Minna Kraatari

THE HERITABILITY AND GENETIC RISK FACTORS OF MODIC CHANGES
ACTA UNIVERSITATIS OULUENSIS
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MINNA KRAATARI

THE HERITABILITY AND GENETIC RISK FACTORS OF MODIC CHANGES

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Abstract

Low back pain (LBP) is a highly prevalent musculoskeletal condition and the leading cause for workplace absenteeism. Lumbar disc degeneration (DD) is considered as a contributing factor to LBP. The role of genetic factors in the development of lumbar DD has been demonstrated to be significant, with heritability estimates ranging from 64% to 81%. Modic change (MC), a distinct phenotype of lumbar DD, is a subchondral and vertebral bone marrow change revealed only by magnetic resonance imaging (MRI). MC has been associated with LBP in both clinical samples and the general population. The genetic background of MC is largely unknown, and the heritability of MC has not previously been assessed.

The aim of this study was to assess the heritability of MC using a twin study, identify predisposing genetic factors for MC in a family-based design using whole-exome sequencing and to identify genetic loci associated with MC using genome-wide association study (GWAS) meta-analysis. An additional aim was to study the prevalence, incidence and morphology of MC. The data consisted of two general population samples, the Northern Finland Birth Cohort 1966 (NFBC1966) and TwinsUK from the United Kingdom, as well as two Finnish families from the Oulu region.

MC was found to be partly heritable with a heritability estimate of 30%. Two novel candidate genes, HSPG2 and MAML1, were found co-segregating with MC in two Finnish families. Both genes are important in the growth and differentiation of chondrocytes. Finally, a genetic locus on chromosome 9 was found to be significantly associated with MC using genome-wide meta-analysis of NFBC1966 and TwinsUK.

These results showed that genetic factors play a role in the development of MC. In conclusion, this thesis increased the knowledge on the genetics of MC. However, the specific roles of these genes need to be studied further.

Keywords: disc degeneration, exome sequencing, genetics, genome wide association study (GWAS), heritability, low back pain, modic change, twin study
Tiivistelmä


Asiasanat: alaselkäkipu, eksomisekvensointi, genetiikka, kaksostutkimus, koko genomin laajuinen assosiaatioanalyysi, Modic-muutos, perinnöllisyys, välilevyrappeuma
To my family
Acknowledgements

This study was carried out in the Faculty of Medicine and in the Faculty of Biochemistry and Molecular Medicine, and I have been privileged to be part of the Medical Research Center Oulu.

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I also greatly appreciate the help of my co-authors Juhani Määttä, PhD, for teaching me to interpret lumbar magnetic resonance images and Sini Skarp, MSc, for her valuable help in whole exome sequencing and genome-wide association data analyses. I also wish to thank Lisa Wobler, PhD, Sam Wadge, BSc, Frances Williams, PhD, FRCP(E), Professor Jaakko Niinimäki, Professor Johannes Kettunen and Maxim Freidin, PhD for their valuable work on my articles.

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18.7.2018

Minna Kraatari
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>adenine</td>
</tr>
<tr>
<td>ACAN</td>
<td>aggrecan</td>
</tr>
<tr>
<td>ADAMTS</td>
<td>a disintegrin and metalloproteinase with thrombospondin motifs</td>
</tr>
<tr>
<td>AF</td>
<td>annulus fibrosus</td>
</tr>
<tr>
<td>ASPN</td>
<td>asporin</td>
</tr>
<tr>
<td>BCL11A</td>
<td>B-cell lymphoma/leukemia 11A</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>CD-CV</td>
<td>common disease – common variant hypothesis</td>
</tr>
<tr>
<td>CHST3</td>
<td>carbohydrate sulfotransferase 3</td>
</tr>
<tr>
<td>CILP</td>
<td>cartilage intermediate layer protein</td>
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<tr>
<td>COL1A1</td>
<td>collagen I alpha 1</td>
</tr>
<tr>
<td>COL2A1</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>collagen IX alpha 2</td>
</tr>
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</tr>
<tr>
<td>COL11A2</td>
<td>collagen XI alpha 2</td>
</tr>
<tr>
<td>COX2</td>
<td>cyclooxygenase-2</td>
</tr>
<tr>
<td>CTCF</td>
<td>CCCTC-Binding Factor</td>
</tr>
<tr>
<td>DD</td>
<td>disc degeneration</td>
</tr>
<tr>
<td>DDSH</td>
<td>dyssegmental dysplasia Silverman-Handmaker type</td>
</tr>
<tr>
<td>DLG2</td>
<td>discs large MAGUK scaffold protein 2</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DZ</td>
<td>dizygotic twin</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
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<tr>
<td>eQTL</td>
<td>expression quantitative trait loci</td>
</tr>
<tr>
<td>ExAC</td>
<td>Exome Aggregation Consortium database</td>
</tr>
<tr>
<td>FDH</td>
<td>Finnish disease heritage</td>
</tr>
<tr>
<td>G</td>
<td>guanine</td>
</tr>
<tr>
<td>GATK</td>
<td>Genome Analysis ToolKit</td>
</tr>
<tr>
<td>GDF5</td>
<td>growth differentiation factor 5</td>
</tr>
<tr>
<td>Gnomad</td>
<td>genome aggregation database</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
</tr>
<tr>
<td>HRC</td>
<td>Haplotype Reference Consortium</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>HSPG2</td>
<td>heparan sulfate proteoglycan 2</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg equilibrium</td>
</tr>
<tr>
<td>indel</td>
<td>insertion and deletion mutation</td>
</tr>
<tr>
<td>IL1A</td>
<td>interleukin 1 alpha</td>
</tr>
<tr>
<td>IL6</td>
<td>interleukin 6</td>
</tr>
<tr>
<td>IL8</td>
<td>interleukin 8</td>
</tr>
<tr>
<td>IVD</td>
<td>intervertebral disc</td>
</tr>
<tr>
<td>LBP</td>
<td>low back pain</td>
</tr>
<tr>
<td>LD</td>
<td>linkage disequilibrium</td>
</tr>
<tr>
<td>LOD</td>
<td>logarithm of the odds</td>
</tr>
<tr>
<td>MAF</td>
<td>minor allele frequency</td>
</tr>
<tr>
<td>MAML1</td>
<td>mastermind-like transcriptional coactivator 1</td>
</tr>
<tr>
<td>MC</td>
<td>Modic change</td>
</tr>
<tr>
<td>MC1</td>
<td>type 1 Modic change</td>
</tr>
<tr>
<td>MC2</td>
<td>type 2 Modic change</td>
</tr>
<tr>
<td>MC3</td>
<td>type 3 Modic change</td>
</tr>
<tr>
<td>MGMT</td>
<td>O-6-Methylguanine-DNA Methyltransferase</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metallopeptidases</td>
</tr>
<tr>
<td>MMP1</td>
<td>matrix metallopeptidase 1</td>
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<tr>
<td>MMP2</td>
<td>matrix metallopeptidase 2</td>
</tr>
<tr>
<td>MMP3</td>
<td>matrix metallopeptidase 3</td>
</tr>
<tr>
<td>MMP9</td>
<td>matrix metallopeptidase 9</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MZ</td>
<td>monozygotic twin</td>
</tr>
<tr>
<td>NFBC1966</td>
<td>Northern Finland Birth Cohort 1966</td>
</tr>
<tr>
<td>NGS</td>
<td>next generation sequencing</td>
</tr>
<tr>
<td>NP</td>
<td>nucleus pulposus</td>
</tr>
<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>OPRK1</td>
<td>opioid receptor kappa 1</td>
</tr>
<tr>
<td>PARK2</td>
<td>Parkinson protein 2</td>
</tr>
<tr>
<td>PBX3</td>
<td>PBX homeobox 3</td>
</tr>
<tr>
<td>PC</td>
<td>principal component</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PGE-2</td>
<td>prostaglandin E$_2$</td>
</tr>
<tr>
<td>PSMB9</td>
<td>proteosome subunit β type 9</td>
</tr>
<tr>
<td>PTPRD</td>
<td>protein tyrosine phosphatase, receptor type D</td>
</tr>
</tbody>
</table>
QQ-plot  quantile-quantile plot
RV-CD    rare variant – common disease hypothesis
RUNX2    runt related transcription factor 2
SISu     Sequencing Initiative Suomi database
SJS      Schwartz-Jampel syndrome
SKT      Sickle tail
SNP      single nucleotide polymorphism
SNV      single nucleotide variation
SPI1     transcription factor PU.1
T        thymine
THSB2    thrombospondin 2
TIMP1    tissue inhibitor of metalloproteinase 1
TIMP3    tissue inhibitor of metalloproteinase 3
TNF-α    Tumor necrosis factor alpha
VDR      vitamin D receptor
VNTR     variable number of tandem repeat polymorphism
WES      whole exome sequencing
WGS      whole genome sequencing
XKR4     XK related 4
ZNF184   zinc finger protein 184
List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:


* These authors contributed equally
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1 Introduction

Low back pain (LBP) is the most disabling health condition worldwide with a lifetime prevalence of up to 84%. It causes significant costs to the society. An estimate of the total costs in Finland in 2007 was 430 million euros. The pathophysiology of LBP is still largely unknown, but disc degeneration (DD) has been recognized as one of the major risk factors. The role of genetic factors in the development of lumbar DD has been demonstrated to be significant, with heritability estimates up to 74%.

Modic change (MC), a distinct phenotype of DD, is a pathological bone marrow signal change adjacent to vertebral endplates visible only on magnetic resonance imaging (MRI). MC can be classified into three types according to the different signal intensities in T1- and T2-weighted MRIs: type 1 (MC1), type 2 (MC2) and type 3 (MC3). The median prevalence of MC has been noted to be 43% among patients with LBP, compared to 6% in non-clinical populations, and to be associated with LBP.

The etiology and pathophysiology of MC are still largely unknown. Based on previous studies using the candidate gene approach, the interleukin 1 alpha (IL1A) and matrix metalloproteinase 3 (MMP3) genes may play roles in the development of MC. However, so far no one has actually assessed the heritability of MC.

The aim of this thesis was to study the heritability of MC in a large twin cohort (N=831), identify rare variants behind the development of MC using whole exome sequencing in two Finnish families with excessive loading of MC and to identify genetic loci associated with MC using a genome-wide association study (GWAS) meta-analysis in two population samples, the Northern Finland Birth Cohort 1966 (NFBC1966) (N=1182) and TwinsUK (N=647).
2 Review of the literature

2.1 Spine

The human spine, also called the vertebral column, is a structure responsible for supporting the body and protecting its vital structures from damage. A normal adult spine is composed of 33 vertebrae. Seven of them are cervical (C1-C7), twelve thoracic (T1-T12), five lumbar (L1-L5), five sacral and four coccygeal (Fig. 1). Sacral and coccygeal vertebrae usually unite in adults forming the sacrum and coccyx, respectively (Putz & Pabst, 2009). The number of vertebrae can vary between individuals due to physiological variation. The spine has four curves, two lordotic that convex anteriorly and two kyphotic that convex posteriorly (Putz & Pabst, 2009). The spinal curves help to absorb the load applied to the spine.

Each vertebra consists of an anterior vertebral body and a posterior vertebral arch. The large vertebral body is responsible for supporting the weight of the human body. Pedicles, transverse and spinous processes, and upper articular processes form the vertebral arch. A synovial joint, called the facet joint, is formed by the articular processes (Fig. 2) (Putz & Pabst, 2009). The bone in both the vertebral body and vertebral arch is composed of an outer layer of compact bone and an inner layer of trabecular bone. An intervertebral disc (IVD) lies between the vertebral bodies bearing axial compression, acting as a shock absorbent and creating a flexible column. The vertebral bodies and intervertebral discs are also connected by stabilizing ligaments.

The spinal cord runs within the vertebral column from the cranium to the L1-L2 level. In total, 31 pairs of spinal nerves emerge through the intervertebral foramen located anterior to the facet joints (Putz & Pabst, 2009). Spinal nerves are responsible for transmitting motor signals from the central nervous system to the muscles, and transmitting sensory information to the central nervous system.
Fig. 1. Lateral view of the spine. This is a public domain image.
2.1.1 Intervertebral disc

IVDs lie between the vertebral bodies anchoring them together and distributing the pressure resulting from the movement of the entire trunk. They are the main joints of the spine making up almost 30% of its height (Raj, 2008). There are normally 25 IVDs in total from the axis to the sacrum (Chan, Sze, Samartzis, Leung, & Chan, 2011). The shape and thickness of the IVDs vary between the different parts of the spine with the thinnest disc in the upper thoracic region and thickest in the lower lumbar level.

The healthy adult disc is an avascular structure while oxygen, energy and nutrients are received and metabolic wastes removed by diffusion through the cartilage endplate and via the outer layers of the disc (Drake, Vogl, & Mitchell, 2010). The IVD is the largest avascular, alymphatic and aneural structure in the adult human body, since the blood vessels and nerves reach only a few millimeters into the outer part of the disc (Roberts, Evans, Trivedi, & Menage, 2006). Overall,
the IVDs are important components of the vertebral column and are vital for the mobility of intervertebral joints (Colombier, Clouet, Hamel, Lescaudron, & Guicheux, 2014).

2.1.2 Intervertebral disc composition

An IVD is composed of a central fluid-like nucleus pulposus (NP), tough laminated annulus fibrosus (AF) located peripherally and cartilaginous endplates that locate both cranially and caudally (Fig. 3 and 4). The three components act synergistically easing the motions of the spine and acting as shock absorbers (Guerin & Elliott, 2007; Schmidt et al., 2007). The principal structural differences between AF and NP are displayed in Table 1.

![Fig. 3. Superior view of the IVD. This image was made by the author.](image-url)
Fig. 4. Lateral view of the IVD. This image was made by the author.

Table 1. Main IVD components.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Outer AF</th>
<th>Inner AF</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM water content</td>
<td>65-75%</td>
<td>75-80%</td>
<td>75-90%</td>
</tr>
<tr>
<td>Dominant collagen type</td>
<td>Type I collagen</td>
<td>Type II collagen</td>
<td>Type II collagen</td>
</tr>
<tr>
<td>Proteoglycan content</td>
<td>10%</td>
<td>20-35%</td>
<td>20-60%</td>
</tr>
<tr>
<td>Other proteins</td>
<td>5-15%</td>
<td>5-40%</td>
<td>15-55%</td>
</tr>
</tbody>
</table>


**Annulus fibrosus**

The AF is the fundamental load-bearing complex of the IVD (Ghannam et al., 2016). It is divided into outer and inner AF that have different compositions and biomechanical properties. The outer AF is highly organized and composed of fibroblasts producing mainly type I collagen. The extracellular matrix (ECM) of the outer AF is arranged into 15-25 concentric rings or lamellae, which are composed of closely arranged collagen and elastin fibers (Chan et al., 2011). Lamellae are connected by proteoglycan aggregates and lubricin as well as collagen IV (Colombier et al., 2014). The inner AF is comprised mostly of chondrocyte-like cells producing mainly collagen II and proteoglycans such as aggrecan making the inner AF less fibrous (Humzah & Soames, 1988).
**Nucleus pulposus**

The NP is described as a gelatinous material bounded by an outer ring and two cartilaginous endplates. It provides support, allows movement, absorbs shocks, permits transient compression and spreads the loads applied to the AF (Lundon & Bolton, 2001). The ECM of the NP is composed of collagen II and proteoglycans, mainly aggrecan, but also small amounts of collagen IX and XI. Due to the hydrophilic nature of the aggrecan, the water content of the NP is high (Roughley, 2004). The collagen II together with the high water content enables the NP to be elastic and distort under stress (Colombier et al., 2014). The cells are a minor component of the ECM and are mainly chondrocyte-like producing other ECM components.

**Endplate**

The third distinct region is the endplate, a thin layer of hyaline cartilage separating the disc from the vertebral bodies. The endplate is composed of ECM, mainly proteoglycans and collagen II synthesized by chondrocytes (Colombier et al., 2014). It acts as a regulator and a source of oxygen and nutrients diffusing from the vertebral bodies to the avascular disc (Urban, Smith, & Fairbank, 2004). It is also responsible for acting as a filter regulating the movement of water between the NP and the vertebral bodies (Ghannam et al., 2016). It is under debate whether the endplate is a part of the disc or the vertebra (Bogduk, 2012; Eyring, 1969).

