

**THE GENETICS AND EPIDEMIOLOGY OF
NEUROPATHIC PAIN-LIKE SYMPTOMS IN
PEOPLE WITH OSTEOARTHRITIS AND
POST-TOTAL JOINT REPLACEMENT**

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ABSTRACT

Background: Osteoarthritis (OA) is the most common form of arthritis and one of the leading causes of pain and disability worldwide. The only long-term treatment for severe, end-stage OA is removal of the damaged tissues by a total joint replacement (TJR). However for some individuals, pain continues to be a concern post-TJR. As well as this, a proportion of individuals with OA and post-TJR suffer with neuropathic pain-like symptoms (NP). NP is caused by changes or damage to the nervous system, which can occur as a result of nerve injury or chronic pain. NP differs from nociceptive pain in that symptoms can occur in response to a non-harmful stimulus, or in the absence of a stimulus. It is also hard to treat NP effectively. The molecular mechanisms underlying pain and NP in OA are still not well understood and therefore require further study. The high prevalence of OA means research in this field has the potential to impact many individuals.

Objectives: The aims of this thesis were a) to describe the effects of NP on post-TJR satisfaction, b) to investigate the genetic contribution to NP in people post-TJR using a genome-wide approach and c) to analyse two candidate genes for an association with pain in people with symptomatic OA or asymptomatic OA.

Methods: Individuals from three independent Nottinghamshire-based cohorts were analysed: 1) the Genetics of OA and Lifestyle (GOAL) study, 2) the Nottingham Genetics of OA Study (NGOAS) and 3) individuals recruited from Nottinghamshire who formed part of the Arthritis Research Council OA Genetics (arcOGEN) Consortium. Participants from all three studies were X-rayed at the time of recruitment and the Kellgren-Lawrence (K/L) grading system was used to classify radiographic severity of OA at the joints of

interest. All participants answered questionnaires containing validated assessment tools. NP was assessed using the painDETECT questionnaire.

Logistic regression analysis was used to identify factors associated with NP in people with OA and post-TJR. A modified LASSO variable reduction method was used to identify the contribution of these factors to post-TJR satisfaction. A genome-wide association scan (GWAS) was used to assess the genetic contribution to NP post-TJR. Candidate gene analysis allowed variants in two genes to be investigated for an association with pain and NP in people with OA and post-TJR.

Results: NP was found to be a very strong predictor of post-TJR satisfaction: AUC=0.79 (0.75-0.82). Other contributing factors were the site of the TJR (knee or hip replacement), history of revision surgery and other measures of pain. The use of opioid medications was also significant associated with a lower likelihood of satisfaction post-TJR: OR=0.54 95% CI 0.36-0.80, $p=0.002$ after adjustment for covariates and pain intensity. Overall, possible NP affected 17.3% of post-TJR patients (10.2% of those satisfied, 41.9% of those partially or not satisfied).

A reproducible genetic effect was found between a single nucleotide polymorphism (SNP) in the protein kinase C alpha (*PRKCA*) gene and the risk of possible NP in people with knee pain, knee OA, hip OA and post-TJR: OR=2.41 95% CI 1.74-3.34, $p=1.29 \times 10^{-7}$ after meta-analysis in three cohorts.

Candidate gene analysis found a consistent genetic effect in the substance P receptor gene (*TACR1*). The G allele at rs11688000 was associated with lower risk of symptomatic OA (OR=0.84 95% CI 0.70-1.00, $p=0.047$). This variant was also associated in four independent cohorts, yielding an overall OR=0.80 95% 0.70-0.91, $p=8.66 \times 10^{-4}$ after meta-analysis in five cohorts (total symptomatic OA n=1,566, total asymptomatic OA n=894).

A SNP in the interleukin-15 receptor alpha (*IL15RA*) gene was found to be significantly associated with the risk of symptomatic OA versus asymptomatic OA: OR=1.56 95% 1.18-2.07, p=0.002. This remained significant (OR=1.43 95% CI 1.04-1.99, p=0.029) after adjustment for covariates (age, sex and body mass index) and tibiofemoral K/L knee OA radiographic grade. Another variant in the *IL15RA* gene was significantly associated with NP post-TJR: OR=0.76 95% 0.63-0.92, p=0.005 after meta-analysis in two cohorts.

Conclusion: The significant contribution of NP to post-TJR satisfaction highlights the importance of identifying cases of NP in people with OA. The PDQ used here to measure NP is a validated tool which could easily be introduced more widely into clinical practice post-TJR. These results also highlight the prevalence of NP symptoms post-TJR. My work also has identified a biologically relevant genetic effect for the risk of NP post-TJR. The SNPs identified in this GWAS provide the first results from a GWAS on NP in OA and could inform future replication studies in additional cohorts. The association of variants in the *TACR1* and *IL15RA* genes with pain in people with OA and, the case of *IL15RA*, with NP pain post-TJR suggests potential treatment targets for future studies into therapies to treat pain and NP in people with OA.

PUBLICATIONS

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Warner SC, van Meurs JBJ, Schiphof D et al. A variant in the protein kinase C alpha (*PRKCA*) gene affects the risk of neuropathic pain-like symptoms post-total joint replacement: a genome-wide association scan (**under review**; *European Journal of Human Genetics*).

Warner SC, Walsh DA, Laslett LL et al. Genetic variation in the neurokinin receptor 1/substance P receptor (*TACR1*) gene is associated with the risk of painful knee osteoarthritis (**under review**; *Arthritis & Rheumatology*)

Warner SC, Nair A, Marpadga R et al. IL-15 in osteoarthritis: Evidence for a role in protease production and symptomatic manifestations (**under review**; *Osteoarthritis and Cartilage*).

Warner SC, Richardson H, Jenkins W et al. Neuropathic pain-like symptoms and pre-surgery radiographic severity predict patient-related outcomes 4.8 years post-total joint replacement (**manuscript prepared**).

DECLARATION

I declare that the work submitted in this thesis is the result of original research. This was conducted primarily by me with the supervision of Ana Valdes (principal supervisor) and Michael Doherty. All authors and their works have been fully acknowledged.

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Table of contents

List of tables	xiv
List of figures.....	xv
CHAPTER 1: INTRODUCTION.....	1
1 Osteoarthritis.....	1
1.1 The nature of osteoarthritis	1
1.2 Histological definition	3
1.3 Diagnosis.....	5
1.3.1 Imaging.....	5
1.3.2 Clinical assessment.....	8
1.4 Risk factors	9
1.4.1 Age	9
1.4.2 Sex	10
1.4.3 Body mass index	10
1.4.4 Biomechanical and trauma	12
1.4.5 Joint morphology.....	13
1.4.6 Index finger to ring finger length ratio	13
1.4.7 Genetic.....	13
1.5 Management.....	14
1.5.1 Non-pharmacological	14
1.5.2 Pharmacological	14
1.5.3 Surgical.....	18
1.5.4 Assessing post-TJR outcomes	19
1.5.5 Comorbidities in osteoarthritis.....	21
2 Neuropathic pain.....	22
2.1 Introduction.....	22
2.2 Diagnosis.....	25
2.2.1 Questionnaire assessment.....	25
2.2.2 Clinical assessment.....	26

2.2.3 MRI and CT scans	27
2.2.4 Other tests	27
2.2.5 Difficulties with diagnosing neuropathic pain	28
2.3 Animal models	28
2.4 Risk factors	29
2.4.1 Age	29
2.4.2 Sex	30
2.4.3 Body mass index	30
2.4.4 Past surgery	30
2.4.5 Pre-existing conditions	31
2.5 Management	32
2.5.1 Non-pharmacological	32
2.5.2 Pharmacological	33
2.5.3 Surgical	36
3 The genetics of osteoarthritis	36
3.1 Introduction	36
3.2 The genetics of osteoarthritis	38
4 The genetics of pain and neuropathic pain	45
4.1 Introduction	45
4.2 Pain and neuropathic pain susceptibility genes	46
5 Aims and objectives	50
CHAPTER 2: CLINICAL COLLECTION AND ASSESSMENT	53
2.1 Introduction	53
2.2 Participants	53
2.2.1 Nottingham Genetics of Osteoarthritis Study (NGOAS)	53
2.2.2 Arthritis Research Council Osteoarthritis Genetics (arcOGEN) Consortium	54
2.2.3 Genetics of Osteoarthritis and Lifestyle (GOAL) Study	55
2.3 Questionnaire data	55
2.3.1 Trait Definitions for statistical analysis	56

2.3.1.1 Kellgren Lawrence grade	56
2.3.1.2 Neuropathic pain-like symptoms.....	56
2.3.1.3 WOMAC pain, stiffness and function.....	56
2.3.1.4 Pain severity	57
2.3.1.5 Chronic widespread pain	57
2.3.1.6 Pain catastrophizing	57
2.3.1.7 Body mass index	57
2.3.1.8 Comorbidities and pre-existing conditions.....	57
2.3.1.9 Medication use	58
CHAPTER 3: NEUROPATHIC PAIN-LIKE SYMPTOMS AND POST- TOTAL JOINT REPLACEMENT SATISFACTION.....	62
3.1 Introduction.....	62
3.2 Methods	62
3.2.1 Participants.....	62
3.2.2 Statistical analysis	63
3.2.3 Definitions for statistical analysis	63
3.2.3.1 Post-TJR satisfaction.....	64
3.2.3.2 Sub-analysis on the factors associated with NP in OA and post- TKR	64
3.3 Results	64
3.3.1 Post-TJR satisfaction	64
3.3.2 Sub-analysis of the factors associated with neuropathic pain-like symptoms in people with OA and post-total knee replacement.....	75
3.4 Discussion.....	79
3.4.1 Post-TJR satisfaction	79
3.4.2 Sub-analysis of the factors associated with NP in OA and post-TKR.	81
3.5 Chapter summary.....	83
CHAPTER 4: GENOME-WIDE ASSOCIATION SCAN OF NEUROPATHIC PAIN-LIKE SYMPTOMS POST-TOTAL JOINT REPLACEMENT AND REPLICATION ANALYSIS	85

4.1 Introduction.....	85
4.1.1 Aims and objectives	85
4.2 Methods	85
4.2.1 Participants.....	85
4.2.1.1 Discovery group	85
4.2.1.2 Replication groups.....	85
4.2.2 Stage 1: GWAS	87
4.2.3 Stage 2: replication cohorts.....	88
4.2.4 Stage 3: meta-analysis.....	88
4.2.5 Phenotype.....	89
4.3 Results	89
4.3.1 Stage 1: GWAS	89
4.3.1.1 Pathway analysis	97
4.3.2 Stage 2: replication cohorts.....	99
4.3.3 Stage 3: meta-analysis.....	99
4.4 Discussion.....	103
4.5 Chapter summary.....	108
CHAPTER 5: CANDIDATE GENE ANALYSIS FOR ASSOCIATIONS WITH PAIN IN OSTEOARTHRITIS.....	110
5.1 Introduction.....	110
5.1.1 Aims and objectives	110
5.2 Methods	112
5.2.1 Analysis of the substance P receptor gene	112
5.2.1.1 Discovery cohort	112
5.2.1.2 Replication cohorts.....	112
5.2.1.3 Genetic analysis.....	112
5.2.2 Analysis of the IL-15 receptor alpha gene	113
5.2.2.1 Study participants	113
5.2.2.2 Binary trait definitions for statistical analysis.....	113
5.2.2.3 Genetic analysis.....	113

5.3 Results	114
5.3.1 Analysis of the substance P receptor gene	114
5.3.2 Analysis of the IL-15 receptor alpha gene	122
5.4 Discussion.....	126
5.4.1 Main findings	126
5.4.1.1 Analysis of the substance P receptor gene	126
5.4.1.2 Analysis of the IL-15 receptor alpha gene	126
5.4.2 Biological relevance and context with other studies.....	127
5.4.2.1 Analysis of the substance P receptor gene	127
5.4.2.2 Analysis of the IL-15 receptor alpha gene	127
5.4.3 Clinical relevance.....	128
5.4.3.1 Analysis of the substance P receptor gene	128
5.4.3.2 Analysis of the IL-15 receptor alpha gene	129
5.4.4 Strengths and limitations.....	129
5.4.4.1 Analysis of the substance P receptor gene	130
5.4.4.2 Analysis of the IL-15 receptor alpha gene	130
5.4.5 Future work.....	131
5.4.5.1 Analysis of the substance P receptor gene	131
5.4.5.2 Analysis of the IL-15 receptor alpha gene	131
5.4.6 Conclusion	132
5.5 Chapter summary.....	132
6 GENERAL DISCUSSION	134
6.1 Introduction.....	134
6.2.1 Neuropathic pain-like symptoms and post-total joint replacement satisfaction	134
6.2.2 The epidemiology of neuropathic pain-like symptoms	135
6.3 Genome-wide association scan of neuropathic pain-like symptoms post- total joint replacement and replication analysis.....	135
6.4 A candidate gene analysis of pain- and inflammation-related genes in osteoarthritis	136

6.6 Other approaches	137
6.7 Limitations.....	140
6.8 Further work	142
6.9 Conclusions	145
7 REFERENCES	146
8 APPENDIX	188

List of tables

Table 1 The Kellgren-Lawrence grading system for classifying the severity of radiographic osteoarthritis (Kellgren, 1957).....	6
Table 2 BMI classifications according to the World Health Organisation (WHO)	11
Table 3 Genetic associations with large joint OA and related traits derived from genome-wide association scans (GWAS) studies with $p < 1 \times 10^{-7}$	41
Table 4 A description of the main questionnaires used for data collection in these studies	60
Table 5 Descriptive statistics categorised by total joint replacement satisfaction status and their contribution to the risk of dissatisfaction post-total joint replacement.....	66
Table 6 Descriptive characteristics including mean painDETECT scores (PDQ) and prevalence of possible and likely neuropathic-like knee pain symptoms..	75
Table 7 Association between comorbidities and possible neuropathic pain-like knee pain symptoms in people with OA and post-TKR, adjusted for age, sex, BMI and OA/post-TKR status, and further adjusted for joint pain intensity ...	78
Table 8 The results of interest from the unadjusted Illumina array NP GWAS	96
Table 9 The list of genes (n=63) containing SNPs of $p < 0.0001$ in the NP GWAS used for pathway analysis	98
Table 10 Overrepresented pathways for the NP GWAS	98
Table 11 Demographic information for the three groups used in this study ..	101
Table 12 The results of replication analysis and meta-analysis	102
Table 13 Descriptive statistics for the discovery group (GOAL) and replication groups used in the analysis of the rs11688000 SNP	117
Table 14 Information about the <i>IL15RA</i> SNPs used in this study	119
Table 15 The results of logistic regression analysis showing the association of each of the six <i>TACR1</i> SNPs with three measures of symptomatic versus asymptomatic OA. All analyses are adjusted for age, sex and BMI	120
Table 16 Descriptive statistics for the groups used in the genetic analysis work	123
Table 17 Information about the <i>IL15RA</i> SNPs used in this study	124
Table 18 Association between knee OA and pain-related traits and two <i>IL15RA</i> SNPs. Odds ratios (OR) are adjusted for age, sex and BMI	125

List of figures

Figure 1 Stages in the development of osteoarthritis, adapted from (Hutton, 1989).....	2
Figure 2 Bilateral medial tibio-femoral OA with varus deformity (source: Michael Doherty, used with permission).....	7
Figure 3 Hands showing the presence of Heberden's nodes at the distal interphalangeal joints (source: Michael Doherty, used with permission)	9
Figure 4 The average BMI in various osteoarthritis and total joint replacement cohorts versus the average BMI for the UK adults population in individuals aged 60-69 years old and 70-79 years old	12
Figure 5 The hierarchy of treatment options for osteoarthritis as recommended by the National Centre for Health and Care and Excellence (NICE, 2014).....	15
Figure 6 Schematic showing the transition which can occur in OA pain between nociceptive or peripheral pain to centrally-mediated pain, as seen in neuropathic pain-like states	32
Figure 7 Flow chart outlining some of the possible pharmacological treatment pathways for NP. Adapted from (Chong and Bajwa, 2003), who reproduced a diagram by (Orza, 2000) with further information from (Finnerup, 2015).....	35
Figure 8 Schematic diagram of the different types of risk factors for osteoarthritis	40
Figure 9 ROC curves adjusted for age, sex and BMI to show the amount of post-surgery satisfaction contributed by a LASSO regression model and painDETECT questionnaire scores (A) post-total joint replacement (total knee and hip replacements combined) (B) post-total hip replacement (C) post-total knee replacement	73
Figure 10 The proportions of individuals reporting satisfaction post-TJR according to PDQ score	74
Figure 11 Study design	86
Figure 12 QQ plot for the results of the GWAS ($\lambda=0.99$).....	90
Figure 13 Regional association plot to show the location of rs887797 and its association with possible neuropathic pain-like symptoms post-total joint replacement.....	92
Figure 14 Regional association plot to show the location of rs12596162 and its association with possible neuropathic pain-like symptoms post-total joint replacement.....	93

Figure 15 Regional association plot to show the location of rs7734804 and its association with possible neuropathic pain-like symptoms post-total joint replacement.....	94
Figure 16 Regional association plot to show the location of rs298235 and its association with possible neuropathic pain-like symptoms post-total joint replacement.....	95
Figure 17 Manhattan plot showing the p value of association tests for SNPs with possible NP in the Illumina array GWAS. P values represent the association of the SNPs with possible NP	97
Figure 18 Forest plot showing the results of an unadjusted Han Eskin analysis on the rs887797 SNP using a recessive model	100
Figure 19 Linkage disequilibrium (LD) plot showing the degree of association between the SNPs used in this study	115
Figure 20 Forest plot showing the results of a fixed-effects meta-analysis of the discovery group (GOAL) and the four replication groups used in the analysis of rs11688000 and its association with symptomatic OA versus asymptomatic OA (A: unadjusted, B: after adjustment for age, sex, BMI and maximum KL grade)	121
Figure 21 Histograms to show A) the normal distribution, B) the distribution of painDETECT questionnaire scores in the discovery cohort of this analysis and C) the log(+1) transformed values of the painDETECT questionnaire scores.....	139
Figure 22 The consent form for enrolment in the Genetics of Osteoarthritis and Lifestyle study	188
Figure 23 The consent form for the Nottingham Genetics of Osteoarthritis Study.....	189
Figure 24 The consent form for enrolment into the Arthritis Research Council Osteoarthritis Genetics study	190

Glossary of abbreviations

OA	Osteoarthritis
K/L	Kellgren-Lawrence grade for radiographic severity of OA
NSAIDs	Non-steroidal anti-inflammatory drugs
OTC	Over-the-counter pain medications
BMI	Body mass index
OR	Odds ratio
TJR	Total joint replacement
TKR	Total knee replacement
THR	Total hip replacement
PDQ	painDETECT questionnaire
NP	Neuropathic pain-like symptoms
WOMAC	Western Ontario and McMancheater Universities Arthritis Index
VAS	Visual analogue scale to measure pain intensity
PCS	Pain Catastrophizing Scale
GWAS	Genome-wide association scan
SNP	Single nucleotide polymorphisms
NGOAS	Nottingham Genetics of Osteoarthritis Study
GOAL	Genetics of Osteoarthritis and Lifestyle Study
arcOGEN	Arthritis Research Council Osteoarthritis Genetics Consortium
NICE	National Institute for Health and Care Excellence
ROC	Receiver operating characteristics
AUC	Area under the curve

CHAPTER 1: INTRODUCTION

1 Osteoarthritis

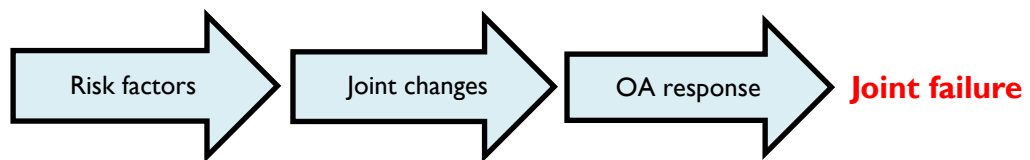
1.1 The nature of osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis and is a major cause of pain and disability worldwide (CDC, 2010, Bitton, 2009). OA is the common outcome of a group of disorders (Valdes, 2013b). As such there are different mechanisms and molecular pathways potentially involved in the pathogenesis of the disease. OA only affects the synovial (diarthrodial) joints and can affect any species which has these joints. Synovial joints are characterised by the presence of the synovium, which produces fluid and provides nutrients to cells in the joint (Altman, 2008). Examples of these joints are seen at the knees, hips and in the hands (Altman, 2008). All tissues of the synovial joint can potentially be affected by OA, such as cartilage and the underlying bone, muscles and nerves (Baugé et al., 2014, Goldring and Goldring, 2007, Samuels et al., 2008). OA can be primary or secondary, depending on whether the disease results from recognised genetic and other individual risk factors, or from obvious joint trauma, respectively (Valdes, 2013a).

OA is a common complex disorder which was previously thought to be a degenerative condition. However evidence suggests that OA is a physiologically active state (Loeser et al., 2012, NICE, 2008). This means OA has potentially regenerative mechanisms, as the processes seen in the condition are identical to the joints' inherent repair mechanisms (Loeser et al., 2012, NICE, 2008). This theory is supported by the fact that OA has been seen throughout our evolutionary history, and since it is also seen in other species suggesting phylogenetic conservation (Hutton, 1987). This may be because the processes which result in OA are potentially regenerative and are inherent in

joint repair (NICE, 2008). Joints are slow to remodel and may be anatomically different after recovery from an insult, but this does not always lead to pain (NICE, 2008). However, an overwhelming insult of flaw in these repair processes may lead to more loss than gain by remodelling, and symptoms are then more likely (see **Figure 1** Stages in the development of osteoarthritis, adapted from (Hutton, 1989)) (NICE, 2008).

Figure 1 Stages in the development of osteoarthritis, adapted from (Hutton, 1989)



Historically, OA has been considered a non-inflammatory condition (Sokolove and Lepus, 2013). However recent findings, such as the presence of synovitis in a proportion of people presenting with OA, have led to the recognition of the important role of inflammation in OA pathogenesis (Scanzello and Goldring, 2012). For example, IL-15 is a pro-inflammatory cytokine that is best characterized for its effects on T-lymphocyte and NK-cell activation, proliferation, and survival (Kennedy et al., 2000, Marks-Konczalik et al., 2000). IL-15 has been detected in many synovial fluid samples from people with knee OA (Sun et al., 2013, Scanzello et al., 2009). IL-15 has also been linked with pain in various rheumatological diseases including rheumatoid arthritis (RA), lupus and OA (Sun et al., 2013, Baslund et al., 2005, Waldmann, 2004, Turkow et al., 1997, Aringer et al., 2001, Baranda et al., 2005). In addition to nociceptive pain, IL-15 has been linked to the

development of neuropathic pain-like symptoms (NP) after nerve injury, due to its role in neuroinflammation (Gómez-Nicola et al., 2008)

Another example relevant to the inflammatory nature of OA is substance P. Substance P is a pain- and inflammation-related neuropeptide which binds to the tachykinin receptor 1 (TACR1; also known as the neurokinin 1 receptor, NK1R) (Szallasi and Blumberg, 1996). Research has linked TACR1 receptors and joint degeneration (Im et al., 2008) and substance P to pain and swelling in OA (Marshall et al., 1990, Lam and Ng, 2010, Lindh et al., 1997), supporting the relevance of the TACR1 receptors and SP in OA research. Substance P is a part of the neurokinin 1 (NK1) pathway and is a pain mediator in the peripheral and central nervous system as well as being involved in pain perception and pain thresholds (Li, 2011, Zubrzycka and Janecka, 2000). More specifically, substance P has been linked to pain and swelling in OA, two major features of the disease (Lam and Ng, 2010). Levels of substance P were found to be higher in the cerebrospinal fluid of individuals with painful OA versus control participants in another study (Lindh et al., 1997). It has also been shown that substance P was elevated in synovial fluid samples from individuals with OA compared to controls (Marshall et al., 1990). Due to the involvement of substance P in pain perception (Zubrzycka and Janecka, 2000), it is thought to be a potential therapeutic target for the treatment of pain and inflammation, including in OA (Marshall et al., 1990). In one study substance P was shown to prevent OA progression in rats, as well as showing an anti-inflammatory effect and signs of tissue repair (Kim et al., 2016).

1.2 Histological definition

OA is a multi-tissue disease (Loeser et al., 2012). The joint damage seen in OA can potentially occur in all tissues of the synovial joint, including hyaline and

fibro-cartilage, underlying bone, synovium/capsule, ligaments, muscles and nerves (Baugé et al., 2014). OA causes changes to the affected joints at a structural and molecular level (Altman, 2008). These changes can affect the ability of the joint to function normally, causing pain and a loss of function (NICE, 2008). Focal loss of hyaline cartilage and bone remodelling are histological features seen only in OA (NICE, 2008, Altman, 2008). There are different aetiologies and risk profiles for the potential sites of OA, but the nature of the disease is similar regardless of the joint affected.

Ossification of fibrocartilage may also be seen (Kawaguchi, 2009). As a result, osteophytes form at the joint margin (NICE, 2008). This is a type of endochondral ossification (new cartilage calcifying to form bone), as seen during growth (Kawaguchi, 2009). Osteophytes can be used as part of the X-ray diagnosis of OA as they are one of the radiographically-visible changes seen in OA (Meulenbelt et al., 1998, Scharstuhl, 2002). The presence of osteophytes may associated with pain (Cicuttini et al., 1996) and changes to the articular cartilage (Boegård et al., 1998).

In OA, cartilage shows a reversion to growth mechanisms (Hutton, 1989). At cartilage maturity, vascularity recedes, cartilage calcification is inhibited and proteins in the cartilage mature (Wollheim, 2008). However in OA neovascularisation, calcification and immature proteins (neo-epitopes) can be seen (Wollheim, 2008). Mature articular cartilage is typically avascular and aneural (Ashraf et al., 2011) however this can change in joints affected by OA (Thakur et al., 2014). This is called neurovascular invasion (Thakur et al., 2014).

1.3 Diagnosis

1.3.1 Imaging

As described in **Section 1.2** Histological definition, OA can be defined in several ways and a diagnosis of OA can be made using these indications (Valdes, 2013b). Radiographic diagnosis of OA is commonly used. The Kellgren-Lawrence (K/L) composite scoring system (see Table 1 The Kellgren-Lawrence grading system for classifying the severity of radiographic osteoarthritis (Kellgren, 1957)) can be used to classify radiographic OA severity (Kellgren, 1957). A higher score means more radiographic changes characteristic of OA (e.g. osteophytes and joint space narrowing (JSN)) have been observed in an individual (Kellgren, 1957) (see **Table 1** The Kellgren-Lawrence grading system for classifying the severity of radiographic osteoarthritis (Kellgren, 1957) and **Figure 2** Bilateral medial tibio-femoral OA with varus deformity (source: Michael Doherty, used with permission)). Research from the Rotterdam Study found that K/L scores were associated with hip pain and can be used to predict total hip replacement (THR) outcomes (Jordan, 2008).

Interestingly, one study noted that the K/L scoring system has not been formally validated (Bhattacharya R., 2007). This system has been altered by Croft to use a scale of 0-5 (instead of 0-4) for grading X-rays in the context of hip OA diagnosis (Jordan, 2008, Reijman, 2004). However, according to some authors, JSN could be seen as more robust and useful radiographic feature than the K/L grade which relies heavily on the presence of osteophytes (Croft, 1990, Lanyon et al., 2003). An association with minimum joint space width (mJSW) pre-surgery and pain post-surgery has been reported in a separate cohort (Valdes, 2011). The use of mJSW is another way of defining OA, with a

minimum JSW $\leq$ 2.5mm (which is a standard cut-off) being classified as radiographically severe (Croft, 1990).

The Nottingham knee OA line drawing atlas is another system used for grading radiographic knee OA (Wilkinson et al., 2005, Nagaosa et al., 2000). This system of line drawings includes scores for tibiofemoral (TF) JSW, patellofemoral JSW and osteophytes in all TF sites. This atlas has advantages over the K/L system in that it is sex-specific and measures the individual radiographic features of OA separately.

It should be noted that radiographs are insensitive for detecting longitudinal structural changes to the joint, including in early OA (Guerhazi et al., 2013).

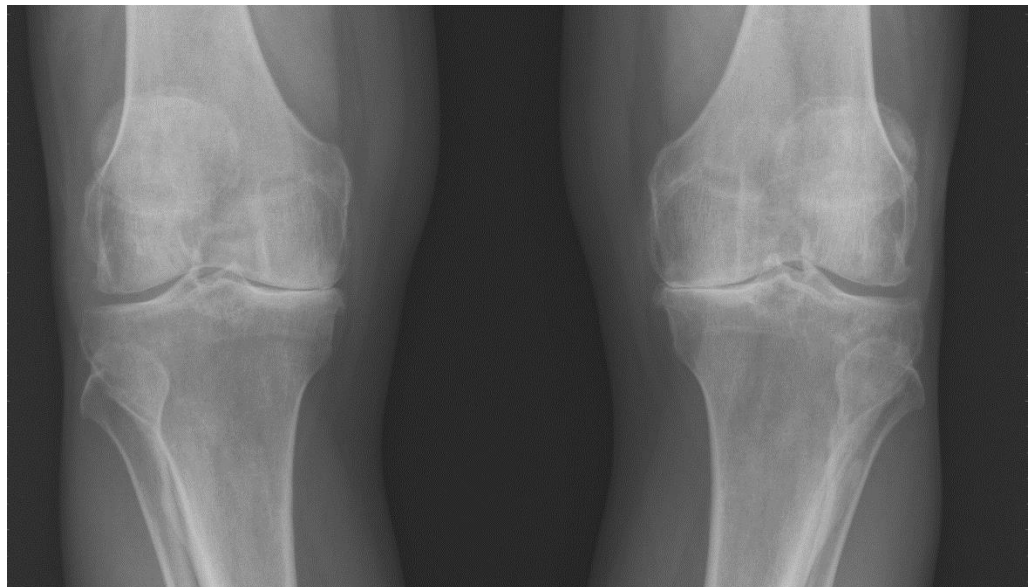
Table 1 The Kellgren-Lawrence grading system for classifying the severity of radiographic osteoarthritis (Kellgren, 1957)

	Joint space narrowing	Osteophytes	Subchondral bone sclerosis	Bone contour deformity
Grade 0	None	None	None	None
Grade 1	Doubtful	Possible	None	None
Grade 2	Possible	Definite	None	None
Grade 3	Definite	Multiple	Definite	Possible
Grade 4	Marked	Large	Severe	Definite

Radiographic severity is not always well associated with pain and function. An individual may feel no pain, but show strong radiographic signs of OA, or vice versa (Valdes, 2013b, Hannan MT, 2000, Valdes, 2013c, Thakur, 2013, Bliddal et al., 2000). This potential for asymptomatic OA can complicate classification

of the disease (NICE, 2008). However, as mentioned above, some studies have suggested that there may be more radiographic evidence for OA seen in painful joints than non-painful ones (Conaghan, 2004). Interestingly, the degree of radiographic changes associated with OA pre-TJR was found by one study to inversely correlated to pain post-TJR (a major outcome measure of TJR satisfaction) (Valdes, 2011). A sophisticated study which used an approach to minimise confounders showed a strong link between structural abnormalities (such as JSN) and pain (Neogi et al., 2009). It concluded that the presence of radiographic knee OA has limited discriminating potential for knee pain as there is a lack of co-occurrence, but radiographic changes do contribute to the understanding of pain in OA (Neogi et al., 2009).

Figure 2 Bilateral medial tibio-femoral OA with varus deformity (source: Michael Doherty, used with permission)



Magnetic resonance imaging (MRI) is another technique which has been used in OA research. MRI allows the soft tissues of the joint to be visualised (Hunter et al., 2011b) and features such as bone marrow lesions, synovial volume and bone volume to be visualised and quantified (Hunter et al., 2011b). Synovitis can also be assessed this way (Pelletier et al., 2008). This technique has been shown in a meta-analysis to have high inter-reader and intra-reader intra-class correlation (Hunter et al., 2011b). Scoring systems have been developed, such as the whole-organ MRI score (WORMS), the Boston Leeds OA knee score (BLOKS) and the MRI OA knee score (MOAKS) (Hunter et al., 2008, Peterfy et al., 2004, Hunter et al., 2011a).

The use of ultrasound to measure the prevalence of effusion, synovial hypertrophy and inflammation is seen in a number of published studies. A higher prevalence of these features is reported in people with knee pain or OA than in the general population and asymptomatic controls (Sarmanova et al.).

At present, MRI and ultrasound are more commonly used in research studies than in clinical practice.

1.3.2 Clinical assessment

There are features of OA which can allow a clinical diagnosis to be made. Pain is the major clinical symptom of OA (Hunter et al., 2013). This pain is worse on use, improved by rest and is often seen alongside a restriction in movement (Zhang et al., 2009). As well as this, short-lived stiffness in the early morning or after inactivity may be reported (Zhang et al., 2009). Examination of the affected joint may show bony enlargement, tenderness at the joint line or near the joint, mild effusion or crepitus (a crunching noise or sensation on movement) (Zhang et al., 2009). Another visible sign of OA includes the presence of nodes on the finger joints (such as Heberden's nodes, which have been linked to radiographic OA; see **Figure 3** Hands showing the presence of

Heberden's nodes at the distal interphalangeal joints (source: Michael Doherty, used with permission)) (Thaper et al., 2005).

