An Association Study of Interleukin 18 Receptor Genes (IL18R1 and IL18RAP) in Lumbar Disc Degeneration

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Abstract: *Objectives*: To examine association of candidate genetic variants in structural, inflammatory, matrix modifying, vitamin D receptor genes and variants associated with osteoarthritis, with surgical candidates and surgical patients with lumbar disc degeneration (LDD), in light of their previously reported susceptibility for LDD.

Methods: Genotyping of 146 Norwegian LDD patients and 188 Norwegian controls was performed for 20 single-nucleotide polymorphisms (SNPs) from collagen, aggrecan, interleukin, VDR, MMP3 and COX2 genes and 7 SNPs from osteoarthritic genes.

Results: The neighboring genes IL18R1 and IL18RAP polymorphisms (rs2287037 and rs1420100), showed a statistically non-significant risk for developing LDD (OR 1.36 [95 % CI 0.99 – 1.87]; p=0.06 and OR 1.33 [95 % CI 0.98-1.81]; p=0.07). Homozygosity of these risk alleles was associated with LDD (p=0.023 and p=0.027). The non-risk alleles at these SNPs were situated on a haplotype negatively associated with LDD (p=0.008). Carriage of at least one non-risk allele at both loci also reduces the risk of developing LDD (OR 0.51 [95 % CI 0.33-0.80]; p=0.003).

Conclusion: Our findings support the polygenic nature of LDD and suggest that variation in interleukin 18 receptor genes could affect the risk of severe LDD and associated low back pain.

Keywords: Candidate genes, interleukin 18 receptor 1, interleukin 18 receptor accessory protein, low back pain, lumbar disc degeneration, Single-nucleotide polymorphisms.

INTRODUCTION

About three quarters of the population of the industrialized western world, occasionally experience low back pain (LBP) in their lifetime, which is one of the commonest causes of activity limitation among the younger age group [1]. Apart from the distress and disability incurred to the patient, it also takes a heavy toll upon the socioeconomic aspect of the society [2]. Its prevalence in Norway has been estimated to be 53 % [3]. Lumbar disc degeneration (LDD) is believed to represent a major cause of LBP [4, 5], but a comprehensive definition and insight into its etiology and pathogenesis are still lacking.

Progressive structural failure can trigger an aberrant response of the disc cells, leading to degeneration of the disc [6]. A series of cellular and morphological age related changes occurs in the discs over time [7]. The prevalence of LDD is about 40 % under 30 years and over 90 % in the fifth decade of life [8]. A degenerated disc causing pain is labeled as degenerative disc disease [6] and not all radiologically assessed changes cause pain [9].

Until the beginning of the nineties, etiology was attributed mainly to the environmental influences on the normally aging disc, such as occupational or leisure time physical loading, postural stresses, vibration, injury, driving and smoking [10-14]. Later, twin studies established a strong familial predisposition and emphasis was laid on major contribution from genetic factors [15-18]. It is quite likely that the LDD has a multifactorial pathogenesis, also involving gene environment interactions [19].

Genes related to the molecular components of the disc and associated biochemical pathways have been explored as candidate genes for LDD. The extracellular matrix of aggrecan containing gelatinous nucleus and fibro cartilaginous annulus fibrosus is mainly composed of Collagen I and II, with Collagen I predominantly in the annulus and Collagen II in nucleus. Collagens V, VI, IX, XI, XII and XIV also contribute to the matrix though scarcely [20]. Disc degeneration involves dehydration due to fragmentation and eventual loss of aggrecan from the nucleus, causing it to behave non-hydrostatically under load [21]. This leads to disc height reduction and eventually osteoarthritis of apophyseal joints. Aggrecan, also prevents large pro inflammatory molecules from entering into the matrix [22] and with its loss, there is an increased inflow of such molecules which can cause degeneration by enzymatic break down and local inflammation [23]. Associated pain can occur due to sensitization of nerve root by these inflammatory molecules secreted by the cells of a degenerated, herniated disc such as prostaglandin E2, phospholipase A2, MMPs, TNF-α and interleukins [24, 25].

Hence all genes coding for the structural proteins of a disc, those involved in inflammation, matrix turnover and

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degradation are ideal candidates to be analyzed for exploring the role of genetics in disc degeneration. So far significant associations for the polymorphisms of aggrecan (AGC), collagens (COL1A1, COL9A1, COL9A2, COL9A3, COL11A1, COL11A2), vitamin D receptor (VDR), matrix metalloproteinases (MMP2, MMP3, MMP9), interleukins (IL1, IL2, IL18R1, IL18RAP), cyclooxygenase-2 (COX2) and cartilage intermediate layer protein (CILP) have been reported with different pathologic changes of disc degeneration and clinical phenotypes [26-44]. To our knowledge, the association of these gene candidates has never been tested in a Norwegian LDD cohort.

Osteoarthritis in general, is another morbid condition that has similarities in clinical phenotypes with LDD [45] which involves OA of facet joints. Genes coding for frizzled-related protein (FZRP), calmodulin (CALM1), growth and differentiation factor 5 (GDF5), prostaglandin-endoper-oxidase synthase-2 (PTGS2) and double von willebrand factor A domains (DVWA) have been observed to be associated with osteoarthritis but have not been studied in LDD [46-49].

