier of disease risk and the EC system may be a mediating factor (Tantimonaco et al., 2014). Future RCTs will therefore explore whether a combination of microbiome-targeted (Vijay & Valdes,

2021) and exercise interventions (Vijay, Kouraki, et al., 2021) may produce a synergistic effect on OA pain relief via activation of EC tone, which in turn regulates anti-inflammatory and antinociceptive pathways.

8.4. Conclusions

The work presented in this thesis has shed new light to key components of the BPS model and their interactions in chronic pain, both in terms of its findings and the implications discussed above. Throughout my thesis, I tested novel hypotheses and evaluated existing theories, while bringing together different disciplines and methodologies. I also contributed new ideas for future research, as discussed above.

I would like to end on revisiting the major reflections this work has inspired. First, I identified novel evidence of a shared genetic link between cognitive and central pain mechanisms traits in OA, which reinforces the idea of a key role of cognition in chronic pain and proposes new targets for investigation. Second, I identified novel relationships between BPS factors, such as social deprivation, cognitive ability, anxiety and IADL, that if recognised early in the disease course could potentially help identify persons at risk or limit the risk for developing disability. Third, I found new evidence in support of the idea that multi-domain interventions, such as a combination of CBT, exercise, and diet, might be better at alleviating anxiety and pain symptoms in older adults with chronic pain, but this will need to be

tested in well-designed RCTs. This approach might be especially beneficial to support chronic pain patients in the lead-up to future lockdowns or for the groups that are the most vulnerable, such as with the lowest levels of education or with comorbid anxiety.

Throughout my thesis, I tackled many questions and ideas from multiple disciplines and I brought my findings together under the umbrella of the BPS model, an approach that is necessary when investigating a complex phenomenon like chronic pain. Nevertheless, this approach favoured breadth over depth and future studies will need to be done to explore in-depth these findings and the new research avenues proposed herein. In conclusion, the results I generated in this thesis show the need for a holistic approach to the study of chronic pain, incorporating both psychosocial and biomedical factors. Such an approach matches the perspective of chronic pain patients, which involves both physiological and psychosocial dimensions.

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STATEMENT OF AUTHORSHIP

The following are statements of authorship for the jointly-authored works in this submission in chronological order. These statements have been authorised by the other authors involved.

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AK is the primary author and analysed the data. AMV advised throughout all stages of study design and data analysis. All authors discussed the results, commented on the manuscript and approved the final version.

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AK wrote the first draft of the manuscript and analysed the data. AMV and AV advised on study design, statistical analysis, presentation of results, edited and critically reviewed the manuscript. SG and AK collected samples and clinical data. All authors have read, critically reviewed, and approved the final version.

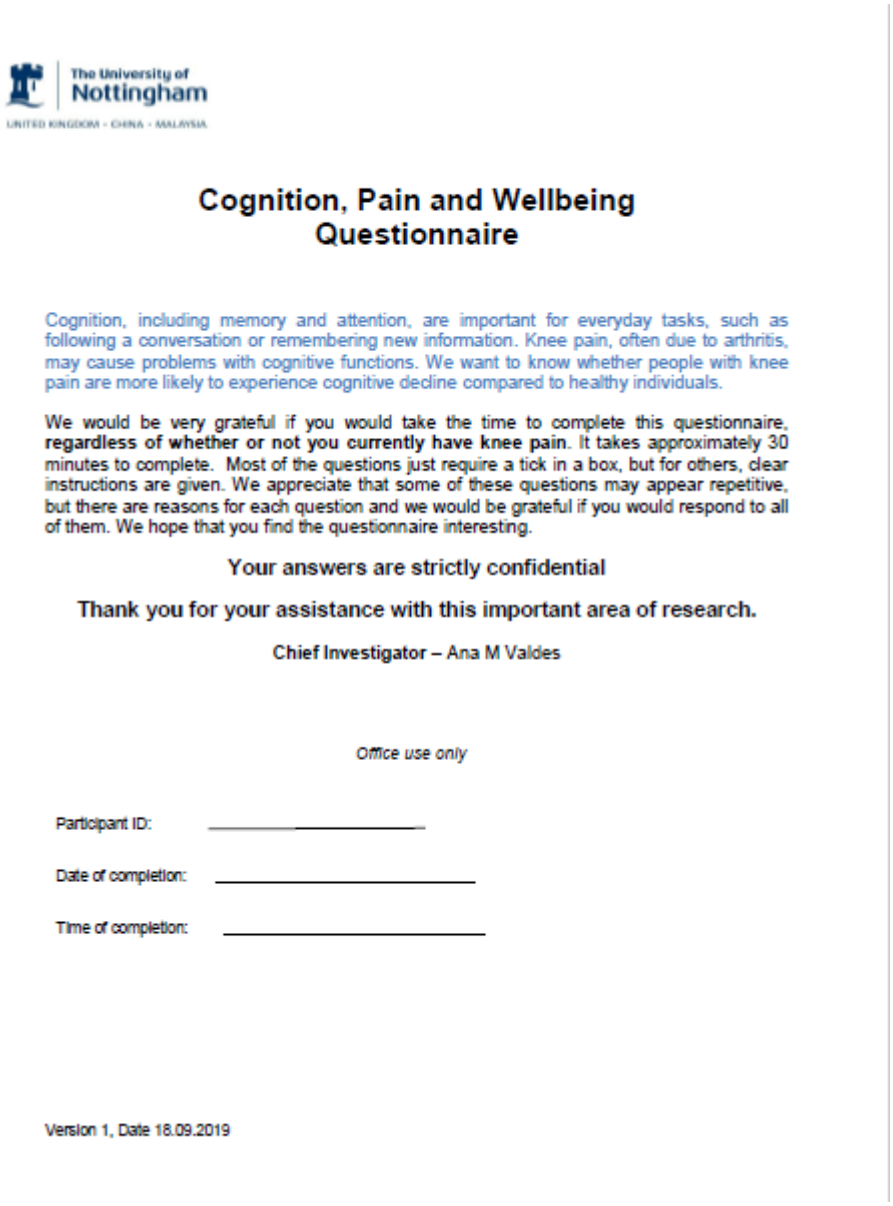
Kouraki, A., Bast, T., Ferguson, E., & Valdes, A. M. Impairments in which cognitive domains are associated with chronic pain in knee osteoarthritis? A study protocol for an observational cohort study. *To be resubmitted.*

AK was responsible for preparing and submitting the ethics application, patient and public involvement and writing the manuscript. AMV, EF and TB advised on the design of this study, edited and critically reviewed the manuscript. All the authors have read, critically reviewed, and approved the final version of this manuscript.

APPENDICES

Appendix 1. Supplementary material supporting chapter 3

Appendix 1.1 Questionnaire booklet for the Cognition, Pain and Wellbeing study (CPW)



The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Cognition, Pain and Wellbeing Questionnaire

Cognition, including memory and attention, are important for everyday tasks, such as following a conversation or remembering new information. Knee pain, often due to arthritis, may cause problems with cognitive functions. We want to know whether people with knee pain are more likely to experience cognitive decline compared to healthy individuals.

We would be very grateful if you would take the time to complete this questionnaire, **regardless of whether or not you currently have knee pain**. It takes approximately 30 minutes to complete. Most of the questions just require a tick in a box, but for others, clear instructions are given. We appreciate that some of these questions may appear repetitive, but there are reasons for each question and we would be grateful if you would respond to all of them. We hope that you find the questionnaire interesting.

Your answers are strictly confidential

Thank you for your assistance with this important area of research.

Chief Investigator – Ana M Valdes

Office use only

Participant ID: _____

Date of completion: _____

Time of completion: _____

Version 1, Date 18.09.2019

SECTION 1: About Yourself

What is your date of birth?

☐☐ Day ☐☐ Month ☐☐☐☐ Year

1. What sex are you?

☐ Male ☐ Female

SECTION 2: About Your Medical History and Medication

2. Have you been diagnosed by your doctor as having osteoarthritis of the knee in the past 24 months?

☐ Yes ☐ No

3. Have you been diagnosed with any new medical conditions in the past 24 months?

_____

4. Have you broken either of your legs in the past 24 months?

☐ Yes ☐ No

If yes, which bone did you break? _____

5. Have you suffered significant injury to either of your knees in the past 24 months that led you to see your doctor?

☐ Yes, *please specify:* _____ ☐ No

6. Have you ever smoked regularly? (at least once a day for at least 3 months)

☐ Yes - age started ☐☐ years old ☐ No

7. Have you stopped smoking?

☐ Yes - age stopped ☐☐ years old ☐ No

8. Please list **all** your **current medication** including those **prescribed by your doctor** and those you **bought yourself** over the counter (*please **include any hormonal medication** such as oestrogen supplements, **vitamin supplements and alternative medicines**).*

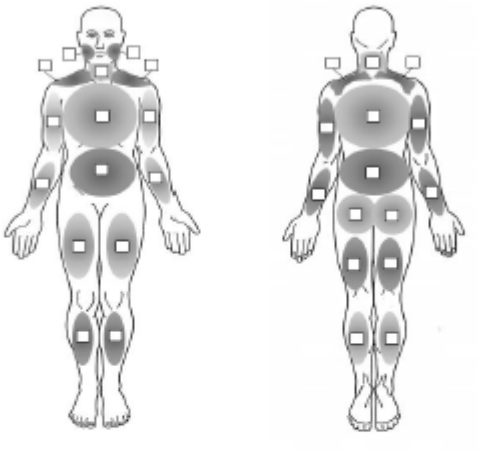
Name of Medication
1)
2)
3)
4)
5)
6)
7)
8)
9)
10)
11)
12)

9. Which, if any, of the following symptoms have you experienced <u>in the past 1 week</u>? Please tick all that apply.

- ☐ Muscle pain
- ☐ Muscle weakness
- ☐ Numbness/tingling
- ☐ Fever
- ☐ Irritable bowel syndrome
- ☐ Pain/cramps in abdomen
- ☐ Constipation
- ☐ Pain in upper abdomen
- ☐ Nausea
- ☐ Diarrhoea
- ☐ Dry mouth
- ☐ Vomiting
- ☐ Heartburn
- ☐ Oral ulcers
- ☐ Loss/change in taste
- ☐ Loss of appetite
- ☐ Fatigue/Tiredness
- ☐ Thinking/ problems remembering
- ☐ Headache
- ☐ Dizziness
- ☐ Depression
- ☐ Nervousness
- ☐ Blurred vision
- ☐ Fits
- ☐ Chest pain
- ☐ Wheezing
- ☐ Shortness of breath
- ☐ Ringing in ears
- ☐ Dry eyes
- ☐ Hearing difficulties
- ☐ Itching
- ☐ Raynaud's*
- ☐ Hives**
- ☐ Rash
- ☐ Sun sensitivity
- ☐ Easy bruising
- ☐ Hair loss
- ☐ Frequent urination
- ☐ Painful urination
- ☐ Bladder pain

**Raynaud's: Sudden painful whitening of several fingers with numbness and tingling*
***Hives: normally raised itchy small pale skin swellings which come and go quickly*

10. This question is about **recent pain** you may have had in **any part of your body**. In the diagrams below, please tick the boxes where you have experienced pain in the region indicated by the shaded areas for **most days in the previous month**. Please tick all relevant boxes (the boxes are either placed in the area itself or joined to the area by a line). By pain we also mean aching, discomfort and/or stiffness. Please **do not include** pain due to feverish illness such as flu. If you **do not have any body pain** that has lasted one day or longer in the last 4 weeks, please tick this box ☐ and move to **the next section**



11. How much physical pain have you had during the **past 4 weeks**? *(please tick one)*

None	Very mild	Mild	Moderate	Severe	Very Severe
☐	☐	☐	☐	☐	☐

12. If you answered "moderate" or "severe" or "very severe", has this pain lasted **more than 3 months?**

☐ Yes ☐ No

SECTION 3: About Your Knee Pain

13. In the past 24 months, have you had any pain in or around a knee on most days for **at least a month**?

☐ Yes ☐ No ***(if No, please go to Section 6)***

14. During the past 24 months, has your knee pain lasted **more than 3 months**?

☐ Yes ☐ No

15. Have you had knee pain on most days of **this past month**?

☐ Yes ☐ No

16. Since it has started, do you think the severity of your knee pain has overall

☐ Greatly improved ☐ Slightly improved ☐ Remained the same ☐ Worsened

17. Which knee(s) do you / did you experience the pain in?

☐ Right ☐ Left ☐ Both

If both, which overall is the worst knee?

☐ Right ☐ Left ☐ Equal

18. Have you ever had any injections into your knee in the past 24 months?

☐ Yes ☐ No

19. If you answered "**yes**", was it:

☐ Steroid injection ☐ Hyaluronic acid injection ☐ Don't know

20. In the past 24 months, have you consulted a healthcare professional (eg your GP, a hospital specialist, a physiotherapist etc) about your knee pain?

☐ Yes ☐ No

21. In the past 24 months, approximately how many different things have you tried for your knee pain (including things like drugs, exercise, changes to diet, footwear modifications etc)? Please include everything you have tried, regardless of whether or not it was recommended by a healthcare professional.

☐

22. Which of the various things you have tried has helped your knee pain the most?

23. Have you had any operation on either of your knees **in the past 24 months**?

☐ Yes ☐ No

If yes, what type of operation did you undergo? *(If you did not undergo one of the specific operations then please leave the related boxes blank)*

Type of operation	**Which knee?** (Right, Left, Both?)	**Age at time of operation** *(years)*
Arthroscopy / Telescope / Keyhole		
Ligament repair		
Meniscus or Cartilage removal		
Joint replacement		
Other, *please specify* _____		

The following questions are to be answered by people who have knee pain.

We now want to ask you about **any kind of knee pain** that you may be currently experiencing

24. **Over the past month**, in your most painful knee, does pain **run up or down** your leg?

☐ Yes ☐ No

Please tell us about the **intensity of discomfort** you experience in or around your **most painful knee**.

25. How would you rate your most painful knee pain on a 0-10 scale at the present time, that is **right now**, where 0 is 'no pain' and 10 is 'pain as bad as could be'? (Please circle only ONE number)

No Pain										**Pain as bad as it could be**
0	1	2	3	4	5	6	7	8	9	10

26. In the **past month**, how intense was your **'worst knee pain'** rated on a 0-10 scale, where 0 is 'no pain' and 10 is 'pain as bad as could be'? (Circle only ONE number)

No Pain										**Pain as bad as it could be**
0	1	2	3	4	5	6	7	8	9	10

27. In the **past month**, on **average**, how **intense** was the pain in your **most painful knee** rated on a 0-10 scale, where 0 is 'no pain' and 10 is 'pain as bad as could be'? (That is, your usual pain at times you were experiencing pain.) (Circle only ONE number)

No Pain										**Pain as bad as it could be**
0	1	2	3	4	5	6	7	8	9	10

28. This next question is on the pattern of your pain in your **most painful knee**. Which of the 4 different options below is the one that best describes the pattern of your worst knee pain over the past month? (Tick one box)

☐  Persistent pain with slight fluctuations

☐  Persistent pain with pain attacks

☐  Pain attacks without pain between them

☐  Pain attacks with pain between them

29. Please select the response that best describes the **type of pain in your most painful** knee over the **past month**. Please tick only one box per question and do not leave any questions blank.