Figure 3 Hands showing the presence of Heberden's nodes at the distal interphalangeal joints (source: Michael Doherty, used with permission)



1.4 Risk factors

1.4.1 Age

Increasing age is a risk factor for the development of OA at all joint sites (Jordan, 2008, NICE, 2008). However, other factors which may be a result of ageing or exacerbated by the ageing processes are responsible (Jordan, 2008); it is not ageing in itself which leads to OA (Loeser, 2011, Jordan, 2008). A 2011 review on the link between OA and ageing makes the point that ageing “promotes the development of OA in conjunction with other risk factors” (Loeser, 2011).

An MRI study on older adults in the community showed a high prevalence of pre-radiographic OA changes (Hayashi et al., 2014). In a Japanese study, it was estimated that Japanese population study estimates that 43% and 62% of men and women, respectively, over the age of 40 had knee OA (Yoshimura, 2011). A study from the USA found a lifetime risk of 44.7% for knee OA and a one in four risk for hip OA in a longitudinal study of people over 45 (Murphy et al., 2008, Murphy et al., 2010).

1.4.2 Sex

OA is more common in women than in men, with female sex being a risk factor for OA (Jordan, 2008, Srikanth et al., 2005, Blagojevic et al., 2010). After the menopause, a woman's risk increases further due to changes in oestrogen and progesterone levels (Jordan, 2008, Wluka et al., 2000). The use of oestrogen replacement therapy has been linked to a higher volume of knee cartilage (Wluka et al., 2001). Further study is needed into the mechanisms underlying the sex-specific differences in risk (Hame and Alexander, 2013) but reasons for this increased risk in women are thought to include anatomical differences in joint size and structure, such as larger quadriceps angles (Conley et al., 2007).

1.4.3 Body mass index

An individual's body mass index (BMI) can play a role in their risk of developing OA, with a higher BMI being associated with a higher risk of OA (Silverwood et al., 2015). **Table 2** BMI classifications according to the World Health Organisation (WHO) describes the criteria used in many studies to categorise BMI. **Figure 4** The average BMI in various osteoarthritis and total joint replacement cohorts versus the average BMI for the UK adults population in individuals aged 60-69 years old and 70-79 years old shows some of the

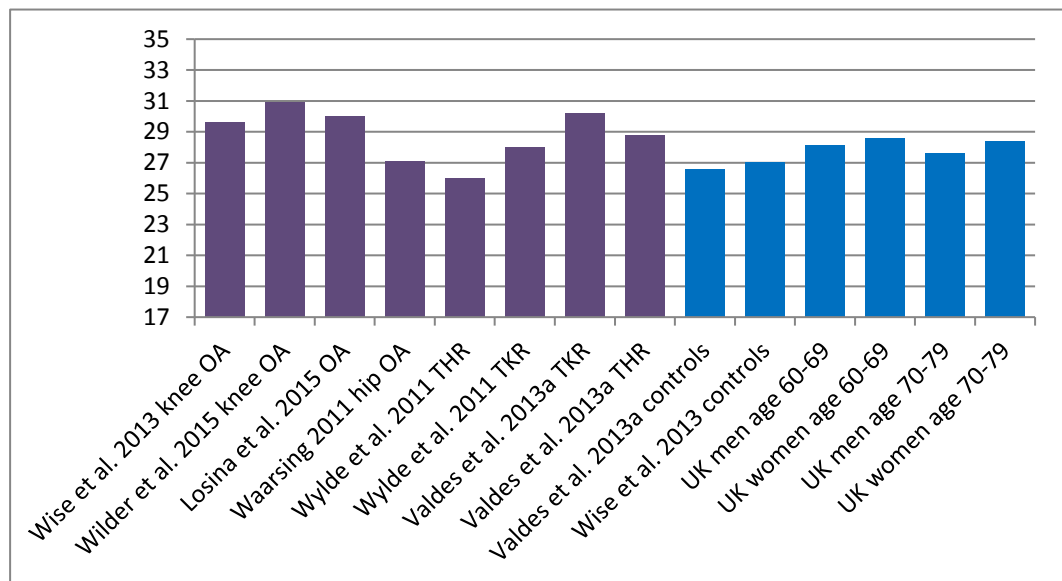
average BMI values reported in studies of people with OA, compared to controls for OA and the UK general population of a comparable age.

Table 2 BMI classifications according to the World Health Organisation (WHO)

	BMI (kgm ⁻²)
Underweight	<18.50
Acceptable	18.50-24.99
Overweight	25.00-29.99
Obese	≥30.00

Data from some studies shows a higher average BMI in TJR patients than in the general population in the same age groups (see **Figure 4** The average BMI in various osteoarthritis and total joint replacement cohorts versus the average BMI for the UK adults population in individuals aged 60-69 years old and 70-79 years old and (Walpole, 2012). Another study showed a linear increase in the risk of knee OA with increasing BMI (Felson, 2008). Increased force and loading on the joint may cause unfavourable changes and/or damage to joint structures. There is also a metabolic component to the increased risk of OA seen in obese individuals, due to the inflammatory nature of obesity as a condition (Jordan, 2008, Das, 2001). However, the literature is not in agreement as to the exact mechanisms by which obesity contributes to OA (i.e. metabolic or biomechanical effects, or both) and how the risk differs according to site (Jordan, 2008).

Figure 4 The average BMI in various osteoarthritis and total joint replacement cohorts versus the average BMI for the UK adults population in individuals aged 60-69 years old and 70-79 years old



1.4.4 Biomechanical and trauma

From a biomechanical perspective, joint damage such as that seen in OA can occur from either overuse or underuse of a joint, leading to damage or atrophy, respectively (NICE, 2008). Trauma has been shown to be a risk factor for OA of the injured joint (Bruyère et al., 2015). This can be caused by sports injuries, occupational injuries, and overuse. A past trauma may also affect the response to treatment in OA (Bruyère et al., 2015). Ligament damage is associated with an increased risk of OA incidence and progression (Hart et al., 2015). If an individual's lifestyle and occupation involve repetitive movements which place a strain on joints, or lead to joint trauma, they may be at an increased risk of developing OA. This was found in a study by Zhang et al. which showed that higher use of the squatting position was associated with a higher risk of knee OA (Zhang et al., 2004).

The biomechanical aspect of OA seems logical when the potentially regenerative nature of the disease is considered. There is a return to growth mechanisms as a result of OA, and it may be a flaw in these processes which causes OA to develop in some individuals (Hutton, 1989).

1.4.5 Joint morphology

Certain types of hip morphology may show an increased risk of hip OA (Waarsing et al., 2011). There are genes which influence bone shape, geometry and development, so variants in these genes may affect OA risk in some individuals. Joint hypermobility increases the risk of OA and sports injuries and the associated trauma of these can in turn lead to an increased risk of OA (Valdes, 2013a).

1.4.6 Index finger to ring finger length ratio

The ratio of the lengths of the second (index finger) and fourth (ring finger) digits of the hand may be associated with the risk of developing OA. This is called the 2D:4D ratio, and there are three possible combinations: 2D>4D (type 1), 2D=4D (type 2) and 2D<4D (type 3). After controlling for other risk factors, a link was seen in some studies between type 3 (the “male” type; in females these fingers are usually the same length) and the risk of knee OA (Zhang et al., 2008, Robertson, 2008).

1.4.7 Genetic

So far, the identified risk factors do not fully explain an individual’s risk of developing OA and the progression of the disease (Sharma, 2016). The genetic contribution to OA has been supported by many studies, and it is thought that at least 30% of the risk of OA is genetically determined (Spector et al., 1996, Dieppe and Lohmander, 2005, Valdes et al., 2010) (see section 3.2 The genetics of osteoarthritis).

1.5 Management

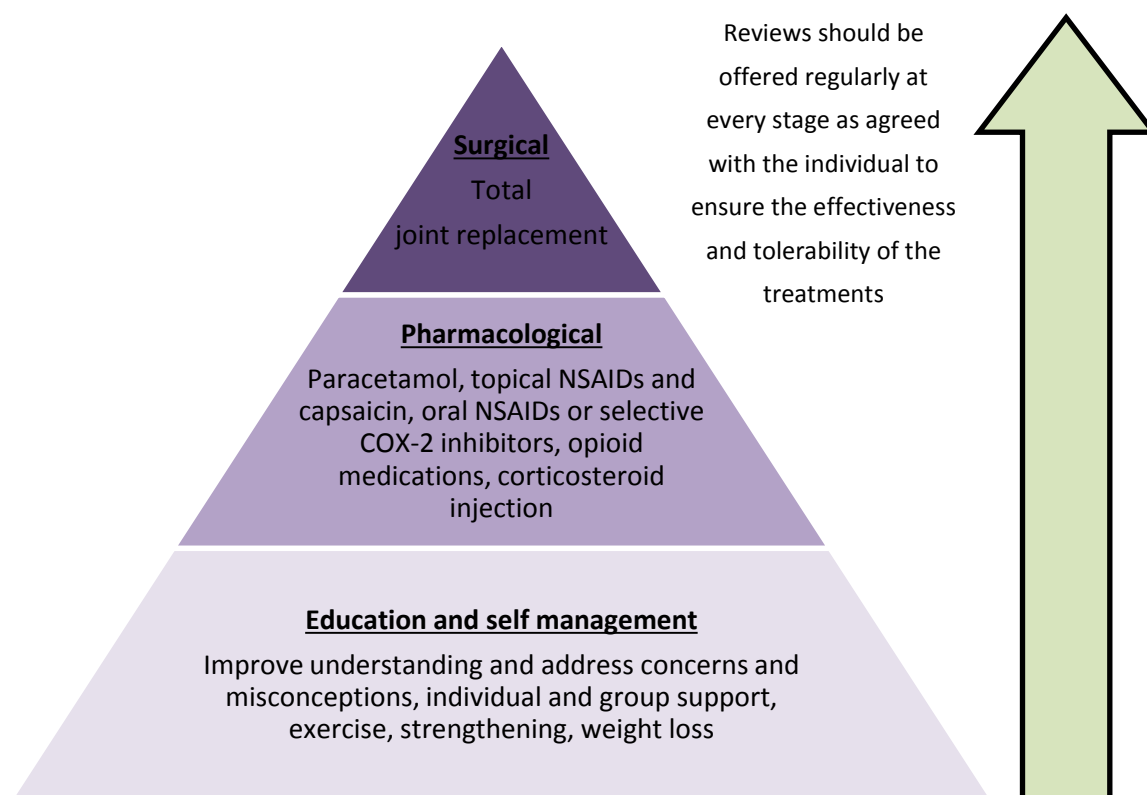
1.5.1 Non-pharmacological

Physical activity has been linked to improvements in health outcomes and quality of life (Spirduso and Cronin, 2001). A dose response effect of PA on quality of life has been seen in older adults, both physically and mentally (Spirduso and Cronin, 2001). The National Institute for Health and Care Excellence (NICE) recommends exercise as a core self-management approach to treat the symptoms of knee OA (NICE, 2014) (see **Figure 5** The hierarchy of treatment options for osteoarthritis as recommended by the National Centre for Health and Care and Excellence (NICE, 2014)).

1.5.2 Pharmacological

Topical non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are the first line of treatment for painful OA (NICE, 2015). Paracetamol, oral NSAIDs such as diclofenac and opioid analgesic medications are also commonly used to treat the pain and inflammation associated with OA (NICE, 2015). A schematic of the currently available and recommended therapies and tiers of treatment for OA can be seen in **Figure 5** The hierarchy of treatment options for osteoarthritis as recommended by the National Centre for Health and Care and Excellence (NICE, 2014).

Figure 5 The hierarchy of treatment options for osteoarthritis as recommended by the National Centre for Health and Care and Excellence (NICE, 2014)



The high prevalence of OA and the pain associated with the condition means there is a high economic cost to managing the disease. In one study, in terms of NSAIDs and analgesics, there was a cost of \$79 per person-year for NSAIDs use in OA, and 4,320 people in the sample studied are taking them (Lanes et al., 1997). A further 2,304 people were taking analgesics, at a cost of £13 per person-year. This was the same cost per person-year as for orthopaedic surgery for OA in the sample (n=140) (Lanes et al., 1997). However, after surgery, there was a decrease in medication costs in this sample of OA patients (Lanes et al., 1997), which supports the use of total joint replacement as a long-term treatment for OA. Medication use has been found to be high in OA patients. One study found that 52% of individuals surveyed were using prescription medications (alone or with OTC medications) for OA (Lawson et al., 2004). This raises issues about possible drug interactions (Lawson et al., 2004), and the possibility of over-medication of individuals.

The side effects of NSAIDs may contraindicate their use in many patients, who as a result may be offered opioid pain medications. This is especially true in older adults, due to an increased prevalence of pre-existing conditions which would make NSAIDs less safe, and a lower prevalence of medication abuse (Becker, 2010). However, this class of medications may have some of the same side effects as NSAIDs. An increased risk of cardiovascular events and other adverse events is associated with NSAID use. These can vary depending on the type of opioid taken, and may actually incur higher risks than those taking NSAIDs. In one study, gastrointestinal bleeding, a well-known side effect of NSAID use, had the same incidence rate in individuals taking NSAIDs or opioids (the incidence rates were 21 (95% CI 14-30) and 21 (95% CI 16-27) for NSAIDs and opioids, respectively) (Solomon, 2010b).

However, opioids may increase risk of fracture, cardiovascular events, and all-cause mortality especially in older adults (Solomon, 2010a, Solomon, 2010b).

This is despite new guidelines which recommend the use of opioids over NSAIDs for older adults with pain (Fauber, 2012). The number of individuals prescribed opioids for long-term use from 2000 to 2005 has increased 35% (Graf, 2010). In one study, people taking NSAIDs actually showed a lower risk of cardiovascular events than opioid user (Solomon, 2010b).

Currently no successful disease-modifying OA drugs (DMAODs) are available (Guermazi et al., 2013). One of the challenges faced in the development of a DMOAD is that radiographic features of the joint are commonly used to assess inclusion into studies, and there are challenges associated with assessing longitudinal changes using radiographs (Guermazi et al., 2013). Structural changes may be hard to visualise over the short time period seen in a clinical trial. As well as this, changes in weight bearing and knee extension as OA progresses or improves can affect the appearance of radiographs taken from the same individuals at different time points (Guermazi et al., 2013). Changes to joint space width (JSW) is the only imaging biomarker used to assess outcomes radiographically (FDA, EMEA, OARSI) when assessing structural changes during clinical trials (Guermazi et al., 2013, Conaghan et al., 2011). MRI could be a better option for use in future studies, as described in this report (Guermazi et al., 2013).

However it has been shown that not all randomised control trials (RCTs) for DMOADs have had negative outcomes. For example doxycycline slowed JSN in the knee (Brandt et al., 2005). As well as this, the “wrong” mechanisms may be being targeted in the development of DMOADs, for example the large focus on chondrocytes (Guermazi et al., 2013). Development and coordination of the joint and remodelling of joint tissues are also targets which could be utilised in future studies (Guermazi et al., 2013).

To get a licence as a DMOADs, a drug must show evidence for slowing structural progression as well as improving pain and other patient-centred outcomes (Marhardt and Muurahainen, 2015). To date this has not been shown by any drug.

1.5.3 Surgical

Minimally invasive arthroscopic surgical treatments are available such as lavage and/or debridement of the affected joint (Dougados, 2008). These procedures, in theory, remove sources of irritation and pain (Dougados, 2008). However these treatments are no longer recommended to treat OA (NICE, 2014) as they are no better than sham lavage and debridement surgeries (Moseley et al., 2002).

A TJR is currently the only long-term treatment for severe large joint OA. There is often an overall improvement in pain and mobility, not just at the site of the replacement (Kirwan et al., 1994). Some factors have been identified which may potentially affect TJR outcomes. For example, success is higher for total hip replacements (THR) than for total knee replacements (TKR) (Valdes, 2011). Some people who have a TJR still have pain and sometimes severe or worse pain following a TJR for OA (Valdes, 2011, Wylde et al., 2011).

However, people post-TJR may have a lower quality of life than people of the same age who have not had this surgery (Boutron et al., 2003). Complications which can follow TJR surgery include bleeding, infection, stiffness, instability and dislocation (Chu et al., 2003, Ugutmen et al., 2008, Healy et al., 2016).

There are a range of patient factors which can influence the perceived outcome of a TJR, and a technically successful surgery can still mean a patient has pain or mobility issues. An underlying medical condition such as cardiovascular disease or diabetes, obesity, psychological conditions and other factors can potentially complicate surgery and/or recovery (Yu et al., 2015, Lash et al.,

2013). Younger age at the time of surgery has also been identified as a possible risk factor for poorer post-TJR outcomes (Meehan et al., 2014). The time point at which post-TJR patients are surveyed may also influence satisfaction, as some studies have shown improvements in pain and mobility up to two years after surgery (Kirwan et al., 1994). Genetic factors are also likely to be involved, as some people feel no improvement in pain and/or function after a TJR, even if other parameters are matched between individuals, and not all post-TJR outcome variation can be explained by the above factors (Valdes, 2011).

1.5.4 Assessing post-TJR outcomes

There are issues when assessing the success and outcomes of any medical procedure or treatment, including a TJR. For example, there may be differences in opinion between the physician and the patient as to the definition of a successful outcome (Janse et al., 2004, Woolhead et al., 2005). If a TJR is technically a success, the surgeon will be satisfied but in practice, the patient may still have pain and/or mobility issues (Woolhead et al., 2005). It may also be possible that a patient may not feel comfortable or not know how to raise concerns about surgery outcomes (Woolhead et al., 2005). It is therefore important to manage patient expectations when discussing TJR as an option (see below) (Woolhead et al., 2005). Patient expectations need to be managed so they are realistic and in line with the surgeon's goals for the surgery (Mancuso et al., 2008). Outcomes are more favourable if the expectations of the patient are met (Hamilton et al., 2013), with patient expectations about the consequences of the surgery being a predictor of TJR satisfaction (Mahomed et al., 2002).

Patient satisfaction can be measured in several ways. Outcome measures are important to assess the success or failure of surgery (Suk et al., 2008). Several

outcome measures are used to assess TJR outcomes, but there is no “gold standard” outcome measure to assess the impact of TJR (Halawi, 2015). Some examples of these measures are Patient Reported Outcome Measures (PROMs). There are a range of outcome measures used (for example, WOMAC scores, other questions about pain and function after TJR, questions about prosthesis failure and revision surgery), so comparisons between studies are more difficult, and therefore the data on surgery outcomes may be unclear (Woolhead et al., 2005). As well as this, there can be differences between the types of outcome reported by physicians versus patients (Halawi, 2015). Potential issues with PROMS to collect data post-TJR include a lack of internal validity and reliability, poor response rates and costs (Olver, 2011, Black, 2013).

Understanding the outcomes of the surgery and managing patient expectations is a key consideration (Mahomed et al., 2002). Demographic factors are not necessarily linked with patient expectations of surgery outcomes (Mahomed et al., 2002). The same is true for pre-operative function (Mahomed et al., 2002). Having high expectations was, in this study, a predictor of better surgical outcomes, such as a large improvement in function, and pain relief (Mahomed et al., 2002). Pre-operative function was not linked to patient expectations (Mahomed et al., 2002).

A qualitative study which interviewed 33 patients post-surgery and then four to eight weeks later concluded that patients need to discuss expectations and be educated about aspects of the surgery and care, and that a holistic approach may benefit patients (Fielden et al., 2003). A similar study undertaken in Japan supports these findings (Fujita et al., 2006). 17 OA patients who underwent TKR surgery were interviewed before and after surgery (Fujita et al., 2006). This included before and after discharge from the hospital post-surgery (Fujita et al., 2006). Patients reported big improvements in pain and mobility but

further questioning in an interview setting revealed issues (Fujita et al., 2006). The results of this study suggest that when preparing a patient for TKR their resources and pain should be accounted for at each stage (Fujita et al., 2006).

As mentioned above expectations are a major determinant of TKR satisfaction (Noble et al., 2006) as opposed to levels of function post-surgery, according to one study (Noble et al., 2006). Pre-operative ideas of the benefits and results of the surgery need to be discussed with patients (Noble et al., 2006).

As well as measuring pain levels post-TJR, including NP, pain medication use after surgery can help gauge satisfaction. Individuals who suffer from chronic pain post-TJR may be taking analgesic medications, so studying the determinants of this may offer information about what factors are associated with TJR outcomes. One study reported that 25.1% of individuals post-TJR were taking an opioid analgesic, which was strongly associated with joint pain and other measures of pain (Valdes et al., 2015). Medication use is a relevant aspect of OA management to study, including post-TJR, as it carries a risk of side effects, and an economic cost to healthcare providers (Fuzier et al., 2014).

1.5.5 Comorbidities in osteoarthritis

Comorbid conditions are commonly seen in people with OA, and people with OA are more likely to develop comorbid conditions (Gabriel et al., 1999, Stang et al., 2006). In one study, 81% of people with arthritis reported at least one comorbid condition (Stang et al., 2006). The pain and loss of function that is associated with OA can lead to a decrease in physical activity levels and therefore increase the risk of comorbidities developing (Fernandes, 2015). Functional limitations, mental health conditions such as depression and cardiovascular disease (CVD) have been reported as common comorbidities in

people with OA (Dunlop et al., 2004, Stang et al., 2006). With regards to CVD, there are thought to be some mechanisms common to both OA and CVD, such as those relating to inflammation (Fernandes, 2015).

As well as depression, anxiety and pain catastrophizing can be seen in people with OA. Pain catastrophizing describes the tendency to magnify or exaggerate the threat value or seriousness of pain sensations (Chaves and Brown, 1987). Pain catastrophizing has been found to be important in the understanding of pain in OA (Somers et al., 2009). Pain catastrophizing is also correlated with a higher use of prescription analgesic medication use post-TJR (Valdes et al., 2015).

2 Neuropathic pain

2.1 Introduction

Neuropathic pain (NP) is currently defined by the International Association for the Study of Pain as “pain caused by a lesion or disease of the somatosensory system” (Jensen et al., 2011). This type of pain differs from nociceptive pain, which is felt in response to an appropriately painful stimulus. Symptoms of NP can include burning and prickling sensations, a loss of sensation, feeling pain in the absence of a harmful stimulus (allodynia) or an increased sensitivity to painful stimuli (hyperalgesia) (Kehlet, 2006). The causes of NP can be primary or secondary (Attal, 2012). Primary NP is found at the site of the injury or insult and mediated by the sensitisation of primary nociceptors (Campbell and Meyer, 2006). Secondary NP is found in the uninjured tissue surrounding the site of the injury, and result from central nervous system sensitisation (Campbell and Meyer, 2006). Secondary NP can result from conditions which are risk factors for acquired NP, such as diabetic neuropathy, neuropathies

caused by cancer, alcohol-related neuropathies, HIV-related neuropathies, degenerative disk diseases, complex regional pain syndrome (type 2), phantom limb pain (all peripheral), and multiple sclerosis, post-stroke pain, spinal cord injury, brain injury, syringomyelia and genetic disorders (all central) (Attal, 2012). NP is an issue in OA patients (Valdes, 2013c). One study found that 19% reported NP symptoms (Hochman, 2011). Although this figure is lower in post-total knee replacement (TKR) patients, NP can be an ongoing concern for some of these individuals. Knee pain is highly prevalent and the knee is a common site of NP in OA patients (Valdes, 2013c, Hochman, 2011).

There are different types of insult the nervous system can experience such as inflammatory and mechanical (Attal, 2012), which results in a large variation in NP presentation (Attal, 2012). Biologically, NP has both positive and negative aspects (Haanpää, 2012, Thakur et al., 2014). Positive aspects include feeling pain from a usually harmless stimulus (allodynia) and an amplified response to a painful stimulus (hyperalgesia) (Haanpää, 2012, Woolf and Thompson, 1991). Negative aspects include altered sensitivity thresholds e.g. temperature and touch (Haanpää, 2012).

Pain is a complex and variable experience involving the somatosensory system. This system is formed of the structures responsible for detecting sensory information (Haanpää, 2012). Different stimuli (inputs) will provoke a different response and lead to the sensation of different types of pain. For example, group C nerve fibres relay impulses from the peripheral nervous system to the central nervous system (Purves, 2004). These small fibres carry impulses relatively slowly and are responsible for slow, burning pain sensations (Purves, 2004). In contrast, A delta fibres respond to cold and pressure stimuli and are faster than group C fibres (Beissner et al., 2010, Voscopoulos and Lema, 2010). The hyperalgesia seen in NP results from and is maintained by central sensitisation due to increased excitability of C fibres

(Baron, 2006). A fibre nociceptors are also thought to have a role in secondary hyperalgesia (increased pain sensitivity away from the site of the original damage/insult) as a result of central sensitisation to A fibre signals in the central nervous system (Ziegler et al., 1999).

Central sensitisation involves changes in the synapses of the central nervous system and can lead to NP (Dimitroulas, 2014, Thakur et al., 2014).

Neurovascular invasion can be a cause of central sensitisation in OA (Thakur et al., 2014). Gene expression and excitability thresholds of central nervous system neurons may be altered in central sensitisation states (Dimitroulas, 2014) and pain perception can be affected as a result of this (Dimitroulas, 2014, Woolf, 1983). For example, sensitisation (increased action potential discharge from synapses) of neurons in the dorsal horn of the spinal cord can lead to pain hypersensitivity (Salter, 2012, Woolf, 2006). Sensitisation of dorsal horn neurons can also be caused by input from peripheral nociceptors, as in peripheral sensitisation (Salter, 2012). The dorsal horn is a processing network which is the somatosensory gateway in the CNS (Salter, 2012, Woolf, 2006, Woolf and Salter, 2000). Additionally, the strength and efficiency of these synapses (how action potentials are produced from synaptic input) can also be affected in central sensitisation (Salter, 2012). Central sensitisation is a prolonged state of hyperexcitability in the peripheral or central nervous system, or both (Woolf). This is relevant to the study of NP as the changes or damage to the nervous system which result in NP can lead to central sensitisation and the development of a chronic pain state (Zambreanu et al., 2005). Changes to these mechanisms can be short-term and then may be more easily reversible, or they may develop over a longer period and remain in place for longer (Salter, 2012).

Peripheral sensitisation involves changes in sensitivity to the peripheral ends of pain-detecting neurons (nociceptors) in the peripheral nervous system (Woolf).

(Woolf). These neurons transmit impulses from peripheral sites to the central nervous system (Woolf). Hyperexcitability is a major cause of peripheral NP. This can occur in dorsal root sensory neurons and at the site of peripheral nerve damage (Campbell and Meyer, 2006, Costigan, 2009). It is now recognised that the hyperalgesia and referred pain seen in some individuals with OA is not solely due to peripheral changes, and that a central processing component is involved (Gwilym, 2009).

It has also been noted that even in nociceptive pain conditions there can be alterations to central nervous system mechanisms, as seen in NP (Clauw and McCarberg, 2012). The potential use of the same medications to effectively treat both nociceptive and neuropathic pain phenotypes highlights the overlap between these two pain states (see **Figure 7** Flow chart outlining some of the possible pharmacological treatment pathways for NP. Adapted from (Chong and Bajwa, 2003), who reproduced a diagram by (Orza, 2000) with further information from (Finnerup, 2015) **and** (Clauw and McCarberg, 2012).

2.2 Diagnosis

2.2.1 Questionnaire assessment

Several questionnaires are available to classify individuals as having possible signs of NP and identify those in need of further investigation (Bouhassira, 2011). The painDETECT questionnaire (PDQ) is one of several validated instruments for neuropathic pain-like symptoms (NP) (Freynhagen, 2006). The PDQ is used to identify individuals with possible and likely NP based on descriptions of the pain experienced (e.g. “burning”, “tingling”, “numbness”) (Freynhagen, 2006). This questionnaire was originally developed for use in lower back pain but has been validated for use in OA (Freynhagen, 2006,

Hochman, 2011). The PDQ has also been used for numerous other conditions which can have a neuropathic component, such as fibromyalgia and RA (Freynhagen et al., 2016).

Other validated questionnaires include the Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) and the “Doleur Neuropathique en 4 questions” (DN4) tools (Bennett et al., 2005, Bouhassira, 2008, Bouhassira et al., 2005). These two tools include a sensory examination (see **Section 2.2.2 Clinical assessment**). The Neuropathic Pain Questionnaire (NPQ) and ID Pain questionnaire resemble the PDQ in that there is no accompanying sensory examination (Krause and Backonja, 2003, Portenoy, 2006).

2.2.2 Clinical assessment

When confirming NP clinically, there needs to be a consistent abnormal response. The nature of the sensitisation also needs to be determined (i.e. if it is peripheral or central). A more specific idea of where the lesion is in the nervous system also needs to be investigated (Haanpää, 2012).

There are three stages of NP testing (Haanpää, 2012):

1. Clinical/”bedside” tests
2. Qualitative sensory testing (QST)
3. Lab-based tests

The following is an overview of some of the clinical, or “bedside” tests which can be done to investigate NP-like symptoms (Haanpää, 2012):

1. Touch –cotton wool, von Frey, sensory evoked potentials (SEPs)/nerve conduction
2. Vibration – tuning fork, a more sophisticated fork, SEPs/nerve conduction

3. Pinprick – cocktail stick, weighted needles, SEPs/nerve conduction
4. Pressure – fingertip, pressure algometer (quantitative sensory testing)
5. Temperature – cold/warm objects, thermotester, laser-evoked potentials

There are tests and observations which can be used to differentiate between a peripheral and central lesion (Haanpää, 2012). Large areas of hypersensitivity can indicate central sensitisation (Haanpää, 2012). Brush tests for altered sensitivity can identify areas where a response is abnormal and the size of this area and how many areas of the body are affected is useful diagnostically (Haanpää, 2012). Summation and hyperpathia (i.e. “wind up”) suggest a central lesion, or central sensitisation as the result of a peripheral lesion (Haanpää, 2012). “Wind up” is caused by repetitive stimulation, which leads to increased action potential discharge, which in turn leads to increased spinal cord neuron excitability (Woolf and Thompson, 1991).

2.2.3 MRI and CT scans

MRI can be used to detect central lesions (Haanpää, 2012). Computerised tomography (CT) scans can also be used for this purpose, but this is less common (Haanpää, 2012). However it must be noted that issues detected by MRI do not necessarily mean that the individual will experience any symptoms of NP. There may be no consequences of the lesions detected by these methods (Haanpää, 2012).

2.2.4 Other tests

SEPs can be used to detect issues in the central nervous system by measuring the response to an electrical stimulus (Gugino and Chabot, 1990). SEPs identify abnormal nerve conduction (Andrew et al., 2015), and may be able to detect changes over time, by acting as a marker of neural plasticity (Andrew et al., 2015).

Similarly, laser-evoked potentials (LEPs) are a way to further the results found by SEPs. A wider range of the somatosensory system can be assessed using LEPs than SEPs. However, the location of a lesion still cannot be ascertained using this method, and increased sensitivity (e.g. hyperalgesia) is hard to detect (Treede et al., 2003).

2.2.5 Difficulties with diagnosing neuropathic pain

A further complication in the diagnosis of NP is that changes to the nervous system may not be seen for some time after the initiating insult (Seminowicz et al., 2009). These changes include changes in volume of brain areas such as the anterior cingulate cortex and primary somatosensory cortex. As well as these visible changes functional changes are seen in chronic NP patients too, as well as in the thalamus and insula (Becerra et al., 2006, Maihöfner et al., 2006, Peyron et al., 2004, Witting et al., 2006). As a result pain can develop into a maladaptive, long-term condition (Hubbard et al., 2015).

However, the original insult or injury may not lead to NP. The complex aetiology of NP makes the link between the causes, mechanisms and symptoms unclear (Woolf and Mannion, 1999).

2.3 Animal models

Animal models allow the aetiology and causes of a condition to be controlled, and can improve the understanding of many processes. For example, surgical repair may be an option after nerve injury and damage (Verdú et al., 1995) but the nature of the injury causing the symptoms may not be clear in patients with NP due to the range of causes and contributing factors which may have led to this condition. Functional MRI (fMRI) and clinical tests are required to assess nerve damage as the exact cause may not be known (Haanpää, 2012). This is in

contrast to animal models of nerve injury, in which nerve damage is induced in a precise and known manner to aid observations. It is therefore not as straightforward to identify the cause and location of the lesion in human participants as animal models and equally it may not be as straightforward when considering the most appropriate treatment options.

2.4 Risk factors

2.4.1 Age

There is an attenuation of neural function with increasing age in both the central and peripheral nervous systems (Verdú et al., 1995). Additionally regeneration and re-innervation may be delayed and less effective over time (Verdú et al., 1995). However, age is not the only factor which predicts recovering from neural injury (Verdú et al., 1995). The nature of the injury will also affect the ability of the nerves to repair themselves (Verdú et al., 1995).

