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# DEGENERATIVE DISC DISEASE AND GENETIC

## *DEJENERATİF DİSK HASTALIĞI VE GENETİK*

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**SUMMARY:**

Although development of the intervertebral disc disease is a multifactorial condition, recent studies associated with genes playing a role in disc dejeneration revealed that the importance of the genetic factors in the development of disc disease. Our purpose of this study is to review current knowledge on genes associated with intervertebral disc degeneration.

**Keywords:** degenerative disc disease, aggrecan, collagene, vitamin D receptor, interleukin, ADAMTS, matrixmetalloproteinase

**Level of evidence:** Review article, Level V

**ÖZET:**

İntervertebral disk hastalığının gelişimi her ne kadar multifaktöriyel bir durum olsa da, disk dejenerasyonunda rol oynayan genlerle ilişkili son zamanda yapılan çalışmalar, genetik faktörlerin disk hastalığı gelişimindeki rollerinin önemini ortaya koymuştur. Bu çalışmadaki amacımız intervertebral disk dejenerasyonunda rol oynayan genlerle ilgili mevcut bilgilerin gözden geçirilmesidir.

**Anahtar Kelimeler:** Dejeneratif disk hastalığı, agrekan, kollajen, vitamin D reseptör, interlökin, ADAMTS, matriksmetalloproteinaz

**Kanıt Düzeyi:** Derleme, Düzey V

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## INTRODUCTION

Low back pain is in the first range among public health problems seen in the active living communities. Low back pain is an important health care problem especially in developed societies in terms of the treatment costs and labor loss. Annual incidence of backache differs between 15% and 20 % in adult population in the industrialized societies. Incidence of the backache in a certain period of life again in the adult population is estimated between 50% and 80% [63]. Inflammatory, metabolic, neoplastic, infectious and traumatic causes play a role in the etiology of low back pain. Degenerative disc disease is one of the leading degenerative causes of low back pain (Fig. 1a,b).