	Never	Hardly noticed	Slightly	Moderate	Strongly	Very strongly
a. Do you suffer from a burning sensation (eg stinging nettles) in or around your most painful knee?	☐	☐	☐	☐	☐	☐
b. Do you have a tingling or prickling sensation in the area of your most painful knee 'pain' (eg like crawling ants or electrical tingling?)	☐	☐	☐	☐	☐	☐
c. Is light touching (clothing, a blanket) in this area painful?	☐	☐	☐	☐	☐	☐
d. Do you have sudden pain attacks in the area of your most painful knee 'pain', like electrical shocks?	☐	☐	☐	☐	☐	☐
e. Is cold or heat (bath water) in this area occasionally painful?	☐	☐	☐	☐	☐	☐
f. Do you suffer from a sensation of numbness in or around your most painful knee?	☐	☐	☐	☐	☐	☐
g. Does slight pressure in this area, (e.g. with a finger), trigger pain?	☐	☐	☐	☐	☐	☐

SECTION 5: Pain Symptoms

Section A – Pain

The following questions concern the amount of **PAIN** you have experienced in your **KNEES**. For each situation please tick (✓) the amount of PAIN experienced in the **last 48 hours**.

How much pain do you have?

	None	Mild	Moderate	Severe	Extreme
1. Walking on a flat surface	☐	☐	☐	☐	☐
2. Going up or down stairs	☐	☐	☐	☐	☐
3. At night while in bed	☐	☐	☐	☐	☐
4. Sitting or lying	☐	☐	☐	☐	☐
5. Standing upright	☐	☐	☐	☐	☐

Section B – Stiffness

The following two questions concern the amount of **JOINT STIFFNESS (not pain)** in your **KNEES**. STIFFNESS is a sensation of restriction or slowness in the ease with which you move your joints. For each situation please tick (✓) the amount of STIFFNESS experienced in the **last 48 hours**.

	None	Mild	Moderate	Severe	Extreme
6. How severe is your STIFFNESS after first wakening in the morning?	☐	☐	☐	☐	☐
7. How severe is your STIFFNESS after sitting, lying or resting later in the day?	☐	☐	☐	☐	☐

Section C – Difficulty Performing Daily Activities
The following questions concern the amount of difficulty you experienced doing the following daily activities due to the arthritis in your **KNEES**. By this we mean your ability to move around and to look after yourself. For each situation please tick (✓) the amount of difficult experienced in the **last 48 hours**.

What degree of difficulty do you have?	None	Mild	Moderate	Severe	Extreme
8. Descending stairs	☐	☐	☐	☐	☐
9. Ascending stairs	☐	☐	☐	☐	☐
10. Rising from sitting	☐	☐	☐	☐	☐
11. Standing	☐	☐	☐	☐	☐
12a. Bending to the floor	☐	☐	☐	☐	☐
12b. Squatting or bending on knees	☐	☐	☐	☐	☐
13a. Walking on a flat surface	☐	☐	☐	☐	☐
13b. Walking on uneven ground	☐	☐	☐	☐	☐
14. Getting in or out of a car, or off a bus	☐	☐	☐	☐	☐
15. Going shopping	☐	☐	☐	☐	☐
16. Putting on your socks or tights	☐	☐	☐	☐	☐
17. Rising from bed	☐	☐	☐	☐	☐
18. Taking off your socks or tights	☐	☐	☐	☐	☐
19. Lying in bed	☐	☐	☐	☐	☐
20. Getting in or out of the bath	☐	☐	☐	☐	☐
21. Sitting	☐	☐	☐	☐	☐
22. Getting on or off the toilet	☐	☐	☐	☐	☐
23. Performing heavy domestic duties	☐	☐	☐	☐	☐
24. Performing light domestic duties	☐	☐	☐	☐	☐
25. Kneeling or reaching the lower part of a cupboard or cabinet?	☐	☐	☐	☐	☐

SECTION 6: The Way You Feel

32. The following questions are designed to help us to know how you feel. Please read each item below and tick (✓) the box next to the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies. Your immediate reaction to each item will probably be more accurate than a long, thought-out response.

a. I feel tense or 'wound up'
- ☐ most of the time
- ☐ a lot of the time
- ☐ from time to time, occasionally
- ☐ not at all

b. I still enjoy the things I used to enjoy
- ☐ definitely as much
- ☐ not quite so much
- ☐ only a little
- ☐ hardly at all

c. I get a sort of frightened feeling as if something awful is about to happen
- ☐ very definitely and quite badly
- ☐ yes, but not too badly
- ☐ a little, but it doesn't worry me
- ☐ not at all

d. I can laugh and see the funny side of things
- ☐ as much as I always could
- ☐ not quite so much now
- ☐ definitely not so much now
- ☐ not at all

e. Worrying thoughts go through my mind
- ☐ a great deal of the time
- ☐ a lot of the time
- ☐ not too often
- ☐ very little

f. I feel cheerful
- ☐ never
- ☐ not often
- ☐ sometimes
- ☐ most of the time

g. I can sit at ease and feel relaxed
- ☐ definitely
- ☐ usually
- ☐ not often
- ☐ not at all

h. I feel as if I am slowed down
- ☐ nearly all the time
- ☐ very often
- ☐ sometimes
- ☐ not at all

i. I get a sort of frightened feeling like 'butterflies' in the stomach
- ☐ not at all
- ☐ occasionally
- ☐ quite often
- ☐ very often

j. I have lost interest in my appearance
- ☐ definitely
- ☐ I don't take as much care as I should
- ☐ I may not take quite as much care
- ☐ I take just as much care as ever

k. I feel restless as if I have to be on the move
- ☐ very much indeed
- ☐ quite a lot
- ☐ not very much
- ☐ not at all

l. I look forward with enjoyment to things
- ☐ as much as I ever did
- ☐ rather less than I used to
- ☐ definitely less than I used to
- ☐ hardly at all

m. I get sudden feelings of panic
- ☐ very often indeed
- ☐ quite often
- ☐ not very often
- ☐ not at all

n. I can enjoy a good book or radio or television programme
- ☐ often
- ☐ sometimes
- ☐ not often
- ☐ very seldom

SECTION 7: About Your Sleep Quality

Sleep and waking up refreshed is an important part of a healthy lifestyle. We would like to ask you a few questions on your **sleeping patterns**. Your answers should indicate the most accurate reply for the majority of days and nights in **the past month**. Please answer all questions.

During the past month:

41. When have you usually gone to bed?

    Time (in hrs)_____

42. How long has it taken you to fall asleep each night?

    Number of minutes per night _____

43. When have you usually gotten up in the morning?

    Time (in hrs)_____

44. How many hours of actual sleep do you get at night? *This may be different than the number of hours you spend in bed.*

    Number of hours per night _____

45. During the past month how often you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes	☐	☐	☐	☐
b. Wake up in the middle of the night or early morning	☐	☐	☐	☐
c. Have to get up to use the bathroom	☐	☐	☐	☐
d. Cannot breath comfortably	☐	☐	☐	☐
e. Cough or snore loudly	☐	☐	☐	☐
f. Feel too cold	☐	☐	☐	☐

g. Feel too hot	☐	☐	☐	☐
h. Have bad dreams	☐	☐	☐	☐
i. Have pain	☐	☐	☐	☐
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):	☐	☐	☐	☐
46. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	☐	☐	☐	☐
47. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	☐	☐	☐	☐
	Not a problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
48. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?	☐	☐	☐	☐
	Very good	Fairly good	Fairly bad	Very bad
49. During the past month, how would you rate your sleep quality overall?	☐	☐	☐	☐

SECTION 8: About Your Quality of Life

We want to know your reviews about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Please answer every question by marking one box. If you are unsure about how to answer, please give the best answer you can.

50. In general, would you say your health currently is:

☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

51. The following questions are about activities you may do during a typical day. **Would your health limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	Not, limited at all
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	☐	☐	☐
Climbing **several** flights of stairs	☐	☐	☐

52. During the **past month**, have you had any of the following problems with work or other regular daily activities **as a result of your physical health?**

	Yes	No
Accomplished less than you would like	☐	☐
Were **limited** in the **kind** of work or other activities	☐	☐

53. During the **past month**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (e.g. feeling depressed or anxious)?

	Yes	No
Accomplished less than you would like	☐	☐
Didn't do work or other activities as **carefully** as usual	☐	☐

54. During the **past month** how much did **pain** interfere with your normal work (including both work outside the home and housework)?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

55. These next questions are about how you feel and how things have been with you **during the past month**. Please give the **one** answer that is closest to the way you have been feeling for **each** item.

How much time **during the last month**...

	None of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
Have you felt downhearted and blue?	☐	☐	☐	☐	☐	☐

56. During the **past month**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives etc).

None of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time
☐	☐	☐	☐	☐	☐

Thank you very much for taking the time to complete this questionnaire.
We would like to emphasise that your contact details and responses will be kept **completely confidential**

The following set of questions and tests are designed to help us understand the way you think and process information and to measure your memory and attention.

SECTION 9: Reading Test

57. Please read slowly down this list of words. After each word please wait until I say "next" before reading the next word. I must warn you that there are many words that you probably won't recognize, in fact most people don't know them, so just have a guess at these.

CHORD	SUPERFLUOUS
ACHE	SIMILE
DEPOT	BANAL
AISLE	QUADRUPED
BOUQUET	CELLIST
PSALM	FACADE
CAPON	ZEALOT
DENY	DRACHM
NAUSEA	AEON
DEBT	PLACEBO
COURTEOUS	ABSTEMIOUS
RAREFY	DETENTE
EQUIVOCAL	IDYLL
NAIVE	PUERPERAL
CATACOMB	AVER
GAOLED	GAUCHE
THYME	TOPIARY
HEIR	LEVIATHAN
RADIX	BEATIFY
ASSIGNATE	PRELATE
HIATUS	SIDEREAL
SUBTLE	DEMESNE
PROCREATE	SYNCOPE
GIST	LABILE
GOUGE	CAMPANILE

SECTION 10: About Your Thinking Style

The following questions are for us to understand the way you think. If you wish, at the end, I will provide you with an answer sheet/feedback form.

58. Please try to answer the following questions:

a) A bat and a ball cost £1.10 in total. The bat costs £1.00 more than the ball.

How much does the ball cost?

_____ pence

b) If it takes 5 machines 5 minutes to make 5 widgets, **how long would it take 100 machines to make 100 widgets?**

_____ minutes

c) In a lake, there is a patch of lily pads. Every day, the patch doubles in size. If it takes 48 days for the patch to cover the entire lake, **how long would it take for the patch to cover half of the lake?**

_____ days

SECTION 11: About Your Memory and Attention

59. The following questions are about minor mistakes which everyone makes from time to time, but some of which happen more often than others. We want to know **how often** these things have happened to you in the **past 6 months**. Please tick the appropriate box.

	Very often	Quite often	Occasionally	Very rarely	Never
Do you read something and find you haven't been thinking about it and must read it again?	☐	☐	☐	☐	☐
Do you find you forget why you went from one part of the house to the other?	☐	☐	☐	☐	☐
Do you fail to notice signposts on the road?	☐	☐	☐	☐	☐
Do you find you confuse right and left when giving directions?	☐	☐	☐	☐	☐

Do you bump into people?	☐	☐	☐	☐	☐
Do you find you forget whether you've turned off a light or a fire or locked the door?	☐	☐	☐	☐	☐
Do you fail to listen to people's names when you are meeting them?	☐	☐	☐	☐	☐
Do you say something and realize afterwards that it might be taken as insulting?	☐	☐	☐	☐	☐
Do you fail to hear people speaking to you when you are doing something else?	☐	☐	☐	☐	☐
Do you lose your temper and regret it?	☐	☐	☐	☐	☐
Do you leave important letters unanswered for days?	☐	☐	☐	☐	☐
Do you find you forget which way to turn on a road you know well but rarely use?	☐	☐	☐	☐	☐
Do you fail to see what you want in a supermarket (although it's there)?	☐	☐	☐	☐	☐
Do you find yourself suddenly wondering whether you've used a word correctly?	☐	☐	☐	☐	☐
Do you have trouble making up your mind?	☐	☐	☐	☐	☐
Do you find you forget appointments?	☐	☐	☐	☐	☐
Do you forget where you put something like a newspaper or a book?	☐	☐	☐	☐	☐
Do you find you accidentally throw away the thing you want and keep what you meant to throw away – as in the example of throwing away the matchbox and putting the used match in your pocket?	☐	☐	☐	☐	☐
Do you daydream when you ought to be listening to something?	☐	☐	☐	☐	☐
Do you find you forget people's names?	☐	☐	☐	☐	☐
Do you start doing one thing at home and get distracted into doing something else (unintentionally)?	☐	☐	☐	☐	☐

Do you find you can't quite remember something although it's "on the tip of your tongue"?	☐	☐	☐	☐	☐
Do you find you forget what you came to the shops to buy?	☐	☐	☐	☐	☐
Do you drop things?	☐	☐	☐	☐	☐
Do you find you can't think of anything to say?	☐	☐	☐	☐	☐

SECTION 12: Decision Making

60. We would like to ask you to make two independent financial decisions. You are playing for real money. Five participants have been selected at random, prior to the study starting, and will paid based on their decisions. So please treat all decisions as real as you may be one of the people who has been chosen. Once you have made your decisions please place these in the sealed envelope provided. This is to keep your decision anonymous. The envelope will be passed onto another researcher, who has not seen you today, who will prepare your payment if you have been selected. You will collect an envelope as you leave. If you have been selected to be paid this will contain the money you have kept for yourself and if you have not been selected it will be empty. The researchers conducting the study with you today will not know if you have been chosen or any of your decisions. The researcher preparing your payment will never have met you.

Decision 1

You have been given £10 by the University and, can give some, none or all to a stranger.

Of £10 I would give a complete stranger £___.____p and keep £___.____p. Whatever money, if any, you decide to give to a stranger will be given to a randomly selected person from another study.

Decision 2

This is a new decision and independent of the previous decision. You have been given £10 by the University, and can give some, none or all to a charity of your choice. The charity you choose will be paid based on your decision.

