Epidemiology and genetics of catastrophizing and pain in OA

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Declaration

All work presented in this thesis is my own. There are no conflicts of interest to declare.

Dedication

To my mum and brother, there are no words to accurately describe how much you mean

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List of author's publications

Valdes A. M., Warner S.C., Harvey H.L., Fernandes G.S., Doherty S., Jenkins W., Wheeler M., Doherty M., *Use of prescription analgesic medication and pain catastrophizing after total joint replacement surgery.* Seminars in Arthritis and Rheumatism, 2015. **45**(2): p. 150-155.

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List of Abbreviations

ACC - Anterior Cingulate Cortex

ACLT- Anterior Cruciate Ligament Transection

ACR - American College of Rheumatology

ANS - Autonomic Nervous System

arcOGEN- Arthritis Research Council Osteoarthritis Genetics

ASPN - Asporin Proteoglycan

BLA- Basolateral Complex of the Amygdala

BMI – Body Mass Index

BML – Bone Marrow Lesion

BMP - Bone Morphogenetic Protein

CBT- Cognitive Behavioral Therapy

CCK- Cholecystokinin

CE- Central Nucleus of the Amygdala

CEN- Central Executive Network

CI- Confidence Interval

CILP - Cartilage Intermediate Layer Protein

CNS- Central Nervous System

COMT- Catechol-O-Methyltransferase

CVD- Cardiovascular Disease

CWP - Chronic Widespread Pain

DLPT- Dorsolateral Pontine Tegmentum

DMM- Destabilisation of the Medial Meniscus

DNA- Deoxyribonucleic Acid

DNIC – Diffuse Noxious Inhibitory Controls

EULAR- European League Against Rheumatism

fMRI - functional Magnetic Resonance Imaging

GDF5 - Growth Dependent Factor 5

GOAL- Genetics of Osteoarthritis and Lifestyle study

GWAS- Genome Wide Association Scan

HGP - Human Genome Project

HPA- axis – Hypothalamus Pituitary Adrenal -axis

IA- Intra-Articular

IL-1 – Interleukin-1

IVU- Intravenous Urogram

K/L - Kellgren-Lawrence

LA- Local Anaesthetic

LD- Linkage Disequilibrium

MIA- Mono-Iodoacetate

MOS- Medical Outcomes Survey

MRI- Magnetic Resonance Imaging

ncRNAs - non-coding RNAs

NGF - Neuronal Growth Factor

NGOAS- Nottingham Genetics of Osteoarthritis study

NICE- The National Institute for health and Care Excellence

NP- Neuropathic-like Pain

NSAIDs- Non-Steroidal Anti-Inflammatory Drugs

nsSNP- Non-synonymous Single Nucleotide Polymorphism

OA – Osteoarthritis

OR- Odds Ratio

PAG - Periaquaductal Gray Area

PCQ- Pain Coping Questionnaire

PCS – Pain Catastrophizing Scale

PDQ- Pain Detect Questionnaire

PET - Positron Emission Tomography

PFC - Pre-Frontal Cortex

PNS - Parasympathetic Nervous System

PT - Post-Traumatic

QQ- Quartile-Quartile

QTL – Quantitative Trait Locus

RA - Rheumatoid Arthritis

rCBF- regional Cerebral Blood Flow

ROA- Radiographic Osteoarthritis

RVM - Rostral-Ventromedial Medulla

SMAD3 - SMAD family member 3

SNP – Single Nucleotide Polymorphism

SNS - Sympathetic Nervous System

SPA- Sensitivity to Physical Exercise

TGFβ – Transforming Growth Factor beta

THR – Total Hip Replacement

TJR – Total Joint Replacement

TKR – Total Knee Replacement

TNF-α - Tumour Necrosis Factor-alpha

VAS – Visual Analogue Scale

WNT - Wingless-Type

WOMAC – Western Ontario McMaster Arthritis Index

Abstract

Background:

Pain is the main reason patients present with Osteoarthritis (OA). There are many risk factors for increased pain but one risk factor that is the focus of this thesis is catastrophizing. There is a discrepancy between the amount of radiographic damage to the joint and the pain intensity felt by the OA patient, which leads to the hypothesis that there must be other non-structural factors such as catastrophizing that could explain this discrepancy.

Objectives:

(1) To assess the effect of catastrophizing on joint and body pain in individuals who have undergone total joint replacement surgery after accounting for sleep quality, anxiety and depression in people diagnosed with large joint OA and controls. X-rays and resulting K/L grade also used to account for joint damage.

(2) To identify if there are specific genes and pathways that could explain the relationship between catastrophizing and pain, also whether catastrophizing is a trait in its own right which is not dependent on depression or bad quality sleep.

(3) To identify genetic factors which contribute to the effect of catastrophizing on pain intensity in OA by comparing those with symptomatic OA to those with asymptomatic OA.

Methods:

Pain post- Total Joint Replacement (TJR) (Chapter 3): This is a case-control study design. Cases were derived from the Nottingham genetics of OA study (NGOAS) and the Genetics of Osteoarthritis and Lifestyle Study (GOAL) cohorts that have undergone joint replacement. Controls were derived from participants in the GOAL cohort that were selected as controls for OA. 1093 individuals from the NGOAS cohort who had been diagnosed with knee or hip OA were recruited from secondary care (82% post-total joint replacement (TJR) none on waiting list) and completed a questionnaire which included items on: joint pain, neuropathic pain like symptoms (using the painDETECT questionnaire), anxiety (using the hospital anxiety and depression scale (HADS) questionnaire), depression (using the HADS questionnaire) and the 13-item pain catastrophizing scale (PCS). A similar questionnaire was sent to participants from a case control OA study known as the GOAL involving 679 cases all with hip or knee OA (59.7% post-TJR, none on waiting list) and 402 non-OA controls. TJR used as cases due to this being the end point of OA with those undergoing TJR most likely in pain from OA.

High pain catastrophizing was defined as being in the top tertile of the PCS (score≥9).

SPSS was then used to calculate descriptive statistics such as percentage of participants that were female, mean age, BMI. This would give a picture of the cohort being used. Then binary logistic regressions were used to calculate odds ratios (ORs) for sleep, depression, anxiety and catastrophizing whilst adjusting for confounding factors such as age, BMI, gender.

Genetics (Chapter 4 and 5): Quantile-Quantile (QQ) and Manhattan plots were produced using R to quality test the genomic data. A Manhattan plot is a type of scatter plot, usually used to display data with a large number of data-points - many of

non-zero amplitude, and with a distribution of higher-magnitude values, for instance in genome-wide association studies (GWAS). The statistics program R (version 3.0.2) was used to create Manhattan and QQ plots using the "ggplot2" library and "qqplot" script.

A genome wide association scan (GWAS) and single nucleotide polymorphism (SNP) extractions, conducted using Plink, were carried out on a subset of 1113 post TJR cases from the NGOAS cohort and pathway analysis was then conducted based on significant SNPs found in the GWAS.

In chapter 4 replication and meta-analysis were then carried out using a separate cohort of 679 participants, taken from the GOAL study.

Results:

Pain post- total joint replacement (TJR) (Chapter 3): The prevalence of high catastrophizing was not significantly higher in OA and post TJR cases (36.8%) than in controls (33.1%)($p<0.07$), however as controls were from intravenous urography (IVU) clinics they would have higher levels of cardio-vascular disease, stroke, kidney disease for example than general population. The main factors associated with catastrophizing were older age, higher body mass index, high pain intensity, anxiety, depression and multiple regional pain. High catastrophizing was associated with increased risk of severe joint (hip or knee) pain after adjustment for sleep quality, anxiety and depression (OR= 2.99, 95% CI= 2.20-4.04, p< 1.54E-12). Similarly, high catastrophizing was associated with body pain (OR=1.47, CI=1.16-1.86, P<0.0002) and neuropathic-like pain symptoms (OR=5.35, CI=4.16-6.90, P<1.42E-35). I also explored if these associations with pain were due to the presence of comorbidities

which are common in OA patients and post-TJR. I did find that pain catastrophizing scores were also associated with cardiovascular disease (CVD) after adjustment for depression and sleep quality as well as BMI, age and gender (OR= 1.44, 95% CI= 1.14-1.81, p< 0.001).

Genetics (Chapter 4 and 5): Several SNPs were linked to high catastrophizing scores with a p-value <0.00005.

ADRA2C was the only gene associated with catastrophizing that replicated in chapter 4 after meta-analysis. This variant was associated with OR=0.67, 95% CI= 0.56-0.80, p-value=7.8x10[-6]. It is involved in the formation of adrenergic receptors and therefore the regulation of possibly many pain pathways including the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis as well as being required for normal presynaptic control of transmitter release in the heart.

PRR20 was another interesting finding in chapter 4. It is involved in dopamine receptors, so possibly influencing that particular pain pathway.

These data demonstrate that catastrophizing does influence pain intensity and implicates certain molecular pathways that may be involved in this relationship.

LEPRE1 and *COL6A1* are both implicated in chapter 5. Both of these genes are involved in collagen formation and destruction thereby could affect the progression of OA and joint structure leading to pain. *LEPRE1* is further known as one of the genetic causes of Osteogenesis imperfecta type VIII another bone formation disorder.

Conclusion:

Pain catastrophizing is associated with joint and body pain in individuals with OA after TJR and this effect is not mediated by sleep quality, anxiety or depression. Therefore catastrophizing can be said to be a trait in its own right.

Many genes and pain pathways were implicated by the genetics studies, mainly involving adrenaline and dopamine, so more focus on these neurotransmitters could be suggested for future study

Another interesting finding that could be investigated further is the link between cardiovascular disorders and catastrophizing as this is a relatively unexplored area, made more interesting by the finding of *ADRA2C* both indicating involvement of the sympathetic nervous system and other central mechanisms.

1 Introduction

1.1 Osteoarthritis (OA)

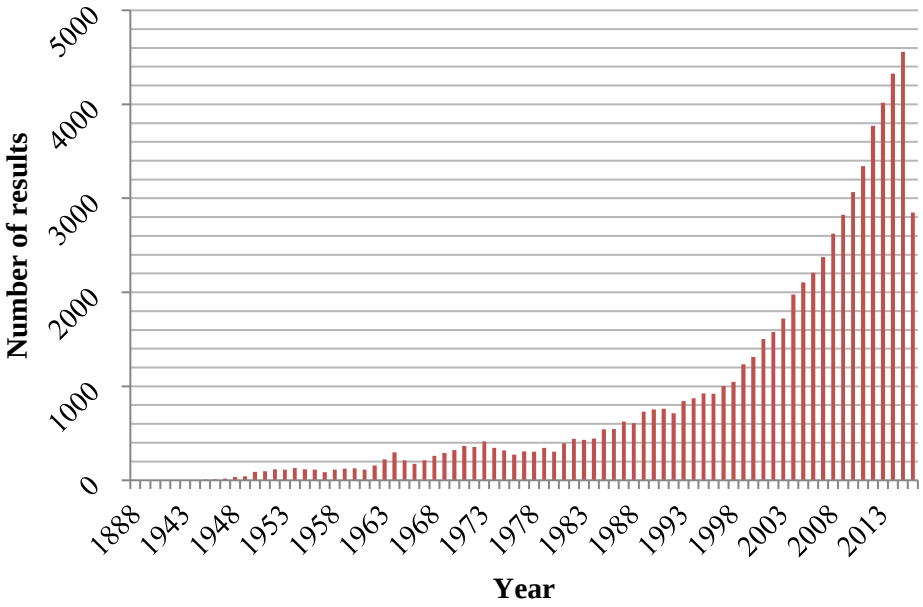
OA is a synovial joint disorder that results in focal loss of articular cartilage, osteophyte formation, and subchondral bone remodelling which can associate with pain (though most is asymptomatic), disability, and a reduction in quality of life.(Valdes, 2013, Aspden, 2008, Evangelou E., 2010, CDC, 2014, Arden N., 2006, Saadat E., 2010, Doherty, 1994)

OA is the most common form of arthritis (Eckstein F., 2006, Jones, 2013, CDC, 2014, Arden N., 2006, Saadat E., 2010) and can occur in any synovial joint in the body, but is most common in the hands (Zhai G., 2009, Kerkhof H. J. M., 2011b, CDC, 2014, Arden N., 2006), knees (Kerkhof H. J. M., 2011b, CDC, 2014, Arden N., 2006), hips (Kerkhof H. J. M., 2011b, CDC, 2014, Arden N., 2006), and spine (CDC, 2014, Arden N., 2006, Saadat E., 2010). A single joint can be involved, but more frequently several joints are affected.

Recent estimates suggest that 250 million people worldwide suffer from knee OA. (Hunter D. J., 2013) Inheritance studies in twins and other family-based studies have assessed the estimated heritability for OA to be in the range of 40–65% depending on the joint site (Evangelou E., 2014, Valdes A. M., 2011b, Kerkhof J. M., 2008, Kerkhof H. J. M., 2010a), demonstrating the genetic impact OA has and why it is a valid field of research.

1.1.1 History of research

Figure 1-1: History of Osteoarthritis Research



Using PubMed a search was conducted for the number of studies per year focused on Osteoarthritis (OA)

OA is a well-developed field of research. As can be seen in figure 1-1 research was first carried out and published as early as 1888, but has grown considerably in recent years.

Research began with a focus on articular cartilage as cartilage loss was seen to be the main contributor to the progression of OA and the cause of most symptoms (McDougall J. J., 2012), due to cartilage being subjected to progressive and accumulative wear and tear during an individual's lifetime. (Schaible, 2012) As articular cartilage is aneural and avascular, this tissue is unlikely to be the source of OA pain (McDougall J. J., 2012), as both neurons and a blood supply are needed for signals to be transmitted and produce pain behaviour. This coupled with the findings that bone remodelling and attrition occur relatively early in the disease (Schaible, 2012) and also no correlation being reported between the damage of articular cartilage

and pain in OA patients, or in preclinical models of OA, caused the focus to shift towards the study of other tissues in the joint such as bone and synovium (McDougall J. J., 2012). All of the above suggests the focus of research now seems to be on the prevention of progression of OA and also genetics as a way to possibly detect more risk factors and systems involved in disease progression.

1.1.2 Aetiology

The aetiology of OA is multifactorial (Evangelou E., 2009, Evangelou E., 2014, Kerkhof J. M., 2008, Warner. S.C., 2016) with a significant genetic component as shown by twin and family studies.(Evangelou E., 2010, Kerkhof J. M., 2008)

OA is heterogeneous and characterized by failure of the synovial joint organ. The disease occurs when the dynamic equilibrium between the breakdown and repair of joint tissues becomes unbalanced, with both degradation and synthesis usually enhanced (Hunter D. J., 2013), with reparative attempts being outmatched by cartilage degradation as disease continues (Lorenz H., 2006) often in a situation where the mechanical loads applied exceed those that can be tolerated by the joint tissues. (Hunter D. J., 2013) Most OA never associates with pain or disability and may remain relatively static if it compensates for the triggering insults.

1.1.3 Risk Factors

Several risk factors affect hip and knee OA, namely higher body mass index (BMI), increasing age, female gender, occupational activity and injury, congenital

abnormalities and joint shape, meniscal tears, presence of OA at other joints, foot and knee alignment in addition to genetic predisposition.(Valdes, 2013, Doherty, 1994)

OA is age-related, being uncommon before 40 years, and then rising in frequency with age, such that it is estimated to affect 40% of people over the age of 70 years continuing to affect 80% of those aged over 75, with most having radiological evidence of osteoarthritis in some joints (Dieppe P. A., 2005, Eckstein F., 2006, Kerkhof H. J. M., 2011a, Arden N., 2006).

Gender is another risk factor for developing OA. Hand OA and knee OA are more common in women and are significantly associated with functional impairment and reduced independence.(Zhai G., 2009) Furthermore oestrogen levels affect bone formation. (Colaianni G., 2014) Moreover the accelerated rise in incidence of OA in women following menopause suggests a possible role for sex hormones, particularly oestrogen deficiency, in the systemic predisposition to OA (Arden N., 2006) a fact which agrees with the above speculation of oestrogen affecting bone formation.

Another risk factor for OA is direct joint injury. An estimated 12% of symptomatic OA may be attributed to a post-traumatic (PT) cause and differences in radiographic patterns have been reported between primary and PT knee OA cases.(Valdes A. M., 2013) Mechanopathology or aberrant loading is yet another risk factor for structural OA.(Schaible, 2012) Periarticular muscles influence joint loading, and impairments in muscle function have been observed in people with OA. Sharma et al (2003) found that reduced absolute quadriceps and hamstrings strength and poor proprioceptive acuity increased the likelihood of poor physical functioning (Sharma L., 2003, Hunter

D. J., 2013). Another risk factor likely to impact mechanopathology is obesity. This together with occupational risk, physical activity risk, trauma and joint alignment highlight the importance of mechanics and structure of joints in OA.

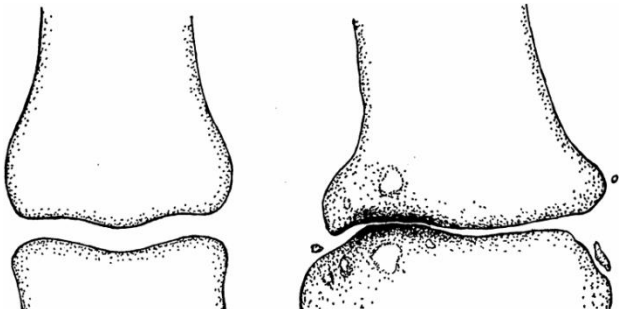
Presence of OA at one site increases the risk of OA at other sites. The presence of Heberden's nodes in cases of hand OA has been reported to confer a 6-fold increased risk of progression of knee OA. Valdes et al (2010) results provide further evidence that the nodal phenotype is associated with a higher prevalence of OA at more than one joint, as shown by firstly the higher risk of undergoing both a hip and a knee replacement in nodal cases of large joint OA, and secondly the higher risk of bilateral TKR in nodal cases. (Valdes A. M., 2010b)

Results of heritability studies in twins, sibling pairs, and families, show genetic factors are estimated to account for about 50% of the risk of developing OAin the hip or knee. (Zeggini E., 2012) This indicates genetic factors contribute to risk of OA, something which is discussed later in this chapter.

1.1.4 Pathology

For the anatomy of the knee and hip joints and how they are affected by OA see Figure 1-2 and Figure 1-3

Figure 1-2: Diagram of knee joint



Showing a normal joint on the left and the changes that can occur with Osteoarthritis (OA) on the right including osteophytes, cysts and joint space narrowing

Figure 1-3: X-ray of a hip joint



This X-ray shows the hip joint is a ball and socket joint unlike the knee. However the same changes occur in Osteoarthritis (OA), specifically focal (superior) joint space narrowing, marginal osteophytes and cysts

Pathologically, OA is characterized by progressive degradation of type II collagen and destruction of articular cartilage, subchondral bone sclerosis, synovitis and osteophyte formation as well as bone marrow lesions (BMLs).(Schaible, 2012, Betancourt M. C. C., 2012, Evangelou E., 2010, Arden N., 2006) Structural changes visible on radiography include narrowing of the joint space, osteophytes and bone remodelling

around the joints. (Aspden, 2008, Valdes, 2013, Evangelou E., 2010) Subchondral bone sclerosis is a reparative change where subchondral new bone is formed (Doherty, 1994). Synovitis is known as the inflammation of the synovium. Osteophytes are bony spurs that develop at the periphery of the joint (Arden N., 2006, Doherty, 1994). BMLs and cysts are lesions formed due to the process of cartilage denudation and subsequent increased intra-articular pressure. (Doherty, 1994)

The synovial layer of OA joints shows an increased number of lining cells and a mixed inflammatory infiltrate, mainly macrophages and lymphocytes, but the percentage of macrophages, T cells, and B cells is much lower in OA than in rheumatoid arthritis (RA) (Schaible, 2012, Valdes A. M., 2011e), as well as synovial changes being more localised in OA but involving the whole synovium in RA. Cytokines in particular, Tumour Necrosis Factor-alpha (TNF-α) and Interleukin (IL)-1 have been suggested to be key factors in OA pathogenesis, both in synovial inflammation and in activation of chondrocytes. Also the presence of activated T-Cells and Th1 cytokine transcripts in chronic joint lesions of patients with OA indicates that T cells contribute to chronic inflammation. (Valdes, 2013, Valdes A. M., 2010a) However, OA is not considered to be inflammatory because of the absence of neutrophils and minimal levels of other pro-inflammatory cells in the synovial fluid and systemic inflammatory responses.(Valdes A. M., 2010a) Therefore it could be said the low degree of inflammation in OA is consistent with breakdown of redundant tissue and production of new tissue in response to tissue injury.

OA is a dynamic process with increased metabolic activity and new tissue production, increased activity and numbers of chondrocytes (Valdes, 2013, Arden N., 2006). Tissue restoration and remodelling in OA are regulated by signalling pathways that are also involved in the development of cartilage and bone. In particular, Transforming Growth Factor beta (TGFβ), Bone Morphogenetic Protein (BMP), and Wingless-Type

(WNT) signalling have been studied in OA and contribute to genetic susceptibility to OA.(Valdes, 2013)

Pain is the most major symptom of OA and is discussed in detail later in this chapter.

1.1.5 Diagnosis

The National Institute for Health and Care Excellence (NICE) set guidelines for the diagnosis, assessment and approach to managing OA (see Figure 1-4). These are periodically updated in accordance with new research. The American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) also publish guidelines on this topic.(Altman R., 1990, EULAR, 2016, Hochburg M.C., 2012).Pain and restricted movement/activity are the main reasons that patients present with OA (Aspden, 2008, Valdes, 2013, Hunter D. J., 2013).

The common clinical features of OA that allow a diagnosis to be made are: increased age (45yrs+), pain, stiffness, bony swelling and crepitus (Fernandes L., 2013). Also when OA is advanced it may be evident on plain radiographs (Dieppe P. A., 2005, Saadat E., 2010). Although radiographs are relatively inexpensive and easily available, they only allow gross visualization of changes in bone and joint space. They also entail exposure to ionizing radiation and offer poor reproducibility for characterizing OA related degeneration. Radiographs are insensitive to the earliest changes associated with OA and to differences between the acetabular as well as the femoral cartilage. Magnetic resonance imaging (MRI) offers greater soft-tissue contrast and allows direct visualization of cartilage, labrum, BMLs, subchondral cysts and other soft-tissue pathologies. (Kumar D., 2013, Mink J. H., 1987). MRI can further be used to identify meniscal abnormalities that may not be detected by X-ray

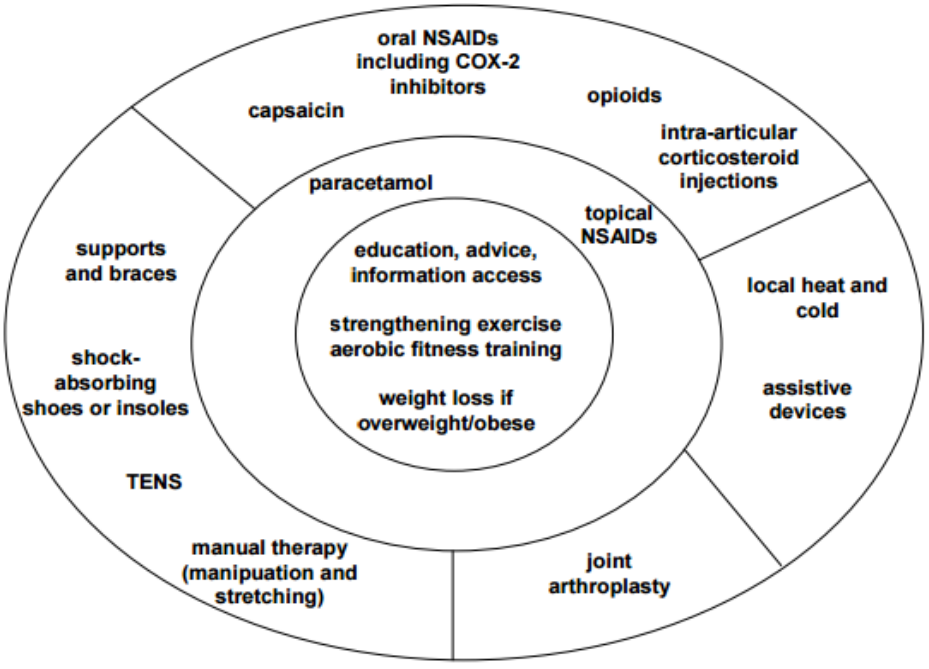
imaging (Mink J. H., 1987), which is important as meniscal damage can lead to secondary OA a fact which is exploited in the DMM animal model.

It is therefore a mixture of clinical presentation and symptoms such as: usage-related pain, slow-to-change; very little early morning or inactivity stiffness, in a middle-aged or older person; and on examination reduced movement, crepitus, bony swelling, possible mal-alignment and only mild/modest signs of inflammation that allow a diagnosis to be made. As NICE states, imaging with X-Rays or MRI is not essential for the clinical diagnosis of OA.

1.1.6 Treatment

NICE guidelines for treatment are shown in Figure 1-4. ACR also updated their guidelines in 2012 to include literature up to 2010 (Hochburg M.C., 2012)

Figure 1-4: NICE guidelines for treatment. This shows the 1st line treatments in the centre and then suggested progressions



Current clinical management of OA focuses on pain control, as this is the major symptom of OA.(Betancourt M. C. C., 2012) The current treatment options for chronic pain are scarce; symptom relief by using analgesics and physical therapy are the most often used therapies (Kerkhof H. J. M., 2014, Hochberg M. C., 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) have been found to be more effective at treating OA pain than paracetamol but have potentially serious side-effects and are often contraindicated in older people with co-morbidity (Mayor, 2016, Excellence, 2014). Opioids are another well-known analgesic used to treat OA pain, but recently misuse of opioids has become evident as an issue in prescribing this medication for long term pain relief (Valdes A. M., 2015, Sehgal N., 2012, Martel M. O., 2013). A further method of pain relief is intra-articular steroid injection (Chou C. L., 2015); for a review of this technique see Su et al (2015). One of the most recent studies in this field by Henricsdotter et al (2016) found no statistically significant effects of steroid injection over placebo injection when this treatment was combined with exercise therapy in all groups using changes in synovial hypertrophy, Doppler activity presence, or presence of Baker's cysts as markers of change (Henricsdotter C., 2016). This would indicate that the injection is no better than the placebo; however this study did also measure exercise as a contributor. A further study by Zhang *et al* (2010) showed from two systematic reviews published between 2006 and 2010 Intra-articular (IA) steroid injections had significant effect on pain but not on physical function or stiffness (Zhang W., 2010).

Despite this, while multidisciplinary pain management strategies help patients cope, there is still a recognised, unmet need for the development of novel, more efficacious treatments for persistent pain (Howard M. A., 2012).

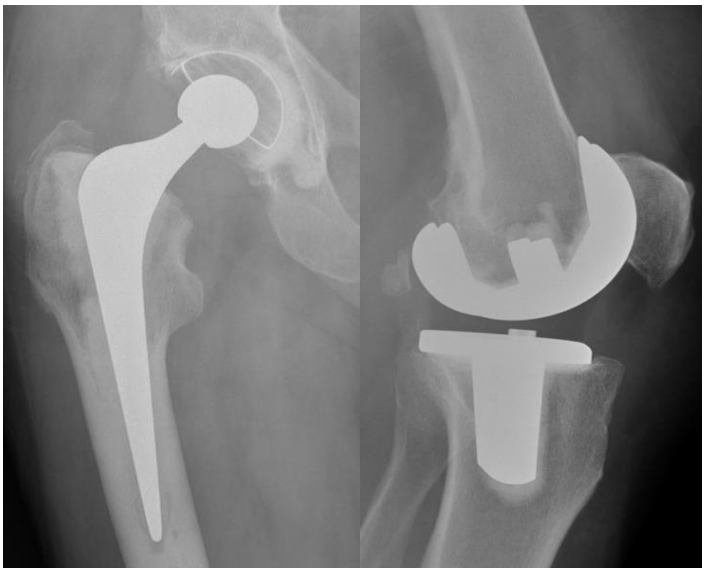
There are currently no targeted drug therapies that maintain homeostasis of the joint or stimulate cartilage repair (Betancourt M. C. C., 2012), which is an issue for OA progression.

Cognitive Behavioural Therapy (CBT) is a possibility in conjunction with other treatments if the patient suffers from other disorders such as depression or catastrophizing (Flor H., 1990).

In severe cases, joint prosthesis surgery may be required (Betancourt M. C. C., 2012) along with other surgical options such as, arthroplasty or osteotomy. (Kerkhof H. J. M., 2014) figure 1-5 shows the results of TJR.

In summary, as of today, there are no disease-modifying drugs for OA and treatment options either address symptoms only or involve joint surgery (Aspden, 2008, Valdes, 2013) with over 50,000 joint replacements for primary OA performed per year in the UK alone (Reynard L. N., 2012)

Figure 1-5: X-ray of a hip and knee joint after TJR



The x-ray on the left shows a hip replacement and the x-ray on the right shows a knee replacement.

1.2 Pain

Persistent pain is a major healthcare problem with as many as 100 million people in Europe alone experiencing an intractable, ongoing condition that affects quality of life. (Howard M. A., 2012)

Chronic pain has been recognized as pain which persists past the normal time of healing, often defined as 12 weeks or more (MedlinePlus, 2011). In practice this may be less than one month, or more than six months; for research purposes six months is preferred. (MedlinePlus, 2011) Chronic pain affects approximately 15% of the population (Valdes A. M., 2011a), with twin study-derived heritability estimates ranging from 35% to 65% being reported for knee pain, low back pain and neck pain (Malfait A. M., 2012)

Chronic Widespread Pain (CWP), as defined by axial plus upper and lower segment plus left- and right-sided pain in a body pain mannequin (Wolfe F., 1990) as marked by the participant is a complex trait since both environmental and genetic factors play a role in the aetiology. (Peters M. J., 2013) CWP is present in 10–15% of the UK population at any one time (Valdes A. M., 2011a) and heritability estimates of twin studies suggest 48–52% of the variance in CWP occurrence is due to genetic factors. (Peters M. J., 2013)

Regional joint pain is defined as a pain that can be confined to one or a limited number of regions in the body (Hardin, 1990). It is even more common than chronic pain with 25% of the population reporting knee pain or back pain in cross-sectional studies. However, there are no valid criteria for epidemiological use that allow us to say which people with regional joint pain have OA. (Dieppe P. A., 2005)

1.2.1 Pain Pathways

Many pathways are involved in producing and inhibiting the sensation of pain. The main ones are listed below:

Sympathetic and parasympathetic nervous system

The term autonomic nervous system (ANS) refers to collections of motor neurons (ganglia) situated in the head, neck, thorax, abdomen, and pelvis, and to the axonal connections of these neurons. The ANS regulates the functions of internal organs (the viscera) such as the heart, stomach and intestines. The ANS is part of the peripheral nervous system and it also controls some of the muscles within the body. The ANS functions are mostly involuntary and reflexively. For example, we do not notice when blood vessels change size or when our heart beats faster. The ANS is most important in "fight of flight" and in the "rest and digest" situations. The sympathetic nervous system (SNS) is part of ANS, which also includes the parasympathetic nervous system (PNS).

