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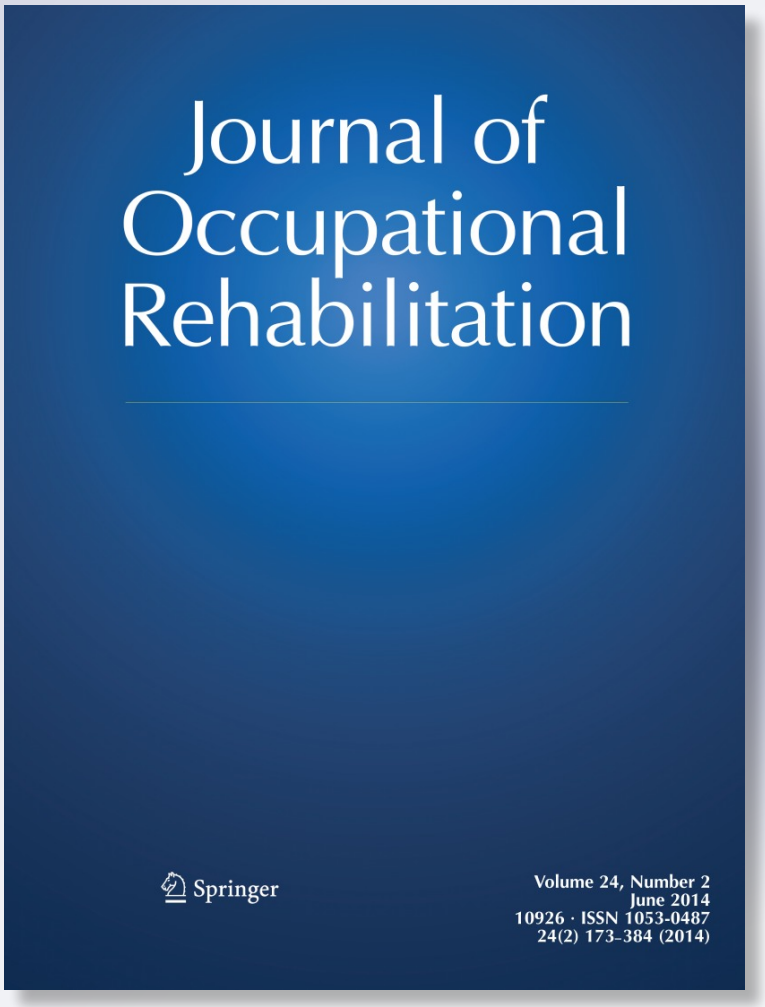

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Matrix Metalloproteinase-3, Vitamin D Receptor Gene Polymorphisms, and Occupational Risk Factors in Lumbar Disc Degeneration

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Abstract *Background* Lumbar disc degeneration (LDD) is a process that begins early in life, contributing to the development of low back pain. LDD is a consequence of a variety of factors, and its etiology remains poorly understood. *Objectives* to investigate occupational and genetic risk factors inducing lumbar disc degeneration, and to evaluate the possible association of genetic polymorphisms of matrix metalloproteinase 3 (MMP-3) and vitamin D receptor (VDR) with the severity of LDD in an Egyptian population. *Subjects and Methods* A case control study involving 84 LDD and 60 controls was carried out. Five types of work related factors were investigated by questionnaire, complete neurological examination for all subjects and MRI for the cases. Polymerase chain reaction and restriction fragment length polymorphism methods were applied to detect polymorphisms in MMP-3 Promoter (−1,171 6A/5A) (rs 731236) and VDR-Apa (rs 35068180). *Results* We found that family history, back injury, smoking, high level of sitting, bending/twisting, physical workload, lifting, whole body vibration, mutant allele 5A of MMP-3 and mutant allele T of VDR were significantly associated with LDD (OR = 2.9, 3.1, 2.1, 11.1, 15.9, 11.7, 8.2, 12.6, 2.5 and 3.1 respectively, $p < 0.05$). Cases that carry allele 5A and/or allele T were associated with LDD severity. *Conclusion* LDD is closely associated in occurrence and severity with occupational, environmental risk factors and susceptibility genes namely MMP-3, and VDR (ApaI). This study throws light on the importance of screening for early detection of susceptible individuals and disease prevention.

Keywords Lumbar disc degeneration · Occupational risk factors · MMP3 · VDR

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Introduction

Low back pain (LBP) affects up to 85 % of people at some point during their lives. It is the single most common cause for disability in individuals aged 45 years or younger and as a result carries tremendous weight in socioeconomic considerations and results in considerable healthcare and related costs [1].

Degeneration of the intervertebral discs is strongly implicated as a cause of low back pain. The intervertebral disc degeneration is best defined as a cascade that begins with changes to the cellular microenvironment within the substructures of the disc that progress over decades to structural breakdown and functional impairment [2].

Lumbar disc degeneration (LDD) is generally multifactorial in origin and its etiology has proven challenging to characterize because it is poorly defined, with complex interaction between occupational and non work-related factors and characteristics, these factors may include age, gender, cigarette smoking, physical fitness level,

anthropometric measures, lumbar mobility and medical history [3].

The importance of genetic factors in LDD etiology has become evident in recent years and probably accounts for greater than 70 % of an individual's risk. Nevertheless, environmental or occupational factors influence on LDD is far from negligible and has been defined in a comprehensive manner by Williams and Sambrook in 2011 [4].

Among the genes suggested to be involved in LDD, are genes that code for collagens I, IX, and XI, interleukin-1 (IL-1), aggrecan, vitamin D receptor (VDR), matrix metalloproteinase 3 (MMP-3), and other proteins [5].

Vitamin D is an important factor in bone metabolism and development. Through activation of VDR, vitamin D facilitates the intestinal absorption of calcium, stimulates renal production of 1,25-$(OH)_2$-vitamin D_3 and also influences osteoblasts, osteoclasts, and PTH secretion [6, 7].

Several biallelic polymorphic sites have been identified in the VDR sequence, both in the coding and noncoding region, the most known and studied are: BsmI, TaqI, ApaI and FokI. BsmI, ApaI and TaqI, are located near the 3′ untranslated region (3′ UTR) of the gene and are in linkage disequilibrium (LD). BsmI and ApaI apparently are not affecting any splicing site and/or transcription factor binding site [8]. TaqI represents a "synonymous" polymorphism in the coding sequence and, as the two polymorphisms mentioned above, it does not determine any change in the amino acid sequence of the encoded protein. On the contrary, FokI is a C/T transition polymorphic site present in the VDR start codon, the allelic variants of this polymorphism code for structural different receptor proteins. This polymorphism can be considered independent and directly associated with pathological conditions, since there is no LD with any of the other cited polymorphisms and the LD area surrounding this polymorphism seems to be very small. Consequently, most functional studies have been performed exclusively on FokI [8, 9].

Apart from FokI polymorphism, increasing focus is on the 3′ UTR of the VDR gene namely the BsmI, ApaI and TaqI restriction fragment length polymorphisms (RFLPs) [10]. The 3′ UTR of genes is known to be involved in regulation of gene expression, especially through regulation of mRNA stability [11, 12]. Moreover this region of the VDR which contains polymorphisms probably not affecting the VDR protein is likely in LD with other nearby functional ones, which, at turn, could be responsible for the observed associations of such VDR polymorphisms with several pathological phenotypes. In particular, the VDR lies downstream from the collagen type II alpha 1 (COL2A1) gene, with a close LD between VDR and a COL2A1 haplotypes [13].

