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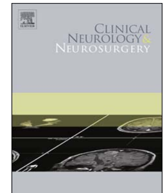

VDR and *GC* gene polymorphisms modulate the risk of lumbar disc degeneration in Iran

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ABSTRACT

Objective: Lumbar disc degeneration (LDD) occurs commonly in humans. Vitamin D metabolic and signaling pathway plays a significant role in intervertebral disc degeneration. The aim of this study was to evaluate the influence of the genetic polymorphism in the two key genes of 1,25-$(OH)_2$-D_3 pathway, *VDR* (vitamin D receptor) and *GC* (group-specific component), in LDD development.
Patients and Methods: Two single-nucleotide polymorphisms, *VDR* rs2228570 (FokI) and *GC* rs7041, were genotyped in 180 patients with LDD and 230 healthy individuals. Genomic DNA was extracted from whole peripheral blood. *VDR* and *GC* genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
Results: A significant difference in genotype distributions of rs2228570 in *VDR* and rs7041 in *GC* gene were observed between cases and controls (P = .01 and. 005, respectively). The *VDR* AA homozygous genotype was seen in 30(16.7%) patients with LDD and 20(8.7%) controls (codominant model: OR = 2.48; 95% CI 1.30–4.73, P = .005) with an estimated approximately 2.5-fold risk of developing LDD in individuals with this genotype. Moreover, higher grades of disc degeneration were more related to *VDR* A allele. The minor allele of *GC* rs7041 was associated with a decreased risk of LDD (OR = 0.69; 95% CI 0.44–0.82, P = .001).
Conclusion: In conclusion, our results suggest for the first time that the genetic variants of *VDR* and *GC* genes contribute to genetic predisposition to LDD in Iran. These findings need further validation in the large multicenter case-control studies.

1. Introduction

Low back pain (LBP) is the main cause of disability and contributor to health care costs [1]. Almost 84% of population is reported to experience LBP at some points of their life [2]. Intervertebral discs have been considered as the most common sources of chronic or recurring axial LBP [2,3]. Intervertebral disc is located between the adjacent two vertebrae, and includes anulus fibrosus and nucleus pulposus. It buffers the vertical pressure to the body to maintain the movement of spine. Lumbar disc herniation is the result of nucleus pulposus protrusion due to anulus fibrosus degeneration [1]. Lumbar disc degeneration (LDD) is a multi-factorial process influenced by bio-mechanisms, lifestyle, behavioral factors such as smoking, occupation, long sitting and genetic factors [4,5].

There is a growing attention in studying the genetic factors involved in acceleration and development of LDD. The identification of relevant candidate genes related to disc degeneration would specify a genetic component of its etiopathogenesis [3]. There are 4 major categories of candidate-genes related to lumbar disc disease as follows: Category 1: the genes related to the structural component of the intervertebral disc, such as the aggrecan gene and the collagen I, collagen IX and collagen XI gene [6,7]. Category 2: the genes producing the extracellular matrix-degrading enzymes such as matrix metalloproteases [4,8]. Category 3: the genes associated to bone structure such as the osteoporosis related genes, estrogen receptor and vitamin D receptor (VDR) genes [7,9]. Category 4: the other genes including the encoding genes of inflammatory cytokines (IL-1 and IL-6) [10,11].

Vitamin D has an essential role in the regulation of skeletal mineral metabolism, modulation of immune system, differentiation and proliferation of different cell types [12]. The biological effects of vitamin D are mediated by its receptor (VDR), a ligand dependent transcription factor encoded by the steroid nuclear receptor gene. VDR is expressed

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in human intervertebral disc nucleus pulposus and annulus fibrosus cells and in chondrocytes and osteoblasts [13,14,15]. VDR binds to the active form of vitamin D and not only plays a key role in normal bone mineralization and remodeling, but also acts in the differentiation, maturation and proliferation of chondrocytes [12]. In addition, vitamin D can induce proteoglycan synthesis by articular chondrocytes *in vitro* [16]. It has been shown that vitamin D affects the amount of inorganic sulfate for intracellular sulfation of proteoglycans through modulation of plasma sulfate concentrations [5,3]. Since the intervertebral disc is also rich in proteoglycan, the matrix gene expression, proliferation and protein production of disc cells are regulated by vitamin D active metabolites [11,14]. The VDR gene is located on chromosome 12q13.11 (OMIM 601769). There are several known genetic polymorphisms in the *VDR* including Apa1 (rs7975232), Bsm1 (rs1544410) and FokI (rs2228570). The genetic variation rs2228570, located in the exon 2, has been regarded as a start codon polymorphism [17]. Several studies examined the association between VDR Fok1 polymorphism and the risk of LDD, but their data were conflicting [18,19,20]. Therefore, further studies in different populations are essentials.