The SNS activates the fight or flight response. In response to noxious stimuli, postganglionic neurons principally release noradrenaline (norepinephrine). (Daily, 2014, Porreca, 2012)

Prolonged activation can elicit the release of adrenaline from the adrenal medulla. Once released, noradrenaline and adrenaline bind to adrenergic receptors on peripheral tissues.

Binding to adrenergic receptors causes the effects seen during the fight-or-flight response. (Daily, 2014) This then acts as part of descending inhibition. (Porreca, 2012)

Barakat et al (2012) showed that lower parasympathetic activity, is associated with higher pain intensity in subjects with CWP. (Barakat A., 2012) This fits with the SNS activating adrenaline release and through that lead to the inhibition of pain.

Hypothalamus–pituitary–adrenal (HPA) axis

The hypothalamus-pituitary-adrenal (HPA) axis connects the central nervous system (CNS) with the hormonal system. The anatomical structures that mediate the stress response are found in both the central nervous system and peripheral tissues. The principal effectors of the stress response are localized in the paraventricular nucleus of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland. This collection of structures is commonly referred to as the hypothalamic-pituitary-adrenal (HPA) axis Glucocorticoid feedback inhibition plays a prominent role in regulating the magnitude and duration of glucocorticoid release. In addition to glucocorticoid feedback, the HPA axis is regulated at the level of the hypothalamus by a diverse group of afferent projections from limbic, mid-brain, and brain stem nuclei. The stress response is also mediated in part by brain stem noradrenergic neurons, sympathetic adreno-rnedullary circuits, and parasympathetic systems (Smith and Vale, 2006). The end product of the HPA axis is cortisol, which has many actions, including: cardiopulmonary, nervous system and perception.(Porreca, 2012) This masks pain by descending inhibition due to the effects of cortisol speeding up metabolism and other systems leading to effects similar to norepinephrine.

Dopamine and cholecystokinin

Dopamine is a neurotransmitter, which works by its action on D2 and D4 receptors. It has been shown to help regulate movement and emotional responses (Finan P. H.,

2013b, Potvin S., 2009). This is verified by D4 receptors being associated with behavioural phenotypes including: autonomic nervous system dysfunction, attention deficit/hyperactivity disorder, and the personality trait of novelty seeking (Coffeen U., 2012). Cholecystokinin (CCK) has two different types of receptor: one is specifically based in the brain whereas the other is widely distributed. The widely distributed receptor (type B) is known to have different reaction to locally released CCK depending on location. For example, CCK has been shown to potentiate dopaminergic activity in the nucleus accumbens but not in the caudate nucleus. Thus, type B CCK receptors located in the brain, protected from circulating CCK and gastrin by the blood-brain barrier, are likely responsive to central neurotransmitter CCK. (Moran T. H., 1986) Despite this other studies argue that CCK action is based in the spine (Faris P. L., 1983), not the brain, though still related to the central nervous system. Faris et al (1983) also found that CCK inhibits the analgesic effect of opioids, therefore indicating that it actually exacerbates pain showing the opposite effect of dopamine though using the same route of action. (Faris P. L., 1983, Nishimura S., 2015, Moran T. H., 1986)

Opioid system

Opioids are strong, addictive painkillers, which are used for pain management in OA. (Sehgal N., 2012)Interestingly, patients reporting high levels of pain are at greater risk for opioid misuse than patients reporting low levels of pain. (Adams L. L., 2004, Grattan A., 2012, Jamison R. N., 2009, Morasco B. J., 2013, Martel M. O., 2013), the same relationship was found with catastrophizing (Valdes A. M., 2015).

Moreover, placebo treatment alters endogenous opioid activity in parts of the endogenous opioid pathway, including the Periaquaductal Gray Area (PAG), Anterior Cingulate Cortex (ACC) and Pre-Frontal Cortex (PFC). (Bushnell M. C., 2013) Many

of these areas are involved in other pain pathways such as the HPA-axis and the sympathetic nervous system.

Diffuse noxious inhibitory controls (DNIC)

Most multi-receptive and some nociceptive-specific neurones can be strongly inhibited by noxious inputs applied outside their receptive fields. DNIC are triggered by conditioning stimuli applied to any part of the body distant from the excitatory receptive field of the neurone under study, provided that the stimuli are noxious. There is a relationship between the intensity of the conditioning stimulus and strength of the resultant DNIC. With strong stimuli, the inhibitory effects are powerful and are followed by long-lasting post-stimulus effects. Since DNIC disappear in animals in which the spinal cord has been sectioned, the underlying mechanisms must involve the brain. It has been proposed that DNIC result from a physiological activation of some of the brainstem structures that produce descending inhibition.(Le Bars, 2002)

1.2.2 Research Methods

As pain is a subjective experience it has to be brought to the individual's attention for it to be perceived. (Crombez G., 1998) Most studies investigating the mechanisms involved in pain generation have used distraction techniques, examining how much a person's performance on a cognitive task is affected by the introduction or threat of an introduction of a mildly painful stimulus. (Crombez G., 2005)

Neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have shown promise in pain research, enabling

non-invasive investigation of the cerebral changes that underpin central pain physiology. (Howard M. A., 2012)

The cold pressor test can also be used as an experimental method to determine an individual's pain threshold. (Harris G., 1983)

Questionnaires are further used to measure perceived pain at a given time and to determine different pain types. Animal models can also be used. Both questionnaires and animal models will be discussed in more detail related to OA in the next section.

1.2.3 Pain and OA

Pain, not tissue damage to the joint, is the main clinical outcome of OA (Chapman K., 2012, Collins J. E., 2014, Saadat E., 2010). However, literature reports discordance between the severity of pain and the degree of radiographic structural OA. (Chapman K., 2012, Valdes, 2013, Keefe F. J., 2000) A recent UK study found that 44% of total knee replacement (TKR) patients and 27% of total hip replacement (THR) patients still had pain 3-4 years after arthroplasty (Valdes A. M., 2012b), further demonstrating the complexity of pain that has been attributed to OA.

In a study by Hawker et al. (2011) individuals with hip and knee OA identified two types of OA pain: one that was intermittent but generally severe or intense and another that was a persistent background pain or aching (Hawker G. A., 2011b). Stages of OA-related pain could be discerned, with early stages characterized by activity-related pain, becoming more constant over time and punctuated by intermittent intense pain. A decrease in participation subsequently occurs in an attempt to avoid triggering such episodes. This would agree with a finding by Neogi et al (2013) that the more intense but less frequent pain that comes and goes (i.e., intermittent), particularly when

unpredictable, had greater impact on quality of life than the ‘background’ (i.e., constant) pain. (Neogi, 2013)

Different pain scales or questionnaires measure different facets of the pain experience in OA and cannot be used interchangeably. (Creamer P., 1999) The visual analogue scale (VAS) (Hawker G. A., 2011b) measures pain intensity and is composed of three questions which ask the participant to rate pain on a scale of 0-10. The questions ask for pain at the present time, worst pain in the past month and average pain in the past month.

The pain detect questionnaire (PDQ) is a questionnaire designed and validated by Freynhagen et al (2006) to diagnose neuropathic pain (NP) (Freynhagen R., 2006, Mathieson S., 2013), therefore focusing on a particular pain type. The PDQ is a reliable screening tool with high sensitivity, specificity and positive predictive accuracy, specifically 84% in a palm-top computerised version and 85%, 80% and 83%, respectively, in a corresponding pencil-and-paper questionnaire. (Freynhagen R., 2006) However it has been found not to be as useful in patients with fibromyalgia as in patients with uncompromised central pain control (Gauffin J., 2013), so knowing the study population and the question being studied is important in determining whether it is a suitable questionnaire to use. Despite this the validation of test–retest properties has shown that the PDQ is reliable and can be used for follow-up (Barona R.).

The Western Ontario McMaster Arthritis Index (WOMAC) measures stiffness, pain and physical function for knee or hip OA and has been much used in clinical trials. (Roos E.M., 1999, Wolfe, 1999)

The quality of pain in OA varies, with approximately one-third of individuals with knee OA using descriptors such as burning, tingling, numbness, and pins and needles to characterize their knee symptoms, (Neogi, 2013) which indicates NP. Hence the

need for various questionnaires to measure the pain experience so the type and quality of pain can be determined.

Animal models such as mono-iodoacetate (MIA) injection, anterior cruciate ligament transection (ACLT)(Glasson S. S., 2007) and destabilisation of the medial meniscus (DMM) (Glasson S. S., 2007) have been used to simulate OA and to thereby study its progression and risk factors. (Glasson S. S., 2007)

1.2.3.1 Risk Factors

Excessive weight is a modifiable factor contributing not only to OA risk, but also to pain. The effect of obesity on pain may be two-fold. For the lower extremities, the effect of excess weight on symptoms may be due to mechanical loading, or relative fat mass in obesity may potentially contribute to pain symptoms related to the elaboration of adipokines. Importantly, weight gain significantly increases pain, highlighting the dose–response relationship of change in weight with change in pain. (Neogi, 2013). Trials have noted reductions in pain with ~10% weight loss, with more substantial effects on pain reduction with greater weight loss.

Similar to obesity, weak muscles, especially quadriceps strength, are independently associated with pain both in cross-sectional and longitudinal studies. In subjects from the OA Initiative, knees with frequent pain demonstrate lower maximal cross-sectional areas and force of the quadriceps compared with contralateral knees without knee pain with the same radiographic stage. (Jones, 2013) There is evidence that both strengthening and aerobic exercises help pain in knee and hip OA. (Jones, 2013) Khayambashi et al (2012) examined the effectiveness of isolated hip abduction and external rotation strengthening on self-reported pain intensity, health status, and hip strength in twenty eight females with patellofemoral pain. They found that a hip-

strengthening program significantly decreased pain and improved health status in cases but not controls. They also reported that this effect was sustained up to 6 months after the participants started the program.(Khayambashi K., 2012) Hamilton el at (1995) found both respiratory and peripheral limb muscle strength were reduced in patients with cardiorespiratory disorders compared with normal healthy subjects.(Hamilton A.L., 1995) Both of these studies indicate that weak muscles can be more likely because of pain or fatigue which is particularly highlighted for joint pain in Khayambashi et al (2012). Hamilton et al (1995) showing this in cardiovascular disease indicates a wider role possibly in the sympathetic nervous system.

A further risk factor is female gender. When responding to pain, girls and women show more of an emotional response and tend to be more worried and irritated about pain. Furthermore, expectations regarding social roles may alter how women and men respond to pain, with women often having multiple roles they may attend to pain sooner so as to reduce its impact. (Keefe F. J., 2000)

1.2.3.2 Mechanical/ Structural

Imaging studies at the knee joint have shown a correlation between pain and both synovitis and subchondral bone changes, suggesting that these two tissues could be sources of pain in osteoarthritis. (Dieppe P. A., 2005, Jones, 2013)

Zhang et al. (2011) showed that changes in the severity of bone marrow lesions (BMLs) and synovitis were associated with fluctuation of frequent knee pain and pain severity, and that of the 2 types of structural lesions, BMLs were the better predictor

for fluctuation of knee pain.(Zhang Y., 2011, Hunter D. J., 2013, Schaible, 2012) Other bone-related causes of pain include periostitis associated with osteophyte formation, subchondral micro fractures, bone attrition and bone angina due to decreased blood flow and elevated intraosseous pressure, which are reflected on imaging as BMLs.(Hunter D. J., 2013)

A difference has been found between those with and without knee pain in the prevalence of moderate or larger effusions and synovial thickening. (Dieppe P. A., 2005, Hill C. L., 2001) It has been demonstrated that synovial thickening is associated with the severity of knee pain (Hill C. L., 2001), with worsening of synovitis and effusions being associated with increased risk of frequent and more severe pain. (Schaible, 2012, Hunter D. J., 2013) A semi-quantitative measure of synovitis from the infrapatellar fat pad also associates with pain severity (Hunter D. J., 2013) The positive associations between synovitis and knee pain indicate that inflammation may play a role in knee pain genesis. Stannus et al (2012) reported that IL-6 and TNFα were associated with radiographic OA and knee cartilage loss in middle-aged to older people with or without knee pain (Stannus O. P., 2012).

Studies that have demonstrated a relation between cartilage damage and pain have traditionally investigated the role of cartilage in predisposing to symptoms in isolation from other tissues and as such cannot provide an overall insight into the independent contribution of cartilage pathology to pain. (Martel-Pelletier J., 2008, Lorenz H., 2006, Kumar D., 2013) The likely mechanism for symptom genesis is through secondary mechanisms such as: exposing the underlying subchondral bone and the inherent symptom genesis (such as exposure of nociceptors) from this structural alteration; vascular congestion of subchondral bone leading to increased intraosseous pressure; and synovitis secondary to articular cartilage damage with activation of synovial

membrane nociceptors. That is, knees exhibiting denuded areas of cartilage are more likely to have concomitant potentially painful tissue pathology such as synovitis/effusion and BMLs. (Hunter D. J., 2013)

Schaible et al (2012) found that knees with frequent pain displayed greater rates of medial cartilage loss. (Schaible, 2012) This explains the finding by Creamer et al (1996) that pain in some cases of OA knee can be abolished by intraarticular (IA) local anaesthetic (LA), suggesting that the structures responsible for pain are in contact with the IA surface (Creamer P., 1996) but, IA saline also does this, so there is a placebo component that acts through central mechanisms. There is also data which show that incident tears and tears involving the outer rim of the meniscus may play a limited role in symptom genesis through angiogenesis and associated sensory nerve growth. Degenerative lesions, described as horizontal cleavages, flap (oblique), or complex tears or meniscal maceration or destruction are associated with older age and are almost universal in persons with OA. In asymptomatic subjects, a tear was found in 67% using MRI, whereas in patients with symptomatic knee OA, a meniscal tear was found in 91% (Hunter D. J., 2013).

Attempts at structure modification have been largely unsuccessful to date with regards to pain. Some recent exceptions include promising results from trials evaluating zoledronic acid targeting bone marrow lesions, with possible additional bone and cartilage effects, and strontium ranelate which may have both bone and cartilage effects (Neogi, 2013).

1.2.3.3 Molecular pathways

OA pain has long been considered as nociceptive pain caused by physiological activation of peripheral nerve terminals by tissue damage or inflammation. (Valdes A.

M., 2014) Since central sensitization is correlated with activation of neural circuits that are implicated in descending facilitation of pain and is therefore a risk factor for the development and maintenance of chronic pain, it is important to identify which patients exhibit abnormal responses to relevant painful stimuli as central pain sensitisation at the spinal or cortical level can occur in OA. (Dieppe P. A., 2005, Finan P. H., 2013a) Patients with higher levels of clinical pain in the absence of moderate-to-severe radiographic changes were consistently more likely to report pain when tested in the distal knee, suggesting evidence of central sensitization. (Finan P. H., 2013a, Valdes A. M., 2014) Continuous and intense input from the OA-damaged knee joint may result in central augmentation of nociceptive processing, which is associated with central and peripheral sensitization of the nervous system, hyperalgesia at the site and beyond the OA joint, and sustained pain potentiation. Central augmentation is also associated with psychosocial aspects of pain perception and symptoms such as fatigue, insomnia, mood disturbances and psychological stress. A further hypothesis is that damage to peripheral nerves resulting in neuropathic pain-like symptoms may be caused by local nerve damage during knee surgery. (Valdes A. M., 2014) Peripheral sensitisation, in which thresholds for activation are diminished, results in enhanced excitability; some evidence indicates that it is a feature of the osteoarthritic joint, perhaps mediated by nerve growth factors or cytokines. (Dieppe P. A., 2005). This can lead to allodynia which is present when normally non-noxious stimuli can now elicit sensations of pain, whilst hyperalgesia refers to exaggerated pain in response to normally painful stimuli. (Porreca, 2012)

In individuals with moderate to severe OA, injections of a monoclonal antibody against NGF, Tenazumab, provided persistent pain relief and improved function up to 56 weeks in a dose-dependent fashion. (Schaible, 2012) Evidence for NGF's role in OA was gathered from mouse OA induced by DMM. Schiable et al (2012) found NGF

was present at day 3 and 16 weeks after the operation, but not in intervals that appeared pain free. A soluble NGF receptor, TrkAd5, suppressed pain in both NGF-positive phases, whereas neutralization of TNF reduced pain only in the first phase, suggesting that OA-associated NGF may not be driven by classical inflammatory mediators. Another study found in the MIA model a late increase of NGF, whereas in the first phase, TNF and IL-6 were elevated. (Schaible, 2012)

1.2.3.4 Placebo effect

The placebo response has been confirmed in OA in regards to pain. Expectation and conditioning are thought to be the main mechanisms involved from research conducted on other diseases such as Parkinson's. The response has been related to *COMT* with patients who have the met/met polymorphism having the strongest placebo response, demonstrating both psychology and genetics play a role in the effectiveness of this response. (Abhishek A., 2013) Anticipation of the relief of pain is a primary contributor to placebo analgesia. Brain imaging studies of expectation-related placebo analgesia find that immediately preceding the presentation of a noxious stimulus, when an individual receives a placebo 'analgesic' and is expecting a reduction in pain, there is activation of the ACC–fronto–PAG pathway. This involves similar brain regions as those that are activated when a positive emotional state reduces pain. Moreover, placebo treatment alters endogenous opioid activity in parts of this pathway, including the PAG, ACC and PFC85. Further evidence that this ACC–fronto–PAG pathway is involved in placebo analgesia comes from findings showing that naloxone (a μ-opioid antagonist) modulates some placebo-induced responses in the PAG as well as the rostral-ventromedial medulla (RVM) and abolishes the coupling between rostral ACC and PAG activity. Conversely, circuitry

involved in the attentional modulation of pain, including the superior parietal cortex and the insula, does not appear to be activated during expectation-related placebo analgesia. This independence of placebo analgesia from the attentional modulation of pain was further confirmed in a study showing additive reductions in pain caused by placebo and distraction, indicating that placebo analgesia does not depend on the active redirection of attention. (Bushnell M. C., 2013)

1.3 Catastrophizing

Catastrophizing has been defined as an individual's tendency to focus on and magnify the threat of painful stimuli as well as negatively evaluate their ability to deal with pain along with feeling helpless. (Keefe F. J., 2000, Benyon K., 2010, Campbell C. M., 2009, Keefe F. J., 2004, Schneider S., 2012, Keefe F. J., 2010, Martel M. O., 2013, Bedard G. B. V., 1997, Sullivan M., 2001) Catastrophizing is therefore viewed as comprising three separate but related dimensions: rumination, magnification and helplessness. (Pavlin D. J., 2005, Bedard G. B. V., 1997) It is measured using the pain catastrophizing scale (PCS) (Sullivan, 2014, Sullivan M., 1995) or pain coping questionnaire (PCQ) (Hirsh A. T., 2007, Reid G.J., 1994, Reid G. J., 1998) both of which are validated questionnaires.

1.3.1 Models of Catastrophizing

There are many theories and models of pain catastrophizing and how it can be studied (for review see (Leung, 2012)). One model is the 'attention bias model' which states that pain catastrophizing results from a preferential and dysfunctional bias of attention toward pain and its related cognition and mental processes. Vancleef et al (2006) used healthy volunteers who had been pre-screened and measured their attentional interference as they performed an auditory discrimination task whilst occasionally distracted by low electrocutaneous stimulations. They found that only pain catastrophizing enhances interference of their attentional ability (Vancleef and Peters, 2006), supporting the attention bias model. In an attempt to tweak attention to modulate pain catastrophizing, Michael et al (2004) subjected volunteers to an initial cold pressor task and then ask them to read out a serious of control, pain-sensory and pain-affect words whilst undergoing a second cold pressor task. As expected, reading affect-related words, but not the other words, led to higher pain catastrophizing in the second task (Michael and Burns, 2004). This study further supports attention bias as affect-related words were given more attention.

Another model used is the appraisal model which states that when confronted with a pain stressor, the subject initially assesses the relevance and levels of harm as the primary appraisal, then contemplates the coping options and formulates beliefs regarding the possibility of success as the secondary appraisal. In the context of pain catastrophizing, researchers proposed that magnification and rumination domain stems from a dysfunctional focus and evaluation in the primary appraisal stage, while helplessness is a maladaptive and negative secondary appraisal (Severeijns et al., 2004).

A further model used is the communal coping model which states that pain catastrophizing is a coping strategy where subjects communicate their painful

experience to elicit emotional and social support from others.(Sullivan M., 2001) Lackner et al (2004) performed circumplex analysis on interpersonal behaviour and proposed that pain-catastrophizing stems from a submissive interpersonal style with high dependency and demand for care and support (Lackner and Gurtman, 2004) which agrees with the communal coping model. However, Severeijns et al (2004) queried the need of this communal coping model for pain catastrophizing as according to the appraisal model, the three domains of ruminative thinking, magnification of pain and feeling of helplessness will impinge upon subsequent interpersonal and support mechanisms, hence making the communal coping model redundant.(Severeijns et al., 2004)

Theories of pain and stress have also been used to explain catastrophizing two good example are the transactional model of stress and neuromatrix theory.

In the transactional model of stress (Lazarus R.S, 1984) stress is viewed as a relational product between the person and the environment, and stress arises when the individual judges that the environmental demands exceed the individual's own resources (Dewe, 2001). It involves two appraisal processes: the primary appraisal occurs when the individual makes sense of the encounter and determines its nature as challenging, harmful or threatening; the secondary appraisal evaluates the coping resources and decides on what can be done (Folkman, 1982), in this respect it can be compared to the appraisal model of catastrophizing.

Melzack updated gate-control theory (Melzack and Wall, 1965, Britton and Skevington, 1989) to the neuromatrix model (Melzack, 1999, Melzack, 2005, Melzack, 2001), which incorporated the essential domains of cognition and emotion with sensory inputs to form a dynamic body-self matrix which in turn generated a neurosignature manifested either as nociception, motor activity or antonomic/immune self-regulatory responses. One important feature of this model is that the neuromatrix

is incessant and sensory input is not essential for generation of a neurosignature. In the context of pain catastrophizing, the cognitive and emotional domains of input become so dominating that the neuromatrix outputs become disproportional and maladaptive.

1.3.2 Risk factors

A relationship between gender and catastrophizing has been found in both clinical and experimental research. In two studies using a cold pressor procedure with undergraduate students and varsity athletes, Sullivan et al. (2000b) found that women scored higher than men on the PCS (Sullivan M., 2000). Bedard et al. (1997) examined gender differences in catastrophizing in a sample of junior high school students and found on the basis of cut-off scores on the PCQ (Reid G.J., 1994, Reid G. J., 1998), more young women than men were classified as catastrophizers, which suggests that gender differences in catastrophizing emerge at a young age. (Keefe F. J., 2000, Bedard G. B. V., 1997) A relation between gender and catastrophizing has further been observed even when subjects are not currently experiencing pain. (Keefe F. J., 2000) One reason for the differences found may be that women and men may differ in their emotional responses to pain. (Unruh, 1996, Keefe F. J., 2000)

Effects of catastrophizing on sensitivity to physical activity (SPA) have been found in symptomatic knee OA. The attentional biases to pain associated with catastrophizing, including vigilance and inability to disengage from pain-related stimuli, appear to partially explain increasing discomfort during physical activity. (Wideman T. H. Finan, 2014) Rapp et al. (2000) found that higher levels of catastrophizing were related to both higher pain intensity and lower physical ability (Benyon K., 2010, Rapp S. R., 2000), emphasising the link between SPA and catastrophizing. Balance between coping strategies and acceptance seems important for engagement in both social and sports activities. Lack of engagement could perpetuate catastrophic ideas,

potentially worsening pain and treatment outcomes. (Keefe F. J., 2004) This suggests that catastrophizing affects lifestyle and the ability to make positive choices which the individual would benefit from in the long term.

Pain somatisation is a frequent manifestation in depressed people and may predispose patients to report pain more often or even to exacerbate it, as is the case in catastrophizing. (Pereira D., 2013) Across numerous studies (Giesecke T., 2005, Hoffman B. M., 2007, Sullivan M., 1990, Velly A. M., 2011), patients with chronic conditions such as back pain and arthritis generally have a twofold to fourfold increase in the risk of being diagnosed with a depressive disorder. (Edwards R. R., 2011a) Furthermore, when patients report three or more pain sites or complaints, the probability of a comorbid depressive disorder increases up to eightfold. (Edwards R. R., 2011a) Moreover those suffering from a chronic illness who also have a partner that suffers from depression are likely to find their partner will not cope as well, which can affect the patient. (Keefe F. J., 2010) Major depression and the number of pain problems elsewhere were found to be significant and independent postoperative determinants of persistent postsurgical pain. (Wylde V, 2011) Depressed mood has been associated with alterations to central pain processing, which render patients more sensitive to particular pain stimuli. Furthermore, prior research has suggested that individuals with chronic pain and comorbid depression may be less likely to adhere to prescribed pain therapies. (Hawker G. A., 2011a)

Pavlin et al (2005) found that high scores on a measure of catastrophizing predicted the degree of pain that individuals experience following surgery and contributed to a higher level of disability in the weeks that immediately followed surgery. (Pavlin D. J., 2005) These findings have been replicated in other studies including patients with rheumatoid arthritis (Keefe F. J., 1989), osteoarthritis (Keefe F. J., 1999),

fibromyalgia (Martin M. Y., 1996), sickle cell disease (Gil K. M., 1992), soft tissue injuries (Sullivan M., 1998, Sullivan M., 2003), neuropathic pain (Sullivan M., 2005) and patients recovering from surgery (Jacobsen P.B., 1996, Sullivan, 2009).

1.3.3 Cultural differences

Other studies (see Table 1-1) have shown that there are cultural differences in the rate and impact of catastrophizing.

Table 1-1: Studies of catastrophizing in different cultures. This demonstrates how widely the Pain Catastrophizing Scale (PCS) is used, samples used and what the study was measuring

Country	**PCS Score out of 52**			**Study details**
	Mean	**Median**	**SD**	
Norway	13.6	12	9.2	Fernandes, L et al (2012) patients with lower back pain 38 men and 52 women Mean age of 47.6 Validating the PCS in that language
South Africa	A-PCS 37 E-PCS 38.2		A-PCS 11.4 E-PCS 11.5	Morris, L et al (2012) patients with fibromyalgia 89 females and 4 males Validating the PCS in that language
Canada				Sullivan M.J.L et al (2004) University undergraduates 26 men, 38 women Mean age of 20.1 years with a range of

				17–30 years Exploring situational effects
	8.65		9.29	Hawker G.A et al (2011) community cohort with hip/knee OA 529 subjects 78.5% of the subjects were women baseline mean age was 75.4 years investigating the link between depression and pain using catastrophizing as an extra component
Brazil				Sehn F et al (2012) chronic nonmalignant pain attending an outpatient multidisciplinary pain center 384 patients, 317 women (82.55%) aged 18–79 years validation of the Brazilian Portuguese version of the PCS
America	Caucasian 13.23 African 15.43 Asian 15.86		Caucasian 8.60 African 11.46 Asian 9.89	Goodin, B.R et al (2012) study flyers were posted throughout the campus of an urban mid-Atlantic university Final sample was 149 individuals (Caucasian Americans, Asian Americans, and African Americans). 78 (52%) participants were female mean age 19.9 years, SD = 2.9 (range = 18 to 38 years). Testing the relation between dispositional optimism and conditioned

				pain modulation: does ethnicity matter?

PIPS used in Spain

1.3.4 Catastrophizing and pain

Evidence exists that catastrophic styles of thinking about pain are related to excessive appraisals of threat and to attentional interference (Sullivan M., 1995, Crombez G., 2005). Those who catastrophize about imminent pain have difficulty performing distraction tasks, and experience less analgesia from distraction (Crombez G., 2005), which supports the point above.

Only the magnification subscale of the PCS was found to be significantly related to satisfaction ratings post-surgery (Pavlin D. J., 2005), suggesting it is pain intensity that is related most to analgesic use which is what would be expected.

Pain somatisation is a frequent manifestation in depressed people and may predispose patients to report pain more often or to exacerbate it. (Pereira D., 2013, Bair M.J., 2003) Depression and pain share biological pathways and neurotransmitters, which has implications for the concurrent treatment of both. (Bair M.J., 2003) Patients with depression often present with a complex set of overlapping symptoms, including emotional and physical complaints. Understanding this relationship has become more important, given that primary care physicians fail to accurately diagnose at least 50% of patients with major depression, and at least 2 studies have shown that patients with depression who present with physical symptoms such as pain are particularly likely to receive an inaccurate diagnosis. People with depression have significantly more unexplained physical symptoms such as pain and fatigue and utilize more health resources than non-depressed people. (Bair M.J., 2003)

Rapp et al. (2000) found that higher levels of catastrophizing were related to both higher pain intensity and lower physical ability (Rapp S. R., 2000, Benyon K., 2010), emphasising the link between SPA and catastrophizing as discussed earlier.

1.3.5 Catastrophizing and OA

One risk factor for pain related to OA is catastrophizing (Velly A. M., 2011). As discussed above, research has demonstrated catastrophizing's strong relationship with pain, physical functioning, psychological functioning, and even health care use. (Campbell C. M., 2009)

It has been found that participants with higher pain scores have higher odds of being affected by depressive symptoms. Additionally, a higher association of knee pain with radiographic knee OA was found in participants without depressive symptoms. (Pereira D., 2013) Major depression and the number of pain problems elsewhere were found to be significant and independent postoperative determinants of persistent postsurgical pain. (Wylde V, 2011) Depressed mood has been associated with alterations to central pain processing, which render patients more sensitive to particular pain stimuli. Furthermore, prior research has suggested that individuals with chronic pain and comorbid depression may be less likely to adhere to prescribed pain therapies. (Hawker G. A., 2011a)

Keefe et al (2000) found that there were significant differences in pain, pain behaviour, and physical disability in men and women having OA. Women had significantly higher levels of pain and physical disability, and exhibited more pain behaviour during an observation session than men. Further analyses revealed that catastrophizing mediated the relationship between gender and pain-related outcomes, even after controlling for depression. These findings underscore the importance of both gender and catastrophizing in understanding the OA pain experience and may have important implications for pain assessment and treatment. (Keefe F. J., 2000)

Effects of catastrophizing on sensitivity to physical activity (SPA) have been found in symptomatic knee OA. The attentional biases to pain associated with catastrophizing, including vigilance and inability to disengage from pain-related stimuli, appear to partially explain increasing discomfort during physical activity. (Wideman T. H. Finan, 2014)

Some evidence suggests that high levels of catastrophizing may produce dysregulation or dysfunction in the endogenous opioid pain-control systems. In several studies, elevated catastrophizing has been associated with a greater need for postoperative opioid analgesics to control postsurgical pain, which suggests that the opioids produced, on a per-unit basis, less analgesic benefits in individuals who reported higher levels of catastrophizing. (Campbell C. M., 2009) An increasing number of studies have shown that patients high in pain catastrophizing are at increased risk for prescription opioid misuse (Edwards R. R., 2011b, Jamison R. N., 2009, Morasco B. J., 2013, Martel M. O., 2013) supporting the previous point. Opioidergic dysfunction was also seen in the cortex and amygdala of mice with chronic inflammation or nerve injury, which was related to increases in anxiety-like behaviours (Bushnell M. C.,

2013). Negative affective processes can also interfere with pharmacologic treatment of pain among patients with discogenic low back pain, and elevated catastrophizing is related to greater need for opioids following surgery. (Edwards R. R., 2011a) All of the above points suggesting mood affects pain intensity.