MMP-3 (stromelysin-1) mediates disc degeneration by degradation of matrix proteoglycans and collagens and also contributes to neuropathic pain after peripheral nerve injury. MMP-3 gene polymorphism 5A/6A (rs72520913) regulates the MMP3 gene expression with 5A allele possessing twice the promoter activity than 6A [14, 15].

Indeed a series of environmental and occupational factors were considered to be risk factors for musculoskeletal degenerative disorders. Additionally, genetic factors were noted for their important role in disease process. The interaction between genetic polymorphisms as modifiers and other factors in LDD occurrence and severity was not sufficiently studied. To our knowledge no such study was done in an Egyptian population. Consequently we undertook this study aiming to investigate some occupational and genetic risk factors as disease modifiers in LDD occurrence and severity in an Egyptian population. Most functional studies on VDR gene have been performed exclusively on FokI gene, so we tried to explore the possible association with one of the 3′ UTR of the VDR gene namely the ApaI and one of the important genes in matrix remodeling MMP-3 gene polymorphisms with LDD. Severity of LDD was assessed from clinical findings and MR imaging.

Subjects and Methods

Subjects

Using a case control design, all low back pain patients diagnosed as having lumbar disc degeneration were recruited from the outpatient clinics of the Rheumatology and Rehabilitation department, Cairo University Hospitals, between January and June 2012.

The total number of lumbar Disc Degeneration cases was 123 and 84 participated in the current research. During the same enrollment period, 89 controls with no medical history of LBP or LDD disorders were randomly selected from residents in the community and from among who visited the same hospitals for a regular medical examination, and matched to the cases as regards gender, age, socioeconomic status, BMI. Of these 89 controls, only 60 subjects agreed to continue through the study and investigations.

Exclusion criteria: (a) subjects with autoimmune or inflammatory diseases, hypertension or diabetes, (b) previous surgical treatment in the lumbar spine, (c) severe osteoporosis, (d) segmental instability.

All the included subjects were treated according to the Helsinki Declaration of biomedical ethics [16]. Informed consent was obtained from all the subjects after proper orientation regarding the objectives of the study, and the data confidentiality as well as the impact of the study.

Methods

Data regarding individual characteristics were collected by a questionnaire that included items on anthropometric measures. The Body Mass Index (BMI = weight in Kg per height in meters squared) was calculated for each subject. Smoking habits, family history of lumbar disc disease and past history of back injury were recorded.

A separate questionnaire, developed by Stockholm Musculoskeletal Intervention Center for assessing manual materials handling, was modified and applied to the assessment of physical and postural loads at work [17]. Questionnaires were implemented via face to face interviewers (occupational medicine specialists). Subjects were asked to recall in details their work history, including descriptions of past jobs. Five potential risk factors for degeneration agreed by US-National Institute for Occupational Safety and Health (NIOSH)—prolonged sitting, whole body vibration, bending/twisting, lifting, and heavy work load- were identified [18].

Subjects were asked to estimate the number of hours spent per day working under each of the five risk factors. The risk factors were classified into three levels by considering the exposure frequency and employment years: low to moderate and high (Table 1)

A complete neurological examination was performed for all the subjects (muscle power, reflexes, sensations, and leg raising). Neurological examination was performed to detect weakness or sensory loss. To test muscle weakness, the patient was asked to walk on his/her heels and toes. Thigh, knee, ankle, and toe muscle groups strength were tested individually. Sensations were checked for superficial sense by light touch in the leg and foot and deep sensation by sense of position of the big toe with closed eyes. In addition, reflexes at the knee and ankle were tested whether present or lost. Straight leg raise (SLR) test (The *test of Lasègue* [19]) was also performed for all subjects. This test is an accurate predictor of the disk herniation. In this test, the patient lies on his/her back with both legs lifted upward and the knees stay straight. The test was considered positive for herniated disk, if pain was felt down the leg and below the knee [19].

Table 1 Two leveled scaled occupational risk factors

Risk factor	Level	Description
Prolonged sitting Bending/twisting	Low/ moderate	≤4 h/day and ≤1–10 years OR >4 h/day and ≤ 5 years
	High	≤4 h/day and >10 years OR >4 h/day and >5 years
Whole body vibration Lifting	Low/ moderate	≤4 h/day and ≤1–5 years OR >4 h/day and ≤2.5 years
Heavy physical load	High	≤4 h/day and >5 years OR >4 h/day and >2.5 years

Magnetic resonance imaging (MRI) of the lumbar spine was performed for all the cases. The degree of degeneration was evaluated from MRI by two radiologists and an independent orthopedic spine surgeon who was not informed about the research.

MR imaging was performed by a 1.5-T MR imaging unit (Intera, Philips medical system). All patients were studied in the supine position with a fixed imaging protocol. Radiologists were asked to report their findings according to a four point scale degeneration which was graded as 0 (no signal), 1 (slight decrease in signal intensity in nucleus pulposus), 2 (distinct decrease in signal intensity in nucleus pulposus, with normal disc height) and 3 (severe decrease in signal intensity in nucleus pulposus with disc space narrowing) (Fig. 1a–d). This scale resulted in a classification of degenerative disorders as non-existent, mild, moderate and severe, respectively [3, 20]. The discs L1/L2, L2/L3, L4/L5, and L5/S1 were examined in every patient for degeneration. A single grade of disc degeneration is assigned for each patient based on the degree of degeneration of lumbar disc; if multiple discs were degenerated we took the worse grade. The final grade presented is a total number (percentage) of all grades mild, moderate and severe of the subjects under study.

Determination of Genotypes

Five mL of blood was collected in EDTA–K3 tubes. The QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) was used for genomic DNA isolation from peripheral blood samples.

Polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) methods were applied to detect polymorphism in MMP-3 Promoter (−1,171 5A→6A) (rs 731236) and VDR (rs 35068180).

MMP-3 and VDR PCR–RFLP [21, 22]

PCR was accomplished by Taq PCR Master Mix (Qiagen, Valencia, CA, USA). The primers for MMP-3 were designed as forward: 5′-GGTTCTCCATTCCTTTGATG GGGGGAAAGA-3′ and reverse: 5′-CTTCCTGGAATT CACATCACTGCCACCACT-3′; and the primers for VDR were designed as Forward—5′ CAG AGC ATG GAC AGG GAG CAA-3′ and Reverse—5′ GCA ACT CCT CAT GGC TGA GGT CTC-3′. In a total volume of 100 μL containing 400 ng DNA, 0.2 μM each primer, 1 × QIAGEN PCR Buffer (200 μM of each dNTP, 2.5 U Taq DNA polymerase and 1.5 mM $MgCl_2$). The reaction was carried out