Some studies report that NP is more prevalent in older adults (Novak, 1999, Werhagen, 2004, Butler, 1979) due to changes in pain processing as a result of ageing of the nervous system (Novak, 1999). Previous work and published literature suggests that age is a risk factor for the development of NP. For example a study by Novak et al. (1999) showed that in older mice, damaged peripheral nerves were slower to regenerate and recover and that even in the control (uninjured) group there was less regeneration with increasing age (Novak, 1999, Verdú et al., 1995). This is not thought to be due to changes in the number of neurons in the dorsal horn but rather an age-related loss of regenerative potential (Bergman and Ulfhake, 1998). Therefore hyperalgesia as seen in NP is more likely (Novak, 1999). This is supported by the results of a study which reported a higher incidence of NP after spinal nerve injury in older adults versus younger adults (Werhagen, 2004).

In contrast to these findings Chung et al. (1995) found that in a study of rats with induced NP-related behaviours were seen more clearly in younger rats than in mature and older rats (Chung et al., 1995). Additionally, younger age at surgery was associated with a higher risk of persistent post-surgical pain NP

A nationwide survey reported the highest prevalence of NP in those of middle age (50-64 years) (Bouhassira, 2008). Another study found that half of all participants reporting chronic NP were aged 65 or older (McDermott et al., 2006). This is in part because the incidence of conditions which can lead to NP, such as diabetic neuropathy, post-herpetic neuralgia, and lower back pain, also increases with age (Schmader et al., 2010, Cummins et al., 2006, Schmader et al., 2008, Schmader, 2002).

2.4.2 Sex

Studies of NP have reported a higher prevalence in women (Toth, 2009) and sex differences could potentially be important in the treatment of NP (Vacca et al., 2014). One reason for this could be the effects of female sex hormones on the glial cells of the immune system, which are involved in the development and maintenance of NP (Svensson and Brodin, 2010, Krishna Temburni and Jacob, 2001).

2.4.3 Body mass index

Research in the literature suggests that a higher BMI is linked with a higher likelihood of NP (Momi et al., 2015). This could be due to the inflammatory nature of obesity (Jordan, 2008, Das, 2001).

2.4.4 Past surgery

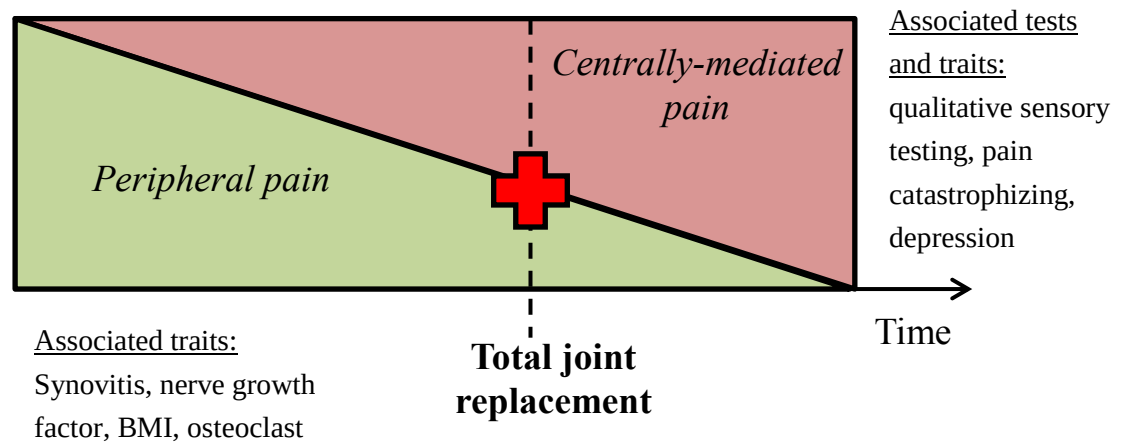
It has been reported that in people with OA a history of arthroscopic knee surgery was associated with higher risk of NP (Valdes, 2013c). This could be due to nerve damage during surgical procedures.

2.4.5 Pre-existing conditions

NP symptoms can result from central sensitisation. A number of chronic conditions such as fibromyalgia (FM), chronic fatigue syndrome (CFS), and irritable bowel syndrome (IBS) show overlapping components and all involve levels of central sensitisation (Aaron, 2000). As well as this NP can be caused by complications from herpes zoster infection and diabetes (Attal, 2012, Jensen, 2001). NP is also commonly seen alongside depression (Jensen, 2006). Due to the emotional aspects of pain (Parks et al., 2011) individuals with a history of anxiety and depression are also at a higher risk of pain including NP (Gore et al., 2011, Pavlin et al., 2005). Chronic pain, as seen in OA, is another potential cause of NP due to changes in the nervous system caused by long-term nociceptive input (Graven-Nielsen et al., 2012). Damage to peripheral somatosensory nerves is also a possibility in OA which can lead to central sensitisation and the development of NP (see **Figure 6** Schematic showing the transition which can occur in OA pain between nociceptive or peripheral pain to centrally-mediated pain, as seen in neuropathic pain-like states and (Thakur et al., 2014)).

There are some common risk factors for both OA and NP such as age, female sex, past joint surgery, and psychological factors (Kehlet, 2006, Hochman, 2011, Freynhagen, 2006, Novak, 1999, Werhagen, 2004, Butler, 1979).

Figure 6 Schematic showing the transition which can occur in OA pain between nociceptive or peripheral pain to centrally-mediated pain, as seen in neuropathic pain-like states



2.5 Management

Several treatment avenues are available for NP. However, the currently available treatments often lack efficacy in a proportion of people with NP (van Hecke, 2014, Torrance et al., 2007, Orza, 2000, Bouhassira, 2011). This can be due to the non-specific nature of the currently available treatments (Mao and Gold, 2011, Vorobeychik et al., 2011).

2.5.1 Non-pharmacological

Physical activity (PA) can be used to improve the symptoms of a range of painful conditions in both adults and children (Spirduso and Cronin, 2001, Sherry et al., 1999, Deyo et al., 1990). PA can improve both pain and function and therefore may lead to decreased costs associated with analgesics as well as improving quality of life. This is in part due to the associated short-term increase in levels of endogenous analgesics such as endogenous opioids (Stagg et al., 2011). Regular exercise may cause upregulation of endogenous opioids, in theory improving pain (Stagg et al., 2011). This suggests that PA may be of benefit to people with NP.

However, the literature shows mixed findings with regards to the use of PA to treat NP (Kuphal et al., 2007). The role of exercise in inflammatory and NP is not clear, and there are few studies of the effect of PA on pain in these conditions (Kuphal et al., 2007). In one study of rats with partial peripheral nerve injury, cold allodynia and thermal hyperalgesia were lower in exercised rats than the control rats (Kuphal et al., 2007). A decrease in behavioural traits associated with hypersensitivity was seen after 18-25 days of 90 mins of swimming per day in 37C water (Kuphal et al., 2007). Another study also used moderate aerobic exercise as a treatment for NP in a rat model (Stagg et al., 2011). During a 3 week period, PA improved hypersensitivity, but this effect did last more than a few days after cessation of the exercise (Stagg et al., 2011). In the periaqueductal grey matter, levels of beta-endorphins and met-enkephalin were higher as a result of exercise (Stagg et al., 2011). This study showed an “exercise-induced reversal of NP” (Stagg et al., 2011). However, so far it is not clear what direct benefits PA could have on NP in people.

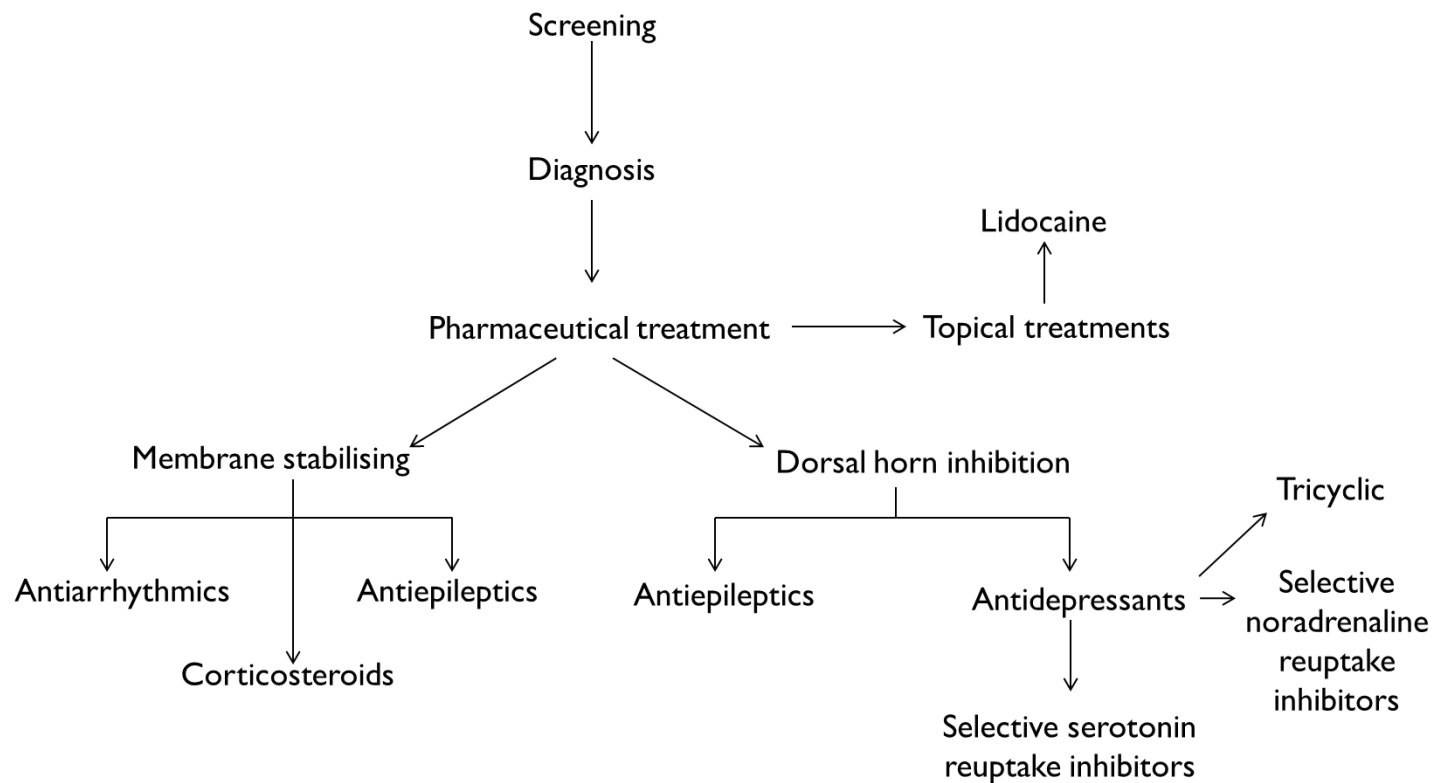
2.5.2 Pharmacological

First-line treatments for NP include tricyclic antidepressants and selective serotonin and noradrenaline re-uptake inhibitors (SSRIs and SNRIs, respectively), as well as gabapentin and pregabalin (see **Figure 7** Flow chart outlining some of the possible pharmacological treatment pathways for NP. Adapted from (Chong and Bajwa, 2003), who reproduced a diagram by (Orza, 2000) with further information from (Finnerup, 2015) and (Finnerup, 2015)). The use of opioid medication to treat NP is recommended as a second-line treatment or for acute NP (Dworkin et al., 2007). Antiepileptics may be used as second- and third-line treatments (Dworkin et al., 2007, Thakur et al., 2014).

For peripheral NP, topical agents such as lidocaine patches are recommended (Finnerup, 2015).

As mentioned above, some treatments for nociceptive pain may also be of use in NP, such as SNRIs (Clauw and McCarberg, 2012). For example, duloxetine is an SNRI and is currently the only treatment for NP in OA to have been examined in a high quality RCT (Chappell et al., 2011). An improvement in pain was seen greater than in the placebo group (Chappell et al., 2011). This was not just because of improvements to mental health and wellbeing (Chappell et al., 2011). Duloxetine has been found to effectively treat chronic pain in other conditions such as diabetic neuropathy, fibromyalgia and lower back pain (Skljarevski et al., 2009, Wernicke et al., 2006, Arnold, 2007, Arnold et al., 2004).

Figure 7 Flow chart outlining some of the possible pharmacological treatment pathways for NP. Adapted from (Chong and Bajwa, 2003), who reproduced a diagram by (Orza, 2000) with further information from (Finnerup, 2015)



2.5.3 Surgical

If self-help and pharmacological treatments for NP have not improved a patient's NP symptoms, there are surgical techniques which may be feasible to treat their pain such as deep brain stimulation (DBS) and motor cortex stimulation (MCS). Neuromodulation such as DBS holds potential for a future treatment of NP (Doan et al., 2015). MCS can give pain relief in 50-75% of cases (Nandi and Aziz, 2004) and should be included as a treatment option for refractory NP (Nguyen et al., 2011). A paper in *Nature Reviews Neurology* discussed that, although findings are mixed, there is sufficient evidence to attest to the use of MCS as a therapy for NP (Nguyen et al., 2011). MCS has advantages over DBS, as it has a wider range of applications and it easier to perform (Nguyen et al., 2011). Controlled trials have been small and need to be confirmed. Also it is not yet clear how to appropriately select patients for this treatment and validated criteria are needed for this (Nguyen et al., 2011).

3 The genetics of osteoarthritis

3.1 Introduction

In recent decades, the technology and techniques behind genetic analysis have developed at an incredibly rapid rate. These developments include, but are not limited to, the developments surrounding the completion of the Human Genome Project (HGP) in 2003 (Collins, 2003). The HGP has uncovered large numbers of polymorphisms which can be used as markers when studying genetic associations (Jorde, 2006). This has made the study of the genetics of OA and other complex diseases easier. Genome-wide association studies (GWAS) use hundreds of thousands of these genetic markers to scan the entire genome, looking for potential associations with a disease phenotype of interest (Jorde, 2006). Markers within genes of interest suggested by the

literature or previous work can also be used to look for associations. This is called the candidate gene approach (Jorde, 2006, Mogil, 2012).

A large proportion of modern genetic research focuses on single nucleotide polymorphisms (SNPs). These variants are responsible for the majority of human genetic differences and are the most common source of genetic variation, with approximately 50 million identified in the human genome (Jorde, 2006). These variants involve a single-letter change at a locus (Jorde, 2006). The genetic locations (loci) where SNPs are located can be in linkage disequilibrium (LD) with each other, meaning that the variants seen at these loci are inherited at a higher incidence than expected purely by chance because they are not randomly associated (Consortium, 2010). So although large numbers of individuals can now be genotyped for many thousands of SNPs using genome-wide arrays such as those made by Affymetrix and Illumina (Neale B.M., 2008), researchers can use the principle of LD to calculate the number of SNPs for which genotype information is needed, in order to represent the variation in the whole genome (Neale B.M., 2008, Consortium, 2010). This means not all loci need to be analysed (Neale B.M., 2008).

Currently, hypothesis-free approaches such as GWAS are considered the best way to identify susceptibility loci for common complex diseases such as OA, due to the small amount of knowledge available so far about the genetic (and therefore molecular) mechanisms involved in OA pathogenesis (Loughlin, 2015). A GWAS uses hundreds of thousands of SNPs to test for an association with a disease or trait of interest (Jorde, 2006). Due to the principle of LD the variation in the entire genome can be represented in the SNPs used in a GWAS. However, a very large sample size (in the region of tens of thousands of individuals) or large genetic effects are needed to reach the genome-wide significance threshold used in GWAS ($p < 5 \times 10^{-8}$). This is to adjust for the huge number of tests run during a GWAS analysis.

As well as GWAS, candidate gene analysis is an approach which can be used to test variants and genes of interest for an association with OA. Candidate gene studies have been responsible for identifying several susceptibility loci for OA. A limitation of this analysis is that *a priori* knowledge is needed about disease aetiology. Another is that only very small regions of the genome can be investigated at a time, meaning that important genes may be overlooked using this method.

3.2 The genetics of osteoarthritis

The genetics of OA have been discussed since at least 1963, when Kellgren and Lawrence hypothesised a heritable component to OA after noticing that relatives of people with OA were more likely to have the disease (Kellgren JH, 1963). As well as this, the estimated heritability of joint pain in classic twin studies using both mono- and dizygotic twins has been found to be 38% for the knee and 38% for the back in one study (Williams et al., 2010). This supports the hypothesis that pain at multiple sites is heritable (Williams et al., 2010). As well as affecting the risk of the incidence of OA, genetic variation may also have an effect on OA progression, for example the genes involved in changes in muscle strength and osteophyte formation may play a part (Williams, 2008). As well as this, cartilage volume and thickness are thought to have a heritable component (Zhai et al., 2004, Castaño Betancourt et al., 2012). It has also been found that after taking age, BMI and other factors into account, the development of osteophytes and JSN have high heritability and so in part may be genetically mediated (Zhai et al., 2007).

Genetic variants implicated in pain sensitivity have been shown to be significantly different between asymptomatic radiographic cases of OA and symptomatic cases. These included amino acid change variants in *COMT*,

SCN9A and *TRPV1* (Diatchenko, 2007, Diatchenko et al., 2005, Phillips, 2013, van Meurs et al., 2009, Neogi et al., 2013, Valdes et al., 2011a, Valdes et al., 2011b). These results suggest that the genetic risk of clinical OA is influenced by the genetic contribution to pain sensitivity. In addition to this, replication analysis of a variant in the *PCSK6/PACE4* gene showed a consistently increased frequency of the minor allele in asymptomatic knee OA cases compared to symptomatic radiographic knee OA cases, and *Pcsk6* knockout mice were significantly protected against pain (Malfait et al., 2012).

The genetic contribution to OA has been supported by many studies (Thakur, 2013) and it is thought that about half of the risk of OA is genetic (Spector et al., 1996, Zeggini, 2012). Unlike some diseases, there is not a single genetic variant responsible for OA. Instead, there are many genes which have been implicated in the incidence and progression of OA, each with a small effect (Valdes, 2013b). The common disease-common variant is not always true for complex diseases with complex aetiologies (Gonzalez, 2013). As well as the numerous intrinsic, extrinsic and systemic factors identified so far in the risk of OA (see **Figure 8** Schematic diagram of the different types of risk factors for osteoarthritis) there may be thousands of loci associated with a complex disease such as OA (Gonzalez, 2013), each with a small effect (Valdes, 2013b). These variants are harder to identify if this is the case (Gonzalez, 2013).

A list of the genetic associations currently reported for large joint OA and related traits derived from GWAS are shown in **Table 3** Genetic associations with large joint OA and related traits derived from genome-wide association scans (GWAS) studies with $p < 1 \times 10^{-7}$.

Figure 8 Schematic diagram of the different types of risk factors for osteoarthritis

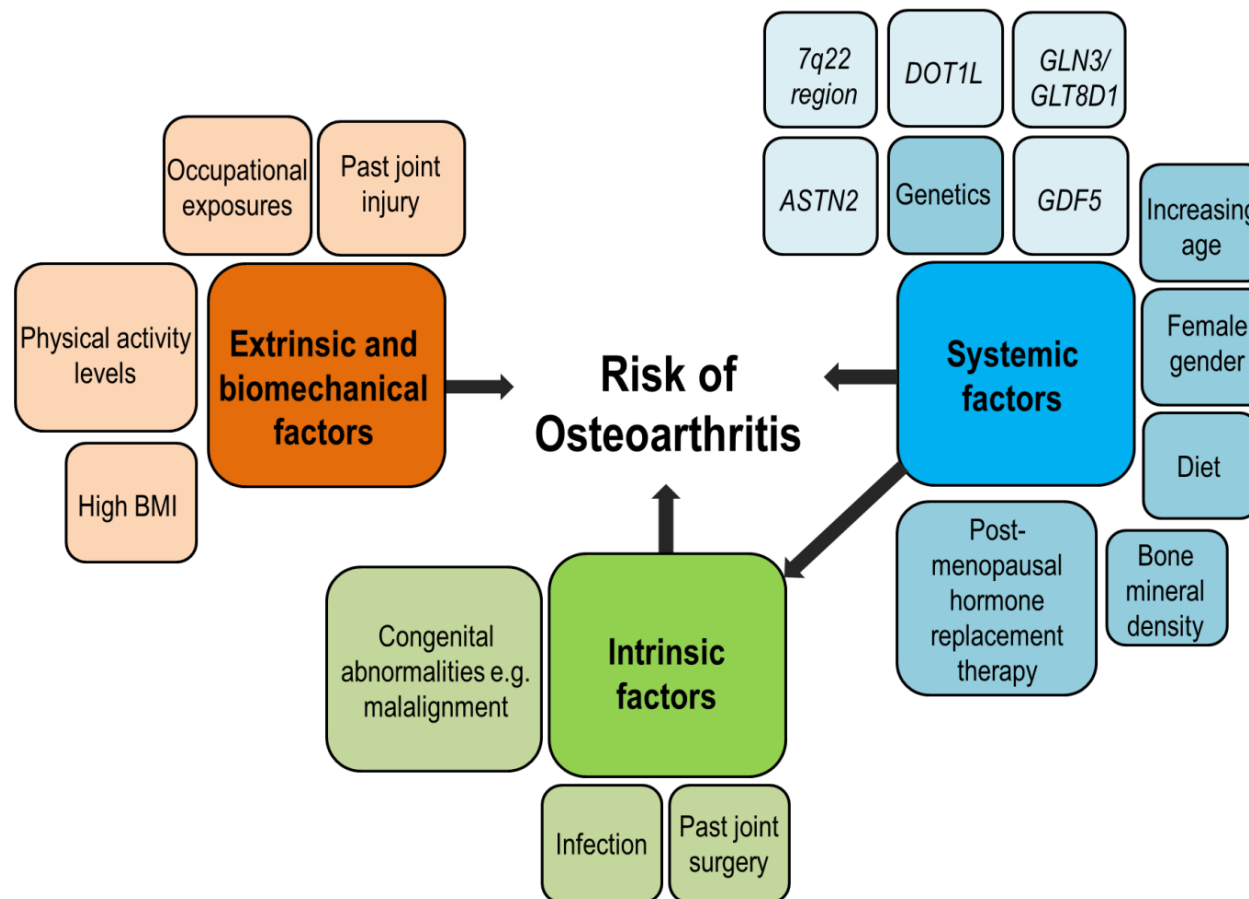


Table 3 Genetic associations with large joint OA and related traits derived from genome-wide association scans (GWAS) studies with $p < 1 \times 10^{-7}$

SNP ID	Gene	Ethnic Group	Trait	Total Sample Size	p value	Putative or Known Function	Ref.
rs11718863	<i>DVWA</i>	Asians	Knee OA	982 cases, 1774 controls	7×10^{-11}	Cartilage-specific tubulin binding	(Yoshinari Miyamoto, 2008)
rs11177/rs6976*	<i>GLN3/GLT8D1</i>	Caucasians	Hip or knee OA	14,883 cases, 53,947 controls	7×10^{-11}	Cell cycle control, tumorigenesis and cellular senescence	(Zeggini, 2012)
rs4836732	<i>ASTN2</i>	Caucasians	Total hip replacement (THR)	5813 cases, 53,947 controls	6.1×10^{-10}	Glial neuronal migration	(Zeggini, 2012)

rs9350591	<i>FILIP1/SENK6</i>	Caucasians	THR	5813 cases, 53,947 controls	2×10^{-9}	Various genes	(Zeggini, 2012)
rs10947262	<i>BTNL2</i>	Asians	Knee OA	906 cases, 3396 controls	5×10^{-9}	Immunomodulatory function, T-cell response	(Nakajima et al., 2010)
rs4730250	<i>COG5/GPR22/D US4L/HBP1</i>	Caucasians	Knee OA	6709 cases, 44,439 controls	9×10^{-9}	Various genes (see (Evangelou et al., 2011))	(Evangelou et al., 2011)
rs11842874	<i>MCF2L</i>	Caucasians	Knee or hip OA	19,041 cases, 24,504 controls	2×10^{-8}	Cell motility	(Day- Williams et al., 2011)
rs10492367	<i>PTHLH</i>	Caucasians	Hip OA	6329 cases, 53,947 controls	1.5×10^{-8}	Chondrogenic regulator	(Zeggini, 2012)

rs835487	<i>CHST11</i>	Caucasians	THR	5813 cases, 53,947 controls	2×10^{-8}	Chondroitin sulfotransferase involved in cartilage metabolism	(Zeggini, 2012)
rs7775228	<i>HLA-DQB1</i>	Asians	Knee OA	906 cases, 3396 controls	2×10^{-8}	Immune response (antigen presentation)	(Nakajima et al., 2010)
rs12107036	<i>TP63</i>	Caucasians	Total knee replacement (TKR) in women	4085 cases, 33,587 controls	7×10^{-8}	Member of the p53 family of transcription factors	(Zeggini, 2012)
rs8044769	<i>Fat mass and obesity- associated (FTO)</i>	Caucasians	TKR in women	4085 cases, 33,587 controls	7×10^{-8}	Control of energy homeostasis	(Zeggini, 2012)
rs10948172	<i>SUPT3H/RUNX2</i>	Caucasians	OA (hip or knee) in men	5617 cases, 20,360 controls	8×10^{-8}	Probable transcriptional activator	(Zeggini, 2012)

rs6094710	<i>NCOA3</i>	Caucasians	Hip OA	11,277 cases, 67,473 controls	7.9×10^{-9}	Nuclear receptor	(Evangelou et al., 2014)
rs788748	<i>IGFBP3</i>	Caucasians	Hip OA	3243 cases, 6891 controls	2×10^{-8}	Cartilage catabolism and osteogenic differentiation	(Evans et al., 2014)
rs12982744	<i>DOT1L</i>	Caucasians	Hip OA	9789 cases, 31,873 controls	8.1×10^{-8}	Wnt signalling	(Evangelou et al., 2013)

*these SNPs are in perfect linkage disequilibrium with each other

4 The genetics of pain and neuropathic pain

4.1 Introduction

Genetics are thought to be responsible for a large proportion of the pain experienced in a variety of chronic pain conditions and the perception of pain (Nielsen et al., 2012, Melzack, 2008). The development of pain has also been found to vary between individuals (Mogil, 2012). In individuals who report chronic pain, there is considerable variation in its severity and response to treatment (Mogil, 2012). It is important to remember when studying genetics that gene and environment interactions also need to be considered (Mogil, 2012).

With regards to the development of chronic pain, an original insult which leads to injury and acute pain is required, as well as other factors such as genetic factors which affect susceptibility to chronic pain (Mogil, 2012). The risk of NP has been shown to be heritable in rodent models (Kehlet, 2006) and as early as 1990 a genetic component to NP was hypothesised (Devor and Raber, 1990). However it was unclear then whether this was due to the genetics associated with the disease causing the pain (such as diabetes) or the genetics of the chronic NP itself.

It is exceptionally difficult to assess the heritability of NP in humans (Devor and Raber, 1990). For example, it is hard to find identical nerve lesions in family members or twins (Devor and Raber, 1990). Similarities in pain responses in families are often linked to shared socialisation versus genetic differences in pain perception (Wolff and Langley, 1968, Violon and Giurgea, 1984, Edwards et al., 1985). Diseases which can lead to neuropathy (such as diabetes) are heritable, but not the “excessive pathological response of nerves after injury” (Devor and Raber, 1990). It should also be noted that although some genetic studies have highlighted genes relevant to pain and NP in mice and some of these are also relevant in human models of pain, some of these genes are only seen in mouse models so far. Despite the many uses and potential to aid research of animal models, genes and variants identified in animal models do not necessarily translate to human pain perception

(Diatchenko et al., 2005). Results from the corresponding human studies may be negative, or may not have been carried out yet (Mogil, 2012).

To date only one GWAS has been published on NP, in individuals with diabetic neuropathy. Results from this GWAS identified SNPs in the *GFRA2* and *ZSCAN20* genes as being suggestively associated with the risk of diabetic neuropathy (Meng et al., 2015a, Meng et al., 2015b). Zinc finger proteins are potentially relevant in the treatment of NP (Krishna et al., 2003). Previous GWAS for other pain phenotypes such as migraine and chronic widespread pain (CWP) have identified susceptibility loci relating to genes involved in synaptic plasticity and some types of neuropathy, respectively (Anttila, 2010, Peters et al., 2013). A GWAS has also been published on acute post-surgical pain. Two intergenic SNPs were suggestively associated with level of acute post-surgical pain, but these results lost their significance after correction for multiple comparisons (Kim et al., 2009).

As well as being implicated in pain perception and sensitivity, genetics are thought to be involved in analgesic medication response (Candiotti, 2011). For example, the *OPRM1*, *COMT* and *MC1R* genes have been linked to differences in analgesic medication response, including post-TKR in some studies (Candiotti, 2011, Sia et al., 2008, Rakvåg et al., 2005, Klepstad et al., 2004, Mogil et al., 2005, Chou et al., 2006, Lötsch et al., 2009).

4.2 Pain and neuropathic pain susceptibility genes

The catechol-O-methyl transferase (*COMT*) gene has been widely studied and associated with pain sensitivity and NP in various cohorts for various conditions. This gene is a popular choice in candidate gene research (Mogil, 2012). There is a strong link between pain perception and *COMT* genotype in humans (Diatchenko et al., 2005, Belfer et al., 2013). It has been shown to have three haplotypes for pain sensitivity (Diatchenko, 2007, Diatchenko et al., 2005) which may be related to pain thresholds (Phillips, 2013, Diatchenko et al., 2005). One study identified three pain haplotypes: low, average, and high pain sensitivity (LPS, APS, HPS) (Diatchenko et al., 2005). These findings

may explain 11% of differences in pain sensitivity in this study (Diatchenko et al., 2005). This is supported by other studies through the use of quantitative trait loci and gene expression (Costigan, 2009, Diatchenko, 2007, Flaherty et al., 2003, Mogil et al., 2003). Different *COMT* haplotypes are associated with different *COMT* enzyme activity levels and different pain scores in this study (and by extension, pain sensitivity) (Diatchenko et al., 2005). Furthermore, its effects may vary in symptomatic versus asymptomatic OA cases because the genetics of pain sensitivity can differ depending on whether an OA patient has symptoms or not (Valdes, 2013b). Although the *COMT* gene is not thought to be an OA risk gene, it may have a role in the progression and disease and/or treatment outcomes in OA patients due to its association with NP, which is reported in people with OA and is a potential complication of TJR surgery (Wylde et al., 2011, Hochman, 2011). The *COMT* gene is therefore of interest in pain genetics and pharmacogenomics (Fernandez Robles et al., 2012).

Other SNPs, such as some found in the *MC1R* gene and neuronal cytochrome P4502D6, can also affect pain sensitivity in humans (Diatchenko et al., 2005, Fernandez Robles et al., 2012). The *MC1R* gene could be a potential target of interest as the CTX-II biomarker (a potential marker of cartilage degradation) expressed in cartilage and bone may be linked to *MC1R* gene expression (El Miedany, 2012) The SNPs rs1805007, rs1805008 and rs1805009 in the *MC1R* gene have also been associated with lower pain thresholds (Belfer et al., 2013).

Another gene containing a variant which has been found to be associated with symptomatic knee OA risk (i.e. painful OA) is the *TRPV1* gene (Valdes et al., 2011b). This gene has also been associated with musculoskeletal pain in candidate gene studies of birds and naked mole rats (Mogil, 2012). Changes in *TRPV1* gene expression and genotype have also been linked to NP and changes to pain thresholds (Belfer et al., 2013).

The genes which code for purinergic receptors (ligand-gated ion channels) (Lucae, 2006), activated by ATP (Tsuda, 2009, Varani et al., 2010) have been implicated in chronic, neuropathic, and inflammatory pain (Tsuda, 2009, Varani et al., 2010), and therefore are of interest in the study of poor post-TJR outcomes. There are numerous processes in the body which involve the P2X

receptors (Varani et al., 2010). They are expressed in high amounts in synovial fibroblasts in OA patients in this study (Varani et al., 2010). P2X1 receptors were found to be anti-inflammatory in this study, with P2X3 receptors having pro-inflammatory effects (Varani et al., 2010). Some research shows a decrease or absence of pain hypersensitivity and some inflammatory pain markers in *P2X4* knockout mice (Ulmann, 2010). These findings were previously reported also by Tsuda et al. (2009), who found that acute pain and tissue damage were not significantly different in *P2X4* knockout mice versus wild type, but nerve damage induced pain (such as that seen in NP states) was significantly less, suggesting that altering these receptors could provide relief from NP (Tsuda, 2009). Involvement of these genes in OA pain is documented in the literature (Dray and Read, 2007). The *P2X7* gene has been positively linked to musculoskeletal pain and NP in some candidate gene studies, and negatively associated with NP in others (Mogil, 2012). It has shown an association in both mouse and human models, and more so in males (Mogil, 2012). One study demonstrates a link between chronic pain sensitivity and P2R7 receptor pore formation (Sorge et al., 2012).