The aims of this study were to examine the allelic diversity of structural, inflammatory, matrix modifying, vitamin D receptor gene variants and variants previously associated with osteoarthritis and their association with surgical candidates and surgical patients with LDD.

SUBJECTS AND METHODS

Cases and Controls

This study included 146 unrelated Norwegian patients, with chronic low back pain and disc degeneration. The age of the patients ranged from 30 to 76 years (mean = 53.2 years). Among whom, 80 were females and 66 were males and their age at the first time experience of back pain ranged from 10 to 61 years (mean [SD], 32.9 [10.3]). Patients were recruited from two randomized controlled trials (RCTs) [50, 51], a pilot study [52] and had been evaluated to have LBP, disc degeneration and as candidates for surgery at least 8-9 years ago. Patients had to have pain duration for at least 1 year and degeneration at L4-L5 or L5-S1, as evaluated by plain radiography and either computed tomography or magnetic resonance imaging. Among them 27 were treated conservatively, 73 had lumbar fusion and 46 underwent discectomy (Table **1**). The control group comprised of 188

healthy Norwegian individuals from the Norwegian Bone Marrow Donor Registry with an unknown history of back pain and age ranging from 24 to 56 years (mean = 39.3 years). Half of the controls were males and half females. Patients were asked about ethnicity, age of onset, duration of pain, family history of back pain, and smoking through a standard questionnaire. Patients were asked if they were smoking at inclusion and the number of cigarettes they smoked per day. 141 patients had one or more first degree relatives with back pain but 5 patients did not have any known family members with back pain history. All subjects received detailed written information about the study procedures and provided informed consent before their participation. The study was approved by the Regional Committee for Medical Research Ethics in Health Region South-East Norway.

Selection of Candidate Gene SNPs

A total of 27 single-nucleotide polymorphisms (SNPs) from 20 different candidate genes were tested (Table **2**). 20 SNPs from 14 genes with a biologic relevance for disc structure, biochemistry and mechanics were selected for genotyping, based upon previously reported association with LDD in different study populations. Variants that have previously shown significant associations with different LDD phenotypes were focused. Variants of some genes that had not been found to be significantly associated, but were biologically relevant were also included. Altogether SNPs from collagen genes (COL1A1, COL2A1, COL3A1, COL9A1, COL9A2, COL9A3, COL11A1 and COL11A2), aggrecan gene (AGC1), vitamin D receptor gene (VDR), interleukin genes (IL1A, IL18R1, and IL18RAP), matrix metalloproteinase-3 gene (MMP3), and cyclooxygenase-2 gene (COX2). Furthermore, seven SNPs from six genes (PTGS2, CALM1, FZRP, DVWA and GDF5) were tested on the basis of their association reported with osteoarthritis, due to its clinical similarities to our phenotype of interest.

Genotyping

Genomic DNA from 140 patients, was extracted manually from 9 ml of peripheral blood leucocytes by the salting out method [53] and from the remaining 6 patients, the DNA was extracted manually from 4 ml of saliva sample using the collection kit (DNA Genotek, Kanata, Ontario, Canada) following the protocol by the manufacturer. DNA

Table 1.   Demographic and Disease Characteristics Cases with Chronic Low Back Pain Given as Numbers N, Unless Stated Otherwise

Total number of cases	146 patients with lumbar disc degeneration
Ethnicity	Norwegian
Gender (Males/Females)	68/78
Age (8 year follow up), years	Range: 30-76, Mean [SD]: 53.2 [8.8]
Age of Onset, years	Range: 10-61, Mean [SD]: 32.9 [10.3]
Duration of pain (baseline), months	Range: 10-480, Mean [SD]: 128.8 [103.04]
Smokers/non-smokers/unknown	68/69/9
Surgical status (None/discectomy/lumbar fusion)	27/46/73
History of back pain in first degree relatives, with no treatment/conservative treatment/surgery/unknown	19/82/40/5