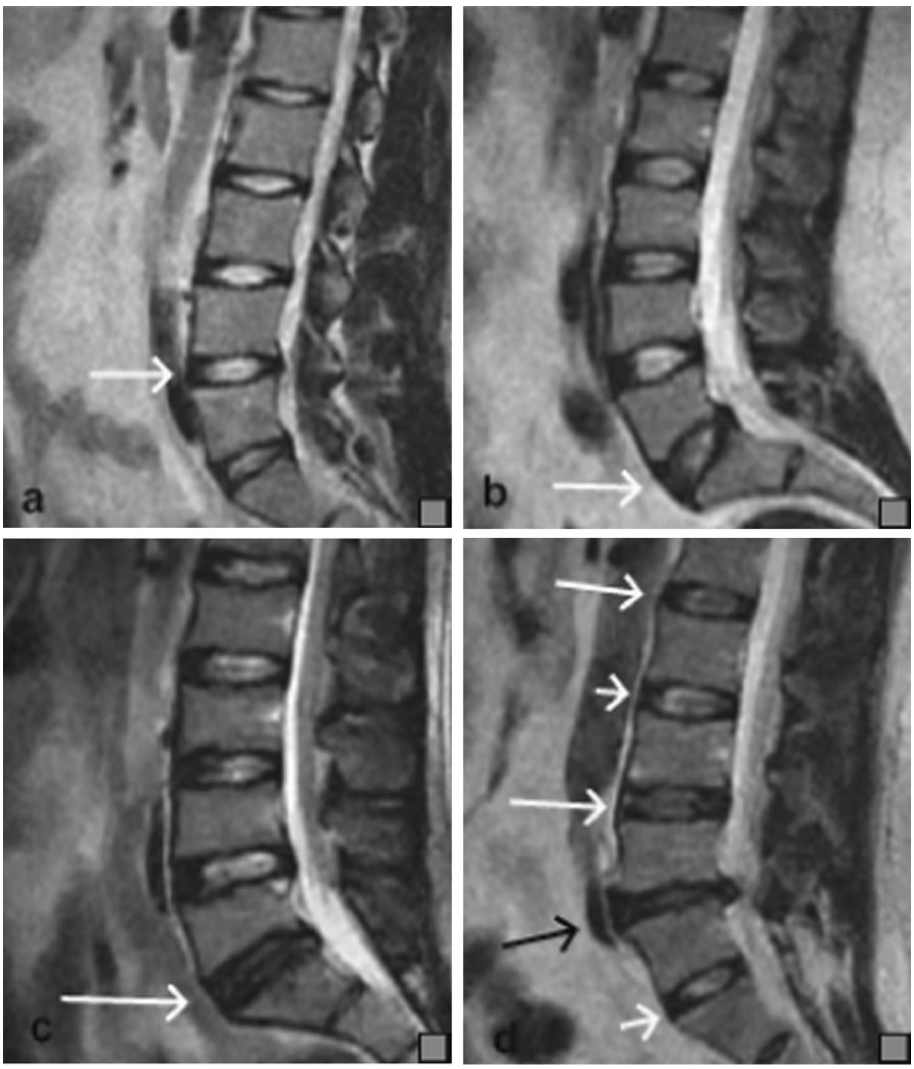

Fig. 1 Four patients complaining of low back pain. Figures (**a**–**d**) are the sagittal T2WI of their Lumbar discs MRI. **a** 33 years old male, showing L4-5 disc of preserved bright T2 signal of nucleus pulposus and disc space (grade 0) (*arrow*), **b** 33 years old female showing slight decrease in the T2 signal intensity of nucleus pulposus at L5-S1 disc (grade 1) (*arrow*), **c** 34 years old male showing distinct decrease in signal intensity with preserved height at L5-S1 disc (grade 2) (*arrow*), **d** 49 years old male showing severe decrease in T2 signal with disc space narrowing at L4-5 disc, (grade 3) (*black arrow*). Also note that L2-3, L5-S1 are (grade 1) degeneration (*short arrows*) and L1-2, L3-4 are (grade 2) degeneration (*long arrows*)

under the following conditions: an initial denaturation for 3 min at 94 °C, samples were subjected to 35 cycles of amplification, consisting of a 45 s denaturating phase at 94 °C, a 45 s annealing phase at (62 °C for MMP-3 and 59 °C for VDR) and a 45 s extension phase at 72 °C. A 10 min 72 °C hold was the final step of the program.

Amplified products (8 µL) were loaded onto 2 % agarose gels previously stained with 0.5 µg/mL ethidium bromide, electrophoresed at 100 V for 30 min and then visualized by UV transilluminator (Figs. 2a, 3a).

PCR products (10 µL) of MMP-3 gene were digested in a 30 µL reaction volume for 10 min with 1 U of Tth111I (PsyI) restriction endonucleases FastDigest (Fermentas, St. Leon-Rot, Germany) at 37 °C. The digested PCR product was separated on 3 % agarose gel and then visualized by UV transilluminator. The genotypes of MMP-3- were 6A/6A (129 bp), 6A/5A(129, 97 and 32 bp) and 5A/5A(97 and 32 bp); 5A was the mutation allele (Fig. 2b).

PCR products (10 µL) of VDR gene were digested in a 30 µL reaction volume for 20 min with 1 U of ApaI restriction endonucleases Fast Digest (Fermentas, St. Leon-Rot, Germany) at 37 °C. The digested PCR product was separated on 2 % agarose gel and then visualized by UV transilluminator. The genotypes of VDR-ApaI were TT (740 bp), GT (740, 520, 220) and GG (520 and 220 bp); T was the mutation allele (Fig. 3b).

Statistical Analysis

Data were computerized and standard statistical methods were applied using SPSS 15.0 for windows (SPSS Inc, Chicago, IL, USA, 2006). Demographic data from the two groups were compared using two tailed student's *t* test and Chi square test as appropriate. For genetic polymorphism, the Chi square test was performed to assess association between LDD cases and control subjects on the basic of

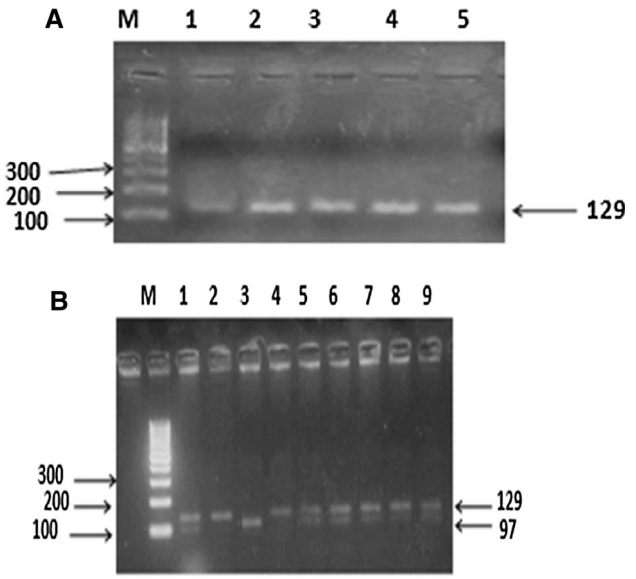

Fig. 2 **a** The expected PCR product size of MMP-3 gene by 100 bp marker [M], *Lane 1–5* shows PCR product followed by separation on 2.0 % agarose gel was confirmed; **b** Genotyping of MMP-3 promoter (−1,171 5A→6A) polymorphism by PCR–RFLP analysis followed by separation on 3 % agarose gel, Lane M = 100 bp marker; lane 1, 5–9 = 5A/6A; lane 2,4 = 6A/6A; lane 3 = 5A/5A

