Siri Bjorland

Genetic variability and persistent low back and lumbar radicular pain
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Oslo, July 2018

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AF</td>
<td>annulus fibrosis</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<td>CDD</td>
<td>change in disc degeneration</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>COMT</td>
<td>catechol-O-methyltransferase</td>
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<td>COL</td>
<td>collagen</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>DD</td>
<td>disc degeneration</td>
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<tr>
<td>DH</td>
<td>disc herniation</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DRG</td>
<td>dorsal root ganglion</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EMG</td>
<td>electromyography</td>
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<td>EP</td>
<td>endplate</td>
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<tr>
<td>GP</td>
<td>genetic polymorphism</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association studies</td>
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<tr>
<td>HIZ</td>
<td>high intensity zone</td>
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<tr>
<td>HSCL</td>
<td>Hopkins symptoms checklist</td>
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<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>IVD</td>
<td>intervertebral disc</td>
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<td>LBP</td>
<td>low back pain</td>
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<td>LRP</td>
<td>lumbar radicular pain</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>NP</td>
<td>nucleus pulposus</td>
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<tr>
<td>ODI</td>
<td>Oswestry disability index</td>
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<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>OPRM1</td>
<td>opioid receptor mu 1</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>SPSS</td>
<td>statistical package for the social sciences</td>
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<tr>
<td>VAS</td>
<td>visual analogue scale</td>
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<tr>
<td>VNTR</td>
<td>variable number of tandem repeats</td>
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<tr>
<td>VDR</td>
<td>vitamin D receptor</td>
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LIST OF PAPERS

The thesis is based on the following papers, which are referred to in the text by their roman numerals I–IV:

**Paper I**  

**Paper II**  

**Paper III**  
Bjorland S, Gjerstad J, Swanson D, Røe C. Persistent lumbar radicular and low back pain; impact of genetic variability versus emotional distress. *Submitted*

**Paper IV**  
ENGLISH SUMMARY

Back pain is a common and major source of disability. Although the majority of patients recover, 10–20% develop persistent pain. Previous data suggest that persistent back pain may be associated with genetic factors. In this thesis, the role of genetic variants regarding persistence of back pain and disc degeneration (DD) was addressed. In patients with low back pain (LBP) and lumbar radicular pain (LRP) the correlation between eight genetic polymorphisms (VDR, COL11, MMP1, MMP9, IL-1α, IL-1RN, OPRM1, COMT) and pain recovery as well as the association between genetic variability and DD were assessed. The present data demonstrated that the rare allele of MMP9 rs17576 A>G was associated with poor recovery and that the rare allele of OPRM1 rs1799971 A>G was associated with better pain recovery at 5-year follow-up. The association between MMP9 rs17576 A>G and pain recovery did not change substantially after adjusting for the level of emotional distress. No association between the genetic factors and change in DD was observed, and no association between disc degeneration and persistent pain was revealed. Age and DD at baseline were associated with development of DD over a 5-year period.
NORSK SAMMENDRAG

Ryggsmarter er et utbredt problem. De fleste blir kvitt sine ryggsmarter men 10-20 % får vedvarende plager, ofte med uttalt funksjonssvikt. Tidligere studier har vist at det kan være en sammenheng mellom genetisk faktorer og vedvarende ryggsmarter. I dette arbeidet ble genetiske varianter av betydning for vedvarende ryggsmerte og isjias samt skivedegenerasjon undersøkt. I pasienter med korsryggsmerte og radikulær smerte studerte vi sammenhengen mellom 8 genetiske varianter (VDR, COL11, MMP1, MMP9, IL-1α, IL-1RN, OPRM1, COMT) og langvarig smerte, samt sammenhengen mellom genetisk variasjon og utvikling av skivedegenerasjon. Våre resultater viste at den genetiske varianten MMP9 rs17576 A>G har sammenheng med vedvarende smerte mens den genetiske varianten OPRM1 rs1799971 A>G har sammenheng med mindre smerte ved 5 års kontroll. Sammenhengen mellom MMP9 rs17576 A>G og smerte forble uendret etter korreksjon for emosjonelt stress. Ingen sikker sammenheng mellom de genetiske variantene og utviklingen av skivedegenerasjon ble observert. Det ble heller ikke funnet noen sammenheng mellom utvikling av skivedegenerasjon og vedvarende smerte. Alder og skivedegenerasjon ved inklusjon var assosiert med utvikling av skivedegenerasjon over en 5 års periode.
1. Introduction

Persistent low back pain (LBP) has a point prevalence of 9-45% and creates a substantial personal and financial public burden globally\(^4\)\(^-\)\(^11\). Lumbar radicular pain (LRP), also referred to as sciatica, account for 5-10% of these LBP conditions\(^12\),\(^13\). The back pain problem has increased markedly during the last 25 years and has recently been listed as the leading global cause of disability\(^5\),\(^14\)-\(^16\).

Approximately 40 years ago, Georg Engel presented the biopsychosocial model, a new way of understanding patient suffering\(^17\). Treatment of back pain has during the last decenniums been based on this biopsychosocial concept, with activity in focus replacing the traditional model with passive rest\(^12\).

Working in the outpatient clinic is challenging and physicians meet patients with biological, psychological and social problems related to their back disorders. Although good evidence exists for the role of biological, psychological, and social factors in the aetiology and prognosis of back pain, synthesis of these components in research and clinical practice has been suboptimal\(^18\). In addition the absence of established biomarkers of back pain has led to increased efforts to identify biological components of such pain that can serve as prognostic markers\(^18\),\(^19\). Estimates of heritability effects shown in twin studies range from 30–45 % and the genetic component seem to be higher for more chronic and disabling LBP than acute and less disabling LBP\(^20\)-\(^24\). However, twin studies need to be followed-up by studies addressing single nucleotide polymorphisms (SNPs), small-scale insertions/deletions and polymorphic repetitive elements.

Previous genetic studies have suggested an association between genetic polymorphisms and persistent pain as well as genetic polymorphisms and disc degeneration. However, cell line experiments are warranted to evaluate causal relationships. A better understanding of the biological mechanism underlying LBP may be important to further improve the management of back pain\(^19\). Longitudinal human studies may uncover specific genetic variants as biological predictors. To
integrate both biological and the psychosocial prognostic factors in a clinical biopsychosocial approach is crucial.

1.1 Pain and nociception

**Definitions and classifications**
Pain is by definition subjective 25. The International Association for the Study of Pain (IASP) defines pain as ‘an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage’ (International Association for the Study of Pain Web site, June 2017; https://www.iasp-pain.org/Taxonomy#Pain). Hence, pain is an experience and a result of complex interaction between sensory, emotional, cognitive and contextual factors. In contrast, nociception refers to the neural process of encoding and processing noxious stimuli 26. Sensory impulses are conducted through myelinated Aβ fibres, thinly myelinated Aδ fibres and unmyelinated C fibers to the dorsal root ganglia (DRG) (Fig 1). The thick Aβ fibres transmit tactile information, while pain transmission is conducted by less myelinated Aδ and C fibres. The activation threshold is higher for the pain-conducting fibres 27.
Increased responsiveness and reduced threshold of nociceptive neurons in the periphery, to the stimulation of their receptive fields, is termed peripheral sensitisation (IASP website, June 2017; https://www.iasp-pain.org/Taxonomy#Pain), whereas increased responsiveness of nociceptive neurons in the central nervous system (CNS) is referred to as central sensitisation.

Pain is often categorised as being nociceptive, inflammatory or neuropathic. Nociceptive pain arises from tissue damage which causes activation of nociceptors without involvement of nerve damage. When the nociceptor is activated from an immune or inflammation response, the term inflammatory pain is used. Neuropathic pain is in contrast defined as pain caused by lesion or disease of the somatosensory system.

**Physiological pain pathways and pain modulation**

Delivering a signal from the periphery to the somatosensory cortex involves activation of peripheral nociceptors and the transmission of nociceptive signals...
from the peripheral nervous system (PNS) to the central nervous system (CNS). On the way, the pain signal is modulated at different levels in the PNS and CNS. Several pathways are involved in the transmission. 

**Figure 2: Pain pathways**

Nociceptors conduct signals in the nociceptive A δ-fibres and C-fibres which synapses with projection neurons located in the superficial lamina (I and II) and deep lamina (V and VI) of the spinal dorsal horn. The signal is conveyed across the synapse through the presynaptic release of glutamate, substance P or other neuropeptides. The response can be modulated by inhibitory or excitatory neurons. Non-neural cells such as astrocytes and microglia give biochemical support and may modulate nociceptive transmission. Dorsal horn neurons transducing pain and temperature belong to the anterolateral system and project to the posterior group of nuclei in the thalamus through a pathway termed the
spinothalamic tract. Axons project further from the ventral posterior thalamus to several cortex areas including the somatosensory cortex. Modulatory effects are largely mediated by descending monoaminergic pathways, inhibiting the transmission of nociceptive inputs at dorsal horn level. Monoamines include serotonin, norepinephrine and dopamine. Previous data have shown that activation of the opioid receptor system may be associated with reductions in the sensory and affective ratings of the pain experience, with distinct neuroanatomical involvements. Moreover, pain may be associated with activity in the amygdala ipsilateral to pain stimulus and in the contralateral ventrolateral portion of the thalamus.

Changes in expression of certain ion channels and synaptic modulators may occur after tissue and nerve damage and this can cause peripheral sensitization. If the stimuli persists, this peripheral sensitisation can lead to neurochemical and structural changes in the CNS and central sensitisation. Such sensitisation is normally an adaptive process that is resolved soon after the inputs from noxious stimuli stops; however, intense abnormal somatosensory processing, which persists beyond the normally expected time course relative to the stimulus, can give rise to neuroplastic changes that perpetuate central sensitization, resulting in chronic pain. Recent evidence suggests that astrocytes, microglia and other central and peripheral immune cells may have a role in initiation of peripheral and central sensitisation. They contribute to the plastic changes occurring within pain pathways that result in sensory dysfunctions. Biochemical and inflammatory factors may contribute to the transition of acute towards chronic pain, and genetic components may modulate any of these factors. The central modulation of pain is complex and assumed to be particularly prominent in chronic pain.

Context including present psychological state as well as prior experiences are of importance. Therefore, the response to pain varies from subject to subject. Furthermore, in the absence of anatomical causes of persistent pain it has been postulated that the condition may be ‘centrally driven’.
1.2 Pathophysiology of the intervertebral disc and vertebral endplate

The normal anatomy of the intervertebral disc (IVD) consists of annulus fibrosis (AF) encapsulating the central nucleus pulposus (NP). The AF comprises Type 1 collagen fibres while NP is a semifluid mucoid material consisting of 70–90% water. Vertebral endplate (EP) comprises two layers of cartilage, covering the top and bottom of each disc 38, 39.

Pathogenesis of the disc is complex and thought to involve changes in biochemistry and metabolic transport mechanisms resulting in alteration of the structure and function of IVDs 40. Decreased extracellular matrix production, increased production of degradative enzymes, and increased expression of inflammatory cytokines contribute to the loss of structural integrity and accelerate IVD degeneration 38.

Enzymes involved in matrix degradation and turnover, specifically aggrecanases and metalloproteinases (MMPs), have been proposed to play a role in the degenerative process 41. Degeneration of the AF is manifested as annular tears.

Figure 3: Intervertebral disc (IVD)
Radial annular tears and fissures have been linked to ingrowth of blood vessels and nerve fibres.\(^\text{42}\) Disc degeneration (DD) is a possible cause of back pain and is identified by MRI. The challenge is that disc bulging, annular tears, narrowing, degeneration, herniation and stenosis are also seen on MRI in asymptomatic individuals.\(^\text{43-46}\) It has been argued that pathological degeneration is different from the normal aging process. During normal aging, the height of the disc is maintained and radial tears or endplate defects are absent, but the disc becomes harder and stiffer and anterior osteophytes may develop.\(^\text{40}\)

Changed signals in the vertebral endplates on MRI (Modic changes) have received much attention in recent years. It has been reported that endplate changes are associated with LBP,\(^\text{47}\) but how these changes influence the clinical time course of back pain is not clear.\(^\text{48}\) Some studies suggest that the changes are signs of vertebral inflammatory processes.\(^\text{49, 50}\) The aetiology and pathobiology of Modic changes are still unknown, but recent data may indicate infection or autoimmune aetiologies, both of which presuppose structural damage of the disc.\(^\text{51}\)

### 1.3 Low back and lumbar radicular pain

**Definition, epidemiology and etiology**

LBP is defined as pain and discomfort, localised below the costal margin and above the inferior folds, with or without referred leg pain.\(^\text{52}\) In contrast radicular pain is a type of pain that radiates into the lower extremity directly along the course of a spinal nerve root. Typically L2–L4 conducted by the n.femoralis and L5–S1 conducted by the sciatic nerve. Mechanical compression of nerve roots and or to local release of biochemical mediators may lead to lumbar radicular pain (LRP). The term persistent or chronic LBP or LRP is used when the duration of pain is more than three months.\(^\text{53}\)

Pain in the lower back is one of the most common medical problems in the adult population and is ranked highest in terms of disability. Heterogeneity exits among
LBP epidemiological studies, with a mean one-year prevalence of 38% \(^4, 8, 13, 54\). LRP, also referred to as sciatica, account for 5–10% of these LBP conditions \(^12, 13\). Although the majority of patients recover, 10–20% develop more persistent pain \(^55\). In addition, about one-third of the recovered patients will experience a recurrent episode of LBP \(^56\).