Neurons in the dorsal root ganglia can become hyperexcitable as a result of mutations in the *SCN9A* gene for Nav 1.7 (Dib-Hajj et al., 2010). A polymorphism in this gene has been associated with increased pain perception in people with OA (Reimanna et al., 2010). This same gene can also host mutations which lead to congenital insensitivity to pain (Harrer et al., 2014). The mutations which cause hyperexcitability (gain-of-function) were found to be more common in individuals suffering from idiopathic small fibre neuropathy (Faber et al., 2012). One polymorphism of this gene (R1150W) is seen in 15% of the Caucasian population. Generally there are no problems associated with this (Estacion et al., 2009), however it may be linked with a predisposition to pain (Reimann et al., 2010). This polymorphism has been identified as a likely cause of neuropathy in a two-generation twin study (Harrer et al., 2014). Some improvement was found with the use of a sodium channel blocker (Harrer et al., 2014). Nav 1.7 has a role in inflammatory pain but not in NP, according to this mouse study (Nassar et al., 2005). However another study disagrees and suggests there is sodium channel involvement in

NP (Rogers et al., 2006). A recent study confirms that Nav 1.7 and 1.8 are important in OA pain (Rahman and Dickenson, 2015).

The opioid receptor (*OPR*) genes are the target for opioid medications. As mentioned above, these can be used to treat pain post-TJR. There are three forms of receptor: mu (μ), delta (δ), and kappa (κ). The corresponding genes are *OPRM1*, *OPRD1*, and *OPRK1*. Some research has suggested an association between opioid use and genetic variation in these receptors (Devor, 2012). However, these results have been conflicting so far (Devor, 2012).

OPRM1 is a gene of interest in the field of pain and pharmacogenomics (Fernandez Robles et al., 2012). Opioid efficacy may also be affected by *OPRM1* genotype, as well as changes to pain and NP thresholds, including in diabetic neuropathy-caused foot ulcer pain (Belfer et al., 2013). Numerous candidate gene studies have used the *OPRM1* gene in studies of pain (Mogil, 2012).

In one study, on the whole *OPRM1* genotype did not affect pain sensitivity but there were significant effects of genotype within groups (Hastie et al., 2012). *OPRM1* genotypes can occur at different frequencies in different ethnicities (Hastie et al., 2012). Ethnic differences in *OPRM1* genotype in experimental pain sensitivity, may explain some of the inter-individual differences in pain sensitivity (Hastie et al., 2012).

The *OPRD1* gene is also involved in heat pain sensitivity in male mice and humans (heat pain sensitivity) (Belfer et al., 2013, Mogil, 2012).

The *CACNG2* gene has also been shown to be involved in a heritable increased predisposition to NP in mice and humans, although further research in other cohorts and in different types of NP is needed (Nissenbaum et al., 2010). The study in question focused only on women who had had a mastectomy (Nissenbaum et al., 2010). The *CACNG2* gene has also shown associations with epilepsy (Nissenbaum et al., 2010) supporting this gene's link to the central nervous system.

A review by the same author in 2012 supported the role of this gene as being involved in NP, using an integrated approach involving model organisms and translational studies (Nissenbaum, 2012).

The genetics of the major histocompatibility complex, such as the genes in the HLA, have also been linked to the risk of NP after peripheral nerve injury in rat models (Dominguez et al., 2013). An association study was carried out in individuals who had undergone inguinal hernia surgery (Dominguez et al., 2013). A variant in the *DQB1* gene was seen at a significantly higher proportion in patients who were classified as having chronic pain post-surgery than those without persistent pain (Dominguez et al., 2013). The same result was seen in patients after lumbar disc herniation surgery (Dominguez et al., 2013). This demonstrates an effect of HLA haplotype on the risk of chronic pain following these two surgeries (Dominguez et al., 2013). One study found that more than four times the number of positive associations and findings were seen in candidate gene studies for HLA genes than for the other risk genes included in that particular review (Mogil, 2012).

5 Aims and objectives

Understanding OA “remains a formidable challenge” (Li, 2007) due to its varying presentations, complex aetiology and the interactions between genetic and environmental factors. The pain and disability which can result from OA have far-reaching implications at an individual and societal level. Productivity and quality of life may be significantly affected due to the pain and loss of mobility caused by OA, even after TJR (Valdes, 2011).

Little progress on new therapies for OA has been made in the last decade compared to other rheumatological conditions, such as gout, RA and lupus (Gelber, 2015). Treatments for OA are still of only limited effectiveness in terms of addressing the changes caused by the disease (Aury-Landas et al., 2016). Thus there is a significant unmet need for better understanding of the molecular mechanisms underlying the development and progression of OA.

It has been suggested that the focus of OA research is shifting towards research into pain relief, as it previously had a “chondrocentric focus” (McDougall and Linton, 2012, Reynard and Loughlin, 2012). This is logical as pain is the

primary outcome of OA (McDougall and Linton, 2012, Valdes, 2011) and an issue which can remain after TJR.

The outcomes of TJR surgery are potentially influenced by a range of factors such as which joint is replaced and pre-existing comorbidities (Valdes, 2011). Further study is needed to better identify those individuals who will derive the most benefit from this surgery and how comorbidities, if present, should be addressed before surgery. Since TJR is currently the only long-term treatment option for severe OA, if its effectiveness can be improved with better understanding of the individual differences in post-operative outcomes, this must be addressed.

It is not straightforward to predict and diagnose OA as it is a condition with a complex aetiology. A definitive test is not available to predict or diagnose OA. The complex aetiology of OA means more research is needed into the determinants and progression of the disease, and into both new and existing treatments and outcomes.

Some of the risk factors for the development of NP are also risk factors for pain, including NP, in OA (Kehlet, 2006, Hochman, 2011, Freynhagen, 2006, Gore et al., 2011). The complex aetiology of NP makes the investigation, diagnosis and treatment of NP more difficult. At present there have been no significant findings from GWAS on the subject of pain in OA. To date only one GWAS on NP exists and further studies of this kind are needed to improve the understanding of both nociceptive and neuropathic pain in OA.

Studying NP in the context of OA is a relevant research focus due to the recognised neuropathic component of OA pain (Hochman, 2011) both pre- and post-TJR. Furthermore, OA with NP may present with a different aetiology and treatment responses than OA without NP (Thakur et al., 2014). NP is a distressing condition (Gilron, 2006, Cohen, 2014) and there is often a loss of quality of life in NP sufferers. The uncertainties regarding the causes, diagnosis, and treatment of NP highlight its relevance as an important research area. There is a high social and economic cost to NP (Gilron, 2006) so a better understanding of the risks and predictors of NP is important and could potentially help society and individuals on several levels.

Further investigation is required to better understand the mechanisms and molecular pathways involved in NP in OA. The mechanisms of pain in humans are not well understood, and human genetic studies of OA pain and NP offer a non-invasive way to investigate the molecular pathways involved in pain.

Pain, including NP, is associated with poor TJR outcomes, lower patient satisfaction, high patient burden, and medical costs (Kehlet, 2006, Gore et al., 2011) making the study of pain in OA and post-TJR a relevant, important and currently unresolved issue for which treatments and further research are necessary (Kehlet, 2006).

There are gaps in the understanding and study of NP in OA, and this research aims to improve this knowledge. The relationships between poor post-TJR outcomes, pain, and genetics in OA will be researched. This will be approached by considering the epidemiological data regarding pain outcomes post-TJR in the context of genetic data to discover potential associations. Results from this work will be further analysed with a view to understanding the pathways and biological relevance of the findings.

CHAPTER 2: CLINICAL COLLECTION AND ASSESSMENT

2.1 Introduction

The aim of this chapter is to describe the cohorts used for the work in this thesis. To avoid repetition of these clinical collection and assessment methods, this information is described here and more specific methodology is detailed in the relevant chapters where applicable. The precise numbers of participants selected from these cohorts for each analysis is detailed in the relevant chapters.

2.2 Participants

2.2.1 Nottingham Genetics of Osteoarthritis Study (NGOAS)

Baseline assessment: OA cases were selected from lists of patients registered in an orthopaedic clinic for clinically severe OA of the knee or hip, sufficient to warrant consideration for TJR. Some of these individuals were post-TJR.

Individuals from the clinic were contacted by post, then telephone if they agreed to participate in the study and subsequently visited by a research nurse for a clinical assessment and to complete a standardised questionnaire.

Exclusion criteria for this study include: not having OA (i.e. being registered at the orthopaedic clinic for another indication), presence of RA, Paget's disease, avascular necrosis, ankylosing spondylitis, psoriatic arthritis, currently on a waiting list for a TJR or less than four months post-TJR.

Follow-up assessment: Participants who consented to further contact completed the follow-up questionnaire. The average duration between the baseline and follow-up questionnaires was 3.3 years. We compared the demographic characteristics of the individuals in the baseline cohort who did not complete the follow-up questionnaire to those who completed it and found no significant difference for age ($p < 0.38$), gender ($p < 0.89$) and body mass index ($p < 0.07$). For those that had pain measures at baseline no difference was seen on baseline pain scores ($p < 0.51$) between individuals who returned the questionnaire and

those who did not. Data from the baseline questionnaire was used in if the necessary data were not available from the follow-up questionnaire data. This is indicated where applicable in the relevant chapters.

This study was approved by the North Nottinghamshire Research Ethics Committee (REC number: 07/Q2501/22).

2.2.2 Arthritis Research Council Osteoarthritis Genetics (arcOGEN) Consortium

The Arthritis Research Council Osteoarthritis Genetics (arcOGEN) study is part of a larger UK-based consortium funded by Arthritis Research UK (ARUK). 7,410 unrelated individuals with clinically severe knee and hip OA sufficient to warrant TJR were recruited for the purpose of genetic studies on risk factors for OA (Zeggini, 2012, Panoutsopoulou et al., 2011). A large proportion of these individuals required TJR. The exclusion criteria for this study were defined as: TJR required due to a fracture, secondary OA (caused by trauma or a damaging condition such as RA) and developmental, vascular or infective causes of joint disease. 1,511 of these participants were recruited at Academic Rheumatology, Nottingham City Hospital, Nottinghamshire and had nurse assessments performed between 2008 and 2010. A postal questionnaire with pain assessments was sent to those who had given consent and were still alive in 2013, and 693 were returned with a return rate of 62%. 613 of these questionnaires provided sufficient information to be used for the pain analysis and work described in this thesis.

Permission was obtained for the use of these genotype data (<https://www.ebi.ac.uk/ega/studies/EGAS000001001017>)

This study was approved by the North Nottinghamshire Research Ethics Committee REC number: 07/Q2501/22).

2.2.3 Genetics of Osteoarthritis and Lifestyle (GOAL) Study

Baseline assessment (Robertson, 2008, Zhang et al., 2008): The Genetics of Osteoarthritis and Lifestyle (GOAL) study is a Nottinghamshire-based study in collaboration with AstraZeneca UK which studied the genetic and environmental factors of hip (n=1,000) and knee OA (n=1,100). Matched controls with no hip or knee OA are also included in this study (n=1,200). Participants were initially contacted and took part in the study between 2002 and 2006. Individuals with clinically severe hip or knee OA were selected from hospital joint replacement waiting lists and a rheumatology clinic to comprise a group of OA cases. Controls were recruited from lists of people referred to the same hospital for intravenous urography. The control individuals were investigated to confirm they had no symptoms, signs or radiographic changes of OA. Hand, knee and hip X- rays were taken as part of this study. The exclusion criteria for this study were having the following conditions: RA, childhood joint disease, TKR, avascular necrosis at the femoral neck or distal condyle and terminal illness (Zhang et al., 2008).

Follow-up assessment: Follow-up questionnaires were sent in March and April 2015. The follow-up questionnaire included questions on pain, function, revision surgery, and cognitive function. NHS data was used to determine how many individuals had changed address or died between the previous follow-up and this most current follow-up. Cause of death information was not available.

This study was approved by the North Nottinghamshire Research Ethics Committee (REC number: EC02/06).

Copies of the consent forms used for recruitment into the above three studies are presented in **Figures 22-24, Chapter 8 APPENDIX**

2.3 Questionnaire data

Participants from all of the three studies described above answered questionnaires containing validated assessment tools (see **Table 4 A**

description of the main questionnaires used for data collection in these studies). Similar questionnaires were sent to all cohorts at both baseline and follow-up.

2.3.1 Trait Definitions for statistical analysis

2.3.1.1 Kellgren Lawrence grade

As previously described, the K/L composite grading system classifies individuals according to the radiographic severity at the joint of interest. This scale ranges from 0-5 and uses the presence of joint space narrowing, osteophytes, subchondral bone sclerosis and bone contour deformity to assess radiographic severity (Kellgren, 1957). Individuals with a K/L score of ≥ 3 were classified as having severe OA in this research and scores of 2 as having OA. mJSW was also used to classify radiographically severe OA in individuals with hip OA, with a mJSW ≤ 2.5 mm (which is a standard cut-off) (Croft, 1990) being classified as radiographically severe OA. For people who had undergone bilateral TJR surgery the joint with the most severe radiographic score was used.

2.3.1.2 Neuropathic pain-like symptoms

Individuals were categorised as having possible or likely NP based on the results of a joint-specific version of the PDQ (see **Table 4** A description of the main questionnaires used for data collection in these studies) which was applied to the OA-affected or replaced joint site (Freynhagen, 2006). Participants used this questionnaire to describe their symptoms at the joints of interest (the knee and/or hip), not the whole body. The PDQ gives more reliable results when used for one specific site (Freynhagen, 2006, Bouhassira, 2011) as in these cohorts.

2.3.1.3 WOMAC pain, stiffness and function

The lower limb OA-specific Western Ontario and McMaster Universities Arthritis Index questionnaire (WOMAC; see **Table 4** A description of the main questionnaires used for data collection in these studies) includes questions

about knee or hip OA-related pain, stiffness and function (Bellamy et al., 1988).

2.3.1.4 Pain severity

A visual analogue scale was used to assess pain severity at the OA-affected or replaced joint (VAS; see **Table 4** A description of the main questionnaires used for data collection in these studies). Score were dichotomised, with scores of 6 or higher representing high pain intensity and scores of 5 or lower representing low pain intensity (Reginster, 2001).

2.3.1.5 Chronic widespread pain

Two line-drawing body mannequins (front and back) were used by participants to indicate areas of pain experienced for one day or longer during the past four weeks. The presence of back pain and chronic widespread body pain (CWP; pain in the upper right, lower right, upper left and lower left quadrants of the body) were determined from these diagrams.

2.3.1.6 Pain catastrophizing

The pain catastrophizing scale (PCS; see **Table 4** A description of the main questionnaires used for data collection in these studies) is used to measure pain catastrophizing in clinical and non-clinical cohorts (Sullivan et al., 1995). Individuals in the top tertile of the PCS scores distribution were classified as high catastrophizers. This questionnaire contains questions about how able participants feel they are to cope with the pain they experience (Sullivan et al., 1995, Campbell, 2009). For example, a person may feel that their pain is overwhelming and that there is nothing they can do to improve it.

2.3.1.7 Body mass index

Participants were classified as overweight if their BMI was >25.00 and obese if their BMI was >30.00, as per the World Health Organisation (WHO) recommendations.

2.3.1.8 Comorbidities and pre-existing conditions

A list of comorbidities of interest was included in every questionnaire relevant to this research. Participants were required to indicate which, if any, of these

conditions they had been previously diagnosed with by a doctor. The rationale for including some of these comorbidities is as follows:

2.3.1.8.1 Cardiovascular disease

This definition included hypertension, coronary heart disease (angina, heart attack), congestive heart failure and congenital heart disease. CVD is a common comorbidity seen in OA patients and there are thought to be some mechanisms common to both conditions (Fernandes, 2015).

2.3.1.8.2 Diabetes

Diabetes was included because peripheral neuropathy is experienced by up to a half of people with diabetes within 10 years of onset (Sun et al., 2005) making it relevant in the context of studying NP.

2.3.1.8.3 Chronic fatigue syndrome, fibromyalgia and IBS

These conditions are thought to have some overlapping components as they all involve levels of central sensitisation (Aaron, 2000), making it relevant to the study of NP.

2.3.1.8.4 Depression

Depression is a common comorbidity in both people with OA and NP (Jensen, 2006, Nicholson and Verma, 2004, Stang et al., 2006).

2.3.1.9 Medication use

Medication use was classified based on questionnaire responses. Participants were classified as over-the-counter (OTC) analgesics, opioids, NSAIDs or other prescription medications which can be used to treat pain using the lists below. This was based on participants' medication use questionnaire responses.

2.3.1.9.1 OTC analgesics

Ibuprofen, aspirin and paracetamol.

2.3.1.9.2 Opioids

Buprenorphine, co-codamol, codeine, co-dydramol, co-proxamol, dihydrocodeine, fentanyl, morphine, nurofen plus (ibuprofen and codeine phosphate), oramorph, oxycodone, tramadol, transtec patch (buprenorphine).

2.3.1.9.3 NSAIDs

Arthrotec, celebrex, diclofenac, meloxicam, naproxen, and piroxicam. None of the patients reported taking other prescription NSAIDs such as indomethacin, nabumetone, etodolac, and tenoxicam. This list includes prescription NSAIDs only. The dose information for medications was not specified in the questionnaire. Although ibuprofen is available on prescription in the UK in higher doses than over the counter, ibuprofen was included in the OTC category as this is its most common indication.

2.3.1.9.4 Other prescription medication which can be used to treat pain (directly or indirectly)

Acupan, amitriptyline, citalopram, citalopram hydrobromide, diazepam, dosulepin, duloxetine, fluoxetine, gabapentin, mirtazapine, nefopam, paroxetine, prozac, pregabalin, sertraline, temazepam, valium, venlafaxine.

Table 4 A description of the main questionnaires used for data collection in these studies

Title	Outcome measure	Type of questionnaire	Range of scores	Validated for use in OA	Validated cut-off point for scoring available	References	Relevant Groups
painDETECT (PDQ)	Neuropathic pain-like symptoms at the joint in question	Likert scale, seven questions	0-39	Yes	Yes	(Freynhagen, 2006)	NGOAS baseline, NGOAS follow-up, arcOGEN, GOAL baseline, GOAL follow-up
Western Ontario and McMaster Universities Arthritis Index (WOMAC)	Pain relating to OA	Likert scale, five questions	0-20	Yes	Yes	(Bellamy, 2012, Bellamy et al., 1988)	NGOAS baseline, NGOAS follow-up, arcOGEN, GOAL baseline, GOAL follow-up
	Stiffness relating to OA	Likert scale, two questions	0-8	Yes	Yes	(Bellamy, 2012,	NGOAS baseline, NGOAS follow-up, arcOGEN,

						Bellamy et al., 1988)	GOAL baseline, GOAL follow-up
	Function relating to OA	Likert scale, 17 questions	0-68	Yes	Yes	(Bellamy, 2012, Bellamy et al., 1988)	NGOAS baseline, NGOAS follow-up, arcOGEN, GOAL baseline, GOAL follow-up
Visual Analogue Scale (VAS)	Pain intensity at the joint in question over the past month: current, worst, average	A visual scale	0-10	No	Yes	(Hawker et al., 2011)	NGOAS baseline, NGOAS follow-up, arcOGEN, GOAL baseline, GOAL follow-up
Pain Catastrophizing Scale	Pain catastrophizing	Likert scale, 13 questions	0-52	No	No	(Sullivan et al., 1995)	NGOAS follow-up, arcOGEN, GOAL follow-up

CHAPTER 3: NEUROPATHIC PAIN-LIKE SYMPTOMS AND POST-TOTAL JOINT REPLACEMENT SATISFACTION

3.1 Introduction

To our knowledge, currently no studies have investigated the role of neuropathic-like pain on patient satisfaction post-TJR. This is despite NP being recognised as a component of the pain experienced by both people with OA and post-TJR (Hochman, 2011, Wylde et al., 2011). So far the identified risk factors do not fully explain the differences in TJR outcomes (Valdes, 2011).

The aim of this chapter was to investigate the predictive factors for long-term patient dissatisfaction post-TJR in people who had undergone knee or hip replacement for OA. Additionally, a sub-analysis of individuals with OA from secondary care and individuals post-TKR were studied in order to investigate what factors were associated with a higher risk of NP.

3.2 Methods

3.2.1 Participants

Participants who had undergone a TJR for OA from the NGOAS and arcOGEN studies (n=1,151; as described in **Chapter 2.2** Participants) were used in this analysis. All participants had symptomatic and radiographic OA prior to TJR surgery. Pre-operative radiographic severity grade has been linked previously to TJR outcomes therefore pre-surgery grades were used as the one available pre-operative component in this study (Valdes, 2011).

For the sub-analysis comparing individuals with clinically severe symptomatic OA (n=230) and post-TKR (n=742), participants were recruited from the NGOAS and arcOGEN studies (see **Chapter 2.2** Participants). The individuals with OA used in this sub-analysis were on a waiting list for TKR or had decided not to have this surgery.

3.2.2 Statistical analysis

The statistics package R (version 3.2.0) was used to run logistic regression analyses to measure associations between 1) TJR satisfaction and potential risk factors and 2) possible NP and potential risk factors.

A least absolute shrinkage and selection operator (LASSO) regression analysis was used to select variables in order to calculate a prediction model for satisfaction. LASSO is a form of regression analysis. The aim of LASSO is to select variables to improve a model's prediction accuracy and interpretability. LASSO resembles the stepwise approach in that it improves prediction accuracy of a model by choosing covariates for inclusion. This method has an advantage over a stepwise approach in that it constrains the coefficients produced, limiting their sizes, meaning they are a better fit to the model and less likely to be overestimates (Tibshirani, 2011). LASSO is a suitable method for fitting lots of predictors to a model and for variables which are highly correlated with one another, as in this data. Models were calculated for a) post-TJR satisfaction, b) post-THR satisfaction and c) post-TKR satisfaction. This analysis was run using the R package "glmnet" (<http://cran.r-project.org/web/packages/glmnet/index.html>) using a logistic link function and the fitted LASSO coefficients derived were used.

Receiver operating characteristic (ROC) analysis was used to visualise the contribution of variables in regression models to the outcome of interest (TJR, THR or TKR satisfaction) using predicted risk (adjusting for age, sex and BMI). The area under the curve (AUC) represents the diagnostic potential of the model. The discrimination ability of the models was examined using the "PredictABEL" package for R (<http://cran.r-project.org/web/packages/PredictABEL/index.html>).

3.2.3 Definitions for statistical analysis

Medication use, WOMAC pain and function scores, the presence of comorbidities and NP status were assessed as described in **Chapter 2.3.1** Trait Definitions for statistical analysis. Medication use in participants who had

completed the follow-up questionnaire was validated against baseline medication responses.

3.2.3.1 Post-TJR satisfaction

Individuals were asked to state how satisfied they felt with their TJR using an ordinal scale of “very satisfied”, “not very satisfied” and “dissatisfied”. For binary logistic regression analysis, individuals were dichotomised between a) “very satisfied” and b) “not very satisfied” and “dissatisfied”. As discussed in **Section 1.5.4** Assessing post-TJR outcomes, satisfaction has been used as an outcome measure to assess TJR outcomes in the literature (Mahomed et al., 2011, Dunbar et al., 2013).

WOMAC function scores were categorised according to an Outcome Measures in Rheumatology (OMERACT)-defined Patient Acceptable Symptom State (PASS) score of “acceptable function” (a score of ≤ 22) to allow a clinical guideline to be used in this study to put the importance of post-TJR satisfaction into a clinical context (Escobar et al., 2012, Maxwell et al., 2014).

3.2.3.2 Sub-analysis on the factors associated with NP in OA and post-TKR

The presence of comorbidities was also of interest in the sub-analysis of the associations with NP, as described in **Section 1.5.5** Comorbidities in osteoarthritis).

3.3 Results

3.3.1 Post-TJR satisfaction

The descriptive characteristics, stratified by TJR satisfaction status are shown in **Table 5** Descriptive statistics categorised by total joint replacement satisfaction status. Possible NP was seen in 17.3% of participants. However, in the dissatisfied group the prevalence of possible NP was 3.8 times higher than in the very satisfied group $OR_{\text{possNP}}=5.91$ (4.22-8.29) and the prevalence of likely NP was 5.4 times higher: $OR_{\text{likNP}}=7.66$ (4.80-12.22) (see **Table 5** Descriptive statistics categorised by total joint replacement satisfaction status).

THR participants reported higher levels of being very satisfied (82.4%) than TKR patients (69.4%) (**Table 5** Descriptive statistics categorised by total joint replacement satisfaction status). Possible NP was also less common in THR than TKR participants (11.9% and 22.3%, respectively) (**Table 5** Descriptive statistics categorised by total joint replacement satisfaction status).

It was investigated whether patient satisfaction was related to measures of healthcare usage, specifically use of analgesic medication. Very strong correlations were found between decreased likelihood of satisfaction and increased likelihood of the use of some prescription analgesics (opioids and other prescription medications which can be used to treat pain) and OTC analgesics, but not prescription NSAIDs (**Table 5** Descriptive statistics categorised by total joint replacement satisfaction status). After adjustment for possible NP, high pain catastrophizing and high pain intensity (VAS) these associations become non-significant except in the case of opioids and OTC analgesics ($p < 0.01$ and $p < 0.05$, respectively).

Table 5 Descriptive statistics categorised by total joint replacement satisfaction status and their contribution to the risk of dissatisfaction post-total joint replacement

	Trait	Very satisfied	Not very satisfied	Dissatisfied	OR not very satisfied/dissatisfied (95% CI)(a)
Demographic and morphometric	Age±SD (years)	73.2±8.6	73.0±8.8	72.2±9.1	1.00 (0.98-1.01)
	% female	57.4	56.8	47.6	0.85 (0.65-1.12)
	BMI±SD (kgm-2)	29±5.2	29.4±5.1	30.7±5.9	1.03 (1.00-1.06)*
Type of surgery	THR (n=494)	407 (82.4%)	74 (15.0%)	13 (2.6%)	0.58 (0.44-0.77)‡
	TKR (n=591)	410 (69.4%)	136 (23.0%)	45 (7.6%)	2.02 (1.50-2.71)‡
	THR+TKR (n=66)	44 (66.7%)	17 (25.8%)	5 (7.6%)	1.63 (0.95-2.78)
	Years since most recent surgery	4.26	3.96	4.58	0.99 (0.96-1.03)
Pre-operative X-ray	Radiographically severe OA	92.1%	93.4%	96.5%	0.49 (0.27-0.87)*

History of surgery	Previous arthroscopic knee surgery(c)	20.0%	26.4%	34.9%	1.65 (1.21-2.25)†
	Revision surgery	5.7%	9.3%	14.3%	2.36 (1.44-3.86)‡
Psychological	% depression	15.9	22.0	28.6	1.64 (1.17-2.30)†
	PCS score (0-52)	8.2	12.8	19.7	1.06 (1.05-1.08)‡
	Top tertile of PCS(b)	20.8%	37.0%	55.6%	3.40 (2.52-4.59)‡
Use of medication	% opioid	21.7	39.5	41.3	2.37 (1.77-3.18)‡
	% OTC	49	64.5	61.9	1.33 (0.84-2.12)
	% NSAIDs	7.8	12.3	3.2	1.83 (1.38-2.42)‡
	% other prescription analgesics	12.2	20.0	23.8	1.85 (1.29-2.66)‡
Measures of pain	PDQ score (0-35)	4.8	10.1	14.3	1.15 (1.13-1.18)‡
	Possible Neuropathic Pain (PDQ>12)	15%	33.9%	57.1%	5.91 (4.22-8.29)‡
	Likely Neuropathic Pain (PDQ≥19)	6.5%	18.1%	34.9%	7.66 (4.80-12.22)‡
	VAS (0-10)	3.1	5.8	7.0	1.35 (1.29-1.41)‡
	highVAS (>5)	30.8%	61.2%	76.2%	6.47 (4.80-8.73)‡

	WOMAC pain (0-20)	5.2	8.5	10.9	1.28 (1.23-1.33)‡
	WOMAC stiffness (0-8)	2.9	4.1	4.4	1.62 (1.49-1.76)‡
	WOMAC function (0-76)	25.7	38.0	47.8	1.07 (1.06-1.08)‡
Comorbidities	% heart disease/angina	16.7	19.4	27.0	1.34 (0.95-1.89)
	% stroke	5.1	9.3	12.7	2.09 (1.26-3.44)†
	% hypertension	52.3	50.2	57.1	0.95 (0.72-1.25)
	% asthma/COPD	13.8	15.4	14.3	1.07 (0.73-1.57)
	% irritable bowel syndrome	10.2	14.5	11.1	1.44 (0.96-2.17)
	% diabetes	11.8	15.0	19.0	1.28 (0.86-1.90)
	% gout	7.5	11.9	11.1	1.50 (0.95-2.37)
	% osteoporosis	11.0	10.1	19.0	1.23 (0.80-1.88)
	% cancer	15.9	19.8	17.5	1.29 (0.91-1.83)
	% current smoker	6.5	11.0	14.3	1.93 (1.22-3.07)†

* p<0.05 † p < 0.01 ‡ p < 0.001

(a) All ORs are adjusted for age, sex and BMI (b) Individuals in the top tertile of scores for the PCS questionnaire were classified as high catastrophizing (c) This classification includes any previous arthroscopic knee surgery

Post-TJR satisfaction is strongly correlated with a measure of acceptable function post-TJR, according to OMERACT-defined PASS scores in the literature (Escobar et al., 2012, Maxwell et al., 2014). This definition of acceptable function, according to a clinical guideline, was a very strong predictor of post-TJR satisfaction in this study after adjusting for age, sex and BMI: $OR_{\text{satisfied}}=9.88$ (95% CI 6.58-14.85). This association remained significant after further adjustment for possible NP, high pain catastrophizing and high pain intensity: $OR_{\text{satisfied}}=4.82$ (95% CI 3.08-7.55).

The post-operative (and one pre-operative) factors that contribute to patient satisfaction were investigated. On univariate analysis, a number of variables appear to contribute to post-TJR satisfaction. In addition to higher BMI and various measures of pain and pain intensity (PDQ, WOMAC, VAS), function and stiffness, a history of a revision surgery and the presence of a high pain catastrophizing score were found to contribute significantly to dissatisfaction post-TJR (**Table 5** Descriptive statistics categorised by total joint replacement satisfaction status).

A history of depression or stroke was associated with an increased risk of dissatisfaction post-TJR ($p<0.01$ for both). The same was true for current smokers (versus ex- and current smokers). The use of a) opioid analgesic medications, b) OTC analgesics and c) other prescription medications which can be used to treat pain was also found to be significantly associated with a higher risk of dissatisfaction. This association remained significant for the use of opioid and OTC analgesics after further adjustment for possible NP, high pain catastrophizing and high joint pain intensity: $OR_{\text{opioids}}=1.05$ (1.20-2.33) and $OR_{\text{OTC}}=1.45$ (1.06-1.96), respectively.

A logistic regression analysis showed that pre-operative radiographic severity was negatively associated and suggestive of a protective effect with possible NP post-TJR although this was not significant: $OR_{\text{possNP}}=0.53$ (0.27-1.03). However, this association became significant after adjustment for pain severity and WOMAC pain scores: $OR_{\text{possNP}}=0.35$ (0.14-0.86) $p=0.022$. PDQ scores were strongly correlated with pre-surgery X-ray scores (Spearman's $\rho=-0.13$,

$p < 0.001$). Higher pre-operative radiographic severity was also significantly associated with increased odds of TJR satisfaction: $OR_{\text{satisfied}} = 2.06$ (1.15-3.69).

The various pain measures are highly correlated with each other. For example, 88.9% of individuals with possible NP pain have a high VAS score (VAS>5) and 70.2% have a WOMAC pain score of >9. This compares to only 21.6% of individuals having high VAS and 12.6% having a WOMAC pain score >9 among people with no NP. A significant correlation was seen between a) PDQ scores and WOMAC pain scores ($\rho=0.70$, $p<2\times10^{-16}$), b) PDQ scores and VAS scores ($\rho=0.86$, $p<2\times10^{-16}$) and c) WOMAC pain scores and VAS scores ($\rho=0.71$, $p<2\times10^{-16}$).