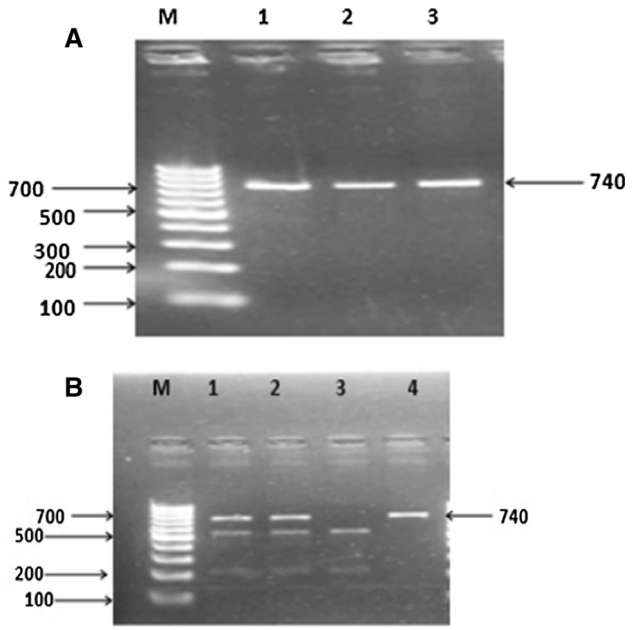

Fig. 3 **a** The expected PCR product size of VDR gene by 100 bp marker [M], *Lane 1–3* shows PCR product (740BP) followed by separation on 2.0 % agarose gel was confirmed; **b** Genotyping of VDR polymorphism by PCR–RFLP analysis followed by separation on 2 % agarose gel, Lane M = 100 bp marker; lane 1, 2 = GT; lane 3 = GG; lane 4 = TT

allelic and genotypic frequencies. The odds ratios (OR), relative risk and 95 % confidence intervals were calculated. Differences were considered to be significant when the p value <0.05.

Results

Demographic Data of the Subjects Table 2

Table 2 shows the characteristics of the cases (n = 84) and the control (n = 60) groups. The majority of the studied subjects were males, the mean age of the cases and the control was 44.2 ± 11.28 and 43.3 ± 10.57 years respectively and the mean BMI was 23.697 ± 3.352 and 23.516 ± 3.614 respectively. There was no significant difference between the two groups regarding gender, age or BMI. Smoking was prevalent in the LDD subjects (42.9 %) compared to 26.7 % in the control group ($p = 0.054$). History of back injury and family history of LDD were statistically significantly higher in cases compared to the control subjects ($p = 0.009$, 0.003 respectively). About one-third of the cases reported past history of regular physical exercise versus 60 % of the control subjects ($p = 0.004$).

Personal and Occupational Risk Factors for Lumbar Disc Degeneration (Tables 1, 2, 3)

Smoking, back injury and family history of LDD and occupational factors (high level): prolonged sitting, twisting/bending, whole body vibration, lifting heavy objects and heavy physical load, were significantly associated with LDD. The OR value for (high level) prolonged sitting reached 11.13 (95 % CI 4.968–24.966), twisting/bending 15.97 (95 % CI 6.76–37.72), whole body vibration 12.692(95 % CI 4.618–34.88), lifting heavy objects 8.257 (95 % CI 3.49–19.512) and heavy physical load 11.701(95 % CI 4.75–28.82), which indicates that people exposed to these factors at high level are more likely to develop lumbar disc degeneration than those who are exposed to the same factors at lower levels (mild/moderate level).

A family history of LDD and back injury were found to be risk factors for LDD, as demonstrated by the OR values of 2.987 (95 % CI 1.43–6.234) and 3.079 (95 % CI 1.285–7.378) respectively.

Indeed exercise seems to be a protective factor for lumbar spine (OR = 0.352, 95 % CI 0.77–0.697).

Genetic Risk Factors for Lumbar Disc Degeneration (Table 4, Figs. 2, 3)

The frequency of mutation genotypes of MMP3 (5A6A and 5A5A) and VDR (GT and TT) genes were significantly higher in LDD cases than the controls. More than half of the LDD cases carried the mutation genotype 5A6A compared to 35 % of the controls (OR = 2.143, 95 % CI 1.083–4.239). Seventeen percent (17.8 %) of the cases

Table 2 Demographic data of the subjects

Variables	Cases (84)	Control (60)	*p*	OR	95 % CI
Age mean ± SD	44.2 ± 11.28	43.3 ± 10.576	0.474[a]		
BMI (kg/m^2) mean ± SD	23.697 ± 3.352	23.516 ± 3.614	0.758[a]		
Females	36 (42.9 %)	25 (41.7 %)			
Males	48 (57.1 %)	35 (58.3 %)	1.000	0.952	0.487–1.863
Smoking	36 (42.9 %)	16 (26.7 %)	0.046	2.100	1.010–4.22
Exercise history	29 (34.5 %)	36 (60 %)	0.002	0.352	0.177–0.697
Back injury	27 (32.1 %)	8 (13.3 %)	0.009	3.079	1.285–7.378
Positive family history	40 (47.6 %)	14 (23.3 %)	0.003	2.987	1.431–6.234

Significance at less than 0.05

BMI body mass index, *OR* odds ratio, *95 % CI* confidence interval at 95 %

[a] *t* test is used

Table 3 Occupational risk factors for lumbar disc degeneration

Variable	Cases N (%)	Control N(%)	*p*	OR	95 % CI
Prolonged sitting					
Low/mod	24 (28.6)	49 (81.7)	<0.001	11.136	(4.968–24.966)
High	60 (71.4)	11 (18.3)			
Bending/twisting					
Low/mod	20 (23.8)	49 (81.7)	<0.001	15.970	(6.760–37.720)
High	64 (76.2)	11 (18.3)			
Whole body vibration					
Low/mod	39 (46.4)	55 (91.7)	<0.001	12.692	(4.618–34.88)
High	45 (53.6)	5 (8.3)			
Lifting					
Low/mod	30 (35.7)	52 (86.7)	<0.001	8.257	(3.494–19.512)
High	54 (64.3)	8 (13.3)			
Heavy workload					
Low/mod	27 (32.1)	53 (88.3)	<0.001	11.701	(4.750–28.820)
High	57 (67.9)	7 (11.7)			

carried the mutation genotype 5A5A compared to 5 % of the control (OR = 4.13, 95 % CI 1.13–1.082).

The 6A6A genotype seems to be protective for lumbar spine with a marked significant difference between the cases (24/84, 28.6 %) and the controls (36/60, 60 %) (OR = 0.267, 95 % CI 0.132–0.537). The frequency of 6A allele in the cases was 56 % (47/84) and in the controls 77 % (46/60) ($p = 0.01$). The value of OR 0.387(95 % CI 0.185–0.808).

The frequency of the mutant allele 5A in the cases was 44 % (37/84) and in the controls 23 % (14/60) ($p = 0.01$). The value of OR 2.587 (95 % CI 1.238-5.406).

More than half (57.1 %) of the LDD cases carried the mutant genotype GT compared to 36.7 % of the controls (OR = 2.303, 95 % CI 1.167–4.546). More than twenty percent (22.6 %) of the cases carried the mutant genotype TT compared to 6.7 % of the control (OR = 4.13, 95 % CI 1.13–1.082).

A marked statistically significant difference was found between the cases and the controls as regards the frequency of the GG genotype (20.2 and 56.7 % respectively, $p < 0.0001$), The GG genotype seems to be protective for lumbar spine (OR = 0.194, 95 % CI 0.93–0.406).

The frequency of G allele in the cases was 49 % (41/84) and in the controls 75 % (45/60) ($p = 0.002$). The value of OR 0.318(95 % CI 0.154–0.656).

The frequency of the mutant allele T in the cases was 51 %, and in the controls 25 % ($p = 0.002$). The value of OR 3.146 (95 % CI 1.525–6.491).