Different structures may cause LBP. Disc and facet joint degeneration has been a particular research focus \(^45\). An experimental study in pigs illustrates the interplay between signals arising from the disc, the facet joint and the multifidus muscles \(^57\). Previous data suggest that LBP may have a muscular genesis \(^58\). Disc herniation may cause a biochemical irritation and/or mechanical compression in a radicular pattern but focal disc abnormalities can be observed in those without LBP, and symptomatic discs can become asymptomatic \(^59\).

Genetic and bio-mechanical models have contributed to the understanding of back disorders and back pain \(^60\). Data from a study of twins suggest that heritability regarding LBP ranges from 30–45% \(^20, 21, 23, 24\). Genetic variability, which is important for degenerative changes, inflammation or pain perception, may play a role in both LBP and LRP \(^23, 61-63\).

Moreover, genetic variability that influences susceptibility to environmental factors may influence the risk of chronic pain \(^64-66\).

**Clinical presentation**

Signs and symptoms of LBP include pain, muscle tension, or stiffness localised below the costal margin and above the inferior gluteal folds, with or without referred and radiating leg pain (sciatica) \(^13\). Pain referred from facet joints, back muscles or structures in the hip and pelvis may radiate to the thigh in a non-radicular pattern and should be discriminated from radicular pain. The variety of clinical presentation reflects the heterogeneity of the LBP and is a challenge in the diagnostic process \(^58, 67\).
LRP is characterised by radiating pain that typically follows the dermatome of the affected nerve root from the lumbar or sacral spine. The clinical presentation is well described and usually involves the L3, L4, L5 or S1 root. The intensity of the radiating pain usually exceeds the pain intensity reported localised in the back but varies from patient to patient. The radiating pain is typically described as shooting and exacerbated by coughing, at least in the acute phase. Although the most common causes are disk herniation and spondylosis causing lateral recess or foraminal stenosis, physicians should be aware of ‘red flags’ described as symptoms and signs that warrant a more comprehensive examination. This examination is conducted to exclude other rare causes such as infection, spinal tumours or metastasis.

**Diagnosis and classification**

The diagnosis of LRP is based on symptoms and clinical findings and may be confirmed by MRI, alternatively by CT or CT myelography. More detailed assessment of nerve dysfunction can be conducted by EMG/neurography, and may be useful when the indication for operation is difficult. For patients with non-specific LBP diagnostic imaging is only recommended when symptoms and signs cannot exclude specific underlying conditions.

A precise patho-anatomical diagnosis is elusive in most LBP patients and therefore the diagnostic process may be frustrating for both physician and patients. European and American guidelines recommend the use of diagnostic triage which classifies LBP into three categories: specific spinal pathology, nerve root pain and nonspecific LBP. Specific pathology should be suspected in the presence of ‘red flags’ in the history and clinical examination. However, the majority of patients (80–90%) have no red flags and are according to diagnostic triage classified as nonspecific LBP, suggesting non-inflammatory, none malignant conditions without nerve root compression. A variety of classification systems have been launched to sub classify this large and heterogenic group of nonspecific LBP patients.
The majority of classification systems are descriptive, but several of them are often applied to assess the prognosis and guide the treatment. A traditional descriptive based approach is illustrated by the Quebec Task Force classification (QTFC) which classifies patients into 1 of 11 diagnostic categories according to the presence of pain, anatomical location of pain, presence of neurological signs, findings from radiological imaging techniques, and surgical history. Strong et al. developed a prognostic classification based on clusters of six dimensions: pain intensity, functional disability, attitude towards pain, pain strategies, depression and illness behaviours. In 1983 the fear avoidance model was presented by Lethal et al. and later elaborated by Vlayem et al. Although a model more than a sub classification – it is today the most widely used single factor to guide prognosis and recovery (see below). McKenzie’s classification of patients into three main groups based on physical signs, symptom behaviours, and lumbar movement tests is also widely used to guide treatment, particularly physical therapy. Several further examples of classification systems exist, but there is no gold standard for sub classification. Yet, it has been stated that without sub classification, research in patients with nonspecific chronic LBP is unlikely to provide useful insights.

**Prognosis and recovery**

The term prognosis refers to the risk of future health outcomes in people with a given disease or health condition. Prognostic factors refer to any measure that among people with a given health condition (a start point), is associated with a subsequent clinical outcome (an end point). Recovery refers to returning to normal health status and equivalents the prognosis for normal health status outcome. Prognosis in the absence of any treatment defines the natural time course of a disease and is very good with respect to acute and subacute (4–12 weeks) LBP. The majority of patients with LRP have spontaneous regression of symptoms. Regression within three months defines the acute/subacute pain conditions whereas pain persisting for longer than three months may be defined as chronic.
pain conditions (International Association for the Study of Pain Web site, June 2017; https://www.iasp-pain.org/Taxonomy#Pain). However, the literature does not present a uniform definition of pain recovery, and measurements as well as levels and thresholds vary. Visual analogues or numeric scales are often applied, but the required threshold for pain-free status varies. Furthermore, decline of pain may also be applied, although strictly speaking this may be evaluated as improvement and not recovery. The advantage of applying improvement is that it can be based on calculations of clinically important differences in the individual patients. It has been suggested that for pain intensity measured by VAS (scale 0–10), the minimal clinical importance change (MCID) is 3.5 points for chronic LBP 91. Yet, also here there is a lack of consensus and other studies have reported a threshold of 2.4 92.

Recovery also reaches beyond the phenomenon of pain. A systematic review by Kamper et al. 93 revealed that 82 included studies used 66 different measures of recovery in LBP. Pain, function based on self-reports of bodily functions and daily activities or physical performance measurements, return to work and participation in leisure activities and sports as well as time to insurance claim closure are examples of measurements of recovery presented in the literature 93. A third concept applied is success rate, where the percentage of patients with 30% improvement in outcomes is calculated 94.

The episodic nature of LBP challenges the definition of improvement and recovery. When the pain level has declined in patients, it may represent recovery, but it can also represent a short pain free-period in the natural course of LBP. The explanation for the episodic nature of back pain is intriguing – and it has been suggested that there is an intrinsic reason for recurrent LBP, and that external factors trigger the onset and severity of episodes 95.

Still, transition from acute to persistent pain (i.e. recovery in low back and lumbar radicular pain patients) may be influenced by demographic, lifestyle, occupational and psychological factors. Age and gender may be of importance regarding persistent back pain. Many earlier studies have shown that back pain problems
increase up to the age of 60–65 years \(^{45, 54}\) and some studies identify gender differences \(^{10, 54, 96, 97}\).

Previous data have also shown that long-lasting LBP may be associated with lifestyle factors such as smoking \(^{5, 98, 99}\). The literature is, however, not consistent regarding dose-response, which challenges the possible physiological causal link between smoking and LBP \(^{98, 100}\). Obesity is also suggested to be related to prolonged duration of back pain, although evidence for a causal link has not been established \(^{96, 101, 102}\).

Occupational mechanical factors such as heavy workload and lifting or body vibration are suggested to be negative prognostic factors that contribute to persistent LBP and LRP \(^{102-104}\). Physical or sporting activity during leisure time has also been suggested to influence recovery but indicate a U-shaped relationship between activity and chronic LBP \(^{102}\).

Psychological factors are deemed to be of major importance for poor recovery and transition into chronic back pain \(^{105-107}\). Psychological factors embrace a broad spectre of factors including personality, mood (i.e. anxiety and depression) and cognitive and behavioural responses to stress. In the back pain field most focus has been put on mood and responses to stress because these factors are deemed to be malleable. Particularly depression, but also anxiety, are associated with chronification of LBP \(^{106}\). It is suggested that only around 10–20\% may fill the diagnostic criteria for anxiety or depression disorder according to DSM IV. The patients themselves may focus more on their bodily symptoms such as palpitations, becoming startled and dizzy or being fatigued and sleepless. Such symptoms are in the absence of specific diseases considered to present somatisation and are in combination with mood changes often termed emotional distress \(^{105}\). According to the cognitive activation theory of stress (CATS), these symptoms may be related to the cognitive and behavioural response to stress \(^{108}\). According to CATS the responses are influenced by previous experience forming expectations regarding outcome. As mentioned above fear avoidance is perhaps the most focused single predictor within back pain research. Fear is closely related to anxiety and assumed
to be influenced by previous experience. Fear is considered to be a normal psychosocial response to acute LBP, but prolonged fear of movement and activities (kinesiophobia and fear avoidance) in chronic pain patients is considered to be maladaptive\textsuperscript{109} and affects the course of LBP condition\textsuperscript{109}. Fear avoidance is observed not only in patients but also in health care providers including specialists\textsuperscript{110, 111}.

1.4 Genetics

Basic concepts
The human genome is our hereditary material. This genome comprises double-stranded deoxyribonucleic acid (DNA). The DNA has four nucleotide bases; A: Adenin, G: Guanin, T:Thymine and C: Cytosine. The human genome comprises approximately $3 \times 10^9$ base pairs of DNA organised into 23 rod-shaped pairs of chromosomes in the cell nucleus. Each individual carries two copies of each chromosome, one from the mother and one from the father. A gene is a locus (region) of DNA encoding functional proteins of an organism (Fig. 4).

![Figure 4: Chromosomes made of DNA located in the cell nucleus](image)
The human genome contains 20,000–25,000 genes, which can be transcribed into messenger RNA (mRNA) and further translated into proteins. Observable traits that appear in an organism as a result of its genetic makeup are referred to as phenotype. Its underlying genetic makeup is termed to as the genotype.

We all carry the same genes; however, the exact base-pair sequences vary among individuals, making every one of us genetically unique. A genetic polymorphism is an allelic variation of a gene that exists in stable form in a population at a frequency of at least 1%. Genetic variants are referred to as common if their minor allele frequency (MAP) is >5%, while rare variants have a MAP of <5%. Millions of genetic polymorphisms are found in the human population. More than 90% of these differences take the form of substitution of single base pairs (i.e. single nucleotide polymorphisms (SNPs)) (Fig. 5). Other types of polymorphism such as insertions, deletions, duplications, repeats and differences in rearrangements may also occur, but are much less frequent.

![Figure 5: Single nucleotide polymorphism (SNP): DNA strand 1 differs from DNA strand 2 at a single base-pair location (C>T polymorphism)](image)

Figure 5: Single nucleotide polymorphism (SNP): DNA strand 1 differs from DNA strand 2 at a single base-pair location (C>T polymorphism)
Genetic nomenclature
Heterogeneity in presenting information about genetic variability is identified in the literature. During recent decades, the National Center for Biotechnology Information (NCBI) in the USA has contributed to clarify the genetic nomenclature. All SNPs in the human genome are listed in a register created by the NCBI. The Rs number stands for Reference SNP cluster ID (June 2017: http://www.ncbi.nlm.nih.gov/). Replacement of one base with another in DNA is termed a base substitution. Information about the position of the replacement can also be found in the NCBI to make it precise and repeatable.

SNPs can occur in the protein-coding region of the gene (i.e. exons), or between exons (i.e. introns). A SNP on the protein-coding region that alters the amino acid sequence in a protein is called a nonsynonymous substitution and may change the protein function or activity. Substitution of one base with another without any amino acid change is called a synonymous substitution (silent substitution) without any functional influence on the protein. SNPs in the promotor region or in the introns may have a role in the transcription process. By influencing gene-regulation, the stability or splicing of messenger RNA (mRNA), such SNPs may affect the expression level of the protein. However, most of the identified SNPs in the human genome have no, or little, effect on protein activity or gene regulation. Only a small percentage of the DNA sequences in the human genome are coding sequences or regulatory sequences, and changes that occur elsewhere usually do not have any impact.

Genetic variation among humans
Between any two humans, the genome varies by approximately 0.1%. Phenotypic variation between individuals is determined by genetic variation and by environmental exposure. Similarly, the risk of diseases may be caused by genetic factors, the environment or both. Genetic variation can result in severe single-gene disorders such as cystic fibrosis, Huntington’s disease and congenital analgesia. In
such monogenic, mendelian, disease, only one or a few mutations in one gene may be necessarily to cause the diseases. Monogenic diseases are caused by genetic variants with a low frequency in the population and often have strong impact.

The risk of more common diseases/disorders, including disc herniation and persistent pain, in contrast, may be influenced by many genetic factors which moderately increase or decrease susceptibility to the disease. The heritability of a disease refers to the proportion of the phenotype variance that is due to genetic variations. For back pain, previous data have shown that the heritability ranges from 30–45%\textsuperscript{24}. Hence, a significant proportion can be explained by genetics. However, common genetic variants in the population explain only a small fraction of the hereditary of disease risk.\textsuperscript{115} Some of the unidentified heritability of complex diseases may be caused by less-studied infrequent gene variants (minor allele frequencies of 5% or less), yet not discovered in association studies.\textsuperscript{116}

Furthermore, it is also important to be aware of the effects of gene-gene interactions\textsuperscript{117} and gene-environmental interactions. For example, in fibromyalgia patients, an antagonistic effect between opioid- and serotonin-related genes has been shown.\textsuperscript{118} Also, in LRP patients, an interaction between a IL-6 haplotype and workload seems likely.\textsuperscript{119}

In addition, physical and psychological environmental exposure can produce epigenetic effects that alter gene expression.\textsuperscript{116} Epigenetic changes represent environmentally caused modifications of chromosomes without changes in the DNA sequences. Epigenetics comprise DNA methylation, histone modification controlled by microRNAs (miRNAs) that can cause changes in gene expression. Changes in methylation of the DNA due to environmental factors may contribute to gene and protein expression. This process will in turn affect the phenotype. Recent works also suggest that epigenetic states can be transmitted from parents to offspring.\textsuperscript{120-122}
Genetic studies for complex diseases
Studies aimed at uncovering genetic predispositions to complex diseases usually attempt to demonstrate an association between the genotype at one or more polymorphic markers and phenotype related to disease susceptibility. There are two main approaches in such association studies; one based on candidate genes and the other based on testing the entire genome (i.e. genome-wide association studies (GWAS)).