1.4 Genetics, epigenetics and OA

1.4.1 What are genetics and epigenetics?

Genetics refers to the primary DNA sequence that we inherit. This is the basic double helix structure which contains base pairs that bind to each other: Adenine (A) with Thymine (T) and Cytosine (C) with Guanine (G). (Carey, 2011, Jorde L. B., 2006) The level of gene expression from the inherited DNA sequence is further controlled by epigenetics. Epigenetics can be defined as the set of modifications to genetic material that alter the levels at which genes are expressed, but do not alter the genes themselves (Carey, 2011, Reynard L. N., 2012); in this respect epigenetics asserts fine control. This is achieved mainly via methylation (Carey, 2011, Reynard L. N., 2012), histone modifications (Carey, 2011, Reynard L. N., 2012) and non-coding RNAs (ncRNAs; e.g. *Xist* and microRNAs) (Reynard L. N., 2012) or by acting post-transcriptionally, leading to changes in the levels of the encoded protein (e.g. microRNAs). Epigenetic patterns undergo dynamic changes during development, cell differentiation and in response to environmental stimuli, leading to changes in temporal and spatial gene expression.(Reynard L. N., 2012)

Molecular pathways already implicated in OA genetic risk include: Bone Morphogenetic Proteins (BMPs)(Valdes, 2013), Growth Dependant Factor 5

(GDF5)(Goldring, 2013, Valdes, 2013, Evangelou E., 2009), Asporin Proteoglycan (ASPN)(Valdes, 2013), SMAD family member 3 (SMAD3)(Goldring, 2013, Valdes, 2013), thyroid metabolism, inflammation and immune response (Ruedel A., 2014, Remst D. F., 2014, Stannus O. P., 2012), as well as Wnt signalling (Valdes, 2013). For details of known genes and studies see Table 1-2

Table 1-2: Table of known genes from the literature that affect Osteoarthritis (OA). A literature review using google, google scholar and PubMed was undertaken and the following data extracted from the studies

SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
rs143383	GDF5	Candidate gene	Asians	Hip OA	1.79	0.74	2×10^{-13}	Bone morphogenetic protein, joint development	Valdes et al, 2012 Valdes et al, 2011 Valdes et al, 2009
rs143383	GDF5	Candidate	Caucasians	Knee OA	1.16	0.61	8×10^{-9}	Bone morphogenetic protein, joint	

SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
		gene						development	
rs11718863	DVWA	GWAS	Asians	Knee OA	1.43	0.64	7×10^{-11}	Cartilage specific tubulin binding	Miyamoto et al 2008
rs10947262	BTNL2	GWAS	Asians	Knee OA	1.31	0.58	5×10^{-9}	Immunomodulatory function, T–cell response	Nakajima et al 2010
rs4730250	COG5/GPR22/ DUS4L/HBP1	GWAS	Caucasians	Knee OA	1.17	0.17	9×10^{-9}	Unknown	Kerkof et al 2010, Reynard, L.N. and J.

SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
									Loughlin 2012 Evangelou et al 2011
rs11842874	MCF2L	GWAS	Caucasians	Knee or hip OA	1.17	0.07	9.2×10^{-9}	Cell motility. Possible involvement in nociception	Reynard, L.N. and J. Loughlin 2012 Day–Williams

SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
									et al 2011
rs7775228	HLA–DQB1	GWAS	Asians	Knee OA	1.34	0.62	2. x 10^{-8}	Immune response (antigen presentation)	Nakajima et al 2010
D14 allele in VNTR	ASPN	Candidate gene	Asians	Knee OA	1.95	0.05	1.3x10^{-6}	TGFβ signalling,	Nakamura 2007
rs12901499	SMAD3	Candidate gene	Caucasians	Knee OA	1.22	0.52	7 x10^{-6}	TGFβ signalling,	Valdes et al, 2010
rs12901499	SMAD3	Candidate gene	Caucasians	Hip OA	1.22	0.52	3x10^{-4}	(see above)	

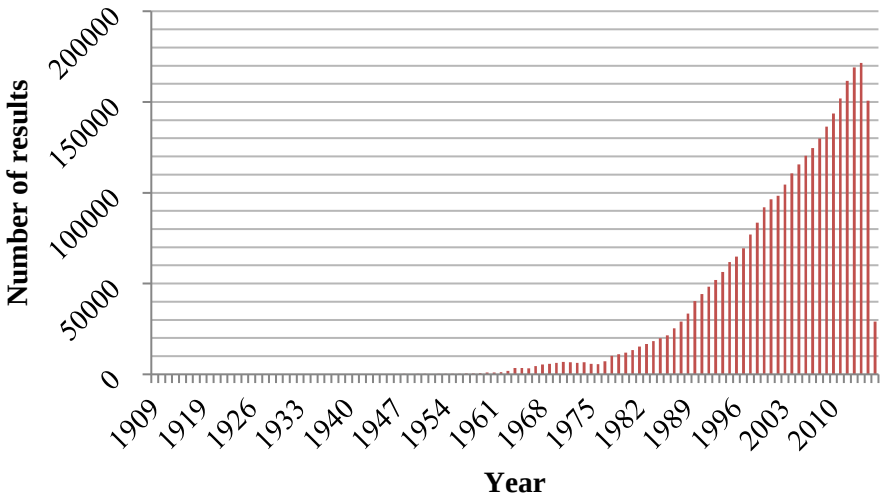
SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
rs10980705	EDG2	Candidate gene	Asians	Knee OA	2.3	0.035	3×10^{-5}	Lysophosphatidic Acid receptor	Mototani 2008
Haplotype rs225014 – rs12885300	DIO2	Candidate gene	Caucasians	Hip OA	1.79#	0.17	2×10^{-5}†	Thyroid metabolism	Goldring 2013 Meulenbelt HMG 2008
Haplotype rs419598– rs315952–	IL1RA	Candidate gene	Caucasians	Knee OA with K/L≥3¶	0.15	0.32	1×10^{-4}	Inflammation	Attur 2010, Kerkhof et al 2011

SNP ID	Gene	Type of Study	Ethnic group	Trait	Odds ratio	Risk variant* frequency in controls	P–value	Putative or known function	Reference
rs9005									
rs8065080	TRPV1	Candidate gene	Caucasians	Symptomatic knee OA	1.33‡	0.86	4×10^{-4}	Nociception	Valdes et al ARD 2011

1.4.2 History of Research

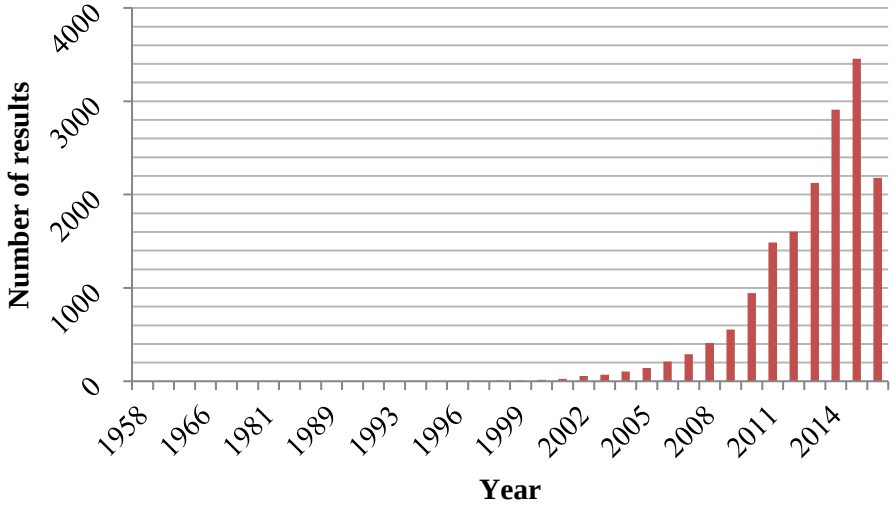
Genetics is a more recent field of research than OA as can be seen when comparing figure 1-1 with figure 1-6. The first piece of research to combine the two fields of genetics and osteoarthritis was done in 1960 as can be seen in figure 1-8. As can be seen in figure 1-7 epigenetics is an even more recent field of research with work first published in 1964.

Figure 1-6: PubMed results for 'Genetics'



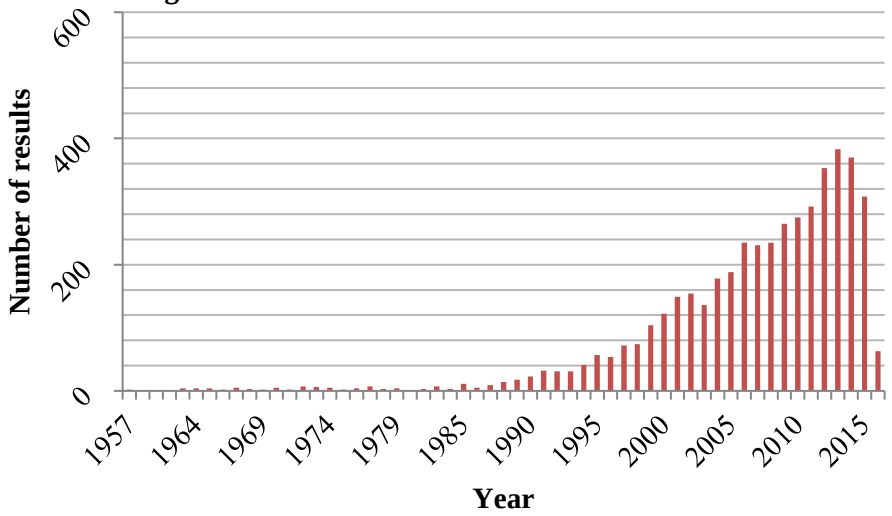
Using PubMed a search was conducted for the number of studies per year focused on Genetics

Figure 1-7: PubMed results for 'Epigenetics'



Using PubMed a search was conducted for the number of studies per year focused on epigenetics

Figure 1-8: PubMed results for 'Genetics in Osteoarthritis'



Using PubMed a search was conducted for the number of studies per year focused on genetics in OA

Possibly the most famous piece of genetics research carried out is the Human Genome Project (HGP) (Sawicki M.P., 1993). This project aimed to map the whole human genome in the hope of being able to map certain genes that would cause susceptibility to certain disease states. This piece of research further highlighted many of the ethical issues involved in genetic research.

1.4.3 Why study using a genetic approach?

Studying OA using genetics allows the investigation of potentially novel molecular pathways involved in disease so potentially facilitating the detection of new risk factors and the possibility of new or improved treatments. Several genes have already been linked with OA, for details of confirmed genes see table 1-2

1.4.4 Research Methods

1.4.4.1 Single Nucleotide Polymorphisms (SNPs)

SNPs are variants of a gene, which can occur due to mutation. Mutations can be due to environmental factors such as UV radiation, chemicals and viruses or they can be inherited. A DNA variation is defined as a polymorphism if it is sufficiently common in a population, usually higher than 1%. This approach is increasingly practical because 4 million of the estimated 13 million common SNPs are already known. Due to their frequency and distribution, SNPs may serve as superior genetic markers for assembly of a high-resolution map, aiding in the identification of phenotype-related loci. (Kim H., 2009)

1.4.4.2 Linkage scan/Quantitative Trait Locus (QTL)/ candidate gene approach

The candidate gene approach works by extracting a particular SNP or SNPs required to focus on a particular gene. (Chapman K., 2012) This therefore means that there are fewer tests ran than if scanning the entire genome, meaning possible better levels of significance as power will be increased and it is less likely to result in a false positive or negative. (Chapman K., 2012)

However this method is limited in that it relies upon a prior understanding of the aetiology of OA and allows for only very small regions of the genome to be targeted for investigation. Therefore, the chances of identifying common OA susceptibility variants are low for three main reasons. The first is sample size. It is accepted that samples in the thousands are essential to detect the effect sizes resulting from common SNP associations. Secondly the sparse coverage of markers employed by the relatively low-density microsatellite maps of linkage means that a large proportion of the genome is untested. Finally too many reports of association are false positives due to a

lack of stringency in the reporting of significant results based on observed p-values. (Chapman K., 2012)

1.4.4.3 Genome Wide Association Scan (GWAS)

A GWAS works by scanning the entire genome looking for common SNPs (Zeggini E., 2012). A p-value of $5x10^{-8}$ is generally said to be a genome-wide significant finding as so many tests are run; the value needs to be this stringent to avoid reporting of false negatives or positives.

One of the advantages of GWAS is that there is no need for prior knowledge of what SNPs could be involved (Zeggini E., 2012) so in this way many new discoveries can be made.

However three important limitations of GWAS studies are: the definition of significant association in view of several correlated stratified analyses, as extra tests will dilute power; effect of phenotype heterogeneity and misclassification in complex diseases such as OA as different phenotypes may have different genotypes and this should therefore be taken into account; and allowing for power constraints when seeking replication of previously identified loci as again the more tests ran the higher the sample has to be in order for the results to be conclusive. (Zeggini E., 2012)

1.4.5 Genes and Pathways Identified in the current literature

Table 1-3: List of genes currently known in the literature for depression, pain and anxiety. A literature review was undertaken using google, google scholar and PubMed then the following data extracted from studies

Gene	Depression	Pain	Anxiety	PCS discovery cohort
COMT	Antypa et al (2013) rs4680 P:0.003 Bosker et al (2011) rs4680 P:0.046 OR:1.79	George S. et al (2008) rs4680 P:0.008 OR:0.06		rs4680 P:0.914, OR:1.016
BDNF	Reitschel et al (2010) rs7713917 P: 7.61E-03 OR :0.74		Chen et al (2006) rs6265 OR:0.335 P:< 0.001	rs6265 P:0.839 OR:0.962
*SLC6A4 (*related to *HTT (Serotonin))*	Su et al (2009) Bosker et al (2011) rs57098334 P:0.005 OR:4.44			P: N/A OR: N/A
TPH1	Bosker at al (2011) rs1799913 P:0.0013			P: N/A OR: N/A

	OR:1.52			
TRPV1		Valdes et al (2011) rs8065080 OR:0.75 P:0.00039		rs8065080 P:0.513 OR:0.902
SMAD3		Valdes et al (2010) rs12901499 P:< 4.0 × 10– 4 OR:1.22		rs12901499 P:0.221 OR:1.201
MCF2L		Day-Williams A.G. et al (2012) rs11842874, OR:1.17 P: 2.1 × 10−8		rs11842874 P:0.314 OR:0.699
BICC1	Lewis C.M. et al (2010) rs9416742, OR: 0.719, P: 1.30E-07			rs9416742 P:0.796 OR:0.953
PREPL			Otowa T. et al (2016) rs786618 P:6.99E-09 OR:	rs786618 P:0.771 OR:0.952

SLC3A1			Otowa T. et al (2016) rs1142523 P: 1.44E-07 OR:	rs1142523 P:0.617 OR:0.923
LBX1			Otowa T. et al (2016) rs11190870 P:9.79E-07 OR:	rs11190870 P:0.164 OR:1.234
HOMER1	Reitschel et al (2010) rs7713917 P: 7.61E-03 OR :0.74			rs7713917 P:0.997 OR:1.001
*PCSK6 (*related to *PACE4)*		Malfait AM et al (2012) rs900414 OR: 1.35 P: $4.3X10^{-5}$		rs900414 P: n/a OR: n/a

The last column displays the results from GWAS study as described in chapter 4, so placing what I have found in the context of the literature

Candidate gene studies found several genes that were potentially involved in the generation of pain in OA (Chapman K., 2012, Valdes, 2013). These include: *COMT* (Peters M. J., 2013, Malfait A. M., 2012, Neogi T., 2013, Thakur M., 2013, Jones, 2013), *TRPV1* (Day-Williams A. G., 2011, Valdes A. M., 2011b, Thakur M., 2013, McDougall J. J., 2012) and *PCSK6*, *ASPN*, *GDF5* (Evangelou E., 2009, Reynard L. N., 2012, Valdes A. M., 2012a, Valdes A. M., 2011c, Valdes A. M., 2009), *FRZB* (Evangelou E., 2009), *PTGS2* and *SMAD3* (Valdes A. M., 2010c, Valdes A. M., 2006). In a candidate gene study in 674 patients with radiographic knee OA with pain

vs 2068 radiographic knee OA/no-pain patients, a SNP in *PCSK6* was strongly associated with protection against pain when radiographic OA was present (Jones, 2013, Thakur M., 2013).

The GWAS of European descent samples and the Rotterdam found a signal in a region on chromosome 7q22, which has an association with combined knee OA and/or hand OA phenotype, that included a large linkage disequilibrium (LD) block. The LD block contained six known genes, including: *PRKAR2B*, *HPB1*, *COG5*, *GPR22*, *DUS4L* and *BCAP29*. The top hit rs4730250 is annotated in intron 3 of *DUS4L*. (Valdes, 2013, Evangelou E., 2010, Reynard L. N., 2012) In the arcOGEN GWAS study the strongest signals were $p = 2.3 \times 10^{-5}$ at SNP rs2277831, located in *MICAL3*, $p = 2.7 \times 10^{-5}$ for rs11280, located in *C6orf130*, and $p = 1.2 \times 10^{-5}$ for rs2615977, located in *COL11A1*. *COL11A1* codes for the α1 polypeptide chain of type XI collagen, a protein that plays an important role in the cartilage extracellular matrix. Further analysis of the stage 1 arcOGEN data resulted in another SNP emerging as a new signal: rs11842874 located in *MCF2L*, with $p = 2.1 \times 10^{-8}$.(Reynard L. N., 2012) As part of its analysis, arcOGEN did look for loci that had stronger association in a joint replacement only subset of patients (i.e., those who had experienced pain associated with OA) vs the broader composite-defined cohort (which included the participants who had asymptomatic radiographic changes). Four such loci were found, corresponding to regions close to the genes: *GLT8D1*, *ASTN2*, *CHST11* and *TP63*. (Thakur M., 2013)

The first GWAS using over 100000 markers identified a signal (rs4140564) between *PTGS2* and *PLA2G4A*. (Valdes, 2013) rs4140564 on chromosome 1 mapping to the *PTGS2* and *PLA2G4A* genes was associated with risk of knee OA in all the cohorts studied. (Valdes A. M., 2008)

COMT is involved in central pain processing (Jones, 2013); it is an enzyme that degrades neurotransmitters including dopamine. The common Val-158-Met polymorphism of rs4680 (or V158M) results in reduced enzymatic activity due to its effect on thermostability, and has been associated with reduced endogenous opioid levels in response to painful stimuli resulting in increased pain sensitivity. (Peters M. J., 2013) This SNP has further been associated with maladaptive coping and pain catastrophizing in fibromyalgia and shoulder pain, as well as influencing acute experimental pain reports in healthy controls and increasing the risk of hip pain among those with hip OA, particularly in women (Malfait A. M., 2012, Neogi T., 2013, Thakur M., 2013, Jones, 2013). For *COMT*, both functional SNP and haplotype were associated with chronic pain in patients with FM, temporomandibular joint disorder (TMJD) and postsurgical pain. (Belfer I., 2013)

TRPV1 primarily has a role in afferent sensory neuron function and has been well demonstrated to have an association with pain but not with radiographic OA. (Day-Williams A. G., 2011) Ile-585-val *TRPV1* variants are reported to confer reduced sensitivity to cold pain (Valdes A. M., 2011b, Thakur M., 2013), whereas the Ile–Ile variant (rs8065080) is more likely to be carried by patients with asymptomatic knee OA than patients with painful OA. (McDougall J. J., 2012, Thakur M., 2013) Further study into the mechanism of action revealed that PAR2-mediated joint pain involves the activation of TRPV1 ion channels and the substance P-preferring NK_1 receptor. Intra-articular injection of a PAR2 agonist caused the local release of the inflammatory cytokine IL-1β. (McDougall J. J., 2012)

The *SCN9A* gene encodes the alpha subunit of voltage gated sodium channel $Na_v1.7$, (Valdes A. M., 2011a, Thakur M., 2013) which is selectively expressed by nociceptors and is essential for transmission of pain-related signals. (Thakur M., 2013) Mutations in the channel can lead to a loss of function conferring a congenital insensitivity to pain. In contrast, gain of function is seen in primary erythromelalgia, fibromyalgia and

idiopathic small fibre neuropathy. (Valdes A. M., 2011a, Thakur M., 2013) A SNP conferring an Arg-1150-Trp substitution in this channel was found to associate with higher pain reports. (Thakur M., 2013)

Growth and Differentiation Factor 5 (*GDF5*) participates in the development, maintenance and repair of synovial joint tissues. The SNP rs143383 maps to the *GDF5* promoter (Valdes A. M., 2012a) and contains a C to T transition. (Reynard L. N., 2012) The predisposing T allele results in lower gene expression (Valdes A. M., 2012a, Valdes A. M., 2009) and is associated with higher risk of knee and hip OA in Asian populations (OR=1.55, $p<1 \times 10^{-6}$). (Valdes A. M., 2009) The A promoter polymorphism (rs143383) in *GDF5* has also been found to be strongly associated with both hip and knee osteoarthritis in Asian individuals. This variant is functional, with the lower gene expression variant having increased genetic risk. (Valdes A. M., 2011c)

Genetic variation in the *SMAD3* gene is involved in genetic susceptibility to large-joint OA. (Valdes A. M., 2010c) Evidence suggests that TGFβ plays an important role in the pathogenesis and progression of OA. This role in OA is likely to derive from its contribution to the maintenance of the stable phenotype in articular chondrocytes. TGFβ signals mainly through the TGFβ type I and type II transmembrane serine/threonine protein kinase receptors and the SMAD signalling cascade. (Valdes A. M., 2010c) Cartilage intermediate layer protein (CILP) has been reported to inhibit the TGFβ1–mediated induction of cartilage matrix genes through direct interaction with TGFβ1 and inhibition of TGFβ1 signalling. The T allele at cilp_395 has been shown to increase the binding and inhibition of TGFβ1, indicating that the extracellular matrix protein CILP regulates TGFβ1 signalling. One of the haplotypes

carrying the C allele at position 395 was associated with a greatly reduced risk of knee OA. (Valdes A. M., 2006)

MCF2L codes for the Rho-specific Guanine nucleotide exchange factor DBS. DBS is phosphorylated by TrkC, a receptor of the Nerve Growth Factor (NGF)-family member neurotrophin NT-3, and enhances Schwann cell migration when activated. (Thakur M., 2013, Valdes A. M., 2012a, Valdes, 2013, Day-Williams A. G., 2011, Chapman K., 2012). Recently treatment of knee OA patients with a humanised monoclonal antibody that inhibits NGF has been found to be associated with joint pain reduction and an improvement in function. This result supports the possible relevance of nociceptive molecular pathways in the genetic susceptibility to OA. (Valdes, 2013, Day-Williams A. G., 2011)

A GWAS of knee OA found two variants. In the locus named double von Willebrand factor A (DWVA), rs11718863 and rs7639618, were confirmed to be strongly associated with risk of knee OA in Japanese and Chinese patients ($OR=1.43$ $p<7 \times 10^{-11}$) (Valdes A. M., 2009), meaning the research may not be extrapolated to other populations. These two SNPs were found to influence the binding of the DVWA protein to beta-tubulin. (Valdes, 2013, Valdes A. M., 2009) Weaker tubulin binding by the wild-type protein was the likely mechanism by which genetic variation in this gene affected the risk of knee OA, therefore tubulins and microtubules might be protective factors in the pathogenesis of OA. (Valdes A. M., 2009) It was later shown that DVWA is part of the human gene coding for the collagen VI alpha4 chain (COL6A4).(Valdes, 2013, Valdes A. M., 2009) A further GWAS on hip-JSW identified the G allele of rs12982744 on chromosome 19p13.3 to be associated with a 5% larger JSW ($P = 4.8 \times 10^{-10}$). The SNP was also strongly associated with a 12%

reduced risk for HOA ($P = 1 \times 10^{-4}$). The SNP is located in the *DOT1L* gene, which is identified as a potentially dedicated enzyme for Wnt target-gene activation in leukaemia. Furthermore, *DOT1L* is expressed in OA articular chondrocytes as well as *DOT1L* knockdown reducing proteoglycan and collagen content, and mineralization during chondrogenesis. (Betancourt M. C. C., 2012) Frizzled-Related protein (*FRZB*) is another key player in the Wnt signaling pathway with regard to cartilage metabolism and OA. The relationship between the rare *FRZB* "Trp^{200}–Gly^{324}" haplotype (frequency of 0.6–5.0%) and OA has been studied. It has been shown that female carriers of this haplotype have an increased risk of THR, severe joint space narrowing (JSN) of the hip and clinical knee OA further supporting a role for *FRZB* variants in OA. (Kerkhof J. M., 2008) All the above indicates that this is a well-studied pathway and that its action on collagen seems to be the main mechanism by which it affects OA.

The significant association of SNP rs1256031 and hip OA was only present in women, not in men. It was hypothesized that significant effects are not observed in men since elderly men have higher estradiol levels compared to postmenopausal women. These higher serum levels of oestrogens in men may mask an impaired *ESR2* signalling caused by the genetic variation and may also explain an association between SNP rs1256031 of the *ESR2* gene and hip OA only in women. (Kerkhof H. J. M., 2010b) Polymorphisms considered as haplotype combinations for the *ESR1* gene, which encodes oestrogen receptor α (ERα), were found to be associated with radiographic OA of the knee in the Rotterdam cohort. In addition, a weak association of the *ESR1* gene with the presence of osteophytes has been previously reported. The oestrogen receptors ERα and ERβ have been identified in normal and OA cartilage, indicating that cartilage can respond to oestrogens. (Valdes A. M., 2006)

The prostaglandin-endoperoxide synthase 2 gene has been found to be associated with knee OA (Kerkhof H. J. M., 2011b). The production of prostaglandin E_2 (PGE_2) is increased in OA cartilage, and cyclooxygenase 2 is the rate-limiting enzyme of PGE_2synthesis. Thus, genetic variations in the *COX2* gene may also contribute to the pathogenesis of knee OA. (Valdes A. M., 2006) The levels of SET-1A are elevated in OA cartilage, which may be one of the mechanisms that lead to increased expression of iNOS and COX-2. Expression of these two proteins may contribute to OA by inducing chondrocyte apoptosis and increasing synthesis of matrix metalloproteinases, as well as inhibiting collagen production. (Reynard L. N., 2012)

The ADAMTS family contains all the known aggrecanases (ADAMTS1, -4, -5, -8, -9, -15). These enzymes cut the aggrecan core protein at a particular site that seems critical for cartilage damage. PACE4 removes the prodomain of the pro-aggrecanases, pro-ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif)-4 and pro-ADAMTS-5, in OA cartilage, thus activating these proteases. Active ADAMTS-4 and ADAMTS-5 are responsible for proteolytic degradation of the major cartilage macromolecule, aggrecan, which is a key pathological event in OA. This suggests that PACE4 represents a novel target for the development of OA therapeutics.(Malfait A. M., 2012) *ADAMTS5* is necessary for cartilage damage in murine models of arthritis. Genetic association of a non-synonymous single nucleotide polymorphisms (nsSNP) in *ADAM12* with knee OA has been reported (Rodriguez-Lopez J., 2009). *ADAM12* was reported to have 1 haplotype that increased the risk of knee OA (CAAT) and 2 haplotypes that reduced the risk of knee OA (CGAT and CGGT), all of which carry the same allele (C) in codon 48, which results in an arginine residue instead of a glycine residue. It also suggests that, by itself, this coding SNP does not affect knee OA susceptibility, but acts only in the presence of other alleles. (Valdes A. M., 2006) An nsSNPs in *ADAMTS14* has been shown to be associated with OA, most notably

knee OA requiring joint replacement. A change of glutamic acid for glycine in *ADAMTS14* was associated with women knee OA requiring joint replacement and with clinical hand OA. (Rodriguez-Lopez J., 2009)

Rs835487 is located in the *CHST11* (carbohydrate sulfotransferase 11) gene, which has a role in skeletal development and signalling pathways. It has substantially higher expression in osteoarthritic cartilage than in normal articular cartilage and mice that are null for this gene have a disorganised growth plate, accelerated chondrocyte differentiation, altered patterns of signalling with transforming growth factor β and bone morphogenetic protein, and a fibrillated cartilage extracellular matrix that is characteristic of osteoarthritic cartilage. (Zhai G., 2009), all of which suggests this is a candidate for study.

rs48804829 produces a proline to leucine change at amino acid 451 (P451L) of *P2X7R*; this mutation is known to affect pore formation in cell membranes. P451L genotype might affect allodynia severity. Consistent with this genetic hypothesis, six of the seven mouse strains with less than average allodynia carried the Leu451 allele. (Sorge R. E., 2012) P2X7 is a purinergic channel expressed on cells of myeloid lineage such as macrophages. A SNP in this gene determines the ability of the channel to form a pore that allows high molecular weight material to pass through the membrane. Individuals with radiographic OA possessing the hypofunctional variant of the channel were significantly less likely to have clinically relevant OA pain when compared with unaffected controls (Thakur M., 2013). This would suggest another target for investigation in terms of symptomatic OA

A number of studies have suggested alterations in DNA methylation patterns may be involved in OA pathogenesis. Reduced methylation of specific CpG sites within the promoters of genes encoding for several metalloproteinase enzymes (e.g. MMP13,

ADAMTS4) has been reported, and this hypomethylation is associated with increased expression of these enzymes in end stage OA chondrocytes. Conversely, decreased expression of the *SOD2* gene is observed in OA chondrocytes, correlating with hypermethylation of several CpG sites within the promoter of this gene. Furthermore, methylation of the *BMP7* promoter increases in chondrocytes with increasing age, and this may underlie the age-related decline in BMP7 levels in cartilage (Reynard L. N., 2012).