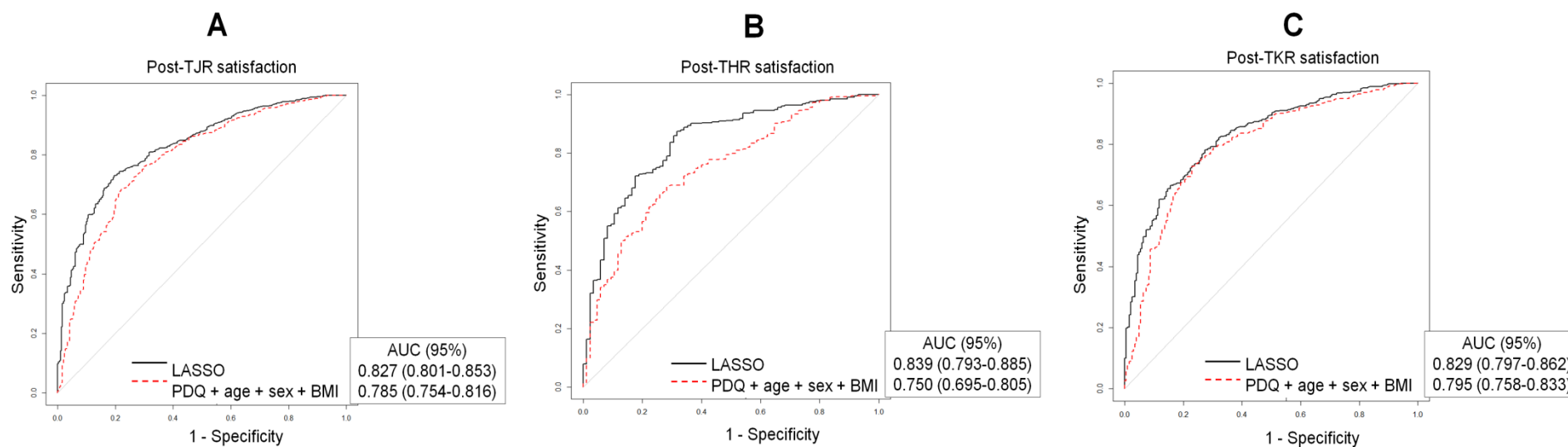
The results of the ROC analyses confirm that PDQ score is a very strong contributor of TJR satisfaction (**Figure 9** ROC curves adjusted for age, sex and BMI to show the amount of post-surgery satisfaction contributed by a LASSO regression model and painDETECT questionnaire scores (A) post-total joint replacement (total knee and hip replacements combined) (B) post-total hip replacement (C) post-total knee replacement). PDQ scores alone achieved AUC values of 0.79, 0.75 and 0.80 in the post-TJR, post-THR and post-TKR logistic regression models, respectively. These values rose to 0.83, 0.84 and 0.83 for the post-TJR, post-THR and post-TKR LASSO regression models, respectively (see **Figure 9** ROC curves adjusted for age, sex and BMI to show the amount of post-surgery satisfaction contributed by a LASSO regression model and painDETECT questionnaire scores (A) post-total joint replacement (total knee and hip replacements combined) (B) post-total hip replacement (C) post-total knee replacement). The LASSO regression model identified the best model for explaining post-TJR, post-THR and post-TKR satisfaction. These models were:

A) TJR satisfaction $\sim$ logit ($2.20671336 + (-0.01308463 \cdot \text{PCS}) + (0.188793354 \cdot \text{sex}) + (-0.398124376 \cdot \text{TKR}) + (0.016286776 \cdot \text{BMI}) + (-0.027476644 \cdot \text{WOMAC function.}) + (-0.0419659 \cdot \text{WOMAC stiffness}) + (-0.011651263 \cdot \text{past knee surgery}) + (-0.056489003 \cdot \text{PDQ}) + (-0.482951687 \cdot \text{highVAS}) + (-0.379943237 \cdot \text{revision surgery}) + (0.351851691 \cdot \text{severe}) + (-0.065600725 \cdot \text{OTC}) + (-0.113353818 \cdot \text{opioid}) + (-0.009913107 \cdot \text{current smoker}) + (-0.218073847 \cdot \text{stroke}) + (0.051460702 \cdot \text{hypertension}) + (0.06200968 \cdot \text{IBS}) + (-0.054783352 \cdot \text{gout}) + (-0.122585504 \cdot \text{depression})$)

B) THR satisfaction $\sim$ logit (2.98457238+(0.014001·years since surgery) + (-0.01838016·age) + (0.03603568·BMI) + (-0.03601472·WOMAC function) + (0.59884905·past knee surgery) + (-0.01780889·PDQ) + (-0.72458977·high VAS) + (-0.72325477·revision surgery) + (0.77886418·severe pre-surgical radiographic OA) + (-0.24831981·OTC) + (-0.11904627·opioid) + (-0.42874346·other medications for pain relief) + (-0.44240106·current smoker) + (-0.01435419·stroke) + (-0.51442047·depression) + (-0.02178514·cancer)

C) TKR satisfaction $\sim$ logit (2.42472895 + (-0.018499448·PCS) + (0.19004356·sex) + (0.000457584·BMI) + (-0.015266675·WOMAC function) + (-0.129503952·WOMAC stiffness) + (-0.075631636·PDQ) + (-0.312667452·high VAS) + (0.008698342·severe pre-surgical radiographic OA) + (-0.142719073·stroke) + (0.071982194·hypertension) + (0.333872701·IBS) + (-0.119930839·gout)

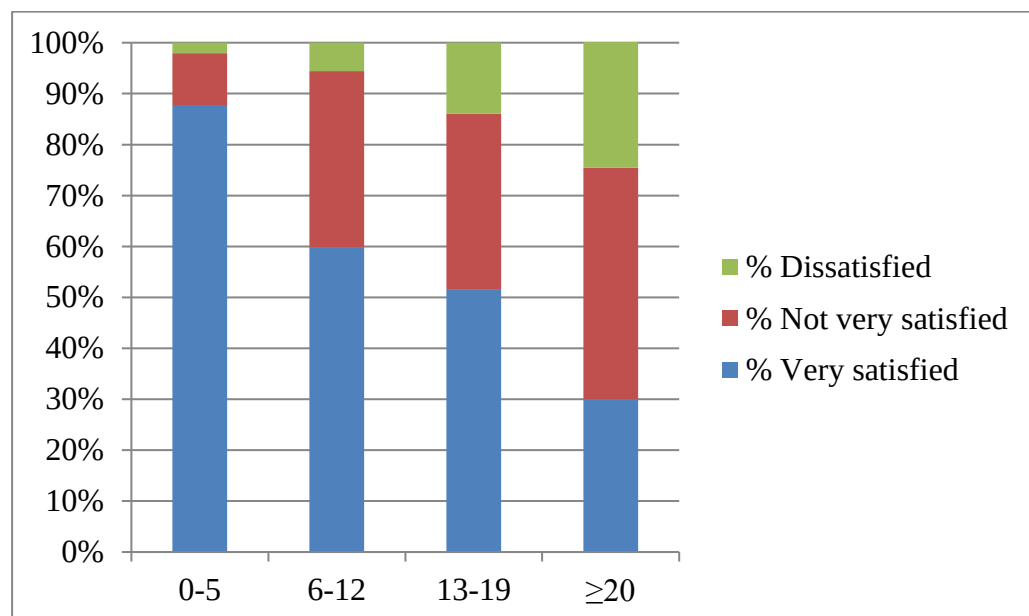
Figure 9 ROC curves adjusted for age, sex and BMI to show the amount of post-surgery satisfaction contributed by a LASSO regression model and painDETECT questionnaire scores (A) post-total joint replacement (total knee and hip replacements combined) (B) post-total hip replacement (C) post-total knee replacement



Some differences were observed in the factors that contribute to satisfaction post-TKR and post-THR, most notably a history of a revision surgery for THR and the stiffness score for TKR. However in both cases PDQ and VAS scores contribute significantly after adjustment for all other factors.

Overall an individual's post-operative PDQ score was found to be a significant contributor to post-TJR satisfaction, reaching AUC values of 0.75 or higher in all cases, even without the inclusion of any of the other pain measures available. Likely NP has been reported previously to be present only in a small proportion of individuals post-TJR (Wylde et al., 2011) using as a definition a PDQ >19. However strong differences exist in satisfaction at various lower cut-offs which explains why painDETECT scores explain such a large proportion of patient satisfaction in these data (**Figure 10** The proportions of individuals reporting satisfaction post-TJR according to PDQ score).

Figure 10 The proportions of individuals reporting satisfaction post-TJR according to PDQ score



3.3.2 Sub-analysis of the factors associated with neuropathic pain-like symptoms in people with OA and post-total knee replacement

The descriptive characteristics of the two groups are presented in **Table 6** Descriptive characteristics including mean painDETECT scores (PDQ) and prevalence of possible and likely neuropathic-like knee pain symptoms. The post-TKR group were older and reported lower pain intensity and pain catastrophizing levels than the OA group. Both possible NP and likely NP were more prevalent in the OA group.

Table 6 Descriptive characteristics including mean painDETECT scores (PDQ) and prevalence of possible and likely neuropathic-like knee pain symptoms

Trait	Secondary care OA	Post-TKR
n	230	742
% possible NP	33.0	21.6
% likely NP	14.8	10.4
knee PDQ score mean (SD)	9.5 (8.0)	6.4 (7.9)
% female	61.7	54.0
Age mean (SD)	65.4 (9.4)	73.4 (8.3)
BMI kgm ⁻² mean (SD)	30.3 (6.0)	30.0 (5.3)
% OTC pain medication	61.3	54.0
% prescription NSAID	10.0	7.4
% other prescription medication for pain relief	17.4	15.1
% on opioid medications	33.9	31.3
% weak opioid medications	23.9	25.7
% strong opioid medications	10.9	5.9
% no analgesic medications	58.3	41.2
% high VAS (≥ 6 out of 10)	73.0	39.8
VAS score mean (SD)	6.7 (3.1)	4.0 (3.7)

% knee/hip pain most days in a month ever	100.0	100.0
% CVD	56.1	62.7
% diabetes	13.5	14.6
% symptoms of depression	23.9	19.1
% cancer	13.0	17.1
% tired often/all the time	61.3	57.6
% chronic fatigue syndrome	0.4	0.8
% fibromyalgia	1.7	1.6
% Irritable bowel syndrome	13.9	10.9
% top tertile of catastrophizing score	62.1	36.9
% back pain y/n	41.3	39.5
% body pain y/n	93.9	49.9
% history of knee surgery (other than TKR)	52.6	30.9
% history of knee trauma	27.8	27.1

High pain intensity (VAS) was the strongest single contributor of NP in both groups (see Error! Reference source not found.). Younger age and higher BMI are also consistently associated with NP.

Comorbidities were tested for an association with possible NP in both groups (see Error! Reference source not found.). No significant associations with possible NP were seen for any of the comorbidities tested in the knee OA group. In the post-TKR group, a significantly increased risk for possible NP was seen in individuals who reported a diagnosis of diabetes, IBS, CFS or FM, body pain or back pain. After combining the groups and adjusting for age, sex, BMI and OA/post-TKR status, the association between possible NP and the presence of IBS, IBS/FM/CFS, body pain and back pain remained significant. This remained true after further adjustment for high pain intensity, except in the case of body pain and back pain.

The use of prescription analgesic medications was also linked to the risk of possible NP. A significantly higher risk of possible NP was seen in individuals in both groups who reported taking opioid analgesic medications. This association remained after adjusting for age, sex, BMI and OA/post-TKR status, but not after further adjustment for high pain intensity. However after this adjustment, the use of over-the-counter pain medications remained significantly associated with a higher risk of possible NP: OR=1.47 (1.03-2.10)

Table 7 Association between comorbidities and possible neuropathic pain-like knee pain symptoms in people with OA and post-TKR, adjusted for age, sex, BMI and OA/post-TKR status, and further adjusted for joint pain intensity

	OA (n=230)	TKR (n=742)	adj age, sex, BMI and status	adj age, sex, BMI, status and VAS
Age	0.97 (0.94-1.00)*	0.96 (0.94-0.98)‡	0.98 (0.96-0.99)†	n/a
Female sex	1.68 (0.94-3.02)	1.72 (1.20-2.47)†	1.56 (1.14-2.14)†	n/a
BMI	1.11 (1.06-1.17)‡	1.05 (1.02-1.09)†	1.05 (1.02-1.08)‡	n/a
High VAS	6.67 (2.72-16.33)‡	23.42 (13.71-40.02)‡	17.02 (10.64-27.24)‡	n/a
Diabetes	0.81 (0.35-1.85)	1.76 (1.12-2.76)†	1.33 (0.87-2.03)	1.27 (0.78-2.07)
CFS	1.81 (0.36-9.02)	1.83 (0.33-10.08)	2.21 (0.48-10.25)	3.83 (0.50-29.21)
FM	2.05 (0.28-14.87)	1.22 (0.33-4.55)	1.07 (0.35-3.25)	0.65 (0.20-2.09)
IBS	1.98 (0.93-4.22)	2.40 (1.47-3.92)‡	2.01 (1.31-3.09)†	1.76 (1.08-2.88)*
CFS or FM or IBS	2.05 (0.99-4.22)	2.05 (1.28-3.30)†	1.81 (1.20-2.73)†	1.62 (1.01-2.59)*
CVD	1.03 (0.59-1.79)	1.14 (0.79-1.64)	1.19 (0.86-1.65)	1.17 (0.81-1.69)
PCS highest tertile	5.62 (0.94-33.55)	6.29 (3.91-10.13)‡	5.80 (3.64-9.24)‡	2.66 (1.52-4.64)†
Knee surgery (excluding TKR)	0.95 (0.55-1.65)	1.31 (0.83-2.06)	1.17 (0.79-1.72)	1.16 (0.76-1.79)
Body pain (CWP1)	1.15 (0.58-2.28)	1.81 (1.19-2.75)†	1.49 (1.03-2.15)*	1.29 (0.85-1.97)
back pain	1.05 (0.60-1.83)	2.04 (1.43-2.90)‡	1.51 (1.11-2.06)†	1.26 (0.89-1.78)

*p<0.05, †p<0.01, ‡p<0.001

3.4 Discussion

3.4.1 Post-TJR satisfaction

This study shows that the presence of possible NP is strongly associated with lower post-TJR satisfaction 4.8 years after surgery. This association remains significant even after adjusting for high joint pain intensity. In this study we also found that self-reported satisfaction post-TJR was strongly linked to WOMAC pain and function scores, pain intensity, use of analgesic medication and pain catastrophizing.

The proportion of NP identified in this study falls within the range reported by previous studies on NP post-TJR (Haroutiunian et al., 2013) particularly when differences in methodology and sample composition are taken into consideration (Wylde et al., 2011, Baker et al., 2007, Brander et al., 2003, Buvanendran et al., 2010, Clarke et al., 2009, Clarke et al., 2010, Wylde et al., 2009, Nikolajsen et al., 2006, Martinez et al., 2007, Remerand et al., 2009, Sanders et al., 2009, Singh and Lewallen, 2010). At first sight the importance of NP post-TJR detailed here appears to contrast with the report by Wylde et al. who suggested that NP is a minor component of post-TJR pain (Wylde et al., 2011). However, a cut-off of 20 was used to classify PDQ scores in their study (Wylde et al., 2011). In the current study (see **Figure 10** The proportions of individuals reporting satisfaction post-TJR according to PDQ score) post-TJR satisfaction is strongly associated with PDQ at various different cut-offs, not only the very high scores. In addition, the participants in our study have a considerably higher BMI (29.1 kgm^{-2}) versus those in the Wylde et al. study (27 kgm^{-2}) which might account for the lower prevalence of likely NP in their study (8.1% overall versus 6% post-TKR and 1% post-THR) given the effect of BMI on likely NP defined using this cut-off: $OR_{\text{likNP}}=1.07$ (95% CI 1.03-1.11).

The importance of NP in defining post-TJR outcomes has been evidenced also by a study reporting that NP 48 hours post-surgery was associated with a higher risk of chronic NP at three months post-surgery (Martinez et al., 2012) although there is evidence that symptoms of central sensitisation can improve post-TJR (Graven-Nielsen et al., 2012).

Patient satisfaction is an outcome measure which is simple to use and accounts for the complex aspects of TJR (Halawi, 2015). Patient satisfaction is recognised as an important tool and is used in the UK to compare hospitals (Hamilton et al., 2014). It has been recommended that patient satisfaction should be incorporated into assessments of post-TJR outcomes (Halawi, 2015). Post-TJR satisfaction has been defined by some as a “nebulous” concept (Dunbar et al., 2013). However these results suggest that although post-TJR satisfaction is influenced by a large number of factors and correlates with use of medication and function, it is well summarised by one single instrument, namely PDQ scores.

The inverse relationship between radiographic severity and pain post-TJR has been reported previously (Dowsey et al., 2012, Valdes, 2011). Keurentjes et al. found that quality of life and physical function improved post-TJR, and that satisfaction was higher post-TKR in those with more severe radiographic changes (Keurentjes et al., 2013). Valdes et al. found the same inverse relationship between radiographic severity and TJR outcomes when using WOMAC pain scores (Valdes, 2011). The results in this chapter suggest that NP plays a role in this, as the inverse association between pre-operative radiographic severity and post-TJR satisfaction remained significant until further adjustment for possible NP.

The current data indicate that people who undergo TJR, with only modest radiographic structural damage are more likely to report NP post-surgery. Although this might suggest that the NP was also present pre-surgery, we lack the pre-operative pain assessments necessary to confirm if that is the case. In addition, pain derived from bone marrow lesions (BMLs) that are not evident on radiographs may still be present post-surgery (Kurien, 2015). BMLs that are not removed during TJR may still produce pain after surgery (Collins et al., 2014, Kurien, 2015).

Central nervous system involvement in OA, such as seen in NP, seems likely when the inconsistent correlation between pain and radiographic severity and the non-linear relationship between nociception and pain experienced are

considered (Graven-Nielsen et al., 2012, Tracey, 2010) supporting the findings in this study.

There are several limitations to the current study. First, is the lack of pre-surgical pain data, which makes it impossible to assess in this cohort whether the pain post-surgery is lower or higher than pre-surgery pain and whether NP symptoms are persistent or originate from the surgery. Phillips et al. found that it was not possible to reliably predict post-TKR outcomes from pre-operative pain intensity and PDQ scores (Phillips et al., 2014), whereas Duale et al., have reported a higher risk of NP post-surgery if peripheral NP is present pre-surgery (Dualé et al., 2014).

Secondly, although the self-administered painDETECT questionnaire allows data collection from a large number of individuals it does not provide definitive evidence of NP (Bouhassira, 2011). A diagnosis of NP requires further tests such as quantitative sensory testing or an MRI scan of the central nervous system to identify nervous system lesions and other possible markers of NP such as central sensitisation (Haanpää, 2012). Without this information it remains uncertain whether true NP was present pre-TJR or developed as a result of the surgery. However one study showed a correlation between PDQ scores and periaqueductal grey matter activation (which is involved in central sensitisation) in people with OA in areas of referred pain in response to punctate stimuli (Gwilym, 2009).

3.4.2 Sub-analysis of the factors associated with NP in OA and post-TKR

High joint pain intensity, younger age and a higher BMI were associated with a higher risk of possible NP in both people with OA and post-TKR. Painful comorbidities such as CFS, FM and IBS were also associated with a higher risk of NP. Pain catastrophizing showed the largest effect on the risk of NP after adjusting for age, sex BMI and joint pain intensity: $OR_{\text{possNP}}=2.66$ (1.52-4.64).

Despite studies which suggest an increase in NP with increasing age (Novak, 1999, Werhagen, 2004, Butler, 1979) one study of OA patients found that the mean age of the participants with possible NP was significantly younger than

those without ($p<0.05$), and that mean BMI was significantly higher in participants with possible NP versus those with no NP ($p<0.05$) (Valdes, 2013c), supporting the results of this study.

The link between analgesic medication use and NP seen here supports the importance of NP on health care usage in patients with chronic musculoskeletal pain. No medications yet exist to slow the structural progression of OA or treat the symptoms directly. Therefore pain relief is the main goal when treating OA (Valdes, 2013b, Barr and Conaghan, 2012). This helps explain the large proportions of individuals taking pain relief medication in the OA groups. After adjustment for confounders, prescription NSAID use was not associated with presence of NP, consistent with other evidence that NSAIDs are not routinely prescribed as an effective treatment for NP (Finnerup, 2015).

Few studies are currently available on the long-term use of opioids post-THR (Singh and Lewallen, 2010). The results in this chapter correspond with the results of another study on post-THR outcomes, which found that opioid use post-THR was associated with a higher risk of revision surgery (Namba et al., 2016). The authors suggested that opioid use could be “an early indicator of potential surgical failures” (Namba et al., 2016).

A high BMI can be both a cause and effect of OA (Oliveria, 1999) therefore the finding that mean BMI was significantly higher in the OA and post-TKR cohorts than the community cohort is relevant. Other studies have found that higher average BMI is seen in post-TKR patients than in the general population in the same age groups (Valdes, 2013a, Walpole, 2012). However, the literature is not in agreement as to the exact mechanisms by which obesity contributes to OA (Jordan, 2008).

Despite studies which suggest an increase in NP with increasing age (Novak, 1999, Werhagen, 2004, Butler, 1979) one study of OA patients found that the mean age of the participants with possible NP was significantly younger than those without ($p<0.05$), and that mean BMI was significantly higher in participants with possible NP versus those with no NP ($p<0.05$) (Valdes, 2013c), showing that this study accords with previous studies.

Although diabetic neuropathy is a potential source of NP, diabetes was not significantly associated with possible NP in this study after adjusting for pain intensity. This may be due to this study's focus on knee NP, whereas the neuropathy seen in diabetes is largely peripheral, affecting the feet and hands (Jensen, 2006).

There are several possible explanations to support the drop in the prevalence of NP with increasing age in this study. Although chronic pain is more prevalent in older populations (Herr and Garand, 2001), ageing affects nociceptive processing (Novak, 1999) and so pain and NP may not be interpreted the same way as a result of neurodegeneration and changes in pain perception with increasing age. One study found that in women, brain deactivation and decreased pain sensitivity were linked with increased age (Arrieta, 2015). Stoicism may also play a role in explaining the effect seen in this study, with some research suggesting that pain may be underreported in older individuals (Yong, 2001).

In summary, this study shows that post-operative painDETECT scores are the strongest predictor of patient satisfaction approximately five years post-TJR. Low pre-surgical radiographic severity is associated with lower post-TJR satisfaction and this is due to a strong association between low radiographic severity and higher pain intensity and NP.

3.5 Chapter summary

A range of demographic, pain-related and health-related data was analysed to test for associations with post-TJR satisfaction in 1,151 individuals. In individuals post-THR, 82.4% were very satisfied. In individuals post-TKR this was lower, at 69.4%. On univariate analysis, the presence of possible NP was one of the strongest single predictors of dissatisfaction post-TJR: OR=5.91 (95% 4.22-8.29) $p<0.001$. Higher pre-operative radiographic severity was significantly associated with lower odds of dissatisfaction post-TJR: OR=0.49 (0.27-0.87) $p=0.015$. This agrees with previously published findings and may suggest a role for patient expectations. After data reduction, factors which

remained significant for both THR and TKR and the combined sample were BMI, WOMAC function, PDQ scores and pre-operative radiographic severity. These results reinforce the importance of managing patient expectations.

A sub-analysis of individuals with knee OA and post-TKR was run to identify factors which were significantly associated with the likelihood of possible NP in each group. The strongest predictor of NP in both groups was high pain intensity at the affected or replaced joint.

CHAPTER 4: GENOME-WIDE ASSOCIATION SCAN OF NEUROPATHIC PAIN-LIKE SYMPTOMS POST-TOTAL JOINT REPLACEMENT AND REPLICATION ANALYSIS

4.1 Introduction

4.1.1 Aims and objectives

The aim of this chapter was to identify genes associated with the risk of NP in individuals post-TJR using a genome-wide approach. The replication analysis aimed to reproduce these findings in other groups containing individuals with knee and hip OA and knee pain.

4.2 Methods

The study design is outlined in **Figure 11** Study design.

4.2.1 Participants

4.2.1.1 Discovery group

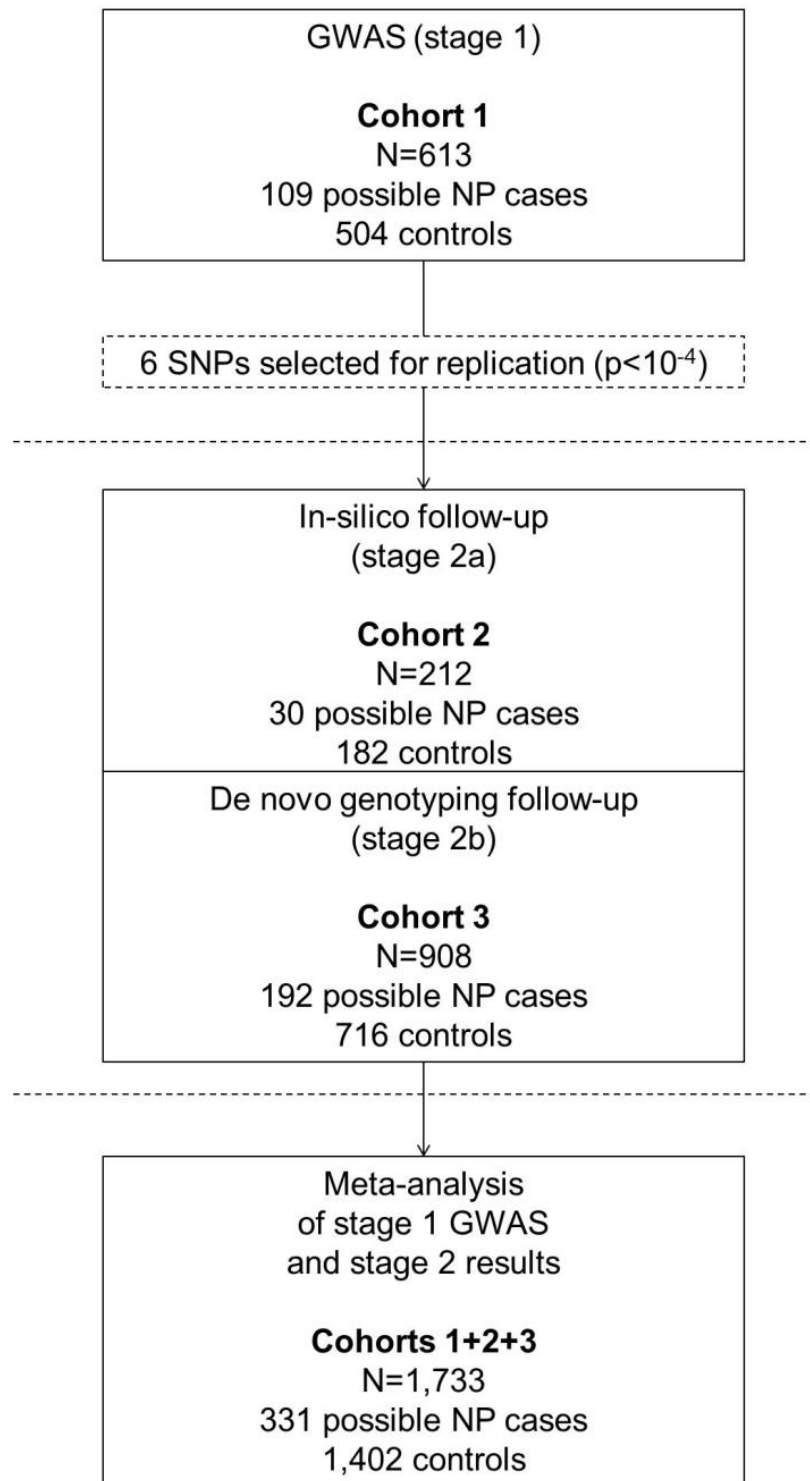
Individuals from the Nottinghamshire portion of the arcOGEN Consortium were analysed in the first stage of this study (n=613).

4.2.1.2 Replication groups

4.2.1.2.1 Nottinghamshire-based replication group

Participants the NGOAS study (n=908) were used as a replication cohort. This group included individuals with knee OA, hip OA, or both and individuals post-TJR.

Figure 11 Study design



4.2.1.2.2 The Rotterdam Study

The selected individuals were part of the Rotterdam Study III (RS-III) which was started in 2006 and comprised in total 3,932 participants. A total of 212 women that reported knee pain had data on PDQ and genetic data. This population-based cohort study has been previously described and was established primarily to study chronic disabling diseases in older adults (Hofman et al., 2007). The Erasmus University Medical School medical ethics committee gave approval for this study. All participants gave written, informed consent.

4.2.2 Stage 1: GWAS

Blood samples from the participants in this study were processed to obtain genotype data. Genotype data were analysed using the Illumina 610k array (<https://www.ebi.ac.uk/ega/studies/EGAS00001001017>). Only directly typed SNPs were used. Genotyping and quality control (QC) were carried out as previously described (Zeggini, 2012, Panoutsopoulou et al., 2011). Briefly, DNA quality and subject identity were validated using a Sequenom iPLEX assay designed to genotype four gender-specific SNPs and 26 SNPs present on the Illumina Human 610-Quad array (Illumina, San Diego, USA) (Zeggini, 2012). A PicoGreen assay and an aliquot assayed by agarose gel electrophoresis were used to quantify DNA concentrations (Panoutsopoulou et al., 2011). A DNA sample was considered to pass quality control if the original DNA concentration was >50ng/ul, the DNA was not degraded, the gender assignment from the iPLEX assay matched that provided in the patient data manifest and genotypes were obtained for over 65% of the SNPs on the iPLEX (Panoutsopoulou et al., 2011).

QC properties (call rate, exact HWE p value in cases and controls, differential missingness between cases and controls) for these independent SNPs with $p < 0.0001$ were investigated. The presence of poorly clustered SNPs can be checked using cluster plots. Cluster plots were studied for the cases and two sets of controls separately (Zeggini, 2012, Panoutsopoulou et al., 2011).

gPLINK software (version 1.07) was used to analyse GWAS data from this array (Purcell, 2007). The results of this association are a list of genetic variants (SNPs) and information about their location in the genome, as well as an odds ratio (OR), chi square value, and p value to indicate the level of association of the variants with the specified phenotype. The statistics program R (version 3.0.2) was used to create Manhattan and QQ plots using the “ggplot2” library and “qqplot” script.

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. The BioCarta and Kegg pathways maps were used for functional annotation.

4.2.3 Stage 2: replication cohorts

Four SNPs with a nominal p value of $p < 5 \times 10^{-6}$ after the stage 1 GWAS analysis and two additional lower-ranking but potentially relevant SNP were selected for replication (rs1133076 and rs4866176; see **Section 4.4** Discussion). Genotype information for these SNPs from in silico and de novo genotype data were used for further analysis. In total six SNPs were selected for replication analysis.

4.2.4 Stage 3: meta-analysis

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 (Han and Eskin, 2011).

4.2.5 Phenotype

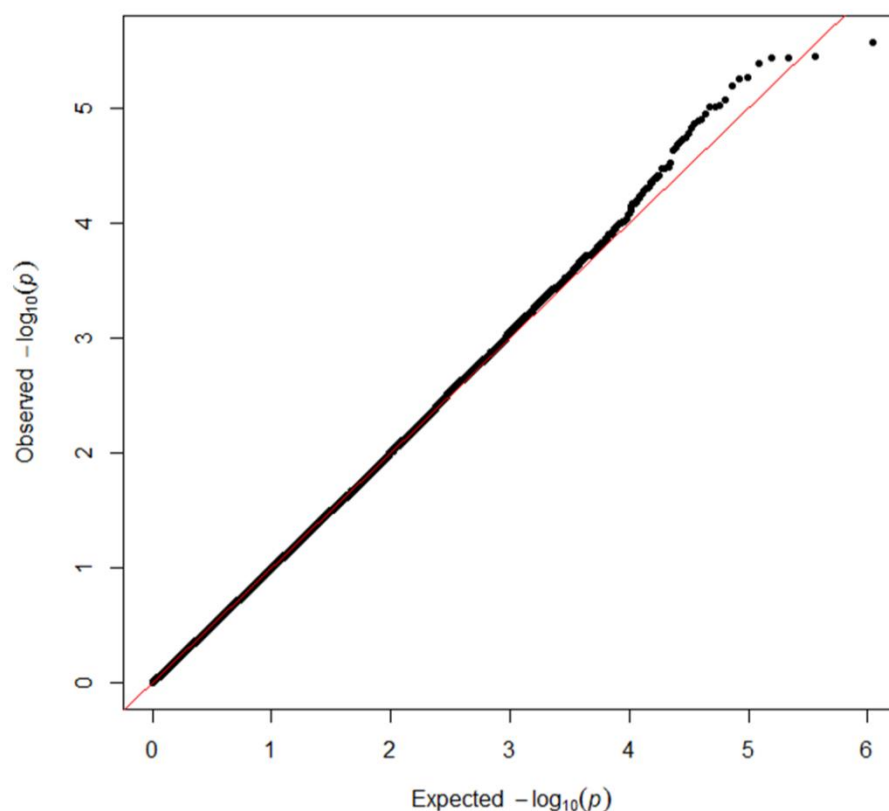
Individuals were assigned a phenotype by classifying them according to their scores on the PDQ (as described in **Chapter 2.3.1.2** Neuropathic pain-like symptoms. In all cohorts, scores of >12 were classified as “possible neuropathic pain” as described by Freynhagen et al. (Freynhagen, 2006).