Of £10 I would give £___.____p to the following charity _____ and keep £___.____p

Thank you very much for taking the time to complete this questionnaire.
We would like to emphasise that your contact details and responses will be kept **completely confidential**

We would be grateful if you could now confirm whether you are happy to continue to receive information about future research projects undertaken by Academic Rheumatology

Yes, I am happy to continue to receive study information ☐

If you have answered '*Yes*' above, please sign and date:

_____ _____
Signature Date

No, I prefer not to receive information in the future ☐

Appendix 1.2 Study forms

Appendix 1.2.1 Research visit checklist and case report form

Research Visit Checklist V1 11/10/19

Participant ID: _____

Appointment Date: _____ **Time**: _____

Title: Cognition, Chronic Pain and Wellbeing study

Activity	**Tick when done**	**Initials**	**Comments**
Consent: ☐ Trial file ☐ Participant			
Bloods: ☐ Serums ☐ DNA ☐ RNA			
Urine			
Faecal sampling kit ☐ given to participant			
Questionnaires: ☐ Body Pain ☐ WOMAC ☐ PD-Q			

☐ HADS ☐ SF-12 ☐ PSQI ☐ CFQ			
CANTAB			
NART			
CRT			
DG			
TuG			
30CST			
QST			
TS			
CPM			
vDMP (optional)			
Data entered onto database			

Comments:

Cognition, Chronic Pain and Wellbeing Study
Case Report Form (CRF) V5

Participant ID _____ **Study ID**_____ **Date** _____ **Time** _____

Bloods_____ **Urine** _____ **Meds** _____ **Weight** (kg) _____ **Height** (cm) _____

QST (KPa)
Prick (256mN)

Most Painful Knee *(Demonstrate probe on Pts nail)*	Right / Left
A. Superolateral Patella 2cm	
B. Superomedial Patella 2cm	
C. Medial Joint Line 3cm	
D. Tibialis Anterior Muscle 5cm	
E. ECRL 5 cm distally of lateral epicondyle	

Temporal Summation Pin

Demo on back of Pts hand Most Painful Knee *(Bent leg. 5cm from centre Patella)*	Right / Left Test 1	Right / Left Test 2
Single measure		
Repeated measure		
TSP Score (R-S=)		

CPM (KPa)

Most Painful Knee *(Demonstrate probe on Pts nail)*	Right / Left		
Tibialis Anterior Muscle 5cm (U)			
Tibialis Anterior Muscle 5cm (C)		CPM score: (C-U):	

Time Up and Go Test

1.		2.		3.		Average	

30CST Score:

National Adult Reading Test (NART)

Answer Sheet

Title: Cognition, Chronic Pain and Wellbeing study

Participant ID: _____

Appointment Date: _____ **Time**:

_____

	Correct	Incorrect		Correct	Incorrect
CHORD			SUPERFLUOUS		
ACHE			SIMILE		
DEPOT			BANAL		
AISLE			QUADRUPED		
BOUQUET			CELLIST		
PSALM			FACADE		
CAPON			ZEALOT		
DENY			DRACHM		
NAUSEA			AEON		
DEBT			PLACEBO		
COURTEOUS			ABSTEMIOUS		
RAREFY			DETENTE		
EQUIVOCAL			IDYLL		
NAIVE			PUERPERAL		
CATACOMB			AVER		
GAOLED			GAUCHE		
THYME			TOPIARY		
HEIR			LEVIATHAN		

RADIX			BEATIFY		
ASSIGNATE			PRELATE		
HIATUS			SIDEREAL		
SUBTLE			DEMESNE		
PROCREATE			SYNCOPE		
GIST			LABILE		
GOUGE			CAMPANILE		

NART total errors = 50 (words) - (number of words read correctly)

=

Appendix 1.2.2 CANTAB performance observations

CANTAB Performance Observations

Participant ID: _____

Appointment Date: _____

Time: _____

Title: Cognition, Pain and Wellbeing study

MOT Voice:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

PAL Recommended Standard Extended:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

RTI Five-Choice:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

SWM Recommended Standard 2.0 Extended:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

RVP 3 Targets:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

IED XF:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

MTT Standard:

Outcome: Aborted ☐ Completed ☐

Observations: Complete and reliable ☐ Distraction ☐

Deviation from instruction ☐ Did not understand instruction ☐

Technical issue ☐ Refusal ☐

Other ☐

Comments:

Appendix 1.2.3 Participant's feedback form

University of Nottingham
UK | CHINA | MALAYSIA

Cognition, Pain and Wellbeing

To help us assess the techniques we carry out and to ensure that we are maintaining a good professional standard within the Nottingham Biomedical Sciences Centre Musculoskeletal Theme/Arthritis Research UK Pain Centre, we would like you to complete the following questionnaire. Please be as honest as possible.

1. STUDY ORGANISATION & PREPARATION

a) Where did you hear about the study?

.....

.....

b) Once you expressed an interest in the trial, did you gladly take part? Y/N

.....

.....

c) What motivated you to take part in the study?

.....

.....

d) Were you dealt with efficiently and politely? Y/N

If 'No', how could this be improved? .....

.....

.....

e) Was it what you expected? Y/N

If 'No', how could this be improved? .....

.....

.....

f) Were the protocol and techniques explained adequately? Y/N

If 'No', how could this be improved? .....

.....

.....

g) Were the questionnaires explained adequately? Y/N

If 'No', how could this be improved?

.....

.....

2. STUDY PROCEDURES

a) During the study visit blood samples were taken.

Was the procedure better, worse or as expected? .....

On a scale of 1 to 10 (1 being painless and 10 being very painful), how uncomfortable would you score the procedure? .....

b) During the study visit computer-based cognitive assessments were carried out.

Was the procedure better, worse or as expected? .....

Any comments? .....

c) During the study visit quantitative sensory testing was carried out.

Was the procedure better, worse or as expected? .....

On a scale of 1 to 10 (1 being painless and 10 being very painful), how uncomfortable would you score the procedure? .....

e) The total experimental time was ....., did you find this too long ? Y/N

If 'Yes' why did it feel too long (please tick any which apply),

i. ☐ Procedures were painful

☐

II. Study was boring
III. ☐ Found it uncomfortable to remain still for this long
IV. ☐ Other

Please give details of 'other' reasons.....

.....

3. FOLLOW-UP

a) Did you incur travel expenses? Y/N
b) Were there any problems with receiving the payment? Y/N

If 'Yes', please detail .....

.....

b) Did you experience any problems with the sample site/s? Y/N

If 'Yes', please detail .....

.....

c) Did you try to contact the study co-ordinator after the study? Y/N
d) Were your concerns adequately addressed? Y/N

.....

.....

If 'No', how could this be improved?

.....

.....

e) Would you recommend others to take part in this research? Y/N

4. TECHNOLOGY

a) Do you use a mobile phone? Y/N
b) Do you use a smart phone? Y/N
c) Do you use apps on your smart phone? Y/N
d) Do you use the internet? Y/N
e) Do you use social media? Y/N

Thank you for answering this questionnaire, please return to Musculoskeletal Recruitment in the envelope provided.

Appendix 1.2.4 CANTAB outcome measures

Measure description	Description	Sense	Min	Max	units
IED EDS Errors	The number of trials for which the outcome was an incorrect response (subject pressed the incorrect button within the response window), calculated across all assessed trials.	-ve	0	50	-
IED Total Errors (Adjusted)	The total number of times that the subject chose a wrong stimulus – i.e. one incompatible with the current rule, adjustment for every stage that was not reached. Lower is better. Total Errors Adjusted = Total Errors (problems reached) + [(number of unreached problems) * (25)] This is a measure of the subject's efficiency in attempting the test. subjects failing at any stage of the test will have had less opportunity to make errors. The adjustment is carried out to compensate for this missing data and provide a more comparable error score to subjects completing all stages of the test.	-ve	0	402	-
MOT Mean Latency	The mean latency for a subject to correctly respond to the stimulus on screen during assessed trials, measured in milliseconds	-ve	0	6000	ms
MTT Incongruency Cost (Median)	The difference between the median latency of response (from stimulus appearance to button press) on the trials that were congruent versus the	cx	-1900	1900	ms

Measure description	Description	Sense	Min	Max	units
	trials that were incongruent. Calculated by subtracting the median congruent latency (in ms) from the median incongruent latency. A positive score indicates that the subject is faster on congruent trials and a negative score indicates that the subject is faster on incongruent trials. A higher incongruency cost indicates that the subjects take longer to process conflicting information.				
MTT Multitasking Cost (Median)	The difference between the median latency of response (from stimulus appearance to button press) during assessed blocks in which both rules are used versus assessed blocks in which only a single rule is used. Calculated by subtracting the median latency of response during single task block(s) from the median latency of response during multitasking block(s). A positive score indicates that the subject responds more slowly during multitasking blocks and indicates a higher cost of managing multiple sources of information.	cx	-1900	1900	ms
MTT Total Incorrect	The number of trials for which the outcome was an incorrect response (subject pressed the incorrect button within the response window). Calculated across all assessed trials.	-ve	0	160	-

Measure description	Description	Sense	Min	Max	units
PAL First Attempt Memory Score	The number of times a subject chose the correct box on their first attempt when recalling the pattern locations. Calculated across assessed trials, omitting 12 box level to provide a direct comparison to Recommended Standard.	+ve	0	20	-
PAL Total Errors (Adjusted)	The number of times the subject chose the incorrect box for a stimulus on assessment problems (PALTE), plus an adjustment for the estimated number of errors they would have made on any problems, attempts and recalls they did not reach. This measure allows you to compare performance on errors made across all subjects regardless of those who terminated early versus those completing the final stage of the task. In this task variant PALTEA does not include 12 box level to provide a direct comparison to Recommended Standard.	-ve	0	70	-
RTI Median Five-Choice Movement Time	The median time taken for a subject to release the response button and select the target stimulus after it flashed yellow on screen. Calculated across correct, assessed trials in which the stimulus could appear in any one of five locations. Measured in milliseconds.	-ve	100	5100	ms

Measure description	Description	Sense	Min	Max	units
RTI Median Five-Choice Reaction Time	The median duration it took for a subject to release the response button after the presentation of a target stimulus. Calculated across correct, assessed trials in which the stimulus could appear in any one of five locations. Measured in milliseconds.	-ve	100	5100	ms
RVP A'	A' (A prime) is the signal detection measure of a subject's sensitivity to the target sequence (string of three numbers), regardless of response tendency (the expected range is 0.00 to 1.00; bad to good). In essence, this metric is a measure of how good the subject is at detecting target sequences.	+ve	0	1	-
RVP Median Response Latency	The median response latency on trials where the subject responded correctly. Calculated across all assessed trials.	-ve	100	1900	ms
RVP Probability of False Alarm	The number of sequence presentations that were false alarms divided by the number of sequence presentations that were false alarms plus the number of sequence presentations that were correct rejections (False Alarms ÷ (False Alarms + Correct Rejections))	-ve	0	1	-
SWM Between	The number of times a subject revisits a box in which a token has previously	-ve	0	114	-

Measure description	Description	Sense	Min	Max	units
Errors 12 Boxes	been found. Calculated across all trials with 12 tokens only.				
SWM Between Errors	The number of times the subject incorrectly revisits a box in which a token has previously been found. Calculated across all assessed four, six and eight token trials.	-ve	0	153	-
SWM Strategy (12 Box Only)	This measure computes the strategy score for the 12-box stage of the task only. The strategy score is calculated based on the number of times a subject begins a new search pattern from the same box they started with previously. If they always begin a search from the same starting point, we infer that the subject is employing a planned strategy for finding the tokens. Therefore, a lower score indicates high strategy use (1 = they always begin the search from the same box), a high score indicates that they are beginning their searches from many different boxes.	-ve	1	12	-

IED, Intra-Extra Dimensional Set Shift; MOT, Motor Screening Task; MTT, Multi-Tasking Task; PAL, Paired Associate Learning; RTI, Five Choice Reaction Time; RVP, Rapid Visual Information Processing; SWM, Spatial Working Memory.

Appendix 2. Supplementary material supporting chapter 4

Appendix 2.1. Model fit indices run with FIML

Model fit indices	**Statistic**
X^2 test of model fit	82.323 (29) p <0.001
RMSEA	0.044
CFI	0.983
TLI	0.957
X^2 test at baseline	3218.297 (90) p <0.001
SRMR	0.026

Appendix 2.2. Model standardised estimates of direct paths predicting pain and cognitive ability at wave 6 and IADL at waves 6 and 7

Measure	Predictor	SD est.	S.E.	95% CI lower	95% CI upper	P	FDR-adj. P
W5 Pain	**W4 Gender**	-0.067	0.032	-0.129	-0.004	0.037*	0.071
W5 Pain	**W4 Age**	-0.019	0.033	-0.083	0.045	0.566	0.692
W5 Pain	**W4 Education**	-0.032	0.031	-0.094	0.029	0.300	0.414
W5 Pain	**W4 BMI**	0.043	0.033	-0.022	0.107	0.192	0.285
W5 Pain	**W4 Chronic diseases**	0.145	0.033	0.080	0.210	<0.001***	<0.001***
W5 Pain	**W4 Alcohol**	-0.008	0.040	-0.085	0.070	0.842	0.872
W5 Cognitive ability	**W4 Gender**	-0.027	0.030	-0.085	0.031	0.360	0.486
W5 Cognitive ability	**W4 Age**	-0.252	0.029	-0.309	-0.195	<0.001***	<0.001***
W5 Cognitive ability	**W4 Education**	0.067	0.029	0.011	0.123	0.019*	0.043*
W5 Cognitive ability	**W4 BMI**	-0.022	0.030	-0.082	0.037	0.459	0.572
W5 Cognitive ability	**W4 Chronic diseases**	-0.090	0.031	-0.151	-0.030	0.003**	0.008**
W5 Cognitive ability	**W4 Alcohol intake**	-0.079	0.039	-0.155	-0.003	0.042*	0.080
W5 Anxiety	**W4 Gender**	-0.054	0.033	-0.118	0.010	0.099	0.161
W5 Anxiety	**W4 Age**	0.005	0.033	-0.060	0.069	0.889	0.913
W5 Anxiety	**W4 Education**	-0.060	0.032	-0.122	0.002	0.058	0.106
W5 Anxiety	**W4 BMI**	0.014	0.033	-0.051	0.080	0.667	0.751
W5 Anxiety	**W4 Chronic diseases**	0.156	0.033	0.091	0.221	<0.001***	<0.001***
W5 Anxiety	**W4 Alcohol intake**	0.002	0.043	-0.083	0.086	0.969	0.977
W5 Social deprivation	**W4 Gender**	-0.059	0.033	-0.124	0.006	0.073	0.125
W5 Social deprivation	**W4 Age**	0.147	0.034	0.081	0.214	<0.001***	<0.001***
W5 Social deprivation	**W4 Education**	-0.017	0.032	-0.079	0.045	0.592	0.707
W5 Social deprivation	**W4 BMI**	0.003	0.034	-0.064	0.069	0.937	0.953
W5 Social deprivation	**W4 Chronic diseases**	0.099	0.035	0.031	0.167	0.004**	0.011*
W5 Social deprivation	**W4 Alcohol intake**	-0.011	0.042	-0.094	0.071	0.785	0.821
W5 IADL	**W4 Gender**	-0.053	0.030	-0.111	0.005	0.072	0.125
W5 IADL	**W4 Age**	0.220	0.029	0.162	0.277	<0.001***	<0.001***
W5 IADL	**W4 Education**	-0.009	0.029	-0.066	0.047	0.750	0.821