### 2.2 Low back pain

LBP is defined by the location of pain between buttock creases and lower rib margins, and the pain may radiate to one or both legs (Dionne et al., 2008). It is the most common musculoskeletal condition throughout the world with a lifetime prevalence of up to 84% (Murray et al., 2013; Walker, 2000). It is the leading cause of years lost to disability worldwide and its burden increases as the population ages and grows (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). LBP causes significant costs to the society, both direct (health care) and indirect (lost productivity and work disability). An estimate of the total costs in Finland in 2007 was 430 million euros (Pohjolainen, Seitsalo, Sund, & Kautiainen, 2007). All age groups are affected by LBP, while it is more common in the 40-69-year age group (D. Hoy et al., 2012; Swain et al., 2014). The prevalence of LBP is
higher in females and in high-income countries (D. Hoy et al., 2012). However, the largest increase during the past few decades in disability caused by LBP has taken place in middle- and low-income countries (D. G. Hoy et al., 2015).

The pathophysiology of LBP is still largely unresolved. DD has been recognized as one of the major risk factors for LBP (Cheung et al., 2009; Kjaer, Leboeuf-Yde, Korsholm, Sorensen, & Bendix, 2005; Livshits et al., 2011; MacGregor, Andrew, Sambrook, & Spector, 2004). The term non-specific LBP is used when the specific pathoanatomical cause of the pain is unknown. It is the most common form of LBP accounting for up to 90% of all patients with LBP (Koes, van Tulder, & Thomas, 2006; Maher, Underwood, & Buchbinder, 2017). Only a small proportion of cases are due to a specific cause such as malignancy, vertebral fracture, infection, inflammatory disease or an intra-abdominal cause that requires targeted treatment.

It is claimed that about 90% of the patients suffering from acute LBP recover within three months (Balague, Mannion, Pellise, & Cedraschi, 2012; Pengel, Herbert, Maher, & Refshauge, 2003), but a considerable proportion of LBP patients have a slower recovery trajectory (Kongsted, Kent, Axen, Downie, & Dunn, 2016) with residual pain and disability at one year (da C Menezes Costa et al., 2012). LBP is considered chronic when it lasts more than three months. It is often persistent or recurrent especially in older patients (Cassidy, Cote, Carroll, & Kristman, 2005; da C Menezes Costa et al., 2012; Kongsted et al., 2016). Known risk factors for the occurrence of LBP episodes or persistent LBP are a previous LBP episode (Taylor, Goode, George, & Cook, 2014), smoking (Shiri, Karppinen, Leino-Arjas, Solovieva, & Viikari-Juntura, 2010), obesity (Shiri et al., 2010), low levels of physical activity (Shiri & Falah-Hassani, 2017), heavy workload (Heneweer, Staes, Aufdemkampe, van Rijn, & Vanhees, 2011), poor mental health (Currie & Wang, 2005) and a chronic disease such as osteoarthritis, asthma and diabetes (Ferreira, Beekenkamp, Maher, Hopper, & Ferreira, 2013).

Treatment of LBP has been assessed widely. New guidelines suggest using non-pharmacological treatments in treating LBP. Emphasis is now placed on self-management, physical and psychosocial therapies and complementary medicine such as pain medication, skeletal muscle relaxants and selective norepinephrine reuptake inhibitors (Foster et al., 2018). LBP is often accompanied by comorbidities like concurrent pain in other sites, general physical and mental health problems typically resulting in a poorer response to treatment (Hartvigsen, Natvig, & Ferreira, 2013). Evidence for prevention of LBP is inadequate and moderate.
evidence exists only for exercise either alone or combined with education (Foster et al., 2018; Steffens et al., 2016).

2.3 Intervertebral disc degeneration

DD affects the disc making it unable to bear physical loads. It is an inevitable part of aging. However, the disc may start to show signs of degeneration as early as the second decade of life (Boos et al., 2002).

2.3.1 Definition and prevalence

DD is characterized as biochemical and morphological changes. Magnetic resonance imaging (MRI) is the current gold standard in assessing the integrity of the IVD (Modic & Ross, 2007). Degenerative findings on MRI are reduced signal intensity representing loss of hydration, reduced disc height, presence of fissures in the disc or dislocation of disc material outside the normal confines of the disc (Haughton, 2006).

The prevalence of DD varies widely from 7% to 85% with a combined estimate of 54% (Endean, Palmer, & Coggon, 2011). Estimates differ between different age groups. In asymptomatic individuals, the prevalence of DD ranges from 37% among 20-year-olds to 96% among 80-year-olds. The prevalence of DD is known to increase with age and the prevalence increases markedly after 50 years of age (Adams & Roughley, 2006; Brinjikji et al., 2015).

The pathogenesis of DD is still not fully understood. The underlying cause of DD is thought to be tissue weakening due to genetic inheritance, aging, nutritional compromise and loading history (Adams & Roughley, 2006). A structural disruption occurring from injury or fatigue is thought to be the precipitating factor (Adams & Roughley, 2006). DD is the most common in the lower lumbar spine (Adams & Roughley, 2006; Rajasekaran, Venkatadass, Naresh Babu, Ganesh, & Shetty, 2008). The role of genetic factors has been demonstrated to be significant, with heritability estimates up to 74% (Bijkerk et al., 1999; Sambrook, MacGregor, & Spector, 1999). Also, the progression of DD is partly controlled by genetic factors. Posterior disc bulge was found to be heritable across all age groups with a heritability estimation ranging from 28% to 53% while changes in anterior osteophytes and disc signal intensity were found to be heritable only in under 50-year-olds (heritability estimations 74% and 76%, respectively) (Williams, Popham, Sambrook et al., 2011). Other suggested risk factors include male gender (Arana et
al., 2011), obesity (Arana et al., 2011; Liuke et al., 2005) and heavy smoking (Battie et al., 1991; Battie, Videman, Levalahti, Gill, & Kaprio, 2008; Kjaer, Korsholm, Bendix, Sorensen, & Leboeuf-Yde, 2006). There is no apparent evidence of an association between DD and heavy occupational loading or leisure time loading (Battie et al., 2009), but an association between DD and high physical activity has been found among athletes (Hangai et al., 2009; A. Ong, Anderson, & Roche, 2003). However, the role of other risk factors in the development of DD has not been shown convincingly.

2.3.2 Structure of degenerated intervertebral disc

The most significant change in DD is the loss of proteoglycans in the NP (Urban & Roberts, 2003). The aggrecan molecules degrade resulting in the loss of glycosaminoglycans that are responsible for maintaining the osmotic pressure in the disc. There is also a change in the type and distribution of collagens with a shift from collagen II to collagen I in the NP (Antoniou et al., 1996). Overall, the biomechanical content of the disc changes from proteoglycans and collagen II to fibrous collagen I, resulting in disorganization of the NP (Urban & Roberts, 2003; Vergroesen et al., 2015). Reduced osmotic pressure is reflected in the reduction of the disc height (Vergroesen, van der Veen, van Royen, Kingma, & Smit, 2014). Furthermore, the lamellae of the AF become disorganized resulting in a reduction of the mechanical strength of the disc. Clefts and fissures formed in the NP further weaken the mechanical integrity of the disc (Urban & Roberts, 2003). Normally, the disc receives its nutrients through the endplates. However, the calcification of the endplates occurring as part of DD reduces the nutrient and cell waste transport. Decreased nutrition accompanied by loss of proteoglycans and water weakens the ability of IVD cells to function properly (Whatley & Wen, 2012). Altogether, the NP becomes dehydrated, fibrous and stiff providing less cushioning effect (Antoniou et al., 1996). Structural changes of the IVD also occur during the normal aging process and it is difficult to distinguish normal aging from DD (Raj, 2008; Roberts et al., 2006).

Degeneration of the disc is described as a vicious cycle. Homeostasis of the disc is contingent on the interactions between the cells, the ECM and biomechanical stress. If this balance is disrupted, the cells start to produce less proteoglycans resulting in reduced osmotic pressure and thus higher shear forces. The increase in shear forces further decreases the proteoglycan production (Vergroesen et al., 2015).
2.4 Modic change

2.4.1 Definition and prevalence

MC is a pathological bone marrow signal change adjacent to vertebral endplates visible only on MRI. It is considered as a distinct phenotype of lumbar DD. De Roos et al. (1987) first described bone marrow signal changes in the vertebral bodies (de Roos, Kressel, Spritzer, & Dalinka, 1987) and Modic et al. classified these signal intensity changes into two groups one year later (Modic, Steinberg, Ross, Masaryk, & Carter, 1988). A third type was classified later by both de Roos et al. and Modic et al. (de Roos et al., 1987; Modic, Masaryk, Ross, & Carter, 1988).

MC can be classified into three types according to the different signal intensities in T1- and T2-weighted MRIs: type 1 (MC1), type 2 (MC2) and type 3 (MC3) (de Roos et al., 1987; Modic et al., 1988). The different types are thought to represent different stages of the same pathological process linked to the adjacent discs (Perilli et al., 2015). MC1 (low T1 and high T2 signals) indicates an ongoing degeneration with disruption and fissuring of the endplates, hypervascularity and high bone turnover (Fig. 5), while MC2 (high T1 and high T2 signals) reflects fatty replacement of the bone marrow and decreased bone formation (Modic et al., 1988; Perilli et al., 2015) (Fig. 6). MC3 (low T1 and low T2 signals) indicates bone sclerosis (Modic et al., 1988; Perilli et al., 2015) (Fig. 7). Also, mixed types have been identified, usually Modic types 1 and 2 or types 2 and 3 (Braithwaite, White, Saifuddin, Renton, & Taylor, 1998; Kuisma et al., 2006).

Usually type, height and width are evaluated when assessing MC on MRI. According to the Nordic Modic Consensus Group classification in 2007, MC is evaluated in terms of height relative to the vertebra, horizontal endplate area and intravertebral volume (T. S. Jensen, Sorensen, & Kjaer, 2007). A more recent classification, however, has included also the localization of MC antero-posteriorly (Määttä, Karppinen, Luk, Cheung, & Samartzis, 2015; Määttä et al., 2016).

It is proposed that the course of MC follows a so-called developmental pathway and all types represent different stages of the same pathological process eventually converging towards MC3 (R. K. Jensen et al., 2012; Kuisma et al., 2006; Mitra, Cassar-Pullicino, & McCall, 2004; Modic et al., 1988; Modic et al., 1988). However, MC can also develop in the opposite direction and can disappear completely (R. K. Jensen et al., 2012; T. S. Jensen et al., 2009; Zhang, Zhao, Jiang, Chen, & Dai, 2008). Large MC has been suggested to be more stable (R. K. Jensen et al., 2012; T. S. Jensen et al., 2009).
Fig. 5. MC1 on MRI. MC1 show decreased signal intensity on T1w (arrow) (right) and increased signal intensity on T2w (arrow) (left) image.

Fig. 6. MC2 on MRI. MC2 show increased signal intensity on T1w (arrow) (right) and increased signal intensity on T2w (arrow) (left) image.
Fig. 7. MC3 on MRI. MC3 show decreased signal intensity on T1w (arrow) (right) and decreased signal intensity on T2w (arrow) (left) image.

MC has been shown to be associated with DD in several studies (T. S. Jensen et al., 2010; Kjaer et al., 2006; Luoma, Vehmas, Grönblad, Kerttula, & Kääpä, 2009; Mok et al., 2016). In addition, it has been linked to other degenerative MRI findings such as intervertebral disc displacement (disc herniation and bulge) (Albert & Manniche, 2007; Albert et al., 2011; T. S. Jensen et al., 2010) and Schmorl’s nodes (el Barzouhi et al., 2014; Määttä et al., 2015). Endplate defects including endplate unevenness and focal defects are associated especially with MC1 (Luoma, Vehmas, Grönblad, Kerttula, & Kääpä, 2008).

MC can be detected in the human lumbar, thoracic and cervical spine and has even been seen in the spine of some animals (Besalti, Pekcan, Sirin, & Erbas, 2006). They are most common in the lower lumbar levels where the depth and extent of MC are also the greatest (Kuisma et al., 2006; Kuisma et al., 2007; Modic et al., 1988; Mok et al., 2016). On the other hand, MC in the upper lumbar level is usually smaller and anteriorly located (T. S. Jensen et al., 2009; Kuisma et al., 2006). MC is usually present in both rostral and caudal endplates of the disc (Kuisma et al., 2006).

The prevalence of MC varies between studies according to the study population and participants’ age. The median prevalence of MC has been reported to be 43% among patients suffering from LBP, while only 6% in non-clinical populations (T. S. Jensen, Karppinen, Sorensen, Niinimäki, & Leboeuf-Yde, 2008). A few studies have found MC1 to be the most frequent (T. S. Jensen et al., 2009; Martinez-
Quinones, Aso-Escario, Gonzalez-Garcia, Consolini, & Arregui-Calvo, 2017) while other studies have found MC2 to be predominant (Arana et al., 2011; Arnbak et al., 2016; Määttä et al., 2015; Teichtahl et al., 2017). In comparison, in both clinical and general populations the prevalence of MC3 is very low (T. S. Jensen et al., 2008; Teichtahl et al., 2017) (Table 2).

There are several lifestyle, occupational and demographic factors associated with MC, but age is the only risk factor that has been consistently associated with MC (R. K. Jensen et al., 2012; Kuisma et al., 2006; Mok et al., 2016; Y. Wang, Videman, & Battie, 2012). Considerable controversy exists in describing the role of other factors. Male gender (Arana et al., 2011; Karchevsky et al., 2005), obesity (Arana et al., 2011; Kuisma et al., 2008; H. L. Wu et al., 2012), heavy smoking (Kjaer et al., 2006; H. L. Wu et al., 2012), previous lumbar injury (Mok et al., 2016) and heavy physical work (Kjaer et al., 2006) have been associated with MC in previous studies. However, in a Danish general population study, gender, high physical work activity, high body mass index (BMI) or heavy smoking did not predict the development of new MC (T. S. Jensen et al., 2010).

2.4.2 Association with low back pain

Various studies have noted the association between MC and LBP (T. S. Jensen et al., 2008; Kjaer et al., 2006; Määttä, Wadge, MacGregor, Karppinen, & Williams, 2015), particularly for MC1 (Kääpä, Luoma, Pitkäniemi, Kerttula, & Grönblad, 2012). The association has been noted in both general (Kjaer et al., 2005) and clinical populations (Albert & Manniche, 2007; Braithwaite et al., 1998; O. K. Jensen, Nielsen, Sorensen, & Stengaard-Pedersen, 2014). However, some studies have provided contradictory results (el Barzouhi et al., 2014; Jarvik et al., 2005; Kovacs et al., 2012). Among English twins, MC was found to be independently associated with episodes of severe and disabling LBP (Määttä et al., 2015). This strengthens the view that MC is not only a part of the DD phenotype but also an independent predictor of both severe disabling and mild non-disabling LBP.
Table 2. Prevalence of lumbar MC in different populations.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Female (%)</th>
<th>Prevalence of MC (%)</th>
<th>Prevalence of MC1 (%)</th>
<th>Prevalence of MC2 (%)</th>
<th>Prevalence of MC3 (%)</th>
<th>Mean age (years)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>34</td>
<td>13.1</td>
<td>8.1</td>
<td>5.0</td>
<td>0</td>
<td>30</td>
<td>Martinez-Quinones et al. 2017</td>
</tr>
<tr>
<td>General</td>
<td>6</td>
<td>-</td>
<td>4.2</td>
<td>27.8</td>
<td>1.3</td>
<td>49</td>
<td>Teichtahl et al. 2017</td>
</tr>
<tr>
<td>General</td>
<td>-</td>
<td>5.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>Mok et al. 2016</td>
</tr>
<tr>
<td>Clinical</td>
<td>5</td>
<td>40.0</td>
<td>15.3</td>
<td>21.8</td>
<td>-</td>
<td>33</td>
<td>Ambak et al. 2016</td>
</tr>
<tr>
<td>General</td>
<td>6</td>
<td>21.9</td>
<td>6.3</td>
<td>15.5</td>
<td>-</td>
<td>49</td>
<td>Määttä et al. 2015</td>
</tr>
<tr>
<td>General</td>
<td>0</td>
<td>55.6</td>
<td>6.4</td>
<td>29.2</td>
<td>2</td>
<td>50</td>
<td>Wang et al. 2012</td>
</tr>
<tr>
<td>Clinical</td>
<td>54</td>
<td>80.9</td>
<td>3.7</td>
<td>51.3</td>
<td>0.6</td>
<td>43</td>
<td>Arana et al. 2011</td>
</tr>
<tr>
<td>Clinical</td>
<td>53</td>
<td>39</td>
<td>32.8</td>
<td>1.2</td>
<td>0</td>
<td>40</td>
<td>Jensen et al. 2009</td>
</tr>
</tbody>
</table>

MC = Modic change, MC1 = type 1 Modic change, MC2 = type 2 Modic change, MC3 = type 3 Modic change

2.4.3 Pathophysiology

The etiology and pathophysiology of MC are still largely unknown. Current literature suggests two hypotheses for the pathogenesis of MC: mechanical and bacterial. Both hypotheses may be involved simultaneously but the exact roles are unknown.