GWAS studies have also been carried out on depression and anxiety (Lewis C. M. and Aitchison K. J., 2010, Smoller J . W., 2009, Otowa T., 2016).

Zubenko et al (2003, 2004) reported several chromosomal loci that may influence the development of recurrent major depressive disorder in 81 families with the highest maximum logarithm of the odds ratio (LOD) score observed occurring at marker D2S2321 (205 cM), located 121 kb proximal to the cyclic adenosine monophosphate (AMP) response element binding protein 1 (CREB1). Nineteen chromosomal regions contained linkage peaks that reached genome-wide statistical significance. Six linkage peaks were revealed after an analysis of covariance controlled for the effects of gender and epistatic interaction with the CREB1 locus (Zubenko G. S., 2004, Zubenko G. S., 2003). However this study could be said to be underpowered so replication would be needed in order to confirm these results.

Abkevich et al (2003) also conducted a genome-wide scan in 1890 individuals from 110 pedigrees with a strong family history of major depression and provided strong evidence for the existence of a sex-specific disposition locus for major depression on chromosome 12q22–12q23.2. (Lesch, 2004, Abkevich V., 2003).

All of the above demonstrates that there is a definite genetic component to mood disorders as well as OA and that this is an area that is well explored. However, the

genetics links between mood disorders and OA and how they interact are yet to be investigated.

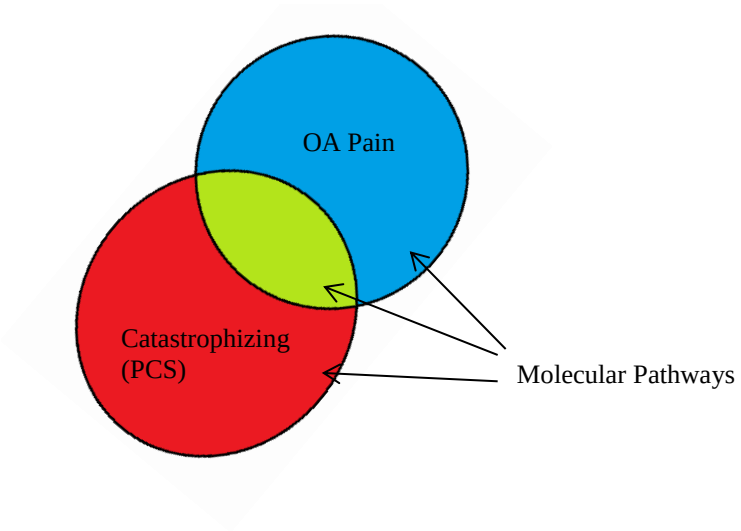
1.5 Hypothesis and Aims

One aim of this thesis is to explore the effects of catastrophizing on post-TJR pain, sleep quality, anxiety and depression. From the above literature mentioned I would hypothesise that there is a link between all aspects. However I would suggest that catastrophizing is independent of sleep and depression in affecting pain intensity.

A further aim is to discover genes as well as possible molecular pathways that link pain and catastrophizing in OA. Again from the above literature I would hypothesis that *COMT* and many genes involving nerve signalling and growth will be evident.

Another aim is to compare symptomatic and asymptomatic individuals to investigate whether there are any genetic differences and also if these link with any genes found in catastrophizing.

Figure 1-9: Aims of study



2 Methods

2.1 Study design

The studies undertaken as part of my thesis are all case control studies.

Case and control selection

In chapter 3 cases were defined as OA/post TJR and controls were defined as non-OA controls, all participants were recruited from secondary care. The OA/post TJR subjects were all recruited from orthopaedic clinics with painful knee or hip OA, but the majority of them had already undergone a TJR at the time of the pain assessment. All controls were x-rayed and were free from OA

Chapter 4 shows a case- control design for genetic association where the case definition was high catastrophizing (top tertile) and the control definition was "low catastrophizing" (bottom two tertiles).

In chapter 5 the cases and controls came from different cohorts. These are individuals were all diagnosed with radiographic knee OA (defined as a Kellgren-Lawrence grade of 2 or higher at the tibiofemoral compartment). I defined "cases" as those with painful OA and "controls" as those with no joint pain.

Exposures and potential confounders.

In chapter 3 I first studied catastrophizing as an exposure contributing to OA status. Having ascertained the lack of difference between OA and controls I then investigated the role of the pain catastrophizing scale as an exposure contributing to other outcomes such as severe joint pain and neuropathic like joint pain. I also took into consideration the role of age, sex, BMI, hip or knee status, K/L grade as potential confounders.

In chapter 4 the exposure are 500,000 genetic variants available in the full GWAS genotyping panel. I have taken into consideration the role of age, sex, and BMI, as potential confounders.

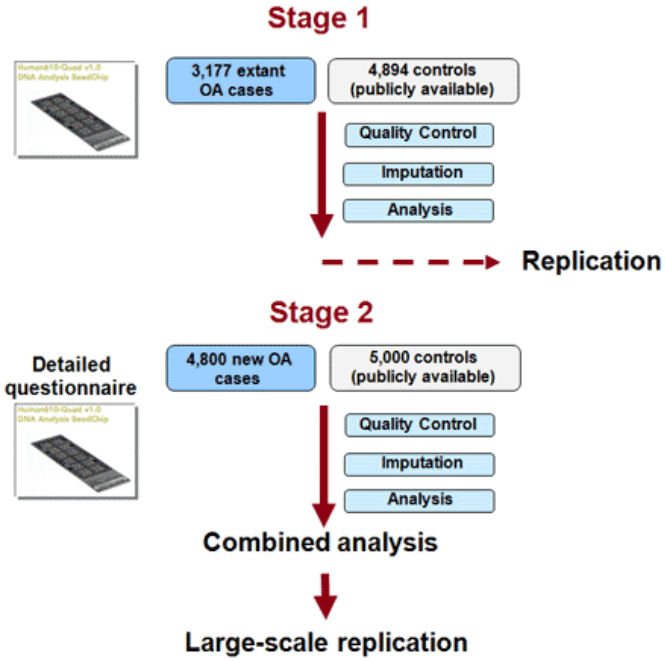
In chapter 5 the exposure are the 80,000 genetic variants present in the exome chip genotyping panel. I have taken into consideration the role of age, sex, BMI, and K/L grade as potential confounders.

2.2 Study cohorts

Described here are the cohorts that were included as part of the various analysis

Discovery/arcOGEN

Figure 2-1: Flow diagram of arcOGEN study design



Adapted from
http://www.arcogen.org.uk/overview.html

The GWAS in this thesis used the samples collected by Academic Rheumatology as part of the arcoGEN study, with a two stage design consisting of discovery in a multicentre collection of 7,410 clinically severe OA cases, 80% ascertained at the time

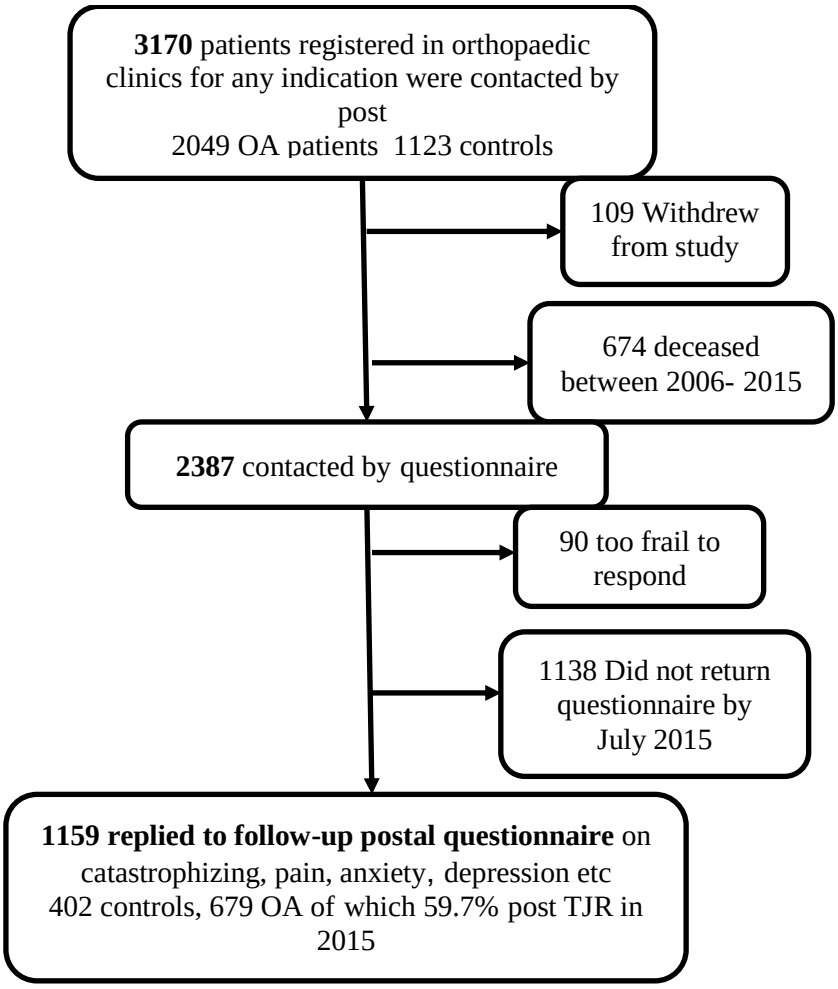
of total joint replacement (TJR) (Zeggini E., 2012) All participants gave written informed consent and the study protocols were reviewed and approved by the appropriate review committees. All participants were unrelated and of European origin, and all had primary OA of the hip or knee of radiographic Kellgren-Lawrence (KL) grade ≥2, or clinical evidence of disease to a level requiring total joint replacement (TJR). The exclusion criteria included the need for joint replacement due to fracture, and developmental, vascular, or infective causes of joint disease.

GOAL

The GOAL (Genetics of Osteoarthritis and Lifestyle Study) cohort is a case–control study undertaken in Nottinghamshire that was originally designed to investigate gene–environment interaction in large-joint OA. (Limer K. L., 2009, Zhang W., 2008) The study recruited between 2002-2006 men and women referred to hospital with clinically severe symptomatic, radiographic OA (ROA) of the knee (n = 1040) or hip (n = 1004), as well as asymptomatic controls (n = 1123) with no ROA of hips or knees referred to hospital for intravenous urography (IVU) between 2002 and 2006. Subjects were excluded if they had any other major arthropathy (e.g., rheumatoid arthritis), childhood joint disease (e.g., Legg-Calvé-Perthes disease, slipped femoral epiphysis, hip dysplasia), TJR, avascular necrosis of the femoral neck or distal condyle, or terminal illness.

Control subjects were recruited from lists of people who had attended hospital for IVU within the last 5 years. Individuals who had no radiographic evidence of hip OA on their screening IVU film and who had no hip or knee symptoms and none of the exclusions listed above for cases were invited to take part in the study. Follow-up questionnaires were sent out in 2015 to live consenting participants and it is the data from the follow-up questionnaires that is used for analysis in this current study. For the recruitment flow chart see Figure 2-2. Approval for the original GOAL study and follow-up questionnaire was obtained from the Nottingham Research Ethics Committee.

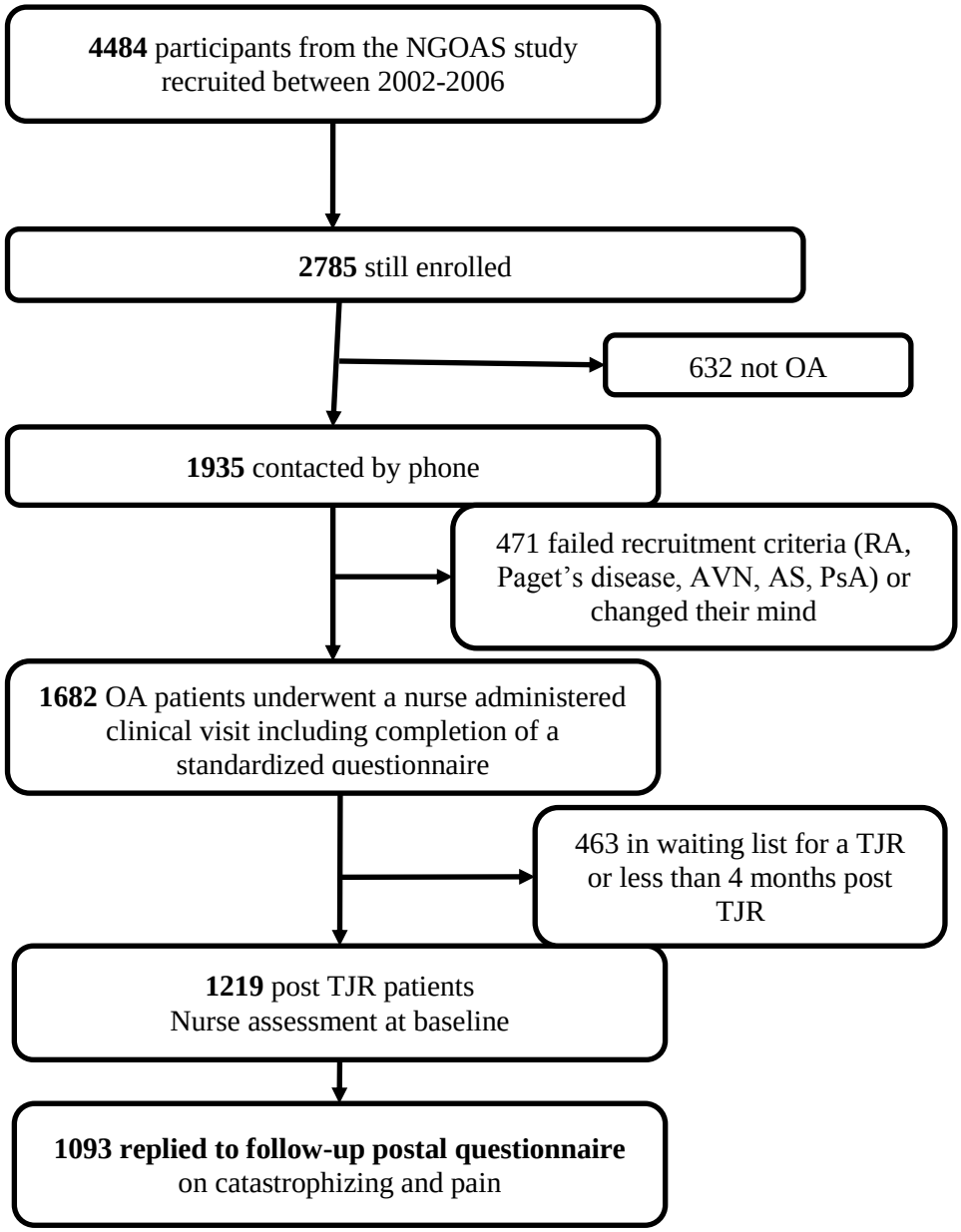
Figure 2-2: GOAL recruitment flow chart



NGOAS

The NGOAS (Nottingham Genetics of OA) study which was originally named The Nottingham case-control study contains individuals affected by knee or hip OA recruited in Nottingham both from families with a history of OA and from clinic populations. All had been referred to the hospital with symptomatic, clinically severe knee OA and the majority had undergone unilateral or bilateral TKR or THR within the previous 5 years. Pre-operative radiographs were examined to confirm the diagnosis. (Valdes A. M., 2012a, Valdes A. M., 2013) Consenting participants underwent a home visit by a nurse between 2008 and 2012 and completed a nurse administered questionnaire. Participants who gave consent to be re-contacted were sent a follow-up postal questionnaire between 2013 and 2014, the data from which is used in the present study. For the recruitment strategy flow chart see Figure 2-3. Approval for the original NGOAS study and the subsequent follow-up questionnaire was obtained from the research ethics committees of Nottingham City Hospital and North Nottinghamshire.

Figure 2-3: NGOAS recruitment flow chart



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2.3 Assessments used

2.3.1 Clinical measures

X-rays were available for the entire arcOGEN cohort (Zeggini E., 2012)

In the GOAL study all participants had standardised radiographs of both knees and pelvis examined and scored. The presurgical radiographs of cases that had undergone

TJR were copied using Hipax Dicom digitising software (Hipax, Vorstetten, Germany) to enable scoring. If cases and controls had not undergone TJR, new radiographs were obtained, unless radiographs of the same joint (using the same standardised method) had been obtained not more than 2 years previously. Cases and controls were further characterised by enquiry, examination and investigation.(Limer K. L., 2009)

In the NGOAS study pre-operative knee or pelvis radiographs of knee or hip OA cases were examined to confirm the diagnosis and to grade for changes of OA severity (Neame R. L., 2004, Lanyon P., 2000, Zhang W., 2008). All pelvis and knee radiographs were scored for individual radiographic features of OA by a single observer and graded 0-3 according to a standard atlas. The Nottingham line drawing atlas (Nagaosa Y., 2000) was used to score joint space narrowing and osteophytes in the knees, whereas the Croft score was used for measurement in the hips (Altman R.D., 1995).

2.3.2 Questionnaires

The following validated questionnaires were included in the questionnaires sent to participants in all cohorts used in this thesis. As well as questionnaires mentioned below a body mannequin was included to measure widespread body pain.

To grade this the participant was asked to shade affected areas these were then converted to sections of the body and from that if 2 or more out of 4 quarters of the body had marks in they would be classified as widespread.

2.3.2.1 Pain Catastrophizing

The Pain Catastrophizing Scale (PCS) is a 13 item questionnaire, with a score range of 0-52, created by Sullivan et al.(1995) to measure the three components of catastrophizing specifically, rumination, magnification and helplessness(Sullivan M., 1995). In this thesis participants were classified as having high catastrophizing if they were in the top tertile with a score of 9 or greater. This cut off was reached on the basis of the histogram displayed in chapter 4 (graph 4-1), data was split into tertiles to make sure the power was still adequate. The top tertile was then compared to the bottom two tertiles leading to the figure of 9 being used.

2.3.2.2 Pain Intensity/ Neuropathic-like Pain

The visual analogue scale (VAS) (Hawker G. A., 2011b) was used to measure pain intensity and is composed of three questions which ask the participant to rate pain on a scale of 0-10; the questions ask for pain at the present time, worst pain in the past month and average pain in the past month. The cut off used for VAS in this study was 6 based on answers to worst pain to account for the spread of data, split into tertiles again and comparing the bottom two to the top tertile, and also we judged any pain above that to possibly affect quality of life. Worst pain was chosen as we hypothesised this would be less variable than present pain due to individuals having 'good' and 'bad' days.

The pain DETECTquestionnaire (PDQ) was used to measure neuropathic-like pain (NP) as it is a questionnaire designed and validated by Freynhagen et al (2006) to diagnose NP, it is scored from −1 to 38, with a score $\leqslant$12 indicating that a neuropathic component is unlikely and a score of $\geqslant$19 indicating that a neuropathic component is likely (Mathieson S., 2013, Wylde V, 2011, Freynhagen R., 2006). The cut-off point used to determine NP in this thesis was therefore 12 or more.

The Western Ontario and McMaster Universities Index (WOMAC) was further used to assess the three dimensions of pain, disability and joint stiffness. It uses

27questions. 5 questions are used to measure pain, 2 questions are used to measure joint stiffness and 20 are used to measure disability. Each question is scored on a scale from 0-4 meaning there is a maximum score of 20 for pain, 8 for joint stiffness and 80 for disability WOMAC has been linguistically validated and is widely used in the evaluation of knee and hip OA.(Bellamy, 2012, Bellamy et al., 1988, Reginster et al., 2001) It has been validated within the context of a double blind randomized controlled parallel trial of 2 non-steroidal anti-inflammatory (NSAID) drugs (Bellamy et al., 1988) and has been used in many studies. This questionnaire assesses pain experienced during 5 different activities: walking, using stairs, sitting or lying, standing upright, and in bed. Response options for each question are on a 5-point Likert Scale, ranging from none to extreme (0 to 4) (Wylde V, 2011).

2.3.2.3 Anxiety and Depression

In the NGOAS cohort this was based on a yes/no question in the medical history section of the follow up questionnaire. In the GOAL cohort depression and anxiety were assessed using the HADS questionnaire (Assessment, 2015), which is a standardised questionnaire that measures both depression and anxiety categories calculated according to the following scores:

1. normal (0-7)
2. borderline abnormal (8-10)
3. abnormal (11-21)

To combine depression in both cohorts the score in the GOAL cohort was converted to yes/no with both situations 2 and 3 in the HADS questionnaire being considered as having depression so a score of 8 or greater used as cut off.

2.3.2.4 Sleep

Four questions drawn from the Medical Outcomes Study (MOS) sleep survey (Hays and Stewart, 1992) were used to assess quality of sleep, specifically: "How often during the past four weeks did you (a) Get enough sleep to feel rested in the morning? (b) Awaken short of breath or with a headache? (c) Have trouble falling asleep? (d) Have trouble staying awake during the day". Each question could have answers ranging from "all of the time" to "never". The sum of responses was scored from 0 (most disturbed sleep) to 100 (optimal sleep). A cut-off of 55 was used for "poor sleep" meaning that the top quartile was considered good sleep. 4 questions were chosen rather than the standard 6 or 9 due to the length of questionnaires sent out, the questions selected are representative. The full MOS questionnaire was applied to a subset of individuals. For space reasons only a subset of questions were sent to most individuals. I have however compared the cut-off that I used and 95% of the time individuals with the full questionnaire would be classified in the bottom quartile as I did with the shorter version. The scores between the reduced version and the full version scores also show a correlation of over 0.65

2.3.2.5 Cardiovascular disease (CVD) and other comorbidities

One section of the questionnaires sent out included questions related to the individuals' medical history. This section included tick-boxes that the participant would mark if they had been diagnosed with a specific illness. To calculate whether a participant had CVD their response in the questionnaire for stroke, hypertension, heart attack/angina and high cholesterol was combined and if they had one or more of these

they were reported as having CVD. Participants were also asked if they were affected by diabetes, cancer and other diseases.

2.4 Genotyping

ArcOGEN used Illumina 610 as the gene chip for genotyping. The Illumina 610 chip tags variation in the whole genome and has more common variants but not necessarily functional (genetics, 2013) See Figure 2-3 derived from Zeggini et al (2012). These data are now in the public domain via the European Genome-phenome Archive accession number EGAD00010000744.

NGOAS uses exomechip as the gene chip for genotyping. Exomechip includes functional or coding variants; this enables an intermediate experiment between genotyping arrays, which focus on relatively common variants, and exome sequencing of very large numbers of samples, which will enable examination of coding variants. (genetics, 2013)

In the GOAL study DNA was extracted from whole blood using the Gentra PureGene kit (Qiagen). All SNPs were genotyped using the TaqMan™ platform (Applied Biosystems). Five nanograms of dried DNA were amplified in 2μl reactions containing TaqMan Universal PCR mastermix (Applied Biosystems) and either assay-by-design or assay-on-demand primer and probe mixes (Applied Biosystems). Reactions were cycled on a KBiosystems thermal cycler for 10 min at 92°C followed by 45 cycles of 15 s at 92°C and 1 min at 57°C. Fluorescence was detected and genotypes assigned to samples using the ABI7900HT Sequence Detection System (Limer K. L., 2009)

2.5 Statistical methods

Pain post-TJR (Chapter 3)

Descriptive statistics were carried out using SPSS package version 22 to summarize the relevant characteristics of the cohorts. Associations between high catastrophizing, severe pain and other binary outcomes with risk factors were carried out using binary logistic regression (as data was non-parametric) adjusting for age, gender and BMI as covariates, again SPSS version 22 was used to carry this out.

Genetics (Chapter 4 and 5)

Descriptive statistics were calculated as described above. However in the case of NGOAS, self-reported ethnicity was assessed by a nurse administered questionnaire and only individuals of European descent were included in the genetic study due to the possibility of cultural differences.

Quantile-Quantile (QQ) plots and Manhattan plots were generated using the software program 'R'. This was done to quality check the data and to ensure there were no outliers or irregularities. A QQ plot shows results expected against results gained, if the plot follows the diagonal line and then tapers off slightly at the top that shows a sound data set with no outliers. A Manhattan plot shows the significance and location of the SNPs tested, if a point falls above the blue line then it is a likely candidate and if a point falls above the red line it can be said to be genome wide significant in that cohort.

To test for associations between SNPs and the trait being studied a Genome Wide Association Scan (GWAS) was undertaken. GWAS scans the entire human genome and tests 1 million SNPs meaning a p-value of 5x10-8 is needed (calculated form a Bon-Ferroni correction) to state genomewide significance has been reached. (Visscher P.M., 2012, Risch N., 1996) A GWAS was then carried out using the software program 'plink'. Plink is a free and open source software that is specifically designed to handle whole genome data (Harvard, 2014).

Replication using the GOAL cohort was then carried out to ensure that the results found in the first GWAS were not false positive or negative.

Meta-analysis was then carried out which combined all cohorts used thereby improving sample size and also the robustness of any results obtained.

The "meta" library in the statistics program R (version 3.0.2) was used to run the meta-analysis using the four cohorts described above. Meta-analysis takes the effect size, standard error and sample size into account to give an overall effect from the different groups studied. If heterogeneity was significant between the cohorts in the meta-analysis, a Han Eskin random effects model was used as an alternative meta-analysis method since compared to traditional models it allows for more heterogeneity in the data as long as the associations are all in the same direction (Han and Eskin, 2011).

Pathway analysis was used following GWAS. This involves using the top genes to identify likely molecular mechanisms and pathways involved; it further gives an indication as to how much impact each mechanism or pathway has. This technique is often used as high-throughput experiments per se do not produce biological findings and also it is well known that genes do not work alone, but in an intricate network of interactions. Pathways analysis therefore helps interpret the data in the context of biological processes, pathways and networks as well as giving a global perspective on

the data and problem at hand (Courses, 2009). Therefore pathway analysis has two main advantages. Firstly, grouping thousands of genes, proteins, and/or other biological molecules by the pathways they are involved in reduces the complexity to just several hundred pathways for the experiment. Secondly, identifying active pathways that differ between two conditions can have more explanatory power than a simple list of different genes or proteins (Khatri P., 2012).

Post-genomic analysis was undertaken using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da et al., 2009). This is an online tool to which a list of genes can be submitted and subsequently results are generated regarding the genes' involvement in biological processes (Huang da et al., 2009). The gene list was comprised of genes corresponding to all SNPs with a p value of $p<0.0001$ in the GWAS analysis

2.6 Statistical power

Chapter 3 compares the prevalence of having a PCS score of 9 or higher in 1172 OA cases (unknown prevalence) to that in 402 controls (prevalence 33%). This type of analysis has not been carried out in the literature, therefore I have assumed that the odds of OA cases of having a "high" pain catastrophizing score (defined as 9 or higher) is 50% higher than for controls. This relatively modest effect of 1.5 given a study with approximately 3 times more cases than controls (as in my study) would require 823 OA cases to be compared to 283 to achieve 80% power at an alpha level of 0.05. This is a sample 30% smaller than the one I have used therefore my study is sufficiently powered.

Chapter 4 focuses on genetic discovery. I did not know a priori what kind of genetic effect sizes could be expected for pain catastrophizing. I have therefore simply estimated the effect sizes that my study is powered to detect for a variant with a minor

allele frequency of 20% to detect as genome wide significant (p< 5 x 10[-8]). My study is an association with an odds ratio of 2.4 or higher. In order to select variants for future replication (p< 1 x 10[-5]) the study has 80% power to detect variants with an odds ratio of 2.05 or higher. For the replication phase, the study has sufficient power to detect at a nominal value of 0.05 in 1093 individuals from the GOAL cohort an association with odds ratios of 1.5 or higher.

For Chapter 5 the situation was the same as for Chapter 4, with no published effect sizes for rare variants associated with symptomatic OA as only a few common variants have been described in the literature. In this case I also have computed the minimum effect size for my study, and found that the study has 80% power to detect variants with a 10% frequency(this chip has only functional variants with a lower mean allele frequency) with p< 1 x 10[-5] for associations with OR >2.4

3 Role of catastrophizing on pain in the post-TJR population, comparison to controls

3.1 Introduction

The aim of this study was to investigate differences in catastrophizing levels between participants with OA, including those who had undergone TJR, and age matched controls without OA. The objectives were then to investigate whether pain catastrophizing was more prevalent in individuals who had undergone TJR than in controls and how much, if any, of the association between catastrophizing on joint and body pain could be explained by anxiety, depression, poor sleep quality or any confounding effect of co-morbidities.

3.2 Methods

Described in this section is the study design and techniques used.

3.2.1 Study Design

A case- control design was used to compare two case groups to a non-OA control group.

The NGOAS (Nottingham Genetics of OA study) cohort, which was used as one of the case groups, contained individuals with severe hip or knee OA on the waiting list for TJR identified from hospital orthopaedic clinic lists in the Nottingham area.

The GOAL (Genetics of Osteoarthritis and Lifestyle Study) cohort is a case–control study undertaken in Nottinghamshire that was originally designed to investigate gene–environment interaction in large-joint OA. (Limer K. L., 2009, Zhang W., 2008) The study recruited men and women referred to hospital with symptomatic, radiographic OA (ROA) of the knee (n = 1040) or hip (n = 1004), as well as asymptomatic controls

(n = 1123) with no ROA of hips or knees referred to hospital for IVU between 2002 and 2006. This cohort was therefore used as another case group as well as a control group.

Antero-posterior and sky-line or lateral knee radiographs of study subjects were examined to confirm the diagnosis and to grade for changes of OA. The intra-observer reproducibility for the grading of the tibio-femoral compartment (repeat readings of 30 randomly selected films 2 weeks apart, blinded to identity and previous scores) was $\kappa = 0.89$ and for the patello-femoral compartment $\kappa = 0.79$. Knee OA was assessed using the scores for the narrowing and osteophytes derived from the Nottingham " knee osteoarthritis line drawing atlas" (Nagaosa Y., 2000) and then given a grade according to the descriptors of grades 1–4 (Kellgren J.H., 1957). An individual was considered to have knee OA if he or she presented as K/L $\geq$ 2 at the tibio-femoral (TF) or the patello-femoral (PF) compartment in either knee based on the above definition.

3.2.1.1 Pain Catastrophizing

The Pain Catastrophizing Scale (PCS) is a 13 item questionnaire created by Sullivan et al.(2009) to measure the three components of catastrophizing: rumination, magnification and helplessness (Sullivan, 2009). In this current study participants were classified as having high catastrophizing if they were in the top tertile with a score of 9 or greater.

3.2.1.2 Pain Intensity/ Neuropathic-like Pain

Two different measures of pain were used. The visual analogue scale (VAS) (Hawker G. A., 2011b) was used to measure pain intensity. The cut off used for VAS in this study was 6 based on answers to worst pain.