Association Between MMP3 and VDR Gene Polymorphism with Neurological Assessment of LDD Cases (Table 5)

The results of the neurological assessment (muscle power, reflexes, sensation and Straight leg raising) performed on

Table 4 The association between lumbar disc degeneration and gene polymorphisms

Genotype	Cases N (%)	Controls N (%)	*p*	OR (Cases/controls)	95 % CI
MMP-3					
6A6A	24 (28.6)	36 (60)	<0.001	0.267	0.132–0.537
5A5A	15 (17.9)	3 (5)	0.02	4.130	1.130–14.979
6A5A	45 (53.6)	21 (35)	0.02	2.143	1.083–4.239
6A	47 (56)	46 (77)	0.01	0.387	0.185–0.808
5A	37 (44)	14 (23)	0.01	2.587	1.238–5.406
VDR ApaI					
GG	17 (20.2)	34 (56.7)	<0.001	0.194	0.093–0.406
TT	19 (22.6)	4 (6.7)	0.01	4.092	1.310–12.743
GT	48 (57.1)	22 (36.7)	0.01	2.303	1.167–4.546
G	41 (49)	45 (75)	0.002	0.318	0.154–0.656
T	43 (51)	15 (25)	0.002	3.146	1.525–6.491

the LDD cases, classified the cases into two groups based on neurological severity, group (1) cases with absent or diminished neurological findings, and group (2) with preserved neurological findings. The number of cases in each group varied according to the examination. The frequency of the severe neurological findings was predominant in the LDD cases. Eighty percent of the cases (68/84) had absent or diminished muscle power, 69 % (58/84), 61.9 % (52/84), 60.7 % (51/84) had absent or diminished reflexes, sensations and straight leg rising respectively.

The frequency of the mutation genes 5A5A, and 5A6A (MMP3) and TT and GT (VDR), were higher in the LDD cases group 1. This was highly statistically significant for mutant gene 5A6A with muscle power ($p = 0.002$) OR = 3.294 (95 % CI 1.168–9.292).The value of the OR for the mutant gene TT reached 4.235(95 % CI 0.609–29.432). No statistically significant association between the mutant genes and the reflexes or leg rising. However a highly significant association between the 5A5A and TT genes with absent or diminished sensations ($p = 0.006$ and 0.001 respectively), OR value reached 8.615 (95 % CI 1.189–62.423) and 11.077(95 % CI 1.553–79.027) respectively.

The 6A6A and GG genes seemed to be protective as evidenced from their higher frequency in the cases with preserved neurological manifestations versus those with absent or diminished manifestations.

Association Between MRI Grading of LDD and Gene Polymorphism (Table 6, Fig. 1)

According to the MRI, 58/84 (69 %) cases were severe, 1(1.2 %) case was mild and 25(29.8 %) cases were moderate. LDD cases were further classified into two groups according to grading of the disc degeneration in the MRI: mild/moderate (n = 26) and severe (n = 58). Frequency of the mutant genes 5A5A, 5A6A, and TT, GT, were higher in the severe degeneration cases which was statistically significant for 5A5A and TT genes ($p = 0.025$, OR = 1.464 95 % CI 1.71–1.830) and ($p = 0.029$, OR = 1.418 95 % CI 1.11–1.806) respectively. The frequency of the 6A6A and GG genes was significantly higher in the mild/moderate grade of LDD ($p = 0.017$, OR = 2.143, 95 % CI 1.66–3.938) 95 % CI 1.71–1.830) and ($p = 0.028$, 2.089, 95 % CI 1.136–3.832) respectively.

Discussion

Several epidemiological studies showed a relationship between lumbar disc diseases and physical workplace factors such as lifting or carrying of loads, forward bending, and whole body vibration [3, 23, 24]. According to our study, high lumbar load (prolonged sitting, twisting/bending, whole body vibration, lifting heavy objects and heavy physical load), contributes significantly to the risk of lumbar disc degeneration, with odds ratios exceeding 10 (except for lifting heavy objects 8.257). Individuals who were exposed to high level bending/twisting were approximately fifteen times and those exposed to high level whole body vibration were approximately twelve times, more likely to suffer from disc degeneration, compared to those who were not.

The effect of occupational factors seems to depend on the combination of their intensity, frequency and duration. As the occupational factor becomes stronger, it is likely to damage the structure of the lumbar disc by narrowing the disc space and breaking annular fibrosus, and by gradually destroying the function of the musculoskeletal system [3]. Several experimental studies suggest that lumbar compressive load which occurs in people engaged in heavy lifting and heavy labor work, can lead to structural changes in intervertebral

Table 5 Association between neurological assessment and gene polymorphisms

Genotype	Group 1	Group 2	*p*	OR[a]	95 % CI
	1. Muscle power				
	68 (80.9 %)	16 (19.1 %)			
6A6A	13 (19.1)	11 (68.8)	<0.001	0.278	0.154–0.502
5A5A	13 (19.1)	2 (12.5)	0.534	1.529	0.383–6.113
6A5A	42 (61.8)	3 (18.8)	0.002	3.294	1.168–9.292
GG	11 (16.2)	6 (37.5)	0.056	0.431	0.188–0.992
TT	18 (26.5)	1 (6.3)	0.082	4.235	0.609–29.432
GT	39 (57.4)	9 (56.3)	0.936	1.020	0.632–1.645
	2. Reflexes				
	58 (69 %)	26 (31 %)			
6A6A	14 (24.1)	10 (38.5)	0.17	0.628	0.322–1.220
5A5A	12 (20.7)	3 (11.5)	0.31	1.793	0.552–5.821
6A5A	32 (55.2)	13 (50.0)	0.66	1.103	0.704–1.729
GG	6 (10.3)	11 (42.3)	0.001	0.245	0.101–0.590
TT	15 (25.9)	4 (15.4)	0.29	1.681	0.618–4.575
GT	37 (63.8)	11 (42.3)	0.06	1.508	0.925–2.459
	3. Sensations				
	52 (61.9 %)	32 (38.1 %)			
6A6A	10 (19.2)	14 (43.8)	0.016	0.440	0.222–0.869
5A5A	14 (26.9)	1 (3.1)	0.006	8.615	1.189–62.423
6A5A	28 (53.8)	17 (53.1)	0.949	1.014	0.672–1.529
GG	8 (15.4)	9 (28.1)	0.158	0.547	0.235–1.273
TT	18 (34.6)	1 (3.1)	0.001	11.077	1.553–79.027
GT	26 (50.0)	22 (68.8)	0.092	0.727	0.508–1.041
	4. Leg raising				
	51 (60.7 %)	33 (39.3 %)			
6A6A	10 (19.6)	14 (42.4)	0.02	0.462	0.233–0.915
5A5A	11 (21.6)	4 (12.1)	0.27	1.779	0.618–5.122
6A5A	30 (58.8)	15 (45.5)	0.23	1.294	0.835–2.007
GG	8 (15.7)	9 (27.3)	0.19	0.575	0.247–1.340
TT	14 (27.5)	5 (15.2)	0.18	1.912	0.720–4.55
GT	29 (56.9)	19 (57.6)	0.95	0.988	0.677–1.441

Group 1: absent or diminished neurological findings

Group 2: preserved neurological findings

[a] For cohort neurological findings = absent or diminished

discs (for example, decreased disc thickness) as well as to changes in intervertebral disc cell metabolism [25, 26].

Health concerns over vibration exposure (frequency, amplitude, and duration) which typically occurs in several exposures including driving automobiles and trucks, and operating industrial vehicles, were first raised in the 1950s. Earlier animal and in vitro studies suggest that vibration could adversely affect the disc through several mechanisms, disc cell matrix metabolism and nutrition supply, exacerbating the lumbar disc pressure, energy consumption and water loss from the nucleus pulposus and annular tears [27, 28].

