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The role of genetic variants in the development and long-term outcome of the treatment of lumbar intervertebral disc degeneration

Ph.D. thesis

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1. LIST OF ABBREVIATIONS

Sd: Danforth's short-tail

Skt: Sickle tail gene

Shh: Sonic hedgehog

NT: neural tube

SC: sclerotome

NC: notochord

VB: vertebral body

CEP: cartilagous endplate

DDD: degenerative disc diseases

LBP: low back pain

IDD: intervertebral disc degeneration

IVD: intervertebral disc

ECM: extracellular matrix

NP: nucleus pulposus

AF: annulus fibrosus

SNP: single nucleotide polymorphism

GWAS: genome wide association study

NSAID: non-steroid anti-inflammatory drug

FBSS: failed back surgery syndrome

ODI: Oswestry Disability Index

ZDS: Zung Depression Scale

SIPS: stress-induced premature senescence

SASP: senescence-associated secretory phenotype

MMP-13: matrix metalloproteinase 13

ADAMTS5: and a disintegrin and metalloproteinase with thrombospondin motifs 5

VDR: Vitamin D receptor

LDD: lumbar disc degeneration

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2. INTRODUCTION

2.1. Degenerative intervertebral disc diseases

2.1.1. *Anatomy and development of the intervertebral discs*

The vertebrate axial skeleton developed to protect the spinal cord and to provide support for the body [1]. The spine as an organ consists of two major parts, the bony vertebral column that develops through endochondral bony formation and the fibrous part that contains the intervertebral discs (IVD) and the spinal ligaments. The IVD functions as a mechanical shock absorber of the spine and consist of two components; the nucleus pulposus (NP) which is the gel like central region and the second part is a fibrous sheet the annulus fibrosus (AF). The IVDs are positioned in between two adjacent vertebral bodies which creates the spine's segmented structure [1].

The NP and AF together form the mature IVD but they are derived from different embryonic structures [2] (Figure 1 The development of NP and AF). The NP comes from the notochord while the AF originates from the sclerotomes of the somites [3]. Somites determine the segmented structure of the spine and forms the vertebral bodies, the AFs, ligaments, and the tendons.

The development of the AF starts with the somitogenesis at the beginning of gastrulation [4]. Most of the skeleton is build up from the mesoderm, precisely the AF is formed from the paraxial mesoderm, which goes through a mesenchymal epithelial transition then it starts to differentiate to dermatome, myotome, and sclerotome. Then from the sclerotomes the vertebrae, the cartilaginous endplates and the AF are formed [5][6]. After the sclerotome has been specified the resegmentation process starts which is necessary for IVD and vertebrae formation, disturbance in this process has clinical implications such as proatlas segmentation anomalies [7][8].

The NP arises from the notochord, and its development begins during gastrulation. Mechanical forces play a role in the growth of notochord and it is hypothesized that they also play a role in the transition of notochord to NP [9]. Not only notochord formation but also notochord maintenance is a vital step during the development of NP, as it affects the

morphology of the NP at the same time it also maintains the boundary between NP and AF [10][11]. This process is affected by Danforth's short-tail (Sd) mutation which is located on the same chromosome as the Sickle tail gene (Skt). Skt gene single nucleotide polymorphism (SNP) have been reported to have an association with IVD herniation in Japanese and Finnish population [11]. The notochord sheath also plays an important role in the formation of NP [1]. Sonic hedgehog (Shh) gene is secreted both from notochord and postnatal NP and it is substantial in the notochord to NP transition [12]. A recent study by Bonavita and his colleagues demonstrated in mice that the collapse of the sacral discs is associated with the down-regulation of Shh signaling in NP which results in the bony fusion and formation of the sacrum [13]. Studying the process of notochord to NP transition is essential for understanding the process of IVD aging and degeneration.

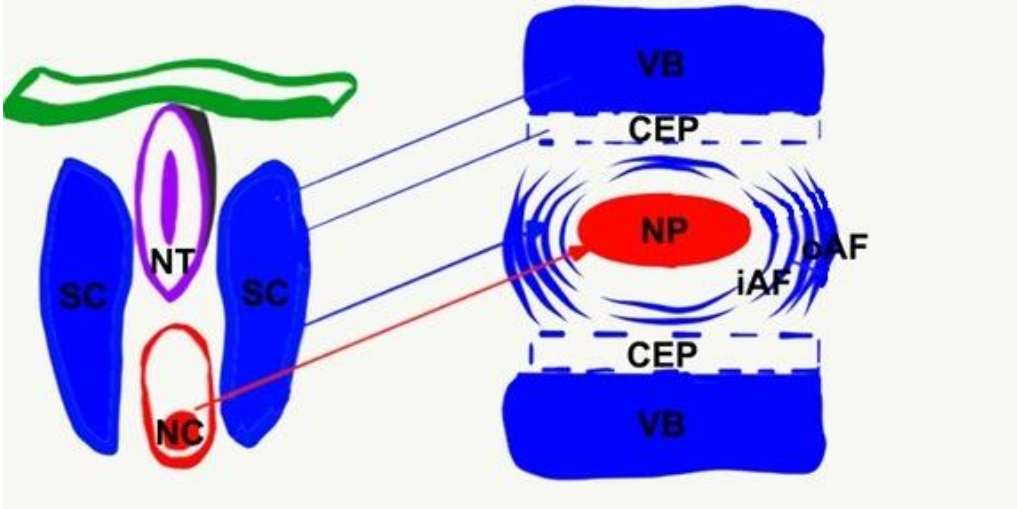

Figure 1 The development of NP and AF

NT: neural tube, SC: sclerotome, NC: notochord, VB: vertebral body, CEP: cartilagous endplate, NP: nucleus pulposus, iAF: inner annulus fibrosus, oAF: outer annulus fibrosus ((Williams et al. 2019)

2.1.2. Epidemiology of intervertebral disc degeneration

Degenerative disc diseases (DDD) and its most frequent manifestation low back pain (LBP) are the leading health care problems which occur in low-, high- and middle-income countries in all age groups [14]. Publications investigating the lifetime prevalence of LBP report it ranging between 75 and 84% [15]. Years lived with disability caused by low back pain increased significantly in the last 3 decades, primarily due to aging and increasing population [14]. It is reported that people with physically demanding occupations, mental or chronic physical comorbidities, obesity and smokers are more susceptible to develop low back pain [14]. In most of the LBP cases it is not possible to accurately identify the exact source of the symptoms – such as vertebral fractures, infections, degenerative disc disease etc. LBP is a complex bio-psycho-social entity that is characterized by biophysical, psychological, and social dimension [14].

However, in some cases the biophysical background of LBP is intervertebral disc degeneration (IDD). IDD starts to develop in adolescence and progress with age [16]. IDD is usually more frequent and severe at the lower lumbar level [17]. Excessive mechanical stress, trauma, smoking and genetics attributes to IDD [18]–[20]. Age-related changes in the IVDs starts earlier compared to other tissues. Pathological changes (IDD) are hard to distinguish from age-related physiological changes. Pathological changes can occur in younger life and it is influenced by factors other than aging, such as trauma, environmental factors and genetic predisposition [21]. IDD can occur only on a single level while physiological aging usually more systematic and is present on all spinal levels [21].

2.1.3. Biochemical and histological changes during intervertebral disc degeneration

The biochemical changes during the degeneration process includes the reduction of IVD cells thus decreasing the production of extracellular matrix (ECM), which tips the scale to catabolic activities over anabolic activities leading to accelerated degeneration [16]. Genomic instability which leads to cell senescence is an important driver of IDD [21]. The risk and frequency of DNA damage overcomes the DNA repair mechanism with aging,

resulting in an accumulation of scrap DNA which can lead to disc aging. In ERCC1-XPF (involved in DNA repair) deficient mice the IVDs showed typical features of IDD such as decreased disc height, loss of proteoglycans, and an abundance of senescent cells. Genotoxic stresses such as smoking or radiation causes disc aging which supports the theory that DNA damages contributes to disc aging [22] [23]. The other possible source of DNA damage is oxidative stress induced by inflammation. Interleukin-1 (IL 1), a cytokine involved in IDD, has been showed to induce cell senescence in NP, furthermore IL-1 receptor knockout mice showed typical features of IDD and its NP cells showed senescence [16].

There are two types of cell senescence, stress-induced premature senescence (SIPS) and replicative senescence. SIPS is caused by genomic and mitochondrial damage. Cells with premature stress-induced senescence acquire a so-called senescence-associated secretory phenotype (SASP), which is characterized by the overexpression of inflammatory cytokines and matrix proteases. The aforementioned cytokines and proteases have a profound catabolic effect on the cells in its vicinity which promotes tissue degeneration [24]–[26]. This mechanism of IDD is supported by previous papers which showed an increased frequency of senescent cells which were marked with senescence-associated beta-galactosidase in human IVD tissue samples. These markers were positively associated with matrix metalloproteinase 13 (MMP-13) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5). A study demonstrated the causal relationship between cellular aging and age-related IDD by using a p16-3MR transgenic mouse model in which the senescent cells could be eliminated by administering ganciclovir. The treated mice showed significantly decreased level of catabolic factors and better histological features of IDD at the age of 2 compared to the non-treated group. This indicates that cell senescence has a direct effect on the development of IDD [27].

As IDD progresses a shift occurs in production of collagen type 2 to collagen type 1 by the nucleus pulposus (NP), also the cross-linking of collagen fibres increases which renders the IVDs more susceptible to mechanical damage [28]. Furthermore in degenerated IVD a localization of collagen X has been observed to cause formation of cell clusters and clefts [16]. A decrease in the proteoglycan content of IVD is seen during the degenerative

process, in addition the keratin sulfate content increases in the glycosaminoglycan chains which cause dehydration. The change observed in type 1 collagen also makes the IVDs less capable to withstand physical stress [29], [30].

The histological changes to IVDs during the degeneration manifest as loss of demarcation between NP and annulus fibrosus (AF), presence of fissures, cell cluster formation, disruption the lamellar structure of AF and increased vascularization and innervation [31], [32].

2.1.4. *Phenotypes of intervertebral disc degeneration*

Back pain does not always correlate with the presence of DDD, however there are some publication that demonstrated that MRI changes in DDD can predict a painful deranged disc [33]. The direct association of back pain and degenerative changes are not consistent, although pain is the most frequent syndrome associated with DDD [33]. Most frequently the changes during the degeneration process are seen in the IVDs, the endplates and in the facet joints. These findings can be observed with MRI in supine position, but a recent publication by Tarantino and his study group states that roughly one third of the physiological supine MRI shows some degree of degeneration with standing MRI [34].

Disc degeneration is mainly characterized by dehydration on the MRI [35], which is linked to the decrease of highly water binding proteoglycans[36]. On MRI the IVD degeneration is visible as loss of hyperintensity in the NP on T2 sequence and a consequential narrowing of disc height. Pfirrmann and his colleagues classified the level of disc degeneration by the distinction between AF and NP, signal intensity on MRI, disc structure and the intervertebral disc height [37] (Figure 2). Another manifestation of the IDD is bulging/herniation. The distinction between bulging and herniation is clinically important, bulging can represent the normal aging of discs and often seen in asymptomatic patients. The AF in disc bulging is intact while in herniation the AF loses its integrity [38] (Figure 3).

Endplate changes on the MRI were classified into three types by Modic and his study group according to the underlying bone marrow signal change [39][40]. Modic I changes are seen as hypointense on T1 and as hyperintense on T2 sequence, where the

underlying cause are subchondral fractures and fibrous tissue replacing the bone marrow due to stress reaction [41]. Modic II manifests as hyperintense T1 and T2 signal, it is seen if the bone marrow undergoes fatty replacement [39]. Modic III changes represent the presence of dense woven bone and are seen as T1 and T2 hypointensity [39]. Modic I and III are more often linked to back pain than type II Modic [42].

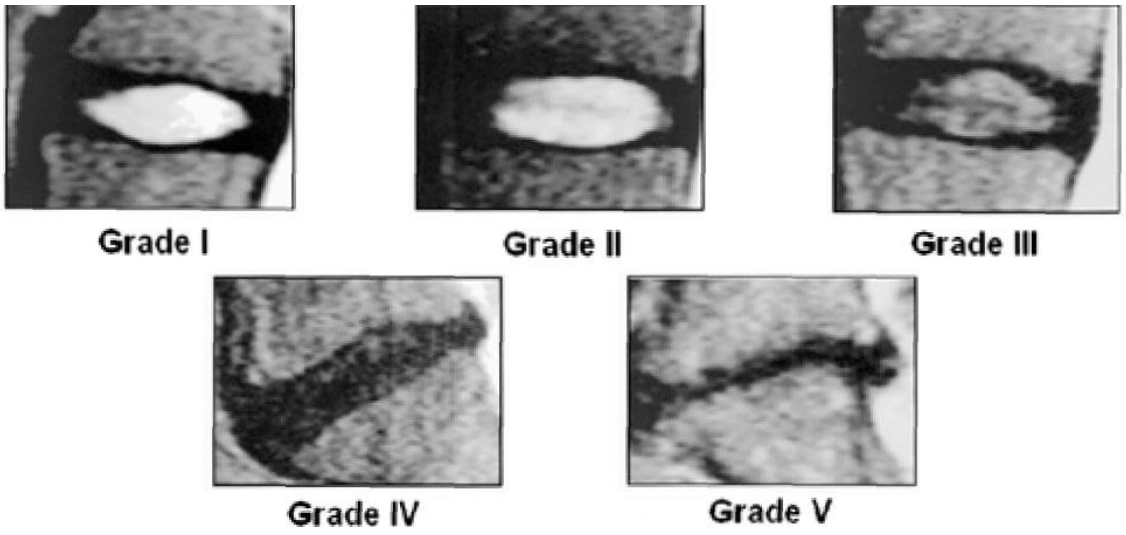

Figure 2 Pfirrmann grading

Grade 1: homogenous structure, white hyperintense signal, clear distinction between NP and AF, normal disc height

Grade 2: inhomogeneous structure, white hyperintense signal, clear distinction between NP and AF, normal disc height

Grade 3: inhomogeneous structure, gray intermediate signal, unclear distinction between NP and AF, normal disc height

Grade 4: inhomogeneous structure, dark gray hypointense signal, lost distinction between NP and AF, normal or moderately decreased disc height

Grade 5: inhomogeneous structure, black hypointense signal, lost distinction between NP and AF, disc space is collapsed

(Pfirrmann et al- 2001)

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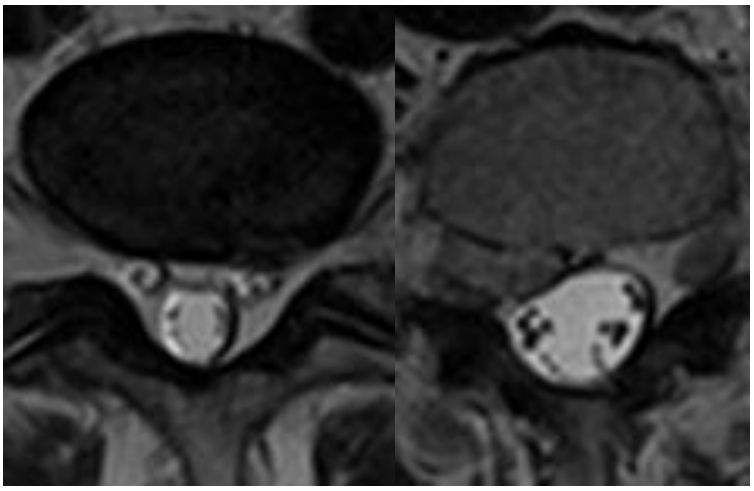

Figure 3 Intervertebral disc protrusion on axial T2 images

IVD bulging (left) and herniation (right) (Li et al. 2015)

2.1.5. Heritability of intervertebral disc degeneration

Investigating and evaluating the IDD heritability is problematic because there are several fundamental obstacles, that need to be addressed. Firstly, there is no standard definition of IDD because the phenomenon is not entirely understood. Conceptually, disc degeneration is physiological lifelong process with synchronized remodelling of discs and adjacent vertebrae which includes response to changing physical load and occasional injuries [43]. Surgically IDD is defined largely by the method of evaluation [43]. The currently preferred method for IDD evaluation is through MRI because it allows to simultaneously observe several phenotypes of degeneration such as disc narrowing, bulging or signal intensity loss. Adams and his colleagues suggested the following definition for disc degeneration: “the process of disc degeneration is an aberrant, cell-mediated response to progressive structural failure. A degenerated disc is one with structural failure combined with accelerated or advanced signs of aging. Early degenerative changes should refer to accelerated age-related changes in a structurally intact disc. Degenerative disc disease should be applied to a degenerate disc that is also painful”[44].

The exact pathomechanism of IDD is still unclear. Age, environmental factors (e.g.: heavy physical loading, smoking, vibration, etc.) have been reported to be risk factors [45][46]. However numerous studies from the early 2000s suggest that the genetic factors

(heredity) play a dominant role in IDD [47]. Adams and his study group defined the underlying cause of IDD as tissue weakening occurring primarily from aging, nutritional compromise, physical load and genetic inheritance [44]. Battié et al. concluded in a review that the genetically determined natural progression of degeneration can be modified to some extent by environmental and behavioural factors [48]. The first systematic analysis of familial aggregation of IDD were carried out on monozygotic twins in 1995 by Battié et al. and their results suggested a substantial genetic influence on IDD [49]. Sambrook conducted a classic twin study to distinguish the hereditary effects from the cultural influences and found that the heritability estimates were 74% for lumbar spine and 73% for cervical spine [50][49] (Figure 4). In more recent twin studies the heritability estimates for back pain were 32-44%.[51][52]. The risk for developing disc herniation before 21 is four to five times greater with positive family history compared to those without [53]. With the technical advancement in the field of genetic sciences studying large variety of genes and its polymorphisms become more and more frequent in the early 2000s. One way to easily examine hundreds of gene polymorphism is the so-called genome wide association study (GWAS). During GWAS a large pool of DNA is created from all the study population then a genotyping is made with previously selected gene polymorphism markers. This way we can get allelic frequency data from thousands of SNP simultaneously.

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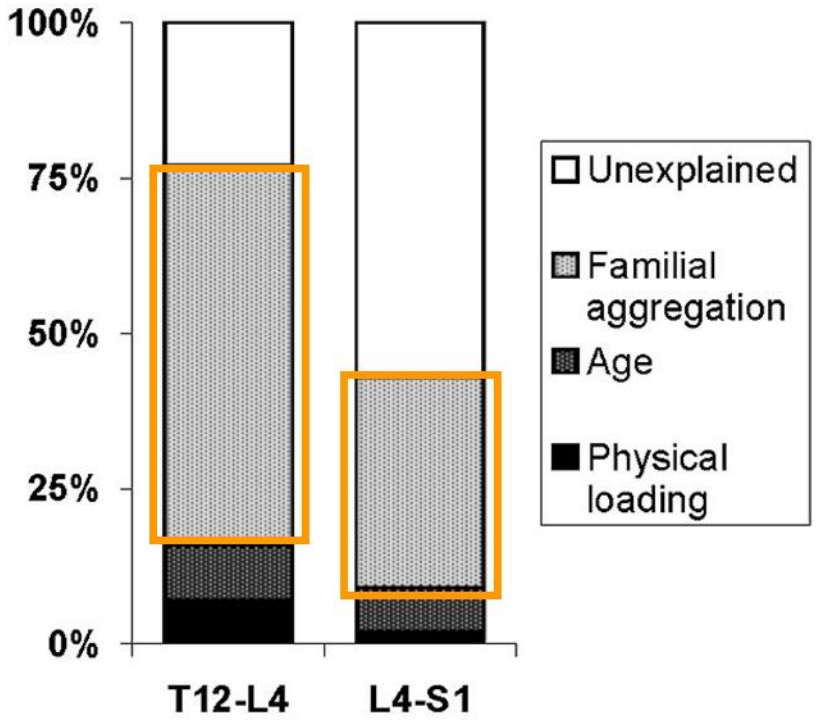

Figure 4 Genetic influence on LDD (Battié 1995)

2.2. Candidate genes and single nucleotide polymorphisms in lumbar disc degeneration

As previously mentioned twin studies have revealed a significant genetic influence in the development of LDD estimated around 40-70% [42] [43]. Over the last decades numerous genes and its SNPs were identified as risk factors in LDD. Vitamin D receptor gene (VDR) polymorphisms was amongst the most studied genes. Its effect were shown in several diseases such as type 1 and 2 diabetes [54] [55], nephrolithiasis [56], prostate cancer [57], breast cancer [58], osteoarthritis [59] and also with degenerative spinal diseases [60]. Interleukin genes and its polymorphisms were also intensively studied in different pathologies. A recent meta-analysis found strong association between IL6 gene SNP and susceptibility to LDD [61]. However, for all reported associations there are some publications that states the opposite, which makes the results questionable. Rajasekaran and his colleagues published a well-rounded meta-analysis which concluded that all GWAS results are incoherent due to the lack of uniform definition of LDD and small population size [60].