Measure	Predictor	SD est.	S.E.	95% CI lower	95% CI upper	P	FDR-adj. P
W5 IADL	**W4 BMI**	0.016	0.030	-0.043	0.075	0.591	0.707
W5 IADL	**W4 Chronic diseases**	0.166	0.031	0.107	0.226	<0.001***	<0.001***
W5 IADL	**W4 Alcohol intake**	-0.072	0.042	-0.154	0.009	0.081	0.135
W6 Cognitive ability	**W5 Cognitive ability**	0.643	0.020	0.604	0.682	<0.001***	<0.001***
W6 Cognitive ability	**W5 Pain**	-0.076	0.022	-0.118	-0.033	<0.001***	0.001**
W6 Cognitive ability	**W5 Anxiety**	-0.006	0.022	-0.050	0.037	0.777	0.821
W6 Cognitive ability	**W5 Social deprivation**	-0.083	0.024	-0.130	-0.036	0.001**	0.002**
W6 Pain	**W5 Pain**	0.248	0.030	0.188	0.307	<0.001***	<0.001***
W6 Pain	**W5 Cognitive ability**	-0.061	0.033	-0.125	0.003	0.060	0.107
W6 Pain	**W5 Anxiety**	0.079	0.032	0.016	0.141	0.014*	0.032*
W6 Pain	**W5 Social deprivation**	0.026	0.034	-0.041	0.092	0.449	0.566
W6 IADL	**W5 Pain**	0.032	0.024	-0.016	0.080	0.187	0.282
W6 IADL	**W5 Cognitive ability**	-0.099	0.027	-0.152	-0.047	<0.001***	0.001**
W6 IADL	**W5 IADL**	0.541	0.024	0.495	0.587	<0.001***	<0.001***
W6 IADL	**W5 Anxiety**	0.072	0.025	0.023	0.121	0.004**	0.010*
W6 IADL	**W5 Social deprivation**	0.033	0.030	-0.025	0.091	0.268	0.375
W7 IADL	**W6 Pain**	0.011	0.021	-0.031	0.052	0.618	0.717
W7 IADL	**W6 Cognitive ability**	-0.072	0.024	-0.119	-0.024	0.003**	0.008**
W7 IADL	**W6 IADL**	0.549	0.026	0.498	0.601	<0.001***	<0.001***
W7 IADL	**W5 Anxiety**	0.040	0.022	-0.004	0.083	0.074	0.125
W7 IADL	**W5 Social deprivation**	-0.001	0.024	-0.048	0.047	0.981	0.981

SD, standardised; SE, standard error; CI, confidence interval; FDR-adj., false detection rate-adjusted; W, wave; BMI, body mass index; IADL, instrumental activities of daily living. * = p < .05, ** = p < .01, *** = p < .001. n = 971

Appendix 2.3. Illustration of direct and indirect paths based on our hypotheses

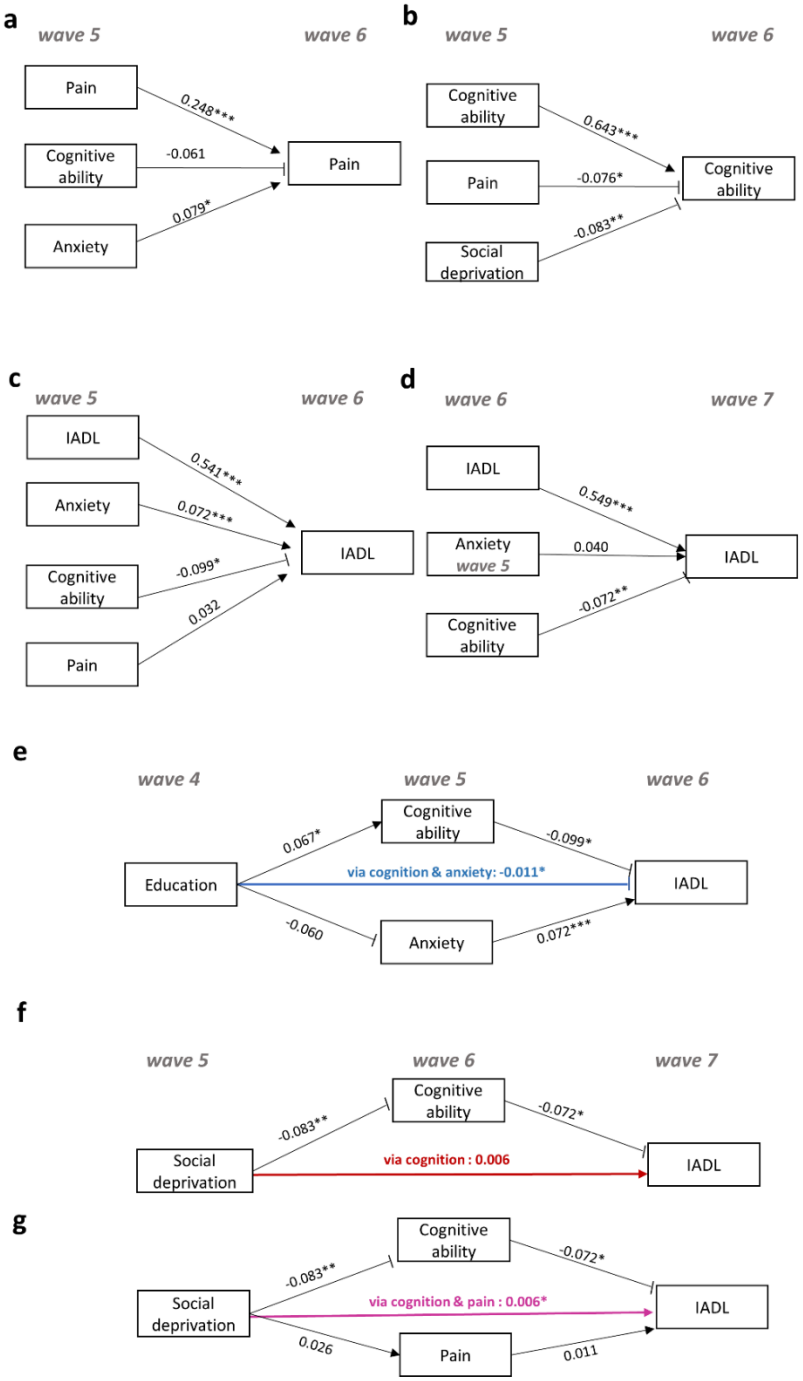


Illustration of direct and indirect paths based on our hypotheses*: (1) higher levels of pain will be related to impaired cognitive ability and higher anxiety after controlling for pain before diagnosis and confounders (panel a), (2) better cognitive ability will be related to reduced pain levels and lower social deprivation after controlling for cognitive ability before diagnosis and confounders (panel b), (3) more limitations in activities of daily living will be associated with higher anxiety and poorer cognitive ability after controlling for limitations in activities of daily living before*

*diagnosis and confounders (panels c and d) and (4) pain, cognitive ability and anxiety will act as the mechanisms indirectly linking socio-economic factors (social deprivation and educational attainment) to limitations in activities of daily living (panels e, f, and g). IADL, instrumental activities of daily living. * = p < .05, ** = p < .01, *** = p < .001. n = 971*

Appendix 2.4. R-Square values for predictor variables

Variable	R-Square
W5 Social deprivation	0.043
W5 Anxiety	0.034
W5 Cognitive ability	0.220
W6 Cognitive ability	0.573
W5 Pain	0.071
W6 Pain	0.128
W5 IADL	0.226
W6 IADL	0.468
W7 IADL	0.590

W, wave; IADL, instrumental activities of daily living. n = 971

Appendix 2.5. Bivariate, zero order correlations between the variables entered into the model.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. W5 Social deprivation	1.000														
2. W4 Education	-0.019	1.000													
3. W4 Age	**0.150**	-0.035	1.000												
4. W4 Sex	0.055	-0.031	-0.012	1.000											
5. W5Anxiety	**0.302**	**-0.064**	0.043	**-0.068**	1.000										
6. W5 Cognitive ability	**-0.311**	**0.088**	**-0.332**	0.015	**-0.199**	1.000									
7. W6 Cognitive ability	**-0.316**	**0.104**	**-0.389**	**0.063**	**-0.177**	**0.735**	1.000								
8. W5 Pain	**0.150**	-0.046	0.036	**-0.089**	**0.168**	**-0.160**	**-0.197**	1.000							
9. W6 Pain	**0.117**	-0.060	**0.084**	**-0.070**	**0.163**	**-0.154**	**-0.160**	**0.300**	1.000						
10. W6 IADL	**0.261**	-0.057	**0.367**	**-0.097**	**0.214**	**-0.348**	**-0.383**	**0.179**	**0.194**	1.000					
11. W7 IADL	**0.237**	-0.044	**0.407**	-0.056	**0.212**	**-0.358**	**-0.392**	**0.148**	**0.181**	**0.743**	1.000				
12. W5 IADL	**0.297**	-0.038	**0.294**	**-0.078**	**0.188**	**-0.305**	**-0.311**	**0.201**	**0.206**	**0.648**	**0.595**	1.000			
13. W4 BMI	0.023	-0.016	-0.017	0.062	0.046	-0.042	-0.021	**0.083**	**0.130**	0.055	**0.073**	**0.070**	1		
14. W4 Chronic diseases	**0.121**	-0.017	**0.239**	**-0.081**	**0.165**	**-0.211**	**-0.155**	**0.188**	**0.215**	**0.271**	**0.307**	**0.284**	**0.217**	1.000	
15. W4 Alcohol intake	0.017	0.026	**0.127**	**-0.198**	0.017	**-0.108**	**-0.091**	0.002	0.022	0.011	0.025	-0.031	**-0.114**	**0.080**	1.000

W, wave; IADL, instrumental activities of daily living. Significant correlations are in bold. n = 971

Appendix 2.6. Bivariate, zero order correlations between items of the social deprivation index and items of the IADL score.

	Isolation	Course	Organisation	Trust	Doctor	Room	Feeling part	Vandalism	Clean area	Help area	Bank access	Shop access	Pharmacy access	IT skills
Using a map	**-0.178**	**-0.084**	-0.051	-0.047	**-0.076**	**-0.183**	-0.015	0.007	0.041	-0.009	**0.183**	**0.173**	**0.173**	**0.235**
Preparing hot meal	**-0.108**	**-0.072**	**-0.061**	**-0.101**	**-0.068**	**-0.095**	-0.009	-0.079	-0.008	-0.019	**0.126**	**0.127**	**0.155**	**0.142**
Grocery shopping	**-0.211**	**-0.076**	-0.008	**-0.091**	**-0.084**	**-0.141**	-0.001	-0.031	0.048	-0.035	**0.217**	**0.220**	**0.213**	**0.184**
Making calls	**-0.186**	-0.036	**-0.058**	**-0.117**	**-0.076**	**-0.129**	-0.055	**-0.074**	-0.030	-0.032	0.054	**0.064**	**0.071**	**0.080**
Taking medications	**-0.131**	**-0.060**	**-0.059**	**-0.058**	**-0.102**	-0.036	-0.042	-0.066	-0.036	-0.013	-0.033	-0.049	-0.040	**0.079**
Doing housework	**-0.171**	**-0.083**	-0.048	-0.052	**-0.099**	**-0.074**	0.024	-0.028	-0.026	0.024	**0.175**	**0.184**	**0.151**	**0.164**
Managing money	**-0.181**	-0.051	-0.047	**-0.088**	**-0.134**	0.027	0.020	-0.034	0.027	0.007	**0.162**	**0.185**	**0.154**	**0.150**
IADL total	**-0.263**	**-0.110**	**-0.068**	**-0.111**	**-0.140**	**-0.141**	-0.003	-0.052	0.012	-0.010	**0.233**	**0.239**	**0.225**	**0.252**

Isolation: Feeling left out of things (Often=1, Sometimes=2, Rarely=3, Never=4), Course: Attended any course in the past 12 months (Yes=1, No=5), Organisation: Taken part in any organization in the past 12 months (Yes=1, No=5), Trust: People cannot be trusted. (0-10), Doctor: Waiting too long to see a doctor. (Yes=1, No=5), Room: How many rooms per person in household?, Feeling part: Feeling part of the local area. (Strongly agree=1, Agree=2, Disagree=3, Strongly disagree=4), Vandalism: Vandalism in the local area. (Strongly agree=1, Agree=2, Disagree=3, Strongly disagree=4), Clean area: Local area clean. (Strongly agree=1, Agree=2, Disagree=3, Strongly disagree=4), Help in area: Helpful people in local area. (Strongly agree=1, Agree=2, Disagree=3, Strongly disagree=4), Bank: Easy access to the nearest bank. (Very easy=1, Easy=2, Difficult=3, Very difficult=4), Shop: Easy access to the nearest shop. (Very easy=1, Easy=2, Difficult=3, Very difficult=4), Pharmacy: Easy access to the nearest pharmacy. (Very easy=1, Easy=2, Difficult=3, Very difficult=4). IT skills: Computer skills or never used a computer. (Excellent=1, Very good=2, Good=3, Fair=4, Poor=5, Never used=6), IADL, instrumental activities of daily living. Note: Literacy from social deprivation index is not reported due to >90 missing values, Significant correlations are in bold, n = 971

Appendix 2.7. Comparison of standardized coefficients and standard errors (S.E.) from analyses based on two indices of deprivation: social and material