The pain detect questionnaire (PDQ) (Mathieson S., 2013) was used to measure NP (Freynhagen R., 2006). The cut-off point used to determine NP in this study was 12 or more based on validated use of the PDQ.

3.2.1.3 Anxiety and Depression

In the NGOAS cohort this was based on a yes/no question in the medical history section of the follow up questionnaire. In the GOAL cohort depression and anxiety were calculated based on the HADS questionnaire (Assessment, 2015), which is a standardised questionnaire that measures both depression and anxiety categories calculated according to the following scores:

1 normal (0-7)

2 borderline abnormal (8-10)

3 abnormal (11-21)

To combine depression in both cohorts the score in the GOAL cohort was converted to yes/no with both situations 2 and 3 in the HADS questionnaire being considered as having depression.

3.2.1.4 Sleep

Four questions drawn from the Medical Outcomes Study (MOS) sleep survey (Hays and Stewart, 1992) were used to assess quality of sleep. This version of the

questionnaire was validated in a subset of participants and showed a concordance of 95% and a correlation of 0.82 with the 10 item questionnaire. The sum of responses was scored from 0 (most disturbed sleep) to 100 (optimal sleep). A cut-off of 55 was used for “poor sleep” (quartile with the most disturbed sleep).

3.2.1.5 Cardiovascular disease (CVD) and other comorbidities

To calculate whether a participant had CVD their response for stroke, hypertension, heart attack/angina and high cholesterol was combined and if they had one or more of the mentioned conditions they were reported as having CVD. Participants were also asked if they were affected by diabetes, cancer and other diseases.

3.2.2 Statistical Analysis

Descriptive statistics were used to summarize the relevant characteristics of the cohorts. Associations between high catastrophizing, severe pain and other binary outcomes with risk factors were carried out using binary logistic regression methods adjusting for age, gender and BMI as covariates. The SPSS package version 22 was used.

3.3 Results

3.3.1 Demographics

The descriptive characteristics of the study participants are shown in Table 3-1. The case and control groups differed significantly in levels of pain, age, BMI and prevalence of comorbidities, but not depression and catastrophizing.

Table 3-1: Participant demographics and clinical details

Characteristic	**GOAL controls**	**GOAL cases**	**NGOAS cases**	**p-value (cases v controls)[1]**
n	402	679	1093	
Women (%)	47.25	49.7	56.45	0.44
Age years (SD)	73.87 ± 7.90	77.48±6.94	73.26 ±9.05	1.20E-13
BMI kgm/ m^2 (SD)	26.80 ±4.19	28.41±4.49	29.27 ±5.15	1.59E-08
13- PCS score (SD)	8.01 ±9.75	9.33 ±10.20	9.38 ±12.14	0.17
Post TJR	0%	59.70%	82%	
High PCS (≥9)	33.10%	39.80%	34.90%	0.07
Severe joint pain (VAS ≥6)	25.60%	36.70%	35.20%	0.001
Depression (yes/no) [2]	21.00%	28.80%	15.40%	0.11
Poor sleep (>55 out of 100, MOS score)	20.30%	29.20%	32.80%	0.02
Diabetes	13.90%	17.80%	14.30%	0.65

CVD [3]	63.40%	69.20%	66.80%	0.85
Body pain [4]	7.70%	16.20%	19.50%	0.003
Possible NP (≥12)	8.70%	17.10%	20.00%	6.09E-04

1- comparing GOAL cases to GOAL controls (not adjusted) n=1081

2- in the GOAL cohort HADs questionnaire was used, in the NGOAS cohort a yes/no question in the medical history section of the questionnaire was used

3- includes high cholesterol, heart attack/angina, hypertension and stroke

4- in the GOAL cohort chronic widespread pain was measured, in the NGOAS cohort body pain was measured, both used a body mannequin but the question regarding pain was phrased differently

Before assessing any relationship between PCS and other exposures, I first determined whether the OA and post TJR case groups could be merged or should be analysed separately. In order to do so I compared the main characteristics between these two groups. As can be seen in Table 3-2 The majority of cases were assessed after they had their TJR. These individuals had significantly lower levels of pain measured as WOMAC, VAS or as the proportion with possible neuropathic-like joint pain than those with ongoing OA on waiting list for a TJR. They were are also on average 3 and a half years older than those on waiting list for a TJR. However, I was unable to detect a significant difference in the levels of catastrophizing, disturbed sleep, CVD, and depression, as well as female sex and BMI between these two groups. For this reason I decided to include both groups together as cases whilst adjusting for OA status in all the analyses.

Table 3-2: Comparison between OA and post TJR participants in the two cohorts.

A t-test was carried out to compare the means of PCS scores, WOMAC pain scores, age and BMI. Proportions of individuals with CVD, Female sex, depression, bottom tertile of sleep, top tertile of PCS, VAS >6 and with possible

	OA N=378					post TJR n=1393					P-value
	median	Q1	Q3	mean	SD	median	Q1	Q3	mean	SD	
PCS score	5	1	15	10.26	12.41	5	1	13	9.12	11.15	0.10
WOMAC pain	7	2	10	6.78	0.96	5	0	9	5.37	0.93	1.02E-06
Possible NP				25.20%					17.23%		4.97E-04
PCS >=9				38.89%					36.25%		0.35
VAS>6				50.14%					31.92%		1.33E-10
age	72.98	66.7	80.2	72.79	9.53	76.45	69.8	81.3	75.43	8.19	1.31E-07
F%				53.72%					53.91%		0.95
BMI	28.4	25.7	32.42	29.28	5.05	28.3	25.6	31.5	28.85	4.89	0.13
Depression Y/N				21.54%		0	0	0	20.22%		0.57
Poor sleep tertile				32.89%		0	0	1	31.01%		0.48
CVD				64.55%		1	0	1	68.56%		0.14

neuropathic pain (PossNP) were compared in a logistic regression model adjusting for cohort of origin.

3.3.2 Significant associations with catastrophizing

Female gender, high BMI, joint pain measured in various ways (VAS, and painDETECT), body pain, sleep quality, depression, anxiety and chronic widespread pain was each independently associated with catastrophizing. Cardio-vascular related health traits were also associated with catastrophizing after adjusting for age, sex and BMI (Table 3-2).

Table 3-3: Risk factors contributing to being in the top tertile of pain catastrophizing. The association with high pain catastrophizing is summarized as an odds ratio (OR) and its 95% confidence intervals (CI). Data refers to both cohorts (adjusted for age, sex, BMI, cohort and OA status)(n= 2174)

Variable	OR	95% CI	p
Age *	0.99	0.98-1.00	0.017
Female Sex *	1.36	1.14-1.63	0.001
Body Mass Index *	1.04	1.02-1.06	3.30E-05
Possible neuropathic pain (>12)	5.35	4.16-6.90	1.42E-38
Severe joint pain	4.8	3.95-5.85	6.59E-55
Diabetes	1.18	0.92-1.52	0.2
Depression (yes/no) [1]	3.84	2.98-4.95	2.33E-25
Body pain [2]	1.47	1.16-1.86	0.002

Cardiovascular disease [3]	1.43	1.17-1.74	3.98E-04
Sleep (bottom Quartile)	3.77	3.09-4.60	4.09E-39

The association with high pain catastrophizing is summarized as an odds ratio (OR) and its 95% confidence intervals (CI). Data refers to both cohorts (adjusted for age, sex, BMI, cohort and OA status)(n= 2174) The NGOAS cohort and the GOAL cases were combined making sure all values were comparable by converting score for depression in the GOAL cohort to a dichotomous variable.

* adjusted for OA and cohort only

1- in the GOAL cohort HADs questionnaire was used, in the NGOAS cohort a yes/no question in the medical history section of the questionnaire was used

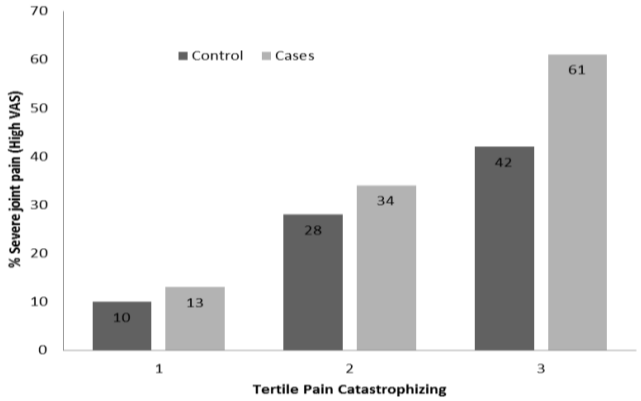
2- in the GOAL cohort chronic widespread pain was measured, in the NGOAS cohort body pain was measured, both used a body mannequin but the question regarding pain was phrased differently

3- includes high cholesterol, heart attack/angina, hypertension and stroke

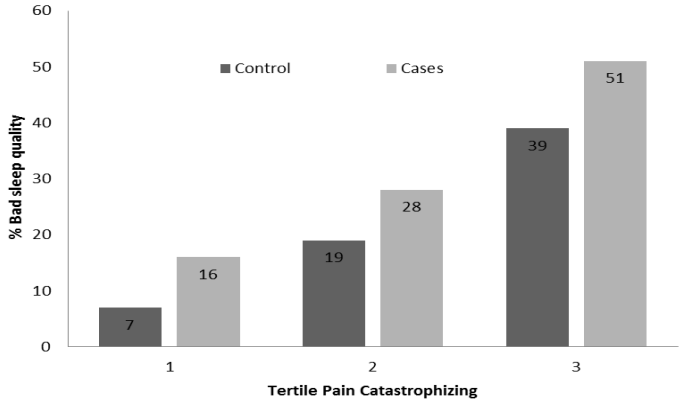
Figure 3-1 below shows a definite upwards linear trend indicating that as levels of catastrophizing increase so do levels of pain severity,bad sleep quality, cardiovascular disease (CVD) and depression. This figure further shows that although catastrophizing affects both cases and controls the effect is exaggerated in the cases. All indicating that these are risk factors for catastrophizing and potentially should be considered further.

Figure 3-1: Effect of PCS on severe joint pain, bad quality sleep, Cardiovascular disease and depression

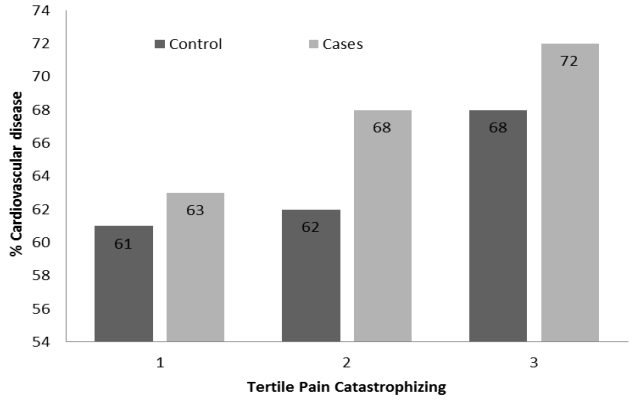
A: Effect of catastrophizing, as defined by Pain Catastrophizing Scale (PCS) score of >=9, on severe joint pain. Effect of Pain Catastrophizing Scale (PCS) on severe joint pain. Severe joint pain was defined as a Visual Analogue Scale (VAS) score of 6 or higher on a scale from 0-10 where 0= no pain. The percentage of individuals within a given PCS tertile category who qualify as having "severe joint pain" is shown in both cases (OA and post TJR) and controls all recruited from secondary care.



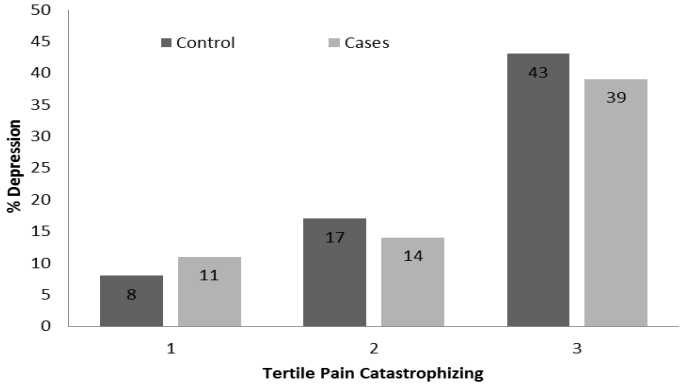
B: Effect of catastrophizing, as defined by Pain Catastrophizing Scale (PCS) score of >=9, on bad quality of sleep. Effect of Pain Catastrophizing Scale (PCS) on disturbed sleep. Disturbed sleep was defined as a Medical Outcomes Score falling within the lowest quartile. The percentage of individuals within a given PCS tertile category who qualify as having "bad sleep" is shown in both cases (OA and post TJR) and controls.



C: Effect of catastrophizing, as defined by Pain Catastrophizing Scale (PCS) score of >=9, on Cardiovascular disease (CVD). Effect of Pain Catastrophizing Scale (PCS) on prevalence of self-reported cardiovascular disease (CVD). Indivduals were considered as being affected with CVD if they reported to have been diagnosed with any of the following: previous history of myocardial infarction, stroke, high blood pressure and high cholesterol The percentage of individuals within a given PCS tertile category who qualify as having CVD is shown in both cases (OA and post TJR) and controls.



D: Effect of catastrophizing, as defined by Pain Catastrophizing Scale (PCS) score of >=9, on depression. Effect of Pain Catastrophizing Scale (PCS) on prevalence of depression. Indivduals were considered as being having depression if they scored >=8 on the HADS questionnaire or self-reported depression. The percentage of individuals within a given PCS tertile category who qualify as having depression is shown in both cases (OA and post TJR) and controls



3.3.3 Catastrophizing, depression, anxiety, poor sleep and impact on pain intensity

A further question to address was whether the strong link between pain catastrophizing and pain intensity is mediated by poor quality sleep, depression or anxiety. Associations between catastrophizing and pain remained significant after adjusting for BMI, age, sex, depression and sleep (Table 3-3). As seen in Table 3-3 after the cohorts were combined and adjusted for BMI, age, sex and OA status, pain catastrophizing remained significant along with depression and sleep. Furthermore, catastrophizing significantly differed between severe joint pain and less severe joint pain after adjusting for depression, sleep and other variables (Table 3-3).

Table 3-4 shows that across all cohorts there is a significant effect of catastrophizing on joint pain, severity, neuropathic pain, CVD, and multiple regional pain even after adjusting for sleep, depression and anxiety using regression models. Table 3-5 then demonstrates the same pattern when only those with hip OA from all cohorts have been analysed. Table 3-6 shows again the same pattern this time when only those with knee OA all cohorts are studied Table 3-7 shows the same pattern when only those in the GOAL cohort with hip OA are analysed Table 3-8 shows the same pattern when only those in the GOAL cohort with knee OA are analysed. Table 3-9 shows the same pattern when only those in the GOAL cohort with hip OA are analysed. Table 3-10 shows the same pattern when only those in the NGOAS cohort with knee OA are analysed

Table 3-4: Role of high catastrophizing as defined by a score of >=9 from the Pain Catastrophizing Scale on severe pain, chronic widespread pain (CWP), cardiovascular disease (CVD), and neuropathic pain (NP). This was conducted for all individuals. Binary logistic regression was carried out in SPSS.

Outcome	**Risk factor**	**PCS**		**Depression**		**Sleep quality**		**Anxiety (4)**	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	4.8	(3.94-5.85) ‡	1.48	1.15-1.91†	2.33	1.91-2.84 ‡	1.81	1.49-2.20‡
	adjusted (2)	4.59	3.52-5.99‡	0.97	0.73-1.29*	1.63	1.28-2.07‡		
	adjusted (3)	2.99	2.20-4.04‡	2.71	1.23-5.96*	1.34	0.96-1.87*	0.68	0.40-1.15*
Multiple regional pain (5)	unadjusted (1)	1.47	1.16-1.86 †	1.64	1.21-2.21 †	1.33	1.04-1.70*	1.74	1.36-2.23‡
	adjusted (2)	1.38	1.03-1.84*	1.38	1.00-1.91*	1.15	0.85-1.55*		
	adjusted (3)	2.01	1.32-3.07†	1.09	0.42-2.85*	1.36	0.88-2.09*	1.27	0.68-2.35*
Cardiovascular Disease (CVD)	unadjusted (1)	1.43	1.17-1.74 ‡	1.48	1.16-1.90†	1.25	1.01-1.54 †	1.21	0.98-1.49*
	adjusted (2)	1.15	0.93-1.41*	1.40	1.08-1.81*	1.12	0.90-1.40*		
	adjusted (3)	1.38	1.01-1.88†	2.65	1.09-6.44†	1.12	0.80-1.59*	0.59	0.33-1.05*
Neuropathic pain	unadjusted (1)	5.34	4.15-6.88‡	2.18	1.67-2.86‡	3.93	3.09-5.01‡	1.94	1.53-2.47‡
	adjusted (2)	3.93	2.87-5.38‡	1.23	0.91-1.66*	2.82	2.17-3.65‡		
	adjusted (3)	2.71	1.76-4.16‡	2.22	0.88-5.62†	2.24	1.47-3.40‡	0.78	0.43-1.42†

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
(4) measured only in GOAL cohort from HADS questionnaire
(5) measured only in GOAL cohort
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-5: Association in individuals with a diagnosis of hip OA or post THR for hip OA between pain catastrophizing, depression, sleep quality and anxiety with severe joint pain, chronic widespread pain (CWP), self-reported cardiovascular disease (CVD), and neuropathic pain. Binary logistic regression was carried out using SPSS

Outcome	Risk factor	PCS		Depression		Sleep quality		Anxiety (4)	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	4.89	3.06-7.83‡	1.44	0.90-2.29*	1.77	1.19-2.63†	1.84	1.27-2.66 †
	adjusted (2)	4.48	2.76-7.27‡	0.97	0.58-1.62*	1.47	0.96-2.24*	-	-
	adjusted (3)	2.43	1.37-4.29†	6.48	1.44-29.21*	2.03	1.14-3.62*	0.4	0.14-3.62*
Multiple regional pain (5)	unadjusted (1)	2.99	1.51-5.88†	1.49	0.72-3.09*	1.34	0.66-2.73*	1.48	0.93-2.35*
	adjusted (2)	2.87	1.36-6.05†	0.91	0.40-2.10*	1.06	0.49-2.32*	-	-
	adjusted (3)	2.77	1.30-5.88†	0.24	0.03-2.05*	1.01	0.45-2.24*	2.51	0.69-9.16*
Cardiovascular Disease (CVD)	unadjusted (1)	1.32	0.93-1.87*	1.32	0.85-2.05*	1.20	0.83-1.74*	1.38	0.92-2.07*
	adjusted (2)	1.28	0.89-1.83*	1.21	0.77-1.91*	1.13	0.77-1.65*	-	-
	adjusted (3)	1.84	1.04-3.27*	0.61	0.12-3.06*	0.95	0.53-1.69*	1.57	0.50-4.88*
Neuropathic pain	unadjusted (1)	3.97	2.33-6.77‡	2.79	1.70-4.57‡	3.92	2.48-6.21‡	2.55	1.63-3.98‡
	adjusted (2)	3.24	1.83-5.74‡	1.73	1.00-2.99*	2.74	1.68-4.47‡	-	-
	adjusted (3)	2.06	0.86-4.94*	9.11	1.62-51.27*	3.48	1.58-7.65†	0.47	0.15-1.51*

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
(4) measured only in GOAL cohort from HADS questionnaire
(5) measured only in GOAL cohort
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-6. Association in individuals with a diagnosis of knee OA or post TKR for knee OA between pain catastrophizing, depression, sleep quality and anxiety with severe joint pain, chronic widespread pain (CWP), self-reported cardiovascular CVD, and neuropathic pain (NP). Binary logistic regression was carried out in SPSS

Outcome	Risk factor	PCS		Depression		Sleep quality		Anxiety (4)	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	5.51	3.90-7.80‡	1.22	0.86-1.72*	2.48	1.85-3.33‡	1.46	1.03-2.09*
	adjusted (2)	4.94	3.45-7.07‡	0.79	0.53-1.17*	1.94	1.41-2.66‡	-	-
	adjusted (3)	4.41	2.37-8.22‡	0.76	0.18-3.23*	0.96	0.51-1.81*	1.07	0.43-2.68*
Chronic widespread pain (5)	unadjusted (1)	2.34	1.23-4.47*	3.57	1.81-7.02‡	2.56	1.33-4.92†	2.29	1.50-3.49‡
	adjusted (2)	1.32	0.62-2.84*	2.52	1.15-5.50*	1.83	0.89-3.75*	-	-
	adjusted (3)	1.24	0.57-2.70*	1.32	0.28-6.29*	1.85	0.90-3.80*	1.61	0.60-4.31*
CVD	unadjusted (1)	1.15	0.87-1.53*	1.46	1.02-2.09*	1.29	0.96-1.73*	1.46	0.95-2.23*
	adjusted (2)	1.04	0.77-1.40*	1.41	0.98-2.04*	1.21	0.89-1.64*	-	-
	adjusted (3)	1.57	0.79-3.13*	23.52	2.54-217.61†	0.89	0.44-1.80*	0.21	0.06-0.74*
Neuropathic pain	unadjusted (1)	6.05	3.98-9.18‡	1.69	1.18-2.41†	4.04	2.94-5.54‡	1.59	1.03-2.46*
	adjusted (2)	4.63	2.98-7.18‡	0.94	0.62-1.41*	3.10	2.22-4.34‡	-	-
	adjusted (3)	4.6	2.04-10.37‡	1	0.18-5.63*	1.51	0.71-3.25*	0.94	0.32-2.76*

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
(4) measured only in GOAL cohort from HADS questionnaire
(5) measured only in GOAL cohort
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-7: Analysis using only individuals in the GOAL cohort with hip OA as defined a diagnosis of hip OA or THR. Binary logistic regression was carried out in SPSS (1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)

Outcome	Risk factor	PCS		Depression		Sleep quality		Anxiety (4)	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	2.89	1.74-4.79‡	3.09	1.78-5.37‡	2.54	1.49-4.33 †	1.84	1.27-2.66 †
	adjusted (2)	2.24	1.28-3.92†	1.86	1.00-3.46*	1.95	1.10-3.47*	-	-
	adjusted (3)	2.43	1.37-4.29†	6.48	1.44-29.21*	2.03	1.14-3.62*	0.4	0.14-3.62*
Chronic widespread pain (5)	unadjusted (1)	2.99	1.51-5.88†	1.49	0.72-3.09*	1.34	0.66-2.73*	1.48	0.93-2.35*
	adjusted (2)	2.87	1.36-6.05†	0.91	0.40-2.10*	1.06	0.49-2.32*	-	-
	adjusted (3)	2.77	1.30-5.88†	0.24	0.03-2.05*	1.01	0.45-2.24*	2.51	0.69-9.16*
CVD	unadjusted (1)	1.78	1.06-2.99*	1.46	0.81-2.62*	1.07	0.62-1.85*	1.38	0.92-2.07*
	adjusted (2)	1.9	1.08-3.36*	1.11	0.57-2.13*	0.96	0.54-1.70*	-	-
	adjusted (3)	1.84	1.04-3.27*	0.61	0.12-3.06*	0.95	0.53-1.69*	1.57	0.50-4.88*
Neuropathic pain	unadjusted (1)	3.4	1.64-7.03†	5.92	2.83-12.37‡	5.41	2.62-11.18‡	2.55	1.63-3.98‡
	adjusted (2)	1.86	0.78-4.40*	3.34	1.44-7.74 †	3.31	1.52-7.24 †	-	-
	adjusted (3)	2.06	0.86-4.94*	9.11	1.62-51.27*	3.48	1.58-7.65†	0.47	0.15-1.51*

(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
(4) measured only in GOAL cohort from HADS questionnaire
(5) measured only in GOAL cohort
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-8: Analysis using only individuals in the GOAL cohort with knee OA as defined by either a diagnosis of knee OA or TKR. Binary logistic regression was carried out in SPSS

Outcome	Risk factor	PCS		Depression		Sleep quality		Anxiety (4)	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	4.22	2.47-7.20‡	1.71	0.99-2.96*	1.51	0.88-2.61*	1.46	1.03-2.09*
	adjusted (2)	4.44	2.39-8.23‡	0.84	0.43-1.65*	0.96	0.51-1.81*	-	-
	adjusted (3)	4.41	2.37-8.22‡	0.76	0.18-3.23*	0.96	0.51-1.81*	1.07	0.43-2.68*
Chronic widespread pain (5)	unadjusted (1)	2.34	1.23-4.47*	3.57	1.81-7.02‡	2.56	1.33-4.92†	2.29	1.50-3.49‡
	adjusted (2)	1.32	0.62-2.84*	2.52	1.15-5.50*	1.83	0.89-3.75*	-	-
	adjusted (3)	1.24	0.57-2.70*	1.32	0.28-6.29*	1.85	0.90-3.80*	1.61	0.60-4.31*
CVD	unadjusted (1)	1.86	1.04-3.32*	2.55	1.30-5.00†	1.32	0.71-2.48*	1.46	0.95-2.23*
	adjusted (2)	1.38	0.71-2.67*	2.22	1.03-4.81*	0.88	0.44-1.77*	-	-
	adjusted (3)	1.57	0.79-3.13*	23.52	2.54-217.61†	0.89	0.44-1.80*	0.21	0.06-0.74*
Neuropathic pain	unadjusted (1)	5.23	2.51-10.88‡	2.07	1.04-4.15*	2.42	1.23-4.76*	1.59	1.03-2.46*
	adjusted (2)	4.58	2.04-10.28‡	0.92	0.40-2.10*	1.51	0.71-3.24*	-	-
	adjusted (3)	4.6	2.04-10.37‡	1	0.18-5.63*	1.51	0.71-3.25*	0.94	0.32-2.76*

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-9: Analysis using only individuals in the NGOAS cohort with hip OA as defined by either a diagnosis of hip OA or THR. Binary logistic regression was carried out in SPSS

Outcome	Risk factor	PCS		Depression		Sleep quality	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	8.87	5.06-15.54‡	1.35	0.68-2.66*	2.19	1.29-3.70*
	adjusted (2)	8.47	4.70-15.27‡	1.17	0.54-2.56*	1.13	0.61-2.10*
	adjusted (3)	-	-	-	-	-	-
CVD	unadjusted (1)	0.96	0.58-1.61*	1.98	0.95-4.16*	1.29	0.77-2.16*
	adjusted (2)	0.89	0.51-1.52*	1.91	0.91-4.02*	1.27	0.74-2.20*
	adjusted (3)	-	-	-	-	-	-
Neuropathic pain	unadjusted (1)	8	3.91-16.35‡	1.33	0.58-3.04*	3.58	1.82-7.03†
	adjusted (2)	3.95	2.29-6.82‡	1.09	0.43-2.76*	1.87	0.88-3.95*
	adjusted (3)	-	-	-	-	-	-

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Table 3-10: Analysis using only individuals in the NGOAS cohort with knee OA as defined by either a diagnosis of knee OA or TKR. Binary logistic regression was carried out in SPSS

Outcome	Risk factor	PCS		Depression		Sleep quality	
		OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Severe joint pain (high VAS)	unadjusted (1)	5.57	3.96-7.84‡	1.12	0.73-1.72*	2.66	1.91-3.72‡
	adjusted (2)	5	3.50-7.14‡	0.67	0.41-1.09*	1.85	1.27-2.68†
	adjusted (3)	-	-	-	-	-	-
CVD	unadjusted (1)	1.63	1.16-2.29*	1.44	0.91-2.26*	1.15	0.82-1.61*
	adjusted (2)	1.6	1.12-2.29*	1.32	0.83-2.11*	0.96	0.66-1.37*
	adjusted (3)	-	-	-	-	-	-
Neuropathic pain	unadjusted (1)	6.41	4.36-9.43‡	1.57	1.01-2.44*	4.43	3.07-6.40‡
	adjusted (2)	5.05	3.37-7.56‡	0.84	0.51-1.41*	3.13	2.10-4.68‡
	adjusted (3)	-	-	-	-	-	-

(1) adjusted only for age, sex, BMI, cohort and OA (includes GOAL and NGOAS)
(2) model includes sleep, depression (y/n used), PCS, age, sex and BMI (includes GOAL and NGOAS)
(3) model includes anxiety, sleep, depression (y/n used), PCS, age, sex and BMI (GOAL cohort only)
* $p < 0.05$; † $p < 0.001$; ‡ $p < 1 \times 10^{-5}$

Another potential confounder is radiographic severity of OA, which was measured in the NGOAS cohorts using the Kellgren-Lawrence grade (Yoshida et al., 2015)

I explored this only in the NGOAS cohort where the X rays had been taken 1 -3 years maximum before the pain and PCS assessment. In the GOAL cohort the X-rays were taken at the initial visit which was >5 years apart from the PCS assessment

In this sub-analysis I first tested whether there was an association between PCS status (top vs bottom two tertiles) with Kellgren-Lawrence (K/L) grade adjusting for age, sex and BMI separating current OA cases from post -TJR cases. I find that there is no association between radiographic severity and being in the top tertile of the PCS distribution (Table 3-10). Moreover, inclusion of K/L as a potential confounder in the relationship between joint pain (as measured by the WOMAC pain score) and catastrophizing status made no difference to the association between these two both in the pre-TJR and post TJR groups.

Table 3-11: Lack of association between catastrophizing status (PCS >=9 vs <9) and radiographic severity measured as Kellgren-Lawrence (K/L) grade.

Association between high catastrophizing and K/L grade	**OR**	**95 CI**		**p-value**
all n=1128	0.96	0.81	1.14	0.66
OA (no TJR) n=271	0.90	0.59	1.36	0.60
OA post TJR =857	1.00	0.83	1.22	0.99
Association joint pain and high catastrophizing	**OR**	**95 CI**		**p-value**
OA (no TJR) n=271	4.64	2.54	8.48	5.9E-07
OA (no TJR) n=271 adj for K/L grade	4.63	2.53	8.48	7.1E-07
assoc of K/L grade with joint pain pre TJR	1.46	0.89	2.38	0.13
OA post TJR =857	10.80	7.32	15.93	3.85E-33
OA post TJR =857 adj K/L grade	10.88	7.37	16.08	3.6E-33
assoc of K/L grade with joint pain post TJR	0.83	0.64	1.09	0.18

*All results are adjusted for age, sex and BMI. Joint pain =WOMAC pain >9, catastrophizing = top tertile of PCS as defined above

3.3 Discussion

In this study pain catastrophizing was strongly correlated with joint pain severity, chronic widespread pain and neuropathic-like joint pain symptoms independently of sleep quality, anxiety and depression. High catastrophizing scores were found to correlate with: female sex, higher BMI, joint pain measured in various ways (VAS, WOMAC, painDETECT), body pain, sleep quality, depression, anxiety and chronic widespread pain (Table 3-2).