Candidate gene studies
The candidate gene approach has been the most widely used approach regarding complex multifactorial diseases. The selection of candidate genes is hypothesis based. Genes are selected on the basis of relevance to the disease with regard to biology and pathogenesis, and in light of associations shown for similar genes and phenotypes in animal models. The candidate gene approach has benefits and limitations; it tends to have rather high statistical power but is incapable of discovering new genes. While genetic association studies have proven successful in exploring the relationship between common genetic polymorphism and common traits and diseases, each identified variant has small effect size. Regarding pain, a number of genes have been identified by using the candidate-gene approach, but since pain is a polygenic disorder, it is likely that there are many novel genes awaiting discovery.

GWAS
GWAS is an observational study of a genome-wide set of genetic variants, typically more than one million SNPs, in different individuals to determine whether any variant is associated with a disease. In this approach, the entire genome is investigated and the most-common study design is a case-control setup which compares two large groups of individuals. GWAS also has a combination of benefits and limitations; this approach can pin-point genes regardless of whether
their function was known before but has low statistical power owing to the high number of independent tests performed. 126-128

Genome-wide association scans for genetic variants have already been conducted adding knowledge to the genetic basis underlying the continuum from personality traits to psychopathology 131. Regarding lumbar disc degeneration (LDD), a few GWAS have shown that methylation of the PARK2 promoter may influence degeneration of the intervertebral disc 132. This gene has not previously been considered a candidate gene in LDD. Further works are however needed to address causality 132. This situation illustrates the challenging work ahead using GWAS in the field of persistent back pain.

Moreover, most clinical studies do not have enough statistical strength for GWAS and this is a challenge in clinical pain research. In addition, the translation from GWAS to clinical application may be controversial (causality is not addressed). Building risk profiles based on contributing single genes controlling for relevant other predictors may facilitate the clinical assessment of the genetic versus environmental impact 133.

Genetic risk factors in persistent low back and lumbar radicular pain
Earlier research suggests that pain sensitivity and risk for chronic pain are complex heritable traits of polygenic origin 130. Genetic variability that influences susceptibility to environmental factors may influence the risk of chronic pain 64, 66.

Current evidence suggest that genetic factors associated with disc degeneration (DD), inflammation and pain perception, play a role in persistent LBP conditions 23, 61-63, 134. In addition, an association between genetic variability and psychological processing has been suggested 24.

Many studies have focused on the association between genetic factors and lumbar DD 24, 129, 135, 136. Genetic variability in genes encoding the aspirin protein (ASPN allele D14), type XI collagen (COL11A1 rs 1676486), growth differentiation factor 5 (GDF5 rs143383), sickle tail (SKT rs16924573), thrombospondin-2
(THBS2) and matrix metalloproteinase 9 (MMP9 rs17576) may be important. Moreover the phenotype of DD may vary among studies. It is suggested that such genetic variants may account for inter-individual differences in disc matrix synthesis and that DD may be a polygenetic condition. Lack of long-term follow-up is still a major challenge in the field of genetics and development of DD. In addition, variations in allele frequencies between different ethnic populations (e.g. Caucasians, Asians) suggest that genetic factors may be involved differently in the development of lumbar DD across ethnic groups. It is suggested that genetic factors associated with inflammation or modulation of pain sensitivity are more important for persistent LBP than genetic factors modulating bone and cartilage structure.

Interestingly, five genetic variants (OPRM1 rs1799971, COMT rs4680, MMP1 rs1799750, IL1α rs1800587, IL1RN rs2234677) have been reported to be associated with reduced recovery in LRP patients. Previous studies suggest that genetic polymorphisms related to inflammation in genes encoding interleukin 1 (IL-1α), interleukin-1 receptor antagonist (IL-1RN) and interleukin-6 (IL-6) may promote persistent LRP. Moreover, genetic variability that is important for opioid, dopaminergic, adrenergic and serotonergic signalling may affect supra-spinal modulation of nociceptive processing.

Variation in modulatory effects mediated by descending monoaminergic pathways can be linked to genetic polymorphisms. In particular, genetic variability related to the opioid receptor mu 1 (OPRM1) and the enzyme catechol-O-methyltransferase (COMT) may affect cortical pain processing and the risk of long lasting pain conditions. Interestingly, results from the clinical genetic study by Omair et al. 2012 suggest that a genetic variant of the COMT gene may contribute to describe the success of treatment in LBP patients.

Genetic risk factors and emotional distress
Heritability may be important for anxiety and depressive disorders. For example, large population-based longitudinal studies indicate that some
individuals have greater susceptibility to depressive affects\textsuperscript{152,153}. Shared genetic basis is suggested as underlying the continuum from personality traits to psychopathology\textsuperscript{131,154}. Interestingly, pro-inflammatory genetic variation is also shown to increase the risk of stress-induced depression\textsuperscript{155}. Moreover, a genetic variant in OPRM1 is associated with attenuated hypothalamic-pituitary-adrenal (HPA) axis responses to stress\textsuperscript{156} and may be linked to alterations in personality traits (Troisi et al., 2011). An interaction between the OPRM1 genotype and sex as well\textsuperscript{157} as subjective health complaints has also been demonstrated\textsuperscript{138}. Hence, the genetic influence of pain recovery may be closely linked to emotional factors.

**Candidate genes**

The candidate genes in this thesis were selected on the basis of relevance to the disease with regard to biology and pathogenesis as well as associations shown in animal models and clinical studies. Due to the test panel available, some limitations were found in the selection of our candidate genes. Genes tested in this thesis, relevant to the field of LBP and LRP research can be divided in three categories (Fig. 6):

1) Degeneration genes: Vitamin D receptor gene (VDR), Collagen gene (COL), matrix-degrading genes (MMPs)

2) Inflammatory genes: Interleukin genes (ILs)

3) Pain modulation genes: Opioid receptor gene (OPRM1), Catechol-O-methyltransferanse gene (COMT)
The primary function of genes tested is clarified in Table 1.

**Table 1: Primary function of genes tested**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Abbreviations</th>
<th>Main function of the protein encoded by the gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR</td>
<td>vitamin D receptor</td>
<td>VDR gene encodes the receptor for Calcitriol, the active form of D vitamin. VDR are also thought to be involved in mineral metabolism and immune responses.</td>
</tr>
<tr>
<td>COL 11A</td>
<td>collagen 11 alpha</td>
<td>COL 11A gene encodes one of the two alpha chains of type XI collagen.</td>
</tr>
<tr>
<td>MMP1</td>
<td>matrix metalloproteinase 1</td>
<td>MMP1 gene encodes for the collagenase enzyme involved in degradation of extracellular matrix, specifically the interstitial collagens type I, II and III.</td>
</tr>
<tr>
<td>MMP9</td>
<td>matrix metalloproteinase 9</td>
<td>MMP9 gene encodes for an enzyme involved in degradation of extracellular matrix, specifically the interstitial collagens type IV and V. MMP9 also integrate multiple immune-regulatory pathways, and play a role in angiogenesis and neovascularisation.</td>
</tr>
<tr>
<td>IL-1α</td>
<td>interleukin 1 alpha</td>
<td>IL-1α encodes for a cytokine responsible for the production of inflammation and plays one of the central roles in the regulation of immune responses.</td>
</tr>
<tr>
<td>IL-1RN</td>
<td>interleukin 1 receptor antagonist</td>
<td>IL-1RN encodes the protein which inhibits the activation of IL-1α and IL-1β and modulates a variety of immune and inflammatory responses.</td>
</tr>
<tr>
<td>OPRM1</td>
<td>opioid receptor mu 1</td>
<td>OPRM1 encodes for a receptor critically involved in modulation of pain. The receptor plays a role in descending pain pathways.</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyltransferase</td>
<td>COMT encodes for an enzyme involved in the activation of the catecholamine neurotransmitters (dopamine, epinephrine and norepinephrine).</td>
</tr>
</tbody>
</table>
2. AIMS OF THE STUDY

The main purpose of this thesis is to provide new knowledge about the relationship between genetic variability and persistent LBP and LRP.

I. To provide an overview of the literature addressing the role of genetic factors and biomarkers predicting pain recovery in LRP patients.

II. To assess the correlation between eight genetic polymorphisms (VDR, COL11, MMP1, MMP9, IL-1α, IL-1RN, OPRM1, COMT) and pain recovery in patients with LBP and LRP.

III. Examine the impact of emotional distress on genetic susceptibility in LBP and LRP patients over a 5-year period.

IV. Investigate whether genetic polymorphism in the genes encoding VDR, COL11, MMP1, MMP9, IL-1α and IL-1RN and disc herniation influence disc degeneration (DD) over a 5-year period and examine the association between DD and pain recovery.
3. METHODS

3.1 Systematic review (Paper I)

Search strategies
Optimised search strategies were performed using mesh words with explore and a combination of words in the title or abstract related to different expression of lumbar radicular pain, genetic polymorphisms and pain biomarkers. Inclusion criteria were prospective studies, including patient with LRP, and assessing genetic factors or pain biomarkers. The selection process is demonstrated in Figure 7 and was conducted by two authors individually followed by a meeting for agreement with access to a third reviewer in cases of disagreement.

Figure 7: Selection process Paper I
Assessment of studies

A checklist based on Sanderson et al. 158, QATSO (Quality Assessment Tool for Systematic Reviews of Observational studies) 159 and the STROBE statement guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) 160 was used. The checklist comprised seven criteria: external validity, sample size, description of sample, follow-up rate, appropriate reporting of outcome, adjustment for confounding factors and correction for multiple testing. The assessments of the two reviewers were compared and if disagreement occurred, a third person was consulted.

3.2 Study population and design (Paper II, III, IV)

The dataset comprises two ongoing prospective cohorts which are merged after inclusion but before 5-year follow-up. The study design is demonstrated in Figure 8.

Figure 8: Design of PhD project
The inclusion of patients at baseline was conducted by three physicians and the 5-year follow-up of all the LBP and LRP patients was performed by one physician.

All subjects were outpatient clinic patients. In total, 418 patients with LBP or LRP were recruited at the outpatient clinic at Oslo University Hospital (OUH) and Haukeland University Hospital (HUH).

The LRP patients were recruited at OUH (Paper II, III and IV) and HUH (Paper II) between 2007 and 2009. The LBP patients (Paper II, III and IV) were recruited at OUH between 2009 and 2011.

The inclusion criteria at baseline for LRP patients were age between 18 and 60 years, lumbar disc herniation on MRI with corresponding distribution of pain in lower limbs and positive straight leg raising test. The exclusion criteria were cauda equina syndrome, lumbar spinal stenosis, previous spinal surgery for a herniated disc at the same level or lumbar fusion at any level, generalised musculoskeletal pain, inflammatory rheumatic disease, diabetic polyneuropathy, cardiovascular disease (NYHA class III and IV), cancer, psychiatric disease, drug misuse and alcoholism, recent surgery (within one month), pregnancy, poor proficiency in the Norwegian language and non-European-Caucasian ethnicity. In Paper II, 270 LRP patients were included and followed over five years. Dropouts in the first year comprised 19 patients. We are not allowed to obtain information about reasons for drop-out due to REK regulations in Norway. Two hundred and fifty-one LRP patients were allocated to five-year follow-up with a response rate of 76% (Figure 9). In Paper III and IV, 127 LRP patients were included and followed over five years. Dropouts in the first year numbered 19 patients. One hundred and eight lumbar radicular pain patients were allocated to 5-year follow-up with a response rate of 88% (Figure 10).

The inclusion criteria at baseline for the LBP patients were age between 18 and 60 year at baseline and persisting LBP. The exclusion criteria were lumbar disc herniation on MRI with corresponding distribution of pain in lower limbs, positive Laseque, cauda equina syndrome, lumbar spinal stenosis, structural deformity of
the vertebral column, previous surgery on the back, generalised muscular and skeletal pain, inflammatory rheumatic diseases, diabetic mellitus with polyneuropathy, comprehensive cardiac disease, cancer or other serious diseases.

In Papers II, III and IV, 148 LBP patients were included and followed over five years. Dropouts in the first year amounted to 12 patients. One hundred and thirty-six LBP patients were allocated to 5-year follow-up with a response rate of 78% (Figures 9 and 10).

Figure 9: Flow diagram of study population paper II

148 low back pain (LBP) patients from Oslo University Hospital (OUH), 127 lumbar radicular pain (LRP) patients from OUH and 143 LRP patients from Haukeland University Hospital (HUH) were included at baseline. Number of dropouts in the first year was 12 patients in the LBP group and 19 patients in the LRP group. 136 LBP and 251 LRP were allocated to 5-year follow-up. 106 LBP and 95 LRP patients participated at 5-year follow-up and 61 patients in LRP group and 30 patients in the LBP group declined to participate.
The study was conducted in accordance with the Helsinki Declaration. The Regional Committee for Medical Research Ethics (reference number 2014/1754) and the Norwegian Social Science Data Service approved the study protocol and all participants gave their written informed consent at baseline and at 5-year follow-up.

* 1-year follow-up data used in previous papers \(^2\), \(^3\) but not in this study.