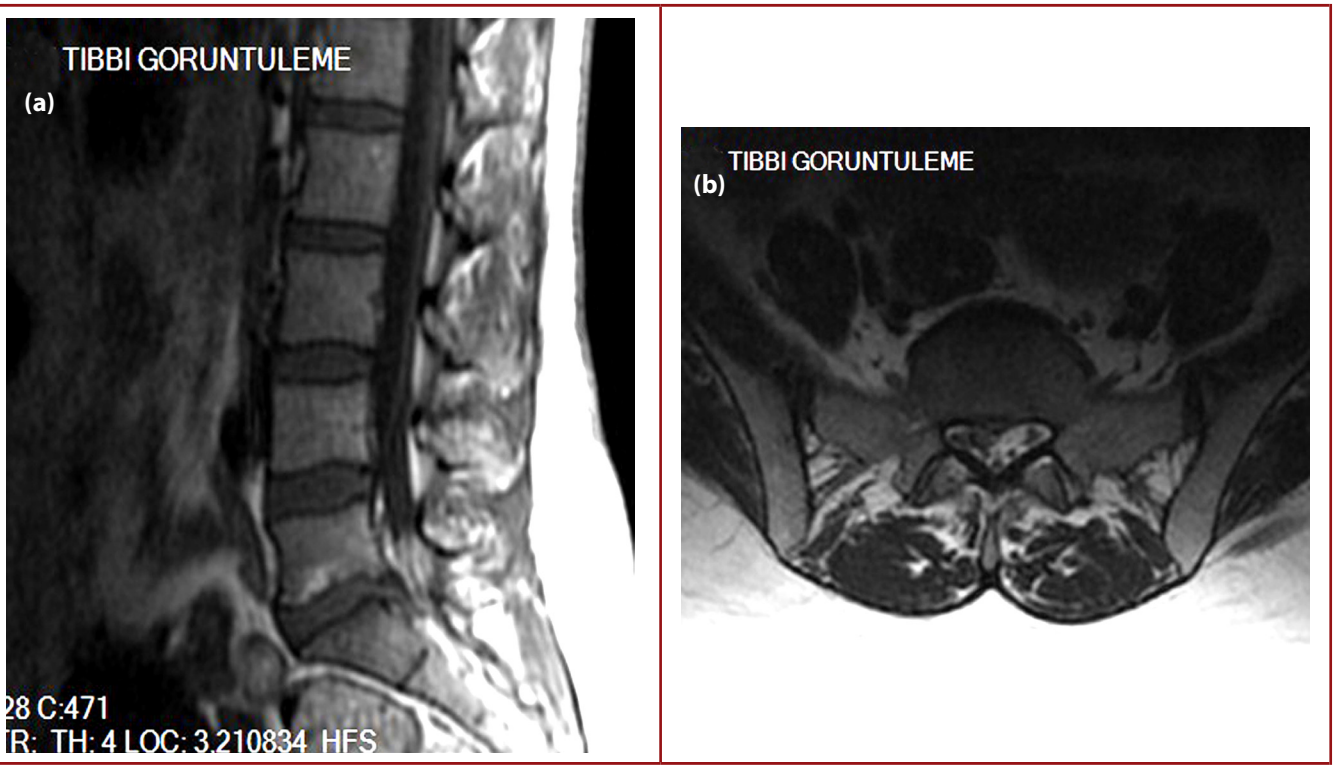


**Figure-1.** Sagittal **(a)** and axial **(b)** MR imaging of a degenerative disc disease.

Besides environmental factors such as exposure to heavy physical load, age, smoking, trauma and infection, genetic predisposition also plays a role in development of degenerative disc disease which naturally emerges during the aging process[17]. Battie et al. described hereditary factors to play a role in disc degeneration for the first time as a result of their magnetic resonance imaging (MRI) guided study in monozygotic twins[4]. In both in vivo and in vitro studies that have been subsequently conducted, numeruous genes have been demonstrated to play a role in development of degenerative disc disease.

Intervertebral disc degeneration is biochemically characterized by degradation of the extracellular matrix. Extracellular matrix primarily consists of proteoglycans and collagens. Metabolism of the extracellular matrix is regulated by the balance between matrix metalloproteinases (MMPs) and aggrecanases which are degrading enzymes and tissue inhibitors of metalloproteinases that are their natural inhibitors. Degenerative disc disease develops in the case of imbalance between these enzymes[34,75]. There is evidence suggesting that besides polymorphisms which code these enzymes, aggregate, collagen, vitamin D receptor and interleukin gen polymorphisms also play a role in intervertebral disc degeneration[2,50,68,71,78]. With the studies conducted in the light of this information, it has been shown that a part of these enzymes which play a role in development of degenerative disc disease may also play a role in the treatment. Treatment of degenerative disc disease with the genes involved in the initial period and the enzymes coded by these genes does not seen to be impossible in the near future (Fig. 2).

Aggrecans are proteoglycans clusters (PG) which consist of the glycosaminoglycan (GAG) chains covalently bound to the protein nucleus and sulfated. Aggrecan is an important proteoglycan, providing resistance of the tissue against compressive loads in the intervertebral disc structure. This structural feature is related to the chondroitin sulfate chains that abundantly present especially in the nucleus protein. Chondroitin sulfate chains present in domains of the nucleus protein that are termed as CS1 and CS2. Chondroitin

sulfate content of the aggrecan in intervertebral disc differs, because aggrecan gene region which codes CS1 domain in humans presents dimensional polymorphism. This situation causing to weakening of the aggrecan structure leads to intervertebral disc degeneration[62]. The relationship between lumbar disc degeneration and aggrecan gene polymorphism was demonstrated first by Kawaguchi et al. They argued that individuals who have a short CS1 domain are in the risk for developing multilevel intervebral disc degeneration at an early age[39]. In a study with 588 mono- and dizygotic twins, Videman et al. reported that, AGC1 gene was associated with surge in the disc, and reduction in the water content and height of the dics[79]. Types and numbers of the aggrecan gene varies among the different societies. Although different results were obtained in the subsequent studies, a correlation has been shown between the short repeated allele frequency and disc degeneration in the Turkish society [13,14].