An association exists between DD, disc herniation and MC (Albert & Manniche, 2007; Elfering et al., 2002; Kjaer et al., 2006). This association gives rise to the mechanical hypothesis suggesting that MC is caused by endplate damage leading to an inflammatory process and chronic inflammation with fibrosis and granulation tissue (Dudli, Fields, Samartzis, Karppinen, & Lotz, 2016). Endplate damage has been identified to occur together with MC and is thought to be a predictive factor for future MC (Modic et al., 1988; Weiner et al., 2015).

Discs adjacent to MC produce higher amounts of cytokines and osteoclast stimulating factors (Chen et al., 2014; Torkki et al., 2016). Structural damage of the
disc or the endplate can enable cytokines to ascend from the disc and the endplate into the bone marrow (Torkki et al., 2016). Higher levels of serum high-sensitivity C-reactive protein were identified in the patients with chronic LBP and MC1 (Rannou et al., 2007). Cytokines ascended into the bone marrow interfere with the cellular composition of the bone marrow and alter trabecular bone mass (Dudli et al., 2016). MC biopsies reveal high bone turnover in MC1 and reduced bone formation in MC2 (Modic et al., 1988). Another possibility is that endplate damage or disc herniation causes a relocation of NP to contact with bone marrow exposing the NP to the immune system where it is recognized as foreign and triggers an autoimmune response (Geiss, Larsson, Junevik, Rydevik, & Olmarker, 2009).

Hyperloading is considered a risk factor for MC. Minor structural changes in the lumbar spine alter the mechanical environment in the disc and cause instability (Adams, Freeman, Morrison, Nelson, & Dolan, 2000; Adams & Roughley, 2006) leading to microfractures in the endplate (Adams et al., 2000). These in turn result in an uneven load distribution across the disc and may contribute to endplate fissures (Gruber & Hanley, 2003; Modic et al., 1988). This enables inflammatory cytokines and ECM catabolites to ascend from the disc into the bone marrow possible leading to the development of MC (Albert et al., 2008). This theory is supported by the fact that histological findings of MC1 exhibit fissuring and disruption of the endplate (Modic et al., 1988; Perilli et al., 2015). Instability might also change the shape of the endplate as an adaptation to restrain abnormal movement of the lumbar segment (Y. Li et al., 2014). MC was frequently seen with the change in sagittal endplates from concave to flat to irregular (Y. Li et al., 2014).

Another theory suggests that the occurrence of MC might involve low virulent anaerobic bacteria. Peripheral disc damage in conjunction with disc herniation could allow access of low virulent bacteria such as Propionibacterium acnes (P. acnes) into the disc causing a low virulent and slowly developing infection (Stirling, Worthington, Rafiq, Lambert, & Elliott, 2001). These bacteria are normally found in the skin and oral cavities, and may enter the circulatory system through a small tear in the skin or the mouth during tooth brushing (Bhanji, Williams, Sheller, Elwood, & Mancl, 2002). The disc is an ideal environment for bacterial growth since it has low oxygen tension and low pH (Urquhart et al., 2015). As a response to the endotoxins released by the bacteria, the disc cells secrete interleukin 6 (IL6), interleukin 8 (IL8) and prostaglandin E2 (PGE-2) (Burke et al., 2003). The constant secretion of cytokines and bacterial metabolites from the disc might cause inflammation to the adjacent bone marrow leading to MC1 (Albert et al., 2008). Positive results after antibiotic treatment have been identified in one randomized
study (Albert, Sorensen, Christensen, & Manniche, 2013). However, this theory has received criticism concerning the sterility and contamination (Rigal et al., 2016; Wedderkopp et al., 2009).

### 2.4.4 Treatment

The treatment of MC is limited due to the unknown etiology and thus, no treatment consensus is established. Clinical trials for non-surgical treatments have focused on suppressing inflammation and infection. Non-steroidal anti-inflammatory drugs are used in the pharmacological treatment of musculoskeletal disorders. Antibiotics (Albert et al., 2013), bisphosphonates (Koivisto et al., 2014), spinal manipulative therapy (Annen et al., 2016) and intradiscal steroid injections (Buttermann, 2004; Fayad et al., 2007) have been shown to reduce the intensity of LBP among patients with MC. In addition, rest from physical activity has been evaluated as a potential treatment, but no difference was identified between rest and exercise groups (R. K. Jensen, Leboeuf-Yde, Wedderkopp, Sorensen, & Manniche, 2012). Tumor necrosis factor alpha (TNF-α) antagonist treatment for sciatic pain was more effective in a subgroup of patients who had MC and symptomatic disc herniation at the same level (Korhonen et al., 2006). The opposite results were observed in a study of patients with MRI confirmed symptomatic lumbar disc herniation. Patients with lumbar disc herniation and the presence of MC at any lumbar level reported significantly lower levels of pain reduction after a lumbar nerve root block compared to patients with no MC (Peterson, Pfirrmann, & Hodler, 2014).

Indications for surgical treatment are instability and disc herniation. MC alone is not an indication for surgical treatment and conservative treatment is the most frequent option (Martinez-Quinones et al., 2017). However, the presence of MC is associated with a higher rate of invasive surgical treatments (Martinez-Quinones et al., 2017). Lumbar spine fusion surgery may lead to better outcomes among patients with MC (Esposito, Pinheiro-Franco, Froelich, & Maitrot, 2006; Vital et al., 2003), but the opposite results have also been obtained (Ghodsi, Rouhani, Abdollahzade, Khadivi, & Faghih Jouibi, 2015). In conclusion, very few randomized controlled trials have been performed on the treatment of MC.
2.5 Genetics of complex diseases

2.5.1 Variation in the human genome

The genome is the complete set of genetic information stored as deoxyribonucleic acid (DNA) in the nucleus of cells. The human genome consists of ca. 3.2 billion base pairs coding about 19,000-20,000 genes (Ezkurdia et al., 2014). Adenine (A), cytosine (C), thymine (T) and guanine (G) form base pairs by binding two nucleobases to each other (A with T and C with G). Genes make up approximately 1-2% of the human genome (ENCODE Project Consortium, 2012). Previously it was viewed that the rest of the human genome is mostly “junk DNA”. This view has been since revised and according to the current information the noncoding DNA is filled with enhancers, promoters and numerous regions encoding RNA transcripts (Ecker et al., 2012; ENCODE Project Consortium, 2012; Hube & Francastel, 2015). However, the function of non-coding DNA regions is only slowly being revealed.

The variation of the human genome between individuals is approximately 0.1%, but still each individual carries millions of variations in their genome. No two humans have identical genomes, with the exception of identical twins. Variations can be major structural variants involving large segments of DNA (>1 kb), smaller variations in the base pair sequence (<1 kb) or single nucleotide variations (SNV). Structural variants include changes in the number of the chromosomes, rearrangement of chromosome parts (translocation), reversed segments of DNA with respect to the rest of the chromosomes (inversion) or copy number variants, which are vast parts of the genome that have been replicated or deleted. Smaller variations in the base pair sequence may be inversions, substitutions, deletions or insertions of one or several base pairs. A variation of one base pair is called a SNV. When a SNV has a minor allele frequency (MAF) >1%, it is called a single nucleotide polymorphism (SNP). A typical human genome differs from the reference genome at 4.1-5.1 million sites (1000 Genomes Project Consortium et al., 2015). Over 99.9% of the variants are SNVs and short indels, while it is estimated that approximately 2100-2500 structural variants affecting about 20 million bases of sequence exist in the human genome (1000 Genomes Project Consortium et al., 2015).

Variants are divided into three groups depending on MAF. Common variants have a MAF over 5% and rare variants have MAF less than 1%. The variants having
a MAF between 1 and 5% are called low frequency variants (Bodmer & Bonilla, 2008).

Variation in the human genome can have a different impact depending on how large it is and where it is located. Most variations have a neutral effect or no effect on human health. Some variations can cause a change in the amino acid sequence resulting in the production of a different protein. For example, a glutamic acid to valine change in the hemoglobin gene is known to cause sickle cell anemia (Habara & Steinberg, 2016). A variation can also alter the reading frame and cause the production of a different protein or a premature stop codon resulting in a truncated protein. Eliminating these truncated proteins is vital because they may possess dominant-negative or deleterious gain-of-function activity. Thus, messenger RNAs (mRNAs) containing premature termination codons are detected and rapidly degraded through nonsense-mediated mRNA decay (Shi et al., 2015).

A variation can have diverse effects if it is located in the noncoding DNA, which contain elements regulating gene expression. A variation can cause an abnormal phenotype by disrupting the function of a regulatory element leading to gene dysregulation. For example, variations in the enhancer elements have been associated with common complex phenotypes such as heart disease, diabetes and cancer (Corradin & Scacheri, 2014). Also, a variation can occur in a gene coding for a transcription factor responsible for regulating gene expression by binding to a specific DNA sequence. Diseases such as T-cell acute lymphoblastic leukemia (Sanda et al., 2012) and prostate cancer (Barbieri et al., 2012) have been associated to variations in the genes coding for transcription factors. The effect of variations on gene expression levels is studied using expression quantitative trait locus (eQTL) mapping (Nica & Dermitzakis, 2013). An eQTL is a locus that explains a fraction of the genetic variance of a gene expression phenotype.

### 2.5.2 Monogenic and complex disorders

Monogenic diseases, i.e., Mendelian diseases, are rare diseases caused by a pathogenic variation in a single, distinct gene with high effect size. According to the Online Mendelian Inheritance in Man (OMIM) database there are up to 5000 monogenic diseases listed (www.omim.org), such as Huntington’s disease (Gusella et al., 1983) and cystic fibrosis (Tsui et al., 1985). The nature of the disease depends on the function of the gene in which the pathogenic variant is located.

It has become apparent that many human diseases cannot be attributed to dysfunction of a single gene, but instead, are thought to result from interactions
between multiple genetic variants, as well as environmental and lifestyle factors. Complex diseases do not follow the standard Mendelian patterns of inheritance since the inherited genes represent only a part of the disease risk. The vast majority of diseases, such as type II diabetes (Ali, 2013) and Alzheimer’s disease (Giri, Zhang, & Lu, 2016), fall into this category.

2.5.3 Genetic approaches

The goal of human medical genetics has long been the understanding of disease development leading to the identification of new treatments. The genetic methods used to study complex diseases have evolved substantially especially during the last years. In the 1990s, the common practice was to use family-based linkage studies followed by candidate gene analyses. Sequencing of the human genome turned out to be a daunting project and the Human Genome Project, launched in 1990, took more than a decade to complete, involved more than 20 institutes all over the world and cost nearly three billion dollars (Lander et al., 2001; Venter, Smith, & Adams, 2015). Another global project, the HapMap project, identified and annotated the sequence location of common SNPs (Collins & McKusick, 2001; International HapMap Consortium, 2003). The HapMap project made possible the emergence of GWAS. More recently, new technologies called next-generation sequencing (NGS) have revolutionized the genetic research of complex traits.

Genetic studies on complex phenotypes are usually designed based upon two hypotheses: common disease – common variant (CD-CV) or rare variant – common disease (RV-CD) hypothesis. The first suggests that complex phenotypes are due to a very large number of common variants with modest effect sizes. The latter suggests that multiple rare variants with large effect sizes are the main driving force in the heritability of the complex phenotypes (Marian, 2012).

Heritability

Heritability measures the proportion of phenotypic variation in a population that is due to genetic variation in a trait. It is expressed as a value between 0 and 1 with the former indicating that the trait studied is 100% due to environmental factors and the latter indicating that the trait is 100% due to genetic factors. Heritability is not a fixed property of a condition since it only applies to a particular population at a particular time. It can also change when the environment changes. Heritability is commonly used in twin studies to study complex diseases (Read & Donnai, 2011).
Linkage analysis was initially developed to study monogenic diseases and it can be used to study either binary traits where two distinct phenotypic values (e.g., affected/unaffected or present/absent) exist (Musolf et al., 2017) or a quantitative trait, which is a measurable phenotype (e.g., blood pressure, height, weight) (Zheng et al., 2001). The method has a high power to detecting rare variants with large effect sizes and is most effective when used to study rare variants in large extended families (Bailey-Wilson & Wilson, 2011). In linkage analysis, multiple variants with known positions in the genome, usually short repeats (microsatellites) or SNPs, are used as genetic markers. Marker alleles are then compared between affected and unaffected members within a large family to detect disease loci. The loci are in linkage when they are inherited together from a parent to a child more often than would be expected with independent inheritance (Ott, Kamatani, & Lathrop, 2011; Schork, 1997). A genetic marker is linked to the disease gene if it is located close enough in a chromosome so that the two genes do not recombine during meiosis (Bailey-Wilson & Wilson, 2011). Linkage analysis enables the identification of candidate regions in the genome for the disease gene.

In order to decrease the chance of false positive findings, accepted thresholds for genome-wide significance and correction for multiple testing are used. A commonly used measurement of the linkage is the logarithm of the odds (LOD) score which is a statistical estimate of whether two loci are likely to be located near each other on a chromosome and thus be inherited as a package. Traditionally, a LOD score over 3.0 is considered as the threshold for genome wide significance indicating that the odds that the loci are linked are 1000 times greater than the odds that they are not (Dawn Teare & Barrett, 2005). To decrease the rate of genome-wide type I error, a higher threshold of 3.3 is commonly used (Dawn Teare & Barrett, 2005).

Linkage studies have been tremendously successful in studying monogenic diseases. In Finland, it has been used to identify genes behind rare disorders overrepresented in Finland (Norio, 2003a; Norio, 2003b; Norio, 2003c). These are rare monogenic diseases belonging to the Finnish disease heritage (FDH). FDH is thought to result from the fact that the Finnish population exhibits low genetic diversity due to being founded by a small number of individuals (Nevanlinna, 1972) and having endured numerous bottlenecks leading to enrichment of some disease-causing genetic variants (Norio, 2003b). Today, FDH comprises 36 disorders that extend to all branches of medicine (Norio, 2003c). Conversely, there are several
diseases, like cystic fibrosis, common throughout the world that are very rarely seen in Finland (Peltonen, Jalanko, & Varilo, 1999). Linkage analysis has also been used to study complex traits such as schizophrenia (Stefansson et al., 2002), Celiac disease (J. Liu et al., 2002) and type II diabetes (Nistico et al., 1996) before the advent of GWAS. However, for most complex diseases, the success has been limited (Rowe & Tenesa, 2012).

**Candidate gene associations**

Candidate gene association analysis was often used as the next step after linkage analysis and was based on a case-control setting. Potential susceptibility genes were selected from the chromosomal regions that were indicated by the linkage signal. In addition, candidate genes could be chosen without prior evidence from linkage studies based on existing knowledge about their function or potential involvement in the pathogenesis of the studied trait. Once candidate genes were identified, the studied variants were chosen for sequencing or SNP genotyping. In many cases, no information about functional variants in the candidate genes existed. Hence, sequencing the whole candidate gene or parts of it including coding regions, splice junctions and the regulatory junctions from cases and controls was performed. Allele frequencies of the genotypes and SNPs were then compared between cases and controls using a Chi Square test. Possible confounding effects of potential differences in the demographics and other determining factors can be taken into account by using various regression analysis methods (Marian, 2012). A P-value is used to determine the certainty of an association as it provides the probability that the obtained result is due to chance. A common cut-off value is 0.05 (Qu, Tien, & Polychronakos, 2010).