Table 2.   Selected SNP Candidates for Lumbar Disc Degeneration

Gene	SNP*	Chromosome	Location	Minor/Major Allele	References
AGC1	rs1042631	15q26.1	Exon (L2141L)	T/C	[26]
AGC1	rs1516797	15q26.1	Intron	G/T	[26]
VDR	rs10735810	12q13.11	Exon (M162T)	C/T	[37] [70]
VDR	rs731236	12q13.11	Exon (I352I)	C/T	[37] [70]
COL1A1	rs2075555	17q21.33	Intron	A/C	[26] [29]
COL2A1	rs917055	12q13.11	Intron	T/C	[26]
COL3A1	rs2056156	2q32.2	Intron	C/T	[26]
COL9A1	rs696990	6q13	Intergenic	G/A	[26]
COL9A2	rs7533552	1p34.2	Exon (Q326R)	G/A	[26] [30] [31]
COL9A3	rs61734651	20q13.33	Exon (R103W)	C/T	[32] [33]
COL11A1	rs1463035	1p21.1	Intron	G/A	[26]
COL11A1	rs1676486	1p21.1	Exon (S1535P)	A/G	[34]
COL11A2	rs2072915	6p21.32	Upstream	T/A	[26]
IL1A	rs2071375	2q13	Intron	A/G	[26] [42]
IL18R1	rs2287037	2q12.1	Upstream	A/G	[26]
IL18RAP	rs1420100	2q12.1	Intron	T/G	[26]
IL18RAP	rs1420106	2q12.1	Upstream	T/C	[26]
IL18RAP	rs917997	2q12.1	Downstream	A/G	[26]
MMP3	rs72520913	11q22.2	Upstream	-/A	[38]
COX2(PTGS2)	rs5277	1q31.1	Exon (V102V)	C/G	[43]
PTGS2(COX2)	rs4140564	1q31.1	Upstream	C/T	[49]
CALM1	rs12885713	14q32.11	5'UTR	C/T	[47]
DVWA	rs11718863	3p25.1	Exon	T/A	[48]
DVWA	rs7639618	3p25.1	Exon	T/C	[48]
FZRP	rs288326	2q32.1	Exon (R200W)	A/G	[46]
FZRP	rs7775	2q32.1	Exon (R324G)	G/C	[46]
GDF5	rs143383	20q11.22	5'UTR	C/T	[48]

* Single-nucleotide polymorphism.

quality and quantity were measured on a DNA spectrophotometer (ND-1000, NanoDrop, Wilmington USA). All the DNA samples were of good quality and quantity and had ratios within 1.8-2.0 at A260/280 and within 1.8-2.2 at A260/230. DNA from the controls was available before hand. Genotyping was performed using a Autoflex instrument and iplex kits from Sequenom[TM] (Hamburg, Germany), at the Centre for interactive genetics; Cigene, Norwegian University of Life Sciences (UMB) Aas. 20 ng DNA was used and one negative control (water) was included with every 95 samples with variable positions, so that it served both as technical and positional control. Real-time genotype calling was followed by manual inspection and where necessary adjustment.

Statistical Analysis

Genotype success rate, Hardy-Weinberg equilibrium (HWE), linkage disequilibrium and allelic associations were calculated using Haploview version 4.2 [54]. Unphased version 3.0.7 [55] was used to estimate haplotypes by the expectation-maximization algorithm and to test for association, as well as to calculate linkage disequilibrium. In accordance with the approach of this statistical programme for haplotype analysis, one allele was compared to a reference haplotype. Odds ratio (OR) with 95 % CI was calculated with respect to minor allele compared to major allele, using the Haldanes modification of Woolf's method [56, 57]. P-value threshold of ≤ 0.05 was chosen based upon the fact that it was a replication study of variants already reported to be significantly associated with LDD. At this threshold value, we had 80 % power to detect a genotype effect of frequency = 0.3 with an OR > 1.9 and an allele effect of frequency = 0.4 with an OR > 1.5. Power calculations were done in PS Power and Sample Size Calculations Version 3.0. The p-value threshold after Bonferroni correction for multiple testing was calculated to be 0.0019, as we tested 27 SNPs (0.05/27).

RESULTS

The Risk of LDD is Associated with IL18R1 and IL18RAP on Chromosome 2q12

The genotype success rate of the 27 candidate SNPs was ≥ 95 % with a mean success rate of 99.2 %. None of the selected SNPs was excluded due to technical problems with genotyping, and no divergence from Hardy-Weinberg equilibrium was observed for any of the markers (p ≥ 0.001). Allele frequencies of the 27 SNPs in both cases and controls are given in Table **3**. Two polymorphisms, rs2287037 from Interleukin 18 receptor 1 (IL18R1) gene and rs1420100 from Interleukin 18 receptor accessory protein (IL18RAP) gene showed a statistically non-significant association (p=0.06 and p=0.07). Both these SNPs were in HWE (p values for rs2287037 and rs1420100 were 0.51 and 0.81 respectively).

Compared to the controls, there was a non-significant increase in risk of LDD for both IL18R1 (OR 1.36, 95 % CI 0.99 – 1.87) and IL18RAP (OR 1.33 95 % CI 0.98-1.81).

The CC genotype at IL18R1 polymorphism (rs2287037) was associated (p=0.023) with LDD and was more frequent among cases (45.2%) compared to controls (33.0 %). For IL18RAP, an association was observed for the AA genotype at rs1420100 (p= 0.027). Both polymorphisms followed a recessive disease model with a risk for LDD in cases which were homozygous for risk allele (OR 1.67 [95 % CI 1.07-2.60] and OR 1.83 [95% CI 1.07-3.14], respectively).

Our associations were not statistically significant after Bonferroni correction.