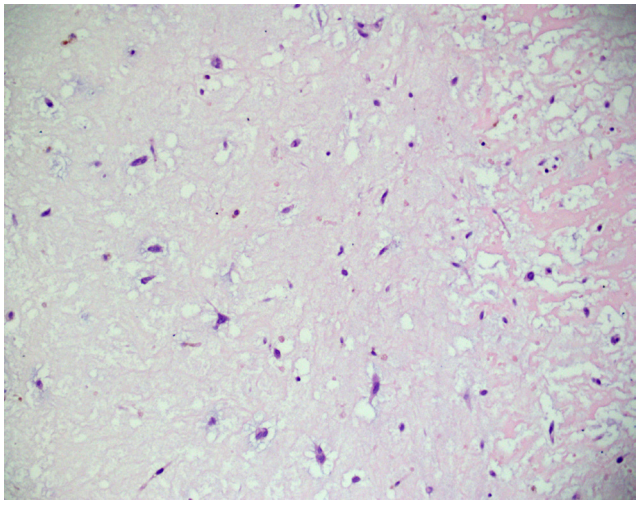

**Figure-2.** Photomicrograph (HEX400) of articular cartilage of degenerated disc tissue.

## COLLAGEN GENE POLYMORPHISMS

Collagens are macromolecules which provide tensile strength of the disc. Collagens form 70% of the annulus fibrosus %70 and 20% of the dry weight of nucleus pulposus. Although there are 16 types of collagens, 80% of those in the human body are Type-1 and Type-2 collagens. Besides those types, there are also Types 3,5,6,9 and 11 collagens in structure of the intervertebral disc. Type-1 collagen is synthesized by fibroblasts and is major callagen of the annulus fibrosus. Type-2 collagen is synthesized by the chondrocytes and mainly presents in the nucleus fibrosus. In addition, Type-3 and Type-5 collagens are synthesized in small quantities in the annulus fibrosus, Type-11 collagen in the nucleus pulposus, Type-6 and Type-9 collagens both in the annulus fibrosus and nucleus pulposus. Type-2 collagen is the major collagen of end-plate at the same time[60]. Although gene polymorphisms causing alterations in the collagen structure have been demonstrated to be associated with the development of disc degeneration, their role in the disc degeneration process is yet to be fully clarified due to the differences observed among societies, even among persons[15].

In the first study regarding the gene polymorphism coding Type-1 collagen, Grant et al. described Sp1 polymorphism which affects Sp1 transcription binding area of the COL1A1 gene. COL1A1 gene codes α1 of Type 1 collagen. As a result of this polymorphism, chain rates in the collagen structure change and durability of the collagen is deteriorated[21]. In another study by Pluijm et al., increase in the frequency of TT (thymine-thymine) allele in the COL1A1 gene was reported to cause increas in the interval disc degeneration[56]. In their study on 24 young Greek soldiers Tilkeridis et al. demonstrated that, TT genotype was associated with the disc degeneration[73]. Videman et al. reported that COL1A1 gene polymorphism led to a loss in the disc signal intensity[79].

In a study by Sahlman et al. showed that inactivation of one of the Col2A1 gene alleles forming the structure of Type 2 collagen caused ossification of the premature vertebral endplate and moderate disc degeneration in experimental mice[65].

Collagen 9 functions as a bridge between collagenous and non- collagenous proteins. Collagen 9 is a heterotrimeric protein which genetically consists of 3 different chains coded by COL9A1, COL9A2 and COL9A3[2,51,55]. In their study on the Finnish population, Karppinen et al. reported that presence of trp2 (tryptophan) allele in the COL9A2 gene was associated with degeneration of the disc and end-plate[38]. In their study with Chinese volunteers, Jim et al. underlined that presence of trp2 allele was effective on development of the disc degeneration at an early age[31]. In a study by Seki et al on Japanese society, presence of trp2 allele was stated to be correlated with the increase in disc degeneration in patients under 40 years of age[67]. Paassilta et al. reported that presence of at least one trp3 allele in α3 chain of the collagen 9 gene increased the disc degeneration by 3 folds[52]. In their study on Greek society, Kales et al. obtained contrary results[33]. Videman et al. reported that, there was an association between COL9A2 and disc generation which especially occurred at the lower lumbar levels, but no such a relationship was found for different alleles of the COL9A3 gene. Again in that study, correlations were demonstrated between allelic variants of the COL1A2 gene and disc degeneration at the lower lumbar level and between allelic variants of the COL11A1 gene and disc degeneration at the upper lumbar level. In the same study, Videman et al. showed the association between COL11A

and COL3A1 genes and disc bulging[79]. Likewise Mio et al. demonstrated the association between allelic variants (rs1676486) of the COL11A1 gene and disc degeneration in Japanese population[48]. Solovieva et al. and Noponen-Hietela et al. showed that COL11A2 polymorphism was associated with disc bulging and degenerative spinal stenosis[50,69]. Boyd et al. demonstrated the association between COL9A1 gene polymorphism and disc degeneration in their study on mice[7]. In their study with compressed discs, Guehring et al. demonstrated increased regulation of the COL1A2 gene in animal model[24].