Vitamin D-binding protein (DBP), also known as group-specific component (GC), plays a key role in vitamin D metabolism. The *GC* gene, which encodes the DBP, maps to chromosome 4q12-q13 (OMIM 139200) [21]. DBP binds to vitamin D metabolites and transports vitamin D to liver, kidney, bone, and other target tissues [22]. A non-synonymous single nucleotide polymorphism (SNP) in the *GC* gene (rs7041) contributes to variation in circulating levels of DBP as well as affect its affinity for vitamin D ligands and circulating concentrations of 25(OH)D [23].

We hypothesize that genetic variants of the key genes in vitamin D pathway will modify the LDD risk. Therefore, this study was intended to ascertain whether SNPs in vitamin D pathway genes (*VDR* and *GC*) are associated with LDD susceptibility in Iran.

2. Materials and methods

2.1. Subjects

One hundred and eighty subjects with LDD were consecutively recruited from the Poursina Hospital of (Rasht, Iran) between October 2014 and December 2016. The presence or absence of degenerated disc was confirmed by 1.5 T Magnetic Resonance Imaging (MRI). The LDD cases with severe osteoporosis, history of spinal trauma, congenital conditions, a profession requiring heavy physical work, inflammatory or autoimmune disorders, malignancy, renal failure or symptomatic kidney stone documented by patient's history and special laboratory tests were excluded from the study. The participants, demographic data and history and imaging findings were entered in a designed checklist. Two hundred and thirty healthy unrelated volunteers were selected by matching healthy individuals with no LDD based on gender and age. They had no history of back problems and had negative MRI findings. All subjects were interviewed using a structured questionnaire to obtain information on demographic factors and health characteristics. A written informed consent was obtained from each participant. The research protocols used for this study were approved by the Ethical Committee of the Guilan University of Medical Sciences, Rasht, Iran (IR.GUMS.REC 1920452611). This study was performed in accordance with the declaration of Helsinki (1989).

2.2. Imaging procedure

All participants underwent an MRI examination using a non-contrast 1.5 T MRI T2 weighted fast spin-echo (2700/110, TR/TE) 12 sagittal and axial scans of the whole spine with 0.5 mm slices. The severity of disc degeneration was assessed according to Schneidermans' classification.

Table 1
Characteristics of investigated polymorphism in *VDR* and *GC*.

Gene Symbol	dbSNP	NCBI Assembly location	Nucleotide change	MAF
VDR	rs2228570	ch 12:47879112	G > A	0.32
GC	rs7041	ch 4:71752617	T > G	0.38

Abbreviation: SNP, single-nucleotide polymorphism.

2.3. Genotyping

We selected two SNPs (rs2228570 in *VDR* and rs7041 in *GC*) for genotyping. The rs2228570 and rs7041 have a Global minor allele frequency (MAF) greater than 10% (0.32, and 0.38, respectively). The basic information of studied SNPs is summarized in Table 1. Blood samples were collected in EDTA-containing tubes and centrifuged at 1500 g for 10 min. Plasma and buffy coat were separated and stored at −20 °C. Genomic DNA was extracted from blood using the Gpp solution kit (Gen Pajoohan, Iran) according to the manufacturer's instructions. The DNA concentration and purity was determined by spectrophotometric measurement of absorbance at 260 and 280 nm by UV spectrophotometer. The samples with a reading below 1.7 for their 260/280 ratio were purified using an ethanol precipitation protocol to guarantee DNA sample purity and samples were stored at −20 °C until a polymerase chain reaction (PCR) was performed.