Conversely the other alleles at these SNPs were negatively associated with LDD, and interestingly they were

Table 3. **Association Analysis of the Structural, Inflammatory, Matrix Degradative, Vitamin D Receptor and Osteoarthritis Associated Gene Variants with LDD**

SNP***	Gene	Risk Allele	RAF** Cases	RAF Control	OR*(95% CI)	p Value
rs1042631	*AGC1*	T	0.212	0.176	1.27 (0.86-1.86)	0.23
rs1516797	*AGC1*	T	0.641	0.622	1.09 (0.79-1.50)	0.60
rs10735810	*VDR*	A	0.401	0.370	1.14 (0.83-1.56)	0.41
rs731236	*VDR*	A	0.603	0.568	1.16 (0.85-1.58)	0.35
rs2075555	*COL1A1*	G	0.873	0.864	1.08 (0.69-1.70)	0.74
rs917055	*COL2A1*	A	0.168	0.168	1.00 (0.67-1.51)	0.99
rs2056156	*COL3A1*	C	0.441	0.420	1.09 (0.80-1.48)	0.58
rs696990	*COL9A1*	C	0.210	0.173	1.27 (0.86-1.87)	0.22
rs7533552	*COL9A2*	T	0.757	0.731	1.14 (0.80-1.63)	0.45
rs61734651	*COL9A3*	C	0.942	0.939	1.05 (0.56-1.97)	0.87
rs1463035	*COL11A1*	T	0.810	0.797	1.08 (0.74-1.59)	0.68
rs1676486	*COL11A1*	A	0.229	0.217	1.07 (0.74-1.55)	0.72
rs2072915	*COL11A2*	T	0.731	0.714	1.09 (0.77-1.54)	0.62
rs2071375	*IL1A*	C	0.700	0.670	1.15 (0.82-1.60)	0.42
rs2287037	*IL18R1*	C	0.668	0.596	1.36 (0.99-1.87)	0.06
rs1420100	*IL18RAP*	A	0.493	0.422	1.33 (0.98-1.81)	0.07
rs1420106	*1L18RAP*	A	0.253	0.239	1.08 (0.76-1.54)	0.68
rs917997	*IL18RAP*	T	0.262	0.235	1.16 (0.81-1.65)	0.43
rs72520913	*MMP3*	A	0.497	0.470	1.11 (0.82-1.51)	0.50
rs5277	*COX2(PTGS2)*	C	0.836	0.832	1.02 (0.68-1.54)	0.91
rs4140564	*PTGS2(COX2)*	A	0.949	0.931	1.35 (0.72-2.56)	0.34
rs12885713	*CALM1*	C	0.451	0.406	1.20 (0.88-1.64)	0.25
rs11718863	*DVWA*	A	0.842	0.824	1.14 (0.76-1.71)	0.54
rs7639618	*DVWA*	C	0.842	0.824	1.14 (0.76-1.71)	0.54
rs288326	*FZRP*	A	0.123	0.109	1.15 (0.72-1.84)	0.57
rs7775	*FZRP*	C	0.083	0.059	1.42 (0.79-2.56)	0.24
rs143383	*GDF5*	A	0.651	0.629	1.10 (0.79-1.52)	0.57

*Odds ratio (95 % Confidence interval); ** Risk allele frequency; *** Single-nucleotide polymorphism.
§rs2287037, allele counts in cases: 66 CC, 63 CT, 17 TT; allele counts in controls: 62 CC, 100 CT and 26 TT.
rs1420100, allele counts in cases: 37 AA, 69 AC, 39 CC; allele counts in controls: 29 AA, 98 AC and 58 CC.

situated on a haplotype rs2287037*T-rs1420100*C that were less frequent in cases compared to controls (23.7 % *vs* 34.3 %, p=0.008; Table **4**). The linkage disequilibrium between these two alleles was modest; D'<0.65 and r^2<0.2. Hence, since carriage of non-risk allele seemed to protect against development of LDD at both loci, we tested the distribution of simultaneously carrying at least one non-risk allele at both loci and found it to reduce the risk of developing LDD (OR 0.51 [95 % CI 0.33-0.80]; p= 0.003).

Table 4.   Association Analysis of Estimated Haplotypes of IL18R1 (rs2287037) and IL18RAP (rs1420100)

Haplotype	Cases n (%)	Controls n (%)	OR** (95 % CI)	p Value
T - C	68.8 (23.7)	126.9 (34.3)	0.63 (0.43-0.93)	0.008
C – A*	114.8 (39.6)	133.9 (36.2)	1.0	0.176
C - C	78.2 (27.0)	87.1 (23.5)	1.05 (0.68-1.61)	0.563
T - A	28.2 (9.7)	22.1 (6.0)	1.49 (0.71-3.10)	0.225

*Reference haplotype; ** Odds ratio (95 % Confidence interval).

Association of IL18RAP-IL18R1 in Non-Smokers

Smoking is an environmental factor that has been shown to influence the risk of LDD according to some studies [58]. The frequency of the protective haplotype, rs2287037*T-rs1420100*C was lowest among the non-smoking patients (19.6 %) compared to LDD smokers (28.5 %) and controls (34.3 %). Hence the haplotype only conferred a reduced risk of LDD among non-smoking patients compared to controls (p=0.007). Cases were considered as smokers if they were smoking at inclusion. Unfortunately, smoking status was not available among controls. Significance was not reached when smokers were compared with non-smokers among LDD patients only (p=0.2).