4.3 Results

4.3.1 Stage 1: GWAS

The results of the unadjusted GWAS on NP can be seen in **Table 8** The results of interest from the unadjusted Illumina array NP GWAS and **Figure 12** QQ plot for the results of the GWAS ($\lambda=0.99$). A total of 548,382 SNPs were tested for association with NP. The genomic control inflation factor for the p values was low ($\lambda=0.99$) and the quantile-quantile (QQ) plot indicated no substantial population stratification due to cryptic relatedness, population substructure or other biases (**Figure 12** QQ plot for the results of the GWAS ($\lambda=0.99$)).

Figure 12 QQ plot for the results of the GWAS ($\lambda=0.99$)



The results of the GWAS are summarised in Manhattan plots of the p values (**Figure 17** Manhattan plot showing the p value of association tests for SNPs with possible NP in the Illumina array GWAS. P values represent the association of the SNPs with possible NP). **Table 8** The results of interest from the unadjusted Illumina array NP GWAS shows the odds ratios and significance of the results from the Illumina array NP GWAS for five of the top-scoring SNPs and a SNP of biological relevance.

Regional association plots were produced for the top four scoring SNPs from the GWAS analyses (see **Figures 13-16**). These plots aim to show individual SNPs in a specified region and their association with the phenotype of interest (possible NP in this analysis). A regional association plot resembles a Manhattan plot but focuses on a small section of the genome. A SNP of interest can be visualised in the context of the surrounding variants in that region

(Broad Institute, accessed 2016). LocusZoom, an online tool, was used to create the regional association plots shown here (Pruim et al., 2010).

Figure 13 Regional association plot to show the location of rs887797 and its association with possible neuropathic pain-like symptoms post-total joint replacement

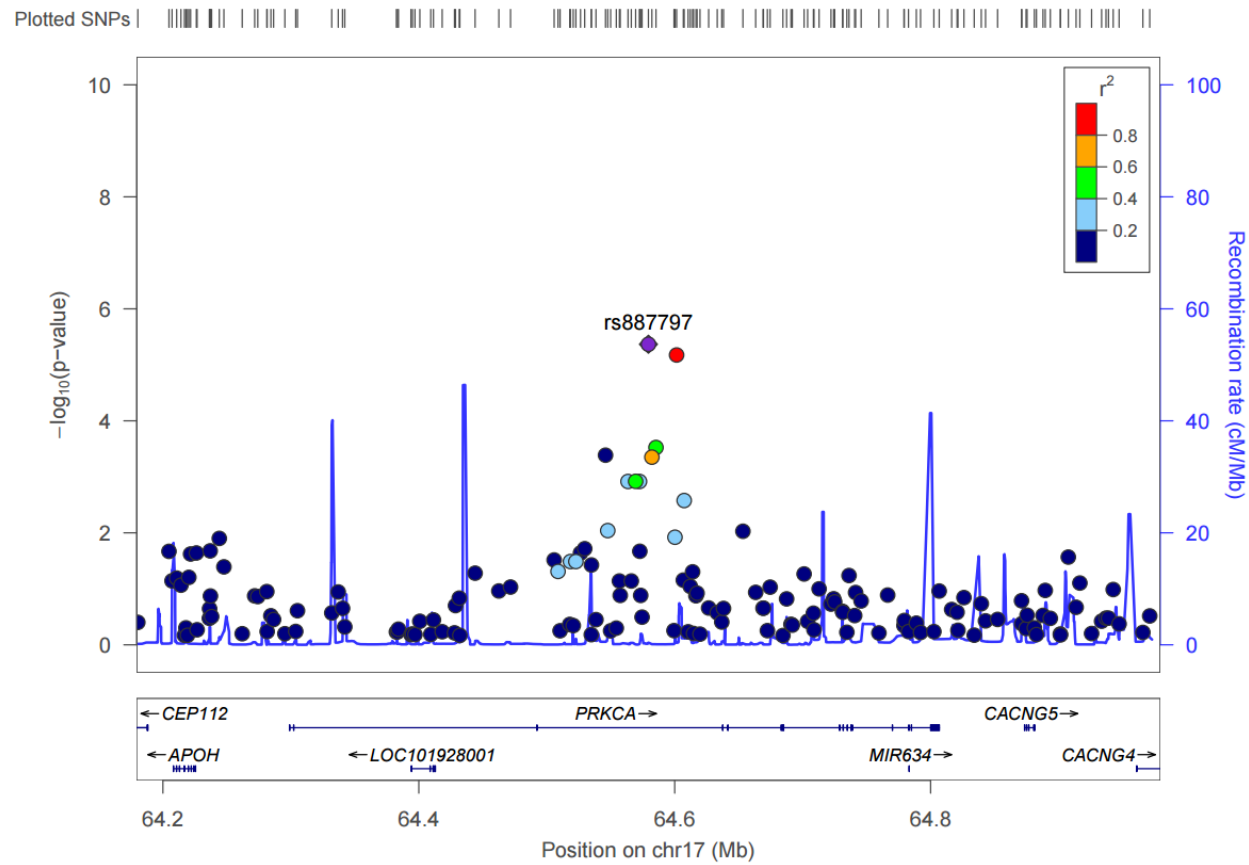


Figure 14 Regional association plot to show the location of rs12596162 and its association with possible neuropathic pain-like symptoms post-total joint replacement

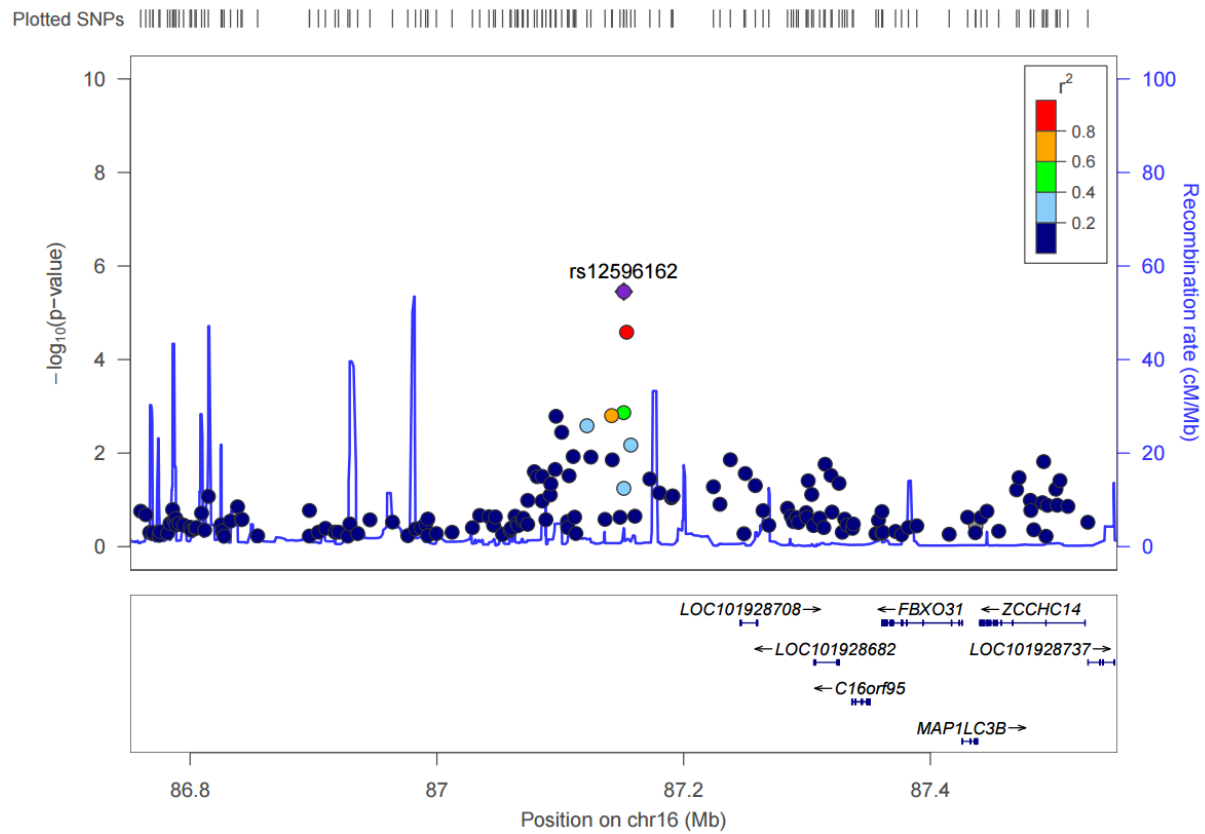


Figure 15 Regional association plot to show the location of rs7734804 and its association with possible neuropathic pain-like symptoms post-total joint replacement

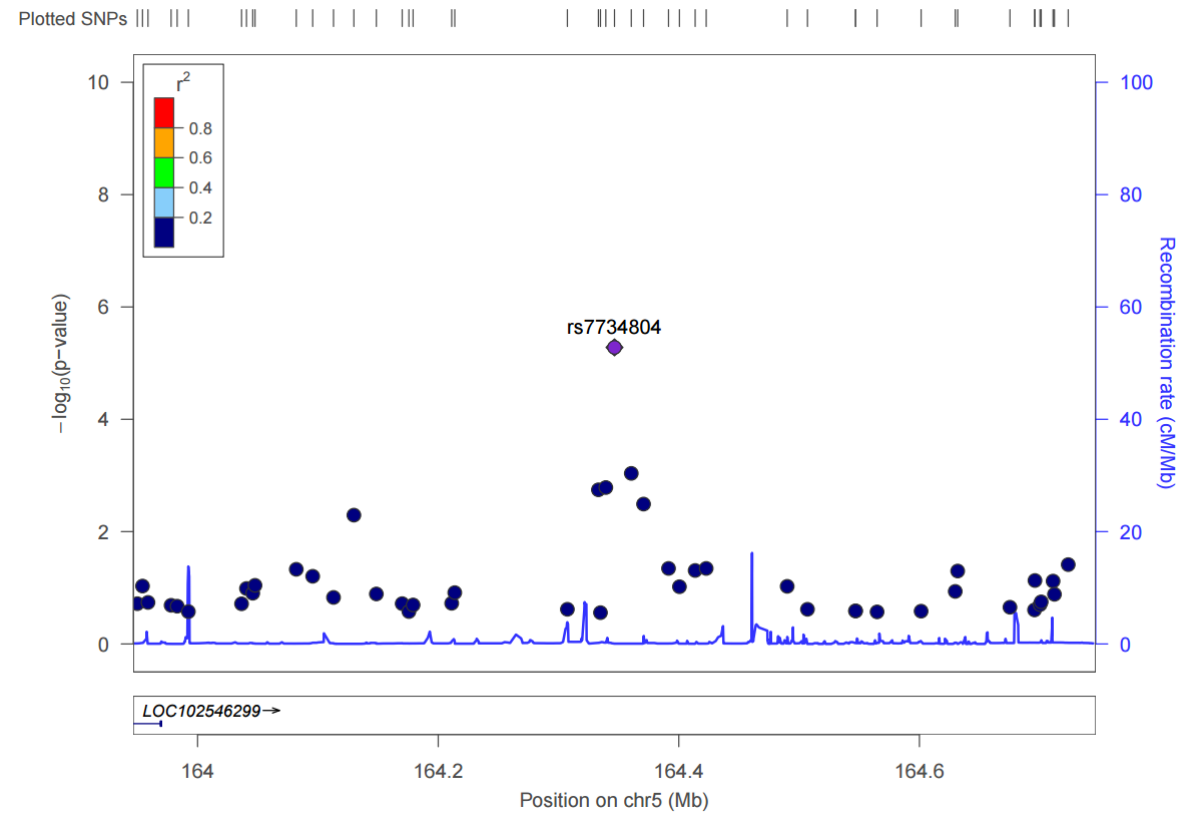


Figure 16 Regional association plot to show the location of rs298235 and its association with possible neuropathic pain-like symptoms post-total joint replacement

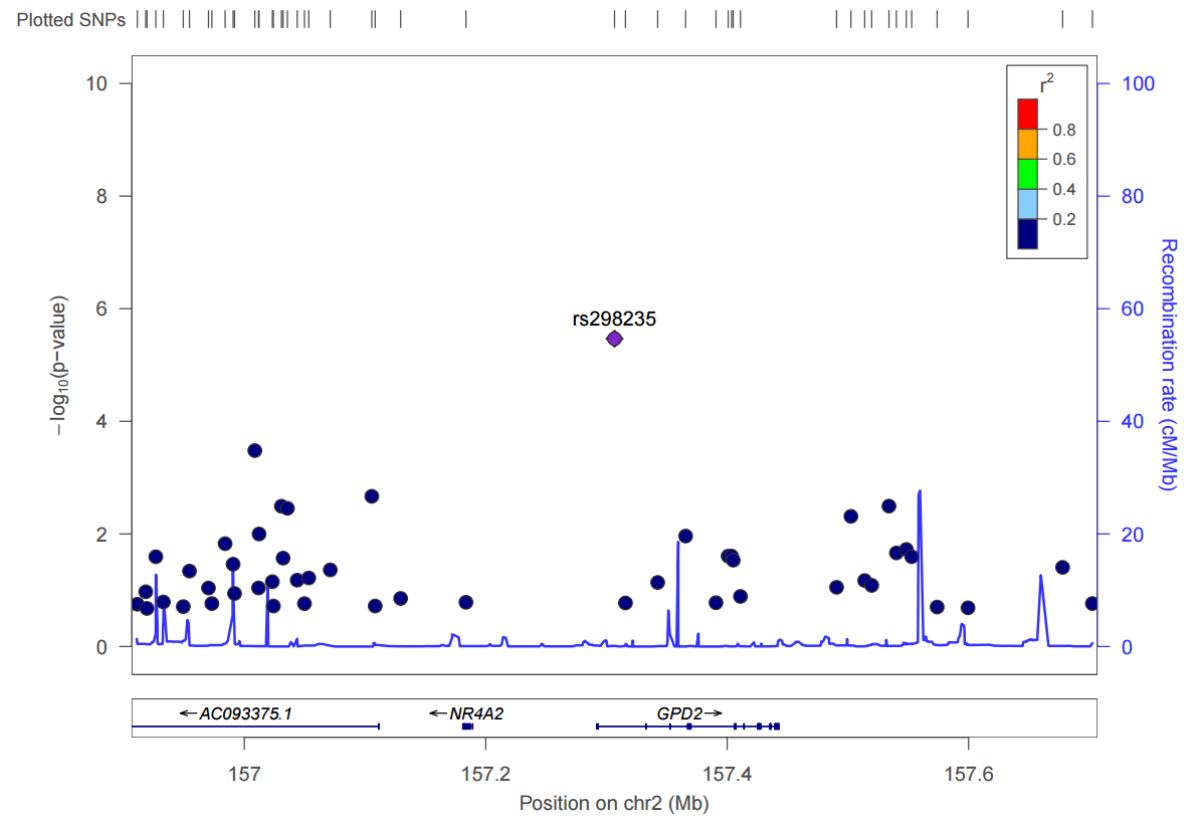
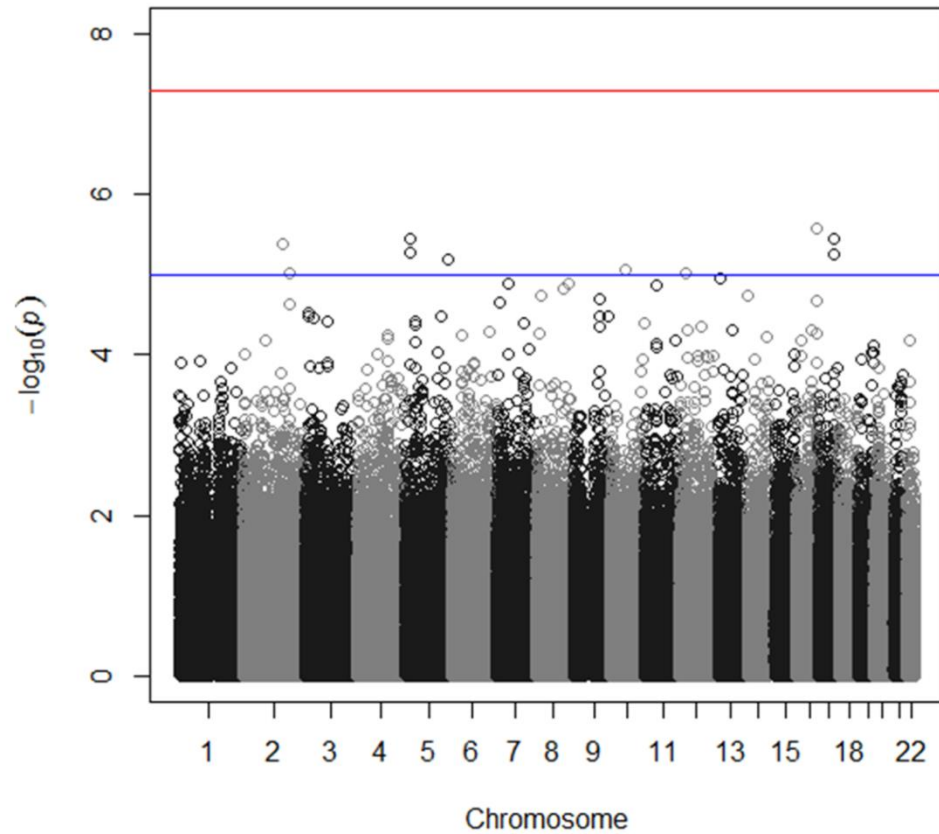


Table 8 The results of interest from the unadjusted Illumina array NP GWAS

SNP ID	Chr	BP	MAF	Effect allele/No effect	Gene or closest gene	Stage 1 (GWAS)	
						GWAS p value	OR (95% CI)
rs887797	17	66,583,327	0.335	A/G	<i>PRKCA</i>	4.29x10 ⁻⁶	2.00 (1.48-2.70)
rs4866176	5	20,245,445	0.064	A/G	<i>CDH18</i>	1.19x10 ⁻⁵	2.86 (1.76-4.66)
rs1133076*	8	133,113,438	0.469	A/G	<i>TG</i>	8.09x10 ⁻⁴	1.66 (1.23-2.24)
rs7734804	5	164,919,530	0.025	A/C	<i>MAT2B</i> (16.09 kbp) <i>ODZ2</i> (690.20 kbp)	5.25x10 ⁻⁶	4.64 (2.26-9.53)
rs298235	2	156,450,176	0.016	A/G	<i>GPD2</i>	3.41x10 ⁻⁶	6.72 (2.67-16.92)
rs12596162	16	87,117,889	0.303	A/G	<i>FOXL1</i> (536.19 kbp) <i>C16orf95</i> (184.91 kbp)	3.53x10 ⁻⁶	2.05 (1.51-2.79)

Chr=chromosome, BP=nucleotide location, MAF=minor allele frequency

Figure 17 Manhattan plot showing the p value of association tests for SNPs with possible NP in the Illumina array GWAS. P values represent the association of the SNPs with possible NP



4.3.1.1 Pathway analysis

Pathway analysis was carried out on the GWAS results using a list of genes corresponding to SNPs with p values less than $p < 0.0001$ in the GWAS ($n=62$; see **Table 9** The list of genes ($n=63$) containing SNPs of $p < 0.0001$ in the NP GWAS used for pathway analysis). If the SNP mapped to an area within a gene, this gene was used. For intergenic SNPs the two closest flanking genes on each side were used. The results of this analysis (see **Table 10** Overrepresented pathways for the NP GWAS) report no significant findings after adjusting for multiple testing with a Bonferroni correction (see **Table 10** Overrepresented pathways for the NP GWAS).

Table 9 The list of genes (n=63) containing SNPs of $p < 0.0001$ in the NP GWAS used for pathway analysis

Gene name	Gene name (continued)	Gene name (continued)
<i>FOXL1</i>	<i>TRPC7</i>	<i>HEATR7B2</i>
<i>PRKCA</i>	<i>SPOCK1</i>	<i>FLJ41423</i>
<i>CDH18</i>	<i>IKZF1</i>	<i>CHST1</i>
<i>GPD2</i>	<i>RNF144A</i>	<i>LILRB1</i>
<i>MAT2B</i>	<i>PRG-3</i>	<i>LILRB4</i>
<i>ODZ2</i>	<i>PDCD6IP</i>	<i>LRRC4C</i>
<i>ZWINT</i>	<i>C6</i>	<i>LRRC4</i>
<i>SSPN</i>	<i>CADM2</i>	<i>SND1</i>
<i>CWC22</i>	<i>PIK3CG</i>	<i>KIR3DX1</i>
<i>USP12</i>	<i>PRKAR2B</i>	<i>LILRA2</i>
<i>IKZF1</i>	<i>TCERG1L</i>	<i>MYH14</i>
<i>FER1L6</i>	<i>XPA</i>	<i>ZNF608</i>
<i>TMEM65</i>	<i>FOXE1</i>	<i>GRAMD3</i>
<i>GSTCD</i>	<i>FLJ35379</i>	<i>CCNG2</i>
<i>GAFA1</i>	<i>CDH5</i>	<i>CXCL13</i>
<i>RNASE10</i>	<i>TMTC1</i>	<i>CDH13</i>
<i>MSR1</i>	<i>IPO8</i>	<i>CPNE5</i>
<i>FGF20</i>	<i>PHACTR2</i>	<i>IL21</i>
<i>DGKB</i>	<i>DLC1</i>	<i>FXVD2</i>
<i>VAV2</i>	<i>KDM3A</i>	<i>FXVD6</i>
<i>SRGAP3</i>	<i>HS3ST2</i>	<i>REEP1</i>

Table 10 Overrepresented pathways for the NP GWAS

Pathway	Genes	p	Bonferroni p
Immunoglobulin	LILRB1, LRRC4, LILRA2, CADM2, LILRB4, LRRC4C	0.013	0.841
Cadherin	CDH13, CDH18 , CDH5	0.002	0.057
Calcium ion binding	PRKCA , GPD2 , CDH13, DGKB, CDH18 , TRPC7, SPOCK1, CDH5	0.024	0.976
Protein kinase C	PRKCA , DGKB, VAV2	0.011	0.809
Protein kinase C-like	PRKCA , DGKB, VAV2	0.019	0.945

4.3.2 Stage 2: replication cohorts

The next stage of the analysis aimed to replicate the 6 selected SNPs for their association with NP in two independent replication cohorts. Demographic information is presented in **Table 11** Demographic information for the three groups used in this study. The results of the replication analysis are shown in **Table 12** The results of replication analysis and meta-analysis. As shown in **Table 12** The results of replication analysis and meta-analysis, two of the SNPs selected from the GWAS in stage 1 for replication analysis show significant p values and effects in the same direction in one of the replication cohorts.

4.3.3 Stage 3: meta-analysis

The discovery and replication results were combined in a joint meta-analysis. The results can be seen in **Table 12** The results of replication analysis and meta-analysis. Heterogeneity of the loci was tested using the Cochran Q test. Due to the significant heterogeneity introduced to the model by the replication data in the rs887797, rs4866176, rs7734804, rs298235 and rs12596162 meta-analyses, a Han Eskin random effects model was used to account for this (see **Table 12** The results of replication analysis and meta-analysis and **Figure 18** Forest plot showing the results of an unadjusted Han Eskin analysis on the rs887797 SNP using a recessive model). The additive model for the rs887797 SNP after this analysis gave a result of: OR=1.47 (95% CI 1.24-1.76), **p=1.33 x10⁻⁵**.

Figure 18 Forest plot showing the results of an unadjusted Han Eskin analysis on the rs887797 SNP using a recessive model

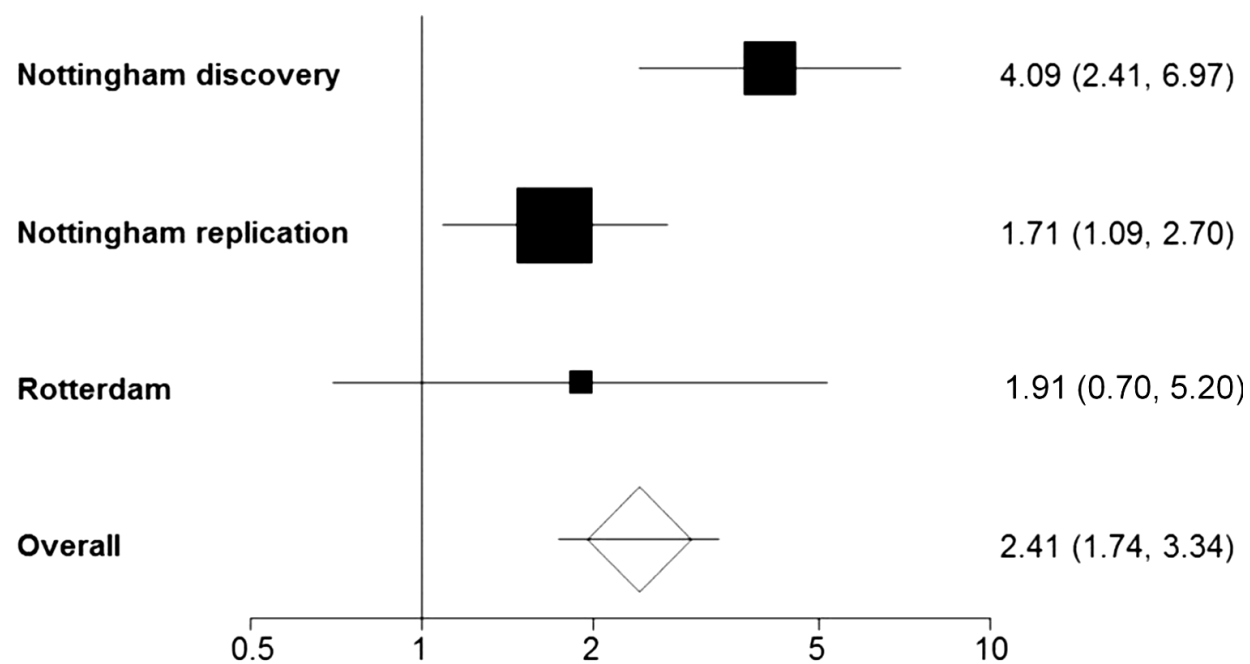


Table 11 Demographic information for the three groups used in this study

	N	Cases	Controls	% possible NP	Mean age (years)	% female	Mean BMI (kgm ⁻²)
Nottingham discovery (arcOGEN)	613	109	504	17.8	74.6	56.8	29.2
Nottingham replication (NGOAS)	930	193	737	20.8	70.8	58.4	29.4
The Rotterdam Study	212	30	182	14.2	60.2	100.0	27.5

Table 12 The results of replication analysis and meta-analysis

	Stage 2a (Rotterdam)	Stage 2b (Nottingham replication)	Stage 3 (Meta-analysis)		
SNP ID	OR (95% CI)	OR (95% CI)	OR (95% CI)	I ²	p value†
rs887797	1.12 (0.64-1.95)	1.28 (1.02-1.61)¹	1.48 (1.23-1.75)	71.3%	1.65x10 ⁻⁵
rs4866176	1.12 (0.36-3.51)	0.88 (0.54-1.43)	1.52 (1.08-2.12)	81.8%	1.39x10 ⁻³
rs1133076*	1.52 (0.88-2.64)	1.18 (0.94-1.48)	1.35 (1.14-1.60)	42.7%	5.45x10 ⁻⁴
rs7734804	12.92 (1.13-147.20)²	1.50 (0.77-2.91)	2.61 (1.62-4.22)	68.4%	7.80x10 ⁻⁵
rs298235		1.12 (0.41-3.08)	2.97 (1.51-5.93)	85.2%	5.32x10 ⁻⁴
rs12596162	1.68 (0.93-3.03)	0.87 (0.67-1.13)	1.26 (1.05-1.52)	88.5%	2.80x10 ⁻⁴

*indicates a SNP within a gene of biological relevance and interest which was hypothesised to be associated with NP

†if the heterogeneity (I²) between the groups was significant, a Han Eskin random effects model was used to calculate this value

1p=0.0374

2p=0.0392

A recessive model for the rs887797 SNP was also used in a meta-analysis. A recessive model was used to test the nature of the effect of the risk allele, that is, to test if two copies of the risk allele were needed to increase the risk of possible NP. After Han Eskin analysis, the recessive model for rs887797 gave a result of OR=2.41 (95% CI 1.74-3.34, **p=1.29x10⁻⁷**) (see **Figure 18** Forest plot showing the results of an unadjusted Han Eskin analysis on the rs887797 SNP using a recessive model).

After adjusting for age, sex and BMI, Han Eskin analysis of the rs887797 SNP gave values of: OR_{possNP}=1.44 (95% CI 1.21-1.73, **p=7.13x10⁻⁵**) and OR_{possNP}=2.33 (95% CI 1.67-3.27, **p=8.67x10⁻⁷**) for the additive and recessive models, respectively. Upon combining the data from the two replication cohorts used, it was found that overall this SNP was significant. The additive model for the rs887797 SNP in the Nottingham replication cohort and Rotterdam Study cohort gave OR=1.25 (95% CI 1.01-1.55), **p=0.040** and the recessive model gave OR=1.75 (95% CI 1.15-2.64), **p=0.008**.

Finally, an attempt was made to replicate two of the top hits from the only published GWAS on NP. These SNPs were reported to be suggestively associated with diabetic neuropathy: rs17428041 (*GFRA2*, OR=0.67, **p=1.77x10⁻⁷**) (Meng et al., 2015b) and rs71647933 (*ZSCAN20*, OR=2.31, **p=4.88x10⁻⁷**) (Meng et al., 2015a). The effect of rs17428041 was not replicated in the results of our GWAS: OR=1.47, **p=0.016**. Similarly, after using a proxy for rs71647933 (rs12565140, r²=0.947) no association was found with NP in the results of this GWAS: OR=0.71 (95% CI 0.46-1.09), p=0.12).

4.4 Discussion

This study reports a suggestive association between a variant in the *PRKCA* gene and NP in people with knee pain, knee or hip OA and post-TJR. Despite not reaching genome-wide significance the replication of effect sizes for four of these SNPs in one or both of the replication cohorts, and the improvement of the p value for one of these SNPs after meta-analysis suggest that these are true associations. The findings are also biologically plausible and supported by

previously published work in the literature. The study did not confirm the recently published association between SNPs in the *GFRA2* and *ZSCAN20* genes and NP from diabetic neuropathy (Meng et al., 2015b, Meng et al., 2015a). However, it should be noted that diabetic neuropathy is not necessarily the same phenotype as neuropathic pain-like joint symptoms. The definition of NP used in these studies was partly based on use of prescription analgesic medication and partly on the results of sensory testing. However, this type of medication is commonly used even by people with no NP, including people post-TJR with no NP. In our study, a validated screening questionnaire (PDQ) was used, the location of pain is exclusively that of the OA-affected joint and further clinical history and demographics have been collected for all participants.

The top hit from this GWAS and replication analysis maps to the *PRKCA* gene. This gene codes for protein kinase C alpha, a protein which has been linked with the nervous system and may contribute to central sensitisation in dorsal horn neurons (Kawasaki, 2004). The *PRKCA* gene has also been found in the literature to be involved in long-term potentiation (LTP). LTP is a process involved in both memory and chronic pain (Price and Inyang, 2015). As well as this, the *PRKCA* gene has been implicated in related processes such as memory capacity and post-traumatic stress disorder (PTSD) (de Quervain et al., 2012) and genetic variation in this gene has been linked to the neural basis of episodic memory (MacLeod and Donaldson, 2014). Although the $p < 5 \times 10^{-8}$ threshold for genome-wide significance was not attained, there were plausible, reproducible genetic effects on NP post-TJR and after replication analysis. The National Human Genome Research Institute (NHGRI) keeps a record of all SNP-trait associations $p < 10^{-5}$ (Welter et al., 2014) which supports the relevance of the findings in this study and their suggestive role in NP, despite not achieving genome-wide significance. More importantly if data from the two replication cohorts used are combined then a significant p value is obtained. A role for the *PRKCA* gene in pain has been previously reported (Olah et al., 2002). The rs887797 variant identified in this paper is a variant already associated with multiple sclerosis (Paraboschi et al., 2014). Therefore, although this association may not reach genome-wide significance it remains highly

biologically plausible, although further work is needed to confirm its role on NP.

In the present GWAS the intergenic rs12596162 SNP near the *FOXL1* gene was associated with NP: OR=1.96 (95% CI 1.45-2.64), **p=1.09x10⁻⁵**. This gene codes for a forkhead/winged helix-box transcription factor (NCBI, 2014). This gene and the rest of the *FOX* gene family are involved in many cellular processes (NCBI, 2014). *FOXL1* in particular was found in one study to be involved in the Wnt/ β -catenin pathway (Jiang, 2013) which is important in the nervous system and has been implicated in NP and hip OA (Zhang et al., 2013, Castaño Betancourt et al., 2012).

Thyroglobulin, encoded by the *TG* gene, is a protein necessary for normal thyroid function. Thyroid function and hypothyroidism have previously been related to NP and central sensitisation in the literature (Penza et al., 2009, Nippoldt, 2014, Orstavik et al., 2006). The rs1133076 SNP mapping to this gene was suggested in this analysis to be associated with possible NP at the discovery stage with **p=8.09 x10⁻⁴**. However this variant did not replicate in the additional cohorts and the evidence for association with NP for this gene is very weak.