Forward flexion can generate compressive forces on the structures of the low back similar to lifting a heavy object. Similarly, rapid twisting can generate shear or rotational forces [29].

The OR value for smoking in the present study was 2.1 (95 % CI 1.01–4.22), indicating an association between smoking habit and LDD. Smoking habit is a further risk to be added to the list of elements that have deleterious effect on intervertebral disc. Many investigators have documented the increasing incidence of lumbar and sciatic pain, as well as disc degeneration in chronic smokers compared to non smokers [30]. Battie and coworkers used MR imaging to study identical twins highly discordant for cigarette smoking, and reported 18 % greater mean LDD scores in smokers than nonsmokers [31]. Experiments conducted in isolated cells of the pulpous nucleus reported that cigarette components, in addition to reducing disc vascularizarion, promote both a decreased glycosaminoglycans production and the expression of type II collagen gene that change the normal disc hydrostatics and damping properties [32].

Table 6 Association between MRI grading of LDD and gene polymorphisms

Genes	Severe (58)	Mild/moderate (26)	*p*	OR[a]	95 % CI
6A6A	12 (20.7)	12 (46.2)	0.017	0.652	0.427–0.996
5A5A	14 (24.1)	1 (3.8)	0.025	1.464	1.171–1.830
6A5A	32 (55.2)	13 (50)	0.660	1.067	0.798–1.425
GG	8 (13.8)	9 (34.6)	0.028	0.631	0.374–1.064
TT	17 (29.3)	2 (7.7)	0.029	1.418	1.110–1.806
GT	33 (56.9)	15 (57.7)	0.940	0.990	0.742–1.321

[a] For cohort degree of degeneration = severe

Moreover, smoking induces biochemical stress to several tissues including intervertebral discs, with catecholamines increase, limiting oxygen supply to tissues [33].

The presence of discogenic disease in the family was found in the majority of the patients in the study group (47.6 %) as compared to the control subjects where the frequency was (23.3 %), the value of OR was 2.987 (95 % CI, 1.431–6.234) which reflects the strong association between family history and LDD. A familial basis for LD herniation was shown earlier by Varlotta et al. [34] in young patients. Our findings reinforce the importance of genetics in the susceptibility to disc degeneration, which was also shown in other studies [3, 33].

We found that history of back injury was highly associated with disc degeneration (OR 3.079, 95 % CI 1.285–7.378). Trauma to the annulus fibrosus can initiate a progressive degenerative process in the disc, and could be interpreted by the theory of accident related trauma [3]. In a cross-sectional study the time sequence between the development of disc degeneration and previous accidental back injuries remains obscure. Thus it is not possible to draw definite conclusions about the role of back accidents as initiators or promoters of disc degeneration. The association between accidental back injuries and disc degeneration should be studied with a prospective design. As a measure of back trauma, we used the number of self-reported back accidents. Although self-reporting is not a very reliable means of obtaining retrospective data because of recall error [35], it was the only possibility. The limit of 2 weeks' absence from work was chosen to improve reliability because such accidents are obviously more serious and will be remembered better.

To date, several gene loci associated with human disc degeneration have been identified. Hereditary factors could affect disc degeneration through several mechanisms, such as an influence on the size and shape of spinal structures that affect the spine's mechanical properties and thus its vulnerability to external forces and biologic processes associated with the synthesis and breakdown of the disc's structural and biochemical constituents, leading to accelerated degenerative changes in some persons relative to others. The identification of specific genetic influences may eventually provide key insights into underlying mechanisms. Furthermore, for specific genes and some environmental factors, gene–gene interactions and gene-environment interactions may exist [5].

One of the important steps in IVD degeneration is the degradation of the disc matrix by enzymes such as matrix metalloproteinases (MMPs). MMPs are responsible for the remodeling of connective tissues under normal physiologic and pathologic conditions. According to their substrate specificity, the classic MMPs fall into three main groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), and stromelysins (MMP-3, -7, -10). MMP-3 (stromelysin-1) is a potent proteoglycan-degrading enzyme [36].

MMP-3 expression is induced in response to local conditions such as mechanical loading and inflammation. In addition, a common polymorphism in the promoter region of the human MMP-3 gene has been identified in which one allele has a run of six adenosines (6A) and the other five (5A). This polymorphism was reported to be involved in the regulation of MMP-3 gene expression with the 5A allele having twice as much promoter activity as the 6A allele. Thus, the 5A allele of the human MMP-3 promoter is a crucial risk factor for the acceleration of IVD degeneration especially in the older population [14]. In our study a similar phenomenon was found. The frequency of the mutant genotypes of the MMP-3 were significantly higher in the LDD cases (5A5A 17.9 %, 6A5A 53.6 %) relative to the control (5A5A 5 %, 6A5A 35 %) $p < 0.05$ and from the odds ratio values they were strong risk factors for LDD. Subjects who carry the mutant allele 5A were two and half times more susceptible for LDD. In a transient transfection experiment, Ye et al., reported that an MMP-3 promoter construct with 5A had an expression which was twice that had a construct with 6A [37]. This suggests that IVDs in the 5A+ group may express a larger amount of MMP-3 than those in the 5A−. Consequently the 5A allele could enhance the degeneration of IVDs associated with environmental conditions resulting from the induction of a higher level of MMP-3 expression in response to such conditions [14].

Interestingly, findings in several studies have led to the speculation that MMP-3 transcriptional regulation in an allele specific manner could affect continuous connective tissue remodelling in arterial walls and in rupture of atherosclerotic plaques [38, 39], consequently the association of MMP-3 promoter polymorphism and IVD degeneration may be due to disease of the lumbar artery.

Alterations in the VDR signaling pathway could be directly involved in the pathophysiology of the degenerated disc. Nucleus pulposus (NP) and annulus fibrosus (AF) cells express VDR and are very sensitive to Vitamin D

active metabolites [40]. Intragenic polymorphisms in the vitamin D receptor gene are linked to disc degeneration features [8]. Indeed the first polymorphisms associated with LDD were two variations in the VDR gene (TaqI and FokI) in a Finnish population [41]. It has been reported that the VDR is expressed not only in osteoblasts but also in the chondrocytes particularly in the fibrillar component, suggesting that vitamin D is directly involved in the differentiation, proliferation, and maturation of cartilage cells. In addition, vitamin D can influence proteoglycan synthesis by articular chondrocytes in vitro [42]. Since the intervertebral disc is also rich in proteoglycan these findings suggest that the vitamin-D receptor may be directly involved in the pathophysiology of the degenerated intervertebral disc. Gruber and co workers found that annulus cells expressed VDR in vivo and in vitro. Exposure to 1, 25 $(OH)_2D_3$ significantly reduced cell proliferation in vitro, but proteoglycan production was unchanged. Moreover 1, 25 $(OH)_2D_3$ or 24, 25(OH) $_2D_3$-treated cells showed variable production of different cellular mediators and specific cytokines [43]. These results suggest that vitamin D/VDR may play roles as regulators of cell proliferation and production of specific cytokines in the lumbar annulus that are possibly related to levels of TNF that are present in the degenerating disc.

Comparable results were found by Colombini et al. [40] who observed that in monolayer cultures, $1,25(OH)_2D_3$, but not $24,25(OH)_2D_3$, determined an inhibition of the proliferation and regulated also the extracellular matrix (ECM) genes expression in nucleus pulposus and annulus fibrosus cells.