2.3. Treatment of lumbar disc degeneration

The clinical manifestation of LDD is versatile it ranges from mild loss of sensory functions to serious vegetative and motoric function loss, but amongst them one of the most significant symptoms in respect of the patient's well-being is pain. Back pain especially chronic pain can be associated with work incapacity, functional disability and affect the everyday quality of life [62]. The treatment of LDD depends on the seriousness of the symptoms, the patient's habit, and pain tolerance and on the regional guidelines of spinal specialists. Pain caused by LDD can be treated conservatively and surgically. Conservative treatments include non-pharmacological treatments such as non-specific exercises (i.e.: yoga, pilates, tai chi, motor control exercise, etc.), or structured exercises (i.e: spinal manipulation, physiotherapy, etc.), education about the causes and self-management of pain [62]. Newer guidelines do not recommend manual traction, electrotherapy, corsets, or foot orthotics neither for acute nor for chronic back pain [62]. Other conservative treatment includes pharmacological treatment such with non-steroid anti-inflammatory drugs (NSAID) or weak opioids. Opioids and duloxetine (serotonin and norepinephrine reuptake inhibitor) are considered second-line medications for LBP if NSAIDs are contraindicated. Around 5% of the patients are not responding to conservative treatment [63]. Surgery shouldn't be used in non-specific LBP [62]. Absolute indication for spinal surgeries are rare, it includes loss of neurologic functions (sensory, motoric and/or vegetative) [63]. There are a lot of surgical techniques for different spinal pathologies and there is no "good for all" gold standard method [64].

2.4. Failed back surgery syndrome

Around 5% of the patients don't respond for conservative treatment, in this population spine surgery can bring some pain relief [63]. With the aging of population the incidence of lumbar surgery for low back pain increases [65]. "Failed back surgery syndrome (FBSS) is defined by the International Association for the Study of Pain as lumbar spinal pain of unknown origin either persisting despite surgical intervention or appearing after surgical intervention for spinal pain originally in the same topographical

location" [65]. The exact estimation of FBSS is difficult due to its heterogenous etiology and its broad definition [66]. The complexity of the surgery increases the incidence of FBSS, failure rates ranges from 30% to 46% for spinal fusion and 19% to 25% for discectomy [67][68]. The pathophysiology of FBSS is complex and hard to assess and is attributable to several factors. Common pathologies include painful disc degeneration, lateral stenosis of the foramina, disc herniation, neuropathic pain and pseudoarthrosis [65][69]. Psychiatric conditions such as depression and anxiety have high comorbidity with FBSS [70]. Therefore, the treatment/management of FBSS should be multi-disciplinary which assesses the biological and the psychological alterations. Conservative treatment should always come first before surgery [71]. Physical therapy, pharmacological agents (classic NSAIDs and off label use of anticonvulsants), neuromodulatory therapy (e.g.: spinal cord stimulation) can be used before surgical intervention [65]. Cognitive behaviour therapy or other psychiatric therapy may enhance the efficacy of the treatment for the patient's pain and can lead to better outcomes [72]. Surgery may be a solution only if there is a clearly identified pain source that can be relieved with surgery. Reoperation generally correlates with worse outcome than conservative treatment [73].

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3. OBJECTIVES

It is crucial to understand the underlying pathophysiological process of a disease to treat it successfully. Intervertebral disc degeneration is a complex entity which starts to develop in early years. There is a possibility that specific anthropometric attributes that lead to degeneration are influenced by gene variations, so our first objective was to find any correlation between quantitative traits and genetic variations.

Numerous studies investigated the possible correlation of the different spinal pathologies and gene variations, however they yielded mostly inconsistent results. These inconsistencies could be the result of small study populations, heterogenous inclusion criteria and the lack of clear definition of degeneration. To rule out the inconsistencies we intended to use endophenotype for each degenerative trait instead of the umbrella term degeneration. Endophenotype is a quantitative biological trait that is reliable in reflecting the function of a discrete biological system and is reasonably heritable, and as such is more closely related to the root cause of the disease than the broad clinical phenotype [74]. Our second objective was to investigate one of the most promising candidate gene VDR and other genes in association with lumbar degenerative pathologies and structural quantitative traits.

On one hand it is crucial to understand the pathophysiology of degeneration to treat low back pain effectively on the other hand it is also very important to identify comorbidities and genetic susceptibility which affect the long-term outcome of treatment. The third objective was to identify specific gene variations which can alter the effect of the long-term outcome of the surgical interventions in spinal pathologies.

Based on the above mentioned we wanted to answer the following questions:

1. **Is there an association between certain quantitative traits and genetic variations?**
2. **Do VDR gene variations influence the development of IDD?**
3. **Do gene variations alter the preoperative physical status of a patient?**

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4. **Can we identify gene variations that alter the long-term outcome of spinal surgeries?**
5. **Can we identify genes that affect the need of subsequent surgery?**

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4. METHODS

4.1. Genetic associations with the development of spinal column

4.1.1. *Genotyping*

An international database (GENODISC) containing radiological, genetical and clinical data of more than 3000 patient from three European country (UK, Italy, Hungary) was used as a base cohort for our investigations. All the database's subject was hospitalized and surgically treated for lumbar degenerative disease. DNA was extracted from venous blood or saliva samples using commercial kits. All candidate gene variations were genotyped at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, USA). Allelic and genotype distributions, Hardy–Weinberg equilibrium, minor allele frequency (MAF) were analysed using the "SNPassoc" and "haplo.stats" R software packages [75].

4.1.2. *Study population, genotyping and statistical analysis*

For the study we used the international GENODISC database which consisted of 2635 patients. Patients with incomplete genetic data (n=754) and incomplete radiologic data (n=266) were ruled out from the study, a total of 1615 Caucasian patient were involved in the study cohort. Mean age was 48.1 years with a range from 18-89years. The male/female ratio was 46.7% and 53.3%. Mean height 170.5 cm (SD 12) and mean weight was 79.7 kg (SD 16.5). In accordance with previous literature Disruptor of telomeric silencing 1-like (DOT1L) gene variant rs12982744 were examined with quantitative attributes such diameter of the bony canal and height [76][77]. The ap diameter of the bony spinal canal were determined previously in the GENODISC project individually at every lumbar level (from L1 to L5) (Figure 5). According to literature we analysed the mean ap diameter of L1-4 and the diameter of L5 [78]. Genetic associations were determined and analysed using the "SNPassoc" and "haplo.stats" R software packages [75]. P values less than 0.05 was considered significant.

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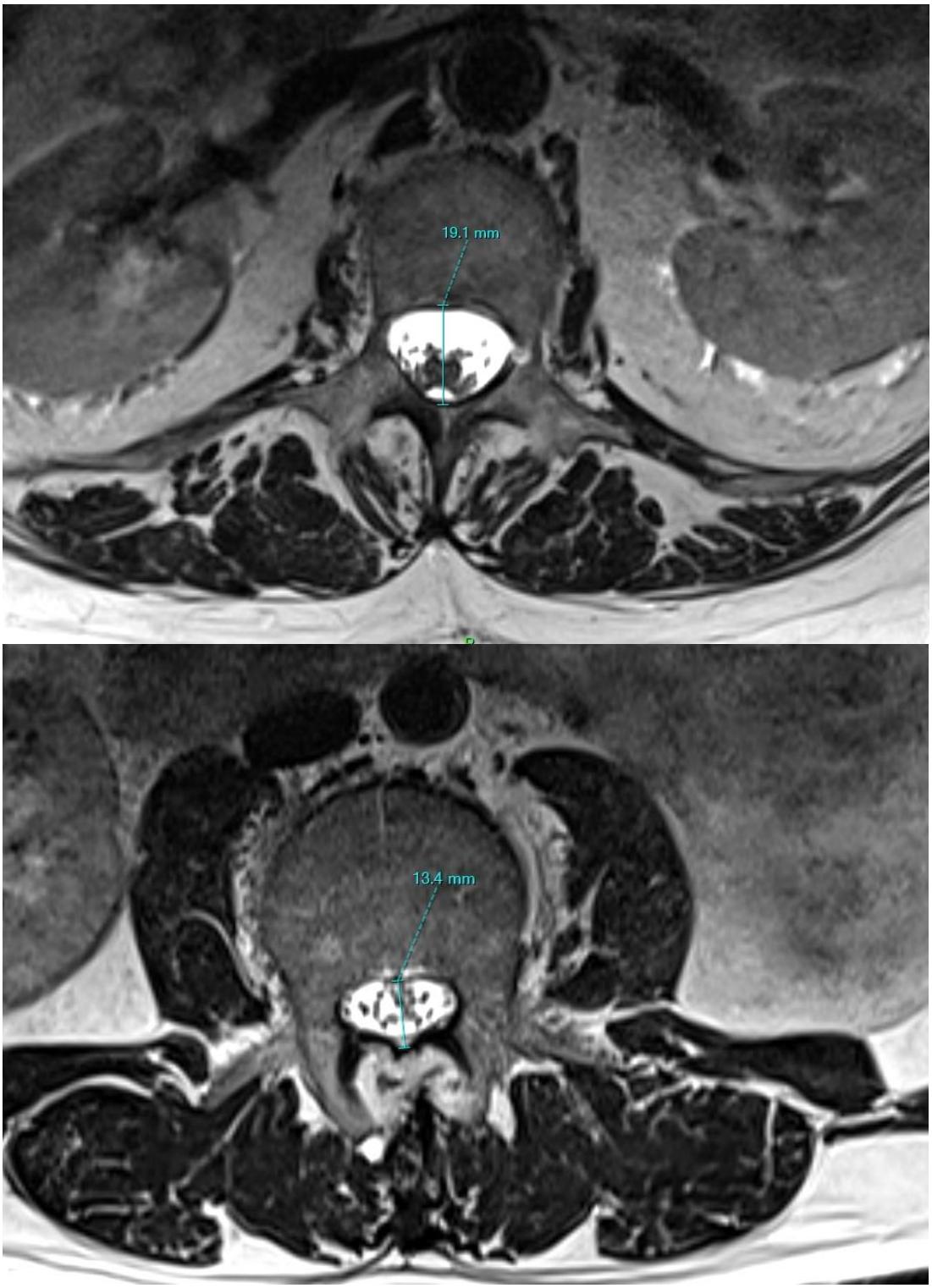

Figure 5 Measurement of AP diameter on axial T2 images

Measurement of diameter is seen at the upper endplate of L.1 level, above there is a normal bony canal below there is a congenitally narrow canal.

4.2. VDR gene variations and lumbar degenerative diseases

4.2.1. *Genotyping*

DNA was extracted from venous blood or saliva samples using commercial kits. Seven candidate VDR SNPs were genotyped at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, USA). Allelic and genotype distributions, Hardy-Weinberg equilibrium, minor allele frequency (MAF) as well as associations between genetic variants and degenerative phenotypes were determined and analysed using the 'SNPassoc' and 'haplo.stats' R software package [75]. Individual genotype-phenotype associations and gene-gene interactions were studied in generalized linear models while haplotype-phenotype association was analysed applying haplo.score tests. In haplo.score analysis, a global test of association as well as individual haplotype-specific tests were carried out using a score function. Significant covariates (age, gender, weight and height, and smoking status) were determined for each phenotype and a p-value less than 0.05 was considered significant. The genetic association analysis was also approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary (431/PI/2007). All the investigated VDR SNPs were in Hardy–Weinberg equilibrium (HWE) (Table 1). At the 3′-end of the gene a haploblock constructed by three candidate SNPs, BsmI, ApaI and TaqI (rs1544410, rs7975232, rs731236), was identified, and another haploblock constructed by two SNPs, Cdx2 and A1012G (rs11568820, rs4516035), was found at the 5′-end of the gene (Figure 6).

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Table 1 Studied VDR SNPs and descriptive statistics of genotyping (Biczo et al. 2019)

rs number	Traditional name	Alleles	Region	Success rate (%)	MAF	HWp
rs11568820	Cdx2	G/A	Promoter	95.7	0.190	0.659
rs4516035	A1012G	T/C	Promoter	99.3	0.415	0.661
rs2228570	FokI	C/T	Exon 2	98.2	0.405	0.867
rs3782905	DdeI	C/G	Intron 2	98.9	0.295	0.522
rs1544410	BsmI	G/A	Intron 8	99.3	0.397	0.505
rs7975232	ApaI	A/C	Exon 9	99.5	0.480	0.456
rs731236	TaqI	T/C	Exon 9	99.6	0.388	0.434

MAF: minor allele frequency, HWp: p-value of Hardy-Weinberg equilibrium

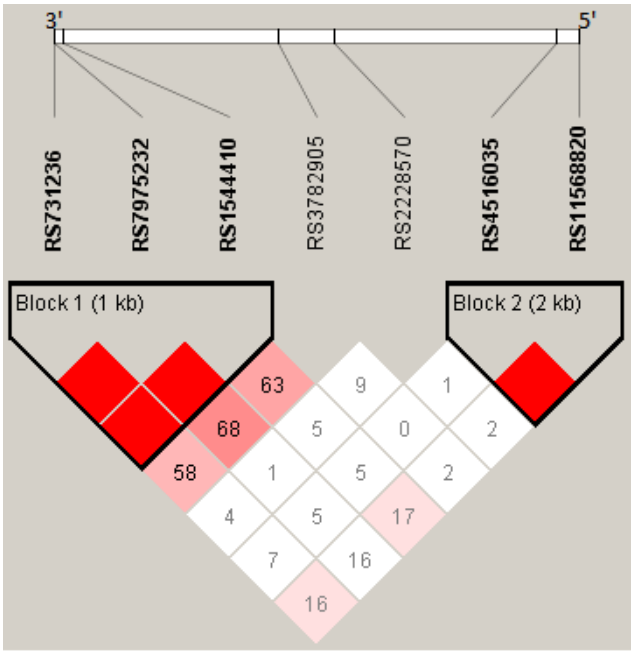

Figure 5 Linkage disequilibrium (LD) map of the seven candidate SNPs

Squares are colored darker if the |D'| value is high, that is, LD is strong. Empty dark squares mean |D'|=1, that is, complete LD between two single nucleotide polymorphisms.

4.2.2. Study population and genotyping

For the study we used the international GENODISC database which consisted of 2635 patients. 455 patients with incomplete radiologic and 754 patients with incomplete genetic data were ruled out from the analysis. 1426 Caucasian subjects were involved from the GENODISC database into the analyses. Mean age was 49.2 years with a range from 18 to 87 years. The male/female ratio was 46% and 54%. Average height was 170.6 cm (SD 10.5) and mean weight was 79.7 kg (SD 16.6) in the cohort. In the study population, there was no data available about the smoking habits of 128 subjects, 593 subjects were never-smoker, and 705 patients were ever-smoker.

4.2.3. Radiographic features

A set of selected degenerative endophenotypes (Pfirrmann grade, Modic change, disc prolapse, endplate defect) were assessed at five lumbar segments. In the subsequent analysis, we determined the genetic association with the phenotypes at any lumbar levels, at L4/5 and L5/S1 levels separately. Pfirrmann grading system was used to determine the level of overall disc degeneration. Mean Pfirrmann grade and dichotomous derivate were analysed statistically. In the latter case, as suggested by others [79][80], discs were scored as "normal" (Pfirrmann 1–2) and "pathologic" (Pfirrmann 3–5) (Figure 7). Degenerative endplate changes, such as Modic I and Modic II changes, were grouped together into the dichotomous Modic change phenotype. Disc prolapse was defined as the presence of disc bulging or herniation at the given spinal segment. Endplate defect was determined as bony defect at either the upper or the lower endplate (e.g.: Schmorl's node). The distribution of the studied phenotypes in the final study population is shown in Table 2.

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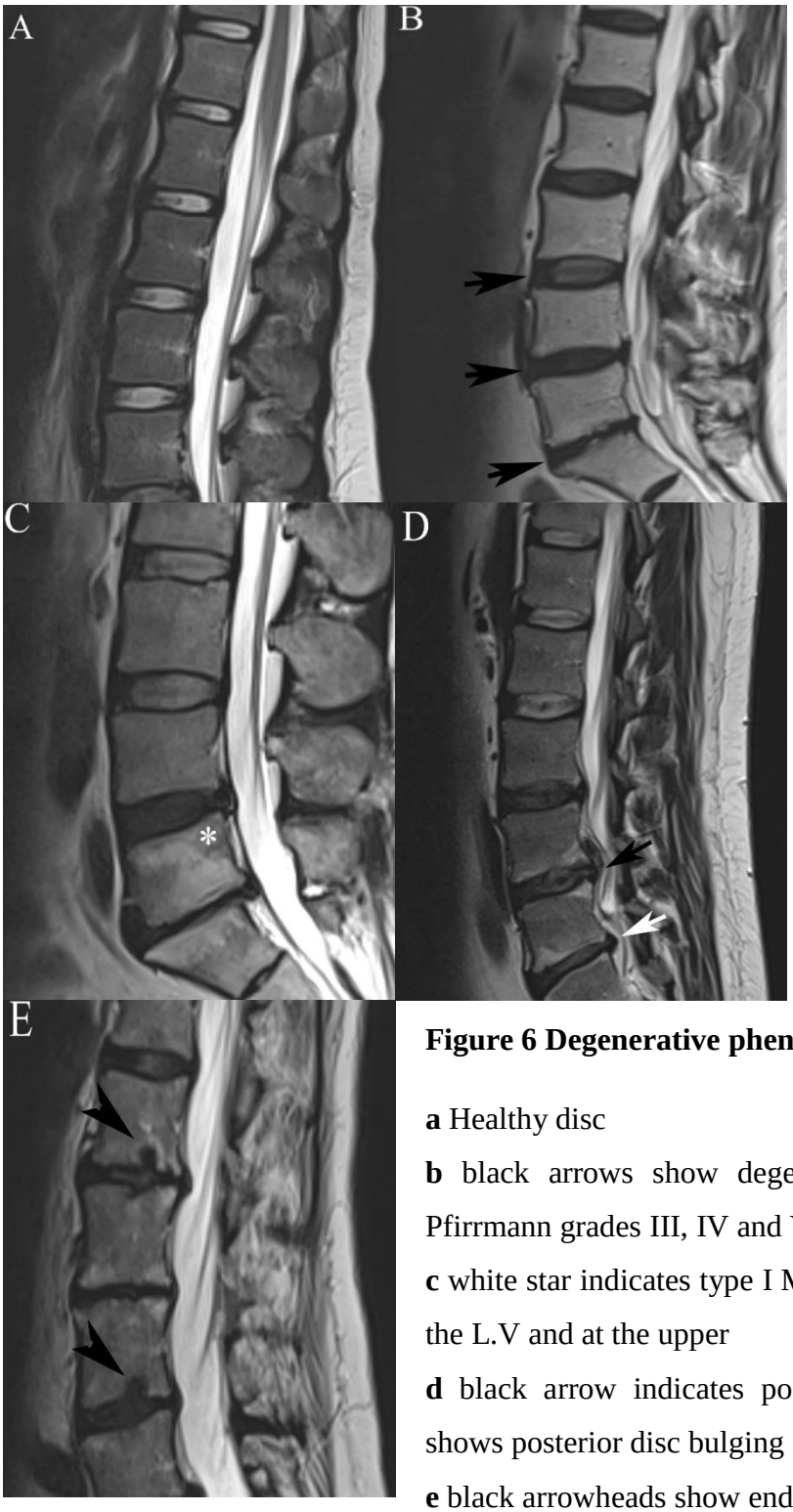

Figure 6 Degenerative phenotypes on sagittal T2 images

a Healthy disc

b black arrows show degenerated discs, from top to bottom Pfirrmann grades III, IV and V

c white star indicates type I Modic change at the lower endplate of the L.V and at the upper

d black arrow indicates posterior disc herniation; white arrow shows posterior disc bulging

e black arrowheads show endplate defects

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Table 2 Prevalence and overlap of degenerative phenotypes in the study population (Biczo et al. 2019)

	Any	L4/5	L5/S1	L4/S1
Modic	873	447	529	782
Pathologic Pfirrmann	1402	1185	1186	1385
Endplate	460	140	40	168
Disc prolapse	1364	1038	1013	1335

4.3. Genetic associations with clinical outcome of surgically treated degenerative disc diseases

4.3.1. *Study population*

Data were collected prospectively from adults (above the age of 18) who underwent routine, elective surgery for lumbar disc degeneration at one or two levels at a tertiary spine center. Prospective clinical data were linked with the subjects' genetic data derived from the GENODISC database. Patients with minimum 2-year follow-up data were included into the final study cohort to explore the long-term outcome of the surgical procedures. Patients reoperated within 2 years due to a surgical site infection, proximal junctional kyphosis (PJK) or adjacent segment degeneration (ASD) as well as subjects undergoing either acute intervention because of neurological emergency or tumour surgery was excluded from the study. Surgeries were performed by board-certified orthopaedic surgeons or neurosurgeons specified in spinal surgery. Applied procedures included microdiscectomy, decompression and instrumented fusion (transforaminal lumbar interbody fusion or posterior fusion). All procedures were carried out using the standard median-sagittal posterior approach. All subjects signed a written consent form describing the scientific purpose of the systematic

collection of their clinical and genetic data. The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council Hungary (431/PI/2007).

For the study we used the Hungarian cohort from the international GENODISC database which consisted of 1181 patients. Out of the 1181 Hungarian patients only 668 patients had at least 2-year follow-up data, amongst these patients 237 of them had insufficient genetic data and clinical data. A total of 431 subjects (all Caucasians) met the study inclusion criteria. Mean age were 52.7 (SD: 13.9y) years (from 20 to 88 years) and male/female ratio was 0.6 (male: 166, female: 265). As the index surgery 171 patients had discectomy, 22 patients had decompression, 142 patients had one level fusion and 96 patients had 2-level fusion. In the final study cohort, 44 patients required a subsequent lumbar surgery at the index level during the follow-up. Eight patients had re-discectomy or decompression, 35 required fusion and in 1 case the implants had to be removed.