The candidate gene approach often includes multiple tests and thus the risk of obtaining a false positive finding is increased. This can be addressed by adding an appropriate correction to the significance threshold. There are several approaches for adjusting the significance threshold including the Bonferroni correction, permutation approach and false discovery rate approaches (Sullivan, 2007). Also, replication of the findings in different study populations is necessary (Marian, 2012).
Genome-wide association studies

The HapMap project made it possible to cost-efficiently assess many of the common variants within an individual in the form of GWAS (Hirschhorn & Daly, 2005). GWAS has been one of the most important technical accomplishments in the study of complex diseases, and has been successful in associating a large number of variants with various complex phenotypes (Marian, 2012). GWAS is based upon the CD-CV hypothesis (Manolio et al., 2009).

One of the achievements that made GWAS possible was the discovery of the linkage disequilibrium (LD) (Gabriel et al., 2002). LD describes the non-random association of alleles at two or more loci in a given population. Due to LD, the prediction of SNP alleles in the genome is possible by genotyping a subset of the SNPs (W. Y. Wang, Barratt, Clayton, & Todd, 2005). The genotyping is done by a commercial genotyping microarray platform. To increase the coverage of the genome and the number of variants, imputation follows the genotyping. In imputation, the genotypes that are not directly assayed are predicted by using a densely genotyped reference panel (Marchini & Howie, 2010). The reference panels frequently used include the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2015), the Haplotype Reference Consortium (HRC) (S. McCarthy et al., 2016) and the Sequencing Initiative Suomi (SISu) (www.sisuproject.fi). Finally, allele frequencies of hundreds of thousands of common variants, usually SNPs, are compared between affected and non-affected individuals to identify variants associating with the trait (Marian, 2012). The statistical level of significance and the possibility of finding false positives due to multiple testing are major issues when interpreting GWAS results (Marian, 2012; M. I. McCarthy et al., 2008). Thus, the threshold for genome-wide significance is p<5 x 10^{-8} (Marian, 2012; M. I. McCarthy et al., 2008). However, the effect sizes of the common variants are usually small and typically explain only a small fraction of variability and heritability of the complex trait studied (Eichler et al., 2010; Marian, 2012).

Next-generation sequencing

The launch of NGS, i.e., high-throughput sequencing instruments, in the mid-2000s revolutionized the study of genetic diseases, offering vastly improved throughput at significantly lower cost (Petersen, Fredrich, Hoeppner, Ellinghaus, & Franke, 2017). This improvement has been achieved by massive parallel sequencing. NGS
provides the opportunity to identify all rare and common DNA sequence variations in the human genome with the exception of large variations.

The different approaches using NGS are whole genome sequencing (WGS), targeted re-sequencing and whole exome sequencing (WES). In WGS, the sequence of the whole genome is obtained, including also mitochondrial DNA and hence, a complete spectrum of genetic variants can be built. It enables testing both the CD-CV and RV-CD hypotheses (Marian, 2012). Still, WGS is an enormous task due to the massive amount of sequencing data, difficulty with data storage and the complexity of data analysis (Marian, 2012). The next breakthrough for NGS in human genetics was the introduction of targeted re-sequencing. In targeted re-sequencing, a more narrowly chosen region of the genome, which has previously been linked to or associated with the phenotype, is sequenced. WES, in which the whole protein-coding region of the genome (exons) is sequenced (Majewski et al. 2011), became the most commonly used targeted re-sequence method. WES enables the detection of both exons as well as splice-site variants while requiring only 2% of the sequencing “load” compared to WGS (Petersen et al. 2017). Overall, NGS is suited for studying both rare and common diseases and has enabled the identification of new genes behind, e.g., Parkinson disease (Zimprich et al., 2011), epilepsy (Lemke et al., 2012), different types of cancer (Ng et al., 2010) and inflammatory bowel disease (Worthey et al., 2011).

2.6 Genetics of DD

DD is considered a condition that is in large part genetically determined with genetic factors contributing up to 74% of the variance in DD in different populations (Battie et al., 1995; Bijkerk et al., 1999; Sambrook et al., 1999). However, there are significant differences regarding individual features of DD as no significant genetic influence was found for disc signal intensity (Sambrook et al., 1999). Several genes have been identified that may play a role in the development of DD and identification of these genes has provided new insight into the molecular mechanism of the disease. The identified genes can be categorized based on their function in the disc: structural genes, catabolic genes, anti-catabolic genes, inflammatory genes and other genes (Table 3). In a systematic review of DD candidate genes none of the variations studied reached the level of strong evidence (Eskola et al., 2012). A moderate level of evidence was found for variants in collagen type XI alpha 1 (COL11A1), asporin (ASPN), thrombospondin 2 (THBS2), matrix metallopeptidase 9 (MMP9), growth differentiation factor 5 (GDF5) and
sickle tail (SKT) genes. The level of evidence in each genetic variation was analyzed according to the Venice interim guidelines by The HuGENet Working Group (Eskola et al., 2012; Ioannidis et al., 2008).

### 2.6.1 Structural genes

Collagens are the most abundant proteins in the human body and are present in the IVD providing mechanical support and interacting with other ECM molecules. Thus, they have been promising candidate genes for DD. Collagen I, II, III, V, IX, X and XI have been studied and polymorphisms in collagen I alpha 1 (COL1A1), collagen IX alpha 2 (COL9A2), collagen IX alpha 3 (COL9A3) and COL11A1 have been found to be associated with DD in different populations (Annunen et al., 1999; Jim et al., 2005; Noponen-Hietala et al., 2003; Pluijm et al., 2004; Tilkeridis, Bei, Garantziotis, & Stratakis, 2005; Videman et al., 2009). Mutations in the collagen II alpha 1 (COL2A1), collagen IX alpha 1 (COL9A1), COL11A1 and collagen XI alpha 2 (COL11A2) have also been associated with a variety of inherited connective tissue disorders affecting cartilage (Jobling et al., 2014).

Other structural genes associated with DD are aggrecan (ACAN), ASPN, cartilage intermediate layer protein (CILP) and THBS2. Aggrecan is a large proteoglycan responsible for the ability to resist compressive loads (Cs-Szabo et al., 2002). ACAN carries a variable number of tandem repeat polymorphisms (VNTR) ranging from 13 to 33 repeats (Doege, Coulter, Meek, Maslen, & Wood, 1997). VNTR with 18, 21 and 26 repeats as well as two ACAN SNPs have been associated with DD (Kawaguchi et al., 1999; Solovieva et al., 2007; Videman et al., 2009). Asporin is a member of the family of small leucine rich proteoglycans and usually contains 13 aspartic acid repeats at the amino-terminal. A polymorphic allele at this site can contain 9-20 aspartic acid repeats and the allele with 14 repeats has been associated with DD in Japanese and Chinese populations (Song et al., 2008). CILP down regulates aggrecan and type II collagen and is widely expressed in the disc (Seki et al., 2005). A SNP in CILP has been associated with DD in Japanese and Chinese populations (Min, Nakazato, Okada, Ochi, & Hiranuma, 2009; Seki et al., 2005). Thrombospondins are extracellular glycoproteins with diverse functions. They bind to collagen and take part in cell-to-matrix and cell-to-cell communications as well as help regulate the levels of matrix metalloproteinase 2 (MMP2) and MMP9 (Bein & Simons, 2000). Polymorphisms in THBS2 have been linked to DD (Hirose et al., 2008).


### 2.6.2 Catabolic genes

MMPs are key proteins involved in various physiological and pathological processes. Currently, there are 18 known members of the MMP family that are involved in the breakdown of the disc matrix components. SNPs in the matrix metallopeptidase 1 (MMP1) and MMP2 genes, and a promoter region polymorphism in MMP9 producing five or six repeats of adenine (5A and 6A, respectively) were linked to DD (Takahashi et al., 2001; Valdes, Hassett, Hart, & Spector, 2005). Other catabolic genes associated with DD are parkinson protein 2 (PARK2), proteasome subunit beta 9 (PSMB9) and carbohydrate sulfotransferase 3 (CHST3) (Song et al., 2013; Williams et al., 2013). PARK2 produces a protein called parkin involved in tagging damaged proteins for proteosomal degradation. PSMB9 is a proteasome that degrades damaged or unneeded proteins and CHST3 is a proteoglycan involved in cell migration and differentiation.

### 2.6.3 Anti-catabolic genes

Anti-catabolic genes linked to DD are tissue inhibitor of metalloproteinases 1 (TIMP1) and GDF5 (Valdes et al., 2005; Williams, Popham, Hart et al., 2011). TIMP is a group of protease inhibitors and GDF5 regulates the development of numerous tissues including cartilage and joints.

### 2.6.4 Inflammatory genes

Interleukins are cytokines that respond to stress and cause a range of systematic responses. Polymorphisms in IL1A and IL6 have been linked to DD (Eskola et al., 2010; Noponen-Hietala et al., 2005; Solovieva et al., 2004). Cylooxygenase-2 (COX2) encodes an enzyme participating in the synthesis of prostaglandins, prostacyclin and thromboxane. It has been linked with Kellgren-Lawrence degeneration grade on plain radiographs (Valdes et al., 2005).

### 2.6.5 Other genes

The vitamin D receptor (VDR) was the first candidate gene found to associate with DD (Videman et al., 1998). It mediates the function of vitamin D and is involved in bone remodeling and mineralization. (Haussler et al., 1998). Two polymorphisms in the VDR gene, FokI and TaqI, have been studied extensively and
their association with DD has been validated in large populations from several ethnic backgrounds (Cheung et al., 2006; Eser et al., 2010; Jones, White, Sambrook, & Eisman, 1998; Kawaguchi et al., 2002; Valdes et al., 2005; Videman et al., 1998).

Another gene associated with DD is SKT expressed in the human IVD. The G allele of the SKT SNP rs16924573 was found to be strongly associated with lumbar disc herniation (Karasugi et al., 2009). Variants associated with DD are shown in more detail in Table 3.

### 2.6.6 Modic change

The genetic basis of MC is still largely unknown. Based on the candidate gene approach, the IL1A and MMP3 genes may play roles in the development of MC (Karppinen et al., 2008; Karppinen et al., 2009). Disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) 4 and 5 and tissue inhibitors of metalloproteases 3 (TIMP3) have been associated with the severity of MC (Perera et al., 2017). However, so far no one has assessed the heritability of MC.
Table 3. Variants associated with DD categorized according to their function.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>Abbreviation</th>
<th>Study population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Structural genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800012</td>
<td>Collagen I alpha 1 chain</td>
<td>COL1A1</td>
<td>Dutch</td>
<td>Pluijm et al. 2004</td>
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<td></td>
<td></td>
<td></td>
<td>Greek</td>
<td>Tikerdlis et al. 2005</td>
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<td>rs2075555, rs1007086</td>
<td>Collagen I alpha 1 chain</td>
<td>COL1A1</td>
<td>Finnish</td>
<td>Tikerdilis et al. 2005</td>
</tr>
<tr>
<td>Trp2 allele</td>
<td>Collagen IX alpha 2 chain</td>
<td>COL9A2</td>
<td>Finnish</td>
<td>Annunen et al. 1999</td>
</tr>
<tr>
<td>Trp3 allele</td>
<td>Collagen IX alpha 3 chain</td>
<td>COL9A3</td>
<td>Finnish</td>
<td>Annunen et al. 1999</td>
</tr>
<tr>
<td>rs1337185, rs1463035</td>
<td>Collagen XI alpha 1 chain</td>
<td>COL11A1</td>
<td>Finnish</td>
<td>Videman et al. 2009</td>
</tr>
<tr>
<td>rs1800587</td>
<td>Collagen XI alpha 2 chain</td>
<td>COL11A2</td>
<td>Finnish</td>
<td>Noponen-Hietala et al. 2003</td>
</tr>
<tr>
<td>rs2072915, rs9277933, rs2076311</td>
<td>Collagen XI alpha 2 chain</td>
<td>COL11A2</td>
<td>Finnish</td>
<td>Videman et al. 2009</td>
</tr>
<tr>
<td>A18, A21</td>
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<td>ACAN</td>
<td>Japanese</td>
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<tr>
<td>A26</td>
<td>Aggrecan</td>
<td>ACAN</td>
<td>Finnish</td>
<td>Solovieva et al. 2007</td>
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<tr>
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<td>ACAN</td>
<td>Finnish</td>
<td>Videman et al. 2009</td>
</tr>
<tr>
<td>D14</td>
<td>Asporin</td>
<td>ASPN</td>
<td>Chinese/ Japanese</td>
<td>Song et al. 2008</td>
</tr>
<tr>
<td>rs2033711</td>
<td>Cartilage intermediate layer</td>
<td>CILP</td>
<td>Chinese</td>
<td>Seki et al. 2005</td>
</tr>
<tr>
<td>rs9406328</td>
<td>Thrombospondin 2</td>
<td>THBS2</td>
<td>Japanese</td>
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<td></td>
<td>Catabolic genes</td>
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<td>Chinese</td>
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<td>rs243865</td>
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<td>5A allele</td>
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<td>MMP3</td>
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<td>Variant</td>
<td>Gene</td>
<td>Abbreviation</td>
<td>Study population</td>
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<tr>
<td>rs926849</td>
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<td>PARK2</td>
<td>English</td>
<td>Valdes et al. 2005</td>
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<tr>
<td>rs2187689, rs7767277, rs1245582, rs4148941</td>
<td>Proteasome subunit beta 9, Carbohydrate sulfotransferase 3</td>
<td>PSMB9, CHST3</td>
<td>North Europeans, Asians</td>
<td>Williams et al. 2013</td>
</tr>
<tr>
<td>C124T</td>
<td>Tissue inhibitor of metalloproteinases 1</td>
<td>TIMP1</td>
<td>English</td>
<td>Valdes et al. 2005</td>
</tr>
<tr>
<td>rs143383</td>
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<td>GDF5</td>
<td>North Europeans</td>
<td>Williams et al. 2011</td>
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<td>Interleukin 1 alpha</td>
<td>IL1A</td>
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<tr>
<td>rs13006435</td>
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<td>IL-6</td>
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</tr>
<tr>
<td>rs1800797, rs1800796, rs1800795</td>
<td>Interleukin 6, Interleukin 6, Interleukin 6</td>
<td>IL-6, IL-6, IL-6</td>
<td>Danish, Finnish, Finnish</td>
<td>Eskola et al. 2010</td>
</tr>
<tr>
<td>rs5277</td>
<td>Cyclooxygenase-2</td>
<td>COX2</td>
<td>English</td>
<td>Valdes et al. 2005</td>
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<tr>
<td>FokI (rs2228570)</td>
<td>Vitamin D receptor</td>
<td>VDR</td>
<td>Finnish</td>
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<tr>
<td>FokI (rs2228570)</td>
<td>Vitamin D receptor</td>
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<td>Eser et al. 2010</td>
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<td>TaqI (rs731236)</td>
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<td>VDR</td>
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<tr>
<td>TaqI (rs731236)</td>
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<td>VDR</td>
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<td>Cheung et al. 2006</td>
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</table>
3  Aims of the thesis

The purpose of this study was to estimate the heritability of MC and to identify genetic factors predisposing to MC. The specific aims were:

1. To estimate the heritability, prevalence and natural history of MC in a large English twin cohort (TwinsUK)
2. To identify novel, rare and pathogenic variants behind development of MC using whole exome sequencing in two Finnish families
3. To identify genetic loci associated with MC using genome-wide meta-analysis in the NFBC1966 and the TwinsUK cohort.
4  Materials and methods

4.1  Study populations

4.1.1  TwinsUK cohort (I)

The participants for study I were recruited from the TwinsUK register (www.twinsuk.ac.uk), which is an adult twin cohort recruited since 1992 from the general UK population (Moayyeri, Hammond, Hart, & Spector, 2013). Between 1996 and 2000, 831 twins attended lumbar MRI, a nurse-led interview and a collection of demographic data. Lumbar MRI was performed on 463 twin pairs approximately a decade later. The twins were neither selected nor excluded for history of LBP, disc disease or neck pain. Appropriate ethics permission had been obtained from the St. Thomas’ Hospital Ethics Committee and twins gave informed written consent.