Involvement in bony and cartilaginous metabolisms could explain why alterations in vitamin D homeostasis are linked to several pathological conditions of knee cartilage and intervertebral disc tissue, in particular osteoarthritis and lumbar disc degeneration [8].

In our study the frequency of the genotypes TT and GT of the VDR- ApaI gene were significantly higher in the LDD cases (22.6 , 57.1 % respectively $p = 0.01$) compared to the control and those who carry the mutant allele T were three times more at risk.

Comparable results were reported in several studies. Wang and co workers noted significant differences in ApaI and TaqI genotype distribution between cases of cervical spondylosis and controls. For ApaI polymorphism, they observed the cases had a marked higher prevalence of TT genotype than controls (19.5 vs. 8.3 %, $p = 0.003$) [44]. Similarly Yaun et al. [3], reported that the mutant genotypes of the ApaI VDR showed a significant effect on disc degeneration (OR = 1.70), indicating that VDR- ApaI gene polymorphism is one of the genetic risk factors involved in LDD.

In our study genetic factors were not only related to the occurrence but also to the severity of disc degeneration. The frequency of the mutation genotype 5A5A, and 5A6A (MMP-3) and TT and GT (VDR, ApaI), were higher in the LDD cases with more severe clinical neurological findings and MRI grading.

The frequency of the mutation homozygotic genotype 5A5A and TT was significantly higher in the cases with severe MRI grading (24.1 and 29.3 %, respectively) compared to 3.8 and 7.7 %, respectively in the cases with mild/moderate MRI grading. The OR values showed that these genes were approximately one and half times associated with severe MRI grading in LDD cases. This suggests that patients prone to disc degeneration have a tendency to have higher transcription of the MMP-3 and VDR genes and that among patients with disc degeneration, those with higher transcription of these genes have more severe disease. Several studies indicate that the 5A allele of the human MMP-3 promoter may be a crucial risk factor for the acceleration and progression of intervertebral disc degeneration, especially in the older population [14, 37].

Indeed, degeneration of the anulus fibrosus of intervertebral discs, is thought to be mediated (at least in part) by MMP-1 and -3. Elevation of ADAMTS-1, -4, -5, and -15 (proteins belonging to the family of metalloproteinases) was detected in degenerated intervertebral discs, with the levels increasing with increasing degree of degeneration [45].

Serum MMP-3 was found to be an independent predictor of radiographic progression 2 years later, particularly in patients with pre-existing radiographic damage [46]. One study [47] found polymorphisms in MMP3, TIMP-1, and COX-2, which encode molecules involved in inflammatory pathways, were associated with radiographic progression of LDD.

Kawaguchi and co workers reported that subjects with the mutant genotypes of the VDR gene were more frequently associated with multilevel and severe disc degeneration and disc herniation. Indeed, polymorphism in the VDR gene was viewed as potential contributor to various conditions that may be related directly or indirectly to severity of lumbar disc diseases [48]. In a meta-analysis study the possibility that VDR polymorphisms play roles in the pathogenesis of joint space narrowing and osteophytes in osteoarthritis was concluded [49]. Polymorphism in the VDR gene was also linked to osteoporosis and lumbar spine bone mineral density, with ApaI genotypes related to circulating levels of osteocalcin and PTH [50].

The molecular and genetic basis of degenerative disc diseases has been an intense focus of research, which has greatly increased our understanding of the biology underlying this process. Alterations in IVD production of ECM, inflammatory cytokines, and degradative enzymes occur in a stepwise cascade leading to the end stage morphological changes evident on routine clinical imaging studies. The

development of alternative biological treatment modalities for disc repair or regeneration will require a detailed understanding of these biological processes to reverse or halt the progression of degenerative changes within the native disc, if components of disc degeneration are found to directly contribute to associated symptoms [51].

Conclusion and Recommendations

LDD is closely associated with occupational risk factors and susceptibility genes namely MMP-3, and VDR (ApaI) polymorphisms. This study also provided evidence that 5Aallele of MMP-3 and T allele of VDR genes are crucial risk factors for the severity of LDD. Workers who carry the mutation genotypes of the above genes are more susceptible for the development of LDD and more severe manifestation, which throws light on the importance of screening in the pre employment settings and periodically for early detection of susceptible individuals and disease prevention. Smoking, family history of LDD and history of back injury are important risk factors especially when considering high physically demanding jobs or jobs exposing the worker to whole body vibration. Therefore, to take preventive measures, it is necessary to consider both environmental and genetic factors in the pre employment and periodic medical examination. The size of the study sample was not large enough to study in depth the synergy between the occupational and genetic risk factors and future studies with larger sample size are needed and matched number between cases and control groups.

References

1. Taher F, Essig DR, Lebl DR, Hughes AP, Sama AA, Cammisa FP, Girardi FP. Lumbar degenerative disc disease: current and future concepts of diagnosis and management. Adv Orthop. 2012;. doi:10.1155/2012/970752.
2. Freemont AJ. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology. 2009;48:5–10.
3. Yaun HY, Tang Y, Liang Y, Lei L, Xiao G, Wang S, Xia Z. Matrix metalloproteinase-3 and vitamin D receptor genetic polymorphism, and their interaction with occupational exposure in lumbar disc degeneration. J Occup Health. 2010;52:23–30.
4. Williams F, Sambrook P. Neck and back pain and intervertebral disc degeneration: role of occupational factors. Best Pract Res. 2011;25(1):69–79.
5. Kalichman L, Hunter DJ. The genetics of intervertebral disc degeneration associated genes. Joint Bone Spine. 2008;75(4):388–96.
6. Gallieni M, Cozzolino M, Fallabrino G, Pasho S, Olivi L, Brancaccio D, et al. Physiology and pathophysiology. Int J Artif Organs. 2009;32:87–94.
7. Michaelsson K, Wolk A, Jacobsson A, Kindmark A, Grundberg E, Stiger F, Mallmin H, et al. The positive effect of dietary vitamin D intake on bone mineral density in men is modulated by the polyadenosine repeat polymorphism of the vitamin D receptor. Bone. 2006;39:1343–51.
8. Colombini A, Cauci S, Lombardi G, Lanteri P, Croiset S, Brayda-Bruno M, Banfi G. Relationship between vitamin D receptor gene (VDR) polymorphisms, vitamin D status, osteoarthritis and intervertebral disc degeneration (Review). J Steroid Biochem Mol Biol. 2013;138:24–40.
9. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature. 1994;367:284–7.
10. Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to vitamin D related disease states. J Steroid Biochem Mol Biol. 2004;89:187–93.
11. Ogunkolade BW, Boucher BJ, Prahl JM, Bustin SA, Burrin JM, Noonan K, North BV, et al. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes. 2002;51:2294–300.
12. Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta. 2006;371:1–12.
13. Priestley L, Fergusson C, Ogilvie D, Wordsworth P, Smith R, Pattrick M, Doherty M, Sykes B. A limited association of generalized osteoarthritis with alleles at the type II collagen locus: cOL2A1. Br J Rheumatol. 1991;30(4):272–5.
14. 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-B: 491–5.
15. Omair A, Holden M, Alexandra ML, Olav Reikeras O, Brox JI. Treatment outcome of chronic low back pain and radiographic lumbar disc degeneration are associated with inflammatory and matrix degrading gene variants: a prospective genetic association study. BMC Musculoskelet Disord. 2013;14:105.
16. World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Revised by the 52nd WMA General Assembly, Edinburgh, Scotland. October 2000. http://www.wma.net/e/policy/b3.htm.
17. Wiktorin C, Hjelm EW, Winkel J, Köster M. Reproducibility of a questionnaire for assessment of physical load during work and leisure time. Stockholm MUSIC 1 study group. MUSculoskeletal Intervention Center. J Occup Environm Med. 1996;38:190–201.
18. NIOSH. National Institute of Occupational Safety and Health. National Occupational Research agenda for musculoskeletal disorders, DHHS (NIOSH) publications No. 2001-117-Cincinnati: DHHS, NIOSH; 2001.
19. Devillé WL, van der Windt DA, Dzaferagić A, Bezemer PD, Bouter LM. The test of Lasègue: systematic review of the accuracy in diagnosing herniated discs. Spine. 2000;25(9):1140–7.
20. Kettler A, Wilke HJ. Review of existing grading systems for cervical or lumbar disc and facet joint degeneration. Eur Spine J. 2006;15(6):705–18.
21. Vairaktaris E, Yapijakis C, Vasiliou S, Derka S, Nkenke E, Serefoglou Z, et al. Association of −1171 promoter polymorphism of matrix metalloproteinase-3 with increased risk for oral cancer. Anticancer Res. 2007;27(6B):4095–100.
22. Carless MA, Kraska T, Lintell N, Neale RE, Green AC, Griffiths LR. Polymorphisms of the VDR gene are associated with presence of solar keratoses on the skin. Br J Dermatol. 2008;159(4):804–10.
23. Seidler A, Bolm-Audorff U, Siol T, Henkel N, Fuchs C, Schug C, et al. Occupational risk factors for symptomatic lumbar disc herniation; a case-control study. Occup Environ Med. 2003;60:821–30.