4.3.2. *Clinical data*

Patients completed standard and validated PROMs to assess their clinical status before the surgery and during the follow-up period. Pain was evaluated by the 10 cm long Visual Analogue Scale. Lumbar spine related function was measured with Oswestry Disability Index (ODI). Psychologic distress was measured by evaluating the level of depression and somatisation and was assessed with the Zung Depression Scale (ZDS) and the Modified Somatic Perception Questionnaire (MSPQ), respectively. Patients were asked to rate the overall outcome of the surgery using a five-category question; ‘‘helped a lot’’, ‘‘helped’’, ‘helped only little’’, ‘‘didn’t help’’, ‘‘made things worse’’. To measure global treatment outcome (GTO) a dichotomous variable was generated based on these given answers. Good outcome was defined if the patient responded by ‘helped a lot, ‘helped’ and poor in case the patient replied by ‘only little’, ‘didn’t help’, ‘made things worse’ [81], [82]. Surgical outcome was considered “good” if no re-operation was performed at the index level within 2 years and “poor” if a subsequent surgery was needed within 2 years.

4.3.3. Genotyping and statistical analysis

DNA was extracted from venous blood or saliva samples. Five SNPs in IL1B and four SNPs in IL6 genes were selected for genotyping based on previous literature data [83]–[88]. Genotyping was performed at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, USA).

Allelic and genotype distributions, Hardy-Weinberg equilibrium, minor allele frequency (MAF) as well as associations between genetic variants and outcomes were determined and analysed using the 'SNPassoc' and 'haplo.stats' R software packages [75]. Genetic associations with preoperative and postoperative pain, disability, and psychological distress as well as global treatment and surgical outcome were investigated. Individual genotype-phenotype associations were studied in generalized linear models (GLM). Genetic subgroups with less than 4 (1%) subjects were excluded from subsequent statistical analyses. Haplotype-phenotype association was analysed applying haplo.score tests and GLM models. In haplo.score analysis, a global test of association as well as individual haplotype-specific tests is carried out using a score function. Significant covariates (age, gender, weight, and height, preop ZDS and preop MSPQ score, type of surgery) were determined and calculated into the models for each outcome. P-values less than 0.05 were considered significant. The genotyping success rate was more than 97% in all cases (Table 3). All studied SNPs were in Hardy-Weinberg equilibrium.

Two haploblock from IL1B gene were identified consisting of 2-2 SNPs (‘rs1143634-rs1143633’and ‘rs1143627-rs16944’) and no haploblock was identified on the IL6 gene as seen on Figure 8.

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Table 3 Descriptive statistics of the genotyped SNPs

Gene	rs number	Position	Alleles	Major allele frequency %	HWE	missing (%)
IL1B	rs3917365	3' UTR	C/T	91.5	0.344	0.2
IL1B	rs1143634	Exon 5	C/T	73.7	1.000	0.5
IL1B	rs1143633	Intron 4	G/A	65.1	0.521	1.2
IL1B	rs1143627	Promoter	T/C	65.6	0.914	0.2
IL1B	rs16944	Promoter	G/A	65.8	1.000	2.1
IL6	rs2069852	3' UTR	G/A	95.1	0.613	0
IL6	rs2069861	3' UTR	C/T	93.6	0.688	0.5
IL6	rs2069835	Intron	T/C	92.7	0.264	1.4
IL6	rs1800796	Promoter	G/C	93.4	1.000	1.2

HWE: Hardy-Weinberg equilibrium

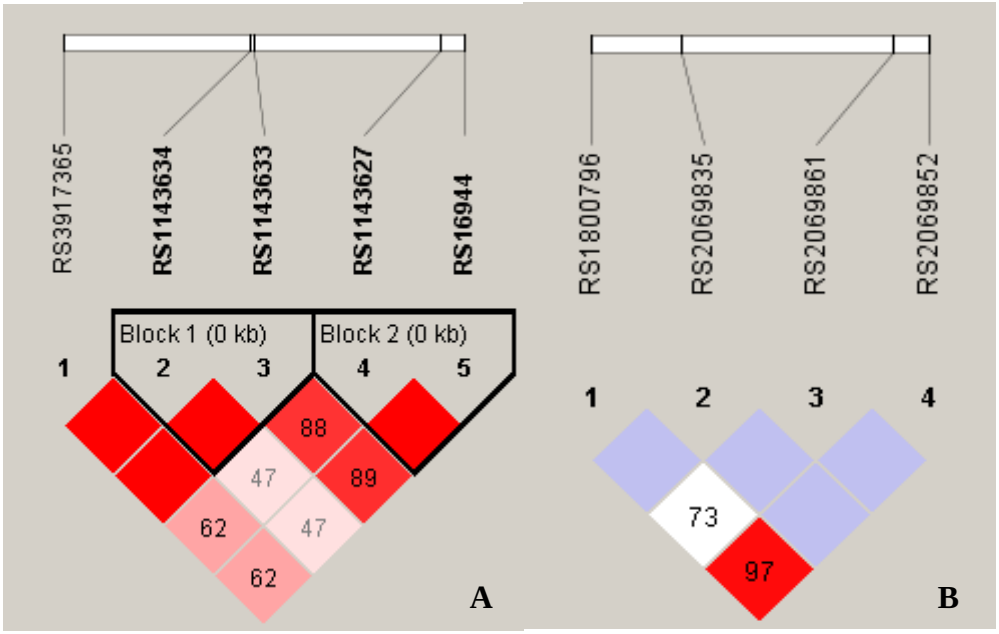

Figure 8 Linkage disequilibrium (LD) maps of IL1B (A) and IL6 (B) SNPs

Squares are colored darker if the |D'| value is high, that is, LD is strong. Empty dark squares mean |D'|=1, that is, complete LD between two single nucleotide polymorphisms.

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5. RESULTS

5.1. Genetic associations with the development of spinal column

5.1.1. *Genetic association of rs12982744 with ap diameter of bony spinal canal*

The mean ap diameter of the L1-4 levels was 1.68 cm (SD 0.24), while the mean diameter of L5 was 1.76 (SD 0.34). DOT1L gene variant rs12982744 was found to be associated with the average L1-4 diameter of the bony canal. The more 'G' allele a patient had the wider the spinal canal was (mean±SD 1.75±0.44, 1.79±0.41, 1.83±0.39, p=0.0005 in log-additive model, for 'C/C', 'C/G' and 'G/G' genotype respectively) (Figure 9). No other association was found.

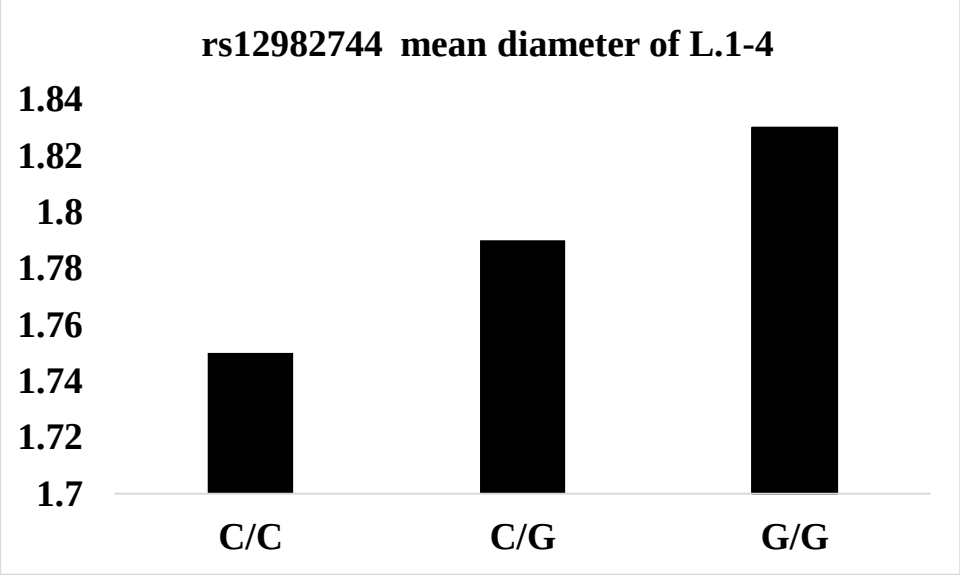

Figure 9 Mean AP diameter of L.1-4 levels in asociation with different rs12982744 genotypes

5.2. VDR gene variations and lumbar degenerative disc disease

5.2.1. *Pfirrmann grade*

Pfirrmann grade was associated with DdeI (rs3782905), FokI (rs2228570), and ApaI (rs7975232) polymorphisms. "G/G" genotype of DdeI was significantly associated with the presence of disc degeneration at level L4/5 (ref=C/C; C/G: OR=0.75, 95%CI=0.55-1.03; G/G: OR=2.01, 95%CI=1.00-4.05; p=0.0064 in codominant model) (Figure 7 10). At L5/S1 level, "C/C" genotype of ApaI was significantly related to the risk for severe degeneration (ref=A/A-C/A; C/C, OR=1.46, 95%CI=1.01-2.13, p=0.0408).

5.2.2. *Disc prolapse*

ApaI was associated with disc prolapse. Homozygous subjects had a higher frequency of disc prolapse at any spinal level (p=0.0458). At L5/S1 region, "C/C" carriers showed the highest risk for disc prolapse (ref=A/A-C/A; C/C: OR= 1.39 95%CI= 1.03-1.88; p= 0.0271, in recessive model) (Figure 811).

5.2.3. *Modic change*

"A/A" genotype of BsmI (rs1544410) was associated with a lower frequency of Modic change at any spinal level (ref=G/G-G/A; A/A, OR=0.67, 95%CI=0.49-0.91, p=0.01, recessive model) and at L4-5 (ref=G/G-G/A; A/A, OR=0.65, 95%CI=0.47-0.91, p=0.0103 in recessive model) (Figure 92). C/C genotype of TaqI (rs731236) polymorphism had also a protective effect against Modic change at any level (ref=T/T-C/T; C/C, OR=0.62, 95%CI=0.45-0.86, p=0.0032) and at L4/5 segment (ref=T/T-C/T; C/C, OR=0.61, 95%CI=0.43-0.86, p=0.0034). FokI (rs2228570) polymorphism was found to have an association with Modic change in codominant genetic model at level L4/5 (ref=C/C; T/C, OR=1.27, 95%CI=0.98- 1.64, T/T, OR=0.83, 95%CI=0.58- 1.20, p=0.0302).

5.2.4. *Endplate defect*

"G" allele of DdeI (rs3782905) polymorphism was associated with endplate defect at any lumbar level (ref=C/C; C/G–G/G, OR 1.38, 95% CI 1.09–1.74, p=0.0064, in

dominant model) (Figure 103). “A/A” genotype of Cdx2 (rs11568820) variant was related to the higher risk of having an endplate defect at L4/5 level (ref=G/G–A/G; A/A, OR 2.32, 95% CI 1.08–4.9, p=0.0444, in the recessive model) (Figure 114).

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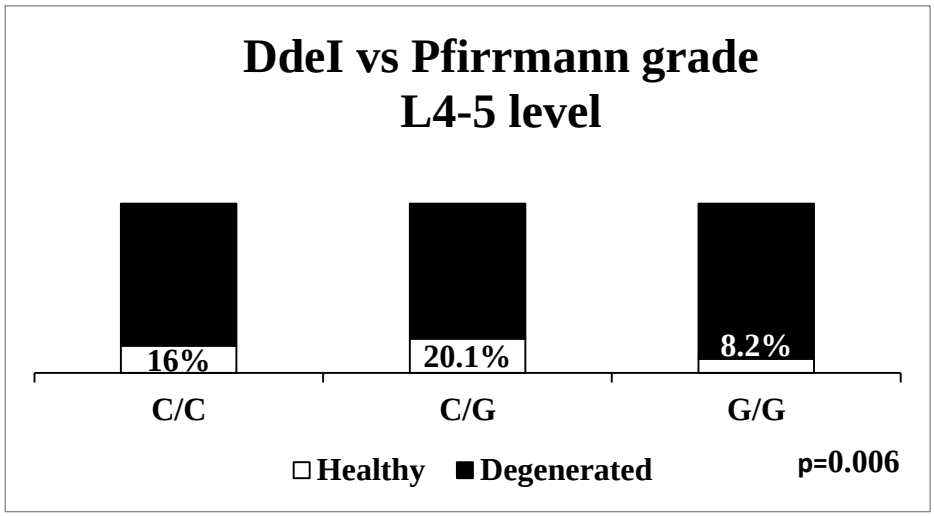

Figure 7 Association of Ddel with Pfirrmann grade distribution of healthy and pathologic endophenotype is represented by genotypes

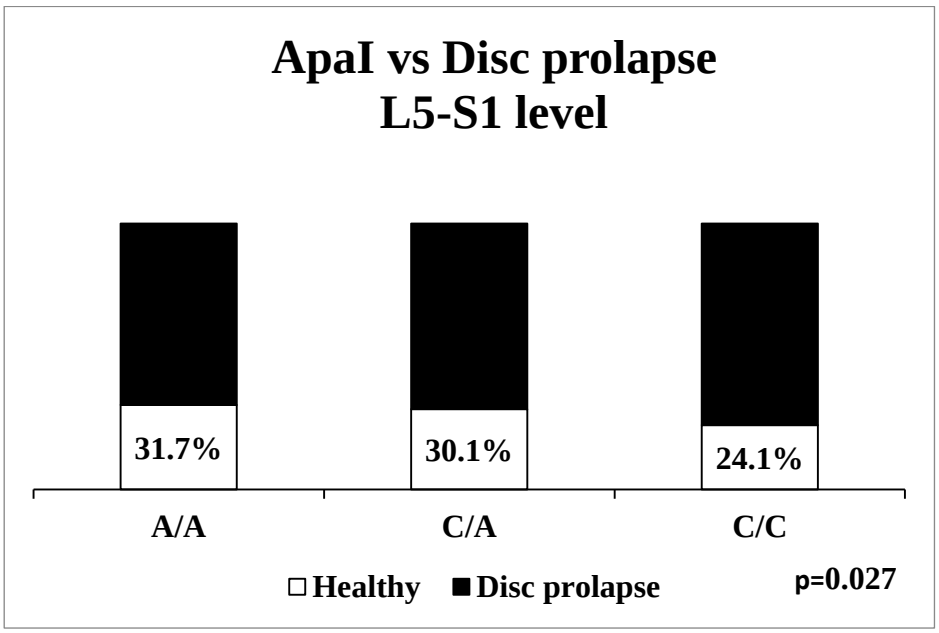

Figure 8 Association of ApaI with disc prolapse distribution of healthy and pathologic endophenotype is represented by genotypes

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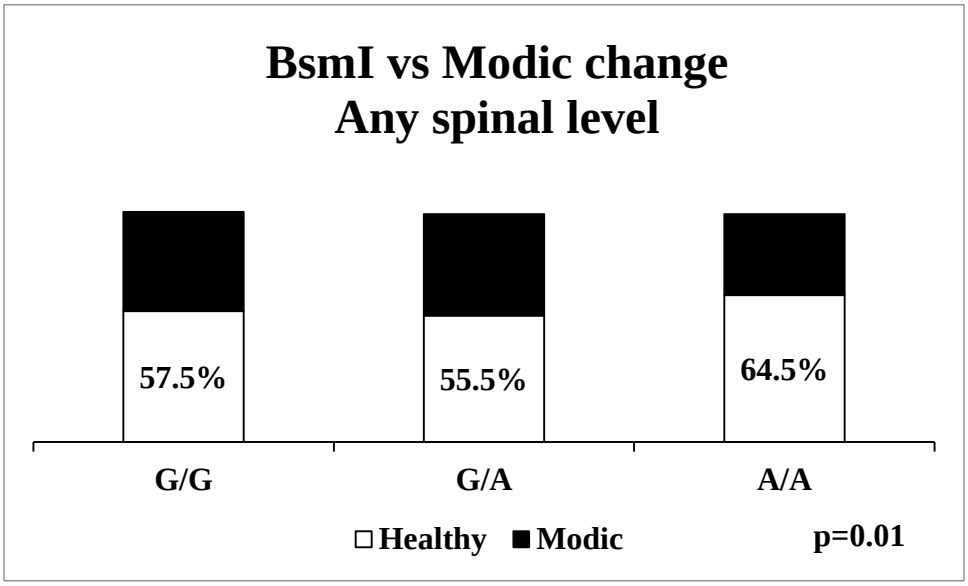

Figure 9 Association of BsmI with Modic change distribution of healthy and pathologic endophenotype is represented by genotypes

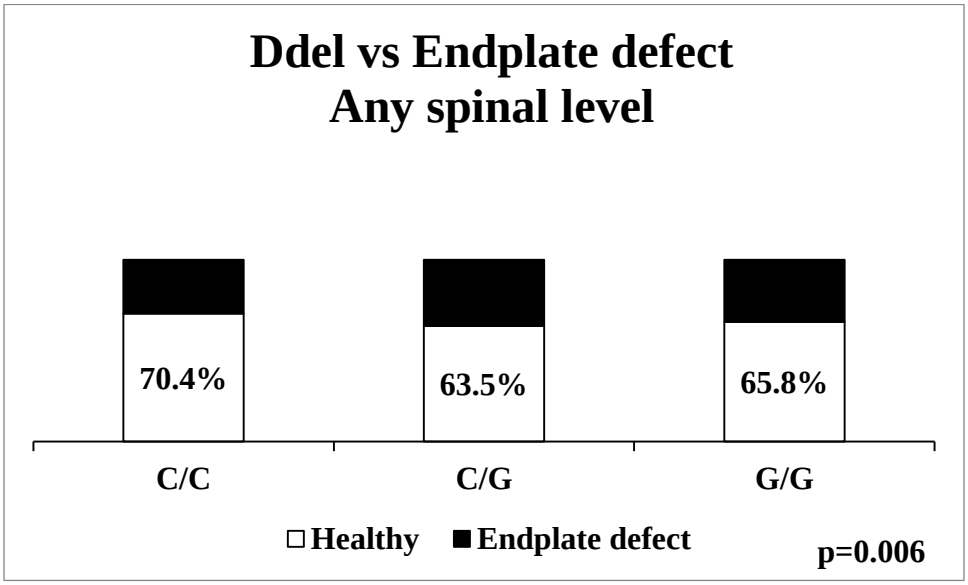

Figure 10 Association of Ddel with endplate defect distribution of healthy and pathologic endophenotype is represented by genotypes

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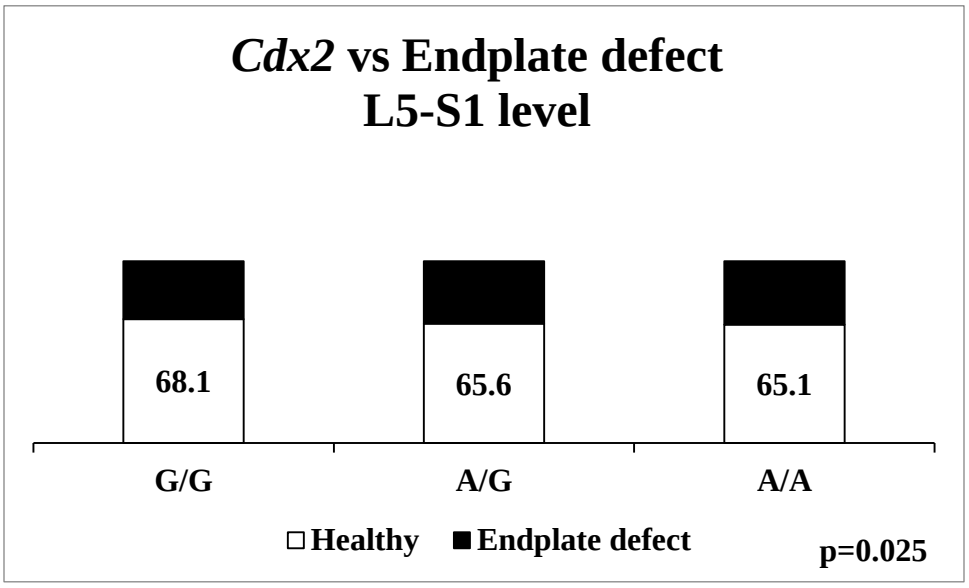

Figure 11 Association of Cdx2 with endplate defect distribution of healthy and pathologic endophenotype is represented by genotypes

5.2.5. Haplotype analyses

Three haplotypes with more than 1% frequency were identified inside the VDR haploblock located at the 3'-end of the gene (BsmI-ApaI-TaqI). The haploblock was significantly associated with the Modic change at L4/5 level (p_{global}=0.0185 in recessive model) where the second most common, "AAC" haplotype was associated with lower risk for Modic change (p=0.0045). Another haploblock with three different haplotypes was identified at the 5'-end (Cdx2-A1012G). It was related to the endplate defect at L4/5 level (p_{blobal}=0.048 in additive model), where the rarest "AT" haplotype was associated with the highest risk for endplate defect (p=0.0055) (Table 4).

Table 4 Association of haploblocks with endophenotypes

Haplotype	Frequency (%)	Hap-Score	p value
(A)			
AAC	38.6%	-2.84	0.0045
GAT	12.6%	-1.21	0.2260
GCT	47.4%	0.42	0.6775
(B)			
GC	41.6%	-1.43	0.1525
GT	39.4%	-0.76	0.4448
AT	19.0%	2.78	0.0055

(A) Association of BsmI–ApaI–TaqI haploblock with Modic change (p_{global}=0.0185) and **(B)** association of Cdx2–A1012G haploblock with endplate defect (p_{global}=0.048)

5.2.6. Gene-gene interaction analysis

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Significant GxG interactions were found between Cdx2 and BsmI ($p_{interaction}$=0.0206) and between Cdx2 and TaqI ($p_{interaction}$=0.0062) on endplate defect at L4/5 level (Figure 15).