The sequences of specific primers were designed based on relevant DNA sequences available in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank) using Oligo-primer analysis software (Version 7.54, Molecular Biology Insights, USA). For PCR, forward primer 5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′ and reverse primer 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′ for rs2228570; forward primer 5′-TACCACAGGTATAGAATTTT-3′ and reverse primer 5′-AGTG GAGGGTTACATTTCCT-3′ for rs7041 were preferred. Primers were synthesized by MWG-Biotech (Ebersberg, Germany). Genomic DNA (30 ng) was added to a PCR mixture, comprising 2 pmol of each primer, 0.1 mM dNTP, 10 × PCR buffer (10 mM Tris–HCl, 50 mM KCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$ and 0.5 unit of Taq polymerase (gene Fanavaran, Iran) in a total volume of 25 μl. The thermal amplification cycling was performed by a programmed protocol: DNA denaturation at 94 °C for 5 min, amplification for 35 cycles (denaturation at 94 °C for 45 s, annealing at 56.2 °C for 45 s, extension at 72 °C for 45 s) and final extension at 72 °C for 3 min followed by a 4 °C hold cycle. The PCR amplicons generated for *VDR* (265-bp) and *GC* (304-bp) were subjected to restriction digestion using *FokI* and *HaeIII*, respectively. The *VDR* genotypes were assessed as follows: a single 265-bp fragment for the AA; 196 and 69-bp for the GG; and three fragments 265, 196 and 69-bp for the GA genotype (Fig. 1). After digestion of *GC* 304-bp amplicon with *HaeIII*, the homozygous GG yielded 183 and 121-bp products, the heterozygous GT yielded 183, 121 and 304-bp products, and the homozygous TT yielded a 304-bp product (Fig. 2). All blood samples were genotyped successfully. To provide quality control, genotyping

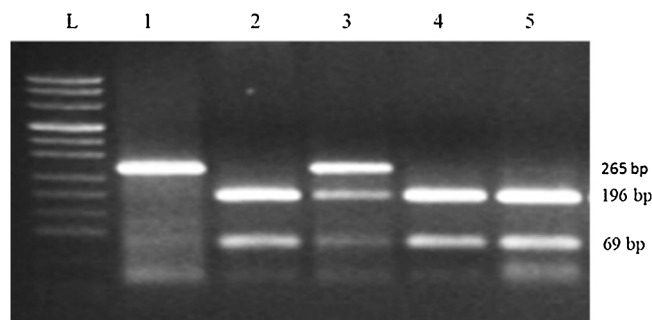

Fig. 1. Agarose gel electrophoresis of *VDR* PCR-RFLP products. Lanes: (L), DNA ladder; (1), AA homozygote; (2), (4) and (5), GG homozygotes; (3), GA heterozygote.

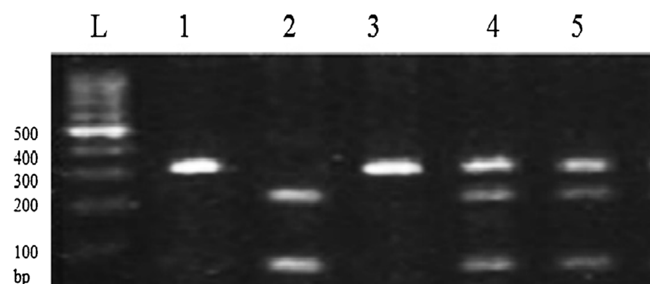

Fig. 2. RFLP pattern of *GC* gene fragments after digestion on 2% agarose gel. Lanes: (L), DNA ladder; (1) and (3), TT; (2), GG; (4) and (5), GT.

was performed without knowledge of the subjects' case/control status and a 20% random sample of LDD and healthy control subjects were genotyped twice by different researchers and the results of the retesting were 100% concordant.

2.4. Statistical analysis

The Chi-square test was used to test for departures of the genotype distribution from the Hardy Weinberg equilibrium. The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. The differences in allele frequencies between case and control subjects were tested using the likelihood ratio χ^2 tests for 2 £ 2 tables (two alleles, case versus control subjects). Odds ratios (OR) and 95% confidence intervals (CI) were used to examine the association between *VDR* and *GC* and polymorphisms and breast cancer occurrence with MedCalc (Version 12.1 Mariakerke, Belgium). We also tested four genetic models including codominant, dominant, recessive and overdominant. A P-value of < .05 was considered statistically significant for all tests.