		Social deprivation			Material deprivation[1]		
Measure	**Predictor**	**SD**	**S.E.**	**P**	**SD est.**	**S.E.**	**P**
W5 Pain	**W4 Gender**	-0.067	0.032	0.037*	-0.066	0.032	0.037*
W5 Pain	**W4 Age**	-0.019	0.033	0.566	0.003	0.032	0.936
W5 Pain	**W4 Education**	-0.032	0.031	0.300	-0.029	0.031	0.344
W5 Pain	**W4 BMI**	0.043	0.033	0.192	0.031	0.033	0.353
W5 Pain	**W4 Chronic diseases**	0.145	0.033	<0.001***	0.135	0.033	<0.001***
W5 Pain	**W4 Alcohol**	-0.008	0.040	0.842	-0.020	0.040	0.611
W5 Cognitive ability	**W4 Gender**	-0.027	0.030	0.360	-0.026	0.029	0.378
W5 Cognitive ability	**W4 Age**	-0.252	0.029	<0.001***	-0.300	0.028	<0.001***
W5 Cognitive ability	**W4 Education**	0.067	0.029	0.019*	0.062	0.029	0.031*
W5 Cognitive ability	**W4 BMI**	-0.022	0.030	0.459	0.001	0.030	0.962
W5 Cognitive ability	**W4 Chronic diseases**	-0.090	0.031	0.003**	-0.075	0.031	0.015*
W5 Cognitive ability	**W4 Alcohol intake**	-0.079	0.039	0.042*	-0.058	0.039	0.136
W5 Anxiety	**W4 Gender**	-0.054	0.033	0.099	-0.053	0.033	0.105
W5 Anxiety	**W4 Age**	0.005	0.033	0.889	0.004	0.033	0.905
W5 Anxiety	**W4 Education**	-0.060	0.032	0.058	-0.060	0.032	0.058
W5 Anxiety	**W4 BMI**	0.014	0.033	0.667	0.014	0.033	0.675
W5 Anxiety	**W4 Chronic diseases**	0.156	0.033	<0.001***	0.156	0.033	<0.001***
W5 Anxiety	**W4 Alcohol intake**	0.002	0.043	0.969	0.007	0.043	0.875
W5 Deprivation	**W4 Gender**	-0.059	0.033	0.073	-0.049	0.033	0.140
W5 Deprivation	**W4 Age**	0.147	0.034	<0.001***	-0.046	0.034	0.172
W5 Deprivation	**W4 Education**	-0.017	0.032	0.592	-0.036	0.032	0.258
W5 Deprivation	**W4 BMI**	0.003	0.034	0.937	0.094	0.033	0.005**
W5 Deprivation	**W4 Chronic diseases**	0.099	0.035	0.004**	0.148	0.034	<0.001***
W5 Deprivation	**W4 Alcohol intake**	-0.011	0.042	0.785	0.076	0.046	0.099
W5 IADL	**W4 Gender**	-0.053	0.030	0.072	-0.057	0.030	0.057
W5 IADL	**W4 Age**	0.220	0.029	<0.001***	0.258	0.029	<0.001***
W5 IADL	**W4 Education**	-0.009	0.029	0.750	-0.006	0.029	0.831
W5 IADL	**W4 BMI**	0.016	0.030	0.591	0.004	0.031	0.905
W5 IADL	**W4 Chronic diseases**	0.166	0.031	<0.001***	0.163	0.031	<0.001***

		Social deprivation			Material deprivation[1]		
Measure	**Predictor**	**SD**	**S.E.**	**P**	**SD est.**	**S.E.**	**P**
W5 IADL	**W4 Alcohol intake**	-0.072	0.042	0.081	-0.081	0.043	0.058
W6 Cognitive ability	**W5 Cognitive ability**	0.643	0.020	<0.001***	0.640	0.020	<0.001***
W6 Cognitive ability	**W5 Pain**	-0.076	0.022	<0.001***	-0.072	0.022	0.001**
W6 Cognitive ability	**W5 Anxiety**	-0.006	0.022	0.777	-0.010	0.022	0.648
W6 Cognitive ability	**W5 Deprivation**	-0.083	0.024	0.001**	-0.083	0.024	<0.001***
W6 Pain	**W5 Pain**	0.248	0.030	<0.001***	0.248	0.030	<0.001***
W6 Pain	**W5 Cognitive ability**	-0.061	0.033	0.060	-0.066	0.032	0.040*
W6 Pain	**W5 Anxiety**	0.079	0.032	0.014*	0.083	0.032	0.009**
W6 Pain	**W5 Deprivation**	0.026	0.034	0.449	0.009	0.033	0.783
W6 IADL	**W5 Pain**	0.032	0.024	0.187	0.029	0.024	0.243
W6 IADL	**W5 Cognitive ability**	-0.099	0.027	<0.001***	-0.094	0.027	0.001**
W6 IADL	**W5 IADL**	0.541	0.024	<0.001***	0.543	0.023	<0.001***
W6 IADL	**W5 Anxiety**	0.072	0.025	0.004**	0.070	0.025	0.004**
W6 IADL	**W5 Deprivation**	0.033	0.030	0.268	0.048	0.028	0.087
W7 IADL	**W6 Pain**	0.011	0.021	0.618	0.010	0.021	0.650
W7 IADL	**W6 Cognitive ability**	-0.072	0.024	0.003**	-0.064	0.024	0.009**
W7 IADL	**W6 IADL**	0.549	0.026	<0.001***	0.547	0.026	<0.001***
W7 IADL	**W5 Anxiety**	0.040	0.022	0.074	0.035	0.022	0.116
W7 IADL	**W5 Deprivation**	-0.001	0.024	0.981	0.028	0.024	0.234

[1]An index of material deprivation was assessed in Wave 5 of the SHARE and includes 11 items that cover aspects of the economic circumstances of households, such as the ability to afford to eat meat or fruit more often than three times per week, the affordability of a number of specific items such as groceries and holidays away from home, the necessity to limit expenses on a number of items, such as shoes or heating to keep living costs down, and the inability to see a doctor because of cost.

SD, standardised; SE, standard error; W, wave; IADL, instrumental activities of daily living. * = p < .05, ** = p < .01, *** = p < .001. n=971

Appendix 2.8. Comparison of standardized coefficients and standard errors (S.E.) from analyses based on two indices of deprivation: social and material

	Social deprivation			**Material deprivation[1]**		
Indirect paths	**SD est.**	**S.E.**	**P**	**SD est.**	**S.E.**	**p**
W5 Anxiety → W6 Cognitive ability → W7IADL	0.000	0.002	0.778	0.001	0.001	0.653
W5 Anxiety → W6 Pain → W7 IADL	0.000	0.001	0.622	0.000	0.001	0.655
W4 Education → W5 Cognitive ability → W6 Cognitive ability	0.043	0.019	0.020*	0.039	0.018	0.032*
W4 Education → W5 Cognitive ability → W6 Pain	-0.004	0.003	0.143	-0.004	0.003	0.138
W4 Education→ W5 Anxiety → W6 Pain	-0.005	0.003	0.134	-0.005	0.003	0.125
W4 Education → W5 Cognitive ability → W6 IADL	-0.007	0.003	0.049*	-0.006	0.003	0.068
W4 Education → W5 Deprivation → W6 IADL	-0.001	0.001	0.631	-0.002	0.002	0.347
W4 Education → W5 Pain → W6 IADL	-0.001	0.001	0.415	-0.001	0.001	0.462
W4 Education → W5 Anxiety → W6 IADL	-0.004	0.003	0.114	-0.004	0.003	0.115
W5 Deprivation → W6 Cognitive ability → W7 IADL	0.006	0.003	0.024*	0.005	0.003	0.036*
W5 Deprivation → W6 Pain → W7 IADL	0.000	0.001	0.677	0.000	0.000	0.814

Table continued on next page

	Social deprivation			Material deprivation[1]		
Total indirect effects	**SD est.**	**S.E.**	**P**	**SD est.**	**S.E.**	**p**
W5 Anxiety → W6 Cognitive ability+W6 Pain → W7 IADL	0.001	0.002	0.630	0.001	0.002	0.543
W4 Education→ W5 Cognitive ability +W5 Anxiety → W6 Pain	-0.009	0.004	0.033*	-0.009	0.004	0.029*
W4 Education → W5 Pain+W5 Deprivation → W6 IADL	-0.002	0.002	0.351	-0.003	0.002	0.226
W4 Education → W5 Cognitive ability+W5 Deprivation → W6IADL	-0.007	0.004	0.040*	-0.007	0.004	0.033*
W4 Education → W5 Anxiety+W5 Deprivation → W6 IADL	-0.005	0.003	0.118	-0.006	0.003	0.079
W4 Education→ W5 Cognitive ability+W5 Pain → W6 IADL	-0.008	0.004	0.032*	-0.007	0.003	0.048*
W4 Education→ W5 Cognitive ability+W5 Anxiety → W6 IADL	-0.011	0.004	0.011*	-0.010	0.004	0.015*
W4 Education→ W5 Pain+W5 Anxiety → W6 IADL	-0.005	0.003	0.072	-0.005	0.003	0.079
W5 Deprivation → W6 Cognitive ability+W6 Pain → W7 IADL	0.006	0.003	0.021*	0.005	0.003	0.034*

[1]An index of material deprivation was assessed in Wave 5 of the SHARE and includes 11 items that cover aspects of the economic circumstances of households, such as the ability to afford to eat meat or fruit more often than three times per week, the affordability of a number of specific items such as groceries and holidays away from home, the necessity to limit expenses on a number of items, such as shoes or heating to keep living costs down, and the inability to see a doctor because of cost.

SD, standardised; SE, standard error; W, wave; IADL, instrumental activities of daily living.* = p < .05, ** = p < .01, *** = p < .001. n=971

Appendix 2.9. One-way ANOVA to compare pain medication use across wave 5, 6 and 7.

	Degrees of freedom	Sum of squares	Mean Square	F value	P>F
wave	2	19.900	9.966	21.100	<0.001***
Residuals	3769	1780	0.472		

Data on pain medication use were collected at all waves by asking participants, “Do you currently take drugs at least once a week for joint pain or for joint inflammation?” The question does not distinguish between over-the counter or prescription drugs. * = p < .05, ** = p < .01, *** = p < .001. n=969 (2 observations were deleted due to missingness)

Appendix 2.10. Post hoc multiple comparisons of mean pain medication use between the different waves with Tukey test

	Mean difference	Lower Limit 95% Confidence Interval	Upper Limit 95% Confidence Interval	FDR-adj. P value
W6 Pain Medication-W5 Pain Medication	0.149	0.084	0.213	<0.001***
W7 Pain Medication-W5 Pain Medication	0.159	0.095	0.223	<0.001***
W7 Pain Medication-W6 Pain Medication	0.011	-0.054	0.075	0.700

Data on pain medication use were collected at all waves by asking participants, “Do you currently take drugs at least once a week for joint pain or for joint inflammation?” The question does not distinguish between over-the counter or prescription drugs. W, wave; FDR-adj., False discovery rate-adjusted, * = p < .05, ** = p < .01, *** = p < .001. n=971

Appendix 2.11. Number of missing data per variable

Variable	Number of missing data
W4BMI	33
W4Chronic diseases	1
W4Alcohol intake	315
W5Social deprivation	83
W4Education	11
W4Age	0
W4Sex	0
W5Anxiety	0
W5Cognitive ability	0
W6Cognitve ability	0
W5Pain	0
W6Pain	0
W6IADL	0
W7IADL	5
W5IADL	0

W, wave; BMI, body mass index; IADL, instrumental activities of daily living. n = 971

Appendix 2.12. Comparison of unstandardized coefficients and standard errors from analyses based on two methods for handling missing data: full information maximum likelihood (FIML) and multiple imputation (MI)

		FIML (n = 971)			**MI (m = 40)**		
Measure	**Predictor**	**Est.**	**S.E.**	**P**	**Est.**	**S.E.**	**P**
W5 Pain	**W4 Gender**	-0.165	0.079	0.037*	-0.168	0.081	0.039*
W5 Pain	**W4 Age**	-0.002	0.004	0.566	-0.002	0.004	0.616
W5 Pain	**W4 Education**	-0.005	0.005	0.300	-0.005	0.005	0.317
W5 Pain	**W4 BMI**	0.009	0.007	0.192	0.009	0.007	0.203
W5 Pain	**W4 Chronic diseases**	0.099	0.023	<0.001***	0.099	0.024	<0.001***
W5 Pain	**W4 Alcohol**	-0.009	0.047	0.842	-0.014	0.041	0.727
W5 Cognitive ability	**W4 Gender**	-0.723	0.791	0.360	-0.691	0.808	0.393
W5 Cognitive ability	**W4 Age**	-0.319	0.038	<0.001***	-0.323	0.039	<0.001***
W5 Cognitive ability	**W4 Education**	0.104	0.045	0.019*	0.104	0.046	0.024*
W5 Cognitive ability	**W4 BMI**	-0.050	0.068	0.459	-0.046	0.068	0.501
W5 Cognitive ability	**W4 Chronic diseases**	-0.668	0.228	0.003**	-0.693	0.234	0.003**
W5 Cognitive ability	**W4 Alcohol intake**	-1.023	0.505	0.042*	-1.030	0.405	0.011*
W5 Anxiety	**W4 Gender**	-0.361	0.219	0.099	-0.359	0.224	0.109
W5 Anxiety	**W4 Age**	0.001	0.010	0.889	0.001	0.011	0.902
W5 Anxiety	**W4 Education**	-0.023	0.012	0.058	-0.024	0.013	0.064
W5 Anxiety	**W4 BMI**	0.008	0.019	0.667	0.008	0.019	0.692
W5 Anxiety	**W4 Chronic diseases**	0.288	0.062	<0.001***	0.290	0.064	<0.001***
W5 Anxiety	**W4 Alcohol intake**	0.005	0.140	0.969	0.008	0.112	0.941
W5 Social deprivation	**W4 Gender**	-1.979	1.108	0.073	-1.934	1.102	0.079
W5 Social deprivation	**W4 Age**	0.232	0.054	<0.001***	0.222	0.053	<0.001***
W5 Social deprivation	**W4 Education**	-0.033	0.061	0.592	-0.028	0.063	0.651
W5 Social deprivation	**W4 BMI**	0.008	0.095	0.937	0.008	0.093	0.928
W5 Social deprivation	**W4 Chronic diseases**	0.907	0.321	0.004**	0.853	0.316	0.007**
W5 Social deprivation	**W4 Alcohol intake**	-0.185	0.680	0.785	-0.284	0.553	0.607
W5 IADL	**W4 Gender**	-0.115	0.064	0.072	-0.125	0.065	0.056
W5 IADL	**W4 Age**	0.022	0.003	<0.001***	0.023	0.003	<0.001***
W5 IADL	**W4 Education**	-0.001	0.004	0.750	-0.001	0.004	0.744

		FIML (n = 971)			MI (m = 40)		
Measure	**Predictor**	**Est.**	**S.E.**	**P**	**Est.**	**S.E.**	**P**
W5 IADL	**W4 BMI**	0.003	0.005	0.591	0.002	0.006	0.654
W5 IADL	**W4 Chronic diseases**	0.100	0.019	<0.001***	0.101	0.019	<0.001***
W5 IADL	**W4 Alcohol intake**	-0.076	0.044	0.081	-0.099	0.033	0.003**
W6 Cognitive ability	**W5 Cognitive ability**	0.622	0.023	<0.001***	0.624	0.023	<0.001***
W6 Cognitive ability	**W5 Pain**	-0.789	0.225	<0.001***	-0.796	0.231	0.001**
W6 Cognitive ability	**W5 Anxiety**	-0.024	0.086	0.777	-0.027	0.089	0.762
W6 Cognitive ability	**W5 Social deprivation**	-0.065	0.019	0.001**	-0.061	0.019	0.001**
W6 Pain	**W5 Pain**	0.152	0.019	<0.001***	0.152	0.020	<0.001***
W6 Pain	**W5 Cognitive ability**	-0.003	0.002	0.060	-0.004	0.002	0.064
W6 Pain	**W5 Anxiety**	0.018	0.007	0.014*	0.018	0.007	0.018*
W6 Pain	**W5 Social deprivation**	0.001	0.002	0.449	0.001	0.002	0.459
W6 IADL	**W5 Pain**	0.045	0.034	0.187	0.046	0.035	0.192
W6 IADL	**W5 Cognitive ability**	-0.013	0.004	<0.001***	-0.013	0.004	<0.001***
W6 IADL	**W5 IADL**	0.880	0.043	<0.001***	0.884	0.044	<0.001***
W6 IADL	**W5 Anxiety**	0.038	0.013	0.004**	0.039	0.014	0.004**
W6 IADL	**W5 Social deprivation**	0.003	0.003	0.268	0.002	0.003	0.423
W7 IADL	**W6 Pain**	0.030	0.060	0.618	0.030	0.061	0.629
W7 IADL	**W6 Cognitive ability**	-0.012	0.004	0.003**	-0.012	0.004	0.003**
W7 IADL	**W6 IADL**	0.669	0.035	<0.001***	0.669	0.035	<0.001***
W7 IADL	**W6 Anxiety**	0.025	0.014	0.074	0.026	0.015	0.079
W7 IADL	**W5 Social deprivation**	0.000	0.003	0.981	-0.001	0.003	0.864