DISCUSSION

Alleles at IL18RAP (rs1420100) and IL18R1 (rs2287037) SNPs did not show a statistically significant association individually, but the tendency of association for IL18R1 (rs2287037) showed a similar trend as in a previous study (p = 0.054) by Videman *et al.* [26]. Homozygosity of risk alleles was associated with the risk of LDD. The non-risk alleles of two polymorphisms were negatively associated with LDD when looking at them together on a haplotype. This haplotype association might be in linkage disequilibrium with a locus involved in predisposition to LDD or it might mark several risk polymorphisms, either including or excluding those tested here in this interleukin gene cluster on chromosome 2q12.

In accordance with our findings, a study of 588 monozygotic and dizygotic male twins from the Finish population, has shown association of IL18RAP (rs1420100) with disc desiccation but not with disc height narrowing or bulging [26]. Videman *et al.* observed an association between individuals with AC genotype and a higher disc signal intensity, and a nearly identical association of AA and CC alleles with a lower disc signal intensity (personal communication), while we found the CC genotype to predispose to LDD. We observed haplotypic association for

across IL18R1 and IL18RAP for rs2287037*T-rs1420100*C. As for many complex disorders, inter study heterogeneity also exists for LDD [59], particularly since the markers tested in studies are likely to be secondary to the causal variants and therefore inter-population differences in LD patterns will influence the single SNP association outcome. Videman *et al.* also found another polymorphism of the IL18RAP (rs917997) to be associated with disc desiccation, but no association was observed for this polymorphism in our sample. They found rs917997 to be tagging with the haplotype of IL1RL1, IL18R1, IL18RAP, and SLC9A4. This gene cluster has been shown to be associated with other inflammatory disorders, like inflammatory bowel disease [60], coeliac disease [61] and asthma. Our findings suggest that inflammation also play a role in patients with chronic LBP and disc degeneration.

IL18RAP is a subunit of IL18 receptor along with IL18R1 [62] and is essential for IL18 signal transduction and ligand binding affinity to IL18Rα and hence plays a functional role of regulation of both innate and adaptive immunity [63]. Activation of T cells and natural killer cells results in secretion of interferon gamma (IFN-γ), which in turn activates macrophages that secrete cytokines TNF-α and IL1, which are involved in increased matrix degradation both directly and through activation of proteinases and have been found to be secreted by the cells of a degenerated and herniated disc [23, 24, 64]. We speculate that polymorphisms of IL18RAP and IL18R1 might play a role by triggering an inflammatory response through one of the above mentioned mechanisms and hence shed light on the role of inflammation in LDD and a possible pathway for associated back pain [24]. While the presence of disc degeneration in many asymptomatic individual indicates an unclear relationship with back pain [65], high levels of cytokines like TNF-α have been found in degenerated discs where the nerve ending is exposed to enzymes and inflammatory mediators, which may explain pain in discogenic patients [66].

The polymorphism of IL18R1 (rs2287037) has previously also showed a tendency towards association with disc desiccation (p=0.054) in the study by Videman *et al.* [26]. Videman *et al.* observed an association between individuals with CC alleles and lowest disc signal intensity and TT alleles were found to be associated with higher disc signal intensity (personal communication). Our results support these finding of Videman *et al.* as in the current study the cases with CC alleles were found to be associated with LDD. To our knowledge, this is only the second time that a similar trend in association of IL18R1 polymorphism rs2287037 with LDD has been reported. The IL18R1 gene, along with four other members of the interleukin 1 receptor family (IL1R2, IL1R1, ILRL2, and IL1RL1) form a gene cluster on chromosome 2q. IL1R1 has shown to be associated with hand OA [67] and a genome wide linkage study have found a region of interest that could harbor genes for both LDD and OA [68]. Over expression of IL18 and IL18R has also been proposed to play a role in pathogenesis of rheumatoid arthritis [69].

We cannot exclude that our results could be false positive because of a small sample size and the fact that our associations did not withstand Bonferroni correction for

multiple testing. On the other hand, the associations were with genetic variants already been reported to be associated with LDD. Hence in the future we intend to replicate our findings in a much larger European population evaluated for disc signal as well as advanced findings of LDD.

It is foremost to define a clear phenotype of disc degeneration [6] because this is a multi stage process progressing from decreased disc signal intensity (disc desiccation) to disc height narrowing, disc bulging, end plate degeneration and osteophytosis of facet joints [70]. Videman *et al.* showed association of IL18RAP and IL18R1 with an early stage of degeneration (disc desiccation). In contrast, our findings suggest associations in a patient population with severe chronic low back pain and disc degeneration, with majority had lumbar fusion and hence this group is considered to have advanced lumbar disc disease.

We however did not find any associations of other structural, matrix degrading, Vitamin D and osteoarthritic genes with advanced stage degeneration in our patients. Videman *et al.* on the other hand did find 10 such associations but most of them with an early stage of degeneration (disc desiccation) [26]. Inability to detect any such associations in our study could be due to limitations of small sample size, not covering all variants of the candidate genes and lack of advanced imaging (no information on disc signal intensity and disc bulging). Videman *et al.* had an enhanced ability to detect the associations with an early stage LDD, due to an accurate method of quantitative measurement of degeneration using cerebrospinal fluid adjusted disc signal intensity, which we lacked and they studied 99 variants compared to 27 in our study. This reduced our power and precision to estimate the association. The DNA from control group had been collected previously, but was not linked to information about back pain and degeneration. Although they were considered healthy at inclusion, we would expect that some of them had disc degeneration and had experienced back pain. The association of IL18RAP-IL18R1 haplotype in non smokers could also be false positive due to a small sample, lack of information on smoking status of controls and that smokers among cases were smoking at inclusion.