3. Results

In the present study, a total number of 410 participants (180 cases and 230 controls) with the age range of 21–50 years were enrolled. The mean age of the case and control groups was 37.32 ± 8.81 and 35.9 ± 7.63 years, respectively. The mean of BMI was 25.66 ± 2.98 and 25.93 ± 2.31 in the case and the control groups, respectively (P = .58). Fifty-six percent of the case group (n = 101) and 58.3% (n = 134) of the control group were male, compared to 44.0% (n = 79) and 41.7% (n = 96) females in the case and control groups, respectively (P = .66). The range of duration of LBP in the group with disc degeneration was 1–120 months. All patients had history of low back pain, with 115 (63.9%) patients had continuous type of pain while 65 (36.1%) had intermittent type of pain. Lumbar nerve root compression was present in 160 (88.9%) patients. One hundred and seventeen (65%) patients had multilevel disc degeneration. The most common levels of disc degeneration were L5-S1 and L4-L5 (38.9% and 36.1%, respectively). The frequencies of disc bulging, protrusion and extrusion were 33.9% (61/180), 53.9% (97/180) and 12.2% (22/180), respectively.

Genotypic frequencies of *VDR* and *GC* did not differ from what was estimated based on the Hardy–Weinberg equilibrium equation (P = .43 and. 08, respectively). So, there was no population stratification and no sampling bias. The allele and genotypes frequencies of the studied genetic variants in LDD cases and healthy subjects are shown in Table 2.

A significant difference was found between genotype frequencies of the *VDR* and *GC* polymorphisms in the control and the patient groups determined by the expected value of the χ^2-test (P = .017 and P = .005, respectively). Genotype frequencies of the *VDR* GG/GA/AA in LDD subjects were 35.5%, 47.8% and 16.7%, respectively; compared to 46.1%, 45.2% and 8.7%, respectively in the controls. The individuals with AA genotype were associated with increased risk of LDD compare to GG (OR = 2.48, 95% CI 1.30–4.73, P = .005). Subsequent grouping of the GG and GA genotypes in the recessive genetic model revealed a significantly increased risk of LDD in AA genotype carriers when compared with that of the wild-type homozygous GG and heterozygous GA genotype carriers (OR = 2.10; 95% CI 1.14–3.83, P = .01). Individuals homozygous for the minor A allele of the rs2228570 SNP in *VDR* gene were overrepresented in the group of cases (41% versus 31%, in controls) leading to approximately 1.5-fold increased risk for LDD (95% CI 1.12–1.99, P = .005). We also observed a tendency for *VDR* AA and GA genotypes to be associated with higher grades of disc herniation including protrusion and extrusion were (P < .05).

Moreover, a risk association was also identified for *GC* rs7041. The frequencies of the genotype TT, TG and GG were 45.7%, 40% and 14.3% and 61.7%, 28.3% and 10% in the controls and cases, respectively. Sixty-nine patients (%38.3) and 125 controls (%54.3) had TG and GG genotypes (dominant model: OR = 0.52; 95% CI 0.35–0.77, P = .001). The association was also significant when analyzing the GG genotype alone (codominant model: OR = 0.51; 95% CI 0.27–0.97, P = .04). The rs7041 T allele was more frequent in patients than in controls (patients vs. controls, 0.76 vs. 0.66). In other words, the G allele carriers seemed to confer protective effects on LDD risk (OR = 0.69; 95% CI 0.44–0.82, P = .001).

We also examined the combined effects of the *VDR* and *GC* genetic variants on LDD risk. As shown in Table 3, the presence of the GG -TG genotype of the VDR and GC genes may decrease the risk of LDD development (OR = 0.15; 95% CI 0.07–0.32, P < .0001). However, none of combined genotype conferred with increased risk for LDD.

4. Discussion

In the present study, we investigated the allelic and genotypic frequencies in the *VDR* and *GC* which affect vitamin D signaling, between healthy controls and LDD patients. The results of present study demonstrated an association between LDD and the rs2228570 SNP of the *VDR* gene as well as the rs7041 of *GC* gene. In addition, the presence of *VDR* rs2228570 polymorphism seems to be related to severity of LDD. There was no previous reports of rs2228570 and rs7041 SNPs in patients with LDD for the evaluation of allelic and genotypic frequencies.