Model fit indices with MI: RMSEA = 0.044, 95% CI 0.033- 0.055, SRMR = 0.031, CFI = 0.980,

TLI = 0.950

Est., estimate; SE, standard error; W, wave; IADL, instrumental activities of daily living. n = 971

Appendix 2.13. Comparison of standardized coefficients and standard errors from analyses based on two methods for handling missing data: FIML and MI

Indirect paths	**FIML SD est.**	**FIML S.E.**	**P**	**MI SD est.**	**MI S.E.**	**P**
W5 Anxiety → W6 Cognitive ability → W7 IADL	0.000	0.001	0.778	0.000	0.001	0.762
W5 Anxiety → W6 Pain → W7 IADL	0.001	0.002	0.622	0.001	0.002	0.617
W4 Education → W5 Cognitive ability → W6 Cognitive ability	0.065	0.028	0.020*	0.065	0.029	0.024*
W4 Education → W5 Cognitive ability → W6 Pain	-0.000	0.000	0.144	0.000	0.000	0.163
W4 Education→ W5 Anxiety → W6 Pain	-0.000	0.000	0.134	0.000	0.000	0.150
W4 Education → W5 Cognitive ability → W6 IADL	-0.001	0.001	0.049*	-0.001	0.001	0.047*
W4 Education → W5 Social Deprivation → W6 IADL	-0.000	0.000	0.631	0.000	0.000	0.801
W4 Education → W5 Pain → W6 IADL	-0.000	0.000	0.415	0.000	0.000	0.419
W4 Education → W5 Anxiety → W6 IADL	-0.001	0.001	0.115	-0.001	0.001	0.109
W5 Social deprivation → W6 Cognitive ability → W7 IADL	0.001	0.000	0.024*	0.001	0.000	0.021*
W5 Social deprivation → W6 Pain → W7 IADL	0.000	0.000	0.677	0.000	0.000	0.597

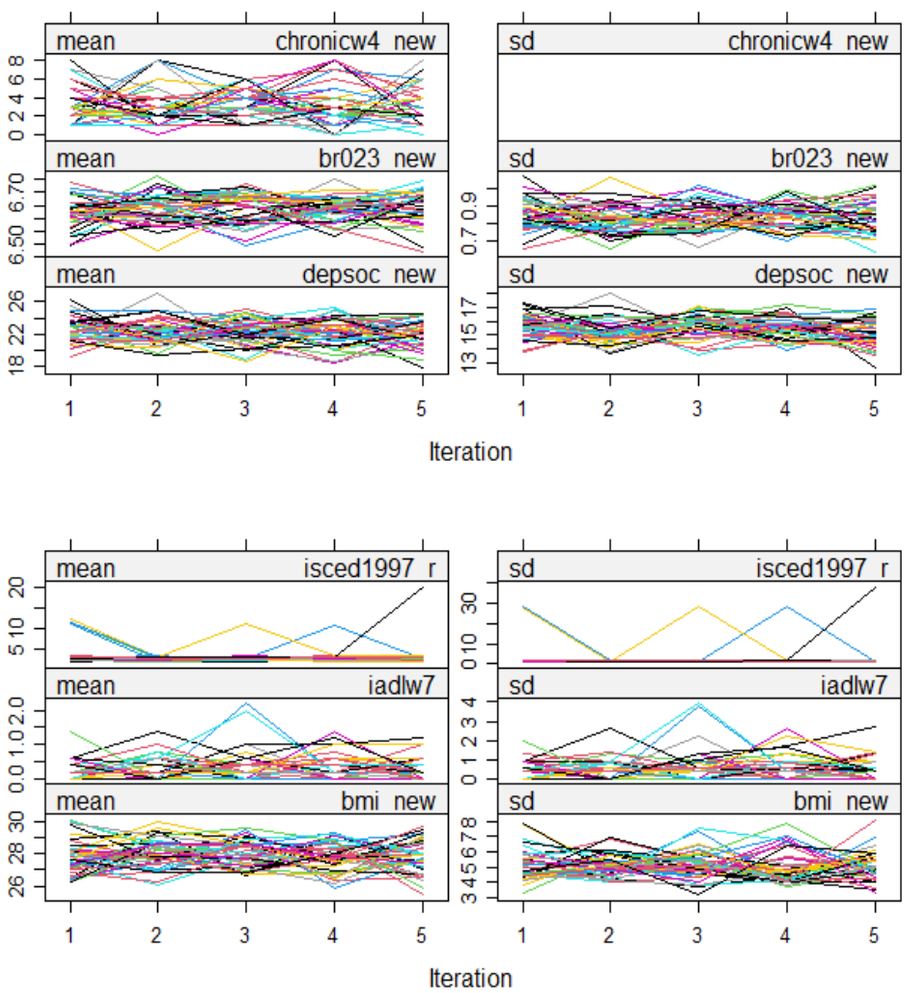
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Model fit indices with MI: RMSEA = 0.044, 95% CI 0.033- 0.055, SRMR = 0.031, CFI = 0.980, TLI = 0.950. Est., estimate; SE, standard error; W, wave; IADL, instrumental activities of daily living. n = 971

Total indirect effects	**FIML SD est.**	**FIML S.E.**	**P**	**MI SD est.**	**MI S.E.**	**P**
W5 Anxiety → W6 Cognitive ability+W6 Pain → W7 IADL	0.001	0.002	0.570	0.001	0.002	0.557
W4 Education→ W5 Cognitive ability+W5 Anxiety → W6 Pain	-0.001	0.000	0.033*	-0.001	0.000	0.040*
W4 Education → W5 Pain+W5 Social deprivation → W6 IADL	-0.000	0.000	0.351	0.000	0.000	0.451
W4 Education → W5 Cognitive ability+W5 Social deprivation → W6 IADL	-0.001	0.001	0.040*	-0.001	0.001	0.048*
W4 Education → W5 Anxiety+W5 Social deprivation → W6 IADL	-0.001	0.001	0.119	-0.001	0.001	0.143
W4 Education→ W5 Cognitive ability+W5 Pain → W6 IADL	-0.002	0.001	0.032*	-0.002	0.001	0.032*
W4 Education→ W5 Cognitive ability+W5 Anxiety → W6 IADL	-0.002	0.001	0.011*	-0.002	0.001	0.009**
W4 Education→ W5 Pain+W5 Anxiety → W6 IADL	-0.001	0.001	0.072	-0.001	0.001	0.069
W5 Social deprivation → W6 Cognitive ability+W6 Pain → W7 IADL	0.001	0.000	0.020*	0.001	0.000	0.017*

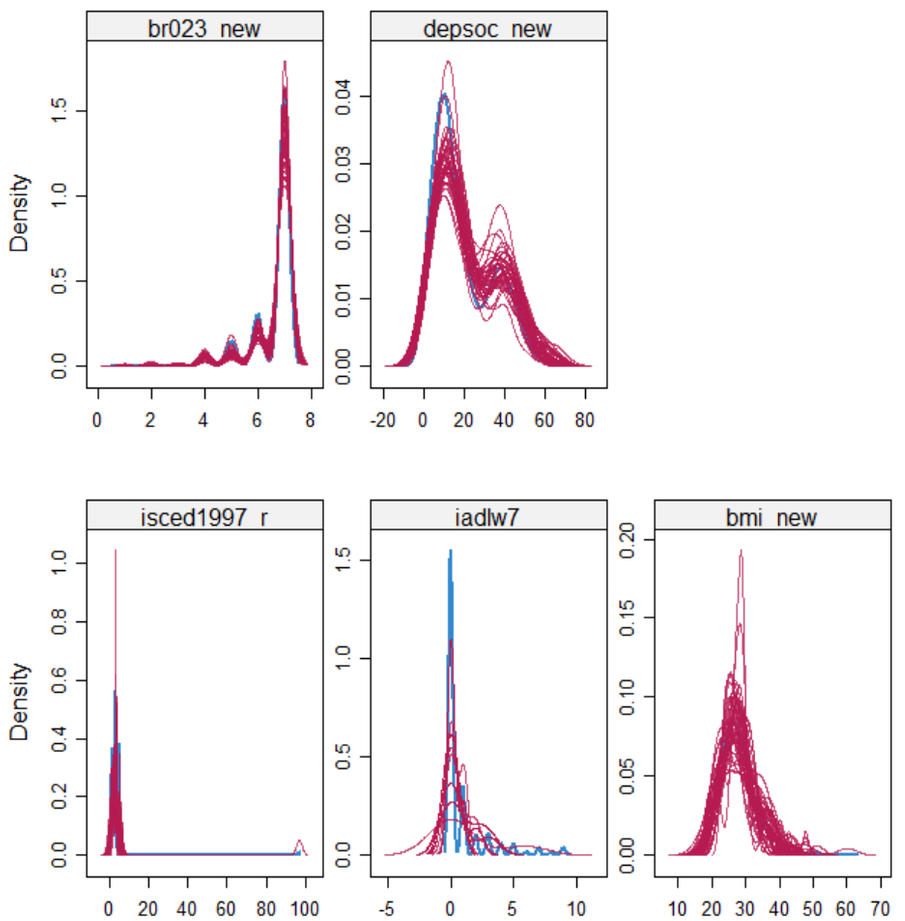
Model fit indices with MI: RMSEA = 0.044, 95% CI 0.033- 0.055, SRMR = 0.031, CFI = 0.980, TLI = 0.950. Est., estimate; SE, standard error; W, wave; IADL, instrumental activities of daily living. n = 971

Appendix 2.14. Convergence plots



Convergence plots. The plots show the mean (left) and standard deviation (right) of the imputed values for each of the imputed variables. w, wave; chronicw4_new, number of diagnosed chronic diseases; br023_new, alcohol intake; depsoc_new, social deprivation; isced1997_r, educational attainment; iadl, instrumental of daily living; bmi_new, body mass index. m=40

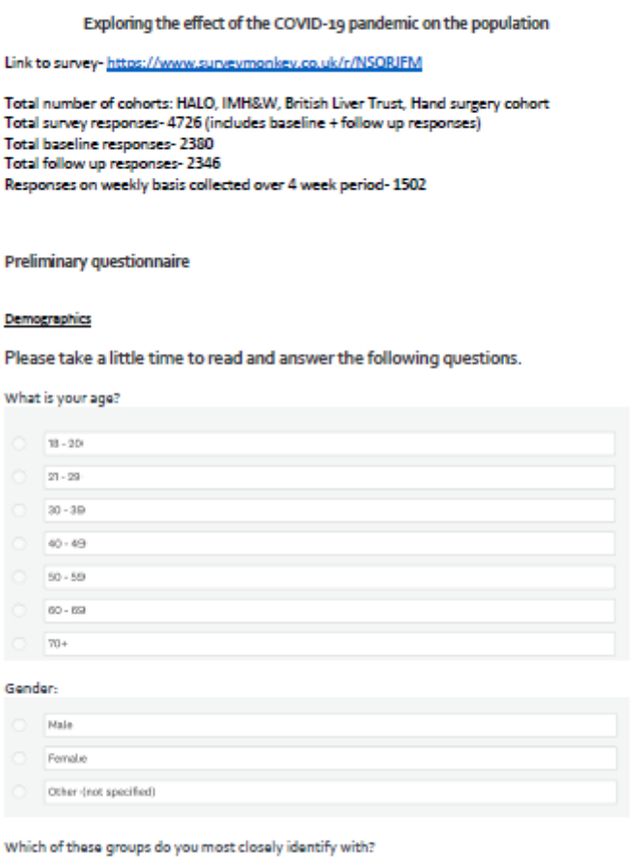
Appendix 2.15. Density plots



Density plots. Red lines depict imputed data while the blue line is the distribution of the original data. br023_new, alcohol intake; depsoc_new, social deprivation; isced1997_r, educational attainment; iadl, instrumental activities of daily living; bmi, body mass index. m=40

Appendix 3. Supplementary material supporting chapter 5

Appendix 3.1 Online survey for exploring the effect of COVID-19



Exploring the effect of the COVID-19 pandemic on the population

Link to survey- https://www.surveymonkey.co.uk/r/NSQRJFM

Total number of cohorts: HALO, IMH&W, British Liver Trust, Hand surgery cohort
Total survey responses- 4726 (includes baseline + follow up responses)
Total baseline responses- 2380
Total follow up responses- 2346
Responses on weekly basis collected over 4 week period- 1502

Preliminary questionnaire

Demographics

Please take a little time to read and answer the following questions.

What is your age?

- 18 - 20
- 21 - 29
- 30 - 39
- 40 - 49
- 50 - 59
- 60 - 69
- 70+

Gender:

- Male
- Female
- Other -(not specified)

Which of these groups do you most closely identify with?



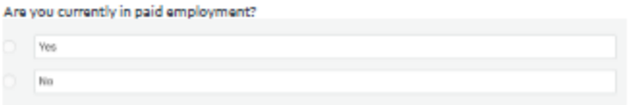
What is your height (in cm for example 180cm)?

What is your weight (in kg for example 72kg)?

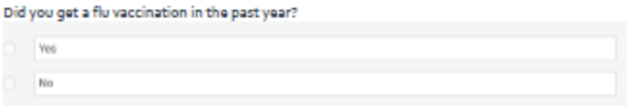
Please provide us with your current postcode

How many people are in your household?

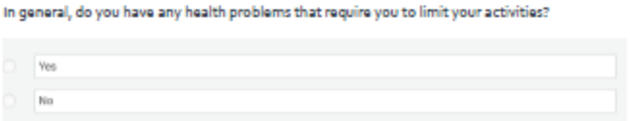
Are you currently in paid employment?



Did you get a flu vaccination in the past year?



In general, do you have any health problems that require you to limit your activities?



Do you suffer from heart disease?

- Yes
- No

Do you suffer from diabetes?

- Yes
- No

Do you suffer from lung disease/ asthma?

- Yes
- No

Do you suffer from kidney disease?