The proportion of LDD and its associated LBP in patients was supposed to be higher than controls because they attended the orthopedic clinic and had either undergone or were candidates for surgery. We could have enhanced our power to detect association for other genes if we compared patients with controls without any degeneration, or even better if we have had access to a larger patient cohort. The study design of Videman *et al*, on the other hand, was not case-control based. Our study had genetic homogeneity (all subjects were of homogenous Norwegian origin) [71], a high genotype success rate (94.9 % to 100 %), no SNP was excluded due to technical issues with genotyping and there was no deviation of any SNP from Hardy Weinberg equilibrium.

Our study is supportive of a polygenic nature of lumbar disc degeneration and suggests that variation in interleukin 18 receptor genes could affect the risk of advanced stage of disc degeneration and its associated LBP. Our findings need to be tested in a larger sample with an accurate phenotype classification.

ABBREVIATIONS

LDD   =   Lumbar disc degeneration

LBP   =   Low back pain

SNPs   =   Single-nucleotide polymorphisms

IL18R1   =   Interleukin 18 receptor 1

IL18RAP   =   Interleukin 18 receptor accessory protein.

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CONFLICT OF INTEREST

Declared none.

REFERENCES

[1]   Andersson GB. Epidemiological features of chronic low-back pain. Lancet 1999; 354(9178): 581-5.

[2]   Katz JN. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am 2006; 88(Suppl. 2): 21-4.

[3]   Natvig B, Nessioy I, Bruusgaard D, Rutle O. [Musculoskeletal complaints in a population. Occurrence and localization]. Tidsskr Nor Laegeforen 1994; 114(3): 323-7.

[4]   Andersson GB, An HS, Oegema TR Jr, Setton LA. Directions for future research. J Bone Joint Surg Am 2006; 88(Suppl 2): 110-4.

[5]   Deyo RA, Weinstein JN. Low back pain. N Engl J Med 2001; 344(5): 363-70.

[6]   Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine (Phila Pa 1976) 2006; 31(18): 2151-61.

[7]   Buckwalter JA. Aging and degeneration of the human intervertebral disc. Spine (Phila Pa 1976) 1995; 20(11): 1307-14.

[8]   Cheung KM, Karppinen J, Chan D, *et al*. Prevalence and pattern of lumbar magnetic resonance imaging changes in a population study of one thousand forty-three individuals. Spine (Phila Pa 1976) 2009; 34(9): 934-40.

[9]   Videman T, Battie MC, Gibbons LE, Maravilla K, Manninen H, Kaprio J. Associations between back pain history and lumbar MRI findings. Spine (Phila Pa 1976) 2003; 28(6): 582-8.

[10]   Heliovaara M. Risk factors for low back pain and sciatica. Ann Med 1989; 21(4): 257-64.

[11]   Kelsey JL, Githens PB, O'Conner T, *et al*. Acute prolapsed lumbar intervertebral disc. An epidemiologic study with special reference to driving automobiles and cigarette smoking. Spine (Phila Pa 1976) 1984; 9(6): 608-13.

[12]   Kelsey JL, Githens PB, White AA, *et al*. An epidemiologic study of lifting and twisting on the job and risk for acute prolapsed lumbar intervertebral disc. J Orthop Res 1984; 2(1): 61-6.

[13]   Pope MH, Hansson TH. Vibration of the spine and low back pain. Clin Orthop Relat Res 1992; (279): 49-59.

[14]   Svensson HO, Andersson GB. Low-back pain in 40- to 47-year-old men: work history and work environment factors. Spine (Phila Pa 1976) 1983; 8(3): 272-6.

[15]   Ala-Kokko L. Genetic risk factors for lumbar disc disease. Ann Med 2002; 34(1): 42-7.

[16]   Kalichman L, Hunter DJ. The genetics of intervertebral disc degeneration. Familial predisposition and heritability estimation. Joint Bone Spine 2008; 75(4): 383-7.

[17]   Matsui H, Kanamori M, Ishihara H, Yudoh K, Naruse Y, Tsuji H. Familial predisposition for lumbar degenerative disc disease. A case-control study. Spine (Phila Pa 1976) 1998; 23(9): 1029-34.

[18]   Richardson JK, Chung T, Schultz JS, Hurvitz E. A familial predisposition toward lumbar disc injury. Spine (Phila Pa 1976) 1997; 22(13): 1487-92; discussion 93.

[19]   Chan D, Song Y, Sham P, Cheung KM. Genetics of disc degeneration. Eur Spine J 2006; 15(Suppl 3): S317-25.

[20]   Eyre DR, Matsui Y, Wu JJ. Collagen polymorphisms of the intervertebral disc. Biochem Soc Trans 2002; 30(Pt 6): 844-8.

[21]   Adams MA, McNally DS, Dolan P. 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. J Bone Joint Surg Br 1996; 78(6): 965-72.