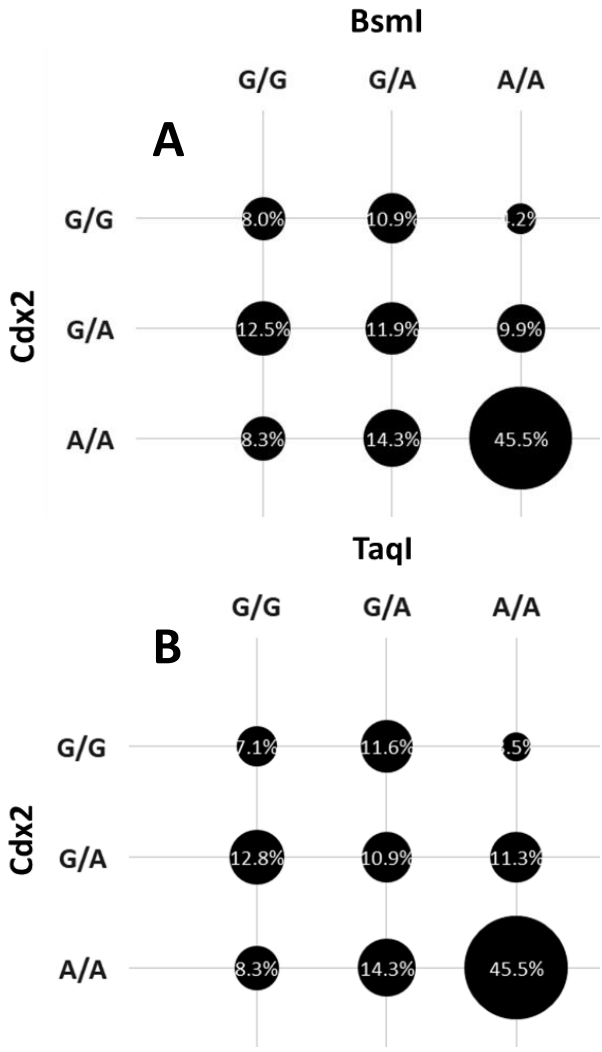

Figure 12 G x G interaction between Cdx2 and BsmI (A) and Cdx2 and TaqI (B) on endplate defect Bubbles represent the percentage of subjects with endplate defect at L4/5 in different genotype combinations, $\boldsymbol{p_{interaction}}$ values are 0.0206 and 0.0062 for Cdx2-BsmI and Cdx2-TaqI respectively

5.3. Association of IL1B and IL6 gene variants with the long-term outcome after lumbar degenerative spinal surgery

5.3.1. *Association of IL1B and IL6 gene variants with preoperative outcome*

In the overall population the mean±SD values of preoperative ODI score was 47.4±18.4 and the mean VAS score was 7.2±1.9, the mean ZDS was 39.6±8.1, MSPQ was 8.3±5.7. No individual SNP was associated with preoperative ODI and pain (Table 5), however both IL genes had SNPs related to the level of depression. 'T' allele of rs1143627 IL1B SNP was associated with higher level of depression (ZDS was 40.6±8.7, 39.2±7.3 and 38.3±8.0 in case of 'T/T', 'T/C' and 'C/C' genotypes, respectively, p-value=0.025 in log additive model). IL1B rs16944 'G' allele carriers also showed higher level of depression (ZDS was 40.6±8.8, 39.2±7.3 and 38.0±8.0 in case of 'G/G', 'A/G' and 'A/A' genotypes, respectively, p-value=0.025 in log additive model). rs1143634 IL1B was associated with ZDS in an overdominant model (p=0.025, "C/T" mean ZDS±SD was 40.8±8.4 and 39.0±7.8 in case of 'C/T' and 'C/C'+'T/T' genotype groups). The 'C' allele of IL6 SNP rs2069835 was linked to increased level of depression (mean ZDS±SD were 39.2±7.8, 42.2±9.2, and 45.3±10.1 in case of 'T/T', 'T/C' and 'C/C' genotypes, respectively, p=0.003 in log-additive model).

IL6 rs2069835 was associated with the level of preoperative somatization (Mean MSPQ±SD was 8.0±5.3 and 10.2±7.0 in case of 'T/T' and 'T/C'+'C/C' genotypes respectively, p=0.010, in dominant model) (Table 5 and Table 6).

IL1B haplotypes were not associated with preoperative ODI, depression, somatization, and pain (data not shown).

SNP	Genotype	Preop ZDS	*p*	Preop MSPQ	*p*	*Preop ODI*	*p*	*Preop pain*	*p*
	(N)	Mean±SD		Mean±SD		Mean+SD		Mean+SD	
IL1B_rs3917365	C/C (358)	39.5+8.2	*0.146*	8.3+5.8	*0.832*	47.5+18.7	*0.78*	7.2+2.0	*0.69*
	T/C (71)	39.6+7.1		8.2+4.9		47.1+16.7		7.2+1.9	
	T/T (1)	63		22		60		8	
IL1B_rs1143634	C/C (233)	39.0+7.8	***0.025*** [**]	8.2+5.7	*0.401*	47.1+17.9	*0.655*	7.3+1.9	*0.253*
	C/T (166)	40.7+8.4		8.3+5.3		47.7+19.0		7.1+2.0	
	T/T (31)	38.4+7.9		9.3+7.4		48.3+18.5		7.0+2.1	
IL1B_rs1143633	G/G (184)	39.4+8.1	*0.526*	8.6+5.6	*0.183*	47.1+17.9	*0.813*	7.1+2.0	*0.170*
	A/G (187)	39.9+7.8		7.9+5.4		47.4+19.2		7.3+1.8	
	A/A (41)	39.7+9.0		8.9+6.9		47.6+16.0		7.4+2.0	
IL1B_rs1143627	T/T (184)	40.6+8.7	***0.025*** [*f*]	8.0+5.5	*0.371*	48.8+18.2	*0.135*	7.1+2.1	*0.338*
	T/C (196)	39.2+7.3		8.5+5.8		46.0+18.8		7.3+1.8	
	C/C (50)	38.0+8.0		8.6+5.6		48.3+16.9		7.2+2.2	
IL1B_rs16944	G/G (182)	40.6+8.8	***0.025*** [*f*]	8.0+5.5	*0.424*	48.6+18.2	*0.207*	7.1+2.1	*0.253*
	A/G (191)	39.2+7.3		8.5+5.9		46.3+19.0		7.3+1.8	
	A/A (49)	38.0+8.0		8.5+5.6		48.5+17.0		7.3+2.2	

Table 5 Associations of IL1B variants with preoperative PROMs

[*]: significant in dominant model, [**]: significant in overdominant model, †: significant in codominant model

[‡]: significant in recessive model, [*f*]: significant in log-additive model

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SNP	Genotype	Preop ZDS	p	Preop MSPQ	p	Preop ODI	p	Preop pain	p
	(N)	Mean±SD		Mean±SD		Mean+SD		Mean+SD	
IL6_rs2069852	G/G (389)	39.7+8.0	0.629	8.4+5.8	0.192	47.2+18.3	0.375	7.2+2.0	0.623
	G/A (42)	39.1+8.7		7.0+4.4		59.8+18.5		7.1+2.0	
	A/A (0)	-		-		-		-	
IL6_rs2069861	C/C (376)	39.7+8.0	0.282	8.5+5.8	0.103	47.9+18.5	0.095	7.2+1.9	0.191
	T/C (51)	39.4+8.5		7.3+4.7		43.5+16.2		7.1+2.1	
	T/T (2)	33.5+0.7		n/a		26+25.5		5.4+4.2	
IL6_rs2069835	C/C (4)	45.3+10.1	**0.003**^{f}	10.0+10.7	**0.010***	39.5+31.8	0.393	8.1+1.3	0.385
	T/C (54)	42.2+9.2		10.2+6.9		48.5+18.4		7.2+1.6	
	T/T (367)	39.2+7.8		8.0+5.3		47.2+18.2		7.2+2.0	
IL6_rs1800796	G/G (371)	39.8+8.0	0.239	8.5+5.7	0.087	47.0+18.1	0.208	7.2+2.0	0.149
	G/C (54)	38.4+8.6		6.8+4.7		50.2+19.3		7.1+1.9	
	C/C (1)	40		14		40		4.4	

Table 6Associations o IL6 gene variants with preoperative PROMs

*: significant in dominant model, **: significant in overdominant model, †: significant in codominant model

‡: significant in recessive model, ^{f}: significant in log-additive model

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5.3.2. Associations of IL1B and IL6 gene variants with postoperative outcome

5.3.2.1. Change in pain and disability

The mean overall improvement in pain intensity was 3.4±3.2 points in the study cohort. IL1B rs1143633 was strongly associated with the change in the reported pain at follow-up, where the 'A' allele carriers had the largest improvement in pain intensity (mean change in pain intensity was -3.7±3.3 in 'A/G'+'A/A' group vs. -2.9 ±3.2 in 'G/G' genotype, p=0.00085 in dominant model) (Table 7). The preoperative pain intensity was associated with the 'C' allele of rs1143634 in the microdiscectomy subgroup (Figure 136). Change in ODI score was not associated with the studied gene variants.

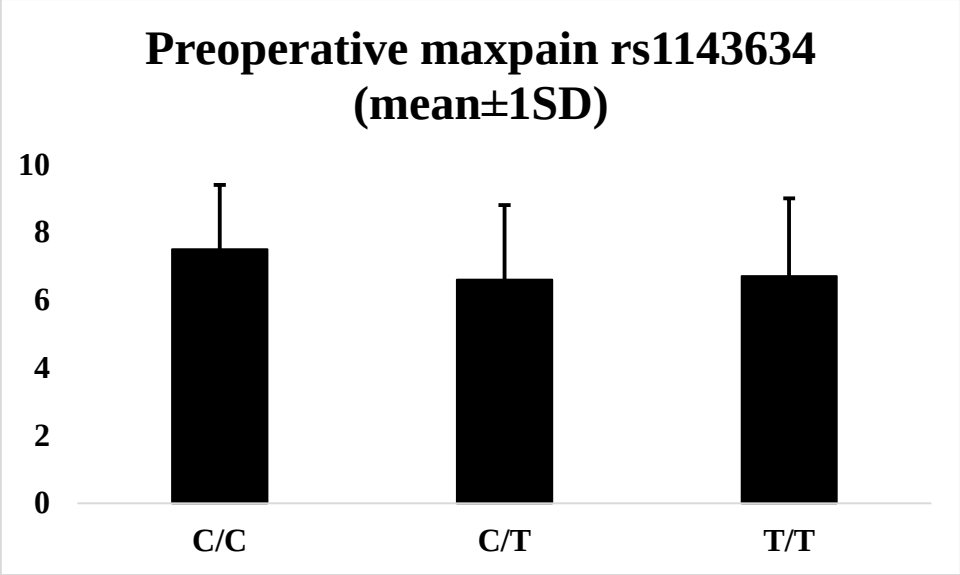

Figure 13 Preoperative pain intensity in the different rs1143634 genotypes

The 'C/C' genotype of rs1143634 is strongly associated with higher pain before surgery (7.5±1.9, 6.6±2.2, 6.7±2.3 for 'C/C', 'C/T' and 'T/T' respectively, p=0.006 in dominant model)

5.3.2.2. Global treatment outcome

In the study cohort 350 patients (82%) reported good outcome while 75 patients (17%) reported poor outcome (6 patients' data were missing). The 'C' allele of IL1B rs1143627 was related with better GTO (OR:1.49, p=0.049 in log-additive model) (Table 7 and Table 8).

5.3.2.3. Surgical outcome

In the overall population 44 patients had poor surgical outcome (10.2%). All 4 IL6 SNPs were associated with the risk of reoperation within 2 years, even after adjusting to type of index surgery. 'G/G' genotype of rs1800796 (OR:6.6, p=0.009, dominant model), 'G/A' genotype of rs2069852 (OR:5, p=0.039 in codominant model) and 'C' allele of rs2069835 (p=0.027, OR:1.27 in log-additive model) were associated with worse outcome. rs2069861 was associated with surgical outcome in an overdominant model (p=0.014) (Table 7and Table 8).

5.3.2.4. Results of haplotype analysis

There was one haploblock in IL1B gene (rs1143634-rs1143633) which was associated with change in pain. 'C-A' haplotype was associated with the greater improvement in pain compared to the most common 'C-G' haplotype (p= 0.001) (Table 9).

Table 7 Associations of IL1B gene variants with postoperative outcome

SNP	Genotype	dODI	p	dPAIN	p	GTO		p	Surgical outcome		p	adjusted p-value
	(N)	Mean±SD		Mean±SD		good N (%)	poor N (%)		good N (%)	poor N (%)		
rs3917365	C/C (358)	23.1+23.6	0.963	-3.3+3.2	0.444	269 (76.2%)	84 (13.8%)	0.861	321 (89.6%)	37 (10.4%)	0.874	0.951
	T/C (71)	24.5+22.23		-3.7+3.3		50 (83.3%)	10 (16.4%)		64 (90.1%)	7 (9.9%)		
	T/T (1)	2.2		-2.2		NA	NA		1	0		
rs1143634	C/C (233)	23.2+23.2	0.353	-3.6+3.2	0.387	175 (83.0%)	36 (17.0%)	0.477	212 (90.99%)	21 (9.01%)	0.334	0.253
	C/T (166)	22.6+23.4		-3.1+3.3		119 (80.0%)	30 (20.0%)		146 (87.95%)	20 (12.05%)		
	T/T (31)	27.3+25.1		-3.4+3.3		24 (80%)	6 (20%)		27 (90%)	3 (10%)		
rs1143633	G/G (184)	22.4+.22.2	0.311	-2.9+3.2	**0.0008**[*]	136 (82.0%)	30 (18.0%)	0.585	166 (90.3%)	18 (9.7%)	0.188	0.249
	A/G (187)	23.2+24.8		-3.6+3.3		138 (80.7%)	33 (19.3%)		165 (88.2%)	22 (11.8%)		
	A/A (41)	25.2+21.2		-4.1+3.2		44 (84.6%)	8 (16.4%)		52 (94.5%)	3 (5.5%)		
rs1143627	T/T (184)	24.4+24.0	0.133	-3.4+3.4	0.412	135 (78.0%)	38 (22.0%)	**0.049**[*f*]	167 (90.8%)	17 (9.2%)	0.535	0.556
	T/C (196)	22.0+23.6		-3.3+3.0		144 (83.2%)	29 (16.8%)		174 (88.7%)	22 (11.3%)		
	C/C (50)	24.3+20.4		-3.4+3.5		40 (88.9%)	5 (11.1%)		45 (90%)	5 (10%)		
rs16944	G/G (182)	24.1+24.0	0.176	-3.4+3.5	0.486	144 (79.1%)	38 (20.9%)	0.056	165 (90.66%)	17 (9.34%)	0.505	0.516
	A/G (191)	22.2+23.7		-3.3+3.0		155 (82.9%)	32 (17.1%)		169 (88.5%)	22 (11.5%)		
	A/A (49)	24.2+20.6		-3.4+3.5		42 (89.4%)	5 (10.6%)		44 (89.8%)	5 (10.2%)		

[*]: significant in dominant model, [**]: significant in overdominant model, [†]: significant in codominant model,

[‡]: significant in recessive model, [*f*]: significant in log-additive model

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Table 8 Associations of IL6 gene variants with postoperative outcome

SNP	Genotype	dODI		dPAIN		GTO			Surgical outcome			
	(N)	Mean±SD	p	Mean±SD	p	good N (%)	poor N (%)	p	good N (%)	poor N (%)	p	adjusted p-value
rs2069852	G/G (389)	22.9+23.0	0.734	-3.4+3.2	0.397	292 (82.5%)	62 (17.5%)	0.144	346 (88.9%)	43 (11.1%)	**0.039**†	0.06
	G/A (42)	26.3+26.5		-3.3+3.4		28 (73.7%)	10 (22.3%)		41 (97.6%)	1 (3.4%)		
	A/A (0)	-				0	0					
rs2069861	C/C (376)	23.7+23.7	0.261	-3.3+3.3	0.360	278 (81.3%)	65 (18.7%)	0.403	334 (88.8%)	42 (11.2%)	**0.014****	**0.004****
	T/C (51)	21.1+21.3		-3.5+2.9		39 (86.6%)	6 (13.4%)		50 (98%)	1 (2%)		
	T/T (2)	13+12.7		-2.4+5.7		2	0		1 (50%)	1 (50%)		
rs2069835	C/C (4)	5.1+9.7	0.351	-4.2+3.0	0.153	3 (75%)	1 (25%)	0.569	2 (50%)	2 (50%)	**0.027**^{f}	**0.05**^{f}
	T/C (54)	19.9+21.6		-2.9+3.1		36 (79.5%)	13 (20.5%)		46 (85.2%)	8 (14.8%)		
	T/T (367)	23.9+23.5		-3.4+3.2		277 (83.1%)	56 (16.9%)		334 (88.7%)	33 (8.9%)		
rs1800796	G/G (371)	22.7+22.8	0.548	-3.4+3.2	0.486	279 (82.5%)	59 (17.5%)	0.165	329 (88.7%)	42 (11.3%)	**0.009***	**0.03***
	G/C (54)	25.7+26.4		-3.4+3.4		36 (75.0%)	12 (25.0%)		53 (98%)	1 (2%)		
	C/C (1)	32		-1.8		1	0		1	0		

*: significant in dominant model, **: significant in overdominant model, †: significant in codominant model

‡: significant in recessive model, ^{f}: significant in log-additive model

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Table 9 IL1B haplotype association (GLM and hapscore) with the change in pain after surgery

Change in max pain GLM model					
Haploblock	**Haplotype**	**Haplotype frequency**	**diff (95% CI)**	**hap score***	**p**
IL1B rs1143634-rs1143633	C-A	0.34	-0.7 (-1.2 – (-)0.3)	-2.46742	**0.001**
	T-G	0.25	-0.2 (-0.7 – 0.3)	1.59408	0.37
	C-G	0.38	-3.8 (reference)	0.38051	-

*: global p-value: 0.051

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6. Discussion

The degenerative spinal diseases play a very important role in the life of the modern society. They are amongst the most common causes for everyday burden that can affect all sex, ethnicity, and age group [14]. The number of spinal surgeries continuously increasing not just in the developed countries but also in the third world countries. There is a huge technical advancement in the medical equipment, surgical techniques, better pharmaceutics, better diagnostics in the field of spine surgery. Before the spinal fixation techniques were developed the treatment of spinal diseases centered around immobilization, bed rest, traction, splinting and bracing [64]. During the 20th century new and new fixational techniques were developed which gave new hope to the spine patients. However, despite the modern techniques the outcome of a spine remained relatively poor. Most of the time the goal of spine surgery is to restore the normal quality of life of a patient but around 5% to 70% of the cases the poor quality of life or at least some disability remains [82]. In the late 20th century, a lot of study examined the basic physiological changes during intervertebral disc degeneration. With the development of genetic examination technologies new type of studies became available to assess the “root of all evil” the aging of the intervertebral disc.

6.1. Genetic association of anthropometric attributes

During the embryonic stage a lot of things can go sideways which can cause different developmental anomalies, such as extra or less limbs, body parts, malformations, loss of different functions. In our study we found that patient who carried the ‘G’ allele of rs12982744 had significantly wider spinal canal in a log-additive model. DOT1L gene and its variants were in the focus of anthropometric studies because it has a role in chondrogenic differentiation and articular cartilage [89]. The gene also has a published association with peak height velocity in puberty and with adolescent idiopathic scoliosis [76]. Sovio et al. reported that the ‘G’ allele of DOT1L gene SNP rsl2459350 resulted in an

increased peak height velocity [77].These effects on the development of the vertebrae could be linked to the DOT1L gene's role in chondrogenesis. In an in vitro mouse model knockdown of DOT1L resulted in reduced chondrogenic differentiation in a cell line. DOT1L influenced chondrogenic differentiation by regulating transcription of Wnt target genes [89]. Wnt signaling is crucial in the formation and development of synovial joint [90]. Mutants in the Wnt signaling pathway have been shown to cause developmental abnormalities [89]. Since DOT1L gene functions is linked to Wnt signaling it can not only cause disruption in the development of synovial cartilage but in the process of endochondral bone formation such as vertebrae formation. However, in the development of degenerative spinal diseases not only anthropometric attributes play role.

6.2. The role of VDR gene variants in the degeneration process

VDR is among the most intensely studied candidate genes in extraskeletal and musculoskeletal conditions. Its association with osteoporosis, muscle function and increased fracture risk has already been showed, but papers on the role of the VDR polymorphisms in the development of LDD have shown conflicting results as discussed by recent meta-analyses about the association of VDR genotypes and LDD [91]–[100][101]–[103]. The cited papers highlight the importance of large-scale, well-designed international investigations to overcome the contradictory results related to the heterogenous phenotype definitions as well as population differences. The direct biological effect of VDR genomic variants is not known in the development of LDD. In a cell line study, it was reported that the 3'UTR haploblock's (BsmI-ApaI-TaqI) "GCT" haplotype resulted in 15% less mRNA and has 30% increased decay rate than "AAC" haplotype [104]. This alteration could cause a decreased quantity of VDR protein in target cells for vitamin D giving such cells an impaired response to vitamin D. The 3'UTR "GCT" haplotype was published in association with increased fracture risk and weaker hand grip strength [92][96]. Polymorphisms in the VDR promoter region can also influence the genetic function. Transcriptional activity of the VDR promoter is 30% less active in case of Cdx2 "G" allele compare to "A" allele

[105]. The “A” to “G” transition in A1012G SNP negatively modifies the GATA-3 transcription factor binding ability in the VDR promoter region [106]. “A” allele (“T” in this thesis) results in an increased promoter activity proved by luciferase activity measurements [104]. These in vitro results support the various possible biological role for VDR variants in the processes of intervertebral disc degeneration. Our results indicate that the distinct phenotypes are differently associated with VDR gene variants, we introduced the use of the “endophenotype” term in LDD genetic association research, which has been already in use in psychiatric genetic association studies. Endophenotype is a quantitative biological trait that is reliable in reflecting the function of a discrete biological system and is reasonably heritable, and as such is more closely related to the root cause of the disease than the broad clinical phenotype [74].