Figure 10: Flow diagram of study population paper III and IV

148 low back pain (LBP) patients from Oslo University Hospital (OUH) and 127 lumbar radicular pain (LRP) patients from OUH were included at baseline. Dropouts in the first year were 12 patients in the LBP group and 19 patients in the LRP group. 136 LBP and 108 LRP patients were allocated to 5-year follow-up. 106 LBP and 95 LRP patients participated at 5-year follow-up and 13 patients in LRP group and 30 patients in the LBP group declined to participate.
3.3 Clinical procedures and outcome measures (Papers II, III, IV)

All patients underwent a standardised clinical examination, which included assessment of sensory and motor functions including straight leg raising and completion of standardised pain and function questionnaires. At baseline, socio-demographic variables, including gender, age, smoking habits and BMI were registered and a baseline MRI was obtained for all patients.

![Figure 11: Measures illustrated in a modified biopsychosocial model](image)

Pain intensity was recorded using the visual analogue scale (VAS) with anchor values from 0 (no pain) to 10 (worst possible pain) at rest in the last week at baseline and at 5-year follow-up. In addition, function was assessed using the validated Norwegian version of the Oswestry Disability Index (ODI), scale 0–
100%, where 0% = no disability at all and 100% = very severe disability at baseline and 5-year follow-up\textsuperscript{162}.

Emotional distress assessed in OUH patients using a short version of the Hopkins Symptoms Check List (HSCL-10), 4-point scale, where 1 = no complaints, 2 = some complaints, 3 = moderate complaints and 4 = many complaints. The scores summarise and divide on the number of answered questions, with a mean score > 1.85 compatible with emotional distress symptoms present\textsuperscript{163}.

**Pain recovery**
The main outcome used in our study was pain intensity during rest and disability at 5-year follow-up (Paper II and III), and change in disc degeneration (DD) (paper IV). Pain recovery was defined as pain intensity at five years adjusted for pain intensity at baseline.

**3.4 Genotyping (Papers II, III, IV)**

In patients with LRP, genomic DNA was extracted from whole blood cells using a FlexiGene DNA isolation kit (Qiagen), whereas in patients with LBP genomic DNA was extracted from saliva using an Oragene DNA sample collection kit (DNA Genotech Inc.) according to the manufacturer’s instructions. SNP genotyping was carried out using predesigned TaqMan SNP genotyping assays (Applied Biosystems). Approximately 10 ng DNA was amplified in a 5 µl reaction mixture in a 384-well plate containing 1x universal TaqMan master mix and 1x assay mix, the latter containing the respective primers and probes. The probes were labelled with the reporter dyes FAM or VIC at the 5’end to distinguish between the two alleles. The reactions were performed on an ABI 7900HT sequence detection system (Applied Biosystems) using the following program: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Negative controls containing water instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Phase v.2.1.1 was
used to define the COMT haplotypes. Approximately 10% of the samples were re-genotyped and the concordance rate was 100%. The SNPs tested are listed in Table 2.

Table 2: Genetic variants tested

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>Base substitution</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR</td>
<td>rs731236</td>
<td>T&gt;C</td>
<td>-</td>
</tr>
<tr>
<td>COL 11A</td>
<td>rs1676486</td>
<td>T&gt;C</td>
<td>Ser1535Pro</td>
</tr>
<tr>
<td>MMP1</td>
<td>rs1792750</td>
<td>1G&gt;2G</td>
<td>-</td>
</tr>
<tr>
<td>MMP9</td>
<td>rs17576</td>
<td>A&gt;G</td>
<td>Gln279Arg</td>
</tr>
<tr>
<td>IL-1α</td>
<td>rs1800587</td>
<td>G&gt;T</td>
<td>-</td>
</tr>
<tr>
<td>IL-1RN</td>
<td>rs2234677</td>
<td>A&gt;G</td>
<td>-</td>
</tr>
<tr>
<td>OPRM1</td>
<td>rs1799971</td>
<td>A&gt;G</td>
<td>Asn40Asp</td>
</tr>
<tr>
<td>COMT</td>
<td>rs4680/rs6269</td>
<td>A&gt;G/A&gt;G/C&gt;G/C&gt;G</td>
<td>Val158Met</td>
</tr>
</tbody>
</table>

The rs number refers to the specific SNP, and rs stands for reference SNP cluster ID, created by the National Center for Biotechnology Information (NCBI). Base substitution refers to replacement of one base with another in DNA. Four of the SNPs cause amino acid substitution. Amino acid substitution: - (no)

3.5 MRI imaging – technique and evaluation (Paper IV)

Imaging technique

At 5-year follow-up, lumbar 1.5 T MRI was performed, including sagittal T2-weighted fast spin echo (FSE) (repetition time (TR)/echo time (TE), 2,376-4,280 ms/88-121 ms) or (in one patient) 3-D turbo spin echo (SPACE) images (TR/TE, 1,500 ms/251 ms), sagittal T1-weighted spin echo images (TR/TE, 400-720 ms/7-14 ms) and axial T2-weighted images of the L3/L4, L4/L5 and L5/S1 levels (TR/TE, 2,209-6,040 ms/93-124 ms). Baseline lumbar MRI (1.5 T in > 85% of the cases) included sagittal T2-weighted FSE (TR/TE, 2,300-4,500 ms/80-125 ms) or (in 10 patients) SPACE images (TR/TE, 1,500 ms/251 ms), sagittal T1-weighted spin echo (TR/TE, 400-750 ms/9-15 ms) or (in 13 patients) fast fluid-attenuated inversion-recovery images (TR/TE, 1,989-2,000 ms/20-21 ms) and axial T2-weighted images (TR/TE, 3,000-7,140 ms/90-131 ms). For this study, all MRIs were de-identified.
**Imaging evaluation**

A radiologist (AE, 33 years’ experience) and a physician specialist in physical medicine and rehabilitation (ES, 10 years’ experience), independently and blinded to clinical data, graded disc degeneration (DD) and the presence of HIZ at baseline and at 5-year follow-up, by comparing initial and follow-up MRIs. In all cases of disagreement, they negotiated a consensus score. ES evaluated the presence of disc herniation (DH) at baseline and at 5-year follow-up with the routine radiology report available.

DD was graded on midsagittal T2-weighted images at each of the five disc levels (L1–S1) using Schneiderman’s qualitative grading system\(^{164}\): a score of 0 indicated no signal change, a score of 1 indicated a slight decrease in signal intensity in the nucleus pulposus, a score of 2 indicated a generalised hypointense nucleus and a score of 3 indicated a hypointense nucleus with disc space narrowing. Thus, the score for each disc level ranged from 0 to 3.

In addition, we calculated a total DD score (0–15) by summing the DD grades for five lumbar discs at baseline and at 5-year follow-up, respectively. For the graphical presentation of DD in figure 2, we grouped DD at baseline and at 5 years according to Jim\(^{165}\) into severe (two or more grade 3 discs, three or more grade 2 discs, or one grade 3 and two grade 2 discs), moderate (one grade 3 disc or two grade 2 discs), mild (only one grade 2 disc and no grade 3 discs), or normal (total DD score of 0 or 1).

As another indicator of disc degeneration, the high intensity zone (HIZ) was evaluated on T2-weighted images as present or not present at each disc level. HIZ was defined as a zone in the posterior annulus fibrosus that was brighter than the nucleus pulposus and was surrounded superiorly, inferiorly and anteriorly by the low-intensity signal of the annulus fibrosus\(^{166}\).

Disc herniation was assessed at each disc level and was noted as present only if there was focal-based herniation, according to a recent definition, involving less
than 25% of the circumference of the disc, or it was an extrusion or sequestration of the nucleus pulposus.  

The radiologist (AE) assessed all MRIs on a clinical picture archiving and communication system (PACS) unit using Agfa Impax 6.5 (Agfa HealthCare, Mortsel, Belgium) software. The other observer (ES) used a dedicated personal computer at the clinic. For decision-making in cases of disagreement, the PACS unit was used. The two observers’ independent MRI evaluations were assessed for inter-observer agreement using the kappa statistic.

3.6 Statistical analysis (Papers II, III, IV)

Descriptive statistics, and a t-test and chi-square test were applied to describe the patients at baseline and 5-year follow-up and evaluate differences between LBP and LRP.

Univariate linear regression analysis was performed to estimate the correlation between the eight genetic variants (VDR, COL11, MMP1, MMP9, IL-1α, IL-1RN, OPRM1, COMT) and pain (VAS) or disability (ODI score) at 5-year follow-up (papers II and III). Univariate linear regression analysis also performed to estimate the correlation between emotional distress and pain (VAS) at 5-year follow-up (paper III). In paper IV, linear regression analysis with six genetic variants (VDR, COL11, MMP1, MMP9, IL-1α, IL-1RN) towards DD change was run. Subsequently, multivariate linear regression analysis (papers II, III and IV) with the covariates baseline pain, LRP/LBP, age, gender, smoking (yes/no), BMI (paper II) and emotional distress (papers III and IV) was performed. In paper IV, a third step adjusting for total DD score (continuous) and DH (yes/no) at baseline was also included. Furthermore, additional analysis for the relationship between radiological changes and ODI or pain at rest and during activity at 5 years was performed using multivariate linear regression (paper IV).

Regarding the OPRM1 variant, additional univariate and multivariable linear regression analyses were run separately in women and men (papers II and III).
All models were checked for collinearity (no collinearity revealed). An allele-dependent model was assumed, such that the effect of the genotype rare/rare was expected to be twice the effect of the genotype rare/common when compared to the genotype common/common.

All statistical analyses were performed using the SPSS (version 22) statistical package. A p-value < 0.05 was set as the level of statistical significance. Variables and statistics performed regarding the main outcome in papers II–IV are listed in Table 3.

**Table 3**: Overview of statistical methods used in the papers in this thesis

<table>
<thead>
<tr>
<th>Statistical analysis</th>
<th>Dependent variable (Main outcome)</th>
<th>Independent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate linear regression</strong></td>
<td>Pain intensity (VAS) rest 5 years (papers II and III)</td>
<td>Genetic factors</td>
</tr>
<tr>
<td></td>
<td>Pain intensity (VAS) rest 5 years (papers III)</td>
<td>Emotional distress</td>
</tr>
<tr>
<td></td>
<td>Disc degeneration (paper IV)</td>
<td>Genetic factors</td>
</tr>
<tr>
<td></td>
<td>Disability (ODI) 5 years (papers II and III)</td>
<td>Genetic factors</td>
</tr>
<tr>
<td><strong>Multivariate linear regression</strong></td>
<td>Pain intensity (VAS) rest 5 years (papers II and III)</td>
<td>Genetic factors</td>
</tr>
<tr>
<td></td>
<td>Pain intensity at baseline</td>
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</tr>
<tr>
<td></td>
<td>Pain location (LBP or LRP)</td>
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<tr>
<td></td>
<td>Age</td>
<td></td>
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<tr>
<td></td>
<td>Gender</td>
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<td></td>
<td>Smoking (yes or no)</td>
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<td></td>
<td>BMI</td>
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<td></td>
<td>+</td>
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<tr>
<td></td>
<td>Emotional distress (paper III)</td>
<td></td>
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<tr>
<td></td>
<td>Disc degeneration change (paper IV)</td>
<td>Genetic factors</td>
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<tr>
<td></td>
<td>Age</td>
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<tr>
<td></td>
<td>Gender</td>
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<tr>
<td></td>
<td>Smoking (yes or no)</td>
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<tr>
<td></td>
<td>BMI</td>
<td></td>
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<tr>
<td></td>
<td>Total DD score at baseline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disc herniation (yes or no) at baseline</td>
<td></td>
</tr>
</tbody>
</table>
(Table 3 continued)

<table>
<thead>
<tr>
<th>Statistical analysis</th>
<th>Dependent variable (Secondary outcome)</th>
<th>Independent variables</th>
</tr>
</thead>
</table>
| **Multivariate linear regression** | Pain intensity (VAS) rest 5 years (paper IV) | DD change  
Disc herniation change  
HIZ change  
Pain intensity (VAS) for pain at rest at baseline  
Age  
Gender  
Smoking (yes or no)  
BMI  
Emotional distress |
4. MAIN RESULTS

4.1 Paper I
The search identified 880 citations, of which 15 fulfilled the inclusion criteria. Five genetic variants (OPRM1 rs1799971 G allele, COMT rs4680 G allele, MMP1 rs1799750 2G allele, IL1α rs1800587 T allele, IL1RN rs2234677 A allele) were associated with reduced recovery of LRP. Three biomarkers (TNFα, IL6 and IFNα) were associated with persistent LRP. The review revealed a variety of pain measurements regarding description/location and no clear definition of pain recovery. Continuous changes as well as dichotomised endpoints were applied to define pain recovery.

4.2 Paper II
Pain intensity at 5-year follow-up was associated with VDR rs731236 (B=-0.5, 95%CI -0.9 to -0.1, p=0.017), MMP9 rs17576 (B=0.5, 95%CI 0.1 to 0.9, p=0.022) and OPRM1 rs1799971 (B=-0.8, 95%CI -1.4 to -0.2, p=0.006) in the univariate analyses. MMP9 rs17576 and OPRM1 rs1799971 remained significant (B=0.4, 95%CI .05 to 0.8, p=0.026 and B=-0.8, 95%CI -1.3 to -0.2, p=0.007) in the multivariable model. The data demonstrated that the rare allele of MMP9 rs17576 was associated with poor pain recovery, whereas the rare allele of OPRM1 rs1799971 was associated with better pain recovery at 5-year follow-up in LBP and LRP patients. In particular, the present study suggests that the OPRM1 rs1799971 A>G in men is associated with better long-term pain recovery. In men, the OPRM1 rs1799971 explained 4.7% of the variance of pain intensity.