24. Seidler A, Bergmann A, Jäger M, Ellegast W, et al. Cumulative occupational lumbar load and lumbar disc disease—results of a German multi-center case-control study (EPILIFT). BMC Musculoskelet Disord. 2009;10:48. doi:10.1186/1471-2474-10-48.
25. Adams MA, McNally DS, Dolan P. Stress distributions inside intervertebral discs. The effects of age and degeneration. J Bone Joint Surg Br. 1996;78:965–72.
26. Iatridis JC, Mente PL, Stokes IA, Aronsson DD, Alini M. Compression- induced changes in intervertebral disc properties in a rat tail model. Spine. 1999;24:996–1002.
27. Keller TS, Hansson TH, Holm S, Pope MH, Spengler DM. In vivo creep behavior of the normal and degenerated porcine intervertebral disc: a preliminary report. J Spinal Disord. 1988;1:267–78.
28. Hadjipavlou AG, Tzermiadianos MN, Bogduk N, et al. The pathophysiology of disc degeneration: a critical review. J Bone Joint Surg Br. 2008;90:1261.
29. Zhang Y, Zhang F, Sun Z, Guo W, Liu J, Liu M, Guoy X. A controlled case study of the relationship between environmental risk factors and apoptotic gene polymorphism and lumbar disc herniation. Am J Pathol. 2013;182:56–63.
30. Mohammed A, Anil K, Bobby Y, Abhinav S, Mike W, Allen G. Effect of nicotine on spinal disc cells: a cellular mechanism for disc degeneration. Spine. 2004;29:568–75.
31. Battie MC, Videman T, Gill K, Moneta A, Giovannil B, Nyman R, Kaprio J, Koskenvuo M. Volvo award in clinical sciences. Smoking and lumbar intervertebral disc degeneration: an MRI study of identical twins. Spine. 1991;16:1015–21.
32. Oda H, Matsuzaki Y, Wakabayash K, Uematsu Y, Iwashi M. Degeneration of intervertebral discs due to smoking: experimental assessment in a rat- smoking model. J Orthop Sci. 2004;9:135–41.
33. Nunes FT, Conforti-Froes ND, Negrelli WF, Souza DRS. Genetic and environmental factors involved in intervertebral disc degeneration. Acta Ortop Bras. 2007;15(1):9–13.
34. Varlotta GP, Brown MD, Kelsey JI, Golden AL. Familial predisposition for herniation of a lumbar disc in patients who are less than twenty one years old. J Bone Joint Surg (Am). 1991;73:124–8.
35. Riihimaki H, Viikari-Juntura E, Moneta G, Kuha J, Videman T, Tola S. Incidence of sciatic pain among men in machine operating, dynamic physical work, and sedentary work: a three-year follow-up. Spine. 1994;19(2):138–42.
36. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. SSE award competition in basic science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. Eur Spine J. 2002;11(4):308–20.
37. Ye S, Eriksson P, Hamsten A, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem. 1996;271:13055–60.
38. Bijerk C, Houwing-Duistermaat JJ, Valkenberg HA. Heritability of radiologic osteoarthritis in peripheral joints and of disc degeneration of the spine. Arthritis Rheum. 1999;42:1729–35.
39. Terashima M, Akita H, Kanazawa K, et al. Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction. Circulation. 1999;99:2717–9.
40. Colombini A, Lanteri P, Lombardi G, Grasso D, Recordati C, Lovi A, Banfi G, et al. Metabolic effects of vitamin D active metabolites in monolayer and micromass cultures of nucleus pulposus and annulus fibrosus cells isolated from human intervertebral disc. Int J Biochem Cell Biol. 2012;44(6):1019–30.
41. Videman T, Leppavuori J, Kaprio J. Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. Spine. 1998;23:2477–85.
42. Balmain N, Hauchecorne M, Pike JW, Cuisinier-Gleizes P, Mathieu H. Distribution and subcellular immunolocalization of 1, 25-dihydroxyvitamin D3 receptors in rat epiphyseal cartilage. Cell Mol Biol (Noisy-le-grand).1993;39:339–50.
43. Gruber HE, Hoelscher G, Ingram JA, Chow Y, Loeffle B, Hanley EN. 1,25(OH)2-vitamin D3 inhibits proliferation and decreases production of monocyte chemoattractant protein-1, thrombopoietin, VEGF, and angiogenin by human annulus cells in vitro. Spine 2008;33(7):755–65.
44. Wang ZC, Chen XS, Wang DW, Shi JG, Jia LS, Xu GH. The genetic association of vitamin D receptor polymorphisms and cervical spondylotic myelopathy in Chinese subjects. Clin Chim Acta. 2010;411:794–7.
45. Pockert AJ, Richardson SM, Le Maitre CL, Lyon M, Deakin JA, et al. Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. Arthritis Rheum. 2009;60(2):482–91.
46. Maksymowych WP, Landewe R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, Poole AR, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis Rheum. 2007;56(6):1846–53.
47. Valdes AM, Hassett G, Hart D, Spector T. Radiographic progression of lumbar spine disc degeneration is influenced by variation at inflammatory genes: a candidate SNP association study in the chingford cohort. Spine. 2005;30:2445–51.
48. 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;84A:2022–8.
49. Lee Y, Woo J, Choi S, Ji J, Song G. Vitamin D receptor TaqI, BsmI and ApaI polymorphisms and osteoarthritis susceptibility: a meta-analysis. Joint Bone Spine. 2009;76:156–61.
50. Lorentzon M, Lorentzon R, Nordström P.Vitamin D receptor gene polymorphism is related to bone density, circulating osteocalcin, and parathyroid hormone in healthy adolescent girls. Bone Miner Metab. 2001;19:302–7.
51. Kepler CK, Ponnappan RK, Tannoury CA, Risbud MV, Anderson DG. The molecular basis of intervertebral disc degeneration. Spine. 2013;13(3):318–30.