The complex etiology of LDD involves a combination of environmental factors and genetic impairments [24,25]. A recently meta-analysis with 74 articles assessed the relationship between genetic polymorphisms and intervertebral disc degeneration. They found *IL-6* rs1800797 and *MMP-9* rs17576 are associated with increased risk of intervertebral disc degeneration [26]. We have previously shown that the effect of the *MMP-3* rs632478 polymorphism in relationship with the occurrence of disc degeneration in northern Iran [8].

Common SNPs in *VDR* and *GC* genes encode variant proteins that generate a different activity, especially in their affinity to vitamin D [27]. FokI polymorphism at translation initiation codon results in a smaller VDR that interacts with transcription factor 2B more efficiently and has greater transcriptional activity than the full length VDR [28]. The *VDR* known FokI and TaqI polymorphisms were investigated in common disorders such as osteoarthritis, disc degeneration disorders and osteoporosis [29,30,31,32].

In the present study, we reported that the rs2228570 AA genotype was related with an increased LDD susceptibility in Iran. In agreement with our results, Colombini et al. reported that the *VDR* rs2228570 FF (AA) genotype had a 2-fold increased risk of discopathies and/or osteochondrosis concomitant with disc herniation, in an Italian population [31,32]. Another Italian case-control study found a notable association between disc herniation and RANKL plasma concentration as

Table 2
Genotype frequency of *VDR* and *GC* SNPs in patients with LDD and controls.

Gene	Accession Number	Model	Genotype	Cases n(%)	Controls n(%)	OR(95% CI)	P-value
VDR	rs2228570	Codominant	GG	64(35.5)	106(46.1)	1.00	
			GA	86(47.8)	104(45.2)	1.36(0.89-2.08)	.14
			AA	30(16.7)	20(8.7)	2.48(1.30-4.73)	.005
		Dominant	GG	64(33.5)	106(46.1)	1.00	
			GA + AA	116(64.4)	124(53.9)	1.54(1.03-2.31)	.03
		Recessive	GG + GA	150(83.3)	210(91.3)	1.00	
			AA	30(16.7)	20(8.7)	2.10(1.14-3.83)	.01
		Overdominant	GG + AA	94(52.2)	126(54.8)	1.00	
			GA	86(47.8)	104(45.2)	1.10(0.74-1.63)	.60
GC	rs7041	Codominant	TT	111(61.7)	105(45.7)	1.00	
			TG	51(28.3)	92(40)	0.52(0.33-0.80)	.003
			GG	18(10)	33(14.3)	0.51(0.27-0.97)	.04
		Dominant	TT	111(61.7)	105(45.7)	1.00	
			TG + GG	69(38.3)	125(54.3)	0.52(0.35-0.77)	.001
		Recessive	TT + TG	162(90)	197(85.6)	1.00	
			GG	18(10)	33(14.3)	0.66(0.36-1.22)	.18
		Overdominant	TT + GG	129(71.7)	138(60)	1.00	
			TG	51(28.3)	92(40)	0.59(0.39-0.90)	.001

Table 3
The combination of the *VDR* and *GC* polymorphism in patients with LDD and controls.

Genotype		LDD n(%)	Controls n(%)	OR(95%CI)	P-value
VDR	*GC*				
GG	TT	43(23.9)	29(12.6)	Ref	
GG	TG	14(7.8)	62(27)	0.15(0.07-0.32)	< .0001
GG	GG	7(3.9)	15(6.5)	0.31(0.11-0.86)	.02
GA	TT	47(26.1)	64(27.8)	0.49(0.27-0.90)	.02
GA	TG	30(16.7)	25(10.9)	0.80(0.39-1.64)	.55
GA	GG	9(5)	15(6.5)	0.40(0.15-1.04)	.06
AA	TT	21(11.6)	12(5.2)	1.18(0.58-2.67)	.70
AA	TG	7(3.9)	5(2.1)	0.94(0.27-3.26)	.92
AA	GG	2(1.1)	3(1.3)	0.44(0.07-2.85)	.39

well as *VDR* FokI variant allele [33]. In a previous relevant meta-analysis on disc degeneration, T allele of *VDR* FokI polymorphism is highly associated with Intervertebral disc degeneration (IDD) in Hispanics and Asians, but not in Caucasians [34]. A recently meta-analysis with nine case control studies, suggested that the association between *VDR* TaqI-ApaI polymorphisms and IDD in Asians is still not clear [30]. In contrast, other meta-analysis did not find any association between the *VDR* TaqI, FokI and ApaI polymorphisms and the risk of intervertebral disc degeneration [20]. Therefore, further studies are warranted among Asian populations. These conflicting results may be a consequence of the number of subjects, the population admixture, the impact of other genetic and environmental factors. The summary of some studies associated with FokI polymorphism and disc degeneration are shown in Table 4.