Even though the pathomechanism of a Modic change is not known Modic change is an excellent example of an endophenotype in LDD as it can be present before any visible damage on the intervertebral disc itself [39]. Some suggest that Modic change is caused by mechanical stress and others suppose that it is related to ongoing inflammation the degeneration process [107]. The mechanical stress model is based on biomechanical studies which found that increased shear force on endplates adjacent to degenerated discs resulted in microtrauma in the endplates with consequential bone marrow oedema like that seen on MRI for Modic I changes [39]. An alternative pathway via elevated levels of proinflammatory mediators such as IL-6 and prostaglandin E2 has been suggested in a study where surgically removed disc tissue from patients undergoing instrumented fusion because of LBP was compared to tissue from patients undergoing discectomy for sciatica [108]. An inflammatory pathway for Modic changes has been also suggested in a study which found higher expression of tumour necrosis factor (TNF), an increase in ingrowth of immunoreactive nerve fibers and elevated cytokine levels in surgically extracted disc tissue of patients with Modic I change [109]. VDR SNPs appears to be more susceptible to inflammatory diseases; the prevalence of TaqI is a relative risk for chronic periodontitis [110], the frequency of the ‘C’ allele of a TaqI is higher in chronic extremity osteomyelitis [111], the “A” allele of BsmI seems to be protective against rheumatoid arthritis [112] and, the “C/C” genotype of FokI has a positive correlation with rheumatoid arthritis [112].

Considering the above-mentioned correlations, it is not impossible that the VDR gene polymorphisms can play a role in the emergence of Modic change through modulation of inflammation in the bone marrow.

In our previous studies the risk for Modic change was significantly lower in carriers of 3'-end "AAC" haplotype, while the promoter haplotype was associated with the presence of structural endplate defects. These two - endplate related - phenotypes were also associated with VDR genetic variants in individual SNP analyses. Since VDR is known affect different bone tissue related physiological processes (e.g.: remodelling, immune response) [98][100] and VDR gene variations have an effect on fracture risk and bone density [104] it is plausible that through these mechanisms the endplates of a vertebrae could be genetically more susceptible to mechanical injuries (fractures, Schmorl's nodes) [113].

We also examined the degenerative changes in the intervertebral disc itself, namely disc prolapse and loss of signal intensity and disc height, classified by the Pfirrmann grade [114]. The intervertebral disc is made of two independent anatomical structures, the outer annulus fibrosus and the inner nucleus pulposus. The nucleus pulposus cells produce extracellular matrix components such as type II collagen or aggrecan which govern the disc's biomechanical behaviour [115]. The resident cells in degenerated discs also produce inflammatory cytokines (TNF-α, IL-1β) which result in an "inflammation-like" state [116] [117] and which stimulate expression of matrix degrading enzymes (ADAMTS, MMPs), resulting in loss of aggrecan which leads through consequent dehydration to a weakened resistance against mechanical loading and fall in disc height [118] [119] [120]. This inflammatory state can be modified by VDR as discussed before. We found some associations between VDR gene variants and these disc related endophenotypes, however they were not supported by haplotype and gene-gene interaction analysis, possibly because of the complexity of the disc degeneration process.

Degeneration not only has a multigenetic background, where several gene and gene variants play small, but significant roles, but it is also influenced by external factors. The influence of environment could explain the findings that genetic influence on the degeneration process differs at different spinal levels (where loading and other

biomechanical factors are also different). Hence, although the exact pathomechanism is unknown, degeneration appears to arise as a consequence of the influences of aging and environmental factors such as mechanical loading on a strong genetic background [121].

6.3. Genetic associations with the long-term outcome of spinal surgery

With a strong genetic background through environmental factors the intervertebral disc starts to disintegrate in the early adult life. This process can lead to symptomatic degeneration that requires treatment. Ultimately some of the symptomatic IDD will require surgical intervention if conservative treatment proves ineffective. Unfortunately, a portion of patients will sustain some pain or disability after spinal surgery, they can even develop chronic pain conditions such as FBSS. Understanding the pathophysiology of chronic pain conditions - such as FBSS - can lead clinicians to develop and apply new therapeutic methods to alleviate pain and improve the quality of life in this large patient group. The well-being of a patient is determined by multiple musculoskeletal, functional, and psychosocial factors [82]. Genetic influence on surgical outcome has been highlighted by previous papers [122]. In the present thesis, polymorphisms of two interleukin (IL1B, IL6) genes in a large cohort of 431 patients who underwent elective lumbar spinal surgery for DDD were investigated in terms of the therapeutical outcome. Relationship between long-term treatment results, psychological factors, pain, and different IL gene variants were supported by individual SNP associations and haplotype analyses. Outcome of routine lumbar degenerative surgeries was analysed in different dimensions. Associations of IL gene variants with change in pain, disability as well as patient-reported global treatment outcome and need for a subsequent surgery were determined to elucidate the potential genetic influence.

IL1B variants were significantly related to the improvement in pain after the spine surgery, ‘A’ allele of rs1143633 as well as ‘C-A’ haplotype of rs1143634-rs1143633 haploblock were associated with greater improvement in pain. No other gene variant was associated with pain relive however when we analysed the microdiscectomy subgroup we

found that patients with ‘C/C’ genotype of rs1143634 had significantly higher preoperative pain compared to the other genotypes. Other IL1B variant (rs1143627) was associated with patient reported global treatment outcome, while majority of the studied IL1B variants were related to the preoperative level of depression. Interestingly IL6 variants were significantly associated with the need for a subsequent surgery during the follow-up period. The ‘C’ allele of rs2069835 IL6 SNP was associated with a higher risk for reoperation and with increased level of preoperative depression and somatization. None of the studied gene variants were associated with preoperative spinal pain and disability level.

Number of studies supported the relationship between intervertebral disc degeneration and IL1, IL6 gene variants [79], [123]–[127]. SNPs of these genes have been showed to be associated with the outcome of different surgical treatment [128]–[131], but only Moen et al. have studied the possible association of IL1 gene family and long-term outcome in patients treated because of lumbar disc herniation [122]. They did not find a significant relationship between rs1143627 IL1B SNP and treatment outcome, however they did not publish the genetic effect of single SNPs but their combinations on a mixed (surgically and non-surgically treated) patient groups. The same SNP (rs1143627) was found to be associated with symptomatic disc herniation [132] and with DDD associated pain [133] by others. IL1B variants have been also described in association with DDD [124], [134]. IL6 variants have not been studied related to the surgical outcome of DDD yet but they were previously associated with the process of lumbar disc degeneration [79], [123], [126].

The association between IL1B, IL6 genetic variants and the therapeutic outcome after lumbar spinal surgeries can be explained by different mechanisms:

1) Progressive degeneration process can lead to persistent spinal pain and a potential indication of a subsequent surgery. IL1B is involved in multiple pathological process of disc degeneration. It stimulates extracellular matrix degradation, accelerates cellular senescence and induces apoptosis [135]. rs1143633 in IL1B was associated with improvement of pain after surgery in our study while this SNP was previously found to be associated with higher occurrence of disc degeneration (high intensity zone) [124]. IL6 variants have been also described in relation to DDD [136][137][126].

2) Tissue damage is often mediated through local inflammation. Inflammatory mediators such as IL6 and IL1B carry an important role in regulating and sustaining inflammation and pain. Different studies showed their potential role in disc degeneration related inflammatory process [138]–[140]. IL6 is crucial in homeostasis maintenance and host defence but its overproduction can cause the development or progression of diseases (such as pathologic pain) [141], [142]. The serum level of IL6 is increased in herniated disc which promotes upregulation of MMPs [142], [143]. Kraychete et al. also showed that patients with chronic low back pain due to disc herniation had higher level of serum IL6 [144]. The tissue level of IL6 can be related to the genetic variant of the gene. For example, rs1800796 IL6 SNP (what we found to be strongly associated with FBSS) is associated with increased promoter activity boosting the local secretion of IL6 [126], [137]. The two genes have a potential influence on each other, while IL1B is described as one of the key local inducers of IL6 production [139], [140], [145]. Not surprisingly, the variants of IL1B and IL6 genes have been associated with other chronic inflammatory conditions such as periodontitis, cancer, osteoporosis, type 2 diabetes and diabetic nephropathy [83]–[86], [146]–[151].

3) Psychological issues are also important in pain response and in the development of chronic pain. Depression and anxiety have been previously described as risk factors of DDD and poor surgical outcome after spine surgeries [82], [152]–[154]. Interleukin genes can significantly influence the patient's psychological profile. Chronic inflammation and dysregulation of the immune response is a key factor in the development of major depressive disorders (MDD) [155], [156]. Patients with MDD show an abnormal profile of pro- and anti-inflammatory circulating cytokines [156]–[159]. In animal models of MDD, increased level of pro-inflammatory cytokines caused central serotonin depletion, hypothalamic–pituitary–adrenal (HPA) axis dysregulation, microglial activation and brain structure alteration [156]. In animal inflammatory MDD (MDD-I) models, IL1B appears to be the initial triggering complex of the inflammatory cascade both centrally and peripherally [156]. In our study, some IL1B variants were significantly associated with the preoperative level of depression. These findings are in accordance with previous report about the positive association between rs1143627 IL1B polymorphisms and MDD [160]. In

accordance with our findings, Yu et al. found that the homozygotic ‘T/T’ patients of rs1143634 had a tendency of suffering from less severe depressive symptoms than ‘T/C’ homozygotes [161]. ‘T/T’ genotype of rs1143627 is reported to have a strong connection with major recurrent depression [162], while we found that patients with this particular genotype had worse scores on the depression scale. Two of the investigated SNPs, rs16944 and rs1143627 are located in the promoter region of the gene. These polymorphisms lead to altered expression of IL1B which results in local inflammation and promotes the production of MMPs [163]. A study suggested that IL1B rs16944 gene polymorphism hinder the pharmacological response in the treatment of MDD by increasing the risk of non-remission over 6 weeks of antidepressant treatment [164]. Another IL1B SNP (rs1143633) was strongly associated with postoperative pain in our study while rs1143634 was strongly associated with the preoperative pain intensity but only in the microdiscectomy subgroup. Previously association of intensity of back pain and rs1143634 have been published in war veterans with posttraumatic stress disorder [165]. Association between rs1143633 and pain have not been published yet, however there are a few studies investigating its relationship in paediatric MDD and schizophrenia [166], [167]. rs2069861 in IL6 was associated with both depression and somatization in our cohort. Somatization is also an important factor in the development of symptomatic DDD [154]. Genetic variants of IL6 were linked to depression, somatization and anxiety in numerous studies [168]–[172].

Recently published data showed the possible role of interleukin agonist drugs in the treatment of pathological pain (e.g., chronic pain, inflammatory pain etc.) [173], therefore novel therapeutic strategies targeting IL6 or its receptors have been developed and successfully used in the treatment of selected diseases. In a paper a single intradiscal injection of tocilizumab (IL6 receptor antibody) provided short-term alleviation of discogenic pain [174]. Variants of the interleukins’ and their receptors’ genes can modify the effect of this targeted anti-inflammatory therapies, however there is no data about that so far.

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6.4. Limitations

There are some important limitations in this thesis. Population selection bias cannot be ruled out because only Caucasian patients who underwent degenerative spinal surgeries were enrolled to the study. All the analysed polymorphisms were in Hardy-Weinberg equilibrium. We did not apply any correction of the alpha-level during the genetic association testing process. We used a hypothesis-driven approach where effect of candidate SNPs on a phenotype/endophenotype was calculated. Genetic associations were analysed with multiple statistical models (haplotype analysis, individual SNP association) to confirm the associations of the thesis. The examined comorbidities can influence the genetic association even if we tried to rule it out by adjusting our models. Another limitation is the size of the studied population which can lead to inconsistent results, we aimed to rule out this kind of population size bias by using a big international large dataset to strengthen our findings. Overall study population selection bias cannot be ruled out completely.

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7. Conclusion

For the questions established in the Objective section of this thesis we conclude the following:

1. Is there an association between certain quantitative traits and genetic variations?

We found association between DOT1L gene variation and the diameter of the bony spinal canal. The more 'G' allele a patient had the wider the spinal canal was (mean±SD 1.75±0.44, 1.79±0.41, 1.83±0.39, p=0.0005 in log-additive model for 'C/C', 'C/G' and 'G/G' genotype respectively).

2. Does VDR gene variations influence the development of IDD?

We found association between VDR genetic variants and intervertebral disc degeneration and support the previously described complexity of the genetic background of this condition. In this thesis, we analysed the genetic and imaging data of a large homogenous sample of subjects treated because of LDD. We determined and analysed associations between VDR genetic variants distinct degenerative disc MRI phenotypes, Pfirrmann grade, disc prolapse, Modic change and endplate defect. Association between LDD phenotypes and VDR gene variants was supported by different level of genetic analyses, namely individual SNP associations, haplotype analyses and gene-gene interactions. We found that each of the specific disc degeneration-linked phenotypes examined was differently associated with VDR polymorphisms; Pfirrmann grade was associated with DdeI, FokI, ApaI; disc prolapse was associated with ApaI; Modic change was associated with BsmI, TaqI, FokI SNPs and the BsmI-ApaI-TaqI haploblock; endplate defect was associated with DdeI, Cdx2 SNPs and the Cdx2-A1012G haploblock. Significant VDR gene-gene interactions were also found to be associated with endplate defects.

3. Do gene variations alter the preoperative physical status of a patient?

We found that IL1B and IL6 gene variations can alter certain patient reported mental status. No individual SNP was associated with preoperative ODI and pain, however both IL genes had SNPs related to the level of depression. 'T' allele of rs1143627 IL1B SNP was associated with higher level of depression. IL1B rs16944 'G' allele carriers also showed higher level of depression. rs1143634 IL1B was associated with ZDS. The 'C' allele of IL6 SNP rs2069835 was linked to increased level of depression. IL6 rs2069835 was associated with the level of preoperative somatization.). The preoperative pain intensity was associated with the 'C' allele of rs1143634 in the microdiscectomy subgroup.
IL1B haplotypes were not associated with preoperative ODI, depression, somatization, and pain.

4. Can we identify genes variations that alter the long-term outcome of spinal surgeries?

IL1B and IL6 gene variants was associated with different postoperative elements. IL1B was strongly associated with the intensity of pain after surgery, and with global treatment outcome . Change in ODI score was not associated with any studied gene variation. Interestingly one IL1B haplotype showed association with the improvement of pain after surgical intervention.

5. Can we identify genes that affect the need of subsequent surgery?

All studied IL6 SNP were associated with surgical outcome but no other postoperative element. rs1800796, rs2069852 and rs2069835 were associated with worse outcome while rs2069861 was associated with better surgical outcome.

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8. Summary

IDD is a complex bio-psycho-social entity that appears to arise because of aging and environmental factors on a strong genetic background. Symptomatic IDD affect all age group all around the globe. The prevalence of FBSS after surgical treatment for lumbar degenerative pathology is more than 20%.

In our examinations we studied the possible role of different genetic variations on the development of some anthropometric trait, the development of degeneration and the possible association with the perioperative patient reported outcomes as well as the long-term outcome of surgical intervention.

We found that the diameter of the bony spinal canal is affected by the DOT1L gene and its variation. Patients with ‘G’ allele of rs1143634 had significantly wider bony canal in the L1-4 region which increased with the number of ‘G’ alleles. This gene has published effect on the Wnt signaling pathway that could lead to different skeletal malformations through endochondral bone formation.

Interleukin 1B variants analysis confirmed association with improvement of pain after surgery, moreover relationship with patient reported outcome and preoperative level of depression was also found. IL6 variants were associated with preoperative depression, somatization and with subsequent surgery.

Haplotype analyses confirmed the association between the 3’-end VDR variants and Modic change as well as the relationship of 5’-end variants with endplate defects. We also found significant interactions between the 3’ and 5’-end regulatory regions and endplate defects. Based on our results, VDR and its gene variants are highly associated with specific degenerative LDD endophenotypes.

In conclusion we state that VDR gene variants are associated with different disc degeneration related endophenotypes moreover; we state that IL1B and IL6 gene variants are associated with the psychological status and the long-term outcome of surgically treated lumbar DDD patients.

Based on our findings and the corresponding literature advanced treatment methods could be established targeting interleukin 1B, interleukin 6 and its genes to successfully prevent/treat FBSS or even primary lumbar degenerative pathologies. On the other hand, the consideration of patient-specific genetic difference can be important to maximize the therapeutic outcome.

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9. Összefoglalás

A degeneratív gerincbetegségek kialakulásában központi szerepet játszik a porckorong degeneráció, mint patológiai alapfolyamat, azonban a tünetes betegséget a környezeti hatásokkal és az öregedéssel interakcióban poligénes örökletes háttér alakítja. Ágyéki gerincműtétek után krónikus fájdalomszindróma, ún. „failed back surgery syndrome" alakulhat ki, amelynek kezelése komprehenzív megközelítést igényel.

A vizsgálataink során különböző génvariációk szerepét vizsgáltuk bizonyos antropometriai jellemzők létrehozásában, degeneratív gerincbetegségek kialakításában továbbá vizsgáltuk a genetikai tényezők szerepét gerincműtétek sikerességének vonatkozásában rövid és hosszútávon. A vizsgálatainkhoz nagy nemzetközi adatbázist használtunk, amely tartalmazta a betegek radiológiai, klinikai és genetikai adatait egyaránt.

A vizsgálatok során azt találtuk, hogy a csontos gerinccsatorna átmérőjének kialakításában a DOT1L génnek és polimorfizmusainak lehet befolyásoló szerepe. Az rs1143634 egypontos nukleotid polimorfizmus 'G' alléljéhez szignifikánsabb tágabb gerinccsatorna társult az ágyéki LI-IV-es szakaszokon. Ennek a génnek korábban leírták módosító hatását a Wnt jelátviteli útvonalon keresztül az endochondralis csontosodásra.

A VDR génpolimorfizmusoknak igazoltuk a szerepét az ágyéki degeneratív endofenotípusok kialakításában. Pfirrman degenerációban a DdeI, FokI, ApaI; a porckorong kiboltosulás vonatkozásában az ApaI; Modic instabilitás kialakulásában a BsmI, TaqI, FokI polymorfizmusok és a BsmI-ApaI-TaqI haploblock; a véglemez sérülés megjelenésében a DdeI, Cdx2 polimorfizmusok, valamint a Cdx2-A1012G haploblock játszottak szerepet.

Összefoglalva azt mondhatjuk, hogy a csontos gerincsatorna átmérőjének kialakulásában a DOT1L gén modulátorként szerepelhet, a VDR gén variációk hatással vannak a különböző degeneratív endofenotípusok kialakításáaban, továbbá az IL1B és IL6 gének befolyásolják a betegek pszichés statusát, fájdalomérzetét, valamint egy ágyéki gerincműtét hosszútávú kimenetelét.

Az eredményeinkre és az ide tartozó nemzetközi eredményekre alapozva modern terápiás módszerek kerülhetnek kifejlesztésre, amely az interleukin gyulladásos citokinek génjeit célozva kezelhetik/megelőzhetik FBSS kialakulását. Továbbá a genotipizálás elterjedésével rizikóbecslő módszerek hozhatók létre, amelyek alapján a terápiás kimenetel javítható.

10.References

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Impact Factor: 4,631 (2016)

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ORIGINAL ARTICLE

Association of vitamin D receptor gene polymorphisms with disc degeneration

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Abstract

Purpose Numerous candidate genes and single-nucleotide polymorphisms (SNPs) have been identified in the background of lumbar disc degeneration (LDD). However, in most of these underpowered studies, definitions of LDD are inconsistent; moreover, many of the findings have not been replicated and are contradictory. Our aim was to characterize LDD by well-defined phenotypes and possible endophenotypes and analyse the association between these and candidate vitamin D receptor (VDR) gene polymorphisms on a large ($N = 1426$) dataset.

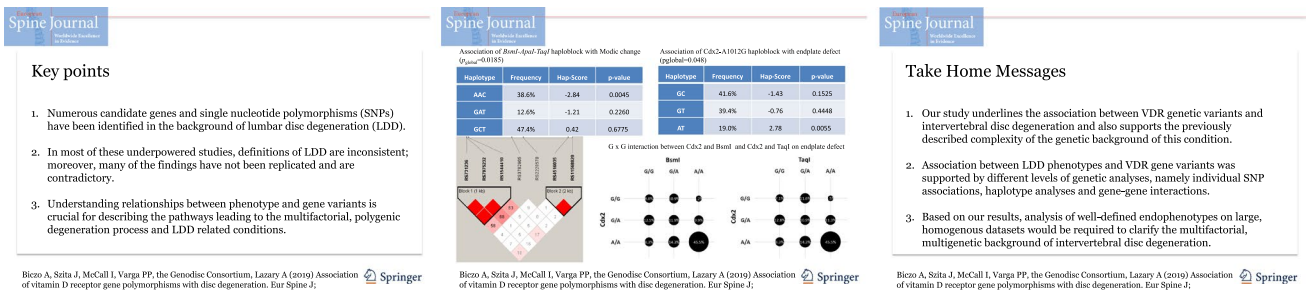
Methods Seven candidate VDR SNPs were genotyped. Individual association, haplotype and gene–gene interaction analyses were performed. All degenerative endophenotypes were significantly associated with one or more candidate VDR gene variants.