- Yes
- No

Do you suffer from any other health conditions, for example Liver disease or cancer?

Do you smoke?

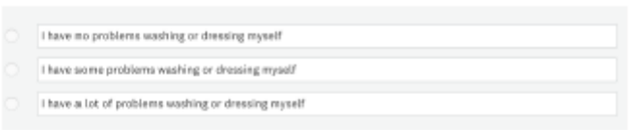
- Yes
- No

Quality of Life Questions

Under each heading, please tick the ONE box that best describes your health TODAY:

- I have no problems walking about
- I have some problems walking about
- I have a lot of problems walking about

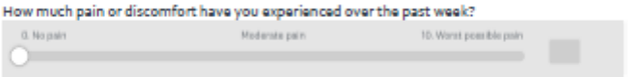
Looking after myself, please tick the ONE box that best describes your health TODAY:



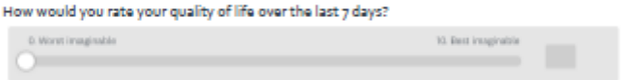
Doing usual activities please tick the ONE box that best describes your health TODAY (for example, work, study, housework, family or leisure activities):



How much pain or discomfort have you experienced over the past week?



How would you rate your quality of life over the last 7 days?



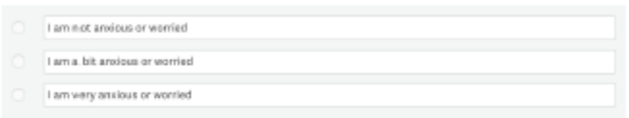
Health and Wellbeing
Measure of general health

How would you rate your general health:



Measure of self-reported mental health

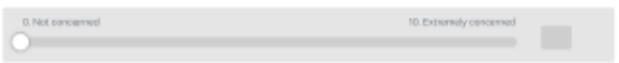
How anxious or worried are you feeling today?



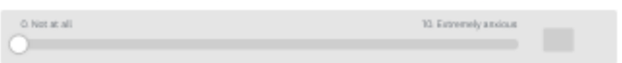
How sad or unhappy are you feeling today?



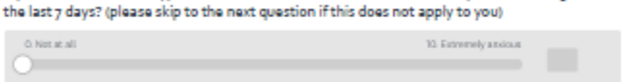
How much have you been concerned about the corona crisis in the past 7 days?



Measures for anxiety and depression
If you suffer from depression, how much has this condition been affected by the COVID-19 crisis in the last 7 days? (please skip to the next question if this does not apply to you)



If you suffer from anxiety, how much has this condition been affected by the COVID-19 crisis in the last 7 days? (please skip to the next question if this does not apply to you)



How would you rate your overall mental health?



COVID-19 infection and symptoms

Do you think you currently have/ had a COVID-19 infection?

- Yes
- No

Have you been tested for a COVID-19 infection using a mouth swab?

- Yes
- No

Have you been hospitalised with a COVID-19 infection?

- Yes
- No

How many days have you been ill?

How often have you experienced the following symptoms in the last 7 days:

- Headache
- Muscle pain/aches
- Difficulty breathing
- Fever/ high temperature
- Sore throat
- Dry cough
- Wet cough
- I felt physically exhausted
- loss of smell and taste

Measure of loneliness:

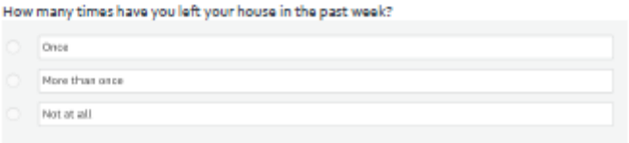
How often do you feel lonely?



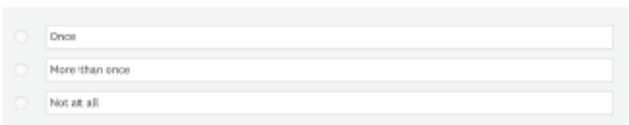
How socially isolated have you felt in the last 7 days?



How many times have you left your house in the past week?

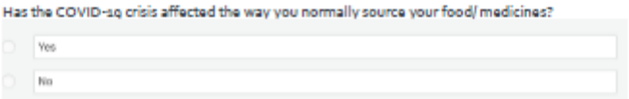


How often have you spoken to someone other than a professional (e.g. a doctor or nurse)?



Lifestyle, Diet and Physical activity

Has the COVID-19 crisis affected the way you normally source your food/ medicines?



Where do you currently source your groceries?

- Local supermarket
- Online shopping
- Local corner shops
- Farmer's market

Do you need help getting your groceries?

- Yes
- No

If yes, who helps you?

On average how many portions of fruit and veg do you currently consume in a day?

- 1-2 servings per day
- 3-4 servings per day
- 5 servings or more per day
- None

On average how many portions of rice, bread and pasta do you currently consume in a day?

- 1-2 servings per day
- 3-4 servings per day
- 5 servings or more per day
- None

On average how many sugary drinks do you currently consume in a day?

- 1-2 servings per day
- 3-4 servings per day
- 5 servings or more per day
- None

Compared to before the lockdown how has your consumption of alcohol changed?

- More alcoholic drinks (one or more drinks per day)
- Fewer alcohol drinks per day
- Same as before

How healthy is your eating pattern compared to the period before the COVID-19 crisis?

- Much less healthy
- Just as healthy
- Healthier

How would you rate your overall diet?

- Excellent
- Very good
- Good
- Fair
- Poor

How many hours per day of moderately intense exercise did you do before the Covid-19 crisis?

- Less than 1 hour
- 1-2 hours
- More than 2 hours
- NA

Has the COVID-19 crisis affected your ability to exercise?

- Yes
- No

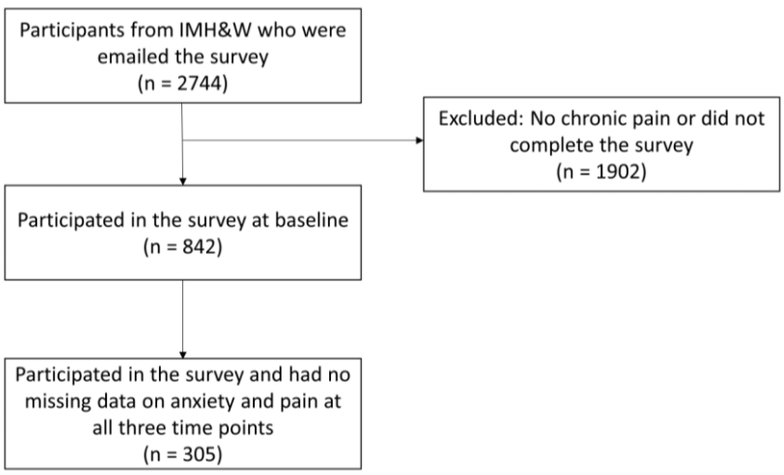
In the past week, on how many days have you done a total of 30 minutes or more of physical activity, which was enough to raise your breathing rate (*This may include sport, exercise, and brisk walking or cycling for recreation or to get to and from places, but should not include housework or physical activity that may be part of your job*)

0-7 as drop down box

Has the COVID-19 crisis changed the way that you use social media?

- No change
- Some small changes
- Significant changes

Appendix 3.2. Study flow chart



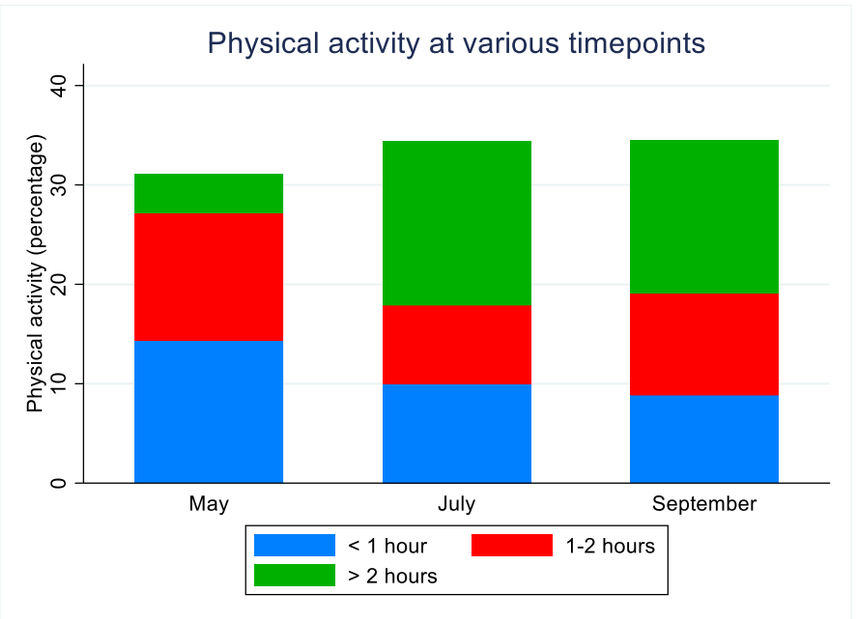
Study flow chart

Appendix 3.3. Correlation coefficients with Spearman's for all three time-points

	Age	**Sex**	**BMI**	**Pain**	**Anxiety**	**Depression**	**Loneliness**	**Physical activity**
Age	1.00							
Sex	0.15**	1.00						
BMI	-0.24**	-0.14**	1.00					
Pain	-0.06*	-0.01	0.05	1.00				
Anxiety	-0.10**	-0.06	-0.02	0.17**	1.00			
Depression	-0.07*	0.02	0.01	0.34**	0.37**	1.00		
Loneliness	-0.09**	-0.04	0.02	0.25**	0.25**	0.50**	1.00	
Physical activity	0.05	0.02	-0.04	-0.17**	-0.06	-0.06	-0.07*	1.00

* = p < .05, ** = p < .01, n=305

Appendix 3.4. Levels of physical activity



Levels of physical activity in May, July and September 2020.

Appendix 4. Supplementary files supporting chapter 6

Appendix 4.1. Figure showing histograms for Pressure Pain Thresholds (PPTs) within the baseline knee pain population (n=320)

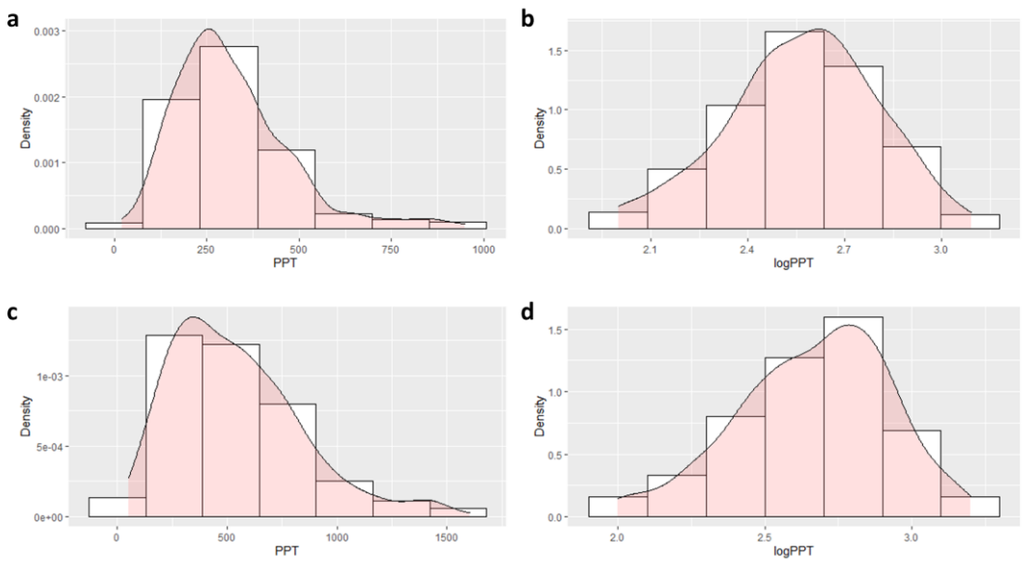
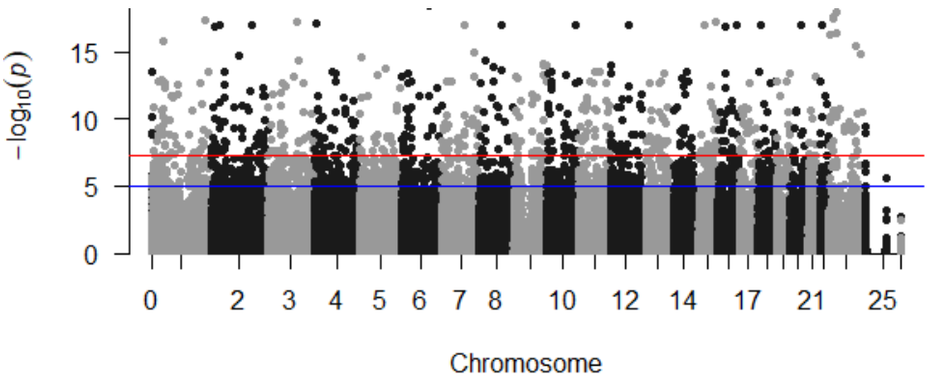
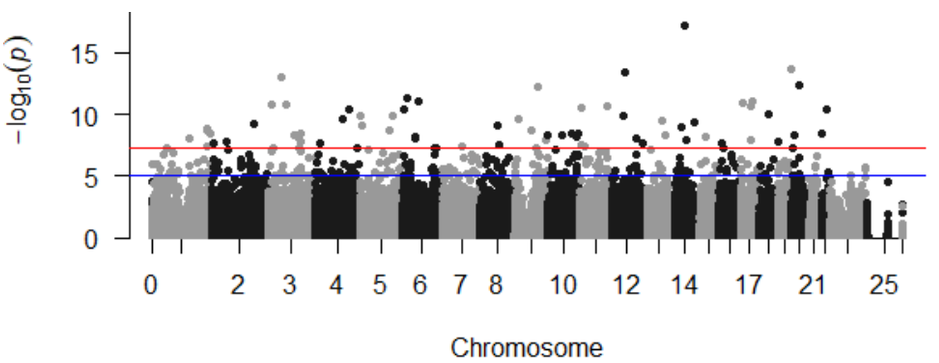


Figure. Histograms of: a. raw anterior tibia PPT, b. log-transformed anterior tibia PPT, c. raw lateral joint line PPT, and d. log-transformed lateral joint line PPT

Appendix 4.2. Manhattan plots of log-transformed anterior tibia PPT and log-transformed lateral joint line PPT phenotypes



Manhattan plot of log-transformed anterior tibia PPT



Manhattan plot of log-transformed lateral joint line PPT

Appendix 4.3. Demographic characteristics of the KPIC subgroup and the Nottingham post-TJR cohort.