The classical function of DBP is to store and prolong the half-life of circulating vitamin D metabolites. DBP binds 88 and 85% of serum 25(OH) D_3 and 1, 25$(OH)_2$ D_3, respectively [35]. Polymorphism of GC (rs7041) has been yet little explored in different diseases related case-control studies such as coronary artery disease [36], non-small cell lung cancer [37] and osteoporosis [38]. There are no previous reports about the prevalence of the GC rs7041 variant in patients with LDD. We found a significantly reduced LDD susceptibility with GG genotype in homozygote comparison (OR = 0.51; 95% CI 0.27–0.97, P = .04), dominant genetic model (OR = 0.52; 95% CI 0.35–0.77, P = .001), and allele comparison (OR = 0.69; 95% CI 0.44–0.82, P = .001).

In our study, no significant differences with respect to gender were observed (p = .66). However, Colombini et al. reported the gender-related association of the functional *VDR*-FokI polymorphism and conventional risk factors with specific spine pathologies in the

Italian white population [19].Unlike western countries, the Iranian women play a key role in agriculture and this can explain the fair equality of genders in our study.

There were some limitations in our study. First, our population was not large enough. Second, some potential confounding factors were not considered in this study, such as sex, occupation, BMI and vitamin D. Third, other polymorphisms of *VDR* and *GC* and their possible interactions were not evaluated. However, numerous factors act individually and together to influence risk of LDD. So, we should involve more factors in our future work.

It is concluded that both *VDR* and *GC* polymorphisms were significantly associated with the altered LDD risk. It is though evident that additional studies are needed to confirm these observations and to evaluate the functional effects of *VDR* and *GC* genetic variants on LDD.

Table 4
Summary of some studies associated with FokI polymorphism and intervertebral disc degeneration.

Authors	Country	Study Population	Results
Videman et al. [9]	Finland	85 Pairs of twins	Ff and ff genotypes associated with reduced disc signal intensity
Colombini et al. [31]	Italy	267 cases/220 controls	FF genotype and F allele represented approximately 2-fold risk factors to develop discopathies
Sansoni et al. [33]	Italy	110 cases/110 controls	F allele associated with disc herniation
Vieira et al. [39]	Brazil	121cases /131controls	T allele OR = 1.58 for developing IVDD
Eser et al. [40]	Turkey	150 cases/150 controls	ff Genotype associated with more severe grades of IVDD (grades III, IV)
Doraiswamy [41]	India	48 cases/47 controls	FF and Ff associated with LDD
Eskola [42]	Denmark	352 children	No association
Cervin Serrano [43]	Mexico	100 cases/100 controls	No association

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No fund.

Conflict of interest

None.