There was also an association between catastrophizing and cardiovascular disease which remained significant after adjustment for anxiety, sleep and depression. Cardiovascular related health traits were further found to be associated with catastrophizing after adjusting for age, sex and BMI (Table 3-2).

A further finding was that catastrophizing contributes to various pain phenotypes, including neuropathic-like pain symptoms, after adjustment for self-reported sleep quality.

There was no significant difference in the prevalence of high pain catastrophizing between cases (OA and post TJR) and controls (Table 3-1). However there were strong significant differences in the levels of pain intensity (Table 3-1) between these two groups and a high correlation between pain intensity and pain catastrophizing (Figure 3-1).

When analysing hip OA and knee OA separately in Table 3-5 and Table 3-6 there was no difference in terms of the effect of catastrophizing on pain between these two groups of patients, indicating that the pooled analyses are valid for these two forms of post-TJR pain.

Following the separate analysis of hip OA and knee OA these groups were then further split into the GOAL and NGOAS cohorts so controls could be compared against cases as shown in Table 3-6 to Table 3-9. In the GOAL cohort PCS was more significant in knees than hips, though still not as significant as both cohorts together.

In the NGOAS cohort PCS was about the same in terms of significance and is about the same as in the GOAL cohort.

The key result, however, is that after adjusting for various potential confounders including anxiety, depression and sleep quality, pain catastrophizing remains a significant risk factor for severe joint pain and also for, neuropathic pain symptoms, chronic widespread pain and cardiovascular disease (Table 3-3).

Additionally, the prevalence of high catastrophizing scores is almost the same in cases and controls (Table 3-1) which is not what would be expected if catastrophizing caused pain. Furthermore, the effects of catastrophizing on joint pain were the same in OA and non-OA controls as shown in figure 3-1. This would seem to demonstrate the catastrophizing is a trait in its own right and is not dependant on depression and sleep to affect pain intensity and therefore should be treated as a separate entity.

When analysing hip OA and knee OA separately in Table 3-4 and Table 3-5 there is no difference in terms of the effect of catastrophizing on pain between these two groups of patients indicated that the pooled analyses are valid for these two forms of post-TJR pain.

This is consistent with the idea that catastrophizing is not a redundant trait captured by other measures of mood (Sullivan M., 1990, Sullivan M., 1998, Geisser M. E., 1994, Martin M. Y., 1996). Although previous studies such as Sullivan et al (2010) have used a cut-off of 16 it is worth noting that this is a cut-off used in the US and that

cultural differences are present with regards to this scale (Table 1-1). In my data I see a very different mean value in the general population in our UK study compared to that seen in the US by Goodin et al (2012) and, further, within the US population there are clear differences between ethnic groups. A study in Canada with similar characteristics (Hawker et al 2011) had much lower values than those reported by the US study. This suggested that cut-offs may not translate easily even between similar cultures (e.g. Canada, US, UK) and I therefore chose to split the cohort data available to me by tertiles. I must also note that within the UK participants that I study there are striking differences in terms of all measures of pain, depression, anxiety, and sleep. Given these cultural differences, it is justified to compare the top and the bottom two tertiles of the pain catastrophizing scale rather than focus on a given cut-off. Moreover, in my work I found that the top and bottom two tertiles of PCS have strikingly different distributions for all pain scores in addition to other measures of negative affect which suggest that the lack of a significant association between OA case and control and pain catastrophizing is not due to the fact that the scale is not measuring something relevant. Rather, it is in agreement with previous studies that suggest that pain catastrophizing is a trait and not just a state that results from pain status

To my knowledge this is the largest study to date to explore the effect of catastrophizing on joint and body pain independent of depression and sleep. Some studies have reported that the effects of catastrophizing and depression cannot be separated and that catastrophizing is not a discrete psychological variable (George S. Z., 2006, Hirsh A. T., 2007, Mounce C., 2010), whereas others have found that catastrophizing uniquely predicts pain levels (Sullivan M., 1990, Sullivan M., 1998, Geisser M. E., 1994, Martin M. Y., 1996, Keefe F. J., 2000). These data add to the body of evidence of studies that have reported a significant contribution by pain

catastrophizing on pain and functional outcomes post TKR after adjusting for depression, indicating that high levels of catastrophizing amplify joint pain in OA and post TJR individuals independently of depression. This is consistent with a study which used fMRI to show that pain catastrophizing, independent of the influence of depression, was significantly associated with increased activity in brain areas related to anticipation of pain, attention to pain, emotional aspects of pain and motor control. (Gorczyca R., 2013) It is also in agreement with a recent study showing that manipulation of pain catastrophizing via hypnosis alters the levels of pain experienced by sufferers of chronic pain and healthy volunteers. (Kjogx H., 2016) Previous literature has shown that better sleep quality can reduce pain intensity (Vitiello M. V., 2014), and that those with insomnia experience greater pain, depression, and pain-related catastrophizing (Campbell C. M., 2015). There was a strong correlation between pain catastrophizing and self-reported sleep quality, which accords with the observation that it is an independent contributor to insomnia in patients with chronic musculoskeletal pain (Wilt J. A., 2016, Vitiello M. V., 2014, Chouchou F., 2014, Campbell C. M., 2015, Byers H.D., 2015). However, the correlation between pain catastrophizing and the various pain traits studied here does not appear to be mediated by its effect on sleep quality. In addition to the association with joint pain intensity, neuropathic-like pain symptoms and chronic widespread pain, there was also a significant correlation between pain catastrophizing and CVD. These data are in agreement with a previous report by Leonard et al (2013) which found that higher blood pressure in those with catastrophizing is possibly due to higher stress levels (Leonard M. T., 2013). Furthermore, individual differences in the extent to which an individual tolerates uncertainty and perceives feared consequences to their worries as severe has been associated with differential autonomic responses to worry and worry catastrophizing, and may partially account for the link between stress and cardiovascular health (Deschênes S. S., 2016, Perlo S., 2016). These data therefore further suggest that pain catastrophizing as a trait may have wider health implications

in addition to its impact on pain, possibly due to effects on the autonomic nervous system.

The observation from figure 3-1A suggests that catastrophizing may amplify the pain intensity in individuals affected by chronic pain but that experiencing higher pain does not necessarily result in higher levels of catastrophizing. The equal prevalence of catastrophizing in cases and controls suggests that catastrophizing as measured here acts as a trait, rather than a state, on joint and body pain, an issue which is debated in the literature currently (Sturgeon J. A., 2013, Sturgeon J. A., 2014, Plazier M., 2015).

A potential link between CVD and catastrophizing is fear, which is regulated by the sympathetic nervous system (SNS).

There are several limitations to this study. Firstly, there are no pre-TJR data and it is possible that in post-TJR cases catastrophizing may change with their levels of pain. However, Sullivan and co-workers found that pain catastrophizing predicts post-surgical pain to the same extent as pre-surgical pain, as well as the contribution of catastrophizing to the prediction of post-surgical pain being independent of the effect of pre-surgical pain. (Sullivan M., 2009)

Secondly, the control group comprised people selected on the basis of having no hip or knee OA which may have introduced bias, and ideally a sample of the general population is required for comparison to the OA cohorts. Further to this ideally to answer the research question for this chapter a case group with pain should be compared to a control group without pain in OA, which would then permit a proper examination of the association between catastrophizing and pain in OA. The current design means that pain is analysed as a covariate so is a less powerful outcome.

Another important limitation is that different measures were used for depression in both cohorts and a measure for anxiety was missing from one of the cohorts. Ideally measures in all groups should have been identical. Finally, this study only included

hospital referred people with OA so both referral and selection bias may be present which limits the generalisability of the data to other people with knee or hip OA.

In conclusion this study provides evidence that catastrophizing has an impact on pain intensity independently of anxiety, depression and sleep quality. This supports the consideration of catastrophizing as an identifiable trait and an entity in its own right rather being an integrated variable within the spectrum of anxiety and depression.

The finding linking CVD and catastrophizing could show avenues for new treatments or improving current treatments by incorporating psychology as it shows catastrophizing has a tangible effect on physiology (Thorn B. E., 2007, Smeets R. J. E. M., 2006, Hoffman B. M., 2007). Furthermore, the association between catastrophizing and cardiovascular outcomes has not been thoroughly investigated in the literature so would be a candidate for further work.

4 Genetics of pain catastrophizing post TJR

4.1 Introduction

Pain catastrophizing has a genetic component. Using a classical study design Trost et al (2015) reported that pain catastrophizing shows significant heritability, suggesting a substantial genetic contribution to this trait. By measuring pain catastrophizing and cold pressor pain responses in 400 twin pairs, roughly half of whom were identical and the other half non identical hey found that cold pressor pain tolerance measures showed heritability estimates slightly above 50%, indicating that slightly more than half of the variance in these pain measures can be attributed to genetic variability. Moreover, for the first time, these investigators demonstrated that pain catastrophizing also showed significant heritability (37%). (Trost et al., 2015) This means that 37% of the variation in pain catastrophizing scores is determined by genetic factors and therefore it should be possible to identify genetic factors associated with it.

Many psychological phenotypes, including personality, depression, and cognitive function, have shown significant heritability (see for example(Bouchard and McGue, 2003)). Importantly however the study by Trost et al (2015) found that the genetic contribution to catastrophizing was largely independent of the genetic contribution to pain responses (Trost et al., 2015). That is, the association between pain catastrophizing and cold pressor pain tolerance appeared to be a direct relationship rather than a reflection of shared genetic variance.

Other studies had previously investigated the link between catastrophizing and genetic factors, George et al (2008) reported a series of studies demonstrating that the catechol-O-methyl-transferase or *COMT* gene interacts with catastrophizing to influence pain.(George et al., 2008)

I therefore hypothesize that investigating the genetic contribution to pain catastrophizing can reveal some of the molecular pathways underlying this trait,

The molecular basis of pain catastrophizing has previously been examined but using a gene expression approach in individuals with fibromyalgia, though with a sample of only nine women all of whom suffered from fibromyalgia and fatigue (Lukkahatai N., 2013). This study by Lukkahatai et al (2013) found that physiologic pathways involving interferon signalling and activation of IRF can potentially distinguish between high and low pain experiences while dendritic cell maturation may possibly delineate between the high and low catastrophizing subjects. This confirms that the molecular pathways involved in high pain catastrophizing but I will use a different approach, taking advantage of the fact that pain catastrophizing is 37% heritable.

After analysis of the GWAS results it will be investigated how associations could relate to pain and pain mechanisms at a molecular level, again an issue that has not to my knowledge been explored.

4.2 Methods

4.2.1 Cohorts used

The data used are derived from the Arthritis Research UK Osteoarthritis Genetics (arcOGEN) study and the ongoing NGOAS study as described in chapter 2.

For replication the GOAL cohort as described in chapter 2 was used. In all the cohorts only subjects that had both catastrophizing and pain ratings were selected from both cases and controls.

4.2.2 Questionnaires used

The Pain Catastrophizing Scale (PCS) questionnaire was used as it is the most widely validated questionnaire for catastrophizing. (Sullivan, 2014, Sullivan M., 1995)

The Western Ontario and McMaster Universities Index (WOMAC) (Bellamy, 2012, Bellamy et al., 1988, Reginster et al., 2001) was used to assess the three dimensions of pain, disability and joint stiffness.

The 9-item PainDETECT questionnaire (PDQ) (Mathieson S., 2013), is another validated questionnaire for the measurement of pain that was used in this study.

There was also a medical history and demographic section that was used to gather basic clinical data on all participants as described in previous chapters.

4.2.3 Design

Data was first quality checked by producing a QQ plot and then a Manhattan plot. QQ and Manhattan plots were generated in R. The QQ plot shows what is expected against what the data shows. If the plot follows the diagonal line and then tapers off slightly at the top that means the data is sound. A Manhattan plot shows the significance and position of snps on the genome. If points are above the blue line they are significant and if they are above the red line they can be said to be genomewide significant. As both showed the data to be sound a GWAS was then carried out using catastrophizing as the phenotype before conducting pathway analysis to gather if any genes could potentially play a role in pain generation. GWAS were conducted using Plink.

For pathway analysis a mix of software and literature review was undertaken. The gene lists generated during data analysis were run through an online piece of software called DAVID (described in Chapter 2). This software analyses the pathways that the specific genes are found in and gives details on how significant these pathways are. Following the production of this data the most significant genes were examined to discover how they could possibly be related to OA, pain, catastrophizing or all three. This was done by performing a literature search using firstly Google, Google Scholar and then PubMed.

I then selected from among the top associations to be further replicated in the GOAL cohort, and the one SNP that replicated in GOAL was then replicated in the NGOAS cohort (see chapter 2 for cohort descriptions).

4.3 Results

4.3.1 Descriptive Statistics

Descriptive statistics of the NGOAS/arcOGEN cohort are displayed in Table 4-1

Table 4-1: Descriptive statistics of Osteoarthritis (OA) patients studied for pain catastrophizing

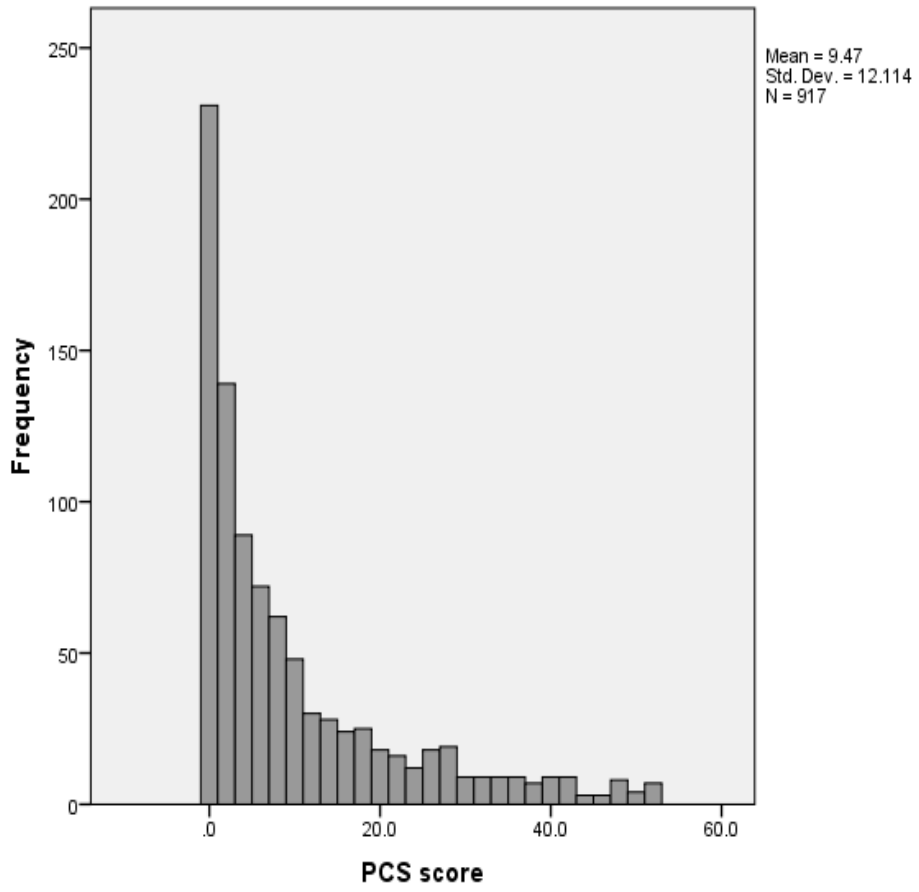
Characteristic		**Number of participants**	**% of cohort**
Gender	male	416	55.9
	female	528	44.1
Moderate WOMAC	no	411	43.5
	yes	508	53.8
Severe WOMAC	no	678	71.8
	yes	241	25.5
VAS	no	603	63.9
	yes	334	35.4
PCS score above nine	no	593	62.8
	yes	324	34.3
Depression medication	no	791	83.8
	yes	153	16.2

Age range from 45- 93 yrs

BMI range from 16.91-53.01

Given the lack of normality in the data (as shown in figure 4-1), the PCS score was transformed into a binary trait using the top tertile with a score of ≥ 9 as the cut off. To transform the data to a binary variable the data was divided into tertiles based on the histogram (figure 4-1) displayed below and then from that the top tertile was compared to the bottom two tertiles.

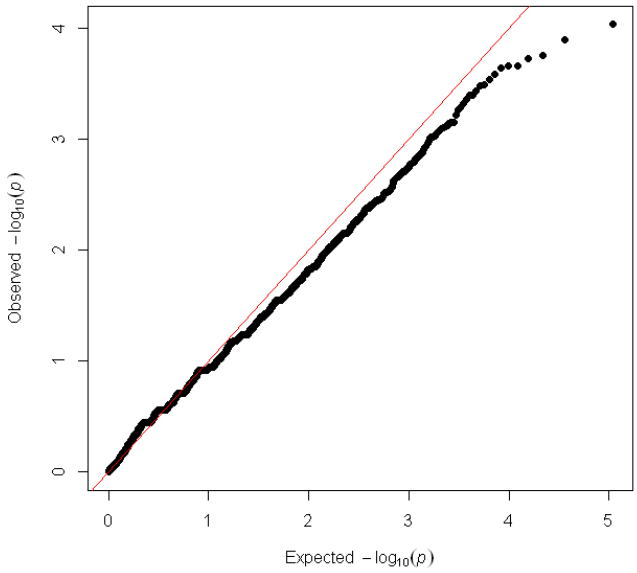
Figure 4-1: Histogram to show the distribution of catastrophizing



4.3.2 Quality control

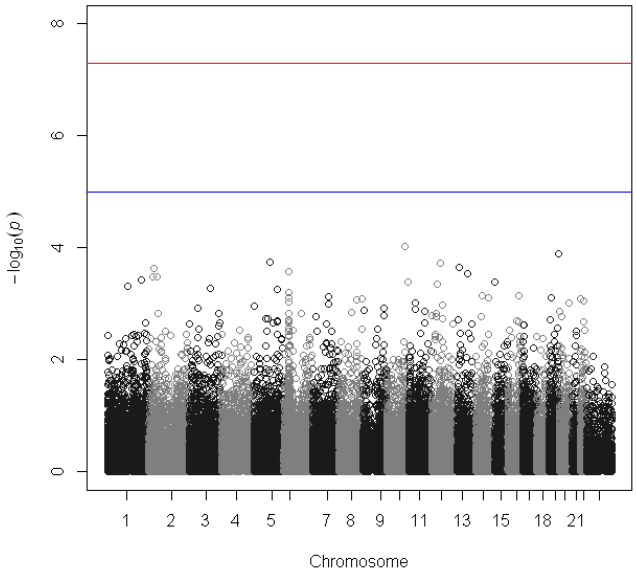
An important aspect of quality control in genetic association studies is ensuring that there is no inflation of association, due to population stratification or other artefacts for example. In order to assess the normality of the data a QQ plot was generated and it is apparent from figure 4-2 that there is no inflation and the results are sound. The QQ plot shows that the data is normal and that there are no outliers or anomalies as the plot follows the trend line. Only one point on the Manhattan plot is close to significance.

Figure 4-2: Quantile-Quantile (QQ) plot for the genome wide association study of pain catastrophizing scores comparing the top to the bottom two tertiles in a case control design using PLINK.



Another important graphic representation for GWAS is a Manhattan plot. This represents where the genes are located and also how significant the association is. If they are above the blue line it is said to be significant and if it is above the red line it is genome-wide significant. Genome-wide significance for a GWAS is above p= 5x10-8. This is due to the fact that this GWAS has tested almost 500,000 variants it is necessary to correct for multiple texts. A significance level of 5 x 10[-8] is commonly accepted as "genome wide significant" (see Appendix I)

Figure 4-3: Manhattan plot for the genome wide association scan of top vs



bottom two tertiles of pain catastrophizing scores

From figure 4-3 it is evident that some SNPs were approaching significance and this cut off was shown to produce the highest significance levels.

From looking at the Manhattan and QQ plots for catastrophizing it was decided the optimum cut off point was at 9 which was subsequently used for the analysis in plink, DAVID and access to produce the data in Tables 4-2 to 4-5.

4.3.3 Genetic Analysis

This binary variable for catastrophizing was used as the outcome of GWAS using both the exome chip and the Illumina 610 array, the results of which are shown in tables 4-2 to 4-4. In all three tables different genes are highlighted with differing OR and P-values those in tables 4-2 and 4-4 being roughly similar whilst those in table 4-3 tend to be higher though none are genome-wide significant.

Table 4-2: Top 10 variants for catastrophizing from analysis with exomechip (NGOAS cohort)

Gene Name	SNP	OR	P-value
FSCN2	rs186367879	0.05473	1.98E-04
MYO1G	rs3735485	0.3664	5.10E-04
LIN28A	rs7532866	0.4489	1.02E-03
PAPPA2	rs11583447	2.204	1.09E-03
Null	rs7219585	0.3424	1.10E-03
Null	rs13280933	0.4532	1.13E-03
C17orf48	rs406446	2.228	1.32E-03
Null	rs210381	2.238	1.33E-03
CCM2	rs11552377	0.4097	1.35E-03
MFHAS1	rs429433	0.203	1.46E-03

Table 4-3: Top 10 variants for catastrophizing from Illumina 610k (arcoGEN cohort)

Gene Name	SNP	OR	P
LOC100287887 | PRR20	rs1360646	0.5276	9.51E-07
SH3RF1	rs6847734	3.06	1.86E-06
LOC100287887 | PRR20	rs1413112	0.5449	3.78E-06
CALD1	rs7803531	1.78	6.14E-06
SLC22A20	rs2904979	2.53	6.71E-06
CALD1	rs3800739	1.774	6.94E-06
GPRIN3	rs6830724	1.991	9.23E-06
LOC100129786 | ADRA2C	rs4916622	0.5376	9.42E-06
MYT1L | LOC729897	rs823243	3.665	1.06E-05
VIPR2	rs6979985	1.678	1.46E-05

There is an overlap of ~ 20,000 variants genotyped by both the Illumina 610 and the exomechip arrays. I further selected SNPs which are in both, which yield a nominally

significant result for both ($p<0.05$) and where the effect of the minor allele is consistent in both sets.

Table 4-4: Top variants for catastrophizing in both exomechip and Illumina 610k

Gene name	**SNPs**	**Odds ratio Exome chip**	**P-value Exome chip**	**Odds ratio arcOGEN**	**P-value arcOGEN**
SIRPB2	rs6042507	0.5063	3.79E-02	0.4053	6.54E-04
TRIM15	rs9368624	0.4266	6.38E-03	0.4929	6.74E-04
TRIM15 | TRIM26	rs9380156	0.4497	1.13E-02	0.4929	1.36E-03
FLJ45422 | HCG18	rs968909	0.6807	1.53E-01	0.5741	1.40E-03
LOC100132345 | KCNH8	rs13073817	1.182	5.02E-01	1.63	1.44E-03
HCG18	rs261949	0.6196	9.30E-02	0.5589	1.45E-03

Although none of the results from the above experiments achieved genome-wide significance, using pathway analysis it is possible to derive some insights into the molecular pathways underlying pain catastrophizing. To do so I used the software DAVID which is designed to identify molecular pathways common to a set of query genes, in this case the genes with $p<0.0001$

The results from pathway analysis are presented in Table 4-5

Table 4-5: Pathway analysis for catastrophizing from both NGOAS and arcOGEN cohorts using DAVID. DAVID is an online, open access piece of software that is used for this type of analysis. Pathway analysis displays the significance of genes found in biological pathways so giving a systemic perspective

Term	**P-Value**	**Genes**
guanine-nucleotide releasing factor	0.014262	*ECT2L, DOCK1, FBXO8*
GO:0005085~guanyl-nucleotide exchange factor activity	0.022536	*ECT2L, DOCK1, FBXO8*
GO:0051960~regulation of nervous system development	0.045081	*VEGFC, POU3F2, FGF2*
GO:0007267~cell-cell signalling	0.02008	*CAMK4, SALL1, ADRA2C, FGF2, VIPR2*

From the pathway analysis it appears that genes involved in cell-cell signalling and regulation of the nervous system development are involved in pain catastrophizing. Another pathway that came up was guanyl-nucleotide releasing factor. This refers to pathways where guanine nucleotide exchange factor (GEF) domain, i,e through the exchange of bound GDP for GTP. This normally activates signalling cascades that are involved in immune cell development, homeostasis and differentiation.

Having investigated some of the potential pathways investigated I then proceeded to examine whether the individual associations were reproducible. Genotyping was carried out in 5 variants in the GOAL cohort by LGC genomics. I selected these 5 variants based on their p-values and on t their possible roles in pain pathways and P-values. I then tested these genotypes for association with pain catastrophizing dividing individuals in the same way as for the discovery cohort (top vs bottom two tertiles). The results are shown in Table 4-6. Only one of the 5 SNPs (rs4916622) was

significantly associated in the replication cohort and in the same direction (i.e. the same allele is "protective" for high pain catastrophizing) in the discovery cohort.

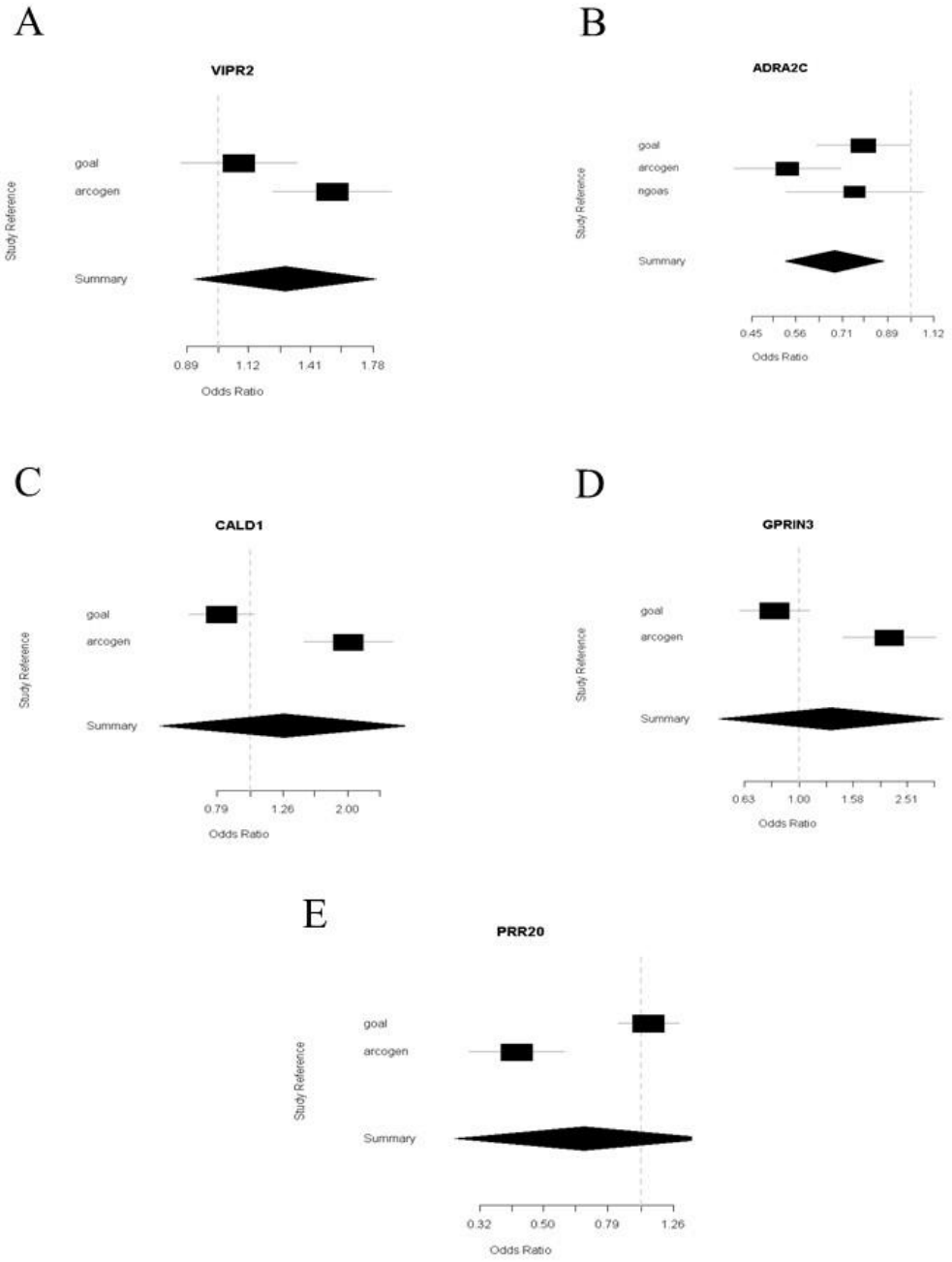
Table 4-6: Genetic association with pain catastrophizing of 5 SNPs selected from GWAS in and independent cohort (GOAL)

Group	Closest gene	SNP	OR	95% CI		p-value
GOAL	*PRR20*	rs1360646	1.05	0.85	1.31	0.637
GOAL	*ADRA2C*	**rs4916622**	**0.79**	**0.62**	**1.00**	**0.049**
GOAL	*GPRIN3*	rs6830724	0.81	0.60	1.10	0.175
GOAL	*VIPR2*	rs6979985	1.08	0.87	1.34	0.481
GOAL	*CALD1*	rs7803531	0.81	0.65	1.03	0.084
Discovery	*PRR20*	**rs1360646**	**0.41**	**0.30**	**0.58**	**3.26E-07**
Discovery	*ADRA2C*	**rs4916622**	**0.49**	**0.35**	**0.69**	**3.70E-05**
Discovery	*GPRIN3*	**rs6830724**	**2.15**	**1.44**	**3.19**	**1.62E-04**
Discovery	*VIPR2*	**rs6979985**	**1.53**	**1.23**	**1.91**	**1.62E-04**
Discovery	*CALD1*	**rs7803531**	**2.01**	**1.46**	**2.76**	**1.82E-05**

I proceeded to meta-analyse the results from the discovery and replication cohorts

Figure 4-4 shows the forest plots for specific SNPs selected for replication. Figure4-4 demonstrates that four out of five SNPs become not nominally statistically significant. Because the SNP mapping to *ADRA2C* was significant in the GOAL cohort, I asked LGC genomics to further genotype this variant in the NGOAS cohort (Figure 4.5) . A fixed effects meta- analysis resulted in an OR = 0.684 (95% CI 0.584- 0.940) p = 2.4 x 10[-6]. I then ran a DerSimonian Lard random effects meta-analysis which revealed that there was some heterogeneity in the three cohorts (I^2= 62% tau^2 = 0.039 p=0.071).