4.1.2  Finnish Families (II)

The study population in study II consisted of Finnish families with a history of LDD characterized by sciatica and collected from the patient population of Oulu University Hospital (Virtanen et al., 2007). In total, 14 families were collected containing a total of 186 family members, 91 males and 95 females. The clinical history of all family members was collected in the beginning of the study and most of them attended lumbar MRI. They were also asked to donate blood samples for DNA analyses. Families with both parents affected were excluded from the study to facilitate the examination of segregation. Out of these 14 families, two were chosen for the study II: Family I and Family II. Family I consisted of 18 family members comprising ten males and eight females, and Family II had a total of 33 family members comprising 16 males and 17 females. In Family I, seven family members had MC (38.9%) while in Family II eight family members had MC (24.2%). Family members were interviewed by telephone and asked about the nature of LBP and if it radiated to the leg below the knee continuously for at least 2 weeks. Also, family members were asked about the number and duration of sciatica including possible back operations. The study was approved by the local ethics committee and family members gave informed written consent.
4.1.3 Northern Finland Birth Cohort 1966 (III)

The study population in study III derives from the NFBC1966 comprised of mothers living in Oulu and Lapland who had expected dates of delivery between January 1st and December 31st, 1966 (12,068 mothers, 12,231 children). NFBC1966 includes 96.3% of all births at that time in Northern Finland. Data collection began around gestational week 24 and data have been collected on the participants’ health, healthcare utilization, medication, social status and lifestyle habits. At ages 14, 31 and 46 years, postal questionnaires were sent to all NFBC1966 participants with a known address. The response rate was 97% at the age of 14, 75% at the age of 31 and 66% at the age of 46. The latest follow-up study at the age of 46, consisting of a wide-scale health examination that included also a clinical examination, was completed in February 2014. A subset of study subjects that had attended the examinations attended lumbar MRI (n=1540). The Ethical Committee of the Northern Ostrobothnia Hospital District has approved the study and the study subjects have given their informed written consent.

4.2 Magnetic resonance imaging

MRI was performed separately in all study populations and was based on previously collected imaging series.

4.2.1 TwinsUK (I)

Study I consisted of lumbar baseline and follow-up MRIs. Baseline MRI was carried out using a 1.0-T equipment (Siemens, Munich, Germany) (Sambrook et al., 1999) and follow-up imaging was performed using a 1.5-T scanner (Siemens, Munich, Germany). Sagittal T2-weighted images of the lumbar spine were used in both baseline and follow-up. Fast spin-echo sequence of time to recovery of 5000-4500 ms and time to echo 112 ms were used with slice thickness of 4 mm.

4.2.2 Finnish families (II)

The MRI examination was performed with a 1.5-T scanner (Signa, GE Medical systems, Milwaukee, Wisconsin, USA). The imaging parameters of the sagittal T1-weighted fast spin-echo sequences with fluid attenuation inversion recovery were repetition time 2274.4–2276.6 ms, inversion time 860 ms and echo time 20.1 ms.
The imaging parameters of the sagittal T2-weighted turbo spin-echo/fast-spin echo sequences were repetition time 3500 ms and echo time 114.7 ms. The imaging parameters of the short tau inversion recovery sequences were repetition time 4400, inversion time 150 and echo time 48.2 ms. The spacing, including slice thickness and slice gap, was 5.0 mm in all sequences.

4.2.3 Northern Finland Birth Cohort 1966 (III)

MRI was performed between 2012 and 2014 using a 1.5-T imaging system (Signa HDxt, General Electric, Milwaukee, Wisconsin, USA). The routine lumbar spine protocol was used with T2w fast-recovery fast spin-echo images in transverse and sagittal planes. Time to echo 112 ms with slice thickness of 3 mm was used in sagittal planes and time to echo 118 ms with slice thickness of 4 mm was used in transverse planes. Also, two family members from Family I attended follow-up lumbar MRI about a decade later. The MRI was performed using this approach.

4.3 Evaluation of magnetic resonance imaging

In all studies, the MR images were evaluated independently. In study I, a single reader coded all available scans without prior knowledge of the clinical status and a second reader re-coded a subset of scans (n=50) for interrater reliability calculation. In study II, two observers (JK and JN) coded all available scans. In study III two observers (MK and JM) each coded half of the scans and a subset of scans (n=66) was coded by both observers for calculation of interrater reliability.

MC in both rostral and caudal endplates were coded. MCs were evaluated as type 1, type 1/2, type 2, type 2/3 and type 3. The type of MC could not be assessed in the TwinsUK cohort since only T2-weighted images were available. The size of the MC was evaluated according to the Nordic Modic Consensus Group Classification (T. S. Jensen et al., 2007). In all studies, the maximum height of every MC was graded from all sagittal slices and compared with the height of the vertebral body as 0) no MC, 1) MC in the endplate, 2) MC less than 25%, 3) MC from 25% to 50% and 4) MC over 50% of the height of the vertebral body (Fig. 8).

In studies I and III, the horizontal width of each MC was evaluated by dividing the vertebra axially into five zones: four peripherally and one central circular zone with width ranging from 0 to 5 (Fig. 9). The size index of each MC was calculated by multiplying the width (0-5) by the height (0-4), thus, obtaining size index values from 1 to 20. All MC size indexes of the lumbar spine were added together to get
the total lumbar MC size index. In study II, the horizontal width of each MC was evaluated in three zones in the anterior posterior direction: anterior, midpoint and posterior (Fig. 10).

Fig. 8. Assessment of vertical height of MC as 0) no MC, 1) MC in the endplate, 2) MC less than 25%, 3) MC from 25% to 50% and 4) MC over 50% of the height of the vertebral body. EP = endplate. This image was made by the author.

Fig. 9. Assessment of horizontal width of MC in five zones: one central and four peripherally. AR = anterior right, AL = anterior left, PR = posterior right, PL = posterior left, C = central. This image was made by the author.
4.4 Statistical analyses

4.4.1 Heritability assessment of MC (I)

Heritability was assessed by probandwise concordance rates which were estimated separately for monozygotic (MZ), i.e., identical, dizygotic (DZ), i.e., non-identical and all twin pairs. The probandwise concordance rate is defined as the probability that one twin is affected with the trait if the other twin is affected. It is calculated by dividing the number of affected individuals with an affected co-twin with the number of affected participants. When the probandwise concordance rate for MZ twins is higher than for DZ twins a genetic influence is indicated.

Standard variance component analysis was used to estimate the heritability of MC. Typically it includes three components: additive genetic factors ($A$), environmental factors shared by the twins ($C$) and unique environmental factors ($E$). The classical twin model states that MZ twin pairs share 100% of their additive genetic factors whereas DZ twin pairs share approximately 50% of their genes like usual siblings and other first-degree relatives. The classical twin model also relies on studying twin pairs born in the same family, thus both MZ and DZ twin pairs are prone to the same environmental factors. Thus, the correlation within MZ and DZ twin pairs can be defined as follows

$$\text{Cov}_{MZ} = A + C$$  \hspace{1cm} (1)
\[ Cov_{DZ} = 0.5A + C \]  

Therefore, the effect of additive genetic factors on a trait examined can be described as double the difference between MZ and DZ twin pair correlations. Estimates for \( C \) and \( E \) can be described as follows

\[ C^2 = C = ICC_{MZ} = -H^2 \]  

and

\[ e^2 = E = 1 - H^2 + C^2 \]

Estimates for \( A \), \( C \) and \( E \) were dictated based on maximum likelihood estimation (Rijsdijk & Sham, 2002) by structural equation modeling software Mx (Neale, Boker, Xie, & Maes, 2006) where the above formulae were applied. Different combinations of \( A \), \( C \) and \( E \) were compared and the best model was selected based on an optimal balance between goodness of fit and parsimony.

4.5 Reliability of evaluation of MC

The interobserver reliability for MC presence was assessed using kappa statistics. In studies I, II and III the interobserver reliability for the presence of MC was 0.92, 0.90 and 0.82, respectively. In study I, the intraobserver reliability for the width and height ranged from 0.61 to 1.00 and the interobserver reliability ranged from 0.69 to 1.00.

4.6 Whole exome sequencing (II)

4.6.1 Capturing the exome data and variant calling

Altogether nine family members (seven affected and two unaffected) from two families were chosen for WES. Blood samples were obtained and genomic DNA was isolated using an Ultraclean Blood DNA Isolation Kit (Mo Bio Laboratories, CA, USA). The WES data were obtained through a commercial service (BGI, Hong Kong, https://www.bgi.com/us/) including exome capture, alignment and allele calling. A SureSelect 51M Capture Kit (Agilent Technologies, Santa Clara, CA, USA) targeting 75 Mb of the human genome with >350,000 exons was used for exome capture. An IlluminaHiSeq2000 100PE platform (San Diego, CA, USA) was used for sequencing exome targets and the reads were aligned to the hg19
human reference genome. Variant calling was carried out using a Genome Analysis ToolKit (GATK) (McKenna et al., 2010).

4.6.2 Variant filtering and annotation

GATK was used to filter the variants according to quality parameters. The hard filters, according to the GATK manual, used with threshold values for SNPs were: genotype quality < 20, the root mean square of the mapping quality < 40 and Haplotype score > 13, and for small insertions and deletions (indels): Phred-scaled P value using Fischer exact test > 200.0, the u-based z-approximation from the Mann-Whitney Rank Sum Test for mapping qualities < -20.0 and quality by depth < 2.0.

Alleles shared by the affected family members were chosen while alleles found in unaffected family members or in the in-house exome set (n=71) or had MAF > 1% in the 1000Genomes database were filtered out. Annotate variation (ANNOVAR) version 2013aug23 was used to annotate the remaining private and rare variants (K. Wang, Li, & Hakonarson, 2010), to identify alleles with harmful pathogenicity estimations (PolyPhen-2, MutationTaster and SIFT) and alleles affecting strong enhancer areas and active promoters (ENCODE database for chromHMM estimations for HSMM and GM12878 cells).

The Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/) and the SISu database (n=6118) (http://www.sisuproject.fi/) were used to obtain the frequency of alleles co-segregating with MC in the general Finnish population. ExAC was also used to acquire MAF in the European population.

4.6.3 Variant validation

Chosen alleles were validated after annotation by Sanger sequencing from all family members to find alleles co-segregating with MC. Primers were designed using Primer3 v.0.4.0 (Untergasser et al., 2012). The regions containing the variants were amplified using the polymerase chain reaction (PCR) and sequenced using capillary sequencing with an ABI3500xL Genetic Analyzer (Applied Biosystems).

4.7 Genome-wide association study meta-analysis (III)

Two population-based cohorts were used for the first ever GWAS meta-analysis on MC: TwinsUK and NFBC1966. Genomic DNA was extracted from whole blood
using a Nucleon BACC 3 Genomic DNA Extraction kit (GE Healthcare, Amersham, UK) in TwinsUK and a phenol-chloroform extraction method in NFBC1966.

4.7.1 Genotyping and imputation

In NFBC1966, the Illumina Human CNV370-Duo DNA bead chip was used for genotyping as described previously (Sabatti et al., 2009). Imputation was performed using IMPUTE (Howie, Donnelly, & Marchini, 2009) and 1000 Genomes Phase 1 version 3 integrated haplotypes as the reference panel. In TwinsUK, HumanHap300, HumanHap610Q, HumanHap1M Duo and HumanHap1.2M Duo, 1M arrays were used for the genotyping as described previously (Ruth et al., 2016). Imputation was performed using the 1000 Genomes phase 3 reference panel.

The sample used for this study comprised altogether 1829 individuals, 1182 individuals from NFBC1966 and 647 individuals from TwinsUK. In NFBC1966, 642 individuals were females and 540 were males, while in TwinsUK, 625 were females and 22 males. The mean age was 47.3±0.7 in NFBC1966 and 54.7±8.9 in TwinsUK, and the mean BMIs were 26.6±4.6 and 25.2±4.7, respectively.

4.7.2 Statistical analyses

Statistical analyses were performed using SPSS Statistics (Chicago, IL, USA). For all variables the descriptive statistics were computed. The groups were compared by parametric and nonparametric tests using the t test, the Mann-Whitney U test and the chi-square test, as appropriate. The threshold for statistical significance was considered a p-value <0.05.

Genome-wide association study

The GWAS analyses were performed in both cohorts separately. The association analyses were carried out using SNPTEST v.2.5.2 in NFBC1966 and using GEMMA v0.9 in TwinsUK. A linear regression analysis model was fit to test for additive effects of SNPs (genotype dosage) and the analysis was adjusted for age, sex and BMI. BMI was adjusted due to contradictory results on the effect of obesity on MC. The first ten principal components (PCs) were used in correcting for population stratification in NFBC1966 while a kinship matrix was used in TwinsUK for correction of family relatedness. We omitted monomorphic SNPs.
**Meta-analysis**

METAL software with an inverse-variance weighting approach was used for the meta-analysis of the results acquired from NFBC1966 and TwinsUK samples (Willer, Li, & Abecasis, 2010). SNPs were excluded from the analysis if there was a lack of Hardy-Weinberg Equilibrium (HWE) \((p<1\times10^{-6})\), if the MAF was <3%, if the call rate was <95% or if the imputation quality was <0.7. In NFBC1966, SNPs with <0.7 model information estimated by SNPTEST were filtered out as well. After the filters, 5,479,070 SNPs remained. A commonly accepted threshold for genome-wide statistical significance level \((p<5\times10^{-8})\) was used to identify significant SNPs. A commonly accepted suggestive association threshold \(p<5\times10^{-5}\) was used. The results were presented as a Manhattan plot and Quantile-quantile plot (QQ-plot).
5 Results

5.1 Incidence and heritability of MC (I)

From the TwinsUK cohort, data were available for 831 twins of confirmed zygosity comprised of 244 MZ twins (122 twin pairs), 446 DZ twins (223 twin pairs) and 141 twins without the co-twin (Table 4). Lumbar MRI data was accessible for 831 twins at baseline and for 436 twins at follow-up. In total, 347 twins attended both baseline and follow-up lumbar MR imaging. Thirty-five (4.2%) twins were male. The mean age at baseline was 57.1 years for MZ twins, 53.0 years for DZ twins, 52.2 years for unpaired twins and 54.1 years for the whole study population.

Table 4. Characteristics of TwinsUK cohort.

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>N</th>
<th>Gender F/M</th>
<th>Age (mean ± SD)</th>
<th>Age range</th>
<th>Baseline MC (control/case)</th>
<th>Probandwise concordance rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ</td>
<td>244</td>
<td>240/4</td>
<td>57.11 ± 8.12</td>
<td>33-73</td>
<td>162/82</td>
<td>0.56</td>
</tr>
<tr>
<td>DZ</td>
<td>446</td>
<td>418/28</td>
<td>53.00 ± 8.06</td>
<td>21-70</td>
<td>303/143</td>
<td>0.39</td>
</tr>
<tr>
<td>Unpaired</td>
<td>141</td>
<td>138/3</td>
<td>52.20 ± 8.49</td>
<td>37-72</td>
<td>99/42</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>831</td>
<td>796/35</td>
<td>54.08 ± 8.38</td>
<td>21-73</td>
<td>564/267</td>
<td>0.45</td>
</tr>
</tbody>
</table>

DZ = dizygotic, F = female, M = male, MC = Modic change, MZ = monozygotic, N = number of individuals, SD = standard deviation

5.1.1 Prevalence of MC

At baseline, the prevalence of MC (individuals manifesting at least a single endplate with MC) was 32.1% and the incidence of new MC during the ten-year follow-up was 21.6% (Table 5). In total, the prevalence of MC at follow-up was 48.4%. MC was most prevalent in the lower lumbar levels (L4-S1) since of all MC, 76.7% were at the lowest lumbar levels at baseline. In addition, the incidence was highest at the lowest lumbar levels and of all MC, 62.5% were at the lowest lumbar levels at follow-up. The prevalence of MC was a little higher in the rostral endplates but the difference was not significant. The volume of MC lesions grew during the ten-year follow-up period as the median size index summary score was 10.0 at baseline and 13.5 at follow-up. MC regressed completely in 12 individuals (3.5%) during follow-up with regression being highest at L5-S1 (Table 5). Half of these had only minor MC with width and height at most 2. The incidence rates did not
deviate between twins with and without baseline MC (31.4% and 31.0%, respectively). Nevertheless, twins with baseline MC had a higher incidence of MC at the three highest lumbar levels (L1-4) compared to twins without baseline MC (25.7% and 14.0%, respectively, \( p = 0.009 \)) (Fig. 11). At the two lowest lumbar levels (L4-S1), no significant difference was observed (25.7% and 22.7%, respectively, \( p = 0.55 \)) (Fig. 11).