Results Haplotype analyses confirmed the association between the 3′-end VDR variants (*BsmI, ApaI, TaqI*) and Modic changes as well as the relationship of 5′-end variants (*Cdx2, A1012G*) with endplate defects. We also found significant interactions between the 3′- and 5′-end regulatory regions and endplate defects. Based on our results, VDR and its gene variants are highly associated with specific degenerative LDD endophenotypes.

Conclusion Understanding relationships between phenotype and gene variants is crucial for describing the pathways leading to the multifactorial, polygenic degeneration process and LDD-related conditions.

Graphic abstract

These slides can be retrieved under Electronic Supplementary Material.



Keywords VDR · Lumbar disc degeneration · Single-nucleotide polymorphism · Haplotype · Endophenotype

Introduction

Low back pain (LBP) is one of the most significant healthcare problems worldwide [1] and imposes a heavy burden on the national health systems in the industrialized countries. Low back pain is thought to be associated with various spinal pathologies (such as disc herniation, spinal stenosis,

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segmental instability) arising from lumbar intervertebral disc degeneration (LDD). The pathomechanism leading to LDD is still unclear. Twin studies showed that up to 70% of LDD could be genetically determined [2]. Candidate gene and genome-wide association studies have identified numerous genes and single-nucleotide polymorphisms (SNPs) in the background of LDD [3]. Vitamin D receptor gene (VDR) has been reported to be one of the first [4] and since then one of the most studied [5] candidate genes. However, studies of the possible role of VDR genetic variants have led to contradictory results with only few findings replicated. Videman et al. [4] in a Finnish population found that "tt" genotype of *TaqI* polymorphism was associated with the most severe degenerative MRI phenotype, yet, also in Finnish populations, Noponen-Hietala et al. [6] and Kelempisioti [6, 7] found no association with disc degeneration and *FokI* and *TaqI* variants. On the other hand, in a Japanese population, Kawaguchi et al. [8], in agreement with Videman et al. [4], found that *TaqI* VDR variant was associated with severe degeneration based on Schneiderman score, though here the risk genotype was "Tt" [8].

Some of the contradictions in the literature could be related to differences in the definition of the LDD phenotype. Rajasekaran et al. [9] critically reviewed the LDD-related genetic studies and examined gene associations with LDD-related morphological phenotypes such as degree of disc degeneration, disc bulging, Modic change, endplate defects and Schmorl's node on a large cohort. They highlighted the importance of standardizing the description of disc degeneration for studies of this topic. The contradictions could also arise from underpowered studies, with only small numbers of subjects examined (e.g. Noponen-Hietala compared only 29 subjects with 56 controls; Kawaguchi's study population consisted of 205 young adults; Toktas compared 75 subjects with 25 controls).

Hence, in the present study, well-defined phenotypes within a homogenous dataset of a large, international cohort were analysed in association with the candidate VDR single-nucleotide gene variants and haplotypes to clarify the possible significance of VDR in LDD. These results point to the existence of endophenotypes in the process of disc degeneration, viz. specific phenotypes (Pfirrmann score, endplate defect, Modic changes, disc prolapse) each with a clear distinct genetic connection underpinning a biological pathway.

Materials and methods

Study population

An international database (Genodisc cohort) containing the clinical, radiological, demographic and genetic data of 2635 low back pain patients from spine hospitals in three European countries (Hungary, Italy, UK) was used [10–14]. All subjects were hospitalized and surgically treated for degenerative lumbar spine pathology. Subjects were involved in the study after signing a written informed consent with the approval of the competent ethical committee. The original dataset of the present study is available upon request.

Phenotype measurements

A set of degenerative phenotypes was qualitatively determined on lumbar spine MRIs by the same radiologist (IM). Four different phenotypes (Pfirrmann grade, Modic change, disc prolapse, endplate defect) were used in the present analysis. All the phenotypes were assessed at five lumbar segments. In the subsequent analysis, we determined the genetic association with the phenotypes at any lumbar levels, at also at L4/5 and L5/S1 levels separately. Pfirrmann grading system was used to determine the level of overall disc degeneration. Mean *Pfirrmann grade* and dichotomous derivate were analysed statistically. In the latter case, as suggested by others [7, 15], discs were scored as "normal" (Pfirrmann 1–2) and "pathologic" (Pfirrmann 3–5) (Fig. 1). Degenerative endplate changes, such as Modic I and Modic II changes, were grouped together into the dichotomous *Modic change* phenotype. *Disc prolapse* was defined as the presence of disc bulging or herniation at the given spinal segment. *Endplate defect* was determined as bony defect at either the upper or the lower endplate (e.g. Schmorl's node). The distribution of the prevalence of the degenerative phenotypes in the Genodisc cohort was the following: 1858 patients had Modic change, 2586 patients had pathologic Pfirrmann grade, 1225 patients had endplate defect and 2541 patients had disc prolapse. The distribution of the studied phenotypes in the final study population is given in Table 1.

Genotyping

DNA was extracted from venous blood or saliva samples using commercial kits. Seven candidate VDR SNPs were genotyped at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, USA). Allelic and genotype distributions, Hardy–Weinberg equilibrium, minor allele frequency (MAF) as well as associations between genetic variants and degenerative phenotypes were determined and analysed using the "SNPassoc" and "haplo.stats" R software packages [16]. Individual genotype–phenotype associations and gene–gene interactions were studied in generalized linear models, while haplotype–phenotype association was analysed applying haplo.score tests. In haplo.score analysis, a global test of association as well as individual haplotype-specific tests was

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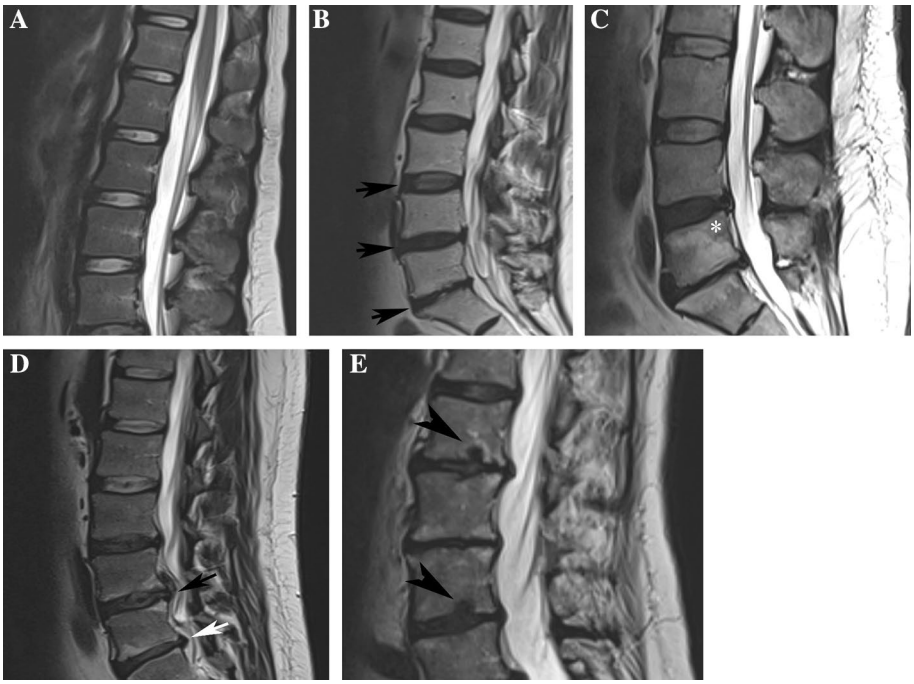

Fig. 1 Degenerative phenotypes. **a** Healthy discs (T2-weighted sagittal MR image); **b** black arrows show degenerated discs, from top to bottom Pfirrmann grades III, IV and V; **c** white star indicates type I Modic change at the lower endplate of the L.V and at the upper endplate of S.I; **d** black arrow indicates posterior disc herniation; white arrow shows posterior disc bulging; **e** black arrowheads show endplate defects

Table 1 Prevalence of the degenerative phenotypes in the study population

	Any	L4/5	L5/S1	L.4/S1
Modic	873	447	529	782
Pathologic Pfirrmann	1402	1185	1186	1385
Endplate	460	140	40	168
Disc prolapse	1364	1038	1013	1335

carried out using a score function. Significant covariates (age, gender, weight and height, and smoking status) were determined for each phenotype, and a p value less than 0.05 was considered significant. The genetic association analysis was also approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary (431/PI/2007).

Results

Descriptive statistics

A total of 1426 Caucasian subjects with a complete dataset were involved in this study (Supplementary Figure 1). Mean age was 49.2 years with a range from 18 to 87 years. The male/female distribution was 46% and 54%. Mean height was 170.6 cm (SD 10.5) and mean weight was 79.7 kg (SD 16.6) in the cohort. In the study population, we had no data about the smoking habits of 128 subjects, while 593 subjects were never-smoker and 705 patients were ever-smoker. Table 2 shows the result of the genotyping process. Genotyping success rate was more than 95% for all variants. All the seven candidate SNPs were in Hardy–Weinberg equilibrium (HWE). A haploblock constructed by three candidate SNPs, *BsmI, ApaI* and *TaqI* (rs1544410, rs7975232, rs731236), was identified at the 3′-end of the gene, and another haploblock

Table 2 Studied VDR SNPs and descriptive statistics of genotyping

rs number	Traditional name	Alleles	Region	Success rate (%)	MAF	HWp
rs11568820	*Cdx2*	G/A	Promoter	95.7	0.190	0.659
rs4516035	*A1012G*	T/C	Promoter	99.3	0.415	0.661
rs2228570	*FokI*	C/T	Exon 2	98.2	0.405	0.867
rs3782905	*DdeI*	C/G	Intron 2	98.9	0.295	0.522
rs1544410	*BsmI*	G/A	Intron 8	99.3	0.397	0.505
rs7975232	*ApaI*	A/C	Exon 9	99.5	0.480	0.456
rs731236	*TaqI*	T/C	Exon 9	99.6	0.388	0.434

MAF minor allele frequency, *HWp p* value of Hardy–Weinberg equilibrium

constructed by two SNPs, *Cdx2* and *A1012G* (rs11568820, rs4516035), was found at the 5′-end of the gene (Fig. 2).

Individual genetic associations

Pfirrmann grade

Ddel (rs3782905), *FokI* (rs2228570), and *ApaI* (rs7975232) polymorphisms were found to be associated with Pfirrmann grade (Supplementary Table 1). "G/G" genotype of *Ddel* was significantly associated with the presence of disc degeneration at level L4–5 (ref = C/C; C/G: OR 0.75, 95% CI 0.55–1.03; G/G: OR 2.01, 95% CI 1.00–4.05; $p = 0.0064$ in codominant model) (Fig. 3a). At L5–S1 level, "C/C" genotype of *ApaI* was significantly related to the risk of severe degeneration (ref = A/A–C/A; C/C, OR 1.46, 95% CI 1.01–2.13, $p = 0.0408$).

Disc prolapse

ApaI was associated with disc prolapse (Supplementary Table 2). Homozygous subjects had a higher frequency of disc prolapse at any spinal level ($p = 0.0458$). At L5/S1 region, "C/C" carriers showed the highest risk of disc prolapse (ref = A/A–C/A; C/C: OR 1.39, 95% CI 1.03–1.88; $p = 0.0271$, in recessive model) (Fig. 3b).

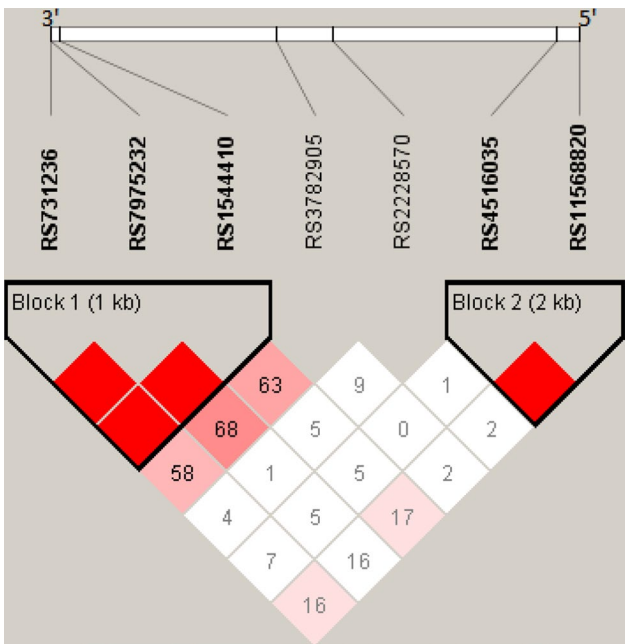

Fig. 2 Linkage disequilibrium (LD) map of the seven candidate SNPs squares that are coloured darker if the $|D'|$ value is high, that is, LD is strong. Empty dark squares mean $|D'| = 1$, that is, complete LD between two single-nucleotide polymorphisms

Modic change

"A/A" genotype of *BsmI* (rs1544410) was associated with a lower frequency of Modic change at any spinal level (ref = G/G–G/A; A/A, OR 0.67, 95% CI 0.49–0.91, $p = 0.01$, recessive model) and at L4–5 (ref = G/G–G/A; A/A, OR 0.65, 95% CI 0.47–0.91, $p = 0.0103$ in recessive model) (Fig. 3c) (Supplementary Table 3). C/C genotype of *TaqI* (rs731236) polymorphism had also a protective effect against Modic change at any level (ref = T/T–C/T; C/C, OR 0.62, 95% CI 0.45–0.86, $p = 0.0032$) and at L4/5 segment (ref = T/T–C/T; C/C, OR 0.61, 95% CI 0.43–0.86, $p = 0.0034$). *FokI* (rs2228570) polymorphism was found to have an association with Modic change in codominant genetic model at level L4/5 (ref = C/C; T/C, OR 1.27, 95% CI 0.98–1.64, T/T, OR 0.83, 95% CI 0.58–1.20, $p = 0.0302$).

Endplate defect

"G" allele of *Ddel* (rs3782905) polymorphism was associated with endplate defect at any lumbar level (ref = C/C; C/G–G/G, OR 1.38, 95% CI 1.09–1.74, $p = 0.0064$, in dominant model) (Fig. 3d) (Supplementary Table 4). "A/A" genotype of *Cdx2* (rs11568820) variant was related to the higher risk of having an endplate defect at L4/5 level (ref = G/G–A/G; A/A, OR 2.32, 95% CI 1.08–4.9, $p = 0.0444$, in the recessive model).

Haplotype analyses

Three haplotypes with more than 1% frequency were identified inside the VDR haploblock located at the 3′-end of the gene (*BsmI–ApaI–TaqI*). The haploblock was significantly associated with the Modic change at L4/5 level ($p_{global} = 0.0185$ in recessive model) where the second most common "AAC" haplotype was associated with lower risk of Modic change ($p = 0.0045$) (Table 3A). Another haploblock with three different haplotypes was identified at the 5′-end (*Cdx2–A1012G*). It was related to the endplate defect at L4/5 level ($p_{blobal} = 0.048$ in additive model), where the rarest "AT" haplotype was associated with the highest risk of endplate defect ($p = 0.0055$) (Table 3B).

Gene–gene interaction analysis

Significant GxG interactions were found between *Cdx2* and *BsmI* ($p_{interaction} = 0.0206$) and between *Cdx2* and *TaqI* ($p_{interaction} = 0.0062$) on endplate defect at L4/5 level (Fig. 4).

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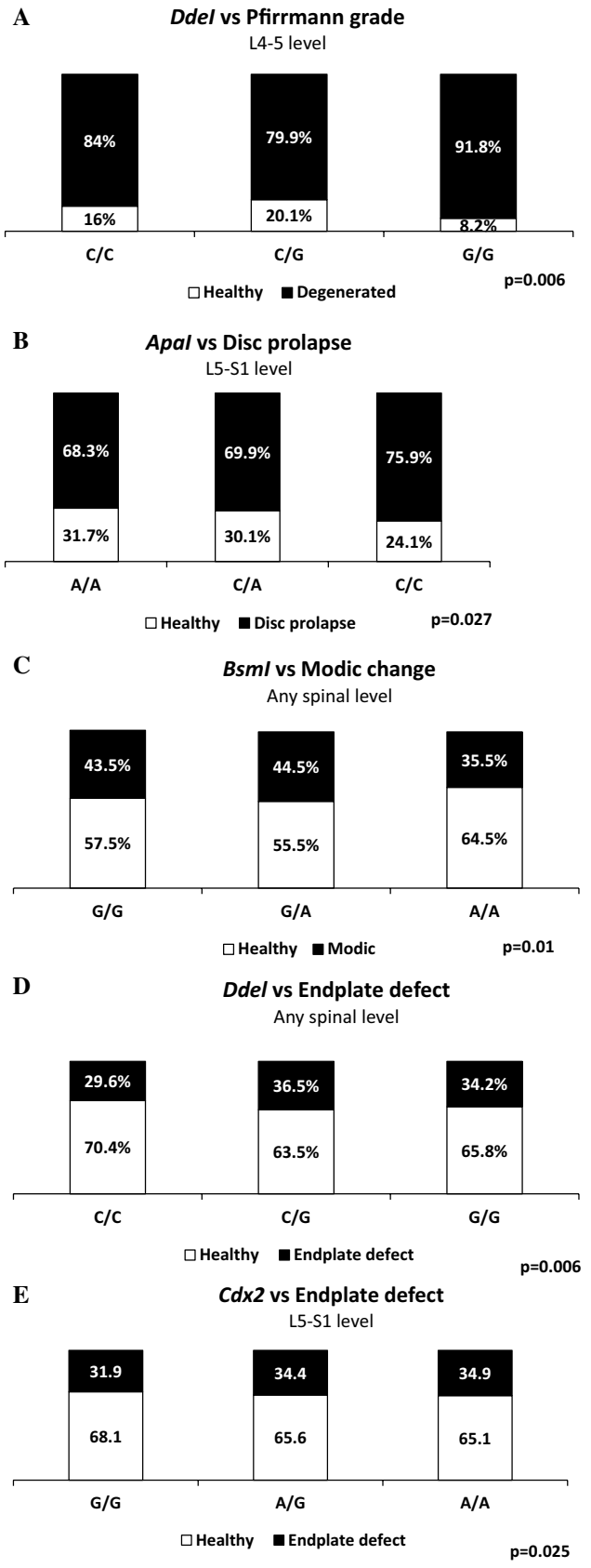

Fig. 3 Association of *DdeI* with Pfirrmann grade (**a**), *ApaI* with disc prolapse (**b**), *BsmI* with Modic change (**c**) and *DdeI* and *Cdx2* with endplate defect (**d**, **e**) distribution of healthy and pathologic endophenotype is represented by genotypes

Discussion

Our study underlines the association between VDR genetic variants and intervertebral disc degeneration and also supports the previously described complexity of the genetic background of this condition. In this study, we analysed the genetic and imaging data of a large homogenous sample ($N = 1426$) of subjects treated because of LDD. We determined and analysed associations between VDR genetic variants and distinct degenerative disc MRI phenotypes Pfirrmann grade, disc prolapse, Modic change and endplate defect. Association between LDD phenotypes and VDR gene variants was supported by different levels of genetic analyses, namely individual SNP associations, haplotype analyses and gene–gene interactions. We found that each of the specific disc degeneration-linked phenotypes was differently associated with VDR polymorphisms; Pfirrmann grade was associated with *DdeI*, *FokI* and *ApaI*; disc prolapse was associated with *ApaI*; Modic change was associated with *BsmI*, *TaqI*, *FokI* SNPs and the *BsmI–ApaI–TaqI* haploblock; endplate defect was associated with *DdeI, Cdx2* SNPs and the *Cdx2–A1012G* haploblock. Significant VDR gene–gene interactions were also found to be associated with endplate defects.

VDR is one of the most intensely studied candidate genes in musculoskeletal and extra skeletal conditions. Its influence has already been shown in osteoporosis [17], muscle function [11] and increased fracture risk [18], but studies on the role of the VDR polymorphisms in the development of LDD have shown conflicting results [8, 19–24] as discussed by three recent meta-analyses about the association of VDR genotypes and LDD [5, 25, 26]. These papers have underlined the importance of large-scale, well-designed international studies to overcome the contradictory research results related to the heterogeneous phenotype definitions as well as gender and ethnic differences.

The direct biological effect of VDR genomic variants is not known in LDD process, some in vitro data can support the genetic results. In a previous cell line study, it was shown that the 3′UTR haploblock's (*BsmI–ApaI–TaqI)* "GCT" haplotype resulted in 15% less mRNA and has 30% increased decay rate than "AAC" haplotype [27]. This alteration likely causes a decreased quantity of VDR protein in target cells for vitamin D giving such cells an impaired response to vitamin D. The 3′UTR "GCT" haplotype was published in association with increased fracture risk [20] and weaker hand grip strength [11]. On the other hand, polymorphisms in the VDR promoter can also influence the genetic function.