The effect sizes in this study are larger than those reported in previous GWAS on pain traits such as migraine and CWP (OR=1.18 and OR=1.23, respectively) (Anttila, 2010, Peters et al., 2013) despite having a smaller sample size. The effect size for the GWAS on NP in diabetes was OR=2.31 for the SNP with the lowest p value, which is consistent with the current finding for the rs887797 SNP in the GWAS analysis (OR=2.04, see **Table 8** The results of interest from the unadjusted Illumina array NP GWAS).

There are a number of limitations to this study. None of the variants identified by this study reach genome-wide significance. This is not surprising given the small discovery and replication sample sizes available for this study. A major issue with the use of GWAS is the potential for inflated associations (Ioannidis, 2008). The statistical power for the rs887797 recessive model with the observed OR=2.41 was 56% for genome-wide significance. For the observed p value the statistical power was 66% given the observed minor allele frequency

and the rare homozygote frequency (which is in HWE). Although the study was underpowered for genome-wide significance the effect size is relatively large. To achieve 80% power with this effect size and the same proportion of cases to controls we would have needed 417 cases and 1,767 controls, a 25% larger sample size, assuming that in the additional sample the effect was the same (Ioannidis, 2008). Only the most extreme p values and effect sizes are selected for further study after a GWAS (Garner, 2007). This is called the “winner’s curse” (Garner, 2007) and means that the effect size reported here is likely to be an overestimate given the small sample size used for the discovery phase, and sample sizes of at least twice those that were used are likely to be needed. Furthermore, heterogeneity between the groups used in the meta-analysis can limit the effects seen in the results though we attempted to address this by the use of a Han Eskin Random Effects analysis (Han and Eskin, 2011).

As above, the large number of statistical tests run in a GWAS means there is a chance of type I error (false positive results). This can reduce confidence in the findings of GWAS analysis if the genome-wide significance threshold is not reached, as in this analysis. However, these results show a suggestive association with possible NP and some of these results showed the same direction of effect in the replication cohorts. In two cases, this was a significant ($p < 0.05$) effect, suggesting the possibility of these results being true effects, despite not reaching genome-wide significance. Replication of these signals improved confidence in the findings, however further replication in larger samples is needed to improve robustness.

After a genetic variant has been associated with a phenotype of interest, functional work is needed to identify the biological processes this variant is involved in, how this relates to disease risk and the variant’s possible functional relevance.

The rs887797 SNP in the PRKCA gene is a non-coding, intronic variant. Non-coding variants such as rs887797 have the potential to influence gene function, in that they can affect the number of splice variants. It has also been reported that non-coding genetic regions can contain variants associated with disease (Cobb et al., 2008). This has been studied in the context of cancer genetics, for

example (Khurana et al., 2013). Non-coding DNA can also potentially influence the binding of transcription factors (Khurana et al., 2016). It has been reported that 88% of tag SNPs for GWAS analysis are intronic (Hindorff), and that many variants identified using a genome-wide approach are noncoding variants (Edwards et al., 2013). This SNP's location in the *PRKCA* gene suggests that it may be involved in processes relevant to this gene's function. This specific variant's role in MS, reported in the literature (Paraboschi et al., 2014), suggests a potential for its involvement in centrally-acting mechanisms. Further genetic replication analysis followed by functional work is needed to ascertain the role of this variant in NP.

Functional work can take the form of transcriptomics. This is the study of the transcriptome, which is the entirety of the RNA transcripts produced by the genome (Wang et al., 2009). This can be undertaken using microarray analysis to compare transcriptomes. Identifying differentially expressed genes (in different cell populations, such as disease or healthy cells, or in response to a treatment) is possible using this method (Wang et al., 2009).

Proteomics is another step in the route from identifying genetic variants to understanding their functional significance. This involves large-scale study of the entire protein complement of a cell or organism (Wilkins et al., 1996).

There is currently very little functional work done on the results from studies on the genetics of NP.

However if OA is used as an example, functional work has shown that variants found in genes of interest can be studied further in numerous ways. For example, cartilage samples have been analysed and it has been shown that *IGFBP3* knockdown (reducing gene expression) in chondrocytes prevented hypertrophy, a change associated with OA pathogenesis (Evans et al., 2014). Similarly, overexpression of *IGFBP3* led to changes to cartilage consistent with those seen in OA pathogenesis (Evans et al., 2014).

If the genes associated with OA in these analyses are expressed in joint tissues, differences in gene expression in OA-affected versus unaffected tissues can be looked for to provide functional validation and a better understanding of the

biological relevance of the results of genetic analyses. Findings from functional validation studies could in theory identify ways to help overcome the genetic deficit seen in individuals with a genetic risk profile for OA at some genetic locations (Ratnayake et al., 2014, Dodd et al., 2013).

These same principles apply to the study of NP. So far, functional work in NP has mostly centred on pre-clinical work. Animal models have advantages in that a more homogeneous sample can be obtained as the disease aetiology is more controlled than it is in human participants. The *CACNG2* gene was identified in animal models studying NP using a candidate gene approach, and then these findings were translated to human models. To achieve this, a three-SNP haplotype in the *CACNG2* gene was studied in individuals post-surgery and was associated with a higher risk of NP following surgery (Nissenbaum, 2012). However, as above, identifying a gene of interest is one of the first steps needed to determine the function and clinical relevance of a variant or gene. Much more research is needed in this area in the field of NP, particularly in human participants.

The absence of a clinical NP diagnosis in these participants is another limitation of this study. However the results of this questionnaire have been shown to correlate with brain activity in areas associated with NP in people with NP and OA (Gwilym, 2009, Hochman et al., 2013).

In summary, this study has found biologically plausible and suggestive genetic effects when analysing possible NP in individuals with knee pain, OA and post-TJR. Replication in further cohorts could improve sample size and p values and it is hoped that this GWAS of neuropathic pain-like symptoms of the joint may encourage the collection of DNA and of PDQ scores and similar instruments in other cohorts.

4.5 Chapter summary

A genome-wide association scan identified signals suggestively associated with the risk of possible NP post-TJR. Upon replication analysis, two of the variants tested showed a significant effect in one of the replication cohorts in the same

direction as seen in the discovery cohort. After meta-analysis of the recessive mode for rs887797 in the *PRKCA* gene, a result of OR=2.41 (95% CI 1.74-3.34) $p=1.29 \times 10^{-7}$ was achieved. Further replication and functional work is needed to investigate the functional significance of this variant, however these results suggest future avenues for study of the genetics of NP in human participants.

CHAPTER 5: CANDIDATE GENE ANALYSIS FOR ASSOCIATIONS WITH PAIN IN OSTEOARTHRITIS

5.1 Introduction

5.1.1 Aims and objectives

The overall aim of this chapter was to test variants from two candidate genes previously associated with pain for an association with OA pain.

As discussed extensively in **Chapter 1, section 4.2** Pain and neuropathic pain susceptibility genes, there are a number of candidate genes which have been linked to pain and NP, including in people with OA, in both human and animal models. This list includes, but is not limited to, the *SCN9A*, *TRPV1* and the *COMT* genes (Valdes, 2013b, Valdes et al., 2011b, Belfer et al., 2013, Reimanna et al., 2010, Harrer et al., 2014). Another target for candidate gene studies on pain is the interleukin family of cytokines. These genes have been the focus of numerous candidate gene studies on a range of pain phenotypes, such as inflammatory pain, back pain, musculoskeletal pain and acute pain (Mogil, 2012). All of these phenotypes are of relevance to the study of OA pain.

The candidate gene approach allows variants in genes of interest to be studied for an association with a disease or trait of interest. The current interest in the role of inflammation in OA pathophysiology and presentation, as discussed in **Chapter 1, section 1.1** The nature of osteoarthritis means that genes involved in inflammatory pathways and processes are relevant targets for candidate gene analysis.

Given the role of substance P in pain perception and pain thresholds (Li, 2011, Zubrzycka and Janecka, 2000) and the involvement of its receptor in joint degeneration (Im et al., 2008), it was hypothesised that the substance P receptor may be involved in pain sensitivity in knee OA. The aim of this study was to investigate genetic variants in the substance P receptor (*TACR1*) gene to test for an association with symptomatic OA.

The association of IL-15 with the emergence of radiographic changes and pain severity in OA, suggest that this cytokine may play multiple roles in disease OA pathogenesis. Importantly, IL-15 signalling has been shown to be dependent on binding to the high specificity IL-15 receptor chain known as interleukin-15 receptor α (IL-15R α), encoded in humans by the *IL15RA* gene (O'Connell and Pistilli, 2015). This analysis therefore aimed to explore the potential effects of IL-15 on OA by investigating whether select *IL15RA* variants were associated with symptoms or radiographic severity in OA by examining two separate cohorts of patients.

5.2 Methods

5.2.1 Analysis of the substance P receptor gene

5.2.1.1 Discovery cohort

Participants from the GOAL study (see **Chapter 2.2.3** Genetics of Osteoarthritis and Lifestyle (GOAL) Study) were used for the discovery group in this analysis (n=1,232).

5.2.1.2 Replication cohorts

Participants from the Hertfordshire Cohort Study (HCS), the Chingford 1,000 Women study, the Tasmanian Older Adult Cohort (TASOAC) and the Clearwater Osteoarthritis Study (COS) were used for replication analysis as described previously (Valdes et al., 2011b). Participants were classified as symptomatic or asymptomatic OA cases using radiographic criteria as described in **Chapter 2.3.1.1** Kellgren Lawrence grade.

5.2.1.3 Genetic analysis

Genotype data for the GOAL samples was obtained from blood samples as previously described (Valdes et al., 2011b). Genotype data were obtained for all participants in the above five cohorts for rs11688000 in the *TACR1* gene. Genotype data for the rs4853119, rs1861457, rs10168354, rs3755456 and rs3771835 SNPs (also from the *TACR1* gene) were available for the GOAL participants only.

Logistic regression analysis was used to test genotype data for an association with a) symptomatic OA versus controls, b) asymptomatic OA versus controls and c) symptomatic OA versus asymptomatic OA.

All analyses were run unadjusted and then adjusted for a) age, sex and BMI and b) age, sex, BMI and maximum knee K/L grade. The statistical package R (version 3.0.2) was used for these analyses. Any further adjustments are indicated in the results tables where applicable.

The “meta” package for R was used to run a meta-analysis to test if any significant findings from the GOAL analysis replicated in the other groups and give an overall effect.

Haploview was used to measure the linkage disequilibrium (LD) between the six SNPs used in this analysis to assess the extent of the variation in the *TACR1* gene which was covered by the SNPs used in this study.

5.2.2 Analysis of the IL-15 receptor alpha gene

5.2.2.1 Study participants

A subset of knee OA cases from the GOAL study (n=795) and from the Nottinghamshire-based individuals from the arcOGEN Consortium (n=613) (see **Section 2.2.2** Arthritis Research Council Osteoarthritis Genetics (arcOGEN) Consortium) were used in this analysis to assess the role of *IL15RA* variation on radiographic severity and on symptomatic vs asymptomatic OA.

5.2.2.2 Binary trait definitions for statistical analysis

The PDQ for NP symptoms of the joint was used as described in **Chapter 2.3.1.2** Neuropathic pain-like symptoms and (Freynhagen, 2006). TF K/L scores were dichotomised as 0=grade 2, 1=grades 3 and 4 (in OA-affected individuals in both groups).

5.2.2.3 Genetic analysis

Blood samples from the participants in this study were processed to obtain genotype data as previously described (Zeggini, 2012). These data was available for all participants in this part of the study. Binary logistic regression analysis was used to test two genetic variants (SNPs) in the *IL15RA* gene, rs2228059 and rs7097780, for an association with TF K/L grade of radiographic severity and possible NP in both groups. Symptomatic versus asymptomatic OA variables were also tested in the GOAL group only. These two SNPs together represent a large proportion of the variation in this gene (<https://snpinfo.niehs.nih.gov/snpinfo/snptag.htm>). The statistics package R

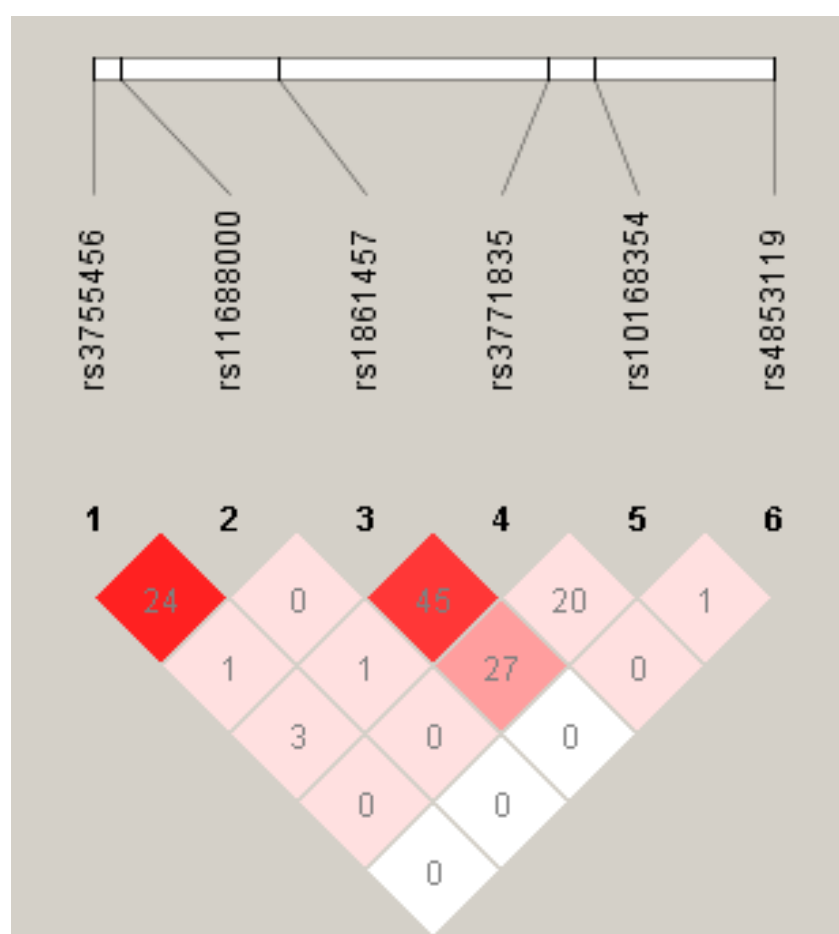
(version 3.0.2) was used for these analyses. All analyses were adjusted for age, sex and BMI.

5.3 Results

5.3.1 Analysis of the substance P receptor gene

The descriptive statistics of the study cohorts are shown in **Table 13**. Descriptive statistics for the discovery group (GOAL) and replication groups used in the analysis of the rs11688000 SNP. A description of the six SNPs used in this analysis can be seen in **Table 14**. Information about the IL15RA SNPs used in this study. The results of Haploview analysis to test the LD between these SNPs and assess the extent of the variation in the *TACR1* covered by these SNPs can be seen in **Figure 19**. Linkage disequilibrium (LD) plot showing the degree of association between the SNPs used in this study.

Figure 19 Linkage disequilibrium (LD) plot showing the degree of association between the SNPs used in this study



A nominally significant association was seen between rs11688000 genotype and symptomatic versus asymptomatic OA in the GOAL group in both the unadjusted analysis: OR=0.84 95% 0.71-0.99, and after adjusting for age, sex and BMI: OR=0.84 95% 0.70-1.00; see **Table 15** The results of logistic regression analysis showing the association of each of the six TACR1 SNPs with three measures of symptomatic versus asymptomatic OA. All analyses are adjusted for age, sex and BMI. This association became non-significant after further adjustment for maximum KL grade. However, a significant association was seen for the rs3755456 SNP after adjusting for age, sex, BMI and maximum KL grade: OR=1.25 95% 1.00-1.57. No significant results were seen for any of these SNPs with regards to an association with symptomatic OA

versus controls, or asymptomatic OA versus controls (see **Table 15** The results of logistic regression analysis showing the association of each of the six TACR1 SNPs with three measures of symptomatic versus asymptomatic OA. All analyses are adjusted for age, sex and BMI).

This SNP was then selected for further replication in four cohorts. Although the association only reached a suggestive association ($p=0.054$) with symptomatic versus asymptomatic OA in the TASOAC group, the direction of the genetic effect was the same in all cohorts with the G (minor) allele being associated with lower risk of symptomatic knee OA. A fixed effects meta-analysis using results from the unadjusted logistic regression analysis in each of the five groups gave a final effect of: OR=0.80 95% 0.70-0.91, $p=8.66 \times 10^{-4}$ (**Figure 20** Forest plot showing the results of a fixed-effects meta-analysis of the discovery group (GOAL) and the four replication groups used in the analysis of rs11688000 and its association with symptomatic OA versus asymptomatic OA (A: unadjusted, B: after adjustment for age, sex, BMI and maximum KL grade)**A**). Using the results from the fully adjusted logistic regression analysis (adjusted for age, sex, BMI and maximum KL grade) gave a result of: OR=0.79 95% 0.68-0.90, $p=9.90 \times 10^{-4}$ (**Figure 20** Forest plot showing the results of a fixed-effects meta-analysis of the discovery group (GOAL) and the four replication groups used in the analysis of rs11688000 and its association with symptomatic OA versus asymptomatic OA (A: unadjusted, B: after adjustment for age, sex, BMI and maximum KL grade)**B**). There was no heterogeneity between the five groups studied ($I^2=0\%$; **Figure 20** Forest plot showing the results of a fixed-effects meta-analysis of the discovery group (GOAL) and the four replication groups used in the analysis of rs11688000 and its association with symptomatic OA versus asymptomatic OA (A: unadjusted, B: after adjustment for age, sex, BMI and maximum KL grade)). After adjusting for multiple testing (six SNPs by three phenotypes) the necessary Bonferroni p-value is 0.0027, therefore this result remains statistically significant.

Table 13 Descriptive statistics for the discovery group (GOAL) and replication groups used in the analysis of the rs11688000 SNP

Cohort name	Genetics of Osteoarthritis and Lifestyle	Hertfordshire Cohort Study	Chingford study	Tasmanian Older Adult Cohort	Clearwater Osteoarthritis Study
Cohort acronym	GOAL	HCS	Chingford	TASOAC	COS
Reference for cohort details	(Valdes et al., 2011b)	(Valdes et al., 2011b)	(Valdes et al., 2011b)	(Valdes et al., 2011b)	(Valdes et al., 2011b)
Country of origin	UK	UK	UK	Australia	USA
Symptomatic knee OA^a	n=1232	n=86	n=71	n=94	n=83
Mean age (SD)	68.4 (7.2)	65.3 (2.8)	55.7 (5.8)	65.0 (7.2)	64.8 (7.8)
% Female	47.89%	45.30%	100%	41.50%	67.50%
Mean BMI kgm ⁻² (SD)	31.2 (5.4)	29.7 (5.4)	26.7 (3.4)	31.3 (6.3)	28.7 (5.0)
% K/L $\geq$ 3	95.62%	39.53%	43.66%	42.55%	38.55%

<i>TACR1</i> effect allele frequency	0.361	0.337	0.324	0.298	0.337
Asymptomatic knee OA^a	n=383	n=44	n=220	n=145	n=92
Mean age (SD)	69.0 (6.8)	65.2 (2.4)	55.7 (5.9)	65.7 (7.6)	63.8 (9.4)
% Female	43.90%	31.80%	100%	56.55%	60.90%
Mean BMI kgm ⁻² (SD)	28.9 (4.9)	28.4 (4.0)	26.3 (4.4)	28.1 (4.3)	26.4 (5.0)
% K/L $\geq$ 3	67.40%	15.90%	28.18%	24.83%	20.65%
<i>TACR1</i> effect allele frequency	0.401	0.398	0.388	0.372	0.413

Table 14 Information about the *IL15RA* SNPs used in this study

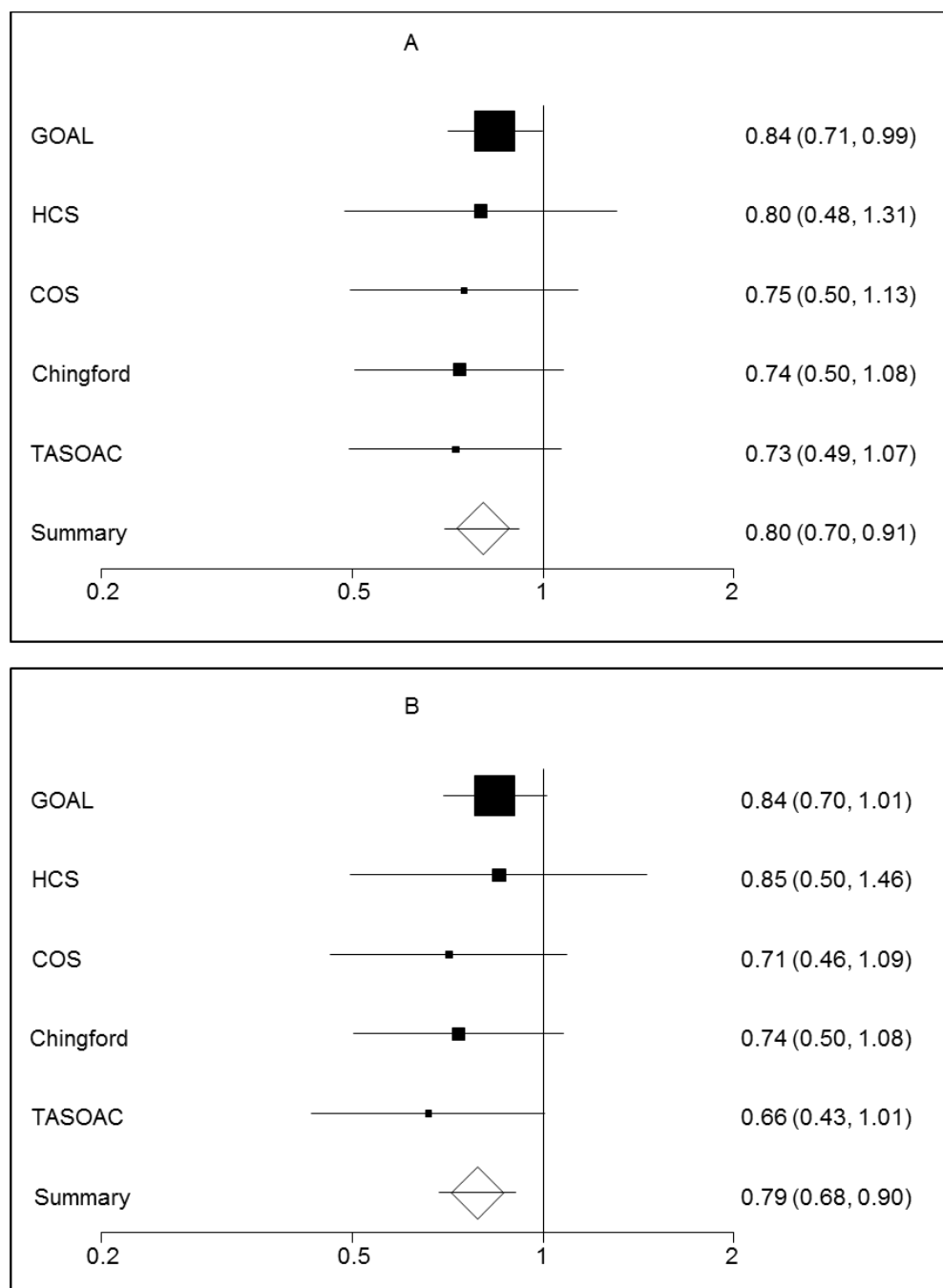
SNP ID	Chr	BP	Minor allele frequency		Effect allele/No effect	Closest gene	Gene location
			GOAL	Nottingham replication group			
rs2228059	10	6002368	0.492	0.498	C/A	<i>IL15RA</i>	Coding
rs7097780	10	6010227	0.323	0.340	G/A	<i>IL15RA</i>	Intron

Chr=chromosome, BP=nucleotide location

Table 15 The results of logistic regression analysis showing the association of each of the six *TACR1* SNPs with three measures of symptomatic versus asymptomatic OA. All analyses are adjusted for age, sex and BMI

SNP ID	BP	MAF	Effect allele/No effect	Gene location	Symptomatic OA versus controls OR (95%)	Symptomatic OA versus asymptomatic OA OR (95%)	Controls versus asymptomatic OA OR (95%)
rs4853119	75416296	0.745	T/C	Intron	0.95 (0.80-1.13)	1.05 (0.88-1.26)	0.93 (0.75-1.16)
rs1861457	75323267	0.33	T/C	Intron	0.89 (0.76-1.04)	0.98 (0.82-1.17)	0.91 (0.74-1.11)
rs10168354	75382710	0.572	T/C	Intron	1.12 (0.96-1.30)	0.95 (0.80-1.12)	1.10 (0.90-1.34)
rs3755456	75287819	0.855	T/C	Intron	0.89 (0.72-1.11)	1.20 (0.96-1.51)	0.77 (0.59-1.00)
rs3771835	75374043	0.453	T/C	Intron	0.92 (0.79-1.07)	1.04 (0.88-1.23)	0.89 (0.73-1.08)
rs11688000	75293157	0.368	G/A	Intron	1.04 (0.89-1.22)	0.84 (0.70-1.00)*	1.19 (0.97-1.46)

Figure 20 Forest plot showing the results of a fixed-effects meta-analysis of the discovery group (GOAL) and the four replication groups used in the analysis of rs11688000 and its association with symptomatic OA versus asymptomatic OA (A: unadjusted, B: after adjustment for age, sex, BMI and maximum KL grade)



5.3.2 Analysis of the IL-15 receptor alpha gene

The effects of IL-15 have been shown to be mediated by its highly specific receptor (O'Connell and Pistilli, 2015) which is encoded by the *IL15RA* gene. We used a genetic approach to assess if there is a relation between *IL15RA* variation and radiographic severity and pain measures in people with OA. The descriptive characteristics of the participants with knee OA and post-TJR in this genetic study are shown in **Table 16** Descriptive statistics for the groups used in the genetic analysis work. Information about the two SNPs tested in this analysis is presented in **Table 17** Information about the *IL15RA* SNPs used in this study.

We found no association between either of the *IL15RA* variants and radiographic severity of knee OA (**Table 18** Association between knee OA and pain-related traits and two *IL15RA* SNPs. Odds ratios (OR) are adjusted for age, sex and BMI). Although there was a trend between the G allele frequency at rs7097780 and tibiofemoral K/L grade (K/L=2: 30.2%, K/L=3: 33.3%, K/L=4: 36.2%, Armitage trend test $p=0.07$) this was not statistically significant. On the other hand the frequency of the G allele, after adjusting for K/L grade, was significantly associated with higher risk of symptomatic knee OA (see below).

A significant association was seen between rs7097780 genotype and the risk of symptomatic versus asymptomatic knee OA. The unadjusted analysis gave a result of: OR=1.56 (1.18-2.07) $p=0.0019$. After adjusting for age, sex and BMI the result was: OR=1.48 (1.10-1.98) $p=0.0098$ (**Table 18** Association between knee OA and pain-related traits and two *IL15RA* SNPs. Odds ratios (OR) are adjusted for age, sex and BMI). Further adjustment for maximum TF knee OA grade gave a result of: OR=1.43 (1.04-1.99) $p=0.029$.

Table 16 Descriptive statistics for the groups used in the genetic analysis work

	GOAL study	Nottingham genetics of OA study
Tibiofemoral OA cases n (%F)	551 (48.3%)	403 (54.8%)
Age (SD)	66.6 (6.7)	69.3 (8.8)
BMI (SD)	30.1 (5.0)	29.8 (5.1)
TF K/L grade (2/3/4)	116/295/140	30/261/112
Symptomatic/Asymptomatic TF OA	403/148	403/0
Post-TJR cases n (F%, % post-TKR)	795 (48.1%, 53.6%)	613 (56.8%, 64.9%)
painDETECT score mean (SD)	4.49 (6.3)	4.99 (7.6)
% possible neuropathic pain	13.8%	17.8%
rs2228059 (C/A)	0.508/0.492	0.502/0.498
rs7097780 (G/A)	0.677/0.323	0.660/0.340

Table 17 Information about the *IL15RA* SNPs used in this study

SNP ID	Chr	BP	Minor allele frequency		Effect allele/No effect	Closest gene	Gene location
			GOAL	Nottingham replication group			
rs2228059	10	6002368	0.492	0.498	C/A	<i>IL15RA</i>	Coding
rs7097780	10	6010227	0.323	0.340	G/A	<i>IL15RA</i>	Intron

Table 18 Association between knee OA and pain-related traits and two IL15RA SNPs. Odds ratios (OR) are adjusted for age, sex and BMI

Trait	<i>IL15RA</i> SNP	
	rs2228059 (effect allele = C)	rs7097780 (effect allele = G)
Radiographic severity:		
K/L grade 2 versus grades 3+4	OR=0.98 (0.75-1.27)	OR=1.19 (0.90-1.57)
Symptomatic versus asymptomatic knee OA	OR=1.02 (0.76-1.36)	OR=1.48 (1.10-1.98) p=0.0098
Possible neuropathic pain post-TJR	OR=0.76 (0.63- 0.92) p=0.005	OR=0.94 (0.76-1.15)

The minor (C) allele in rs2228059 was associated with a lower risk of NP joint symptoms post-TJR. Before adjusting for covariates, the effect of rs2228059 genotype on the risk of NP in the GOAL cohort was: OR=0.75 (0.58-0.96). A very similar effect size is seen in the Nottingham replication cohort: OR=0.81 (0.60-1.09). After adjustment for covariates and meta-analysis of both cohorts, this achieves: OR=0.76 (0.63-0.92), $p=0.005$ (**Table 18** Association between knee OA and pain-related traits and two IL15RA SNPs. Odds ratios (OR) are adjusted for age, sex and BMI).

5.4 Discussion

5.4.1 Main findings

5.4.1.1 Analysis of the substance P receptor gene

This study found a reproducible genetic effect on the risk of symptomatic OA versus asymptomatic OA given by the rs11688000 SNP in the *TACR1* gene. Another SNP in the *TACR1* gene (rs3755456) also showed a significant association with symptomatic versus asymptomatic OA in the discovery group, supporting the role of this gene in OA pain.

5.4.1.2 Analysis of the IL-15 receptor alpha gene

Genetic variation in the IL-15 receptor is associated with symptomatic OA and NP post-TJR.

We find that a variant in the *IL15RA* gene is associated with higher risk of symptomatic OA, but not with radiographic severity. A role for cartilage in OA pain has been recently shown in OA models. Driscoll et al. have found that pain related molecules are largely regulated in articular cartilage in animal models of OA (Driscoll et al., 2016). The lack of association here with radiographic severity suggests that the links to pain may be independent of its effect on cartilage.

We also observed a significant association between one of the *IL15RA* variants and joint NP. This is unlikely to be related to the effect of IL-15 on cartilage as

it is seen in replaced joints and suggests that there is a role to be investigated in more detail for IL-15 in joint pain that is not dependent on its effects on cartilage.

5.4.2 Biological relevance and context with other studies

5.4.2.1 Analysis of the substance P receptor gene

This study supports previously published work on the relevance of substance P in pain, and specifically in OA pain (Li, 2011, Zubrzycka and Janecka, 2000, Marshall et al., 1990, Lam and Ng, 2010, Lindh et al., 1997) by showing significant effects of variants in the substance P receptor gene on the risk of painful (symptomatic) OA.

The results of this study link with the results from previously published candidate gene studies on the genetic differences in pain perception and sensitivity. Variants in the *TRPV1*, *COMT*, *SCN9A* and *PACE4* genes have been implicated in the risk of symptomatic versus asymptomatic OA in the literature (Diatchenko, 2007, Diatchenko et al., 2005, Phillips, 2013, van Meurs et al., 2009, Neogi et al., 2013, Valdes et al., 2011a, Valdes et al., 2011b, Malfait et al., 2012). The results of studies such as these highlight the role of genetics in the risk of clinical OA (Warner and Valdes, 2016).

5.4.2.2 Analysis of the IL-15 receptor alpha gene

The association we found between specific *IL15RA* SNPs and symptoms in OA is particularly novel and interesting. These results support a small number of previous studies suggesting IL-15 could potentially be a new serum or synovial fluid biomarker to help assess disease severity (Sun et al., 2013).

The role of IL-15 in OA pain as described in the literature (Sun et al., 2013) is supported by these results as there was a significant association for *IL15RA* genotype when comparing symptomatic OA and asymptomatic OA individuals in terms of *IL15RA* genotype in the GOAL cohort.

The link between IL-15 levels and radiographic severity is still unclear (Sun et al., 2013). Our results showed no consistent effect of *IL15RA* genotype on radiographic severity.

The protective effect seen in terms of rs7097780 genotype with symptomatic OA suggests that in terms of IL-15 expression, asymptomatic cases of OA have lower IL-15 expression than controls (OR<1) and symptomatic cases of OA have higher levels than controls (OR>1).