Table 5. The prevalence, incidence and regression of MC in each rostral and caudal endplate.

<table>
<thead>
<tr>
<th>Disc level</th>
<th>MC at baseline (N=831) (%)</th>
<th>MC at follow-up (n=347) (%)</th>
<th>New MC at follow-up (%)</th>
<th>Regressed MC at follow-up (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-2</td>
<td>16 (1.9)</td>
<td>21 (6.1)</td>
<td>18 (5.2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>14 (1.7)</td>
<td>16 (4.6)</td>
<td>13 (3.7)</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>11 (1.3)</td>
<td>20 (5.8)</td>
<td>18 (5.2)</td>
</tr>
<tr>
<td>L2-3</td>
<td>32 (3.9)</td>
<td>43 (12.4)</td>
<td>36 (10.4)</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>31 (3.7)</td>
<td>38 (11.0)</td>
<td>31 (8.9)</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>26 (3.1)</td>
<td>33 (9.5)</td>
<td>27 (7.5)</td>
</tr>
<tr>
<td>L3-4</td>
<td>40 (4.8)</td>
<td>36 (10.4)</td>
<td>28 (8.1)</td>
<td>5 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>37 (4.5)</td>
<td>32 (9.2)</td>
<td>24 (6.9)</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>32 (3.9)</td>
<td>31 (8.9)</td>
<td>25 (7.2)</td>
</tr>
<tr>
<td>L4-5</td>
<td>114 (13.7)</td>
<td>80 (23.1)</td>
<td>47 (13.5)</td>
<td>4 (1.2)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>110 (13.2)</td>
<td>76 (21.9)</td>
<td>43 (12.4)</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>101 (12.2)</td>
<td>73 (21.0)</td>
<td>43 (12.4)</td>
</tr>
<tr>
<td>L5-S1</td>
<td>151 (18.2)</td>
<td>92 (26.5)</td>
<td>40 (11.5)</td>
<td>18 (5.2)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>149 (17.9)</td>
<td>89 (11.2)</td>
<td>39 (11.2)</td>
</tr>
<tr>
<td></td>
<td>Caudal</td>
<td>135 (16.2)</td>
<td>81 (23.3)</td>
<td>35 (10.1)</td>
</tr>
<tr>
<td>Total</td>
<td>267 (32.1)</td>
<td>168 (48.4)</td>
<td>75 (21.6)</td>
<td>12 (3.5)</td>
</tr>
</tbody>
</table>

MC = Modic change, N = number of individuals, SD = standard deviation
5.1.2 Heritability of MC

At baseline, the prevalence of MC was 33.6% in MZ, 32.1% in DZ and 29.8% in unpaired twins. In the case of MZ twins, the probandwise concordance rates were higher than in DZ twins indicating familial influence (0.56 and 0.39, respectively). In total, the risk of MC in a co-twin of an affected twin was 0.45 (Table 5). To raise the variance in MC phenotype, also unpaired twins were included. Chi square statistics were used to determine the model fit for the full ACE model and to compare reduced models (CE, AE, E) to it under the principle of parsimony. Additive genetic factors \[A (95\% CI) = 30 (1-43\%)\] and unique environmental factors \[E (95\% CI) = 70 (57-84\%)\] accounted for most of the variance in MC while shared environmental factors \[C (95\% CI) = 0 (0-20\%)\] had only a small effect in the full ACE model. While comparing the full ACE model to reduced models, the AE model provided a corresponding model fit but demanded fewer
latent parameters ($\Delta df = 1$, AIC = $-2.00$) and was chosen as it provided the most parsimonious description of the data with additive genetic factors [A (95% CI) = 30 (16–43%)] and unique environmental factors [E (95% CI) = 70 (57–84%)] best describing the variance in MC.

5.2 Association of HSPG2 and MAML1 and MC (II)

Family I consisted of 18 family members comprised of ten males and eight females aged 35 to 76 years (mean = 55.6), and Family II had a total of 33 family members comprised of 16 males and 17 females aged 30 to 78 years (mean = 54.1). Clinical characteristics and family demographics are displayed in the Table 6.

Table 6. Clinical characteristics and family demographics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Family I</th>
<th>Family II</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>Mean age (years) [range]</td>
<td>55.6 (35-76)</td>
<td>54.1 (30-78)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>10 (55.6)</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>8 (44.4)</td>
<td>17 (51.5)</td>
</tr>
<tr>
<td>Interviewed by telephone (%)</td>
<td>16 (88.9)</td>
<td>26 (78.8)</td>
</tr>
<tr>
<td>Lifetime prevalence of sciatica (%)</td>
<td>12 (66.7)</td>
<td>12 (36.4)</td>
</tr>
<tr>
<td>Sciatica during past year (%)</td>
<td>5 (27.8)</td>
<td>5 (15.2)</td>
</tr>
<tr>
<td>Operated (%)</td>
<td>4 (22.2)</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>MC (%)</td>
<td>7 (38.9)</td>
<td>8 (24.2)</td>
</tr>
</tbody>
</table>

N = number of individuals, MC = Modic change

5.2.1 Variant calling, filtering and annotation

WES resulted in an average of 80,456 SNPs and 9,151 indels per sample. An average sequencing depth of 67X in the target area was achieved. After filtering for quality an average of 72,682 SNPs and 9,073 indels remained. In Family I, the affected family members shared 35,522 SNPs and 4,337 indels. Of them, 182 SNPs and 16 indels were found only in the affected individuals, but not in the unaffected ones or in the in-house exome set. Five of these variants fulfilled the annotation criteria (harmful pathogenicity estimations or affecting strong enhancer areas and active promoters) In Family II, affected family members shared 43,999 SNPs and 5,323 indels. In total, 478 SNPs and 53 indels were found only in the affected individuals, but not in the unaffected ones or in the in-house exome set. Of these,
23 fulfilled the annotation criteria (harmful pathogenicity estimations or affecting strong enhancer areas and active promoters).

### 5.2.2 Variant validation

The 23 (Family I) and five (Family II) variants fulfilling the annotation criteria were genotyped in the extended families. One rare and pathogenic allele was found co-segregating with MC in each family (Table 7). The c.4829_4831delAGCinsCACTGTG (NM_005529) allele leading to an insertion and deletion mutation p.Q1611delfsX20 (NP_005520.4) in heparin sulfate proteoglycan 2 (HSPG2) co-segregated with MC in Family I with complete penetrance. The mutation is located in exon 38 responsible for coding the third domain of the protein. It alters the open reading frame resulting in a premature stop codon and was considered deleterious by MutationTaster (Table 7).

The SNP c.1657G>A (NM_014757.4) (rs61753465) allele causing a glutamic acid to lysine change p.Glu553Lys (NP_055572.1) in the mastermind-like transcriptional coactivator (MAML1) gene co-segregated in Family II with incomplete penetrance. The variant was estimated probably damaging by PolyPhen-2 (Table 7). It was found from eight affected family members but was also carried by two unaffected family members.
Table 7. Variants co-segregating with MC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>p.Q1611delfsX20</th>
<th>p.Glu553Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Gene</td>
<td>HSPG2</td>
<td>MAML1</td>
</tr>
<tr>
<td>Location</td>
<td>chr1: 22188515–22188517</td>
<td>chr5:179193668</td>
</tr>
<tr>
<td>Rs-number</td>
<td>NA</td>
<td>rs61753465</td>
</tr>
<tr>
<td>1000G</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td>ExAC</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td>SISu</td>
<td>NA</td>
<td>0.0003024</td>
</tr>
<tr>
<td>SIFT</td>
<td>NA</td>
<td>Tolerated</td>
</tr>
<tr>
<td>PolyPhen-2</td>
<td>NA</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>MutationTaster</td>
<td>Disease causing</td>
<td>Polymorphism</td>
</tr>
</tbody>
</table>

NCBI Human Genome Build 37/hg19. 1000G = 1000 Genomes database, ExAC Fin = Finnish population in Exome Aggregation Consortium database (n=3307), MC = Modic change, NA = missing information, SISu = Sequencing Initiative Suomi database (n=6118).

5.3 Prevalence of MC and associating locus (III)

5.3.1 Prevalence of MC

MR images were available from 1509 participants. In total, 949 subjects had MC (62.8%). Altogether, 15,090 vertebral bodies adjacent to endplates were evaluated. MC was observed in 2756 (18.2%) endplates; of them 292 (10.6%) were type 1, 1015 (36.8%) were type 1/2, 1398 (50.7%) were type 2, 63 (2.3%) were type 2/3 and 2 (0.07%) were type 3 (Table 8). Of all MC, 173 (6.3%) were at L1-L2, 275 (10.0%) were at L2-L3, 382 (13.9%) were at L3-L4, 465 (27.6%) were at L4-L5 and 1166 (42.3%) were at L5-S1. MC was most frequent (1632, 59.2%) at lower lumbar levels (L4-S1).
Table 8. The distribution of types of MC by endplate.

<table>
<thead>
<tr>
<th>Disc Level</th>
<th>Type 1 (%)</th>
<th>Type 1/2 (%)</th>
<th>Type 2 (%)</th>
<th>Type 2/3 (%)</th>
<th>Type 3 (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>12 (4.1)</td>
<td>16 (1.6)</td>
<td>41 (3.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>69 (2.5)</td>
</tr>
<tr>
<td>Caudal</td>
<td>13 (4.5)</td>
<td>18 (1.7)</td>
<td>72 (5.2)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td>104 (3.8)</td>
</tr>
<tr>
<td>L2-L3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>15 (5.1)</td>
<td>46 (4.5)</td>
<td>40 (2.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>101 (3.7)</td>
</tr>
<tr>
<td>Caudal</td>
<td>20 (6.8)</td>
<td>47 (4.6)</td>
<td>107 (7.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>174 (6.3)</td>
</tr>
<tr>
<td>L3-L4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>19 (6.5)</td>
<td>49 (4.8)</td>
<td>64 (4.6)</td>
<td>3 (4.8)</td>
<td>0 (0)</td>
<td>135 (4.9)</td>
</tr>
<tr>
<td>Caudal</td>
<td>23 (7.8)</td>
<td>67 (6.6)</td>
<td>154 (11.1)</td>
<td>3 (4.8)</td>
<td>0 (0)</td>
<td>247 (9.0)</td>
</tr>
<tr>
<td>L4-L5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>43 (14.7)</td>
<td>152 (15.0)</td>
<td>146 (10.6)</td>
<td>9 (14.3)</td>
<td>0 (0)</td>
<td>350 (12.7)</td>
</tr>
<tr>
<td>Caudal</td>
<td>38 (13.0)</td>
<td>144 (14.2)</td>
<td>218 (15.6)</td>
<td>10 (15.9)</td>
<td>0 (0)</td>
<td>410 (14.9)</td>
</tr>
<tr>
<td>L5-S1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral</td>
<td>56 (19.1)</td>
<td>256 (25.2)</td>
<td>260 (18.8)</td>
<td>15 (23.8)</td>
<td>0 (0)</td>
<td>587 (21.3)</td>
</tr>
<tr>
<td>Caudal</td>
<td>53 (18.2)</td>
<td>220 (21.7)</td>
<td>282 (20.4)</td>
<td>22 (34.9)</td>
<td>2 (100)</td>
<td>579 (21.0)</td>
</tr>
<tr>
<td>Total</td>
<td>292 (100)</td>
<td>1015 (100)</td>
<td>1384 (100)</td>
<td>63 (100)</td>
<td>2 (100)</td>
<td>2756 (100)</td>
</tr>
</tbody>
</table>

MC = Modic change

5.3.2 Size of the MC

The median size index was 5.44 and the median of the MC size index summary score was 16.0. Of all MC, 1550 (56.2%) affected 1-2 horizontal zones, 238 (8.6%) affected three horizontal zones and 968 (35.1%) affected 4-5 horizontal zones of the vertebrae. When comparing type 1 and type 2 MC groups, type 2 MC more frequently affected the entire horizontal plane compared to type 1 MC (13.7% vs. 17.9%, p=0.05). Of all MC, 440 (16.0%) were along the endplate only, 1462 (53.0%) had a maximum height of less than 25%, 666 (24.2%) had a maximum height from 25% to 50% and 188 (6.8%) had a maximum height of more than 50% of the vertebral body (Table 9).
Table 9. The height and width of types of MC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Type 1 n (%)</th>
<th>Type 1/2 n (%)</th>
<th>Type 2 n (%)</th>
<th>Type 2/3 n (%)</th>
<th>Type 3 n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal zones affected by MC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 zones</td>
<td>213 (72.9)</td>
<td>429 (42.3)</td>
<td>888 (64.1)</td>
<td>19 (30.2)</td>
<td>1 (50.0)</td>
<td>1550 (56.2)</td>
</tr>
<tr>
<td>3 zones</td>
<td>18 (6.2)</td>
<td>120 (11.8)</td>
<td>95 (6.9)</td>
<td>5 (7.9)</td>
<td>0 (0)</td>
<td>238 (8.6)</td>
</tr>
<tr>
<td>4-5 zones</td>
<td>61 (20.9)</td>
<td>466 (45.9)</td>
<td>401 (29.0)</td>
<td>39 (61.9)</td>
<td>1 (50.0)</td>
<td>968 (35.1)</td>
</tr>
<tr>
<td>Height of MC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Along the endplate</td>
<td>63 (21.6)</td>
<td>69 (6.8)</td>
<td>306 (22.1)</td>
<td>2 (3.2)</td>
<td>0 (0)</td>
<td>440 (16.0)</td>
</tr>
<tr>
<td>Less than 25%</td>
<td>160 (54.8)</td>
<td>543 (53.5)</td>
<td>741 (53.6)</td>
<td>18 (28.6)</td>
<td>0 (0)</td>
<td>1462 (53.0)</td>
</tr>
<tr>
<td>From 25% to 50%</td>
<td>51 (17.7)</td>
<td>315 (31.3)</td>
<td>271 (19.6)</td>
<td>28 (44.4)</td>
<td>1 (50.0)</td>
<td>666 (24.2)</td>
</tr>
<tr>
<td>More than 50%</td>
<td>18 (6.2)</td>
<td>88 (8.7)</td>
<td>66 (4.8)</td>
<td>15 (23.8)</td>
<td>1 (50.0)</td>
<td>188 (6.8)</td>
</tr>
<tr>
<td>Total</td>
<td>292 (100)</td>
<td>1015 (100)</td>
<td>1384 (100)</td>
<td>63 (100)</td>
<td>2 (100)</td>
<td>2756 (100)</td>
</tr>
</tbody>
</table>

MC = Modic change

5.3.3 Locus on chromosome 9 associated with MC

A meta-analysis comprising 1829 individuals (994 cases and 835 controls) of Northern European ancestry revealed a single locus with a genome-wide significance on chromosome 9 (Fig. 12). Heterogeneity between the two studies was not observed (Fig. 13). The lead SNP rs1934268 is situated in an intron of the protein tyrosine phosphatase, receptor type D (PTPRD) gene (Fig. 14). The lead SNP rs1934268 or any other SNP in strong LD with it (r2>0.7) have not previously been significantly associated with any trait according to Phenoscanner (Staley et al., 2016).
Fig. 12. Manhattan plot showing the p-values of the meta-analysis. The threshold for genome-wide significance (p<5×10^-8) is marked with the upper line and the threshold of suggestive significance (p<5×10^-5) with the lower line. The negative logarithm of the p-value is presented in the y-axis and chromosomal position in the x-axis.