Figure 4-4: Forest plots showing the discovery and replication results for five SNPs



A) Forest plot for *VIPR2.* This shows that replication was successful due to odds ratios going in the same direction, but the error bar for GOAL does go in to less than one. B) Forest plot for *ADRA2C.* Showing that the SNP successfully replicated in three cohorts with odds ratios in the same direction C) Forest plot for *CALD1*. This shows that replication was unsuccessful due to odds ratios going in opposite directions D)

Forest plot for *GPRIN3*. This shows that replication was unsuccessful due to odds ratios going in opposite directions. E) Forest plot for *PRR20*. This shows that replication was unsuccessful due to odds ratios going in opposite directions

Because the data of a meta-analysis can be heterogeneous, referring to the differences in effect sizes between the collected studies, what is often done in the literature is to apply both the fixed-effects model under an assumption of the same effect size between studies and the random-effects model under an assumption of varying effect size between studies. However, surprisingly, random-effects models gives less significant p values than fixed effects at variants that actually show varying effect sizes between studies in spite of being designed specifically for the case in which there is heterogeneity. Han and Eskin (2011) have shown that the underlying reason for this phenomenon is that random-effects models implicitly assume a markedly conservative null-hypothesis model, have developed a new random-effects model that relaxes the conservative assumption. This method achieves higher statistical power than fixed effects when there is heterogeneity, indicating that the new method has practical utility for discovering associations in the meta-analysis of GWASs.

I have therefore used this random-effects model to derive the statistical significance of my meta-analysis and the Han-Eskin p-value achieved is 3.26 x 10[-6]

Importantly, when both of the replication cohorts were combined excluding the discovery cohort the genetic association at the *ADRA2C* SNP achieved an OR=0.78 95% CI 0.64-0.94 $p<0.0112$. There was no heterogeneity between the two discovery cohorts ($I^2=0\%$)

4.4 Discussion

The molecular pathways identified in the genetic analysis of pain catastrophizing highlighted genes related to the regulation of nervous system development and cell-cell interactions.

There is no evidence in this work of rare genetic variants with strong genetic effect. The analysis of exomechip (low frequency functional variants) achieved only modest p-values very far from genome-wide significance (Table 4-1). On the other hand, 8 different SNPs with p< 1 x 10[-5] were observed using the Illumina 610 array (Table 4-2).

Among these we note SNP rs1360646 found to be the most significant in Table 4-3 mapping near gene *PRR20*. The predicted protein from this gene is proline-rich and contains several dopamine D4 receptor signatures, suggesting that dopamine related pathways may influence pain catastrophizing. In previous literature dopamine has been found to be associated with sleep and mood disorders such as depression (Finan P. H., 2013b). It has further been reported by Bodenmann et al (2009) that the val158met SNP of *COMT* has been associated with dopamine metabolism by causing alterations to the thermostability of the COMT enzyme (Finan P. H., 2013b, Bodenmann S., 2009). However this study was conducted using an exclusively male cohort with 88 participants (Bodenmann S., 2009) therefore results may need replication. Interestingly, microinjections of dopamine agonists in the rostral agranular insular cortex (RAIC) and the ACC have been shown to produce antihyperalgesic effects using a neuropathic pain model (Coffeen U., 2012, López-Avila A., 2004, Potvin S., 2009), possibly demonstrating another mechanism that dopamine can act through and how *COMT* ,by controlling dopamine metabolism, can contribute.

The *CALD1* gene encodes a calmodulin- and actin-binding protein which acts as a potent inhibitor of the actin-tropomyosin activated myosin MgATPase, and serves as a mediating factor for Ca(2+)-dependent inhibition of smooth muscle contraction (Compendium, 2014e, NCBI, 2014g). Recently there have been a few studies on this genes role in endometriosis (Gupta S., 2015, Gupta D., 2016), which is a painful disorder that occurs in women, but very little is reported on its role in OA (Zhang R.K., 2012).

Neither of these SNPs however replicated in an independent dataset. On the other hand we did find a SNP that was associated with pain catastrophizing in three independent sets. This is the SNP rs4916622 in Table 4-3 being related to the *Alpha-2-adrenergic receptor C (ADRA2C)* gene. *ADRA2C* is a member of the G protein-coupled receptor superfamily. These receptors have a critical role in regulating neurotransmitter release from sympathetic nerves located in the heart and from adrenergic neurons in the central nervous system.(Compendium, 2014f, NCBI, 2014f) This clearly links with the HPA axis and sympathetic nervous system, so again showing a possible link to pain. To my knowledge these links have been shown in the literature but not at a genetic level. Furthermore this implies specific mechanisms that catastrophizing could affect, thereby indicating mechanisms that could be targeted in treatment possibly leading to more individualised and effective pain management.

This is the first study to explore the genetics of pain catastrophizing in musculoskeletal pain , but the sample is still relatively small to be used for a genetics study so could be improved upon by further replication.

A major limitation of the current finding is that it does not reach the gold standard of $p< 5 \times 10[-8]$. However, I would like to argue that the replication of the genetic variant and the improvement of the p values for this SNP as a result of analysis on other

cohorts and meta-analysis, as well as the biological relevance of my findings, support the likelihood that these results may be a true association, despite not reaching genome-wide significance.

Lack of replication in genetic association studies had been notoriously common in the candidate-gene era. (Hirschhorn et al., 2002) and false-positives due to type I error may be the most likely explanation. The importance of genome-wide significance (GWS) thresholds has been discussed in the literature (Panagiotou O.A., 2012) and it has been shown that the standard for genome-wide significance can actually be relaxed based on the high reproducibility of genetic associations that achieve at least p< 1 x 10[-6]. An empirical evaluation has shown that a substantial proportion, but not all, of the associations with borderline genome-wide significance represent replicable, possibly genuine associations suggesting a possible relaxation in the current GWS threshold. (Panagiotou O.A., 2012)

Although my results did not reach the p<5x10-8 cut-off, I have shown a reproducible genetic effects on pain catastrophizing after replication analysis. It should be noted that the National Human Genome Research Institute (NHGRI) keeps a record of all SNP-trait associations p<10-5.(Welter D., 2014) importantly if I combine the data from the two replication cohorts used a p-value of 0.011 was still achieved. Therefore, although this association may not reach genome wide significance it remains plausible and worth pursuing in further studies. Replication in further cohorts could improve sample size and p values and it is hoped that this first GWAS of pain catastrophizing may encourage the collection of DNA and of pain catastrophizing scores in other cohorts.

Future research could include more in depth study into the mechanisms indicated in this work and how they affect pain catastrophizing particularly in individuals with chronic musculoskeletal pain..

5 Genetics of pain in OA: comparison of symptomatic and asymptomatic OA

5.1 Introduction

Another research question to address is whether there are genetic variants associated with symptomatic vs asymptomatic knee OA. The definition of symptom was pain as recorded by the WOMAC, VAS and painDETECT questionnaires. This was the only deciding factor in the grouping.

Up to 40% of patients with severe radiographic changes in knee joints are symptom-free, indicating that joint pathology does not always cause symptoms. (Kc R., 2016) Despite this, structural differences have been noted in symptomatic OA, specifically in levels of synovitis. (Stoppiello L. A., 2014) Other risk factors reported in literature included: female sex (Englund M., 2004), high BMI (Englund M., 2004) and a degenerative type of meniscal tear as opposed to a traumatic tear (Englund M., 2004).

There are several genes known to be associated with pain that are described in the current literature (see table 1-2). Therefore one hypothesis could be that the genes already identified show different rates between symptomatic and asymptomatic OA demonstrating genetics has an influential role over pain in OA

Therefore this study aims to investigate if there are any genetic differences between asymptomatic vs symptomatic OA and what possible mechanisms these genes could affect.

5.2 Methods

5.2.1 Cohorts used

Data from 692 knee OA patients (480 symptomatic patients and 212 asymptomatic patients) recruited as part of the Twin UK and GOAL baseline studies were used for this analysis. These individuals were all genotyped with the exomechip array (as described in methods in chapter 2 and 4) and symptomatic OA was used as the outcome of a GWAS. In these two studies there was no measure of catastrophizing.

5.2.2 Questionnaires used

The PCS questionnaire was used in the questionnaires that were sent out as it is the most widely validated questionnaire for this phenotype. (Sullivan, 2014)

The WOMAC Index (Bellamy, 2012, Bellamy et al., 1988, Reginster et al., 2001) was used to assess the three dimensions of pain, disability and joint stiffness. The 9-item PDQ (Mathieson S., 2013), is another validated questionnaire that was used in this study.

5.2.3 Study design

For data analysis a combination of several pieces of software developed for genetic and general data analysis were used. This included: R, SPSS, Plink, and Microsoft excel and access. First Microsoft access was used to store the data collected from the questionnaires as they were returned. BMI for the participants was calculated using the self-reported weight and height as kg/m2.

GWAS are used to scan several genes at once and find associations between disorders and specific genes. Pathway analysis is used to determine the molecular pathways of interest based on the genes found in GWAS. These studies examine the effects of specific genetic variants on disease occurrence. Regions identified by linkage analysis can be investigated, or candidate gene association studies can be conducted to identify the genetic and allelic variants involved in disease risk. Typical GWAS are carried out on a sample of cases and controls, and these samples are genotyped for hundreds of thousands of SNPs. After several quality control steps and SNP imputation, each SNP is tested for an association with the disease and the top SNPs, usually those with the smallest *P*-values, are selected for further testing in replication cohorts. (Valdes A. M., 2011d)

Genotyping with the Exome BeadChip array was carried out in 458 knee OA cases with a K/L score of 2 or higher in the tibiofemoral compartment. Of these 212 were asymptomatic reporting no knee pain, 67% of them had a K/L of 3 or higher. 246 reported pain in the knee at least 15 days during the past month, most of them were either on waiting list for a TKR or had undergone a TKR already, 81% of them had a K/L of 3 or higher.

For pathway analysis a mix of software and literature review was undertaken. The gene lists generated during data analysis were run through an online piece of software called DAVID. This software analyses the pathways that the genes from the list can be found in and gives details on how significant these pathways are. Following the production of this data the most significant genes were examined to discover how they could be related to OA, pain, catastrophizing or all three. This was done by performing a literature search using firstly Google and then Google Scholar.

5.3 Results

5.3.1 Descriptive statistics

The descriptive characteristics of participants are shown in Table 5-1. As can be seen in the table there are more women than men in this cohort, a majority have symptomatic OA and this is over a wide BMI and age range.

Table 5-1: Descriptive statistics for the symptomatic vs asymptomatic cohort

Characteristic		**Number of participants**	**% of cohort**
Gender	male	278	40.2
	female	414	59.8
Symptomatic	yes	480	69.4
	no	212	30.6
K/L	2	144	20.8
	3	407	58.8
	4	140	20.2

Age range: 39-91

BMI ranges:18.29 - 53.01

5.3.2 Quality checking

The data was also quality checked, as in the previous chapter; a QQ plot and Manhattan plot were drawn (see figure 5-1 and figure 5-2). The QQ plot shows that the data is normal and that there are no outliers or anomalies as can be seen in that the plot follows the trend line. Only one point on the Manhattan plot is close to significance.

Figure 5-1: **Quantile-Quantile plot for symptomatic vs asymptomatic. A QQ plot displays the expected against the data used. As can be seen in this plot the data follows the trend line so can be said to be sound**

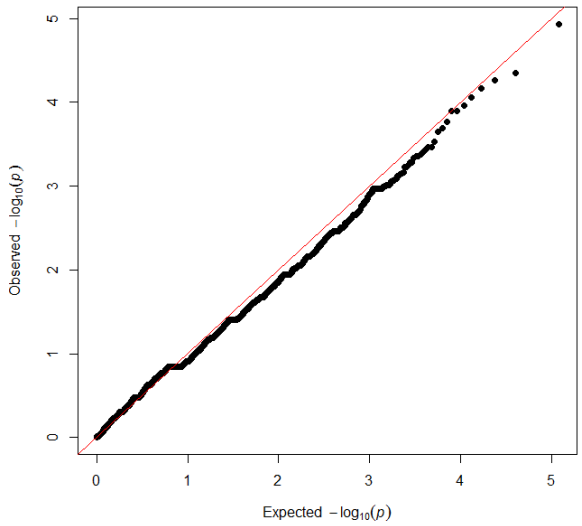
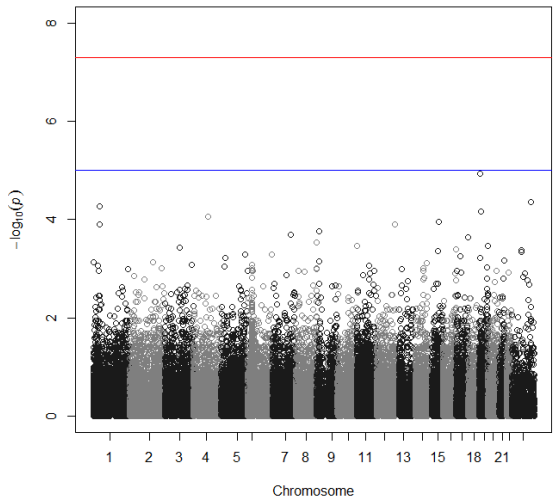


Figure 5-2 Manhattan plot for symptomatic vs asymptomatic OA. A Manhattan plot displays the location in the genome and significance of SNPs. If above the blue line it is approaching significance and if above the red line it is genome-wide significant. From this plot there is one SNP that is approaching significance



5.3.3 Genetic Analysis

The results of the GWAS are shown in Table 5-2. Of particular interest was the SNP rs6700677 which is indicated to play a role in the *LEPRE1* gene which is implicated in bone formation. Also from this table you can see that modest P-values were gained and none were genome-wide significant.

Table 5-2: Top 20 variants for asymptomatic vs asymptomatic. Below are the top 20 SNPs based on p-value after Genome-wide association scan (GWAS)

rsID	**Gene Name**	**P-value**	**OR**
rs145561195	*S1PR4*	1.16E-05	3.505
rs6700677	*LEPRE1*	5.43E-05	0.3517
rs968502	*PRAM1*	6.91E-05	1.952
rs17029215	*MTTP*	8.73E-05	0.3436
rs142345927	*MYO9A*	1.09E-04	0
rs9660973	*CLDN19*	1.26E-04	0.3614
rs61940807	*TMEM132B*	1.27E-04	0.092
rs10961700	*FREM1*	1.70E-04	0.561
rs117070316	*SND1/LRRC4*	2.05E-04	0.3536
rs61743692	*FN3KRP*	2.28E-04	0.1762
rs11136341	*PLEC*	2.94E-04	1.57
rs35598292	*FAM55C*	3.73E-04	0.4076
rs4785763	*AFG3L1P*	4.03E-04	0.6543
rs17258507	*DIS3L*	4.38E-04	2.733
rs34848112	*TIPIN*	4.38E-04	2.733
rs6925614	*DACT2*	5.18E-04	1.541
rs2125343	*GABRG2*	5.22E-04	0.6406
rs11568466	*SLC13A2*	5.60E-04	0.6242
rs79266675	*ACSBG2*	5.91E-04	0.2291
rs2839077	*COL6A1*	6.80E-04	2.171

I then carried out pathway analysis based on results in Table 5-2. Interestingly, the pathways identified in Table 5-3 are related to extracellular matrix biology, suggesting that this may be playing a part in nociception in OA. Again modest P-values can be seen with none reaching significance as they are all greater than 0.005.

Table 5-3: Pathway analysis for symptomatic vs asymptomatic knee OA using DAVID. DAVID is an online, open access piece of software used for this type of analysis. Pathway analysis displays the significance of genes found in biological pathways so giving a systemic perspective

Term	**P Value**	**Genes**
GO:0007155~cell adhesion	0.014789	*CDH12, CLDN19, FREM2, FREM1, COL6A1*
GO:0005578~proteinaceous extracellular matrix	0.0148	*LEPRE1, FREM2, FREM1, COL6A1*
GO:0022610~biological adhesion	0.014861	*CDH12, CLDN19, FREM2, FREM1, COL6A1*
GO:0044420~extracellular matrix part	0.015573	*FREM2, FREM1, COL6A1*
GO:0031012~extracellular matrix	0.018078	*LEPRE1, FREM2, FREM1, COL6A1*
Cell adhesion	0.022881	*CDH12, FREM2, FREM1, COL6A1*
Extracellular matrix	0.047734	*LEPRE1, FREM1, COL6A1*
GO:0043167~ion binding	0.020542	*CDH12, ZC3H3, GABRG2, CLDN19, LEPRE1, CYP27A1, FREM2, FREM1, SLC13A2, SYTL2, MEGF6, MYO9A*
GO:0046872~metal ion binding	0.048664	*CDH12, ZC3H3, CLDN19, LEPRE1, CYP27A1, FREM2, FREM1, SLC13A2, SYTL2, MEGF6, MYO9A*
GO:0005626~insoluble fraction	0.045094	*ACSBG2, TCL1A, SLC13A2, SYTL2, PLEC*

5.4 Discussion

The investigation of symptomatic versus asymptomatic OA found weaker associations than those identified in the previous chapter. However some of the genes identified suggest similar molecular mechanisms.

LEPRE1, which features in Table 5-2 and Table 5-3, encodes an enzyme that is a member of the collagen prolyl hydroxylase family. These enzymes are required for normal collagen synthesis and assembly. Mutations in *LEPRE1* are associated with osteogenesis imperfecta (OI) type VIII which is a bone dysplasia characterized by reduced bone mass, bone fragility, and growth deficiency (Barnes A. M., 2010, Genetics, 2014a, NCBI, 2014e, Compendium, 2014g, Genetics, 2014b) The product of the *LEPRE1*, hydroxylates a single proline, pro986 of the collagen type I alpha-1 chain (COL1A1) and forms a complex with cartilage-associated protein (CRTAP) and cyclophilin B. (OMIM, 2013, Willaert A., 2009) Therefore mutations in either *CRTAP* or *LEPRE1* disrupt the normal folding, assembly, and secretion of collagen molecules. These defects then weaken connective tissues, leading to severe bone abnormalities and problems with growth.(Genetics, 2014a) Further to this *LEPRE1* levels have been reported in the literature to be elevated 2.6 fold in OA synovial fibroblasts, decreasing to 2.4 fold in end-stage OA synovium (Remst D. F., 2014) , indicating they do play a role in OA and can act in the synovium. Therefore findings in this study would strengthen the argument for its role in bone and joint disorders

COL6A1 again featured in Table 5-2 and Table 5-3. The basic structural unit of collagen VI is a heterotrimer of the alpha1(VI), alpha2(VI), and alpha3(VI) chains.

The protein encoded by this gene is the alpha 1 subunit of type VI collagen (alpha1(VI) chain). (Compendium, 2014b, NCBI, 2014c) Martel-Pelletier et al (2008) reported that mice lacking type VI collagen exhibit accelerated development of hip osteoarthritis, as well as a delayed secondary ossification process and reduced BMD. (Alexopoulos L. G., 2009, Martel-Pelletier J., 2008) This further implies the importance of collagen in OA and further supports the effect of collagen in regards to osteogenesis imperfecta.

GABRG2 featured once in Table 5-2 and Table 5-3. This gene encodes the gamma 2 subunit of the gamma-aminobutyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the mammalian brain, where it acts at GABA-A receptors, which are ligand-gated chloride channels. (Compendium, 2014d, NCBI, 2014b) As GABA is inhibitory and a neurotransmitter this is a logical candidate for involvement in pain pathways, so causing symptomatic rather than asymptomatic OA.

The Solute Carrier Family 13 (sodium-dependent dicarboxylate transporter), member 2 (*SLC13A2*) gene featured three times in Table 5-3 **Table 5-3** and was also in Table 5-2. *SLC13A2* is a protein-coding gene, and is affiliated with the ncRNA class. (Compendium, 2014h) Again as sodium is involved in nerve transmission this points to involvement in pain pathways.

The *S1PR4* gene is the most statistically significant gene in Table 5-2. This gene is a member of the endothelial differentiation, G-protein-coupled (EDG) receptor gene family. EDG receptors bind lysophospholipids or lysosphingolipids as ligands, and are

involved in cell signalling in many different cell types (Compendium, 2014a, NCBI, 2014a) again implying nerve signalling and therefore numerous pain pathways.

CDH12 featured frequently in Table 5-3, with the best p-value being 0.015. This gene encodes a type II classical cadherin that helps mediate calcium-dependent cell-cell adhesion. This particular cadherin appears to be expressed specifically in the brain and its temporal pattern of expression would be consistent with a role during a critical period of neuronal development, perhaps specifically during synaptogenesis. (Compendium, 2014c, NCBI, 2014d)

The main limitation of this study is that none of the genes found achieved genome-wide significance, due to the small sample size in the original cohort meaning that the effect sizes for this trait are fairly modest and require much larger discovery sample sizes. Moreover, no replication of these genes was possible in an independent cohort.

Future research could include replication and if there is any positive replication the genes implied in that may be studied in greater detail, of particular interest would be the association with collagen and *LEPRE1*.

6 Discussion

My thesis has dealt both with pain catastrophizing (chapters 3 and 4) and with OA pain directly (chapter 5) seeking to understand some of the molecular mechanisms underlying chronic joint pain. Because other negative affect measures such as depression have a genetic component I have hypothesized that so does pain catastrophizing and I have tested this in my thesis.

In Chapter 3 I aimed to evaluate the contribution of pain catastrophizing to pain augmentation in people diagnosed with OA, and to determine whether this dimension of negative affect contributes to chronic joint pain independently of other measures of negative affect, particularly anxiety and depression.

High catastrophizing scores were found to correlate with: female sex, higher BMI, joint pain measured in various ways (VAS, WOMAC, painDETECT), body pain, sleep quality, depression, anxiety and chronic widespread pain (Table 3-2). This all agrees with current literature and helps to strengthen current thinking in this area. It is also the largest study to my knowledge adding yet more power to the findings.

Cardiovascular related health traits were further found to be associated with catastrophizing after adjusting for age, sex and BMI (Table 3-2). A potential link between CVD and catastrophizing is fear, which is regulated by the sympathetic nervous system (SNS). This finding could show avenues for new treatments or improving current treatments by incorporating psychology as it shows catastrophizing has a tangible effect on physiology (Thorn B. E., 2007, Smeets R. J. E. M., 2006, Hoffman B. M., 2007).

The key result, however, is that after adjusting for various potential confounders including anxiety, depression and sleep quality, pain catastrophizing remains a

significant risk factor for severe joint pain and also for, neuropathic pain symptoms, chronic widespread pain and cardiovascular disease (Table 3-3).

Additionally, the prevalence of high catastrophizing scores is almost the same in cases and controls (Table 3-1) and is virtually identical in post TJR cases as in patients with ongoing OA, in spite of the fact that these two groups have strikingly different levels of pain. This is not what would be expected if pain caused higher levels of catastrophizing.. Rather, this finding would seem to confirm that catastrophizing is a trait in its own right and is not dependant on depression and sleep to affect pain intensity and therefore should be treated as a separate entity.

Having confirmed that the relationship between catastrophizing and pain is not merely a consequence of anxiety or depression I further carried out genetic association studies in an attempt to establish the possible molecular pathways involved in pain catastrophizing to better understand how it may influence pain. The aim of these studies was to assess whether improved understanding of pain catastrophizing can help better understand joint pain in OA.

The key finding from this analysis was that variants near *ADRA2C* are associated with pain catastrophizing. As discussed in Chapter 4 this result should be taken with caution. Although I found that the same variant mapping to the *ADRA2C* region was nominally significantly associated with pain catastrophizing in three cohorts, the result does not achieve genome wide significance (see Appendix I). However, another way of looking at this result is to accept the GWAS results as hypothesis generating. Having selected a handful of SNPs for replication and finding *ADRA2C* SNP associated in two independent cohorts strongly suggests that this is unlikely to be merely a false positive.

In addition to the study of pain catastrophizing I also aimed to study directly the genetic influence on OA pain. I did this by running a “mini-GWAS” comparing gene

variant frequencies in symptomatic vs asymptomatic OA. This analysis revealed several regions potentially implicated in symptomatic vs non-symptomatic knee OA, but it also highlighted that genetic effects are only modest and require much larger sample sizes. Of interest were the findings in pathway analysis that indicated the involvement of bone in pain generation.

First I approached the notion of whether catastrophizing's effect on pain reflects that of other negative affect related traits, particularly anxiety or depression, or whether catastrophizing in itself constitutes a risk factor for joint pain severity. Mounting evidence supports a complex interplay between joint pathology and processing of sensory inputs by the central nervous system, which leads to both pain facilitation and a spread of pain to remote, non-diseased areas for example (Woolf, 2011).

Anxiety and depression are overlapping, inter-correlated constructs which together contribute to negative affect. Negative affect is common in people with OA (Axford et al., 2010). Negative affect predicts worse pain outcomes in OA, including more severe pain (Smith and Zautra, 2008), greater disability (Smith and Zautra, 2008), and worse outcomes from treatment, including arthroplasty (McHugh et al., 2013). These findings support the view that negative affect is not only a consequence of OA, but may also predispose people with OA to worse pain.

Overwhelming evidence identifies catastrophizing, which is a set of negative emotional/cognitive processes that involve rumination and pessimism, perceptions of helplessness, and magnification of pain-related symptoms as a critically important risk factor for adverse pain-related outcomes. (Edwards R. R., 2011a). However, the association between catastrophizing and cardiovascular outcomes has not been thoroughly investigated in the literature so would be a candidate for further work.

With regards to chapter 4, the molecular mechanisms underlying pain catastrophizing and negative affect which influence OA and musculoskeletal chronic pain are poorly understood.

Better understanding of the mechanisms by which negative affect can augment chronic joint pain could lead to novel treatments that reduce the impact of musculoskeletal pain, at least in a subgroup of sufferers. As I presented in the introduction, although pain catastrophizing is known to have a genetic component, the exact genetic contribution has not been studied and my work is the first genome wide association scan of pain catastrophizing.

In chapter 5 I also attempted a genome wide analysis of the genetic contribution to symptomatic vs asymptomatic OA. Unlike pain catastrophizing, several candidate gene studies have already been published linking genetic variants to pain in OA and unfortunately my work was unable to unravel any new strong associations with this trait.

The potential implications of my findings on the genetic contribution of variation at an adrenergic receptor gene to pain catastrophizing are quite relevant. It is well known that adrenergic neurotransmission is an integral part of the pathways that regulate the response to pain and stress (Drummond, 2014). An anti-nociceptive effect of both novel (burpanolol) and off-patent beta-blockers has been demonstrated recently in a mouse model of chronic pain (Martin et al., 2015).

Although the receptor that I found associated with pain catastrophizing is an alpha adrenergic receptor and not a beta receptor it is important to note that variants in this gene have also been consistently associated with different response to beta-adrenergic antagonists (or beta-blockers) (Cresci et al., 2012). A role for beta-adrenergic modulation in pain has been reported already. For example, the beta-blocker propanolol reduces the number of painful body sites and self-reported pain severity in

people with fibromyalgia and in those with temporomandibular joint disorder compared to placebo (Light et al., 2009). These data suggest that use of adrenergic receptor antagonists might reduce the prevalence and severity of chronic pain, which is consistent with my finding of a role for adrenergic modulation being implicated in pain catastrophizing.

Importantly, beta-adrenergic agonists have been shown to modulate TNF-α induced astrocytic inflammatory gene expression and brain inflammatory cell populations (Laureys et al., 2014).

Adrenal receptors are expressed among other brain regions in the amygdala which further has a role in fear and fear conditioning (Beesdo K., 2009, Orsinia C. A., 2012, Johansen J. P., 2011, Jiang Y., 2016) as well as pain response (Balleine B. W., 2006, Rauch S. L., 2003, Orsinia C. A., 2012, Jiang Y., 2016). The amygdala is one of the key areas on which anxiety works and one of the target regions for adrenergic modulation.(Baliki and Apkarian, 2015).

Adrenal receptors have an impact on the amygdala which further has a role in fear and fear conditioning (Beesdo K., 2009, Orsinia C. A., 2012, Johansen J. P., 2011, Jiang Y., 2016) as well as pain response (Balleine B. W., 2006, Rauch S. L., 2003, Orsinia C. A., 2012, Jiang Y., 2016).

To generate a fear response, information must flow from the basolateral complex of the amygdala (BLA) to the central nucleus of the amygdala (CE), which is thought to be the output centre of the amygdala. Support from electrophysiological studies confirms the BLA's role in associative plasticity in fear memory formation. (Orsinia C. A., 2012) The amygdala's role in fear and fear conditioning has also been shown to

be associated with the short (S) allele of the 5-HTTLPR heightening the response to adverse stimuli (Murphy S. E., 2013). An effect which has been replicated in healthy controls (Bertolino A., 2005), depression (Dannlowski U., 2006), social phobia (Furmark T., 2004) and panic disorder (Domschke K., 2006).

Current literature reports that the prefrontal cortex of the amygdala is more activated in patients with chronic pain than in healthy subjects along with a positive association between pain catastrophizing and neural responses in bilateral, prefrontal and parietal cortices as well as the extended amygdala in response to mild pain found in patients with fibromyalgia. Jiang et al (2016) found that helplessness and rumination drove the relationship between catastrophizing and exaggerated amygdala-central executive network (CEN) connectivity. The amygdala and lateral prefrontal cortex are also more active during anticipation of pain in patients with depression than in healthy controls, and amygdala activation has also been associated with both the helplessness and rumination subscales of the pain catastrophizing scale in patients with depression but not in healthy controls (Jiang Y., 2016), demonstrating a link with depression also.

Pathways from CE to downstream areas control defensive behaviour (freezing) and autonomic and endocrine responses, studies suggest that dopamine receptor activation (both D1 and D2 receptor subtypes) in the amygdala contributes to the acquisition of fear conditioning (Johansen J. P., 2011), as these are areas of the amygdala that dopamine is active in. Therefore the finding that genetic variation at *ADRA2C* and also CVD risk are associated with pain catastrophizing suggests that amygdala mechanisms play a role in connecting catastrophizing to pain responses.

The suggestive associations from chapter 5, on the other hand, suggest that genetic variation linked to genes involved in extracellular matrix biology and specifically bone biology may be involved in genetic susceptibility to painful knee OA.

Although OA affects articular cartilage, it is increasingly recognised as a disease of the whole joint. Changes in subchondral bone are key in the pathogenesis of knee OA, and associated with knee pain (Felson et al., 2001) and radiographic progression (Tanamas et al., 2010). Bone remodelling and increased pain mediators (cyclooxygenase 2, substance P, TNF-α) in the subchondral bone may occur before overt OA cartilage degeneration (Ogino et al., 2009). Subchondral bone is densely innervated by sensory nerves (Kwan Tat et al., 2010), and may be a key source of OA pain.

Animal models of OA and imaging studies in man support associations between pain and subchondral structural pathology (Strassle et al., 2010). In particular, increased osteoclast activity indicative of subchondral bone turnover might be associated with OA and pain (Sagar et al., 2014).