[22]   Maroudas A. Biophysical chemistry of cartilaginous tissues with special reference to solute and fluid transport. Biorheology 1975; 12(3-4): 233-48.

[23]   Rannou F, Corvol MT, Hudry C, et al. Sensitivity of anulus fibrosus cells to interleukin 1 beta. Comparison with articular chondrocytes. Spine (Phila Pa 1976) 2000; 25(1): 17-23.

[24]   Cavanaugh JM. Neural mechanisms of lumbar pain. Spine (Phila Pa 1976) 1995; 20(16): 1804-9.

[25]   Kang JD, Georgescu HI, McIntyre-Larkin L, Stefanovic-Racic M, Donaldson WF, 3rd, Evans CH. Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. Spine (Phila Pa 1976) 1996; 21(3): 271-7.

[26]   Videman T, Saarela J, Kaprio J, et al. Associations of 25 structural, degradative, and inflammatory candidate genes with lumbar disc desiccation, bulging, and height narrowing. Arthritis Rheum 2009; 60(2): 470-81.

[27]   Solovieva S, Noponen N, Mannikko M, et al. Association between the aggrecan gene variable number of tandem repeats polymorphism and intervertebral disc degeneration. Spine (Phila Pa 1976) 2007; 32(16): 1700-5.

[28]   Cong L, Pang H, Xuan D, Tu GJ. Association between the expression of aggrecan and the distribution of aggrecan gene variable number of tandem repeats with symptomatic lumbar disc herniation in Chinese Han of Northern China. Spine (Phila Pa 1976) 2010; 35(14): 1371-6.

[29]   Pluijm SM, van Essen HW, Bravenboer N, et al. Collagen type I alpha1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. Ann Rheum Dis 2004; 63(1): 71-7.

[30]   Jim JJ, Noponen-Hietala N, Cheung KM, et al. The TRP2 allele of COL9A2 is an age-dependent risk factor for the development and severity of intervertebral disc degeneration. Spine (Phila Pa 1976) 2005; 30(24): 2735-42.

[31]   Seki S, Kawaguchi Y, Mori M, et al. Association study of COL9A2 with lumbar disc disease in the Japanese population. J Hum Genet 2006; 51(12): 1063-7.

[32]   Paassilta P, Lohiniva J, Goring HH, et al. Identification of a novel common genetic risk factor for lumbar disk disease. JAMA 2001; 285(14): 1843-9.

[33]   Solovieva S, Lohiniva J, Leino-Arjas P, et al. Intervertebral disc degeneration in relation to the COL9A3 and the IL-1ss gene polymorphisms. Eur Spine J 2006; 15(5): 613-9.

[34]   Mio F, Chiba K, Hirose Y, et al. A functional polymorphism in COL11A1, which encodes the alpha 1 chain of type XI collagen, is associated with susceptibility to lumbar disc herniation. Am J Hum Genet 2007; 81(6): 1271-7.

[35]   Koshizuka Y, Ogata N, Shiraki M, et al. Distinct association of gene polymorphisms of estrogen receptor and vitamin D receptor with lumbar spondylosis in post-menopausal women. Eur Spine J 2006; 15(10): 1521-8.

[36]   Kawaguchi Y, Kanamori M, Ishihara H, Ohmori K, Matsui H, Kimura T. The association of lumbar disc disease with vitamin-D receptor gene polymorphism. J Bone Joint Surg Am 2002; 84-A(11): 2022-8.

[37]   Videman T, Gibbons LE, Battie MC, et al. The relative roles of intragenic polymorphisms of the vitamin d receptor gene in lumbar spine degeneration and bone density. Spine (Phila Pa 1976) 2001; 26(3): E7-12.

[38]   Takahashi M, Haro H, Wakabayashi Y, Kawa-uchi T, Komori H, Shinomiya K. The association of degeneration of the intervertebral disc with 5a/6a polymorphism in the promoter of the human matrix metalloproteinase-3 gene. J Bone Joint Surg Br 2001; 83(4): 491-5.

[39]   Dong DM, Yao M, Liu B, Sun CY, Jiang YQ, Wang YS. Association between the -1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults. Eur Spine J 2007; 16(11): 1958-61.

[40]   Yuan HY, Tang Y, Liang YX, et al. Matrix metalloproteinase-3 and vitamin d receptor genetic polymorphisms, and their interactions with occupational exposure in lumbar disc degeneration. J Occup Health 2010; 52(1): 23-30.

[41]   Sun ZM, Miao L, Zhang YG, Ming L. Association between the -1562 C/T polymorphism of matrix metalloproteinase-9 gene and lumbar disc disease in the young adult population in North China. Connect Tissue Res 2009; 50(3): 181-5.

[42]   Solovieva S, Kouhia S, Leino-Arjas P, et al. Interleukin 1 polymorphisms and intervertebral disc degeneration. Epidemiology 2004; 15(5): 626-33.

[43]   Valdes AM, Hassett G, Hart DJ, Spector TD. Radiographic progression of lumbar spine disc degeneration is influenced by variation at inflammatory genes: a candidate SNP association study in the Chingford cohort. Spine (Phila Pa 1976) 2005; 30(21): 2445-51.