Fig. 13. QQ-plot. The negative logarithm of the expected p-values is presented on the x-axis and the negative logarithm of the observed p-values on the y-axis. For the meta-analysis, the test-statistic inflation was 1.02 suggesting the absence of essential population stratification in the datasets.
Fig. 14. Regional association plot. The chromosomal position is indicated on the x-axis and the negative logarithm of the p-value is presented on the y-axis. The SNP (rs1934268) with the lowest p-value is marked with a diamond. The LD between the lead SNP and neighboring SNPs are represented by the shading of the circles. The plot was generated using the LocusZoom software (Pruim et al., 2010).

According to RegulomeDB (Boyle et al., 2012), the lead SNP rs1934268 is situated in a binding region for transcription factors including transcription factor PU.1 (SPI1), PBX homeobox 3 (PBX3) and B-cell lymphoma/leukemia 11A (BCL11A). However, its potential to affect the binding is minimal (RegulomeDB score 5). According to the GTEx database (GTEx Consortium, 2015), the lead SNP is not known as an eQTL. Furthermore, according to LDlink (Machiela & Chanock, 2015) and RegulomeDB, no SNP in strong LD with the lead SNP is known as functionally significant. Yet, a rare SNP rs186163656 in perfect LD with the lead SNP (D’ = 1) shows a probable effect on the binding of transcription factor CCCTC-Binding Factor (CTCF) (RegulomeDB score 2c). 34 SNPs reached a threshold of p<5×10^{-5}, which is commonly held as a threshold for suggestive association (Michailidou...
et al., 2017; Savage et al., 2018) (Table 10). These SNPs are located in or in close proximity of the *PTPRD*, XK related 4 (*XKR4*), O-6-Methylguanine-DNA Methyltransferase (*MGMT*), discs large MAGUK scaffold protein 2 (*DLG2*), zinc finger protein 184 (*ZNF184*), and opioid receptor kappa 1 (*OPRK1*).
Table 10. SNPs with p-value $5 \times 10^{-8} < p < 5 \times 10^{-5}$.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Chr:Pos</th>
<th>Effect allele</th>
<th>Other allele</th>
<th>EAF ± SE</th>
<th>Beta ± SE</th>
<th>P</th>
<th>$P^f$</th>
<th>Het. P</th>
<th>Overlapped gene</th>
<th>Nearest gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs36160095</td>
<td>4:32845987</td>
<td>A</td>
<td>G</td>
<td>0.938 ± 0.003</td>
<td>0.158 ± 0.034</td>
<td>3.4×10^{-6}</td>
<td>0</td>
<td>0.510</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs140476053</td>
<td>4:32843085</td>
<td>C</td>
<td>G</td>
<td>0.938 ± 0.003</td>
<td>0.155 ± 0.034</td>
<td>3.7×10^{-6}</td>
<td>0</td>
<td>0.522</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs143907932</td>
<td>4:32847206</td>
<td>T</td>
<td>C</td>
<td>0.062 ± 0.002</td>
<td>-0.155 ± 0.034</td>
<td>4.0×10^{-6}</td>
<td>0</td>
<td>0.528</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs150196231</td>
<td>4:32844355</td>
<td>T</td>
<td>C</td>
<td>0.063 ± 0.004</td>
<td>-0.151 ± 0.034</td>
<td>6.6×10^{-6}</td>
<td>0</td>
<td>0.634</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs201475493</td>
<td>6:27455134</td>
<td>A</td>
<td>AATG</td>
<td>0.073 ± 0.010</td>
<td>-0.143 ± 0.031</td>
<td>3.0×10^{-6}</td>
<td>0</td>
<td>0.762</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs150644047</td>
<td>6:27457523</td>
<td>T</td>
<td>C</td>
<td>0.073 ± 0.010</td>
<td>-0.141 ± 0.031</td>
<td>4.2×10^{-6}</td>
<td>0</td>
<td>0.806</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs16667806</td>
<td>6:27463684</td>
<td>T</td>
<td>C</td>
<td>0.928 ± 0.010</td>
<td>0.142 ± 0.031</td>
<td>4.2×10^{-6}</td>
<td>0</td>
<td>0.807</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs78950897</td>
<td>6:27471298</td>
<td>T</td>
<td>G</td>
<td>0.072 ± 0.010</td>
<td>-0.142 ± 0.031</td>
<td>4.3×10^{-6}</td>
<td>0</td>
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<td>rs74293985</td>
<td>6:27472488</td>
<td>T</td>
<td>C</td>
<td>0.928 ± 0.010</td>
<td>0.142 ± 0.031</td>
<td>4.3×10^{-6}</td>
<td>0</td>
<td>0.809</td>
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<td>ZNF184</td>
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<tr>
<td>rs77052921</td>
<td>6:27473455</td>
<td>A</td>
<td>C</td>
<td>0.928 ± 0.010</td>
<td>0.142 ± 0.031</td>
<td>4.3×10^{-6}</td>
<td>0</td>
<td>0.810</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs77380993</td>
<td>6:27474646</td>
<td>T</td>
<td>C</td>
<td>0.928 ± 0.010</td>
<td>0.142 ± 0.031</td>
<td>4.3×10^{-6}</td>
<td>0</td>
<td>0.810</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs80183551</td>
<td>6:27454446</td>
<td>A</td>
<td>G</td>
<td>0.928 ± 0.010</td>
<td>0.138 ± 0.031</td>
<td>4.4×10^{-6}</td>
<td>0</td>
<td>0.820</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs76458558</td>
<td>6:27480547</td>
<td>A</td>
<td>G</td>
<td>0.928 ± 0.010</td>
<td>0.138 ± 0.031</td>
<td>4.4×10^{-6}</td>
<td>0</td>
<td>0.997</td>
<td>-</td>
<td>ZNF184</td>
</tr>
<tr>
<td>rs1017404</td>
<td>7:12695507</td>
<td>A</td>
<td>G</td>
<td>0.783 ± 0.015</td>
<td>0.088 ± 0.019</td>
<td>3.9×10^{-6}</td>
<td>53.2</td>
<td>0.144</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs1036372</td>
<td>7:12695169</td>
<td>A</td>
<td>G</td>
<td>0.783 ± 0.015</td>
<td>0.088 ± 0.019</td>
<td>4.0×10^{-6}</td>
<td>55.2</td>
<td>0.135</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs6473810</td>
<td>8:54244458</td>
<td>A</td>
<td>G</td>
<td>0.682 ± 0.002</td>
<td>-0.078 ± 0.017</td>
<td>4.6×10^{-6}</td>
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<td>OPRK1</td>
</tr>
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<td>rs7831674</td>
<td>8:54224521</td>
<td>A</td>
<td>T</td>
<td>0.683 ± 0.001</td>
<td>-0.078 ± 0.017</td>
<td>4.9×10^{-6}</td>
<td>0</td>
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<td>OPRK1</td>
</tr>
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<td>rs10107460</td>
<td>8:54247311</td>
<td>A</td>
<td>G</td>
<td>0.681 ± 0.003</td>
<td>-0.077 ± 0.017</td>
<td>4.9×10^{-6}</td>
<td>0</td>
<td>0.716</td>
<td>-</td>
<td>OPRK1</td>
</tr>
<tr>
<td>rs2658912</td>
<td>8:56280631</td>
<td>A</td>
<td>G</td>
<td>0.127 ± 0.015</td>
<td>-0.106 ± 0.024</td>
<td>9.1×10^{-6}</td>
<td>0</td>
<td>0.869</td>
<td>XKR4</td>
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</tr>
<tr>
<td>rs1174582</td>
<td>9:9763539</td>
<td>C</td>
<td>G</td>
<td>0.821 ± 0.037</td>
<td>0.104 ± 0.022</td>
<td>1.2×10^{-6}</td>
<td>0</td>
<td>0.552</td>
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<td>-</td>
</tr>
<tr>
<td>rs2761762</td>
<td>9:9733974</td>
<td>A</td>
<td>G</td>
<td>0.768 ± 0.044</td>
<td>0.089 ± 0.019</td>
<td>3.2×10^{-6}</td>
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<td>0.337</td>
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<td>-</td>
</tr>
<tr>
<td>rs4742607</td>
<td>9:9513109</td>
<td>A</td>
<td>G</td>
<td>0.462 ± 0.043</td>
<td>-0.071 ± 0.016</td>
<td>9.3×10^{-6}</td>
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<tr>
<td>rs4742606</td>
<td>9:9513039</td>
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<td>G</td>
<td>0.462 ± 0.043</td>
<td>-0.071 ± 0.016</td>
<td>9.4×10^{-6}</td>
<td>0</td>
<td>0.634</td>
<td>PTPRD</td>
<td>-</td>
</tr>
<tr>
<td>rs1174587</td>
<td>9:9767971</td>
<td>A</td>
<td>G</td>
<td>0.829 ± 0.036</td>
<td>0.097 ± 0.022</td>
<td>9.5×10^{-6}</td>
<td>0</td>
<td>0.524</td>
<td>PTPRD</td>
<td>-</td>
</tr>
<tr>
<td>SNP ID</td>
<td>Chr:Pos</td>
<td>Effect allele</td>
<td>Other allele</td>
<td>EAF ± SE</td>
<td>Beta ± SE</td>
<td>P</td>
<td>I²</td>
<td>Het. P</td>
<td>Overlapped gene</td>
<td>Nearest gene</td>
</tr>
<tr>
<td>--------------</td>
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<td>------------</td>
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<td>-------------</td>
<td>--------</td>
<td>-----------------</td>
<td>--------------</td>
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<tr>
<td>rs74778840</td>
<td>10:131489527</td>
<td>C</td>
<td>G</td>
<td>0.902 ± 0.039</td>
<td>-0.131 ± 0.030</td>
<td>8.8×10⁻⁶</td>
<td>0</td>
<td>0.792</td>
<td>MGMT</td>
<td>-</td>
</tr>
<tr>
<td>rs117285296</td>
<td>11:83055365</td>
<td>A</td>
<td>G</td>
<td>0.153 ± 0.028</td>
<td>0.110 ± 0.022</td>
<td>8.2×10⁻⁶</td>
<td>0</td>
<td>0.329</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs2043208</td>
<td>11:83184839</td>
<td>A</td>
<td>G</td>
<td>0.235 ± 0.029</td>
<td>0.084 ± 0.019</td>
<td>6.5×10⁻⁶</td>
<td>0</td>
<td>0.913</td>
<td>DLG2</td>
<td>-</td>
</tr>
<tr>
<td>rs9787813</td>
<td>11:83184256</td>
<td>A</td>
<td>C</td>
<td>0.229 ± 0.030</td>
<td>0.084 ± 0.019</td>
<td>8.1×10⁻⁶</td>
<td>0</td>
<td>0.783</td>
<td>DLG2</td>
<td>-</td>
</tr>
<tr>
<td>rs183727937</td>
<td>11:83178853</td>
<td>T</td>
<td>C</td>
<td>0.650 ± 0.038</td>
<td>-0.076 ± 0.017</td>
<td>8.4×10⁻⁶</td>
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<td>DLG2</td>
<td>-</td>
</tr>
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<td>11:83181164</td>
<td>T</td>
<td>G</td>
<td>0.319 ± 0.028</td>
<td>0.076 ± 0.017</td>
<td>9.5×10⁻⁶</td>
<td>0</td>
<td>0.492</td>
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</tr>
<tr>
<td>rs143569491</td>
<td>11:83195804</td>
<td>T</td>
<td>TG</td>
<td>0.230 ± 0.031</td>
<td>0.084 ± 0.019</td>
<td>9.8×10⁻⁶</td>
<td>0</td>
<td>0.864</td>
<td>DLG2</td>
<td>-</td>
</tr>
<tr>
<td>rs17461216</td>
<td>11:83193813</td>
<td>A</td>
<td>G</td>
<td>0.230 ± 0.031</td>
<td>0.084 ± 0.019</td>
<td>9.8×10⁻⁶</td>
<td>0</td>
<td>0.864</td>
<td>DLG2</td>
<td>-</td>
</tr>
<tr>
<td>rs1432050</td>
<td>11:83201563</td>
<td>T</td>
<td>C</td>
<td>0.229 ± 0.032</td>
<td>0.084 ± 0.019</td>
<td>9.9×10⁻⁶</td>
<td>0</td>
<td>0.873</td>
<td>DLG2</td>
<td>-</td>
</tr>
</tbody>
</table>

Chr: pos = chromosome:position, DLG2 = discs large MAGUK scaffold protein 2, EAF = effect allele frequency, Het. P = heterogeneity p-value, I² = heterogeneity statistics, MGMT = O-6-Methylguanine-DNA Methyltransferase, OPRK1 = and opioid receptor kappa 1, P = p-value, PTPRD = protein tyrosine phosphatase, receptor type D, SNP = single nucleotide polymorphism, ZNF184 = zinc finger protein 184, XKR4 = XK related 4.
6 Discussion

6.1 Prevalence and natural history of MC (I, III)

In TwinsUK, the prevalence of MC was 32.1% at baseline and 49% at follow-up in female twins with a mean age of 54 years at baseline and 64.1 at follow-up. The prevalence was also observed to increase with age, an observation in line with previous studies (T. S. Jensen et al., 2008; Tarukado et al., 2017). Among 46-year-old Finnish participants, the prevalence of MC was 62.9%. The prevalence of MC observed in this thesis is higher than the median prevalence reported in a systematic review (43% among patients with LBP) (T. S. Jensen et al., 2008). This is possibly due to the older age in TwinsUK as the systematic review also comprised working age non-clinical populations. In the NFBC1966, even the small MCs were included as MC thus raising the prevalence. In comparison, in a study of Finnish male twins with a mean age of 49 years, the prevalence of MC was 55.6% (Y. Wang et al., 2012) and 56% among Finnish train engineers and male paper mill workers with a mean age of 46.0 years among train engineers and 47.8 years among paper mill workers (Karppinen et al., 2008).

In TwinsUK the prevalence of MC increased during the ten-year follow-up period with an incidence of 21.6%. Previously, the natural course of MC has been studied over three-year and four-year periods. In a study of Finnish sciatica patients, the incidence of MC was 6% during a three-year follow-up (Kuisma et al., 2006), and in a study of the Danish general population the incidence was 10% during a four-year follow-up (T. S. Jensen et al., 2009). We also observed regression of MC in TwinsUK as MC totally disappeared in 12 twins (3.5%). The finding supports previous observations that MC can be reversible (Albert & Manniche, 2007; R. K. Jensen et al., 2012; T. S. Jensen et al., 2009). To our knowledge, the TwinsUK study is the longest follow-up study of MC and the follow-up period was long enough to enable the assessment of the natural course of MC over time.

MC2 was found to be most prevalent MC type in the NFBC1966 cohort. The most prevalent MC type varies between studies and no clear consensus on the most prevalent type has been established. MC1 is thought to reflect active disc degeneration and instability and is more common among patients with LBP (Albert & Manniche, 2007; Järvinen et al., 2015; Rahme & Moussa, 2008), whereas MC2 is thought to be more stable and thus more prevalent in the general population. We found only two MC3 in NFBC1966. This is in line with previous studies showing...
Previous studies have suggested that the DD phenotype comprises two different phenotypes (Adams & Dolan, 2012; Y. Li et al., 2014). DD in the upper lumbar levels and thoracic spine occurring at a younger age is suggested to be endplate-driven. In comparison, DD in the lower lumbar levels increases with age as fatigue damage accumulates and age-related biomechanical changes occur in the annulus. Thus, it is thought to be annulus-driven (Adams & Dolan, 2012). A possible link between irregular endplate shape, abnormal disc slippage and DD was observed in a study of LBP and sciatica patients who attended kinetic MRI supporting the hypothesis of endplate-driven DD (Y. Li et al., 2014).

Modestly higher heritability estimates for upper lumbar levels (for disc height narrowing $h^2=0.45$, for signal intensity $h^2=0.51$) compared to lower lumbar levels (for disc height narrowing $h^2=0.41$, for signal intensity $h^2=0.30$) were observed in a study of Finnish male twins (Battie et al., 2008). However, distinct differences were not apparent. Although some genetic influences were shared, the majority of genetic and environmental effects on DD were unique to upper or lower lumbar levels (Battie et al., 2008). Similar results were also obtained from the TwinsUK as twins with baseline MC developed MC more often in the three highest lumbar levels. No significant difference was found at the two lowest lumbar levels. This is in line with endplate-driven DD (Adams & Dolan, 2012).