Table 3 (A) Association of *BsmI–ApaI–TaqI* haploblock with Modic change ($p_{global}=0.0185$) and (B) association of *Cdx2–A1012G* haploblock with endplate defect ($p_{global}=0.048$)

Haplotype	Frequency (%)	Hap-Score	*p* value
(*A*)			
AAC	38.6	−2.84	0.0045
GAT	12.6	−1.21	0.2260
GCT	47.4	0.42	0.6775
(*B*)			
GC	41.6	−1.43	0.1525
GT	39.4	−0.76	0.4448
AT	19.0	2.78	0.0055

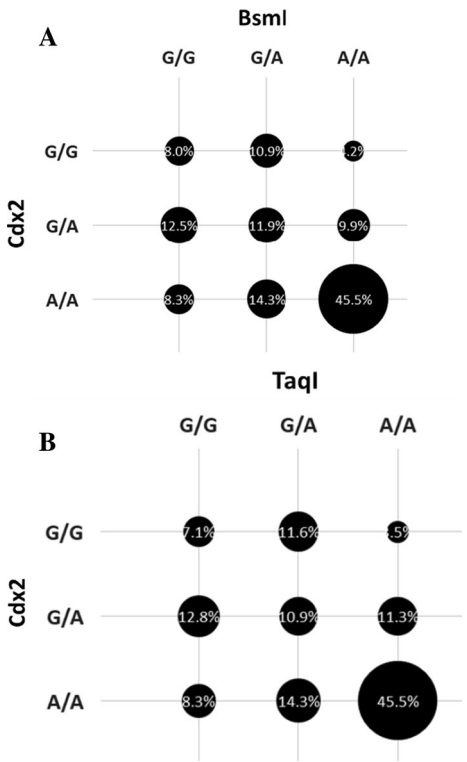

Fig. 4 GxG interaction between *Cdx2* and *BsmI* (**a**) and *Cdx2* and *TaqI* (**b**) on endplate defect (bubbles represent the percentage of subjects with endplate defect at L4/5 in different genotype combinations)

Transcriptional activity of the VDR promoter is 30% less in case of *Cdx2* "G" allele compared to "A" allele [28]. The "A" to "G" transition in A1012G SNP negatively modifies the GATA-3 transcription factor-binding ability of the VDR promoter [29]. "A" allele ("T" in our paper) results in an increased promoter activity proved by Fang et al. [27] using luciferase activity measurements. These in vitro results thus support the role of various possible biological roles for VDR variants in the processes of disc degeneration.

As our results indicate the distinct phenotypes are differently associated with VDR genetic variants, we introduce the use of the "endophenotype" term in LDD genetic association research, which has been already applied in psychiatric genetic association studies. Endophenotype is a quantitative biological trait that is reliable in reflecting the function of a discrete biological system and is reasonably heritable, and as such is more closely related to the root cause of the disease than the broad clinical phenotype [30].

A Modic change is an excellent example of an endophenotype in LDD as it can be present before any visible damage on the intervertebral disc itself [31] even though the pathomechanism of a Modic change is not known. Some authors suggest that it is caused by mechanical stress while others suppose that it is related to ongoing inflammation in the degeneration process [32]. The mechanical stress model is based on biomechanical studies which found that increased shear force on endplates adjacent to degenerated discs resulted in microtrauma in the endplates with consequential bone marrow oedema similar to that seen on MRI for Modic I changes [31]. An alternative pathway via elevated levels of proinflammatory mediators such as IL-6 and prostaglandin E2 has been suggested in a study where surgically removed disc tissue from patients undergoing fusion because of LBP was compared to tissue from patients undergoing discectomy for sciatica [33]. An inflammatory pathway for Modic changes has been also suggested in a study which found higher expression of tumour necrosis factor (TNF), an increase in ingrowth of immunoreactive nerve fibres and elevated cytokine levels in surgically extracted disc tissue of patients with Modic I change [34]. VDR SNPs appear linked to elevated susceptibility to inflammatory diseases; the prevalence of *TaqI* is a relative risk of chronic periodontitis [35], the frequency of the "C" allele of a *TaqI* polymorphism is higher in chronic extremity osteomyelitis [36], the "A" allele of *BsmI* seems to be protective against rheumatoid arthritis [37], and the "C/C" genotype of *FokI* has a positive correlation with rheumatoid arthritis [37]. Considering the above-mentioned correlations, it is not impossible that the VDR gene polymorphisms can play a role in the emergence of Modic change through modulation of inflammation in the bone marrow.

In our study, risk of Modic change was significantly lower in carriers of 3′-end "AAC" haplotype, while the promoter haplotype was associated with the presence of structural endplate defects. These two endplate-related phenotypes were also associated with VDR genetic variants in individual SNP analyses. Since VDR is known to have an effect on different bone tissue-related physiological processes (e.g. remodelling, immune response) [19–22, 24] and VDR SNPs have an effect on fracture risk and bone density [27], it is plausible that through these mechanisms the endplates of a

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vertebrae could be genetically more susceptible to mechanical injuries (fractures, Schmorl's nodes) [38] too.

Besides the endplate changes in degenerative spinal disorders, we examined the degenerative changes in the intervertebral disc itself, namely disc prolapse and also loss of signal intensity and disc height, classified by the Pfirrmann grade [39]. The intervertebral disc is made of two independent anatomical structures, the outer annulus fibrosus and the inner nucleus pulposus. The nucleus pulposus cells produce extracellular matrix components such as type II collagen or aggrecan which govern the disc's biomechanical behaviour [40]. In human degenerative discs, the resident cells also produce inflammatory cytokines (TNF-α, IL-1β) which result in an "inflammation-like" state [41, 42] and which stimulate expression of enzymes able to degrade the matrix (ADAMTS, MMPs), resulting in loss of aggrecan leading to consequent dehydration to a weakened resistance against mechanical loading and fall in disc height [43–45]. This inflammatory state can be modified by VDR as discussed above [22, 24, 32]. We found some associations between VDR gene variants and these disc-related endophenotypes; however, they were not supported by haplotype and gene–gene interaction analysis, possibly because of the complexity of the disc degeneration process.

Degeneration not only has a multigenetic background, where several gene and gene variants play small, but significant, roles, but it is also influenced by external factors. The influence of environment could explain the findings that genetic influence on the degeneration process differs at different spinal levels (where loading and other biomechanical factors are also different). Hence, although the exact pathomechanism is unknown, degeneration appears to arise as a consequence of the influences of ageing and environmental factors such as mechanical loading on a strong genetic background [46].

There are some important limitations of the present study. We could not take into account possibly relevant environmental effects such as physical loading history or diet. Also, there could be an overlapping between the phenotypes even with the use of the endophenotype approach. We did not apply any correction of the alpha level during the genetic association testing process. We followed this method because we used a hypothesis-driven approach where effect of candidate SNPs on a phenotype was calculated. Moreover, genetic associations were tested on different levels with different statistical models (individual SNP association, haplotype analysis, gene–gene interaction) to confirm the associations of the study even if the type I error rate was not conservatively reduced. Moreover, some of our results showed a different association with that reported in previous papers; whether this arises from differences in study population phenotype definitions or even selection bias cannot be ascertained. These limitations above can influence the reliability of our findings; therefore, independent replications of the study on different populations are strongly recommended.

In conclusion, we state that VDR gene variants are associated with different disc degeneration-related endophenotypes. The most plausible explanation of these associations is related to the influence of vitamin D on modulating inflammation and the immune response, but this assumption needs more in vitro and in vivo studies to confirm it. Based on our results, analysis of well-defined endophenotypes on large, homogenous datasets would be required to clarify the multifactorial, multigenetic background of intervertebral disc degeneration.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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RESEARCH

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Genetic variants of interleukin 1B and 6 are associated with clinical outcome of surgically treated lumbar degenerative disc disease

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Abstract

Background: Successfully surgically treating degenerative disc diseases can be challenging to the spine surgeons, the long-term outcome relies on both the physical and mental status of the patient before and after treatment. Numerous studies underlined the role of inflammatory cytokines – like interleukin 1B and 6 – in the development of chronic diseases such as failed back surgery syndrome (FBSS) and major depressive disorder (MDD) which alter the outcome after spinal surgery. Our aim was to evaluate the associations of IL6 and IL1B gene polymorphisms with the long-term outcome of degenerative lumbar spine surgeries.

Methods: An international genetical database (GENODISC) was combined with our institute's clinical database to create a large pool with long term follow up data. Altogether 431 patient's data were analysed. Patient reported outcome measures and surgical outcome was investigated in association with IL1B and IL6 SNPs with the help of 'SNPassoc' R genome wide association package.

Results: Interleukin 1B variants analysis confirmed association with improvement of pain after surgery on individual SNP level and on haplotype level, moreover relationship with patient reported outcome and preoperative level of depression was found on individual SNP level. IL6 variants were associated with preoperative depression, somatization and with subsequent surgery.

Conclusion: Understanding the complexity of spinal surgery patients' long-term well-being is crucial in effectively treating chronic debilitating somatic diseases and the associated mental illnesses. Further studies should investigate more comprehensively the linkage of chronic physical and mental illnesses focusing on their simultaneous treatment.

Keywords: Interleukins, Degenerative disc diseases, Long term outcome, Single nucleotide polymorphism

Introduction

Degenerative disc disease (DDD) is a chronic and debilitating condition, which leads to loss of workdays in an active adult's life [1]. Conservative treatments are mainly effective but occasionally surgical treatment is inevitable [2]. The surgical intervention in DDD aims to reduce pain and restore function. Surgical outcome is complex and multifactorial. It can be measured with objective (e.g.: muscle strength, vegetative functions) and subjective (patient reported outcome measures 'PROMs') assessment tools. Not uncommonly, despite the perfect surgical technique for an obvious pathology, the patient reports no significant improvement and continues to suffer from pain and even failed back surgery syndrome (FBSS) can develop [3].

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Pain processing is a complex multifactorial pathway with different regulatory molecules. It has a biomedical component defined by tissue damage, an evaluative component which is influenced by coping mechanisms and an affective component which is altered by psychological disorders like depression and anxiety [4]. Cytokines and interleukins – such as interleukin 6 (IL6) and interleukin 1B (IL1B) - have an important role in regulating local pain response [5–8]. IL1B also act as an upregulator of other nociceptive agents and cascades such as IL6, prostaglandins, substance-P and matrix metalloprotease (MMP) 9 [9].

Single nucleotide polymorphisms (SNPs), the most common genetic variants have been identified in the genetic background of the development and outcome of several multifactorial diseases [10–14]. DDD is also a multifactorial entity with a strong genetic background [15] and high number of genes and their SNPs are linked to its pathomechanism [16–18]. Also genetic variations occurring in COL1A1, COL9a3 and VDR genes seems to be associated with the development of LDD [19]. DDD related pain is also influenced by different genes and their variations such as catechol-O-methyltransferase (COMT) [20] and β2-adrenergic receptor genes (ARDRB2) [5, 21]. According to published data the IL1 gene family (IL1A, IL1B, IL1RN) and the IL6 gene variations have connection with degenerative spinal pathologies [22–24], DDD related pain (low back pain, leg pain) [12, 25, 26] and the outcome of conservative treatment [27], but there is limited amount of data available about the associations of these gene variants in relation to spinal surgery outcome [28].

The current study focuses on evaluating the relationship between IL1B and IL6 gene polymorphisms and the long-term outcome of degenerative lumbar spine surgeries.

Materials and methods

Study population

Data were collected prospectively from adults (above the age of 18) who underwent routine, elective surgery for lumbar disc degeneration at one or two levels at a tertiary spine center. Prospective clinical data were linked with the subjects' genetic data derived from the GENODISC multicenter international collaboration. Patients with minimum 2-year follow-up data were included into the final study cohort to explore the long-term outcome of the surgical procedures. Patients reoperated within 2 years due to a surgical site infection, proximal junctional kyphosis (PJK) or adjacent segment degeneration (ASD) as well as subjects undergoing either acute intervention because of neurological emergency or tumour surgery were excluded from the study. Surgeries were performed by board-certified orthopaedic surgeons or neurosurgeons specified in spinal surgery. Applied procedures included microdiscectomy, decompression and instrumented fusion (transforaminal lumbar interbody fusion or posterior fusion). All procedures were carried out using the standard median-sagittal posterior approach. All subjects signed a written consent form describing the scientific purpose of the systematic collection of their clinical and genetic data. The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council Hungary (431/PI/2007).

Clinical data

Patients completed standard and validated PROMs to assess their clinical status before the surgery and during the follow-up period [29, 30]. Pain was evaluated by the 10 cm long Visual Analogue Scale. Lumbar spine related function was measured with Oswestry Disability Index (ODI). Psychologic distress was measured by evaluating the level of depression and somatisation and was assessed with the Hungarian versions of Zung Depression Scale (ZDS) [30] and the Modified Somatic Perception Questionnaire (MSPQ) (Supplementary Material), respectively. Patients were asked to rate the overall outcome of the surgery using a five-category question; "helped a lot", "helped", 'helped only little", "didn't help", "made things worse". To measure global treatment outcome (GTO) a dichotomous variable was generated based on these given answers. Good outcome was defined if the patient responded by 'helped a lot, 'helped' and poor in case the patient replied by 'only little', 'didn't help', 'made things worse' [31, 32]. Surgical outcome was considered "good" if no re-operation was performed at the index level within 2 years and "poor" if a subsequent surgery was needed within 2 years.

Genotyping

DNA was extracted from venous blood or saliva samples using commercial. Five SNPs in IL1B and four SNPs in IL6 genes were selected for genotyping based on previous literature data [11, 33–37]. Genotyping was performed from 2007 to 2013 at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, USA).

Statistics

Allelic and genotype distributions, Hardy-Weinberg equilibrium, minor allele frequency (MAF) as well as associations between genetic variants and outcomes were determined and analysed using the 'SNPassoc' and 'haplo.stats' R software packages [38]. Genetic

associations with preoperative and postoperative pain, disability, and psychological distress as well as global treatment and surgical outcome were investigated. Individual genotype-phenotype associations were studied in generalized linear models (GLM). Genetic subgroups with less than 4 (1%) subjects were excluded from subsequent statistical analyses. Haplotype-phenotype association was analysed applying haplo.score tests and GLM models. In haplo.score analysis, a global test of association as well as individual haplotype-specific tests are carried out using a score function. Disc herniation subgroup (patients underwent microdiscectomy) was also analysed separately to investigate the role of IL SNPs in sciatica. Significant covariates (age, gender, weight, height, preop ZDS and preop MSPQ score, type of surgery) were determined and entered into the models for each outcome. *P*-value less than 0.05 was considered significant.

Results

Study population

A total of 431 subjects (all Caucasians) met the study inclusion criteria. Mean age were 52.7 (SD:13.9y) years (from 20 to 88 years) and male/female ratio was 0.6 (male:166, female:265). As the index surgery 171 patients had microdiscectomy, 22 patients had decompression, 142 patients had one level fusion and 96 patients had 2-level fusion. In the final study cohort, 44 patients required a subsequent lumbar surgery at the index level during the follow-up. Eight patients had re-discectomy or decompression, 35 required fusion and in 1 case the implants had to be removed.

Descriptive statistics of genotyping

Table 1 shows the results of the genotyping process. The genotyping success rate was more than 97% in all cases. All studied SNPs were in Hardy-Weinberg equilibrium.

Two haploblock from IL1B gene were identified consisting of 2-2 SNPs *('rs1143634-rs1143633'*and '*rs1143627-rs16944'*) and no haploblock was identified on the IL6 gene as seen on Fig. 1.

Associations of IL1B and IL6 gene variants with preoperative PROMs

In the overall population the mean±SD values of preoperative ODI score was 47.4±18.4 and the mean VAS score was 7.2±1.9, the mean ZDS was 39.6±8.1, MSPQ was 8.3±5.7. No individual SNP was associated with preoperative ODI and pain (Table 2), however both IL genes had SNPs related to the level of depression. 'T' allele of *rs1143627* IL1B SNP was associated with higher level of depression (ZDS was 40.6±8.7, 39.2±7.3 and 38.3±8.0 in case of 'T/T', 'T/C' and 'C/C' genotypes, respectively, *p*-value=0.025 in log additive model). IL1B *rs16944* 'G' allele carriers also showed higher level of depression (ZDS was 40.6±8.8, 39.2±7.3 and 38.0±8.0 in case of 'G/G', 'A/G' and 'A/A' genotypes, respectively, *p*-value=0.025 in log additive model). *rs1143634* IL1B was associated with ZDS in an overdominant model (p=0.025, "C/T" mean ZDS±SD was 40.8±8.4 and 39.0±7.8 in case of 'C/T' and 'C/C'+'T/T' genotype groups). The 'C' allele of IL6 SNP *rs2069835* was linked to increased level of depression (mean ZDS±SD were 39.2±7.8, 42.2±9.2, and 45.3±10.1 in case of 'T/T', 'T/C' and 'C/C' genotypes, respectively, p=0.003 in log-additive model).

IL6 *rs2069835* was associated with the level of preoperative somatization (Mean MSPQ±SD was 8.0±5.3 and 10.2±7.0 in case of 'T/T' and 'T/C'+'C/C' genotypes respectively, p=0.010, in dominant model) (Table 2).

IL1B haplotypes were not associated with preoperative ODI, depression, somatization, and pain (data not shown).

Table 1 The descriptive statistics of the genotyped SNPs

Gene	rs number	Position	Alleles	Major allele frequency %	HWE	missing (%)
IL1B	rs3917365	3'UTR	C/T	91.5	0.344	0.2
IL1B	rs1143634	Exon 5	C/T	73.7	1.000	0.5
IL1B	rs1143633	Intron 4	G/A	65.1	0.521	1.2
IL1B	rs1143627	Promoter	T/C	65.6	0.914	0.2
IL1B	rs16944	Promoter	G/A	65.8	1.000	2.1
IL6	rs2069852	3'UTR	G/A	95.1	0.613	0
IL6	rs2069861	3'UTR	C/T	93.6	0.688	0.5
IL6	rs2069835	Intron	T/C	92.7	0.264	1.4
IL6	rs1800796	Promoter	G/C	93.4	1.000	1.2

The descriptive statistics of the genotyped SNPs, HWE: Hardy-Weinberg equilibrium

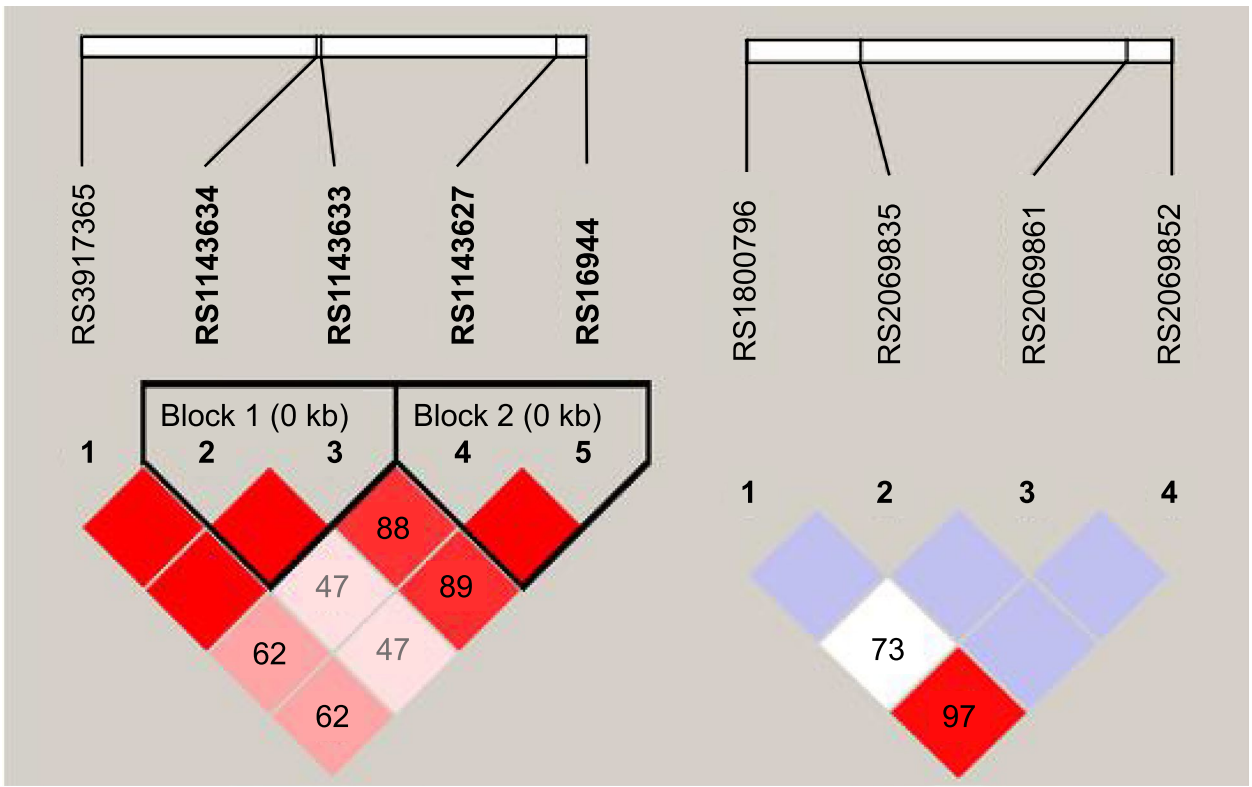

Fig. 1 Linkage disequilibrium (LD) map of IL1B (**A**) and IL6 (**B**) SNPs. Squares are colored darker if the |D'| value is high, that is, LD is strong. Empty dark squares mean |D'|=1, that is, complete LD between two single nucleotide polymorphisms

Associations of IL1B and IL6 gene variants with postoperative outcome

Change in pain and disability

The mean overall improvement in pain intensity was 3.4±3.2 points (overall 61% improvement) in the study cohort. IL1B *rs1143633* was strongly associated with the change in the reported pain at follow-up, where the 'A' allele carriers had the largest improvement in pain intensity (mean change±SD (%) in pain intensity was -3.7±3.3 (50%) in 'A/G'+'A/A' group vs -2.9 ±3.2 (40%) in 'G/G' genotype, p=0.00085 in dominant model) (Table 3). Another IL1B SNP (*rs1143634*) was associated with sciatica in the disc herniation subgroup. In this cohort, the level of preoperative pain was significantly higher in the 'CC' genotype (VAS=7.5±*1.9, 6.6±2.2 and 6.7±2.3* for 'C/C', 'C/T' and 'T/T' genotypes respectively, p=0.006 in dominant model) (Fig. 2). Change in ODI score was not associated with the studied gene variants.