There are a number of study limitations to each of the three research chapters which I have covered in the individual chapter discussions. Briefly these are the cross-sectional nature of the work in chapter 3 and the fact that the case control is not really using pain free controls to assess the role of pain catastrophizing

One limitation of this work is that all of the cohorts used are cross-sectional studies that do not contain both pre and post-surgery data, but only post-surgery pain and catastrophizing information. Therefore there was no pre-surgical data available to compare to investigate differences that occur as a result of surgery.

A further limitation of this work is that it lacked a community based cohort to use as a true comparison between secondary care and the general population, though OA controls were used in the GOAL cohort.

For chapter 4 the main weakness of the study is that it did not achieve genome wide significance but as I have argued, a p-value in the range of 10[-6] which has replicated in two independent cohorts is likely to replicate in further studies.

The major weakness of the genetic results presented for symptomatic vs asymptomatic OA is that they are inconclusive. Unlike the pain catastrophizing GWAS where I was able to replicate in two independent cohorts the *ADRA2C* finding, I was unable to provide replication for the genetic association study of symptomatic vs asymptomatic OA. As such the data presented in Chapter 5 are in my view less compelling and less likely to have an impact than those in Chapter 4.

In conclusion, this work is consistent with the notion that catastrophizing affects pain intensity by autonomic system activation; specifically the sympathetic system is implied earlier in chapter 3 with cardiovascular disease as well as in chapter 4 with *ADRA2C.*

The results presented here suggest that in-depth investigation into the association between catastrophizing and the cardiovascular system should be carried out in order to determine the exact mechanism generating this association

Further work into how amygdala related mechanisms are linked to joint pain could open new avenues for therapy of this very common condition.

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Appendix I: Genetic Study Methodology

To facilitate reading specifically for chapters 4 and 5 of this thesis I have summarized the main genetic study methods and gold standards. This covers candidate gene

studies, Genome-wide association scans (GWAS), replication and then meta-analysis. It also details the normal flow of a genetic study.

Summary of relevant sections and gold standard Genome-Wide Association Study procedure from Bush et al (2012). (Bush W.S., 2012)

Study Designs: Case Control versus Quantitative Designs

There are two primary classes of phenotypes: categorical (often binary case/control) or quantitative. For some disease traits of interest, quantitative disease risk factors have already been identified. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels are strong predictors of heart disease, and so genetic studies of heart disease outcomes can be conducted by examining these levels as a quantitative trait. Genetic variants that influence these levels have a clear interpretation – for example, a unit change in LDL level per allele or by genotype class. Their results were also easily combined to conduct an extremely well-powered massive meta-analysis, which revealed 95 loci associated to lipid traits in more than 100,000 people (Teslovich et al., 2010).

Other disease traits do not have established quantitative measures. In these circumstances, individuals are usually classified as a binary categorical variable. For example, multiple sclerosis is a complex clinical phenotype that is often diagnosed over a long period of time by ruling out other possible conditions. However, despite the "loose" classification of case and control, GWAS of multiple sclerosis have been successful, implicating more than 10 new genes for the disorder (Habek et al., 2010).

Single Locus Statistical Analysis

The *standard* analysis of genome-wide association data is a series of single-locus statistic tests, examining each SNP independently for association to the phenotype.

Dichotomous case/control traits are generally analysed using either contingency table methods or *logistic regression*. Contingency table tests examine and measure the deviation from independence that is expected under the null hypothesis that there is no association between the phenotype and genotype classes. Logistic regression is an extension of linear regression where the outcome of a linear model is transformed using a logistic function that predicts the probability of having case status given a genotype class. Logistic regression is often the preferred approach because it allows for adjustment for clinical covariates (and other factors), and can provide adjusted odds ratios as a measure of effect size.

For both quantitative and dichotomous trait analysis, there are a variety of ways that genotype data can be encoded or shaped for association tests. The choice of data encoding can have implications for the statistical power of a test, as the degrees of freedom for the test may change depending on the number of genotype-based groups that are formed. *Allelic* association tests examine the association between one allele of the SNP and the phenotype. *Genotypic* association tests examine the association between genotypes (or genotype classes) and the phenotype. The genotypes for a SNP can also be grouped into genotype classes or models, such as dominant, recessive, multiplicative, or additive models (Lewis, 2002). Each model makes different assumptions about the genetic effect in the data – assuming two alleles for a SNP, A and a, a dominant model (for A) assumes that having one or more copies of the A allele increases risk compared to a (i.e. Aa or AA genotypes have higher risk). The recessive model (for A) assumes that two copies of the A allele are required to alter risk, so individuals with the AA genotype are compared to individuals with Aa and aa genotypes. The multiplicative model (for A) assumes that if there is 3× risk for having a single A allele, there is a 9× risk for having two copies of the A allele: in this case if the risk for $Aa$is k, the risk for AA is k^2. The additive model (for A) assumes that there is a uniform, linear increase in risk for each copy of

the A allele, so if the risk is 3× for Aa, there is a 6× risk for AA - in this case the risk for $Aa$is k and the risk for AA is $2k$. A common practice for GWAS is to examine additive models only, as the additive model has reasonable power to detect both additive and dominant effects, but it is important to note that an additive model may be underpowered to detect some recessive effects (Lettre et al., 2007).

Adjustment for Covariates and correcting for multiple testing

In addition to selecting an encoding scheme, statistical tests should be adjusted for factors that are known to influence the trait, such as sex, age, study site, and known clinical covariates. Covariate adjustment reduces spurious associations due to sampling artifacts or biases in study design, but adjustment comes at the price of using additional degrees of freedom which may impact statistical power. One of the more important covariates to consider in genetic analysis is a measure of population substructure. There are often known differences in phenotype prevalence due to ethnicity, and allele frequencies are highly variable across human subpopulations, meaning that in a sample with multiple ethnicities, ethnic-specific SNPs will likely be associated to the trait due to *population stratification*.

One of the simplest approaches to correct for multiple testing is the Bonferroni correction. The Bonferroni correction adjusts the alpha value from $\alpha=0.05$ to $\alpha=(0.05/k)$ where k is the number of statistical tests conducted. For a typical GWAS using 500,000 SNPs, statistical significance of a SNP association would be set at 1e-7. This correction is the most conservative, as it assumes that each association test of the 500,000 is independent of all other tests.

Permutation testing is another approach for establishing significance in GWAS and is a straightforward way to generate the empirical distribution of test statistics for a given dataset when the null hypothesis is true. This is achieved by randomly reassigning the phenotypes of each individual to another individual in the dataset,

effectively breaking the genotype-phenotype relationship of the dataset. Each random reassignment of the data represents one possible sampling of individuals under the null hypothesis, and this process is repeated a predefined number of times N to generate an empirical distribution with resolution N, so a permutation procedure with an N of 1000 gives an empirical p-value within $1/1000^{th}$ of a decimal place. Several software packages have been developed to perform permutation testing for GWAS studies, including the popular PLINK software (Purcell et al., 2007), PRESTO (Browning, 2008), and PERMORY (Pahl and Schafer, 2010).

Multi-Locus Analysis

In addition to single-locus analyses, genome-wide association studies provide an enormous opportunity to examine interactions among genetic variants throughout the genome. *Multi-locus analysis*, however, is not nearly as straightforward as conducting single-locus tests, and presents numerous computational, statistical, and logistical challenges (Moore and Ritchie, 2004).

Because most GWAS genotype between 500,000 and one million SNPs, examining all pair-wise combinations of SNPs is a computationally intractable approach, even for highly efficient algorithms. One approach to this issue is to reduce or filter the set of genotyped SNPs, eliminating redundant information. A simple and common way to filter SNPs is to select a set of results from a single-SNP analysis based on an arbitrary significance threshold and exhaustively evaluate interactions in that subset. However, a specific combination of markers incurs a significant change in disease risk. The benefits of this analysis are that it performs an unbiased analysis for interactions within the selected set of SNPs. It is also far more computationally and statistically tractable than analysing all possible combinations of markers.

Another strategy is to restrict examination of SNP combinations to those that fall within an established biological context, such as a biochemical pathway or a protein family. For example, the Biofilter approach uses a variety of public data sources with logistic regression and multifactor dimensionality reduction methods (Grady et al., 2011, Bush et al., 2009). Similarly, INTERSNP uses logistic regression, log-linear, and contingency table approaches to assess SNP-SNP interaction models (Herold et al., 2009).

Replication and Meta-Analysis

Statistical Replication

The gold standard for validation of any genetic study is replication in an additional independent sample. That said, there are a variety of criteria involved in defining "replication" of a GWAS result. (Chanock et al., 2007).

Replication studies should have sufficient sample size to detect the effect of the susceptibility allele. Often, the effects identified in an initial GWAS suffer from winner's curse, where the detected effect is likely stronger in the GWAS sample than in the general population (Zollner and Pritchard, 2007). This means that replication samples should ideally be larger to account for the over-estimation of effect size. With replication, it is important for the study to be well-powered to identify spuriously associated SNPs where the null hypothesis is most likely true – in other words, to confidently call the initial GWAS result a false-positive.

Replication studies should be conducted in an independent dataset drawn from the same population as the GWAS, in an attempt to confirm the effect in the GWAS target population. Once an effect is confirmed in the target population, other populations may be sampled to determine if the SNP has an ethnic-specific effect.

Identical phenotype criteria should be used in both GWAS and replication studies. Replication of a GWAS result should be thought of as the replication of a specific statistical model – a given SNP predicts a specific phenotype effect.

A similar effect should be seen in the replication set from the same SNP, or a SNP in high LD with the GWAS-identified SNP. Because GWAS typically use SNPs that are markers that were chosen based on LD patterns, it is difficult to say what SNP within the larger genomic region is mechanistically influencing disease risk. With this in mind, the unit of replication for a GWAS should be *the genomic region*, and all SNPs in high LD are potential replication candidates. If SNPs in high LD are used to demonstrate the effect in replication, the direction of effect must be determined using a reference panel to determine two-SNP haplotype frequencies. For example, if allele A is associated in the GWAS with an odds ratio of 1.5, and allele T of a nearby SNP is associated in the replication set with an odds ratio of 1.46, it must be demonstrated that allele A and allele T carry effects in the same direction. The most straightforward way to assess this is to examine a reference panel, such as the HapMap data, for a relevant population. If this panel shows that allele A from SNP 1 and allele T from SNP 2 form a two-marker haplotype in 90% of the sample, then this is a reasonable assumption. If however the panel shows that allele A from SNP 1 and allele A from SNP 2 form the predominant two-marker haplotype, the effect has probably flipped in the replication set. Mapping the effect through the haplotype would be equivalent to observing an odds ratio of 1.5 in the GWAS and 0.685 in the replication set.

In brief, the general strategy for a replication study is to repeat the ascertainment and design of the GWAS as closely as possible, but examine only specific genetic effects found significant in the GWAS. Effects that are consistent across the two studies can be labelled *replicated effects*.

Meta-Analysis of Multiple Analysis Results

The results of multiple GWAS studies can be pooled together to perform a meta-analysis. With the development of large academic consortia, meta-analysis approaches allow the synthesis of results from multiple studies without requiring the transfer of protected genotype or clinical information to parties who were not part of the original study approval – only statistical results from a study need be transferred. For example, a recent publication examining lipid profiles was based on a meta-analysis of 46 studies (Teslovich et al., 2010).

A fundamental principle in meta-analysis is that all studies included examined the same hypothesis. As such, the general design of each included study should be similar, and the study-level SNP analysis should follow near-identical procedures across all studies. Quality control procedures that determine which SNPs are included from each site should be standardized, along with any covariate adjustments, and the measurement of clinical covariates and phenotypes should be consistent across multiple sites. The sample sets across all studies should be independent – an assumption that should always be examined as investigators often contribute the same samples to multiple studies. Also, an extremely important and somewhat bothersome logistical matter is ensuring that all studies report results relative to a common genomic build and reference allele.

With all of these factors to consider, it is rare to find multiple studies that match perfectly on all criteria. Therefore, study heterogeneity is often statistically quantified in a meta-analysis to determine the degree to which studies differ. The most popular measures of study heterogeneity are the Q statistic and the I^2 index (Huedo-Medina et al., 2006), with the I^2 index favoured in more recent studies. Coefficients resulting from a meta-analysis have variability (or error) associated with them, and the I^2 index represents the approximate proportion of this variability that can be attributed to heterogeneity between studies (Higgins, 2008). I^2 values fall into low (<25), medium

(>25 and <75), and high (>75) heterogeneity, and have been proposed as a way to identify studies that should perhaps be removed from a meta-analysis.

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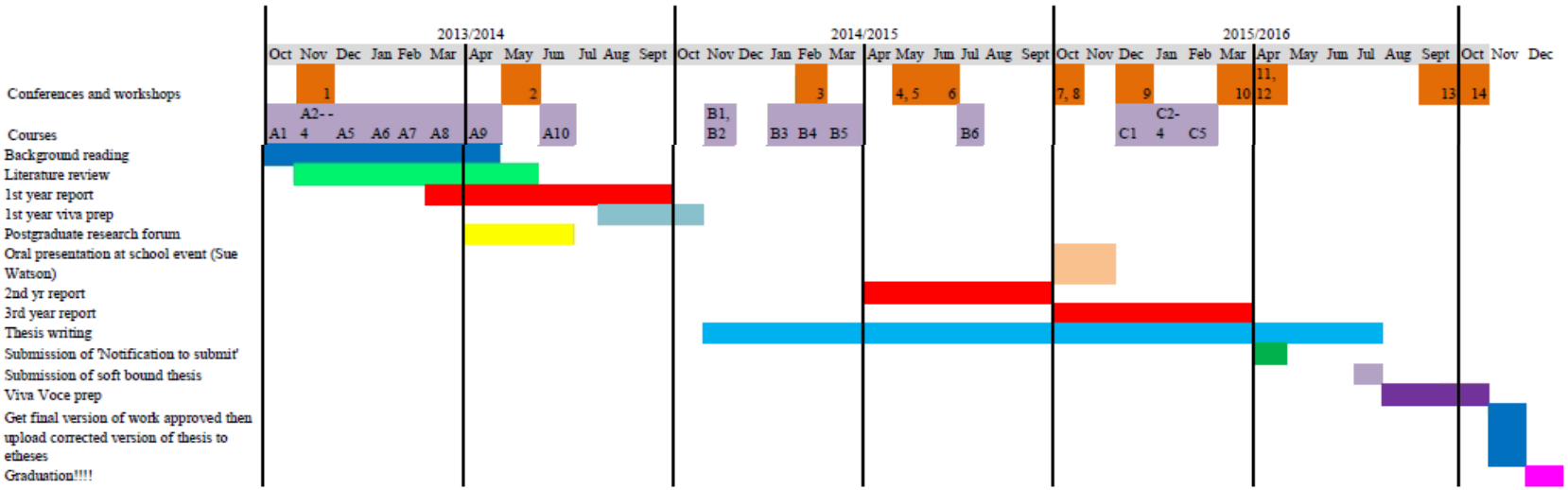
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Appendix II: Documentation covering the history of the studies involved in this thesis

This appendix includes: Gantt chart, progress reports and training credits. Ethics and questionnaires not included as this had been done before I joined the department.

PhD Gantt Chart



Conferences, meetings and workshops

1	Early OA workshop (31/10/13)
2	Risk factor theme day (8/5/14)
3	ROD meeting (9/2/15)
4	Pain after knee surgery talk (15/5/15)
5	Pain in OA talk (18/5/15)
6	Cutting edge OA conference in Oxford (23/6-25/6)
7	Writing better papers (6/10/15)
8	Scientific Advisory Board meeting (30/10/15)
9	Internal scientific meeting (4/12/15)
10	External scientific meeting (9/3/16)
11	OARSI (31/3/16-3/4/16)
12	Epidemiology in OA seminar (11/04/16)
13	IASP (26/09/16-30/09/16)
14	UK-Rime (07/10/16)

Courses

A1	Nature of the doctorate 30/10
A2	Getting into the habit of writing 12/11
A3	Planning your research 19/11
A4	Basic stats in SPSS 20/11, 27/11
A5	Post-genomics and bioinformatics 9/12,10/12,11/12
A6	Critical appraisal 2 22/1
A7	Preparing 1st year report 26/2
A8	advanced stats in SPSS 5/3, 12/3, 19/3
A9	Writing an impact statement 10/4
A10	Postgraduate Research Forum 25/7
B1	Research ethics and the ethics review process for doctoral research 12/11
B2	Getting going on your thesis 24/11
B3	Exploiting scientific and technological innovations 15/1
B4	Introduction to the literature review process (moderated online learning course) 2/2-13/2
B5	Creating and managing long documents in microsoft word 9/3
B6	Further presentation skills for researchers 1/7
C1	Applying for academic jobs (3/12/15)
C2	Finishing your thesis (13/01/16)
C3	Career action planning for final year PhD students (15/1/16)
C4	Applying for jobs outside academia (18/01/16)
C5	Preparing for the viva (29/2/16)

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has completed the following training course

Nature of the doctorate and the supervision process on the 30/10/2013

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

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Getting into the habit of writing on the 12/11/2013

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Basic Statistics with SPSS on the 20/11/2013, 27/11/2013

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Post-genomics and bioinformatics on the 09/12/2013, 10/12/2013, 11/12/2013

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Critical Appraisal of the scientific literature 2 (Clinical) on the 22/01/2014

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Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

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Miss Hollie Louise Harvey
has completed the following training course

Advanced Statistics in SPSS (M&HS Faculty) on the 05/03/2014, 12/03/2014, 19/03/2014

and receives 3.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Writing an Impact Statement (Medicine and Health Sciences) on the 10/04/2014

and receives 0.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Faculty postgraduate Research Forum (Medicine and Health Sciences Faculty) on the 25/06/2014

and receives 4.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Research ethics and the ethics review process for doctoral research on the 12/11/2014

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Getting going on your thesis on the 24/11/2014

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Exploiting scientific and technological innovations on the 15/01/2015

and receives 2.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Introduction to the literature review process (moderated online learning course) on the 02/02/2015, 03/02/2015, 04/02/2015, 05/02/2015, 06/02/2015, 09/02/2015, 10/02/2015, 11/02/2015, 12/02/2015, 13/02/2015

and receives 3.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

D. A. Burns

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Creating and Managing Long Documents in Microsoft Word on the 09/03/2015

and receives 2.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Further presentation skills for researchers on the 01/07/2015

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Applying for academic jobs - PhD students on the 03/12/2015

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Finishing your thesis on the 13/01/2016

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Career action planning for final year PhDs on the 15/01/2016

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

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Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Applying for jobs outside academia - PhD students on the 18/01/2016

and receives 1.00 training unit(s)

Professor Jeremy Roberts
Dean of the Graduate School

Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

The University of Nottingham

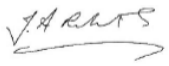
UNITED KINGDOM • CHINA • MALAYSIA

Certificate of Attendance

Miss Hollie Louise Harvey
has completed the following training course

Preparing for the viva on the 29/02/2016

and receives 1.00 training unit(s)



Professor Jeremy Roberts
Dean of the Graduate School



Mr David Burns
Director of Professional Development

This Cerficate of Attendance is issued by the University of Nottingham, for attendance at short courses. One training point is equivalent to half a day of tutor contact time or independent study.

UNIVERSITY OF NOTTINGHAM
School of Medicine
Research Student Annual (confirmation) Review Form
(To be completed for the First Year Annual Review)

Section 1: All students registered on higher degrees by research are subject to review annually. During the first year of registration, the status of research degree students is probationary and they are therefore subject to Confirmation Review by the end of that year.

Name of student: Hollie Harvey Date of initial registration: 1/10/2014

Current registration (PhD/MPhil/DM): PhD Year of study: End of 1st year

Mode of study (FT/PT): FT

Title of project: Epigenetics and genetics of pain in osteoarthritis

Main supervisor (name/School/Division): Dr Ana Valdes, SoM, ROD.

Additional supervisor(s) (name/School/Division): Prof Mike Doherty, SoM, ROD.

Internal Assessor(s) (name/School/Division): Dr Weiya Zhang, SoM, ROD

Section 2: Internal Assessor's report:

Please state the date the Viva was held (dd/mm/YYYY)	21 Oct 2014

Please comment specifically on EACH element of the annual review, e.g. the student's written report and the viva voce. Detailed comment is required on any inadequacies in the student's performance):

Hollie has made a very good progress in her first year. She has carried out a literature review, has learnt how to use SPSS for statistical analyses, has undertaken

some preliminary analyses based on the several databases where catastrophizing, OA pain and genetic data have been documented. She has picked up some signals for catastrophizing. She is now involved in a new study, where more data for the association by design will be collected to validate her results in the next year or so.

The written report follows the School format and word limit. It is well written and clear, which demonstrates that Hollie has a good knowledge of the literature about osteoarthritis (OA), its genetic risk and pain mechanism. She is also aware of the gaps in research into catastrophizing, its genetic predisposition and association with OA pain.

During the viva, Hollie showed considerable enthusiasm and commitment to conduct research in this filed. She demonstrated her interests and involvement in the development and execution of the project. She answered most of my questions well, showing good understanding of the project and research questions. We discussed some ways of restructuring section of the methods according to study design, participants, case and control ascertainment, genotyping, catastrophizing and other outcomes, power analysis (if possible) and statistical analysis. We discussed the potential caveats of the existing cohorts and approaches to get over the problems for the study questions. We also discussed the possible improvement of the presentation, such as using standard tables and figures rather than raw outputs from SPSS and how to put them in the context of the thesis.

Hollie has attended several relevant training courses in her first year and has gained more than required training credits. She has also had sufficient supervision meeting records with her main supervisor during the first year of her study. More supervision meetings with both supervisors in the future would be a bonus.

RECOMMENDATION

Satisfactory progress:

☒ that the student's current MPhil/PhD/DM registration is confirmed

☐ that the student be upgraded from MPhil to PhD status (with agreement of student)

☐ that the student transfer from DM to PhD (with agreement of student)

Borderline candidates

☐ Continue as probationary (full confirmation review required in 12 months time in year 2) with an agreed plan of supportive or corrective action being put into place.

Unsatisfactory progress

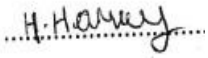
☐ current PhD/DM registration be altered to MPhil (student has a right of appeal)

☐ that the student's course be terminated (to be supported by evidence that the student has received written warnings on the lack of progress during the period of study – appeal opportunity exists)

Signed:  Internal Assessor Date: 22 Oct 2014

Section 3: Student's comments on progress: (*please complete and sign this section and return the form to your Internal Assessor who will pass this to the supervisor*): NB: **The student is responsible for submitting all the relevant documentation (see section 5) to the School Office by the published deadline**

I am pleased with my progress so far. I have already learnt so much and enjoyed attending workshops as well as courses. I am really happy that I have been able to do some preliminary analysis and run some GWAS which produced some good results. As per the discussion in the report I am now aiming to continue with planned future work.

Signed:  (Student) Date: 22/10/2014

Section 4: University (lead) Supervisor's comments:

How many documented progress meetings have taken place between you and the student in the past 12 months?	Insert number here: 10
Please confirm that supervision records are available	Yes/No
Have you discussed the Internal Assessor's report with the student?	Yes/No
Have you agreed an appropriate research plan in the light of the Internal Assessor's comments?	Yes/No
Please state the number of training points achieved (Note: The student should have completed 15 points if doing N-Trans or 20 points from the Postgraduate Training Programme (the portfolio of courses offered by the Faculty of Medicine and Health Sciences & Graduate School) **	Insert number here: 20
Please confirm that in year 1 the student has attended the compulsory "**Faculty Postgraduate Research Forum"** **	Yes

** **Note: students can access their training certificate at**: https://training.nottingham.ac.uk/cbs-notts/Guests/ADLogin.aspx?ReturnUrl=%2fcbs-notts%2fRegisteredGuest%2fGuestBookingList.aspx

Please comment on the student's written report, on his/her general progress and attendance, and on the Internal Assessor's report).

RECOMMENDATION: (please tick as appropriate*)

Satisfactory progress:

☐ that the student's current MPhil/PhD/DM registration is confirmed

☐ that the student be upgraded from MPhil to PhD status (with agreement of student)

☐ that the student transfer from DM to PhD (with agreement of student)

Borderline candidates

☐ Continue as probationary (full confirmation review required in 12 months time in year 2) with an agreed plan of supportive or corrective action being put into place.

Unsatisfactory progress

☐ current PhD/DM registration be altered to MPhil (student has a right of appeal)

☐ that the student's course be terminated (to be supported by evidence that the student has received written warnings on the lack of progress during the period of study – appeal opportunity exists)

In signing this form I also confirm that I have seen a copy of this students' training record confirming H&S training has been completed and signed by both the trainer and the student (not applicable if the student is a "study away" student).

Signed: (University lead Supervisor) Date:

Section 5: The student is to submit the following documentation together with this report (Please tick)

☐ A copy of the student's annual review report should be emailed to:

scs-pgenquiries@nottingham.ac.uk

☐ A signed copy of the plagiarism form (this can be found on the School workspace at http://workspace.nottingham.ac.uk/display/SCS/Postgraduate+Research+Students+-+Current+Information

Please return this form together with the documentation required to:

Course Administrator, University of Nottingham

School of Medicine, D Floor, East Block

Queen's Medical Centre, Nottingham NG7 2UH

Scs-pgenquiries@nottingham.ac.uk

(This form must be completed as part of the annual review of the progress of research students and lodged in the student's School file where it must be available for monitoring and appeals purposes).

For School Office use only

The Head of School recommends that: (please delete as appropriate*)

Satisfactory progress:

☐ that the student be upgraded from MPhil to PhD status;

☐ that the student transfer from DM to PhD

☐ that the student's current MPhil/PhD/DM registration be confirmed

Other comments/conditions:

……

……..

Unsatisfactory progress

☐ that the student's current PhD/DM registration be altered to MPhil

☐ that the student's course be terminated (to be supported by evidence that the student has received written warnings on the lack of progress during the period of study)

Signed: **(PP, Head of School)** **Date:**

Form updated – April 2015

UNIVERSITY OF NOTTINGHAM
School of Medicine - Research Student Annual Review Form
(To be completed for the FINAL Annual Review)

Section 1:

Name of student: Hollie Louise Harvey Date of initial registration: Oct 2013

Current registration (PhD/MPhil/DM): PhD Year of study: end of 3rd year

Mode of study (FT/PT): FT

Title of project: Epidemiology and Genetics of Catastrophizing and Pain in Osteoarthritis (OA)

Training programme (please delete as appropriate): MHS Faculty Training Programme

Main supervisor (name/School/Division): Dr Ana Valdes, School of Medicine, Rheumatology, Orthopaedics & Dermatology.

Additional supervisor(s) (name/School/Division): Professor Michael Doherty, School of Medicine, Rheumatology, Orthopaedics & Dermatology.

Internal Assessor(s) (name/School/Division): Professor Weiya Zhang, School of Medicine, Rheumatology, Orthopaedics & Dermatology.

Section 2: University (lead) Supervisors comments:

The final annual review of all research students should include a detailed consideration of progress, including progress made with writing up and a plan to ensure the thesis is submitted within a maximum of 4* years from initial registration. *Detailed comment is required on any inadequacies in the student's performance:*
*

A written report from the student on progress received?	Yes
A satisfactory thesis plan provided?	Yes/No
A detailed plan for submission within the maximum 4 year period?	Yes/No
How many documented progress meetings have taken place between you and the student in the past 12 months?	Insert no here: 10
Please confirm that supervision records are available	Yes
Please state the number of training points achieved in the last 12 months (Note: The student should have completed 5 points if doing N-Trans or 5 points from the MHS Faculty Postgraduate Training Programme **)	Insert number here: 5

Please comment on the student's progress, attendance, thesis plan and remaining experimental work and in particular the time-frame for completion.

RECOMMENDATION (please tick as appropriate)

Satisfactory progress:

☐ The student will continue as registered until the end of their programme period after which they will automatically enter the "thesis pending period"

☑ The student is ready to enter the thesis pending period before the end of their programme (i.e. early). They will enter thesis pending on (insert date) 30/06/2016

Unsatisfactory progress

☐ The student's current PhD registration may need to be exceptionally extended to a fourth year (please state how many more months) ____

☐ The current PhD/DM registration be altered to MPhil without the agreement of the student (student has a right of appeal)

☐ The current PhD/DM registration be altered to MPhil with the agreement of the student

☐ That the student's course be terminated (to be supported by evidence that the student has received written warnings on the lack of progress during the period of study – appeal opportunity exists)

Signed: [signature] (Supervisor) Date: 30/6/16.

Section 3: Student's comments on progress: NB: **The student is responsible for submitting all the relevant documentation (see section 4) to the School Office by the published deadline**

Please comment on thesis plan and experimental plan, do you agree that deadlines are realistic or can you foresee any problems or extenuating circumstances which might prevent you from completing your work in the time-frame set out in your report?

I believe that the thesis plan and deadlines are realistic and feel I have made good progress this year

There are no circumstance that I can foresee which would prevent me from keeping to plans and deadlines

Signed: [signature] (Student) Date: 30/06/16

Section 4: The student is to submit the following documentation together with this report (Please tick)

i) ☑ A copy of the student's annual review report, including thesis plan, should be emailed to: medicine-pgr@nottingham.ac.uk

ii) ☐ A signed copy of the plagiarism form (this can be found on the School workspace at: http://workspace.nottingham.ac.uk/display/medicine/Postgraduate+Research+Programme

Form updated – April 2015

Please return this form together with the documentation required to:
Doctoral Programmes Administrator
Medical Education Centre
School of Medicine
Nottingham, NG7 2UH
medicine-pgr@nottingham.ac.uk

(This form must be completed as part of the annual review of the progress of research students and lodged in the student's School file where it must be available for monitoring and appeals purposes).

For School Office use only

The Head of School recommends that: (please delete as appropriate*)

Satisfactory progress:

- ☐ The student will continue as registered until the end of their programme period after which they will automatically enter the "thesis pending period"
- ☐ The student is ready to enter the thesis pending period before the end of their programme (i.e. early). They will enter thesis pending on (insert date) _ _/_ _/_ _ _ _.

Unsatisfactory progress

- ☐ The student's current PhD registration may need to be exceptionally extended to a fourth year (please state how many more months) ____
- ☐ The current PhD/DM registration be altered to MPhil without the agreement of the student (student has a right of appeal)
- ☐ The current PhD/DM registration be altered to MPhil with the agreement of the student
- ☐ That the student's course be terminated (to be supported by evidence that the student has received written warnings on the lack of progress during the period of study – appeal opportunity exists)

Other comments/conditions:

.....

.....

Signed: **(PP, Head of School)** **Date:**