[44]   Seki S, Kawaguchi Y, Chiba K, et al. A functional SNP in CILP, encoding cartilage intermediate layer protein, is associated with susceptibility to lumbar disc disease. Nat Genet 2005; 37(6): 607-12.

[45]   Fujiwara A, Tamai K, An HS, et al. The relationship between disc degeneration, facet joint osteoarthritis, and stability of the degenerative lumbar spine. J Spinal Disord 2000; 13(5): 444-50.

[46]   Loughlin J, Dowling B, Chapman K, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci USA 2004; 101(26): 9757-62.

[47]   Poulou M, Kaliakatsos M, Tsezou A, Kanavakis E, Malizos KN, Tzetis M. Association of the CALM1 core promoter polymorphism with knee osteoarthritis in patients of Greek origin. Genet Test 2008; 12(2): 263-5.

[48]   Valdes AM, Spector TD, Doherty S, Wheeler M, Hart DJ, Doherty M. Association of the DVWA and GDF5 polymorphisms with osteoarthritis in UK populations. Ann Rheum Dis 2009; 68(12): 1916-20.

[49]   Valdes AM, Loughlin J, Timms KM, et al. Genome-wide association scan identifies a prostaglandin-endoperoxide synthase 2 variant involved in risk of knee osteoarthritis. Am J Hum Genet 2008; 82(6): 1231-40.

[50]   Brox JI, Reikeras O, Nygaard O, et al. Lumbar instrumented fusion compared with cognitive intervention and exercises in patients with chronic back pain after previous surgery for disc herniation: a prospective randomized controlled study. Pain 2006; 122(1-2): 145-55.

[51]   Brox JI, Sorensen R, Friis A, et al. Randomized clinical trial of lumbar instrumented fusion and cognitive intervention and exercises in patients with chronic low back pain and disc degeneration. Spine (Phila Pa 1976) 2003; 28(17): 1913-21.

[52]   Brox JI, Friis A, Holm I, et al. [Patients with chronic degenerative spinal disease--can conservative treatment reduce the waiting list for surgery?]. Tidsskr Nor Laegeforen 1999; 119(12): 1784-7.

[53]   Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3): 1215.

[54]   Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21(2): 263-5.

[55]   Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003; 25(2): 115-21.

[56]   Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 1956; 20(4): 309-11.

[57]   Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955; 19(4): 251-3.

[58]   Battie MC, Videman T, Gill K, et al. 1991 Volvo Award in clinical sciences. Smoking and lumbar intervertebral disc degeneration: an MRI study of identical twins. Spine (Phila Pa 1976) 1991; 16(9): 1015-21.

[59]   Wang X, Ria M, Kelmenson PM, et al. Positional identification of TNFSF4, encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. Nat Genet 2005; 37(4): 365-72.

[60]   Zhernakova A, Festen EM, Franke L, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. Am J Hum Genet 2008; 82(5): 1202-10.

[61]   Koskinen LL, Einarsdottir E, Dukes E, et al. Association study of the IL18RAP locus in three European populations with coeliac disease. Hum Mol Genet 2009; 18(6): 1148-55.

[62] Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc Natl Acad Sci USA 1999; 96(5): 2256-61.

[63] Fiszer D, Rozwadowska N, Rychlewski L, Kosicki W, Kurpisz M. Identification of IL-18RAP mRNA truncated splice variants in human testis and the other human tissues. Cytokine 2007; 39(3): 178-83.

[64] Doita M, Kanatani T, Ozaki T, Matsui N, Kurosaka M, Yoshiya S. Influence of macrophage infiltration of herniated disc tissue on the production of matrix metalloproteinases leading to disc resorption. Spine (Phila Pa 1976) 2001; 26(14): 1522-7.

[65] Jensen MC, Brant-Zawadzki MN, Obuchowski N, Modic MT, Malkasian D, Ross JS. Magnetic resonance imaging of the lumbar spine in people without back pain. N Engl J Med 1994; 331(2): 69-73.

[66] Lee S, Moon CS, Sul D, *et al.* Comparison of growth factor and cytokine expression in patients with degenerated disc disease and herniated nucleus pulposus. Clin Biochem 2009; 42(15): 1504-11.

[67] Nakki A, Kouhia ST, Saarela J, *et al.* Allelic variants of IL1R1 gene associate with severe hand osteoarthritis. BMC Med Genet 2010; 11(1): 50.

[68] Williams FM, Kato BS, Livshits G, Sambrook PN, Spector TD, MacGregor AJ. Lumbar disc disease shows linkage to chromosome 19 overlapping with a QTL for hand OA. Ann Rheum Dis 2008; 67(1): 117-9.

[69] Shao XT, Feng L, Gu LJ, *et al.* Expression of interleukin-18, IL-18BP, and IL-18R in serum, synovial fluid, and synovial tissue in patients with rheumatoid arthritis. Clin Exp Med 2009; 9(3): 215-21.

[70] Videman T, Leppavuori J, Kaprio J, *et al.* Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. Spine (Phila Pa 1976) 1998; 23(23): 2477-85.

[71] Novembre J, Johnson T, Bryc K, *et al.* Genes mirror geography within Europe. Nature 2008; 456(7218): 98-101.