4.3 Paper III
A significant association between pain intensity at 5-year follow-up and MMP9 rs17576 (B=0.71, 95% CI 0.18 to 1.24, p=0.009) as well as OPRM1 (B=-0.85, 95% CI -1.66 to -0.05, p=0.038) was found. In multivariate analysis adjusting for pain intensity at baseline, age, gender, smoking, body mass index, pain localisation and
emotional distress (HSCL-10) only MMP9 remained significant (B=0.68, 95% CI 0.18 to 1.18, p=0.008). In addition, pain location (i.e. LRP or LBP) showed a highly significant association with pain at 5 years (B=0.79, 95% CI 0.09 to 1.49, p=0.027). Hence, we demonstrated that MMP9 rs17576 and emotional distress have an additive predictive effect for persistent back pain. However, regarding OPRM1 rs179971, a clear dependence between the two predictors was revealed. LBP patients had poorer pain recovery than LRP patients also after controlling for differences in emotional distress.

4.4 Paper IV
Univariate linear regression analysis of six genetic factors (CDR, COL11, MMP1, MMP9, IL-1α and ILRN) as predictors for changes in disc DD revealed no genetic impact on development of disc degeneration (DD) during 5 years.

Lumbar disc herniation (DH) was present in 112 patients (77.8%). No significant difference in DD was found between DH and non-DH groups either at baseline or 5-year follow-up (p=0.07). Higher age and less DD at baseline were associated with DD progression in the lumbar spine (p<0.001 and p<0.001). Increased number of herniated discs over 5 years was associated with reported pain intensity at rest at 5-year follow-up (B=0.44, 95% CI 0.07 to 0.81, p=0.019) but no association between change in DD and pain intensity was observed.
5. DISCUSSION

In this thesis, the genetic susceptibility for persistent pain over a 5-year period was investigated. Previous studies discussed in our review have suggested that five genetic variants (OPRM1 rs1799971 G allele, COMT rs4680 G allele, MMP1 rs1799750 2G allele, IL1α rs1800587 T allele, IL1RN rs2234677 A allele) may be associated with reduced recovery of lumbar radicular pain (LRP) (Paper I). In low back pain (LBP) as well as LRP patients, we found that the rare allele G of MMP9 rs17576 was associated with poor pain recovery, whereas the rare allele G of OPRM1 rs1799971 was associated with better pain recovery at 5-year follow-up (Paper II). Further on, the data suggested a clear link between OPRM1 rs1799971 and emotional distress but not for MMP9 rs1799971. In addition, LBP patients had poorer pain recovery than LRP patients also after controlling for differences in emotional distress (Paper III). Finally, our data demonstrated that age and disc degeneration (DD) at baseline, but not genetic factors, influenced the development of DD over a 5-year period (Paper IV).

5.1 Discussion of main results

Genetic variants
Previous data suggest that several genetic markers may be linked to degenerative disc phenomenon and persistent LBP. In addition, our research in the literature regarding genetics and pain recovery in LRP patients indicated that some genetic factors may predict slow recovery in these patients. Our data suggest a link between VDR rs731236 and pain intensity at 5-year follow-up in both LBP and LRP patients, but regarding change in DD or pain recovery in LBP and LRP patients, no relationship was observed. A previous review reported weak evidence for most of the associations between genetic markers and lumbar DD. We found that the MMP-9 rs17576 A>G was associated with poor pain recovery in LBP and LRP patients, but no significant association between genetic factors and development of degenerative changes was observed. Hence, these data suggest that
the genetic factors examined contribute little to development of disc degeneration in the spine. However, some may argue that this conclusion does not take into account the heterogeneity of LBP patients. For example, a possible link between genetic factors and disc degeneration in a subgroup of these patients cannot be excluded. Nevertheless, the relationship between recovery of pain and MMP9, which is also involved in immunomodulation, neuromodulation, and activation of the epieregulin - PI3K/AKT/mTOR pathway, might be independent of DD. Regarding the matrix metalloproteinase family earlier data also suggest that MMP-1 rs1799750 1G>2G may be associated with painful degenerative conditions. However, no relationship between genetic variability in this gene and change in DD or pain recovery was observed in our study.

COL11 rs1676486 G>A has also previously been linked to increased risk for lumbar disc herniation, and several lines of evidence suggest that genetic variability in genes encoding inflammatory cytokines may be associated with persistent back pain. For example, IL-1α rs1800587 C>T may be associated with more pain at one year in LRP patients. However, the present study did not support a relationship between genetic variability in these genes and change in DD or pain recovery.

OPRM1 may be crucial for sensory processing and modulation of back pain and other pain conditions. Two previous studies that emanate from our LRP cohort reported a positive association between the OPRM1 rs179971 A>G and better recovery of pain in men at 1-year follow-up. Moreover, earlier observations show that the OPRM1 rs179971 A>G may be associated with lower cortical responses to experimental pain stimuli. In accordance with these earlier observations, the present data suggest that the OPRM1 rs179971 A>G in men is associated with better long-term pain recovery. However, our data also suggest a clear impact of emotional distress on the association between OPRM1 rs179971 and pain intensity. This result may be related to OPRM1 having a role in a common supra-spinal neural network processing the affective component of pain. Earlier data have demonstrated that brain regions particularly related to the
somatosensory component of pain processing are modulated by variations in OPRM1.  

Similar to the OPRM1 genotype, the genetic variability related to the COMT enzyme may be important for pain. The COMT enzyme metabolizes catecholamines and thus modulates adrenergic, noradrenergic and dopaminergic signalling in the CNS as well as in the peripheral tissue. Therefore, many supraspinal processes including nociceptive modulation may be affected by genetic factors that influence the COMT enzyme. Previous data suggest that the COMT haplotype rs6269/rs4633/rs4818/rs4680 ACCG may be associated with increased pain. No relationship between the COMT rs6269/rs4633/rs4818/rs4680 haplotype and pain recovery was, however, observed in our present study.

Genetic polymorphism may influence the susceptibility of an individual to environmental factors. This susceptibility may explain why persistent pain or severe change in DD develops in just some of the patients exposed to the same risk factor. In addition, the frequency of the particular polymorphism is different in various ethnic groups. Hence, the clinical relevance of a genetic polymorphism may vary among populations.

Some findings, including the sex dependent role of OPRM1, are controversial and need to be replicated in a larger population. Testing for several other pain related genes is also warranted. In addition, to highlight the gap between genetic markers thought to have a role in persistent pain, as well as development of DD and current knowledge of how polymorphisms may affect protein expression, is crucial. The causal relationship between genetic factors and persistent back pain remains to be examined.

Proving associations between genetic variants and persistent back pain could nevertheless clinically help to target a high-risk group and provide the basis for the development of new forms of prevention and treatment.
Emotional distress

Our data showed that emotional distress explained 9.0% of the variation of pain at five years. The LBP patients reported significantly more emotional complaints than the LRP patients both at baseline and 5-year follow-up. However, the data suggested that poorer pain recovery in LBP cannot be explained by the emotional factor alone. Psychological factors at baseline are shown to correlate with persistent LBP \textsuperscript{107}. Earlier data suggest that emotional distress may not be a strong predictor for persistence of low back disability in persons having their first episode of LBP \textsuperscript{181}. Nevertheless, to prevent persistent back disability, emotional distress should definitely be considered and treated \textsuperscript{181}.

Emotional distress was implemented in our analysis of a subgroup of our study population to evaluate its impact on the association between genetic factors and pain recovery. Our data suggest an independence between emotional distress and MMP9 rs17576 regarding the association to pain recovery at five years. Emotional distress may be a mediator but also a moderator. Our data showed an impact of emotional distress on the association between OPRM1 rs179971 and pain intensity. This result may suggest that OPRM1 rs179971 has a role in neural network processing of affective components of pain. Hence, emotional distress may play a mediator role regarding OPRM1 and pain. In addition, we found that controlling for emotional distress changed the size of the association between OPRM1 and pain intensity, hence; a moderator role cannot be excluded.

Our data contain information about emotional distress in general, and do not distinguish between anxiety, depression, somatisation or other psychological symptoms. To differ between different psychological traits regarding the associations between genetic factors and persistent pain would definitely be of interest. For example, some pain-related genetic polymorphisms known to be associated with supra-spinal neuronal activity in stress-induced depression \textsuperscript{155} may not be of importance regarding anxiety. In addition, differentiation between moderator and mediator roles of different psychological traits regarding the genetic
influence on pain recovery may contribute to uncovering the mechanisms and provide targets for treatment.

**Pain location and disc degeneration**

Our data revealed poorer pain recovery in LBP compared to LRP. The pain level was statistically significantly higher in the LBP at 5-year follow-up and mean pain at group level >3 compared to < 3 in LRP. The decline in pain was also larger in the LRP compared to the LBP, although not statistically significant. However, controlling for a higher level of baseline pain in LBP the difference was statistically significant. Hence, our data do not support earlier studies with shorter follow-up demonstrating poorer recovery in LRP than LBP patients. Differences in other risk factors between LBP and LRP may explain these results. Emotional distress is one of the strongest predictors for recovery in back pain and the LRP patients showed a significantly better pain recovery than the LBP patients also when controlling for this factor. We have to consider that factors other than emotional distress may also differ between LBP and LRP patients referred to specialised healthcare. We cannot exclude that different traditions in primary care regarding referring patients with LBP and LRP could be of importance.

Our statistical models including clinical and genetic factors explained approximately 25–30% of the variation in pain intensity at five years. In addition, sociodemographic factors including education, occupation and sick-leave are relevant predictors for pain recovery. Hence, many possible related factors were not investigated and adjusted for in the present studies and we do not know how these factors may influence the relationship between genetic factors and pain recovery. We would however argue that by including both LBP and LRP, our study population may be representative for populations of chronic pain in the lower back in general. We only assessed about 70% of the study population in the long term and this reduced the internal validity of the study and may have influenced the results.
By conducting a review, we explored how the literature assesses pain. The review revealed a wide variety of pain recovery definitions. Continuous changes as well as dichotomised endpoints were applied. In addition, the setting (rest/activity) and recall period of the measurement (present pain up to pain over last four weeks) varied 119, 185-187. Because activity may vary considerably, we selected pain at rest with a recall period of one week. The latter may be less influenced by recall bias than longer time periods 188. The choice of only one measure of pain intensity and pain recovery is a limitation of our study. Our conclusions are only valid for pain at rest. Pain in activity and pain at rest are different.

About 80% of the included patients did have disc herniation at baseline. In contrast to previous studies 189, 190 no significant difference in disc degeneration (DD) was found between disc herniation and non-disc herniation groups at either baseline or 5-year follow-up. Increased number of herniated discs over five years was associated with pain intensity. No association between changes in DD and pain intensity was observed. These findings support previous studies postulating that degenerative changes do not constitute a diagnosis because there is little correlation with pain 45. In contrast, some research groups suggest an association between intervertebral DD and back pain 191-193. Disc space narrowing is reported to be associated with LBP 193. Our results showed that higher age and less DD at baseline were associated with DD progression in the lumbar spine.

5.2 Methodological considerations

Systematic review
To our knowledge, the review in this thesis is the first attempting to provide an overview over genetic variants linked to development of persistent lumbar radicular pain. Our review has several limitations which should be emphasised.

Selection and assessment of the studies were performed by two researchers followed by discussion for a common agreement. However, the review process was not blinded, which may have affected the methodological quality of the
review. A new purpose-made checklist based on Sanderson et al. QATSO (Quality Assessment Tool for Systematic Reviews of Observational studies) and the STROBE statement guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) was used to assess the quality of the included observational studies. This approach may be a limitation regarding assessment of the genetic studies. For such studies, current guidelines suggest that the level of evidence for genetic association should be assessed regarding the amount of evidence (number of studies), replication (consistence or conflicting findings) and protection for bias (independent evaluation). The checklist we applied may have neglected some of the quality items described by Eskola et al. In addition, the checklist we used did not include assessment of the inclusion criteria. By example, radicular pain is defined as leg pain in a dermatome with consistent MRI findings, and a classification based on self-report is likely to be different from one based on clinical examination.

Study design

The strength of our study is the prospective longitudinal cohort design with extended follow-up over a 5-year period allowing the evaluation of the predictive value of candidate polymorphism for long-term pain recovery. No control group was assigned in this study, which may weaken our conclusions. In addition, most of the settings were already decided at baseline and this challenged the conduction of the 5-year follow-up. Two separate studies regarding LRP and LBP, respectively, have been merged which give both strength but certainly limitations. We aimed to evaluate the recovery of LBP and LRP patients and hypothesised that the mechanism underlying development of persistent pain may be the same regardless of LBP or LRP diagnosed five years earlier. The mechanism for acute LRP is considered different from acute LBP, and also the mechanism in persisting pain regarding these two conditions may differ. We did not perform any further subgrouping of patients based on clinical examination or social-demographic factors, which means that our conclusions cannot be extended to certain groups of
patients. Extensive research has been conducted to classify disc related pain, but even the discography is not helpful for classification according to well-conducted studies 195,196.

**Study population**
The patients included had been referred for specialised medical care from the general practitioner, which challenges the external validity of our results. The included patients are referred to a public multidisciplinary clinic including physical medicine and rehabilitation, neurology, orthopaedic surgery and neurosurgery at Oslo University hospital and Haukeland University hospital. It is likely that the patients referred for secondary care have more severe complaints than those treated in primary care. The dropout rate was high, respectively 30% and 28% in the LRP and LBP) groups, which may bias the results and threaten their validity.