### 6.2 The heritability of MC (I, II)

The heritability of MC has not been evaluated before. In study I, we estimated that the heritability of MC ranges from 16% to 43% supporting the hypothesis that MC is not entirely environmental but does have at least a small heritable component. Higher heritability estimates have been found for lumbar DD as classical twin studies estimated the heritability to range from 34% to 74% (Battie et al., 1995; Williams et al., 2011). In comparison, lower heritability estimates have been found for sciatica with a heritability estimation of 20.8% (Heikkilä et al., 1989).

Originally, 14 families with a history of DD characterized with sciatica were collected from the Oulu and Jyväskylä regions. Out of these, two large families with several affected family members in at least two generations were chosen for WES in the study II: Family I and II. The prevalence of MC was 38.9% in Family I and 24.2% in Family II. The occurrence of MC in at least two generations and in
multiple individuals in family data suggests that there are hereditary components behind the development of MC or that some form of MC is hereditary.

6.3 **HSPG2 as a novel candidate gene for MC (II)**

In study II, we identified an insertion and deletion mutation in *HSPG2* (p.Q1611delfxX20) co-segregating with MC. *HSPG2* encodes a protein called perlecan, which is a heparan sulfate proteoglycan expressed in mammalian cartilage and basement membranes (Costell et al., 1999; Iozzo, Cohen, Grassel, & Murdoch, 1994). *HSPG2* has 94 exons and encodes a protein comprised of five structural core domains to which long chains of glycosaminoglycans are attached (Murdoch, Dodge, Cohen, Tuan, & Iozzo, 1992).

Previously, mutations in *HSPG2* have been associated with two rare autosomal recessive diseases: Schwartz-Jampel syndrome (SJS) and dyssegmental dysplasia Silverman-Handmaker type (DDSH) (Arikawa-Hirasawa et al., 2001; Nicole et al., 2000; Stum et al., 2006). Mutations causing SJS occur throughout the *HSPG2* gene and the mutated proteins have varying degrees of functionality. SJS is a non-lethal condition characterized by skeletal dysplasia and myotonia (Nicole et al., 2000; Stum et al., 2006). In contrast, DDSH is caused by functional null mutations resulting in a truncated protein core that is not secreted (Arikawa-Hirasawa et al., 2001) and is a fatal condition characterized by skeletal dysplasia (Stum et al., 2006). Similarly, *Hspg2* knockout mice are embryonic lethal and the few mice that have survived exhibited significant kyphoscoliosis and skeletal defects and died shortly after birth (Costell et al., 1999). Also 21 rare and potentially damaging variants in *HSPG2* have been associated with another skeletal condition, idiopathic scoliosis, among Northern American individuals (Baschal et al., 2014).

6.4 **MAML1 as a novel candidate gene for MC (II)**

In study II, we identified a glutamate to lysine amino acid change (p.Glu553Lys) in the *MAML1* gene co-segregating with MC. MAML1 has been shown to be a co-activator of the Notch signaling pathway (L. Wu et al., 2000), which regulates a variety of biological functions including cartilage development and homeostasis (Dong et al., 2010; Kohn et al., 2012; Mead & Yutzey, 2009; Shang et al., 2016). Loss of Notch signaling in postnatal murine joints leads to osteoarthritis (Z. Liu et al., 2015; Z. Liu et al., 2016; Mirando et al., 2013). MAML1 has also several functions in a variety of biological processes independent of Notch signaling.
A study of *MAML1* knockout mice revealed that the mouse homolog Maml1 enhances the transcriptional activity of runt related transcription factor 2 (Runx2) (Watanabe et al., 2013). Maml1 knockout mice had impaired chondrocyte maturation (Watanabe et al., 2013). RUNX2 is a transcription factor necessary for the proliferation and differentiation of chondrocytes and osteoblasts in humans (Komori et al., 1997). It has been shown to be highly expressed in degenerated discs (Rutges et al., 2010) and is upregulated in discs with MC (Torkki et al., 2016).

### 6.5 Locus on chromosome 9 associated with MC (III)

In study III, we conducted the first ever GWAS on MC and discovered a genome-wide significant locus on chromosome 9. This locus has earlier been associated with genome-wide significance with numerous complex traits such as restless legs syndrome (Schormair et al., 2008), type 2 diabetes (Tsai et al., 2010) and personality and mood traits (Kim et al., 2013; Ward et al., 2017). The association region is probably functional and at least SNP rs186163656, which is in strong LD with the lead SNP, is able to affect the binding of transcription factors including SPI1, PBX3, BCL11A and CTCF. SPI1 takes part in osteoclast differentiation (Tondravi et al., 1997), PBX3 and CTCF participate in numerous pathways including the development of embryonic limb buds (Capellini, Zappavigna, & Selleri, 2011; Soshnikova, Montavon, Leleu, Galjart, & Duboule, 2010) and BCL11A takes part in development of the spinal cord (John et al., 2012).

The identified locus harbors *PTPRD*, which is important for neural growth (Uetani, Chagnon, Kennedy, Iwakura, & Tremblay, 2006) and acts a tumor suppressor (Veeriah et al., 2009). According to the GTEx database (https://www.gtexportal.org/home/), the gene is expressed in the central nervous system. However, cartilage and bone tissues are not currently included in the database. *PTPRD* is a member of the protein tyrosine phosphatase family that has been observed to be involved in numerous cellular processes including cell growth, cell differentiation, mitotic cycle and oncogenic transformation. Elevated expression of *PTPRD* has been associated with successful autologous chondrocyte implantation in knee osteoarthritis patients (Stenberg et al., 2014). This may indicate that these patients were less affected with degeneration since *PTPRD* has been seen to be upregulated in intact cartilage compared to cartilage that has been damaged by osteoarthritis (Tsuritani et al., 2010). In mice, the homologous *Ptprd*
gene was observed to be expressed during spinal development (Schaapveld et al., 1998).

6.6 Methodological considerations

6.6.1 Study I

For unavoidable reasons, the baseline and follow-up MRI was performed using different equipment as baseline imaging was performed using a 1.0-T and follow-up using a 1.5-T scanner. This may have had an impact on the results as MC are more frequently observed in a higher-field MRI (Bendix et al., 2012). Also, for funding reasons the study lacked T1w images.

The study sample was predominately female and thus did not contain study subjects reporting heavy manual occupations. However, there was not a significant difference in age or MC prevalence between men and women, and thus both genders were included. The twinsUK cohort has previously been shown to represent the general UK population (Andrew et al., 2001).

We estimated the heritability of MC using a twin study approach. The advantages of twin studies are that researchers can estimate the proportion of variance in a trait attributable to genetics and environment. Twin studies can improve the statistical power of a study as well as reduce the amount of genetic and environmental variability (Sahu & Prasuna, 2016). There are however some limitations to twin studies. Results obtained from twin studies cannot be directly generalized to the general population due to the lack of randomization. Twin registers usually rely on voluntary participation of twins. This can lead to volunteer bias or recruitment bias and may lead to overinclusion of female and MZ twins resulting in overestimation of the heritability of the trait studied (Sahu & Prasuna, 2016).

In study I, the AE-model was used to estimate the heritability of MC. The model has two components: additive genetic factors and unique environmental factors. The use of the full ACE-model has been criticized as the model relies on the assumption that common environmental factors in MZ and DZ twins are equal. This assumption is controversial as MZ twins usually encounter more similar environments than DZ twins (Moore & Shenk, 2017).
6.6.2 Study II

The benefit of using a family-based study design is the enrichment of genetic effects; studies based on familial cases have more power than unrelated cases to detect genetic effects (M. Li, Boehnke, & Abecasis, 2006). In addition, in family-based studies, all the study subjects are of the same ethnic origin thus decreasing the population heterogeneity (Ott et al., 2011). The family based method has previously been used to identify genes behind Mendelian forms of complex disorders such as early-onset breast cancer and Alzheimer’s disease (Levy-Lahad et al., 1995; Miki et al., 1994). However, complex traits have proven more difficult with the family-based method due to complex inheritance, laborious task of family collection and small sample sizes. Thus, the research leads towards population-based designs that assume that much of the phenotypic variance in complex traits might be explained with common genetic variants (Botstein & Risch, 2003).

Investigation of rare variants is the current frontier of human genetic studies. In many common complex diseases such as osteoporosis and osteoarthritis, the common alleles usually explain only a modest portion of the heritability (arcOGEN Consortium et al., 2012; Estrada et al., 2012; Wellcome Trust Case Control Consortium, 2007). It is thought that alleles with low frequency (0.01<MAF<0.05) and rare alleles (MAF<0.01) explain a substantial part of the heritability in complex diseases (Manolio et al., 2009). In order to identify rare variants using GWAS, the sample size would need to increase substantially. Large pedigrees, which usually have 20-25 family members, may offer a solution as they provide a design that has considerable power in the search of rare variants behind a common complex trait (Wijsman, 2012). When combined with NGS, a small amount of sequencing added to a well-characterized large pedigree can yield considerable information (Wijsman, 2012). WES has been used successfully in family based studied in the search for rare variants for example in diabetes (Jambaljav et al., 2017), cone dystrophy (Gupta et al., 2017) and primary open angle glaucoma (Micheal et al., 2017). Our families consisted of 18 and 33 family members and may thus be considered large enough for identifying rare variants behind the development of MC.

NGS data analysis also encounters some challenges. The filtering of a massive number of variants produced by NGS is still a major challenge. Several large-scale sequencing studies for genomes and exomes have been performed, and population specific allele frequencies are provided in databases like ExAC (http://exac.broadinstitute.org/) or genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/). Several in silico prediction tools are also
available to aid in the interpretation of rare missense mutations in order to find mutations altering the protein structure. We used SIFT (Kumar, Henikoff, & Ng, 2009), Polyphen-2 (Adzhubei et al., 2010) and MutationTaster (Schwarz, Rodelsperger, Schuelke, & Seelow, 2010) as at the time they were the most commonly used tools and were integrated in the ANNOVAR (Charlesworth et al., 2012; Ishida et al., 2013). The interpretation of non-coding variants has proven to be very challenging. The understanding of functional elements in the genome was facilitated by the ENCODE project (Rosenbloom et al., 2010).

The role of a causal variant is difficult to prove without massive and expensive functional experiments. In studying MC, the fibrous cartilage tissue causes additional challenges. In the NGS era, the production of large datasets from transcriptomes has yielded large and valuable datasets like ENCODE containing the expression data from many human cell lines and tissues. The datasets can be used to assess the alteration of single cells and tissues in different pathological states (Casamassimi, Federico, Rienzo, Esposito, & Ciccodicola, 2017). However, cartilage and bone tissues are not currently included in the databases. Thus, chondrocytic cell lines were not available. Instead, data from GM12878 and HSMM cell lines were used to identify alleles affecting strong enhancer areas and active promoter. This may have affected our results since the activity of the enhancer and promoter regions can vary between different tissues and cell lines (C. T. Ong & Corces, 2011).

6.6.3 Study III

In study III, we utilized a well-characterized birth cohort sample with data from prenatal time to midlife, broad sociodemographic coverage and a large MRI population. Previously, NFBC1966 has been used to identify genetic loci associated with, for example, post-term birth (Schierding et al., 2018) and susceptibility to psychosis (Ortega-Alonso et al., 2017). NFBC1966 has also been used as a part of a meta-analysis to identify genetic loci associated with, for example, cardiac stroke (Malik et al., 2018) and gastroesophageal reflux disease (Bonfiglio et al., 2017).

In GWAS, the statistical power to detect disease susceptibility loci depends mainly on the size of the study sample (Hong & Park, 2012) and the precision of the phenotype (MacRae & Vasan, 2011). Also, the genetic model tested has an impact on the statistical power. The maximum power is reached when the genetic model used and the model of inheritance of the trait studied are consistent (Lettre, Lange, & Hirschhorn, 2007). Possible models are additive, dominant and recessive.
Each model makes different assumptions about the genetic effect in the data. The dominant model assumes that one copy of the risk allele is required to increase the risk whereas the recessive model assumes that two copies of the risk allele are required to alter the risk. The additive model assumes that the increase in the risk is linear for each copy of the risk allele.

A common practice is to examine the additive model only, as it has reasonable power to detect both additive and dominant effects but it may be unable to detect some recessive effects (Lettre et al., 2007). In contrast, the recessive model performs poorly for traits that act additively or dominantly (Lettre et al., 2007). If the model used is inappropriate, the statistical power is reduced. This was shown in a study of the association between lactase persistence and BMI as authors compared the additive model used by the previous GWASs and the correct dominant model (Kettunen et al., 2010). We tested additive, recessive and dominant models. The loci identified in the additive model disappeared in the dominant and recessive models. Thus, we concluded that the additive model best describes the genetic effect in our data.

In study III, we joined two cohorts (NFBC1966 and TwinsUK) for one meta-analysis. Meta-analysis increases the sample size and thus increases the power and reduces the false-positive rate compared to a single GWA study (Evangelou & Ioannidis, 2013). Meta-analysis also increases the precision of the effect estimates for the identified association and reduces the so-called winner’s curse phenomenon (overestimation of the disease risk of novel genetic association when the statistical power of the original study is not adequate). Furthermore, it evaluates and measures the degree of consistency and heterogeneity of the genetic effects across the combined studies (Ioannidis, Patsopoulos, & Evangelou, 2007). It has become increasingly common for the detection of subtle genetic effects on complex traits (Panagiotou, Willer, Hirschhorn, & Ioannidis, 2013) and has been used successfully to identify genetic loci behind adolescent idiopathic scoliosis (Ogura et al., 2018), cardiac stroke (Malik et al., 2018) and blood pressure (Sung et al., 2018).

### 6.7 Future possibilities

The progress in human genetic studies has been enormous in the last decades especially after the development of GWAS and NGS methods. Now, sequencing of the whole human genome or the exomes, or more targeted sequencing have become more popular methods, especially as the costs decline. Previously, the genetic
background and the genes affecting the development of MC were unknown. This thesis provided new insight into the etiology of MC, but further work is required.

Studying common variants behind common complex phenotypes only explains a modest portion of the heritability, and low frequency and rare variants are thought to explain a substantial portion of the heritability. For this reason, using family-based methods and large pedigrees in the future combined with NGS methods could help to identify more of the genetic background behind MC. In addition, confirmation and validation of results is important to avoid false positive findings. In the future, studying the rest of the Finnish families that were collected previously using whole exome sequencing, or even whole genome sequencing could help to shed more light on the etiology of MC. Also, it would be beneficial to perform genetic studies using GWAS in larger MC cohorts. We discovered an indel mutation in HSPG2 and SNP in MAML1. Further research to study these genes and variants is required in order to establish whether our observations are separate or do these genes play a larger role in MC development.

To date, no definite or specific diagnostic test for MC exists and the diagnosis is established solely by imaging. Possible tools in the future could be blood biomarkers of MC and novel imaging technologies like ultrashort echo time imaging that recognizes endplate pathologies (Lotz, Fields, & Liebenberg, 2013). These could help in the detection of MC and provide more information on the development of MC.
The main purpose of this thesis was to estimate the heritability of MC and to identify predisposing genetic factors for MC. The results of this thesis indicate that MC is not completely environmental, but has a small heritable component. To the best of our knowledge, no GWAS or NGS had previously been performed for MC. Here we identified two novel candidate genes, *MAML1* and *HSPG2*, using exome sequencing in families, and with a population-based analysis we identified a novel locus for MC. Our results provide new insight into the etiology of MC development, although the exact role of the candidate genes needs to be studied further.

To conclude, understanding the genetic background of degenerative MRI findings and their development might provide clinicians with more powerful tools in treatment, prevention or even reversion of DD.
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Original Publications


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1473. Lantto, Ulla (2018) Etiology and outcome of PFAPA (periodic fever, aphthous stomatitis, pharyngitis and adenitis) syndrome among patients operated with tonsillectomy in childhood
1474. Hintsala, Heidi (2018) Cardiovascular responses to cold exposure in untreated hypertension
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THE HERITABILITY AND GENETIC RISK FACTORS OF MODIC CHANGES