Global treatment outcome

In the study cohort 350 patients (82%) reported good outcome while 75 patients (17%) reported poor outcome (6 patients' data were missing). The 'C' allele of IL1B *rs1143627* was related with better GTO (OR:1.49, p=*0.049* in log-additive model) (Table 3).

Surgical outcome

In the overall population 44 patients had poor surgical outcome (10.2%). All 4 IL6 SNPs were associated with the risk of reoperation within 2 years, even after adjusting to type of index surgery. 'G/G' genotype of *rs1800796* (OR:6.6, p=0.009, dominant model), 'G/A' genotype of *rs2069852* (OR:5, p=0.039 in codominant model) and 'C' allele of *rs2069835* (p=0.027, OR:1.27 in log-additive model) were associated with worse outcome. *rs2069861* was associated with surgical outcome in an overdominant model (p=0.014) (Table 3).

Results of haplotype analysis

There was one haploblock in IL1B gene (*rs1143634-rs1143633*) which was associated with change in pain. 'C-A' haplotype was associated with the greater improvement in pain compared to the most common 'C-G' haplotype (p= 0.001) (Table 4).

Table 2 Associations of IL1B and IL6 gene variants with preoperative PROMs

SNP	Genotype (N)	Preop ZDS Mean+SD	*p*	Preop MSPQ Mean+SD	*p*	*Preop ODI* Mean+SD	*p*	*Preop pain* Mean+SD	*p*
IL1B_rs3917365	C/C (358) T/C (71) T/T (1)	39.5+8.2 39.6+7.1 63	*0.146*	8.3+5.8 8.2+4.9 22	*0.832*	47.5+18.7 47.1+16.7 60	*0.78*	7.2+2.0 7.2+1.9 8	*0.69*
IL1B_rs1143634	C/C (233) C/T (166) T/T (31)	39.0+7.8 40.7+8.4 38.4+7.9	***0.025*** **	8.2+5.7 8.3+5.3 9.3+7.4	*0.401*	47.1+17.9 47.7+19.0 48.3+18.5	*0.655*	7.3+1.9 7.1+2.0 7.0+2.1	*0.253*
IL1B_rs1143633	G/G (184) A/G (187) A/A (41)	39.4+8.1 39.9+7.8 39.7+9.0	*0.526*	8.6+5.6 7.9+5.4 8.9+6.9	*0.183*	47.1+17.9 47.4+19.2 47.6+16.0	*0.813*	7.1+2.0 7.3+1.8 7.4+2.0	*0.170*
IL1B_rs1143627	T/T (184) T/C (196) C/C (50)	40.6+8.7 39.2+7.3 38.0+8.0	***0.025*** ^{f}	8.0+5.5 8.5+5.8 8.6+5.6	*0.371*	48.8+18.2 46.0+18.8 48.3+16.9	*0.135*	7.1+2.1 7.3+1.8 7.2+2.2	*0.338*
IL1B_rs16944	G/G (182) A/G (191) A/A (49)	40.6+8.8 39.2+7.3 38.0+8.0	***0.025*** ^{f}	8.0+5.5 8.5+5.9 8.5+5.6	*0.424*	48.6+18.2 46.3+19.0 48.5+17.0	*0.207*	7.1+2.1 7.3+1.8 7.3+2.2	*0.253*
IL6_rs2069852	G/G (389) G/A (42) A/A (0)	39.7+8.0 39.1+8.7 -	*0.629*	8.4+5.8 7.0+4.4 -	*0.192*	47.2+18.3 59.8+18.5 -	*0.375*	7.2+2.0 7.1+2.0 -	*0.623*
IL6_rs2069861	C/C (376) T/C (51) T/T (2)	39.7+8.0 39.4+8.5 33.5+0.7	*0.282*	8.5+5.8 7.3+4.7 n/a	*0.103*	47.9+18.5 43.5+16.2 26+25.5	*0.095*	7.2+1.9 7.1+2.1 5.4+4.2	*0.191*
IL6_rs2069835	C/C (4) T/C (54) T/T (367)	45.3+10.1 42.2+9.2 39.2+7.8	***0.003*** ^{f}	10.0+10.7 10.2+6.9 8.0+5.3	***0.010*** *	39.5+31.8 48.5+18.4 47.2+18.2	*0.393*	8.1+1.3 7.2+1.6 7.2+2.0	*0.385*
IL6_rs1800796	G/G (371) G/C (54) C/C (1)	39.8+8.0 38.4+8.6 40	*0.239*	8.5+5.7 6.8+4.7 14	*0.087*	47.0+18.1 50.2+19.3 40	*0.208*	7.2+2.0 7.1+1.9 4.4	*0.149*

*: significant in dominant model, **: significant in overdominant model, †: significant in codominant model, ‡: significant in recessive model, ^{f}: significant in log-additive model

Discussion

Number of spine surgeries because of DDD is continuously increasing. The rate of patients with poor outcome is between 5-70% in different surgical cohorts, and chronic pain condition because of failed back surgery syndrome (FBSS) is also not uncommon in this population. Understanding the pathophysiology of chronic pain conditions - such as FBSS - can lead clinicians to develop and apply new therapeutic methods in order to alleviate pain and improve the quality of life in this large patient group. The well-being of a patient is determined by multiple musculoskeletal, functional, and psychosocial factors [32]. Genetic influence on surgical outcome has been also highlighted by previous studies [28]. In the present study, polymorphisms of two interleukin (IL1B, IL6) genes in a large cohort of 431 patients who underwent elective lumbar spinal surgery for DDD were investigated in terms of the therapeutical outcome. Relationship between long-term treatment results, psychological factors, pain, and different IL gene variants were supported by individual SNP associations and haplotype analyses. Outcome of routine lumbar degenerative surgeries was analysed in different dimensions. Associations of IL gene variants with change in pain, disability as well as patient-reported global treatment outcome and need for a subsequent surgery were determined to elucidate the potential genetic influence.

IL1B variants were significantly related to the improvement in pain after the spine surgery, 'A' allele of *rs1143633* as well as 'C-A' haplotype of *rs1143634-rs1143633* haploblock were associated with greater improvement in pain. No other gene variant was associated with pain relieve however when we analysed the microdiscectomy subgroup we found that patients with 'C/C' genotype of *rs1143634* had significantly higher preoperative pain compared to the other genotypes. Other IL1B variant (*rs1143627*) was associated with patient reported global treatment outcome, while majority of the studied IL1B variants were related to the preoperative level of depression. Interestingly IL6 variants were significantly associated with the need for a subsequent surgery during the follow-up period. The 'C' allele of *rs2069835* IL6 SNP was associated with a higher risk for reoperation and also with increased level of preoperative depression and somatization. None of the studied gene variants were associated with preoperative spinal pain and disability level.

Table 3 Associations of IL1B and IL6 gene variants with postoperative outcome

SNP	Genotype	dODI	*p*	dPAIN	*p*	GTO		*p*	Surgical outcome		*p*	*adjusted p-value*
	(N)	Mean±SD		Mean±SD		good N (%)	poor N (%)		good N (%)	poor N (%)		
IL1B_rs3917365	C/C (358)	23.1+23.6	*0.963*	-3.3+3.2	*0.444*	269 (76.2%)	84 (13.8%)	*0.861*	321 (89.6%)	37 (10.4%)	*0.874*	*0.951*
	T/C (71)	24.5+22.23		-3.7+3.3		50 (83.3%)	10 (16.4%)		64 (90.1%)	7 (9.9%)		
	T/T (1)	2.2		-2.2		NA	NA		1	0		
IL1B_rs1143634	C/C (233)	23.2+23.2	*0.353*	-3.6+3.2	*0.387*	175 (83.0%)	36 (17.0%)	*0.477*	212 (90.99%)	21 (9.01%)	*0.334*	*0.253*
	C/T (166)	22.6+23.4		-3.1+3.3		119 (80.0%)	30 (20.0%)		146 (87.95%)	20 (12.05%)		
	T/T (31)	27.3+25.1		-3.4+3.3		24 (80%)	6 (20%)		27 (90%)	3 (10%)		
IL1B_rs1143633	G/G (184)	22.4+.22.2	*0.311*	-2.9+3.2	***0.00085****	136 (82.0%)	30 (18.0%)	*0.585*	166 (90.3%)	18 (9.7%)	*0.188*	*0.249*
	A/G (187)	23.2+24.8		-3.6+3.3		138 (80.7%)	33 (19.3%)		165 (88.2%)	22 (11.8%)		
	A/A (41)	25.2+21.2		-4.1+3.2		44 (84.6%)	8 (16.4%)		52 (94.5%)	3 (5.5%)		
IL1B_rs1143627	T/T (184)	24.4+24.0	*0.133*	-3.4+3.4	*0.412*	135 (78.0%)	38 (22.0%)	***0.049**^f^*	167 (90.8%)	17 (9.2%)	*0.535*	*0.556*
	T/C (196)	22.0+23.6		-3.3+3.0		144 (83.2%)	29 (16.8%)		174 (88.7%)	22 (11.3%)		
	C/C (50)	24.3+20.4		-3.4+3.5		40 (88.9%)	5 (11.1%)		45 (90%)	5 (10%)		
IL1B_rs16944	G/G (182)	24.1+24.0	*0.176*	-3.4+3.5	*0.486*	144 (79.1%)	38 (20.9%)	*0.056*	165 (90.66%)	17 (9.34%)	*0.505*	*0.516*
	A/G (191)	22.2+23.7		-3.3+3.0		155 (82.9%)	32 (17.1%)		169 (88.5%)	22 (11.5%)		
	A/A (49)	24.2+20.6		-3.4+3.5		42 (89.4%)	5 (10.6%)		44 (89.8%)	5 (10.2%)		
IL6_rs2069852	G/G (389)	22.9+23.0	*0.734*	-3.4+3.2	*0.397*	292 (82.5%)	62 (17.5%)	*0.144*	346 (88.9%)	43 (11.1%)	***0.039**^†^*	*0.06*
	G/A (42)	26.3+26.5		-3.3+3.4		28 (73.7%)	10 (22.3%)		41 (97.6%)	1 (3.4%)		
	A/A (0)	-				0	0					
IL6_rs2069861	C/C (376)	23.7+23.7	*0.261*	-3.3+3.3	*0.360*	278 (81.3%)	65 (18.7%)	*0.403*	334 (88.8%)	42 (11.2%)	***0.014****	***0.004****
	T/C (51)	21.1+21.3		-3.5+2.9		39 (86.6%)	6 (13.4%)		50 (98%)	1 (2%)		
	T/T (2)	13+12.7		-2.4+5.7		2	0		1 (50%)	1 (50%)		
IL6_rs2069835	C/C (4)	5.1+9.7	*0.351*	-4.2+3.0	*0.153*	3 (75%)	1 (25%)	*0.569*	2 (50%)	2 (50%)	***0.027**^f^*	***0.05**^f^*
	T/C (54)	19.9+21.6		-2.9+3.1		36 (79.5%)	13 (20.5%)		46 (85.2%)	8 (14.8%)		
	T/T (367)	23.9+23.5		-3.4+3.2		277 (83.1%)	56 (16.9%)		334 (88.7%)	33 (8.9%)		
IL6_rs1800796	G/G (371)	22.7+22.8	*0.548*	-3.4+3.2	*0.486*	279 (82.5%)	59 (17.5%)	*0.165*	329 (88.7%)	42 (11.3%)	***0.009****	***0.03****
	G/C (54)	25.7+26.4		-3.4+3.4		36 (75.0%)	12 (25.0%)		53 (98%)	1 (2%)		
	C/C (1)	32		-1.8		1	0		1	0		

*: significant in dominant model, **: significant in overdominant model, †: significant in codominant model, ‡: significant in recessive model, *f*: significant in log-additive model

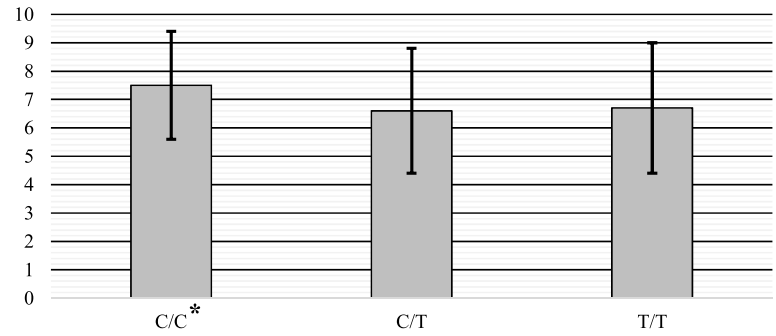

Fig. 2 Preoperative pain in microdiscectomy subgroup (mean ±SD)

Table 4 IL1B haplotype association (GLM and hapscore) with the change in pain after surgery

Change in max pain GLM model					
Haploblock	**Haplotype**	**Haplotype frequency**	**diff (95% CI)**	**hap score[a]**	***p***
IL1B rs1143634-rs1143633	C-A	0.34	-0.7 (-1.2 – (-)0.3)	-2.46742	***0.001***
	T-G	0.25	-0.2 (-0.7 – 0.3)	1.59408	*0.37*
	C-G	0.38	-3.8 (reference)	0.38051	-

[a] global *p*-value: 0.051

Number of studies supported the relationship between intervertebral disc degeneration and IL1, IL6 gene variants [23, 24, 39–42]. SNPs of these genes have been showed to be associated with the outcome of different surgical treatment [43–46], but only Moen et al. have studied the possible association of IL1 gene family and long-term outcome in patients treated because of lumbar disc herniation so far [28]. They did not find a significant relationship between *rs1143627* IL1B SNP and treatment outcome, however they did not publish the genetic effect of single SNPs but their combinations on a mixed (surgically and non-surgically treated) patient groups. The same SNP (*rs1143627)* was found to be associated with symptomatic disc herniation [26] and with DDD associated pain [25] by others. IL1B variants have been also described in association with DDD [22, 40]. IL6 variants have not been studied related to the surgical outcome of DDD yet but they were previously associated with the process of lumbar disc degeneration [24, 39, 42].

The association between IL1B, IL6 genetic variants and the therapeutic outcome after lumbar spinal surgeries can be explained by different mechanisms:

1) Progressive degeneration process can lead to persistent spinal pain and a potential indication of a subsequent surgery. IL1B is involved in multiple pathological process of disc degeneration. It stimulates extracellular matrix degradation, accelerates cellular senescence and induces apoptosis [47]. *rs1143633* in IL1B was associated with improvement of pain after surgery in our study while this SNP was found to be associated previously with the higher occurrence of disc degeneration (HIZ) [40] what can be a potential chronic pain source. IL6 variants have been also described in relation to DDD [48, 12, 42].

2) Tissue damage is often mediated through local inflammation. Inflammatory mediators such as IL6 and IL1B carry an important role in regulating and sustaining inflammation and pain. Different studies showed their potential role in disc degeneration related inflammatory process [49–51]. IL6 is crucial in homeostasis maintenance and host defence but its overproduction can cause the development or progression of diseases (such as pathologic pain) [52, 53]. The serum level of IL6 is increased in herniated disc which promotes upregulation of MMPs [53, 54]. Kraychete et al. also showed that patients with chronic low back pain due to disc herniation had higher level of serum IL6 [55]. The tissue level of IL6 can be related to the genetic variant of the gene. For example, *rs1800796* IL6 SNP (what we found to be strongly associated with FBSS) is associated with increased promoter activity boosting the local secretion of IL6 [12, 42]. The two genes have a potential influence on each other, while IL1B is described as one of the key local inducers of IL6 production [50, 51], [56]. Not surprisingly, the variants of IL1B and IL6 genes have been associated with other chronic inflammatory conditions such as periodontitis, cancer, osteoporosis, type 2 diabetes and diabetic nephropathy [33–36, 57–62].

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3) Psychological issues are also important in pain response and in the development of chronic pain. Depression and anxiety have been previously described as risk factors of DDD and poor surgical outcome after spine surgeries [4, 32, 63, 64]. Interleukin genes can significantly influence the patient's psychological profile. Chronic inflammation and dysregulation of the immune response is a key factor in the development of major depressive disorders (MDD) [65, 66]. Patients with MDD show an abnormal profile of pro- and anti-inflammatory circulating cytokines [66–69]. In animal models of MDD, increased level of pro-inflammatory cytokines caused central serotonin depletion, hypothalamic–pituitary–adrenal (HPA) axis dysregulation, microglial activation and brain structure alteration [66]. In animal inflammatory MDD (MDD-I) models, IL1B appears to be the initial triggering complex of the inflammatory cascade both centrally and peripherally [66]. In our study, some IL1B variants were significantly associated with the preoperative level of depression. These findings are in accordance with previous report about the positive association between *rs1143627* IL1B polymorphisms and MDD [70]. In accordance with our findings, Yu et al. found that the homozygotic 'T/T' patients of *rs1143634* had a tendency of suffering from less severe depressive symptoms than 'T/C' homozygotes [71]. 'T/T' genotype of *rs1143627* is reported to have a strong connection with major recurrent depression [72], in the meantime we found that patients with this particular genotype had worse scores on the depression scale. Two of the investigated SNPs, *rs16944* and *rs1143627* are located in the promoter region of the gene. These polymorphisms lead to altered expression of IL1B which results in local inflammation and promotes the production of MMPs [73]. A study suggested that IL1B *rs16944* gene polymorphism hinder the pharmacological response in the treatment of MDD by increasing the risk of non-remission over 6 weeks of antidepressant treatment [74]. Another IL1B SNP (*rs1143633)* was strongly associated with postoperative pain in our study while *rs1143634* was strongly associated with the preoperative pain intensity but only in the disc herniation subgroup. Previously, association of intensity of back pain and this SNP have been published in war veterans with posttraumatic stress disorder [75]. Association between *rs1143633* and pain have not been published yet, however there are a few studies investigating its relationship in paediatric MDD and schizophrenia [76, 77]. *rs2069861* in IL6 was associated with both depression and somatization in our cohort. Somatization is also an important factor in the development of symptomatic DDD [64]. Genetic variants of IL6 were linked to depression, somatization and anxiety in numerous studies [78–82].

Recently published data showed the possible role of interleukin agonist drugs in the treatment of pathological pain (e.g., chronic pain, inflammatory pain etc.) [83], therefore novel therapeutic strategies targeting IL6 or its receptors have been developed and successfully used in the treatment of selected diseases. In a paper a single intradiscal injection of tocilizumab (IL6 receptor antibody) provided short-term alleviation of discogenic pain [84]. Variants of the interleukins' and their receptors' genes can modify the effect of this targeted anti-inflammatory therapies, however there is no data about that so far.

In genetic association studies the sample size is highly important, as it can significantly alter the results. However, the sample size varies in the published studies, hence inconsistent genome wide association study results with non-reproducible results exist [16]. Thus, in our study we aimed to avoid sample size related study bias by using a prospective international large dataset to strengthen the findings. There are some limitations of the present study. Selection and regional population bias cannot be ruled out fully because only Caucasian patients were enrolled to the study. We did not apply any correction of the alpha-level during the genetic association testing process. We followed this method because we used a hypothesis-driven approach where effect of candidate SNPs on a phenotype was calculated. Moreover, genetic associations were tested with different statistical models (individual SNP association, haplotype analysis) to confirm the associations of the study even if the Type I error rate was not reduced. Comorbidities (e.g.: psychiatric disorders) can influence the genetic associations even if we have adjusted the statistical analyses for individual level of depression and anxiety. Therefore, study population selection bias cannot be ruled out completely. These limitations above can influence the credibility of our findings, therefore independent replications of the study are strongly recommended on different populations.

In conclusion we can state that IL1B and IL6 gene variants are associated with the psychological status and the long-term outcome of surgically treated lumbar DDD patients and these associations can be related to each other. The most plausible explanation to these findings could be linked to the major role of these cytokines in local and systemic chronic inflammation. Based on our findings and the corresponding literature advanced treatment methods could be established targeting interleukin 1B, interleukin 6 and its genes to successfully prevent/treat FBSS or even primary lumbar degenerative pathologies. On the other hand, the consideration of patient-specific genetic difference can be important to maximize the therapeutic outcome.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-022-05711-0.

Additional file 1.

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Code availability
Not applicable

Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and genotyping were done by the members of GENODISC consortium. The first draft of the manuscript was written by Adam Biczo and Aron Lazary and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during the current study are not publicly available due to there is still ongoing research on the study data, but raw data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The research protocol was in accordance with the Helsinki Declaration of 1975 on human subjects testing revised in 1996. The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council Hungary (431/PI/2007)
Subjects were included after signing a written informed consent. The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council Hungary (431/PI/2007).

Consent for publication
Not applicable

Competing interests
The authors declare they have no financial interests.

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