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References

[1] D. Hoy, L. March, P. Brooks, et al., The global burden of low back pain: estimates from the global burden of disease 2010 study, Ann. Rheumatol. Dis. 73 (2014) 968–974.
[2] A. El-Metwally, M. Mikkelsson, M. Ståhl, et al., Genetic and environmental influences on non-specific low back pain in children: a twin study, Eur. Spine J. 1 (7) (2008) 502–508.
[3] P.P. Vergroesen, I. Kingma, K.S. Emanuel, et al., Mechanics and biology in intervertebral disc degeneration: a vicious circle, Osteoarthr. Cartil. 23 (2015) 1057–1070.
[4] H.Y. Yuan, Y. Tang, Y.X. Liang, et al., Matrix metalloproteinase-3 and vitamin D receptor genetic polymorphisms, and their interactions with occupational exposure in lumbar disc degeneration, J. Occup. Health 52 (2010) 23–30.
[5] Y. Zhang, Z. Sun, J. Liu, et al., Advances in susceptibility genetics of intervertebral degenerative disc disease, Int. J. Biol. Sci. 4 (2008) 283–290.
[6] F. Mashayekhi, G. Shafiee, M. Kazemi, et al., Lumbar disk degeneration disease and aggrecan gene polymorphism in northern Iran, Biochem. Genet. 48 (2010) 684–689.
[7] Z.O. Toktaş, M.Ş Ekşi, B. Yılmaz, et al., Association of collagen I, IX and vitamin D receptor gene polymorphisms with radiological severity of intervertebral disc degeneration in southern European ancestor, Eur. Spine J. 24 (2015) 2432–2441.
[8] A. Saberi, Z. Salehi, B. Naderinabi, et al., Genetic dimension of intervertebral disc degeneration: polymorphism of matrix metalloproteinase 1 and 3 in the North Iranian population, Turk. Neurosurg. (2017), http://dx.doi.org/10.5137/1019-5149.JTN.19978-17.0 In press.
[9] T. Videman, J. Leppävuori, J. Kaprio, et al., Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration, Spine 23 (1998) 2477–2485 Phila Pa 1976.
[10] Z. Wang, Z. Qu, C. Fu, et al., Interleukin 1 polymorphisms contribute to intervertebral disc degeneration risk: a meta-analysis, PLoS One 11 (2016) e0156412.
[11] S. Kalb, N.L. Martirosyan, M.Y. Kalani, et al., Genetics of the degenerated intervertebral disc, World Neurosurg. 77 (2012) 491–501.
[12] S. Christakos, P. Dhawan, A. Verstuyf, et al., Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects, Physiol. Rev. 96 (2016) 365–408.
[13] N. Amizuka, H. Ozawa, Intracellular localization and translocation of 1 alpha, 25-dihydroxyvitamin D3 receptor in osteoblasts, Arch Histol. Cytol. 55 (1992) 77–88.
[14] A. Colombini, P. Lanteri, G. Lombardi, 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. 44 (2012) 1019–1030.
[15] H.E. Gruber, G. Hoelscher, J.A. Ingram, et al., 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 33 (2008) 755–765 Phila Pa 1976.
[16] M.T. Corvol, M.F. Dumontier, L. Tsagris, et al., Cartilage and vitamin D in vitro (author's transl), Ann. Endocrinol. (Paris) 42 (1981) 482–487.
[17] A.G. Uitterlinden, Y. Fang, J.B. Van Meurs, et al., Genetics and biology of vitamin D receptor polymorphisms, Gene 338 (2004) 143–156.
[18] A. Colombini, S. Cauci, G. Lombardi, P. Lanteri, S. Croiset, M. Brayda-Bruno, G. Banfi, Relationship between vitamin D receptor gene (VDR) polymorphisms, vitamin D status, osteoarthritis and intervertebral disc degeneration, J. Steroid Biochem. Mol. Biol. 138 (2013) 24–40.
[19] A. Colombini, M. Brayda-Bruno, L. Ferino, et al., Gender differences in the VDR-FokI polymorphism and conventional non-genetic risk factors in association with lumbar spine pathologies in an Italian case-control study, Int. J. Mol. Sci. 16 (2015) 3722–3739.
[20] G. Xu, Q. Mei, D. Zhou, et al., Vitamin D receptor gene and aggrecan gene polymorphisms and the risk of intervertebral disc degeneration - a meta-analysis, PLoS One. 7 (2012) e50243.
[21] A. Braun, R. Bichlmaier, H. Cleve, Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types, Hum. Genet. 89 (1992) 401–406.
[22] F.F. Safadi, P. Thornton, H. Magiera, et al., Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein, J. Clin. Invest. 103 (1999) 239–251.