5.4.3 Clinical relevance

5.4.3.1 Analysis of the substance P receptor gene

The significant association of two of the SNPs in this study with symptomatic OA highlights the role of substance P in pain and OA pain specifically. This association is biologically plausible according to previously published findings. Levels of substance P were found to be higher in the cerebrospinal fluid of individuals with painful OA versus control participants in one study (Lindh et al., 1997). As well as this it has been shown that substance P was elevated in synovial fluid samples from individuals with OA compared to controls (Marshall et al., 1990).

A clinical trial involving substance P for pain in OA has not yet been undertaken and the results of this study suggest such a trial could be worthwhile in understanding pain in OA. Substance P shows potential as a therapeutic agent for pain in OA.

TACR1 and substance P antagonists are of interest in research into pain and other conditions relating to the nervous system, such as stress, anxiety and post-traumatic stress disorder (Pantaleo et al., 2010). Numerous TACR1 antagonists have been researched in the context of migraine pain specifically, and some were found to effectively treat acute pain due to migraine (see (Peroutka, 2005) for a review of this). A role for an substance P antagonist and the prevention of a severe form of epilepsy has also been described (Pantaleo et al., 2010, Liu et al., 1999).

5.4.3.2 Analysis of the IL-15 receptor alpha gene

These studies have identified potential targets for therapeutic agents to treat pain in OA, for example IL-15 receptor and substance P receptor antagonists

These results also suggest that IL-15 could potentially be a new serum biomarker to help assess disease severity (Sun et al., 2013). Biomarkers for early OA could potentially have a huge impact in the diagnosis and treatment of OA (Sun et al., 2013).

It has been suggested that NP may be improved by inhibition of IL-15 (Austin and Moalem-Taylor, 2010). A proportion of individuals with OA suffer from NP even post-TJR (Hochman, 2011, Valdes, 2013c) so this cytokine may prove to have a relevant role in both nociceptive and NP in OA which requires further investigation.

The therapeutic potential of IL-15R α has been suggested (Wei et al., 2001). A soluble IL-15R α treatment has been reported to have possible applications in the treatment of collagen-induced arthritis (in an animal model of RA) (Ruchatz et al., 1998) and was found in an animal model to reduce acute inflammation (Wei et al., 2001). These results show the relevance of further study of IL-15 and its receptor in understanding the aetiology and treatment of OA.

5.4.4 Strengths and limitations

A limitation of these studies is that the candidate gene approach carries a risk of bias due to the nature of the study design. Potential genes of interest may be overlooked if they have not previously been linked with OA pain or NP, or do not have an obvious association with these traits.

The use of a candidate gene approach in both studies has advantages in that variants located in genes of interest can be studied directly. This saves costs on genotyping the entire genome of an individual. Although the costs of genotyping have decreased and continue to decrease, replication analysis is more straightforward if only small amounts of genetic data are required (Kwon

and Goate, 2000). Additionally, if there is already some understanding about the pathophysiology of the trait being tested (OA pain and NP in these studies) a candidate gene approach is an appropriate methodology (Kwon and Goate, 2000). As described in **Chapter 1.1** The nature of osteoarthritis there was a clear rationale supported by previously published studies for the selected genes' involvement in the phenotypes of interest in these studies.

The pathogenesis of OA pain and NP is still unclear in many cases. The different types of nerve injury or insult which can lead to NP have been demonstrated to have their own distinct genetic mechanisms (Young et al., 2014). A limitation of the methodology in these studies is that the precise cause of NP and location of any somatosensory lesions was unknown.

5.4.4.1 Analysis of the substance P receptor gene

A large proportion of the variation within the *TACR1* gene was covered by the six SNPs used in this analysis. Although only six SNPs were used, comprehensive cover of the *TACR1* gene was achieved using the available SNPs in this study.

5.4.4.2 Analysis of the IL-15 receptor alpha gene

There are logistic and ethical difficulties surrounding the recruitment of asymptomatic OA cohorts, such as possible selection bias. This may explain the small effect sizes seen here, which can result from a small sample size or a less clearly defined phenotype when compared to more clearly defined phenotypes such as WOMAC scores, for example.

The same effect of rs7097780 genotype on NP was seen in both two cohorts from the same recruitment area, but the lack of asymptomatic cases in the second cohort prevents us from doing the same for symptomatic versus asymptomatic OA. Thus, these results require further replication. In addition neither of the variants tested has a known functional role e.g. in terms of ligand binding or expression so it is not possible to directly link the genetic association seen here to increased or decreased IL-15.

5.4.5 Future work

5.4.5.1 Analysis of the substance P receptor gene

These results support findings in the literature which suggest the potential of substance P receptor antagonists in the treatment of pain. The role of SP in pain and inflammation, shown in the literature and these results, highlights its potential as a therapeutic target for future research.

The previously published association of rs11688000 with symptoms of alcohol dependency (Blaine et al., 2013) suggests a potential for a centrally-acting role of this variant. All six of the six of the SNPs studied here were intronic, that is, noncoding variants. However, as previously described in Chapter 4.4, intronic variants still have the ability to influence gene function and therefore are of interest for further studies. Functional work could be used to ascertain the roles of rs11688000 in symptomatic OA. It has previously been reported that NK1 knockout mice show changes to their nociceptive responses after tissue or nerve injury. This includes decreased wind-up and reduced central sensitisation (Weng et al., 2001).

5.4.5.2 Analysis of the IL-15 receptor alpha gene

The results of this analysis suggest that IL-15 may be important in NP. NP is a concern for many people with OA at all stages of the disease and treatment process (Hochman, 2011, Valdes, 2013c) therefore it is important to improve the understanding of the molecular mechanisms underlying NP in OA.

The *IL15RA* gene has not been well-studied in the context of pain or OA. More research is needed to identify any potential effects of this gene on rheumatological diseases. As well as this, the role of IL-15 activation in the pathogenesis of OA and the molecular pathways involved require further investigation (Sun et al., 2013).

A relationship with variants in the *IL15RA* gene and NP scores was identified. Whether IL-15 activity or levels predict emergence of peripheral or central sensitization is an important question that will need to be addressed in future, longitudinal studies.

Of the two SNPs studied, two SNPs, one is a coding variant (rs2228059) and one is intronic (rs7097780). A coding variant can potentially change the amino acid sequence of a protein if it is a nonsynonymous SNP. However, without further investigation, the impact of this mutation on protein structure cannot be hypothesised (Schaefer and Rost, 2012). Tools exist, such as SNaP (Broad Institute) which aim to predict the impact of such mutations (Bromberg and Rost, 2007).

5.4.6 Conclusion

The results of these analyses contribute to the knowledge of the genetic aetiology of painful OA. OA is a complex disorder and the lack of strong associations between radiographic severity and pain experienced by individuals is still unclear (Pincus and Block, 2009).

The results of this study support the role of substance P in OA pain in human participants and the genetic involvement in pain sensitivity in OA. This paper suggests a role for substance P in the treatment of arthritis. It is also suggested by these results that if such treatments are developed, there may be possible differences in treatment responses to substance P therapies for OA.

Variants in the two candidate genes tested here are associated with pain in OA. The use of a candidate gene approach was an appropriate choice of methodology for these studies. Reproducible genetic effects were found supporting the genetic contribution to symptomatic OA.

5.5 Chapter summary

In this chapter, two candidate gene analyses are presented. Firstly, an analysis of variants in the substance P receptor gene (TACR1) was run to test for an association with symptomatic versus asymptomatic knee OA. The results of this analysis showed a significant association between rs11688000 in the TACR1 gene and the likelihood of symptomatic versus asymptomatic OA: OR=0.84 (95% CI 0.70-1.00) p=0.045. This association was tested in four

additional cohorts and the direction of effect was the same, supporting the validity of this result. Meta-analysis of the discovery group and four replication groups improved the p value, achieving: OR=0.80 (95% CI 0.70-0.91) $p=8.66 \times 10^{-4}$.

Secondly, variants in the IL-15 receptor alpha (IL15RA) gene were tested for an association with symptomatic versus asymptomatic knee OA and possible NP post-TJR. It was found that rs7097780 was significantly associated with the likelihood of symptomatic OA, and that rs2228059 was significantly associated with the likelihood of possible NP post-TJR.

6 GENERAL DISCUSSION

6.1 Introduction

The pathogenesis of OA remains unclear and more research is needed to understand the causes of pain and NP. OA is the most common form of arthritis and therefore improvements in the understanding of OA have the potential to improve quality of life for many people worldwide. The aim of this chapter is to summarise the main findings of this thesis and further discuss and put the results into context.

In this thesis results were presented which highlight the importance of NP when assessing post-TJR outcomes. These results also suggest that there are reproducible genetic effects on the risk of NP post-TJR, in OA and in people with knee pain. Finally, variants in candidate genes of interest in inflammation and pain were found to be significantly associated with NP and symptomatic OA in two candidate gene analyses.

6.2.1 Neuropathic pain-like symptoms and post-total joint replacement satisfaction

The work in this thesis identified NP as the strongest single predictor of satisfaction post-TJR. The significant association between NP and post-TJR satisfaction remained after adjustment for confounding factors such as age, sex, BMI and other pain traits such as pain intensity. An important point to note is that NP was identified in this work using the PDQ, a seven-item questionnaire which classifies individuals as having possible NP or likely NP. If this questionnaire is administered pre-surgery it could help identify individuals who currently suffer with NP and so may be less likely to achieve the maximum benefit from a TJR. Individuals who are found to have NP either pre- or post-TJR could be indicated using this short questionnaire and referred for further testing and treatment to improve their outcomes at every stage of their OA treatment process.

6.2.2 The epidemiology of neuropathic pain-like symptoms

Although it is currently difficult in many cases to find the cause of NP in a patient, this study has identified some common risk factors to consider when assessing an individual's risk of NP. The strongest predictors of NP in people with knee OA and post-TKR were high pain intensity at the affected or replaced joint. Even after adjustment for pain intensity, the use of over-the-counter pain medications remained significantly associated in both groups with a 47% higher risk of possible NP for the sample as a whole. Interestingly, history of knee surgery (excluding TKR) was not significantly associated with NP in either group.

These results highlight the need for more research into and greater understanding of the aetiology of NP in people with OA and post-TJR throughout their treatment and at every stage of the progression of the disease.

These findings support the link between low pre-surgical radiographic severity and higher levels of pain, including NP, and poorer satisfaction post-TJR (Dowsey et al., 2012, Valdes, 2011, Keurentjes et al., 2013). These results show lower post-TJR satisfaction in people with lower pre-surgical radiographic severity. A reason for this could be that the pain felt pre-surgery was due to the presence of NP or BMLs, neither of which would be addressed by a TJR.

6.3 Genome-wide association scan of neuropathic pain-like symptoms post-total joint replacement and replication analysis

This study was the first GWAS on NP in people post-TJR. NP is difficult to assess and diagnose accurately in humans due to individual differences in risk factors and NP aetiology. Therefore genetics is a very good way to study risk factors for NP as it uses non-invasive techniques which can be automated and which are becoming increasingly cheaper and available to run in large scale studies. Suggestive and reproducible findings were identified with one result approaching genome-wide significance. These effects suggest that these are

true associations, although further replication and other analyses are required to confirm the role in NP. Several variants were identified and suggested an increased likelihood of NP. Some of these variants map to genes of interest in the nervous system, highlighting the relevance of these findings. These variants, if found to replicate in additional cohorts, could in theory be a future target for OA therapies and potentially identify individuals at risk of NP. At present, the factors which have been identified as potentially influencing post-TJR outcomes do not fully explain why there are differences in TJR outcomes in patients who are matched for epidemiological factors. The results of this research support the hypothesis there is a genetic component to this issue.

These results help to improve the understanding of the molecular pathways involved in NP, which is a source of pain for many people with OA. Additional research is needed to ascertain the biological and functional relevance of these genetic findings which could help put the research into a clinical context to benefit people with NP in OA (Ramos and Meulenbelt, 2015).

6.4 A candidate gene analysis of pain- and inflammation-related genes in osteoarthritis

The role of inflammation in OA has become an area of interest in recent years. The inflammatory components to OA are recognised as being important contributors to the pain experienced. The results of this thesis highlight the importance of inflammation in pain and OA pathogenesis, due to the effects of IL-15 on cartilage and the association of variants in the IL-15 receptor gene.

The role of SP as an inflammatory neuropeptide is also reinforced, showing an association with symptomatic knee OA in GOAL which replicated in four additional groups. The replication of this association suggests a true association between SP and the likelihood of symptomatic OA. Antagonists to the SP receptor, TACR1, have been found to improve pain in people with migraine (Peroutka, 2005) and this study of symptomatic OA suggests that this may also be a possibility to consider when developing future therapies for pain in OA.

Further work is needed in this area. As is always the case in genetic research, additional replication will improve the understanding of the molecular pathways involved in pain and inflammation in OA. In future it may be possible to identify which individuals are at risk of symptomatic OA and therefore more likely to require intervention than an individual with asymptomatic OA. As pain is the major outcome of OA (McDougall and Linton, 2012, Valdes, 2011) therapies which benefit individuals with symptomatic OA and therefore pain symptoms are of great interest for future studies.

6.6 Other approaches

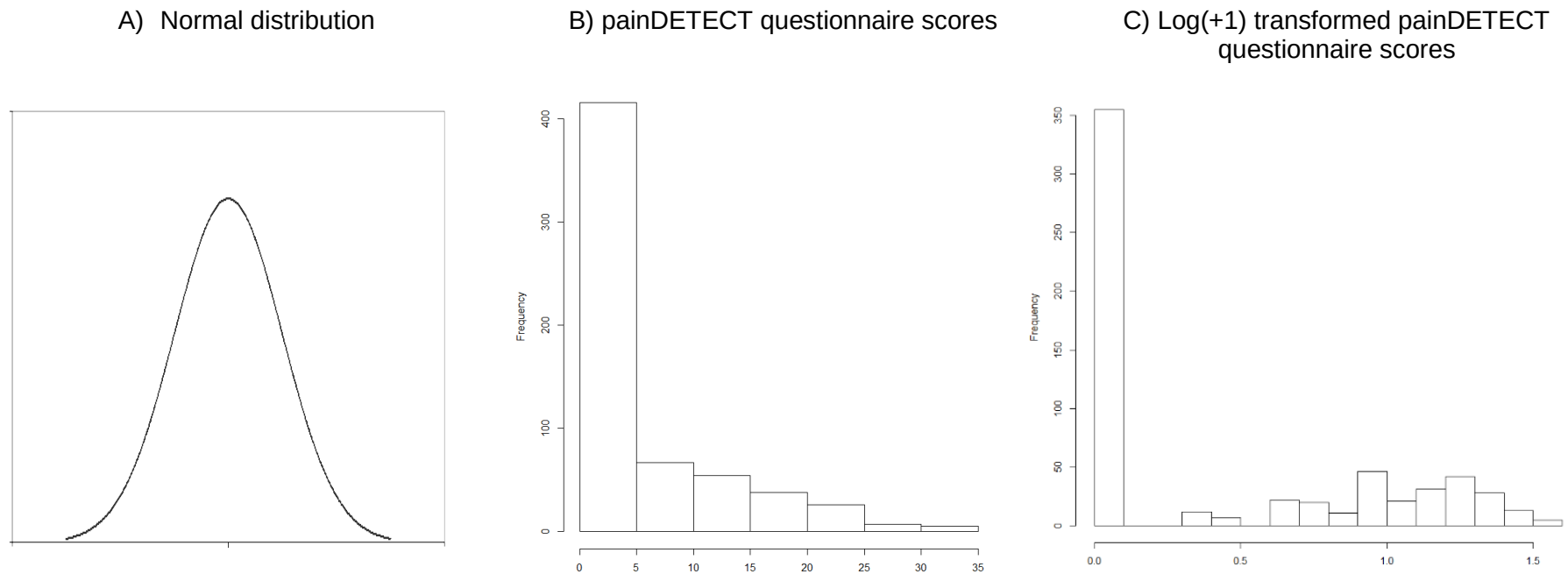
In this analysis, PDQ scores were dichotomised according to validated cut-offs (Freynhagen, 2006). The distribution of PDQ scores was highly positively skewed (see Figure 21) i.e. there was a disproportionately large proportion of scores in the 0-5 range. A log+1 transformation of the PDQ data, as shown in Figure 21, did not improve the distribution of the data sufficiently for use as a continuous variable. The data here does not lend itself to transformation. Although the skew and kurtosis values improved after a log+1 transformation, a disproportionate peak still appears for the lower PDQ scores, and as such the shape of the data distribution, shown in Figure 21, does not make this data suitable for continuous analysis.

Although generally speaking quantitative traits, such as continuous measures, are useful in genetic analyses and more powerful, in this case using continuous PDQ scores would not be a statistically appropriate analysis.

An alternative way to approach this analysis could have been to use the higher validated cut-off point for NP, whereby scores of ≥ 19 are classified as likely NP (Freynhagen, 2006). It could be said that this is a slightly more relevant phenotype, in that it may be more relevant for clinical purposes. However, to utilise a higher cut-off, much larger sample sizes would be needed to achieve the necessary statistical power for this analysis. For example, in the discovery group in this GWAS analysis, the likely NP cut-off would only result in 46

cases (7.5% of the group). If larger sample sizes were available this higher cut-off would be considered.

Figure 21 Histograms to show A) the normal distribution, B) the distribution of painDETECT questionnaire scores in the discovery cohort of this analysis and C) the log(+1) transformed values of the painDETECT questionnaire scores



However, when comparing participants from this study who have possible NP and likely NP, it was found that individuals with possible NP closely resemble those with likely NP in terms of demographic factors and pain medication use. The likely NP phenotype is just a more extreme version of the possible NP phenotype. Both phenotypes reflect a lower age, higher proportion of females, higher BMI, higher PCS, higher pain levels and intensity, poorer stiffness and function, reduced QoL, higher usage of OTC and opioid analgesics, as well as much higher use of other medications which can be used to treat pain, including NP (such as gabapentin and pregabalin). Additionally, both NP phenotypes report much lower levels of satisfaction post-TJR compared to those individuals without possible or likely NP.

6.7 Limitations

For a more detailed discussion of the limitations of the methods and work used and described in this thesis, please see the relevant chapters.

Various questionnaires can be used to assess pain post-TJR, the use of which has both advantages and disadvantages. The WOMAC questionnaire, developed in 1982 for use in OA patients, has been widely used and tested for reliability (Bellamy, 2012, Bellamy et al., 1988). A Likert or visual analogue scale (VAS) of varying lengths can be used to answer the questions (Reginster, 2001). However, there are difficulties when comparing WOMAC scores between studies, as there is not a standard way of presenting the scores. Some papers have recommended that the scores are scaled from 0-100 to allow easier comparisons between studies (Ackerman, 2009a), and this is seen in several studies (Wylde et al., 2011, D'Agostino et al., 2005, Ackerman, 2009b). When categorising the scores, there are different cut offs and methods used by several studies. One study by Wylde et al., 2011 used arbitrary cut-offs using scores on a scale of 0-100 (Wylde et al., 2011). These cut-offs were the ones used in the research described in this report.

It should be reiterated that a key limitation of this study is the lack of pre-operative and PROMS data. This study was designed to investigate the genetics of pain in OA and the questionnaire design reflects this. However, although we do not have PROMS data available, the questionnaire used in this study accounts for 89% of the material covered in the PROMS. I believe that this questionnaire also addressed some aspects of patient health which are not covered by PROMS. The questionnaire used in this study reflects numerous aspects of health such as prescription analgesic medication use, the presence of comorbidities and smoking behaviour. Additionally, the standard PROMS do not contain any questions relating to NP, which as shown here to be a strong contributor to post-TJR outcomes. These factors are significant contributors to post-TJR satisfaction.

Pre-operative data would be a valuable addition to this data. However, with regards to PDQ, one study found that it was not possible to reliably predict post-TKR outcomes from pre-operative pain intensity and PDQ scores (Phillips et al., 2014) whereas Dualé et al. have reported a higher risk of NP post-surgery if peripheral NP is present pre-surgery (Dualé et al., 2014).

The PDQ used to classify individuals as having unlikely, possible and likely NP in this work has been validated for use in OA and is widely used in studies on NP (Hochman, 2011, Gwilym, 2009). It has been shown to have high sensitivity, specificity, and positive predictive accuracy at both the individual and cohort level (Freynhagen, 2006). As discussed previously in this thesis, the PDQ is a self-administered questionnaire and as such does not contain an element of supplementary clinical examination. Although this methodology allows data collection from a large number of individuals it does not provide definitive evidence of NP (Bouhassira, 2011). Due to the self-administered nature of the PDQ, physical tests of NP are not possible to supplement the questionnaire scores, as they are in clinician-administered measures (Bouhassira, 2011). Because NP is the result of changes to the nervous system, a diagnosis of NP needs to be confirmed by investigation to assess the lesion or issues in the nervous system (Bouhassira, 2011). Despite not having the ability to confirm or locate somatosensory lesions, as is possible with clinical tests (as described in **Section 2.2.2 Clinical assessment**) the results of this questionnaire

have been found to correlate well with the results from fMRI scans and clinical tests for NP (Hochman et al., 2013, Gwilym, 2009). More specifically, one of these studies showed a correlation between PDQ scores and periaqueductal grey matter activation (which is involved in central sensitisation) in people with OA in areas of referred pain in response to punctate stimuli (Gwilym, 2009). The other reported that the presence of possible NP (classified using the PDQ) was associated with a higher likelihood of central sensitisation, assessed using QST (Hochman et al., 2013). A recent study reports that the PDQ showed “satisfactory hypothesis testing and criterion validity” (Mathieson et al., 2015).

Although added clinical examination would help obtain a more definitive diagnosis of NP, the results of these studies support the use of the PDQ in people with painful OA. The PDQ provides a way to identify individuals with possible NP, and so at a higher risk of post-TJR dissatisfaction, according to the results of this analysis.

6.8 Further work

Although a number of the GWAS-identified signals in this analysis showed a consistent direction of effect in the two available replication cohorts, further replication is still required to aim for genome-wide significance. Further replication analysis would ideally strengthen the association, and potentially improve the p value for the variant to levels reaching genome-wide significance. Even for well-powered studies (i.e. large discovery cohorts) replication of the findings is essential if there is to be confidence in the results (Kraft et al., 2009).

Follow-up studies should have sufficient power to reliably detect the observed effect, whether this is when analysed individually or in a meta-analysis. Differences in the population, which could in theory be caused by differences in methodology or study design, or heterogeneity in the population (for example, individuals of different ethnic backgrounds) can also confound the results of genetic and replication studies, and should be considered when

identifying possible replication cohorts (Kraft et al., 2009). The use of large datasets for replication is dependent on collaboration and data sharing, such as through Consortia (Kraft et al., 2009).

For the analysis undertaken in this thesis specifically, ideal replication cohorts would be sourced from large studies on individuals with knee or hip pain, knee or hip OA or post-TJR, for whom joint-specific PDQ scores were available.

Functional analysis has been undertaken in some studies of pain, regarding the functional effects of sodium channel mutations on DRG neuron excitability (described in (Dib-Hajj et al., 2015)). Variants in the SCN11A/Nav1.9 gene have identified and functional characterisation has been done on these variants in human participants with peripheral NP. In one study, participants with peripheral NP were recruited (Huang et al., 2014). Mutations were identified, and functional profiling by electrophysiological recordings ascertained the role of these variants (Huang et al., 2014). These mutations were associated with a gain-of-function and changes to the activity of DRG neurons (Huang et al., 2014).

One way to study the functional relevance of the genes identified by my work on OA or post-TJR centrally augmented pain is to use a knockout approach on animal models of OA pain, such as the one used by Miller et al. to show the involvement of CCR2 receptors on the development of pain in knee OA. In this study the surgical destabilization of the medial meniscus (DMM) model of OA was used, leading to mechanical allodynia. An increase in CCR2 mRNA and protein was seen in the weeks following surgery, that is, this gene's activity was upregulated (Miller et al., 2012). The pattern of pain behaviours related to the DMM procedure correlated with this trend (Miller et al., 2012). *Ccr2*-null mice (versus the wild-type mice) developed allodynia and joint damage, but not movement-provoked pain (a proxy here for OA pain) and macrophage infiltration into the DRG was also not seen in these particular mice (Miller et al., 2012). These findings support the role of CCR2 signalling in OA pain (Miller et al., 2012). *Prkca* knockout mice are already available (Braz et al., 2004) and have been characterised for other disease phenotypes

A pharmacological approach is also feasible with the *PRKCA* gene and inhibitors against PKC alpha with various specificities are commercially available (http://www.scbt.com/chemicals-table-pkc_alpha_inhibitors.html). It is therefore possible to assess the effect of *PRKCA* antagonists on pain phenotypes for example in DMM mice.

Another way to influence gene expression of a gene of interest is via gene silencing. This method has an advantage over gene knockout in that no DNA is removed from the genome, rather the expression of the gene of interest is greatly reduced. Clustered regularly interspaced short palindromic repeats (CRISPR) can be used for this purpose, and more specifically, the CRISPR-Cas9 system. This involves the use of the Cas9 nuclease (Jinek et al., 2012). This method allows the genome to be edited (Sander and Joung, 2014). As changes in gene expression can be associated with the development of diseases, this is a relevant area of study.

This approach has been used in the study of chronic pain. Variants in the *FKBP51* gene have been previously linked to an increased likelihood of pain in response to trauma (Bortsov et al., 2013). A recent study has shown that *FKBP51* knockout mice, when compared to wild-type mice, cope better with chronic pain induced by arthritic joints and nerve damage (Maiarù et al., 2016). RT-qPCR showed upregulation of *FKBP51* in the dorsal horn after inducing ankle joint inflammation. Secondly, *FKBP51* knockdown using small interfering RNA (siRNA) in the spinal cord also led to reduced hypersensitivity in wild-type mice (Maiarù et al., 2016). Finally, spinal administration of an *FKBP51* inhibitor (SAFit2) (Gaali et al., 2015) showed the same effect. Knockout mice were still sensitive to acute pain, however, which supports the theory that acute and chronic pain may differ in their mechanisms (Maiarù et al., 2016).

An *FKBP51* antagonist showed anti-inflammatory effects in both wild-type and knockout mice, however after injury, increased inflammatory signalling was seen in the wild-type mice, but not in the knockout mice (Maiarù et al., 2016). This type of work may be feasible on some of the molecules identified by my research as relevant to NP pain.

In the future, CRISPR-Cas9 analysis could be used as an approach to test the effects of suppressing the genes identified in the analyses described in this thesis. Indeed, changes in gene expression have been the subject of some studies on the genetics of NP in animal models. For example, changes in gene expression in the DRG were studied after peripheral nerve injury in a rat model (Costigan et al., 2002). Some of the identified genes have known functions, and so could be classified into functional classes (Costigan et al., 2002). If the putative function of a gene is known, potential effects of gene editing or knockout can be hypothesised and then tested.

6.9 Conclusions

The work described in this thesis has contributed to the understanding of NP in OA by identifying reproducible genetic effects on the risk of NP in people with knee pain, OA and post-TJR. This was the first GWAS on NP in OA and one of the few which has used a self-reported measure of pain as the phenotype (versus using a disease diagnosis, such as a diagnosis of OA, to classify individuals). As well as this, the role of NP on post-TJR satisfaction has been clearly demonstrated. NP was found to be the single strongest predictor of post-TJR satisfaction in this work and was also linked to the likelihood of taking prescription analgesic medications, even after adjustment for other risk factors. Variants in genes of interest in pain and inflammation in OA have also been identified and reinforced, with a suggestion towards their therapeutic potential.

It is hoped that the results from this thesis will inform future genetic studies into NP and highlight the importance of taking into account the presence of NP in people with OA, such as by testing for NP symptoms at every stage of OA and undertaking tests to confirm its presence, when making treatment decisions for people with OA and when assessing the success of treatments.

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8 APPENDIX

Figure 22 The consent form for enrolment in the Genetics of Osteoarthritis and Lifestyle study

 The University of Nottingham	Nottingham City Hospital  NHS Trust
THE GOAL STUDY (Genetics of Osteoarthritis and Lifestyle) CONSENT FORM (Principal Investigator: Professor Michael Doherty, Academic Rheumatology)	
In order to participate in the GOAL Study, you MUST circle yes to show that you agree with ALL of the following statements.	
I have read and understood the information leaflet and consent form	Yes
I have had the opportunity to ask questions about the study, and my questions have been answered satisfactorily	Yes
I understand that my participation in the study may involve x-rays of my hands, knees and hips solely for the purposes of this study	Yes
I understand that the study will involve taking up to 20 ml (4 teaspoons) of my blood for genetic testing (DNA). I understand the DNA will be stored at the City Hospital, Nottingham and at AstraZeneca for up to 20 years	Yes
I understand that I will not receive the results of genetic testing	Yes
I understand that the study will involve taking up to 30 ml (6 teaspoons) of blood for bone markers, part being stored at the City Hospital, Nottingham and part at AstraZeneca for up to 20 years	Yes
I understand that the study will involve additional tests, to assess my bones, which will involve an x-ray scan of my heel (DEXA) and a urine test	Yes
I understand that information from my study records may be needed for the study and I agree to approved personnel from Government or Ethical bodies or from AstraZeneca having access to these records.	Yes
I understand that by taking part in this genetic research I agree that AstraZeneca and the University of Nottingham may apply for and use patents relating to the research	Yes
I understand that I am free to withdraw from the study at any time, without having to give a reason and without it affecting my future medical care	Yes
I agree to take part in this study	Yes

By signing this consent form you agree to **all** of the above statements.

Research Subject's Name	Subject's Signature	Date
Witness' Name	Witness' Signature	Date

Version 2 – 13/10/04

Figure 23 The consent form for the Nottingham Genetics of Osteoarthritis Study

ID number:

Nottingham University Hospitals 
NHS Trust



The University of
Nottingham

**NOTTINGHAM GENETICS OF OSTEOARTHRITIS STUDY
CONSENT FORM**
(Principal Investigator: Professor Michael Doherty)

	Please initial below
I confirm that I have read and understand the information sheet dated 16/06/10 (version 7) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without my medical care or legal rights being affected.	
I understand that participating in this study will involve me being seen in my home by a researcher who will ask me some brief questions about my lifestyle and who will then take a blood sample (15 ml). This blood sample will be used for genetic testing. I understand that I will not receive the results of this genetic testing.	
I understand that samples taken for this study will be stored in Academic Rheumatology and/or St Thomas' Hospital, London for up to 20 years. The cells in my blood will be used to examine DNA and may also be tested for biomarkers (blood components such as enzymes, hormones, proteins) that might identify risk or severity of osteoarthritis. However, St Thomas' Hospital will not be provided with any personal details.	
I understand that Academic Rheumatology and/or St Thomas' Hospital, London may pass on my samples to other academic centres or commercial companies in order to assist in this research, including processing of samples and analysis of data. However, no such third party will have access to any of my personal details.	
I understand that relevant sections of my study notes may be looked at by responsible individuals from the University of Nottingham or the City Hospital (Nottingham University Hospitals NHS Trust) or from regulatory authorities. I give permission for these individuals to have access to my study records.	
I agree to participate in the above study.	

Name of Participant

Name of Person taking consent

Date

Date

Signature

Signature

Figure 24 The consent form for enrolment into the Arthritis Research Council Osteoarthritis Genetics study

ID number:

Nottingham University Hospitals
NHS Trust





0870 850 5000
www.arc.org.uk
Committed to curing arthritis



The University of
Nottingham

GENETICS OF OSTEOARTHRITIS – THE arcOGEN STUDY

CONSENT FORM
(Principal Investigator: Professor Michael Doherty)

	Please initial below
I confirm that I have read and understand the information sheet dated 11/2/10 (version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without my medical care or legal rights being affected.	
I understand that participating in this study will involve me being seen in my home by a researcher who will ask me some brief questions about my lifestyle and who will then take a blood sample (15 ml). This blood sample will be used for genetic testing. I understand that I will not receive the results of this genetic testing.	
I understand that samples taken for this study will be stored in Academic Rheumatology and either St Thomas' Hospital, London or the University of Manchester for up to 20 years. The cells in my blood will be used to examine DNA and may also be tested for biomarkers (blood components such as enzymes, hormones, proteins) that might identify risk or severity of osteoarthritis. However, St Thomas' Hospital or the University of Manchester will not be provided with any personal details.	
I understand that Academic Rheumatology and/or the St Thomas' Hospital, London or the University of Manchester may pass on my samples to other academic centres or commercial companies in order to assist in this research, including processing of samples and analysis of data. However, no such third party will have access to any of my personal details.	
I agree to a copy of my ~~anonymised~~ pre-surgical x-rays being sent to Sheffield for further analysis.	
I understand that relevant sections of my study notes may be looked at by responsible individuals from the University of Nottingham or the City Hospital (Nottingham University Hospitals NHS Trust) or from regulatory authorities. I give permission for these individuals to have access to my study records.	
I agree to participate in the above study.	

Name of participant

Name of researcher

Signature of participant

Signature of researcher

Date

Date

Version 5 (11 February 2010)