**Pain recovery**
The literature comprises a variety of ways to measure pain recovery in patients with back disorders 93. Some studies dichotomise and group patients into recovered and non-recovered using a reduction of 20% 185,197 or 50% 141 in pain measured by VAS between baseline and follow-up to define pain recovery. Other authors rather use improving expressed as mean reduction in pain measured from baseline to follow-up. Lack of consistency regarding definitions of LBP and LRP recovery challenge a standardised measurement of recovery. The literature does not present one established definition of recovery, and the level of pain or pain reduction necessary for recovery is seldom defined 93. Due to a lack of gold standard cut-off and statistical fit, we selected pain intensity at five years adjusted for pain intensity at baseline as a proxy for pain recovery. Some may argue and be right, that this is a measure of improvement rather than pain recovery. To dichotomise our patients in two groups, recovered or not, might have been a better choice regarding a definition of recovery but would have reduced data variation.
Selection of candidate genes and sources of genomic DNA

The success of candidate gene studies relies on the correct choice of genes that are relevant in the disease investigated, and a priori hypothesis about biological function is required. Hence, in the LBP and LRP patients, genes encoding proteins that effect the degeneration, the inflammatory response or the activity in the pain pathway may be of importance. In addition, the genes of interest must exist in several variants, preferable with known alternations in protein levels or function (i.e. functional variants). Although many genetic factors may be associated with LBP and LRP, we here choose to emphasise the variants in the gene encoding VDR, COL11a, MMP1, MMP9, IL-1α, IL-1RN, OPRM1 and COMT. The selection of these candidate genes was based on relevance to the disease with regard to biology and pathogenesis as well as associations shown in animal models and clinical studies.

It is important to be aware that several other candidate genes may be of relevance. For example, disc degeneration could also be affected by genetic variants in genes encoding the aspirin protein (ASPN allele D14), growth differentiation factor 5 (GDF5 rs143383), sickle tail (SKT rs16924573) and thrombospondin-2 (THBS2)\textsuperscript{61}. Unfortunately, these genes were not in our test panel.

When performing genotyping, re-genotyping it is also important to reduce potential genotyping errors because an error rate of 1% can obscure medical findings. Typically, sources to genotyping errors include problems with DNA quality or quantity, errors with the DNA sequence itself, biochemical artefacts or errors due to human factors\textsuperscript{198}.

Different sources of genomic DNA were used, respectively blood samples in the LRP patients and saliva in the LBP patients. However, this difference was not a problem with regard to genotyping. To avoid human genotyping errors, an automated program performed all allelic discriminations, as performed in previous
studies 3, 138-140, 142, 143. As a reliability control, 10% of the samples were re-
genotyped, always giving the same result.

**Evaluation of emotional distress versus genetic susceptibility**

Emotional distress is known to be a major predictor for pain and may have been controlled for in Paper II ‘Genetic predictors of recovery in low back and lumbar radicular pain patients’. Unfortunately, however, data on emotional distress at baseline were not available for LRP patients included at Haukeland University Hospital (HUH) and the analyses including emotional distress had to be performed in a subgroup of patients, the LRP and LBP patients from Ullevål University Hospital (UUH) (paper III). By evaluating the change in beta value (B) when adjusting for emotional distress, we wanted to address the relationship between the genetic variants, emotional distress and pain intensity. The purpose was to evaluate whether an adjustment for emotional distress would have influenced the results of paper II. The strength in this analysis might be debated with respect to our small sample of patients.

In addition, using the short version of Hopkins Symptom Checklist (HSCL-10) for identification of mental symptoms and measuring emotional distress has been shown to be a valid instrument 199, 200. However, it does not distinguish between anxiety, depression, somatisation or other psychological symptoms. Hence, HSCL-10 may be a useful instrument for the measurement of mental health in general but does not specify the type or severity of the mental problem in our patients. Moreover, to include data regarding fear avoidance beliefs, which might be a strong predictor within back pain research, would certainly be of major interest and might have affected our conclusions. Also, we cannot exclude the possibility that more specific evaluation of anxiety and depression would have a different impact.
General methodological considerations

The strength of this study is its prospective design with extended follow-up over 5-year period allowing the evaluation of the predictive value of candidate polymorphisms for long-term pain recovery. However, the candidate gene approach can be questioned. A restricted number of candidate genes was selected in our study as well as in previous genetic association studies. Therefore, many genetic factors that could influence pain perception or change in DD may still not have been examined. Hence, the current literature does not represent all possible genetic contributors. GWAS would shed light on other genetic factors related to the same phenomena. Unfortunately, however, our clinical study would not have enough statistical strength for GWAS. This may be a major challenge in clinical research. In addition GWAS have to be followed up to uncover functional SNPs. The problem is that inheritance of one SNP from one generation to the next is always accompanied by other SNPs as well (linkage equilibrium). This issue has to be addressed in future studies.

Previous studies show that the explained variance of the genetic variants is rather low, even for inherited characteristics such as human height and the clinical value can be questioned \(^{201}\). Regarding a multifactorial disorder such as persistent back pain a genetic variant explains approximately 1–2% of the variance. However, some SNPs may have a more pronounced effect on a subgroup of patients. For example, the current literature as well as our data suggest that the effect of the OPRM1 G allele may be different in men and women. Our subgroup analysis demonstrated that the OPRM1 rs1799971 in men explained 4.7% of the variance in pain intensity at 5-year follow-up. This result emphasises the clinical relevance of such genetic studies. However, we did not check for use of analgesics which may influence the pain experience and therefore influence the association between pain and genetic variants studied in this thesis. In particular, the use of opioids may affect the influence of the OPRM1 SNP. In addition, our study and also previous candidate studies in pain may provide only weak evidence due to lack of replication \(^{202}\).
Differences in psychosocial risk profiles were observed among the hospital populations. Nevertheless, independence for MMP9 rs1799971 regarding pain recovery was found in the OUH population suggesting that this genetic factor has a direct effect on pain recovery. On the other hand, our data showed a clear impact of emotional distress on the association between OPRM1 rs179971 and pain intensity. This association may be related to the role of OPRM1 in a common supra-spinal neural network processing the affective components of pain. Hence, regarding OPRM1 rs179971, emotional distress is certainly of major relevance.

A p-value <0.05 was set as the level of statistical significance. Since we had one hypothesis for each SNP Bonferroni correction to compensate for multiple testing was not performed. However, this approach can be questioned. In our study, eight genetic variants was tested and according to the Bonferroni correction, we only should reject a null hypothesis if the p-value is <0.05/n. On the other hand, the Bonferroni correction may be too conservative. Further on, when using Bonferroni correction, one has to consider the reduction of false positives at the expense of false negatives.

Taken together, our study illustrates that MMP9 rs17576 and OPRM1 rs1799971 may affect 5-year recovery in patients with LBP and LRP but regarding OPRM1, an impact on emotional distress was revealed. We found a significant association between MMP9 rs17576 and poor pain recovery. Hence, our findings suggest that MMP9 rs17576 may be a useful prognostic factor in clinical approaches. However, it remains to assess the value of a genetic risk score (GRS) based on several genetic factors found to be linked to pain recovery. This approach would be of major interest for exploration in larger longitudinally studies. A strong predictive GRS could facilitate better clinical assessment and choice of treatment in LBP and LRP patients. Aggregating the contribution of SNPs into GRS has been found useful in several fields of medicine and predicts complex diseases better than traditional risk factors alone.\textsuperscript{203, 204}

In this work, we did not explore the relationship between physical work-load and genetic variants regarding change in DD or pain recovery. Also, research about the
interplay between environmental factors and genes could open a new window for understanding how genes may be transformed by such environmental factors\textsuperscript{205}. There is a clear need for future research in this field based on large study populations in a longitudinal setting implementing an optimal set of covariates covering psychosocial and biological factors important for poor pain recovery in back pain patients. It would certainly be of major interest to explore the genetics and possible shared physiology and nerve matrix between psychological disorders and persistent pain.

5.3 Ethical consideration
In general, genetic studies may be associated with many ethical considerations. For example, individual genetic information may have consequences for both the patient and their family members. Although information about genetic susceptibility may promote concerns and emotional distress in the patient such information could help to guide treatment.

However, in the present study, genetic information was anonymised and the data handled at a group level. Moreover, information about genetic susceptibility was only handled anonymised by two researchers, and did not have any consequences for clinical treatment. This information was also given to the patients.

Questionnaires and clinical examination performed both at baseline and at 5-year follow-up were similar to routine assessments in our outpatient clinic. The MRI at 5-year follow-up was, however, not part of the routine at OUH. Hence, patients included in the study underwent one extra MRI investigation. Notably, most patients reported that they had had a positive experience regarding the MRI investigation. We did emphasise the lack of correlation between MRI findings and recovery. We also informed patients that the MRI findings would probably not have any consequences for their treatment, but potentially could improve clinical practice in the future.
This study was conducted in accordance with the Helsinki Declaration, and approved by the regional committee for Medical Research Ethics (reference number 2014/1754). All participants gave their written informed consent at baseline and at 5-year follow-up.
6. CONCLUSION

6.1 Conclusions paper I-IV

- Our systematic search revealed that five genetic variants (OPRM1 rs1799971 G allele, COMT rs4680 G allele, MMP1 rs1799750 2G allele, IL1α rs1800587 T allele and IL1RN rs2234677 A allele) were associated with reduced recovery of LRP. Three protein biomarkers (TNFα, IL6 and IFNα) were associated with persistent LRP. Methodological quality was found satisfactory in most of the studies but several studies emanated from the same cohort and replication of the findings will be crucial. A stricter use of nomenclature is also highly necessary.

- The rare allele of MMP9 rs17576 was associated with poor pain recovery, whereas the rare allele of OPRM1 rs1799971 was associated with better pain recovery in low back and lumbar radicular pain patients. Hence, our results suggest that genetic factors involved in tissue degeneration, inflammation and pain perception may predict long-term recovery in these patients. The two genes only explained approximately 2–5% each, which means that most of the variance was not explained by the candidate genes examined.

- The present results also suggested that both genetic factors and emotional distress predicted persistent back pain. Emotional distress did not impact the association between MMP9 rs17576 and pain recovery. In contrast, we found a clear impact of emotional distress on the association between OPRM1 rs179971 and pain intensity, suggesting that the impact of emotional distress on the association between genetic factors and persistent pain depends on the candidate gene being tested.

- Age and disc degeneration (DD) at baseline, rather than genetic factors, influenced DD at follow-up. These findings underscore the age-related development of DD in the lumbar spine.
6.2 Overall conclusion

- In summary, the present thesis shows that the rare allele of MMP9 rs17576 A>G is associated with poor recovery, whereas the rare allele of OPRM1 rs1799971 A>G is associated with persistent low back and lumbar radicular pain. The genetic variants examined in the present study explained no more than 2–5% of the pain intensity variance. The negative finding regarding genetic factors and MRI changes might suggest that the MMP9 rs17576 A>G affect pain recovery but not disc degeneration. Findings from analyses of the relationship between genetics, MRI changes and pain can point to the mechanisms, but also help to identify new targets for future clinical trials.
7. CLINICAL RELEVANCE AND FUTURE PERSPECTIVES

The mechanisms behind low back and lumbar radicular pain are complex and multifactorial. Presently, alleles predicting 2–5% of the variance in pain do not have direct clinical relevance. However, uncovering new genetic variants can point to the mechanisms, and new targets for pharmaceutical treatments. Genome-wide association studies (GWAS) may add to the candidate gene approaches, but need to be followed up with cell-line experiments and clinical studies in order to document functionality of the genetic variants. Improvement in clinical diagnosis of back pain and subgroup identification may be needed in order to uncover the influence of the genetic factors in patients. Appropriate classification of low back and lumbar radicular pain, proper matching of controls and a large sample size must be considered mandatory for future candidate gene studies.
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Genes associated with persistent lumbar radicular pain; a systematic review

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Abstract

Background: The aim of the present study was to provide an overview of the literature addressing the role of genetic factors and biomarkers predicting pain recovery in newly diagnosed lumbar radicular pain (LRP) patients.

Methods: The search was performed in Medline OVID, Embase, PsycInfo and Web of Science (2004 to 2015). Only prospective studies of patients with LRP addressing the role of genetic factors (genetic susceptibility) and pain biomarkers (proteins in serum) were included. Two independent reviewers extracted the data and assessed methodological quality.

Results: The search identified 880 citations of which 15 fulfilled the inclusion criteria. Five genetic variants; i.e., OPRM1 rs1799971 G allele, COMT rs4680 G allele, MMP1 rs1799750 2G allele, IL1α rs1800587 T allele, IL1RN rs2234677 A allele, were associated with reduced recovery of LRP. Three biomarkers; i.e., TNFα, IL6 and IFNα, were associated with persistent LRP.

Conclusion: The present results indicate that several genetic factors and biomarkers may predict slow recovery in LRP. Still, there is a need for replication of the findings. A stricter use of nomenclature is also highly necessary.

Trial registration: The review is registered PROSPERO 20th of November 2015. Registration number is CRD42015029125.

Keywords: Lumbar radicular pain, Genes, Biomarkers

Background

Low back pain (LBP) has a lifetime prevalence of 70% [1]. The annual prevalence of lumbar radicular pain (LRP) in the population is estimated to 2–3% [2, 3]. Hence, LRP, also referred to as “sciatica”, account for 5–10% of the low back pain conditions. However, the disability is worse and the recovery is slower for LRP than for other low back pain conditions [3, 4]. Low back disorders constitute an important source of disability and are among the most cost-intensive health problems [5].