[23] T.J. Wang, F. Zhang, J.B. Richards, et al., Common genetic determinants of vitamin D insufficiency: a genome-wide association study, Lancet 376 (2010) 180–188.
[24] N.L. Martirosyan, A.A. Patel, A. Carotenuto, et al., Genetic alterations in intervertebral disc disease, Front. Surg. 3 (2016) 59.
[25] M.N. Stienen, N.R. Smoll, G. Hildebrandt, et al., Influence of smoking status at time of surgery for herniated lumbar disk on postoperative pain and health-related quality of life, Clin. Neurol. Neurosurg. 122 (2014) 12–19.
[26] J. Rigal, A. Léglise, T. Barnetche, et al., Meta-analysis of the effects of genetic polymorphisms on intervertebral disc degeneration, Eur Spine J. (May 27) (2017), http://dx.doi.org/10.1007/s00586-017-5146-z.
[27] J. Arnaud, J. Constans, Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP), Hum. Genet. 92 (1993) 183–188.
[28] P.W. Jurutka, L.S. Remus, G.K. Whitfield, et al., The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB, Mol. Endocrinol. 14 (2000) 401–420.
[29] Z.H. Zhu, X.Z. Jin, W. Zhang, et al., Associations between vitamin D receptor gene polymorphisms and osteoarthritis: an updated meta-analysis, Rheumatol. (Oxf.) 53 (2014) 998–1008.
[30] L. Chen, S. Zhao, F. Niu, et al., Association between vitamin D receptor gene polymorphisms and intervertebral disc degeneration: A meta-analysis, J. Orthop. Sci. 22 (2017) 184–189.
[31] A. Colombini, M. Brayda-Bruno, G. Lombardi, et al., FokI polymorphism in the vitamin D receptor gene (VDR) and its association with lumbar spine pathologies in the Italian population: a case-control study, PLoS One 9 (2014) e97027.
[32] A. Colombini, M. Brayda-Bruno, G. Lombardi, et al., BsmI, ApaI and TaqI polymorphisms in the vitamin D receptor Gene (*VDR*) and association with lumbar spine pathologies: an Italian case-control study, PLoS One 11 (2016) e0155004.
[33] V. Sansoni, S. Perego, A. Colombini, et al., Interplay between low plasma RANKL and VDR-FokI polymorphism in lumbar disc herniation independently from age, body mass, and environmental factors: a case-control study in the Italian population, Eur. Spine J. 25 (2016) 192–199.
[34] J. Zhao, M. Yang, J. Shao, et al., Association between VDR FokI polymorphism and intervertebral disk degeneration, Genom. Proteom. Bioinform. 13 (2015) 371–376.
[35] P. White, N. Cooke, The multifunctional properties and characteristics of vitamin D-binding protein, Trends Endocrinol. Metab. 11 (2000) 320–327.
[36] D. Stakisaitis, V. Lesauskaitė, M. Girdauskaitė, et al., Investigation of vitamin D-binding protein polymorphism impact on coronary artery disease and relationship with longevity: own data and a review, Int. J. Endocrinol. 2016 (2016) 8347379.
[37] X. Wu, J. Cheng, K. Yang, Vitamin D-related Gene polymorphisms, plasma 25-hydroxy-vitamin D, cigarette smoke and Non-Small Cell Lung Cancer (NSCLC) risk, Int. J. Mol. Sci. 17 (10) (2016).
[38] Y. Fang, J.B. van Meurs, P. Arp, et al., Vitamin D binding protein genotype and osteoporosis, Calcif. Tissue Int. 85 (2009) 85–93.
[39] L.A. Vieira, P.L. De Marchi, A.A. dos Santos, et al., Analysis of FokI polymorphism of vitamin D receptor gene in intervertebral disc degeneration, Genet. Test. Mol. Biomarkers 18 (2014) 625–629.
[40] B. Eser, T. Cora, O. Eser, et al., Association of the polymorphisms of vitamin D receptor and aggrecan genes with degenerative disc disease, Genet. Test. Mol. Biomarkers. 14 (2010) 313–317.
[41] R. Doraiswamy, K. Ramaswami, R. Subramanian, et al., Association of vitamin-D receptor polymorphisms with degenerative disc disease in Indian population, FASEB J. 31 (2017) 902–919.
[42] P.J. Eskola, P. Kjaer, I.M. Daavittila, et al., Genetic risk factors of disc degeneration among 12-14-year-old Danish children: a population study, Int. J. Mol. Epidemiol. Genet. 29 (1) (2010) 158–165.
[43] S. Cervin Serrano, D. González Villareal, M. Aguilar-Medina, et al., Genetic polymorphisms of interleukin-1 alpha and the vitamin d receptor in mexican mestizo patients with intervertebral disc degeneration, Int. J. Genom. 2014 (2014) 302568.