Characteristics	Age	Sex (%, female)	BMI	Knee pain*	Opioids (%)	Anti-neuropathics (%)	Anxiety** % high/moderate	Depression** % high/moderate
KPIC (n=320)[1]	60.66 (9.40)	60.63	28.80 (5.79)	4.67 (3.35)	12.78	3.75	6.71 (4.32) 17.18	4.78 (3.53) 7.81
Radiographic knee OA (n=133)[1]	63.04 (8.78)	41.24	30.06 (6.49)	6.71 (2.49)	22.56	6.77	7.76 (4.51) 24.81	5.76 (3.73) 9.77
Controls (n=187)[1]	58.96 (9.48)	58.76	27.89 (5.05)	3.21 (3.13)	8.56	1.6	5.96 (4.03) 11.76	4.08 (3.22) 6.42
Nottingham post-TJR (n=613)[2]	70.98 (7.01)	54.16	30.44 (5.40)	4.15 (3.69)	30.69	6.12	-	- 19.01
Neuropathic-like pain (n=109)[2]	69.79 (7.34)	63.19	31.50 (5.78)	7.85 (1.77)	38.5	11	-	- 29.86
Controls (n=504)[2]	71.35 (6.87)	51.28	30.12 (5.24)	3.02 (3.37)	28.99	5.06	-	- 16.67

[1]The KPIC cohort includes 320 participants—133 individuals with radiographic knee OA (Kellgren–Lawrence radiographic score >2 in the tibiofemoral or patellofemoral compartments of either knee) and current knee pain (present most days, lasting more than 3 months), plus 187 controls (i.e. without radiographic knee OA and without persistent, lasting pain). For the KPIC cohort, no binary classification was carried out, pressure pain thresholds were analysed as a continuous variable adjusting for radiographic knee OA status.

[2]The Nottingham post-TJR cohort includes 613 individuals with severe OA who had undergone a joint replacement and pain where present refers to persistent joint pain after one or more years of surgery. For this cohort, individuals were classified as having possible or probable neuropathic-like pain (i.e. PainDETECT score > 12 on a scale from 0 to 39) or not (lower PainDETECT scores) making up 109 neuropathic-like pain participants versus 504 controls. The dichotomised value for the classification of participants to neuropathic-like pain versus controls was used for analysis and the results can be found in Warner, van Meurs, et al. (2017).

**Knee pain was measured with the Visual Analogue Scale*

***Anxiety and Depression were assessed with the Hospital Anxiety and Depression scale (HADS). Depression and anxiety were categorised using established clinical cutoffs for this instrument: moderate/high if HADS score > 10 and absent/low if HADS ≤ 10. Anxiety was not measured in the Nottingham cohort and thus is not reported.*

Appendix 4.4. The results of the top 10 SNPs from the adjusted Illumina array anterior tibia PPT GWAS and their corresponding genes

SNP ID	**rs140673111**	**rs2099135**	**rs113274983**	**rs79567074**	**rs77959805**	**rs71524349**	**rs3805048**	**rs112247130**	**rs112942946**	**rs113993320**
Location	17:69194427-69194427	8:111438263-111438263	10:44876147-44876147	19:390200-390200	12:14893636-14893636	4:6289024-6289024	X:32488618-32488618	6:44301434-44301434	18:39220166-39220166	4:99420661-99420661
CHR	17	8	10	19	12	4	23	6	18	4
BP	67190568	112450492	45371595	390200	15046570	6290751	32506735	44269171	36800130	100341818
Effect allele	G	G	G	A	G	C	C	A	A	G
Gene or closest gene	ABCA10	LINC02237	TMEM72-AS1	AC010641.2	C12orf60	WFS1	DMD	AARS2	MIR924HG	ADH7
Beta	-2.56	-2.56	-2.56	-2.56	-2.56	-2.56	-1.28	-2.56	-2.56	-2.56
STAT	-10.42	-10.34	-10.33	-10.32	-10.31	-10.31	-10.31	-10.3	-10.3	-10.3
GWAS P	5.22E-22	1.01E-21	1.02E-21	1.12E-21	1.18E-21	1.19E-21	1.21E-21	1.21E-21	1.21E-21	1.21E-21
Adjusted P*	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18	2.86E-18

*adjusted P value after correction for multiple testing with B–H

Appendix 4.5. The results of the top 10 SNPs from the adjusted Illumina array lateral joint line PPT GWAS and their corresponding genes

SNP ID	**rs146956711**	**rs201446493**	**rs112246679**	**rs6128548**	**rs116877798**	**rs76603930**	**rs72839866**	**rs115198029**	**rs145320435**	**rs75600413**
Location	18:75287913-75287913	6:116925456-116925456	1:91976319-91976319	20:59363367-59363367	13:98853444-98853444	3:38059757-38059757	17:6018700-6018700	3:33132671-33132671	14:60125773-60125773	12:56004018-56004018
CHR	18	6	1	20	13	3	17	3	14	12
BP	72999868	117246619	92441876	57938422	99505698	38101248	5922020	33174163	60592491	56415348
Effect allele	A	A	A	A	G	A	A	A	G	A
Gene or closest gene	TSHZ1	RFX6	BRDT	AL035250.2	DOCK9	DLEC1	AC007846.2	CRTAP	PCNX4	IKZF4
Beta	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-1.71	-1.51
STAT	-10.96	-10.96	-10.96	-10.96	-10.96	-10.96	-10.96	-10.94	-9.195	-7.945
GWAS P	6.88E-24	6.88E-24	6.88E-24	6.88E-24	6.88E-24	6.88E-24	6.88E-24	7.93E-24	5.32E-18	3.47E-14
Adjusted P*	2.25E-19	2.25E-19	2.25E-19	2.25E-19	2.25E-19	2.25E-19	2.25E-19	2.32E-19	1.34E-13	7.71E-10

*adjusted P value after correction for multiple testing with B–H

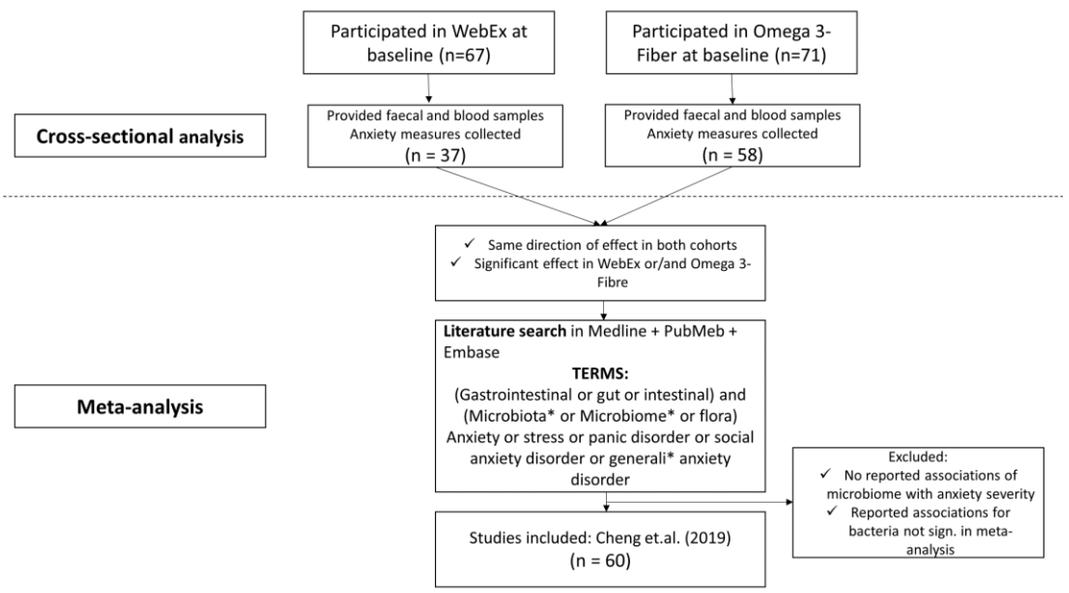
Appendix 4.6. The results from the fixed-effects gene meta-analysis of anterior tibia PPT (KPIC cohort) and neuropathic-like pain (Nottingham post-TJR cohort).

Gene name	CHR	ZSTAT	P	FDR-BH P
Wnt family member 9A (WNT9A)	1	5.71	5.79E-09	9.83E-05
RNA polymerase III subunit E (POLR3E)	16	5.25	7.58E-08	4.39E-04
absent in melanoma 2 (AIM2)	1	5.25	7.75E-08	4.39E-04
hdc homolog, cell cycle regulator(HECA)	6	5.03	2.49E-07	1.06E-03
interferon gamma receptor 1(IFNGR1)	6	4.95	3.67E-07	1.06E-03
dynein axonemal heavy chain 7(DNAH7)	2	4.95	3.74E-07	1.06E-03
coiled-coil domain containing 14(CCDC14)	3	4.80	8.04E-07	1.95E-03
transcription factor B1, mitochondrial(TFB1M)	6	4.48	3.67E-06	7.71E-03
cell division cycle 27(CDC27)	17	4.45	4.24E-06	7.71E-03
DEAD-box helicase 59(DDX59)	1	4.43	4.70E-06	7.71E-03
WD repeat domain 25(WDR25)	14	4.42	4.99E-06	7.71E-03
rhophilin associated tail protein 1(ROPN1)	3	4.38	5.92E-06	8.38E-03
low density lipoprotein receptor class A domain containing 4(LDLRAD4)	18	4.33	7.57E-06	9.89E-03
claudin 20(CLDN20)	6	4.22	1.23E-05	1.48E-02
histamine receptor H1(HRH1)	3	4.20	1.31E-05	1.48E-02
E74 like ETS transcription factor 1(ELF1)	13	4.16	1.59E-05	1.69E-02
cell adhesion molecule 2(CADM2)	3	4.11	1.94E-05	1.94E-02
nuclear cap binding subunit 3(NCBP3)	17	4.06	2.46E-05	2.33E-02
GTPase, IMAP family member 8(GIMAP8)	7	4.03	2.77E-05	2.48E-02
monoacylglycerol O-acyltransferase 2(MOGAT2)	11	3.97	3.56E-05	3.02E-02
cadherin 18(CDH18)	5	3.92	4.43E-05	3.58E-02

*adjusted P value after correction for multiple testing with B–H

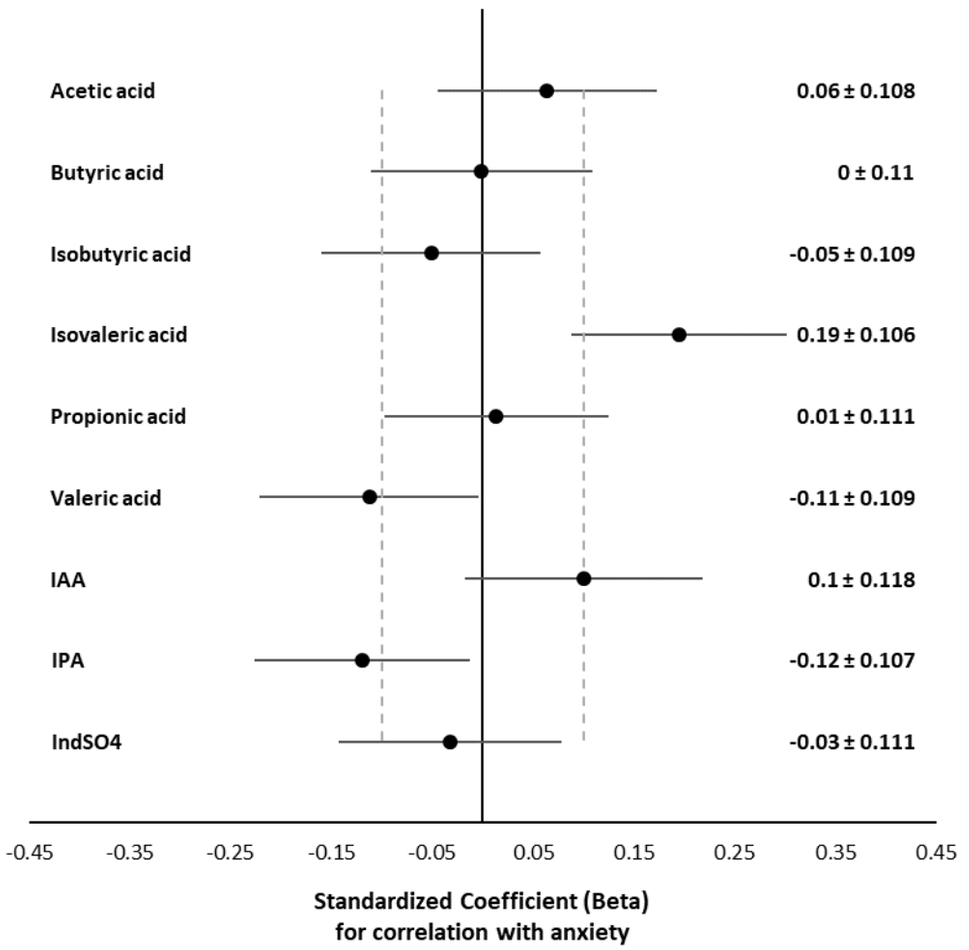
Appendix 5. Supplementary material supporting chapter 7

Appendix 5.1. CONSORT flow diagram



CONSORT flow diagram

Appendix 5.2. Forest plot of meta-analysis of linear regressions of anxiety with gut-derived metabolites (SCFAs and indoles).



Forest plot of meta-analysis of linear regressions of anxiety with gut-derived metabolites (SCFAs and indoles). No association reached statistical significance. Abbreviations: IAA: Indole-3-acetic acid, IPA: 3-indolepropionic acid, IndSO4: Indoxyl sulfate

Appendix 5.3. Linear regressions with anxiety

Bacteria	**Omega 3/Fibre (n = 58)**			**WebEx (n = 37)**		
	Beta	SE	P	Beta	SE	P
Butyricicoccus	-0.014	0.13	0.91	-0.52	0.15	0.00097
Escherichia-Shigella	0.047	0.17	0.78	0.45	0.15	0.005
Lachnospiraceae ambiguous taxa	-0.20	0.13	0.13	-0.46	0.15	0.005
Paeniclostridium	0.16	0.13	0.22	0.37	0.16	0.0225
Eubacterium	0.10	0.13	0.43	0.38	0.17	0.0353
Lachnospiraceae ND3007 group	-0.17	0.13	0.18	-0.35	0.16	0.036
Ruminococcaceae UCG-005	-0.28	0.13	0.032	-0.26	0.17	0.14
Ruminococcaceae UCG-010	-0.38	0.13	0.004	-0.26	0.18	0.15
Lactobacillus ambiguous taxa	0.40	0.15	0.012	0.028	0.17	0.87
Prevotellaceae NK3B31 group	-0.45	0.20	0.032	-0.011	0.17	0.94
Christensenellaceae R-7 group	-0.36	0.15	0.025	-0.28	0.17	0.10
Lachnospiraceae UCG-001	-0.27	0.14	0.045	-0.18	0.17	0.28
Ruminococcaceae UCG-002	-0.28	0.14	0.045	-0.088	0.17	0.37
Ruminococcus 2	-0.29	0.14	0.038	-0.094	0.17	0.58

Abbreviations: SE, standard error.