Development of persistent low back pain and sciatica may be associated with ergonomic strains, but also psychosocial aspects. Risk factors such as age, smoking, body weight, height, occupational load and mental stress contribute to LRP [2, 3, 6–8]. Clearly, many psychosocial factors predict poor recovery in LRP [6, 9]. In addition, genetic variability may influence the risk of a chronic outcome [10, 11].

LRP is characterized by radiating pain that typically follows the dermatome of the affected nerve root from the lumbar or sacral spine [12]. Previous data suggest that discharges emanating from the dorsal nerve roots or their ganglions explain the radiating nature of this form of back disorder [13]. LRP may be induced by mechanical compression of the nerve root, but also by the biochemical influence on the neuronal tissues caused by a local inflammatory process. Moreover, leak of nucleus pulposus from herniated discs may have many effects on the nerves inducing histological changes and increased neuronal excitability. Microvascular changes close to the dorsal ganglion, spinal nerve roots and spinal cord is a part of the pathogenesis [14, 15].

Environmental factors including heavy work load is assumed to contribute to acceleration of degeneration of the spinal joints and discs, but also genetic factors are of importance [10]. It has been postulated that heritability for back pain range from 30 to 45% [16].

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The genetic susceptibility for LBP and LRP may be associated with genetic variability in genes related to modulation of nociceptive processing, tissue degeneration and local or systemic inflammation.

In particular genetic variability important for opioid, dopaminergic, adrenergic and serotonergic signaling may affect modulation of nociceptive processing [17–19]. Several previous studies demonstrate a link between genetic variability in the gene encoding opioid receptor mu 1 (OPRM1) and LRP [20, 21]. Earlier reviews, for example Detchenko et al. [22], highlight that genetic factors related to the enzyme catechol-O-methyltransferase (COMT) affect cortical pain processing and the risk of chronic LBP.

Genetic variability in the gene encoding the sodium ion channel (SCN9A) [23] and the GTP cyclohydrolase 1 (GCH1) [24] gene may affect LRP, indicating that genetic factors may affect peripheral nerves as well. GCH1 is an enzyme involved in production of tetrahydrobiopterin (BH4). BH4 is an essential cofactor for catecholamine, serotonin and nitric oxide production. Earlier data also suggest that disc degeneration and the clinical outcome after sciatica may be associated with the large molecule collagen type IX alpha 2 (COL9A2) [25]. Thus, previous data show possible association between genetic markers and lumbar disc degeneration. However, the relationship between degenerative changes and persistence of pain is still controversial [26, 27].

Interestingly, previous findings [28] suggest that patients with lumbar disc herniation (LDH) have more peripheral Th17 cells and enhanced IL-17 expression in blood compared with healthy controls. Some studies also indicate an association between genetic variability in genes encoding interleukin 1 (IL-1α), interleukin 6 (IL-6) and the human leukocyte antigen II (HLA II) regarding persistent LRP [29–33]. Hence, back pain after disc herniation seems to be associated with activation of the immune system.

From a clinical point of view, slow recovery is a major challenge in LRP – the disability is worse and the recovery is slower for LRP than for LBP. Still, previous reviews have only addressed the relationship between genetic variability and LBP. In the present study, however, we provide an overview of the literature presenting genetic factors and biomarkers predicting pain recovery in LRP patients. The present review emphasizes that several genetic factors and biomarkers described in the literature may predict slow recovery in LRP.

**Methods**

**Search strategy**

The Medline OVID, Embase, PsyCInfo and Web of Science were searched using optimized systematic search strategies including mesh words with explore and a combination of words in the title or abstract related to different expressions of Lumbar radicular pain, Genetic variation and Pain biomarkers. The main key words for the search included “lumbar radicular pain”, “sciatica”, “pain and lumbar disc herniation”, “pain and lumbar prolapse” OR “lumbar radiculopathy”, AND “genetic variability”, “genetic polymorphism”, “allele”, “haplotype”, “micro-RNA”, “pain biomarker”, “cytokines”, “chemokines”, “interleukins” OR “interferons”. The search was performed from 2004 up to 12th of January 2015.

**Selection of studies**

Inclusion criteria were prospective studies, including patients with lumbar radicular pain, and assessing genetic factors or pain biomarkers. Exclusion criteria were non English language, lumbar radicular pain due to tumor, infection or systemic disorders.

**Procedure**

Based on screening of the titles and abstracts eligible articles for full text reading by two of the authors were identified.

**Assessing the quality of the studies**

A checklist based on Sanderson et al. [34], QATSO (Quality Assessment Tool for Systematic Reviews of Observational studies) [35] and the STROBE statement guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) [36] was used. The checklist compromised seven criteria namely: external validity, sample size, description of sample, follow up rate, appropriate reporting of outcome, adjustment for confounding factors (No = not adjusted for any covariates. Yes = adjusted) and correction for multiple testing. The assessments of the two reviewers were compared. If disagreement a final evaluation of the paper was performed.

**Results**

The systematic search identified 880 relevant publications, of which 791 were excluded after screening of titles and abstracts. Thus, 89 studies were found eligible, but after full-text screening only 15 publications met the inclusion criteria (Fig. 1).

**Methodological quality**

A summary of methodological quality is shown in Table 1. External validity found to be satisfactory in 11 of the 15 included studies and number of cases >100 in 10 of the studies. The studies comprise a total of 872 LRP patients and mean age ranged from 41 to 47 years. Seven of the studies emanates from the same patient population that affect the total number of patients included (Table 1). Although all the studies provided a short description of the sample, several shortcomings in this description were identified. Just one study, Karpinnen et al. [29], included
BMI and work load in the analyzes. Moreover, Gebhardt et al. [37] controlled for smoking and BMI. In only 6 studies the data were evaluated after correction for their multiple testing.

Assessment and definition of pain recovery
All but one study reported VAS (Visual analog scale) as assessment tool for pain. Tegeder et al. [24] did not describe how pain was measured, but provide us the z-score from several time points to express the development in pain intensity over time. Detailed information about pain intensity was not present in most of the included studies. Moreover, we found a variety of different pain descriptions/locations and procedures for pain testing. In two studies, the pain score was based on pain during activity, in two of the studies at rest, whereas in the rest of the studies this was not clearly described. Pain duration at baseline were described in five studies where two reported duration ≥3 months, one study <3 months and the two last both ≥3 and <3 months.

Even if the follow up time was 12 months or more in 12 of the studies, the presentation of the development of pain over time was not clear. Gebhardt et al. [37] measured pain at 12 time points and gave a detailed description of how high-sensitive C-reactive protein (hsCRP) declined corresponding to decreased pain first 3 weeks, but did not emphasized what happened after the subacute phase.

Pain recovery was described in 4 of the studies. Andrade et al. [38, 39] used >20% reduction in VAS while Rut et al. [40] and Takeuchi et al. [41] used >50% reduction in VAS between baseline and follow to define recovery. Specific description of change in pain state during the follow up period was reported in just two of the studies. Both Olsen et al. [20] and Schistad et al. [30, 42] described a significant decrease in pain the first year after herniation.

Genetic variability and pain recovery
In 9 of the studies the association between genetic variability and LRP were studied (Table 2). The roles of 20 genetic polymorphisms were addressed. Only 1 study

<table>
<thead>
<tr>
<th>Study</th>
<th>Externally validity</th>
<th>Findings can be generalised</th>
<th>Sample size cases</th>
<th>Description sample</th>
<th>Follow up rate</th>
<th>Appropriate outcomes reported</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrade et al. (2013) [39]</td>
<td>No</td>
<td>n = 10</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Andrade et al. (2011) [38]</td>
<td>No</td>
<td>n = 10</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Gebhardt et al. (2006) [37]</td>
<td>Yes</td>
<td>n = 31</td>
<td>Yes</td>
<td>88%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hasvik et al. (2014) [21]</td>
<td>Yes</td>
<td>n = 118a</td>
<td>Yes</td>
<td>95%</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Jacobsen et al. (2013) [44]</td>
<td>Yes</td>
<td>n = 260a</td>
<td>Yes</td>
<td>91%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Jacobsen et al. (2012) [43]</td>
<td>Yes</td>
<td>n = 258a</td>
<td>Yes</td>
<td>89%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Karppinen et al. (2008) [29]</td>
<td>Yes</td>
<td>n = 153</td>
<td>Yes</td>
<td>97%</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Moen et al. (2014) [31]</td>
<td>Yes</td>
<td>n = 252a</td>
<td>Yes</td>
<td>91%</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Olsen et al. (2012) [20]</td>
<td>Yes</td>
<td>n = 258a</td>
<td>Yes</td>
<td>92%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rut et al. (2014) [40]</td>
<td>Yes</td>
<td>n = 176</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Schistad et al. (2014) [42]</td>
<td>Yes</td>
<td>n = 108a</td>
<td>Yes</td>
<td>90%</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Schistad et al. (2014) [30]</td>
<td>Yes</td>
<td>n = 121a</td>
<td>Yes</td>
<td>91%</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Scuderi et al. (2009) [45]</td>
<td>No</td>
<td>n = 47</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Takeuchi et al. (2007) [41]</td>
<td>No</td>
<td>n = 27</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tegeder et al. (2006) [24]</td>
<td>Yes</td>
<td>n = 168</td>
<td>Yes</td>
<td>88%</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable, ND, not described
*aemanates from the same patient population
addressed the relationship between genetic variability and tissue degeneration seen on MRI.

Olsen et al. [20] and Hasvik et al. [21] demonstrated that a genetic variant, OPRM1 rs1799971 SNP, in the gene encoding OPRM1 receptor is associated with both pain and subjective health in LRP patients. The OPRM1 rs1799971 G allele increased the pain score in women, but reduced the pain score in men. Thus, the data revealed a significant interaction between sex and OPRM1 genotype regarding the pain intensity.

Jacobsen et al. [43] showed that the COMT rs4680 SNP affects pain recovery after disk herniation. In both men and women, carriers of COMT rs4680 2G alleles had more pain than carriers of two A alleles at 6 months after disc herniation. Conversely, Rut et al. [40] reported that carriers of two COMT rs4680 G alleles may be associated with significant positive improvement in pain recovery one year after surgery.

Jacobsen et al. [44] addressed the relationship between MMP1 rs1799750 SNP and tissue degeneration. The data indicated that the MMP1 rs1799750, in the gene encoding the MMP1 enzyme, may affect the long-term outcome in disc herniation patients. Carriers of two MMP1 rs1799750 2G alleles had a reduced pain recovery rate, but not increased MRI disc changes.

Moen et al. [31] and Schistad et al. [30, 42] found increased risk of persistent pain in carriers of the IL1α rs1800587 T allele. Moreover, Karppinen et al. [29] demonstrated a significant association between the IL-6 haplotype rs1800797 G/rs1800796 C/rs13306435 A and days of leg pain 3 years after disc herniation in men with high physical work load. Finally, Tegeder et al. [24] showed that the GTP cyclohydrolase (GCH1) haplotype rs8007267 A/rs3783641 T/rs8007201 C/rs752688 A could be protective and be associated with less pain following discectomy.

Six of the studies emanates from the same patient population (Table 1). None of these association studies included data on protein expression.

### Table 2 Genetic variability and pain recovery

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>Base substitution</th>
<th>Position in DNA</th>
<th>Amino acid substitution</th>
<th>Reference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPRM1</td>
<td>rs1799971</td>
<td>A→G</td>
<td>118</td>
<td>Asn40Asp</td>
<td>Hasvik et al. (2014) [21]</td>
<td>↑ (W) ↓ (M)</td>
</tr>
<tr>
<td></td>
<td>rs1799971</td>
<td>A→G</td>
<td>118</td>
<td>Asn40Asp</td>
<td>Olsen et al. (2012) [20]</td>
<td>↑ (W) ↓ (M)</td>
</tr>
<tr>
<td>COMT</td>
<td>rs4680</td>
<td>A→G</td>
<td>472</td>
<td>Val158Met</td>
<td>Jacobsen (2012) [43]</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>rs4680</td>
<td>A→G</td>
<td>158</td>
<td>Val 158Met</td>
<td>Rut et al. (2014) [40]</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>rs6269</td>
<td>G→A</td>
<td>–98</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs4633</td>
<td>T→C</td>
<td>186</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs4818</td>
<td>G→C</td>
<td>408</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MMP1</td>
<td>rs1799750</td>
<td>1G allele→2G allele</td>
<td>–1719</td>
<td>–</td>
<td>Jacobsen (2013) [44]</td>
<td>↑</td>
</tr>
<tr>
<td>IL1A</td>
<td>rs1800587</td>
<td>C→T</td>
<td>–949</td>
<td>–</td>
<td>Moen et al. (2014) [31]</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>rs1800587</td>
<td>C→T</td>
<td>–949</td>
<td>–</td>
<td>Schistad et al. (2014) [30]</td>
<td>↑</td>
</tr>
<tr>
<td>IL1B</td>
<td>rs1143627</td>
<td>T→C</td>
<td>–118</td>
<td>–</td>
<td>Moen et al. (2014) [31]</td>
<td>–</td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs2234677</td>
<td>G→A</td>
<td>–87</td>
<td>–</td>
<td>–</td>
<td>↑</td>
</tr>
<tr>
<td>IL-6</td>
<td>rs1800797</td>
<td>A→G</td>
<td>–661</td>
<td>–</td>
<td>Karppinen et al. (2008) [29]</td>
<td>↓ (Haplotype GGGA)</td>
</tr>
<tr>
<td></td>
<td>rs1800796</td>
<td>G→C</td>
<td>–636</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs1800795</td>
<td>G→C</td>
<td>–237</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs13306435</td>
<td>T→A</td>
<td>486</td>
<td>Asp162Glu</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GCH1</td>
<td>rs8007267</td>
<td>G→A</td>
<td>–9610</td>
<td>–</td>
<td>Tegeder et al. (2006) [24]</td>
<td>↓ (Haplotype ATCA)</td>
</tr>
<tr>
<td></td>
<td>rs3783641</td>
<td>A→T</td>
<td>343 + 8900</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs8007201</td>
<td>T→C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rs752688</td>
<td>G→A</td>
<td>509 + 1551</td>
<td>627–708</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The rs number refers to a specific SNP and rs stands for Reference SNP cluster ID, created by National Center for Biotechnology Information (NCBI) (Ref.: http://www.ncbi.nlm.nih.gov/). Base substitution refers to replacement of one base with another in DNA. Position on DNA based on information from NCBI. Only two of our SNP causes a direct amino acid substitution. A change of nucleotide in the exon is a prerequisite for change in amino acid. A replacement of nucleotide in the intron does not cause such a substitution but may have a role in the transcription process. Polymorphism located in the promotor part of the gene is expressed by adding minus prior to the position on the DNA. ↑ (positive association with poor recovery), ↓ (negative association with poor recovery), - (no change in amino acid), - (no association with poor recovery), W (Women), M (Men).

Biomarkers and pain recovery

Six studies presented data on biomarkers linked to pain recovery (Table 3). As many as 28 biomarkers have been assessed: IL1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10,
<table>
<thead>
<tr>
<th>Study</th>
<th>Cases</th>
<th>Pain Local</th>
<th>Pain time points</th>
<th>Gene</th>
<th>Biomarker</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrade et al. (2013) [39]</td>
<td>n = 10</td>
<td>Leg</td>
<td>1 day preoperative</td>
<td>(Tissue: PM, AF, NP)</td>
<td>IL-1beta, IL-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 weeks postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 month postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andrade et al. (2011) [38]</td>
<td>n = 10</td>
<td>Leg</td>
<td>1 day preoperative</td>
<td>(Tissue: PM, AF, NP)</td>
<td>TNF alpha, TNF R1, TNF R2</td>
<td>↑ (6 week r = 0.54, 12 month r = 0.65) ↑ (6 week r = 0.75, 12 month r = 0.80) ↓ (6 week r = -0.60, 12 month r = -0.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 week postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 month postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gebhardt et al. (2006) [37]</td>
<td>n = 31</td>
<td>ND</td>
<td>0 day, 3 day, 7 day, 10 day, 14 day, 17 day, 21 day, 23 month, 6 month</td>
<td>(Blood)</td>
<td>hsCRP</td>
<td>↓ (first 3 weeks p &lt; 0.05) - (after 3 weeks)</td>
</tr>
<tr>
<td>Hasvik et al. (2014) [21]</td>
<td>n = 118</td>
<td>Leg</td>
<td>Baseline</td>
<td>OPRM1</td>
<td></td>
<td>↑ (Women p &lt; 0.008) ↓ (Men p &lt; 0.008)</td>
</tr>
<tr>
<td>Jacobsen et al. (2013) [44]</td>
<td>n = 260</td>
<td>Leg Back</td>
<td>Baseline</td>
<td>MMP1</td>
<td></td>
<td>↑ (6 week p = 0.004, 12 month p = 0.004)</td>
</tr>
<tr>
<td>Jacobsen et al. (2012) [43]</td>
<td>n = 258</td>
<td>ND Activity</td>
<td>Baseline</td>
<td>COMT</td>
<td></td>
<td>↑ (6 month p = 0.028)</td>
</tr>
<tr>
<td>Karpinnen et al. (2008) [29]</td>
<td>n = 153</td>
<td>Leg Back</td>
<td>Baseline</td>
<td>IL-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moen et al. (2014) [31]</td>
<td>n = 252</td>
<td>Activity</td>
<td>Baseline</td>
<td>1L-1a + IL-1RN</td>
<td></td>
<td>↑ (12 month p = 0.049)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-1b + IL-1RN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olsen et al. (2012) [20]</td>
<td>n = 258</td>
<td>Leg Back Activity</td>
<td>Baseline</td>
<td>OPRM1</td>
<td></td>
<td>↑ (12 month Women p = 0.002) ↓ (12 month Men p = 0.002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rut et al. (2014) [40]</td>
<td>n = 176</td>
<td>Leg Back</td>
<td>Preoperative</td>
<td>COMT</td>
<td></td>
<td>↑ (12 month p = 0.0042)</td>
</tr>
<tr>
<td>Schistad et al. (2014) [42]</td>
<td>n = 108</td>
<td>Leg Back Activity</td>
<td>Baseline</td>
<td>(Blood)</td>
<td>IL-6</td>
<td>↑ (12 month p = 0.004)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistad et al. (2014) [30]</td>
<td>n = 121</td>
<td>Leg Back Activity Present</td>
<td>Baseline</td>
<td>IL-1a</td>
<td></td>
<td>↑ (12 month p = 0.002)</td>
</tr>
<tr>
<td>Study</td>
<td>n</td>
<td>Region</td>
<td>Time Points</td>
<td>Sample Type</td>
<td>Preinjection Measurements</td>
<td>Postinjection Measurements</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------</td>
<td>------------</td>
<td>----------------------</td>
<td>-------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Scuderi et al. (2009)</td>
<td>47</td>
<td>ND</td>
<td>Preinjection 3 month postinjection</td>
<td>(CSF)</td>
<td>↓ (3 month p = 0.001)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IFNa</td>
<td></td>
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<tr>
<td></td>
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<td>G-CSF</td>
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<td>GM-CSF</td>
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<td>TNFa</td>
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<td>IL1b</td>
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<td>MCP-1b</td>
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<td>MIP-1b</td>
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<td>Takeuchi et al. (2007)</td>
<td>27</td>
<td>Leg</td>
<td>Preoperative 3 week postoperative</td>
<td>(Blood)</td>
<td>↑ (Preoperative p = 0.01)</td>
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<td>CGRP1</td>
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<td>Galanin</td>
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<td></td>
<td>Neuro-peptide4</td>
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<td>SubstP</td>
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<td>Tegeder et al. (2006)</td>
<td>168</td>
<td>ND</td>
<td>4 time points a</td>
<td>GCH</td>
<td>↓ (p &lt; 0.05)</td>
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</table>

1 (positive association with poor recovery), ↓ (negative association with poor recovery), - (no association with poor recovery), ND (not defined, SD(z-score). All pain measures reported by VAS except Tegeder et al. PM (Paravertebral muscle), AF (Annulus fibrosis), NP (Nucleus pulposus).
IL-12, IL-13, IL-17, G-CSF, GM-CSF, MCP-1b, MIP-1b, TNFα, TNF R1, TNF R2, CGRP1, Galanin, Neuropeptides4, SubstP. Most of the biomarkers examined are members of the cytokine family, but also the role of some neuropeptides is among the studied molecules. In addition low levels of the C-reactive protein (hsCRP) were assessed in the study by Gebhardt et al. 2006 [37].

Andrade et al. [39] was unable to detect any link between IL-6 and pain recovery while Schistad et al. [30, 42] demonstrated from the results that high level of IL-6 correlate with less favorable pain recovery 1 year after disk herniation. Regarding recovery, Andrade et al. [38] and Scuderi et al. [45] found a link to tissue and CSF level of TNFα at one year and tissue IFNα level at 3 month – whereas Takeuchi et al. [41] found that plasma level of the neuropeptide CGRP was associated with the extent of sciatica. In acute lumbosacral patient, hsCRP declined with decreased pain the first 3 weeks after disc herniation, but no clear relationship between pain and level of hsCRP was observed after that (Gebhardt et al. 2006 [37]). Specific results from the studies assessing biomarkers and pain recovery are listed in Table 3.

Discussion

In the present review, we identified nine studies addressing the relationship between genetic polymorphism and LRP. The data analyzed in these studies were limited to eleven DNA base substitutions. In all these studies, polymorphisms of genes encoding proteins expected to affect the phenotype were studied [46]. Some of the SNPs were located in the promoter region, whereas others were located in the coding regions of the genes.

Two studies reported a positive association between the OPRM1 SNP rs179971 and poor recovery of pain in women with LRP [20, 21]. These data support the previous observation that some individuals, in particular in females, carrying the ORPM1 G allele have increased pain sensitivity [47, 48]. OPRM1 is crucial for processing and modulation of pain. Moreover, several studies addressed the association between COMT SNP rs4680 G allele and pain. This enzyme metabolizes catecholamines and thus modulates adrenergic, noradrenergic and dopaminergic signaling in the CNS as well as in the peripheral tissue. However, while Jacobsen et al. found a positive correlation between the COMT rs4680 G allele and long lasting pain, Rut et al. reported that the same SNP may be associated with better clinical outcome [40, 43].

Although the data may be debated, most experimental studies support a positive correlation between the COMT haplotype rs4680 G, rs6269 A, rs4633 C, rs4818 C and pain hypersensitivity [49]. Moreover, several of these COMT SNPs may be associated with increased postoperative pain. For example, the COMT haplotype rs4680 G, rs6269 A, rs4633 C, rs4818 C is associated with slower recovery after surgical treatment for lumbar degenerative disc disease [50].

Only one study addressed the relationship between genetic variability, tissue degeneration and persistent pain [44]. Previous data suggest that the enzyme MMP influences tissue degradation or inflammation [51]. Surprisingly, however, no relationship between the MMP1 SNP rs1799750 and disc degeneration shown on MRI was observed in the systematic search performed for this review. Still, the study of Jacobsen et al. [44] showed that the MMP1 SNP rs1799750, i.e., the 2G allele insert, may be associated with poor pain recovery after lumbar disc herniation. Previous studies show that other painful degenerative inflammatory conditions may be associated with the MMP1 SNP rs1799750 2G allele [52, 53].

Several lines of evidence suggest that genetic variability in genes encoding inflammatory cytokines may be associated with persistent LBP [16]. The present review shows data that the IL1α rs1800587 T allele and the IL6 haplotype rs180077 G, rs1800796 C, rs1800795 C, rs13306435 A may be associated with slower recovery in LRP patients [29–31]. Moreover, data exists that the rare allele of the gene encoding the GTP cyclohydrolase, could be associated with reduced pain following discectomy in LRP patients [24]. However, more recent reports questions these data [54].

Six studies in the present review show correlations between protein levels and recovery of pain [37–39, 41, 42, 45]. However, only IL-6, TNFα and IFNα seem to be associated with persistence of LRP. Schistad et al. [30, 42] showed that higher serum level of IL-6 predicts a less favorable clinical outcome. Moreover, Scuderi et al. [45] and Andrade et al. [38] showed that TNFα and IFNα may be associated with persistent LRP. In addition, previous studies suggest a correlation between TNFα and recovery of pain in chronic LBP and lumbar radiculopathy patients [55, 56].

Development of persistent pain is multifactorial. It is now well established that psychosocial factors, such as depressive mood, distress and somatization, may contribute to chronic LRP [57]. Together with individual factors as gender, age, smoke, obesity and education level, genetic predisposition may be crucial prognostic factors in LBP patients as well as LRP patients [6, 57].

Strength and limitation

To our knowledge, this is the first paper attempting to provide an overview over genetic variants linked to the development of persistence LRP. Still, many of the findings, including the role of the GTP cyclohydrolase, are controversial and need to be replicated [54]. In addition few researchers present genetic data together with changes in protein expression. In further studies this knowledge gap need to be highlighted.
Therefore, the interpretation of the data, but also the heterogeneity in the nomenclature, might be challenging. In the present review we have listed the genetic variants by number, base substitution, position on DNA and if applicable amino acid substitution. Position of base replacement refers to position found in National Center for Biotechnology (NCBI). The majority of the nucleotide replacements listed is located in the intron or promoter region. Only two of the SNPs cause amino acid substitution. Regarding the interpretation of the data, the link between the genetic variability, protein expression and function is therefore definitely challenging.

External validity in all but one of the genetic association studies is fair. The sample size was >100 patients in nine studies, however, as many as six of the samples emanate from the same cohort, and the methodological quality of the studies may still be debated. A bias towards only positive findings being published cannot be excluded. Moreover, the external validity is poor in the six studies about biomarkers – and in only two studies correction for multiple testing was performed. The strength of this review, however, is the optimized systematic search in several databases and the strict inclusion criteria. Unfortunately, the studies were too few and too heterogeneous to perform meta-analyses, and many of the studies emanate from the same cohort. Further on, GWAS would shed light on other genetic factors related to the same phenomena. Unfortunately, however, most clinical studies do not have enough statistical strength for GWAS. This may be a major challenge in clinical research. None of the included studies were GWAS. Moreover, no studies addressed the interaction between environmental factors and genetic markers. An extensive systematic review by Eskola et al. 2012 regarding LBP and genetics evaluated that the credibility of reported genetic associations were mostly not applicable as this is a systematic review of previously published studies.

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Authors’ contributions
SB, JG and CR were involved in the design of the review paper. SB performed the systematic search for articles. SB and CR performed reading, assessed and included the relevant articles, assessed methodological quality and analyzed the results. SB, AM, ES, JG and CR participated in interpretation of the data and drafting of the manuscript. SB, JG and CR wrote the paper. All authors have read and approved the final manuscript and stand by the integrity of the entire work. We declare no conflict of interest.

Authors’ information
No additional comments.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

References


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