## VITAMIN D RECEPTOR GENE POLYMORPHISMS:

Vitamin D plays a crucial role in sulfatation of glycosaminoglycans during the proteoglycan synthesis. Sulfatation mechanism and vitamin D receptor gene (VDR) must be stable for proteoglycans can normally function. VDR bind to the active form of vitamin D and help the control of the calcium balance. Furthermore, it is also effective on differentiation, proliferation and maturation of chondrocytes. Chondrocytes play an important role in proteoglycan synthesis. As a conclusion, VDR indirectly influence the lumbar disc degeneration due to its effect on chondrocytes. However, the exact mechanism is still not fully known[6,16].

The association between VDR and disc degeneration was demonstrated for the first time by Videman et al. in 1998. In that study, two iatrogenic polymorphisms were analyzed using MRI in 85 couples male monozygotic twins. Taq 1 was one of these polymorphisms. Individuals having Taq 1 tt and Tt genotypes were found to show predisposition to disc degeneration. Similarly, in ff and Ff genotypes of the second polymorphism Fok1, predisposition to disc degeneration was reported[77]. In a study with 205 persons in the 20-29 age range in Japanese population, Kawaguchi et al. reported that Taq 1 Tt genotype caused multilevel prominent disc degeneration[37]. Cheung et al. found a correlation between t allele of Taq 1 polymorphism of the VDR gene and, bulging and degeneration of the disc in persons under 40 years of age[9]. Yuan et al. demonstrated the association between VDR-Apa A allele mutation and disc degeneration[82]. In their study on Australian society, Jones et al. reported predisposition to disc degeneration in the persons with VDR Taq 1 tt genotype[32]. In a study by Eser et al. on Taq 1 and Fok 1 polymorphisms, Ff, ff and tt genotypes were shown to be associated with heavy disc degeneration[14].

## INTERLEUKIN GENE POLIMORPHISMS:

Inflammatory cytokines are produced in the body in cases of stimulation of the antigenic response such as trauma, infection etc. Their contribution to backache is well-known. In addition, inflammation is one of the factors playing an important role in catabolic process of degenerative disc disease. Solovieva et al. demonstrated the association between IL-1 polimorphism and disc degeneration using MRI in actively working Finnish population. They reported that in presence of IL-1b + 3954 T and IL-1a - 889 T alleles, disc surge increased by 2.4 and 3 folds, respectively[68]. In their study, Videman et al. noted that IL1A, IL18RAP and IL18R1 gene polymorphisms were associated with disc dehidration[79].

## ADAMTS GENE POLYMORPHISMS:

ADAMTS (a metalloproteinase with disintegrin and thrombospondin motifs) is a sub family of metalloproteinase having degradation feature, affecting a part different from matrix metalloproteinases. There are 19 subtypes of ADAMTS which are divided into 4 groups in human genome[58]. Aggrecanases (ADAMTS-1,-4,-5,-8,-9,-15 ve -20) have a proteoglycanolitic activity. In a recent study by Pockert et al., elevation of ADAMTS-1, -4, -5 and -15 were shown to be associated with disc degeneration and, the increase in ADAMTS-4, -5 and -15 to be correlated with increment in the degree of disc degeneration[57]. In their animal model of the intervertebral disc degeneration, Furtwangler et al. reported that in that injection of ADAMTS-4 was associated with reduction in the proliferation and survival of the cells[19]. Majumdar et al. and Patel et al. demonstrated the relationship between ADAMTS-4 and intervertebral disc degeneration[47,53]. In their study without a control group, Hatano et al. showed the association between transligamentous disc sequestration and mRNA expression of ADAMTS-4[28]. Roberts et al. reported the association between increase of the severity of disc degeneration and aggrecanase production [61].

## MATRIX METALLOPROTEINASE GENE POLYMORPHISMS:

Matrix metalloproteinases are an enzyme family capable to degrade major structural components of the intervertebral disc, having 28 types described until today. In addition, matrix metalloproteinases plays a role both in the natural production – degradation process and pathological destruction of the extracellular matrix of other several connective tissues. Main factor in intervertebral disc degeneration is impaired balance between matrix metalloproteinases and tissue metalloproteinase inhibitors that inhibit them. Of 28 types of matrix metalloproteinases, especially collagenase (MMPs 1, 8, 13), gelatinase (MMPs 2, 9), matrilysin (MMP-7) and stromelysin (MMP-3) are thought to play a prominent role in intervertebral disc degeneration. Matrix metalloproteinases are secreted as the inactive precursors and then become active. TIMPs inhibit the matrix metalloproteinases through zinc binding region of the enzyme. There are 4 types of TIMP

identified with only TIMP-4 showing a high sensitivity to the cardiac tissue. TIMPs 1 and 2 among the tissue metalloproteinase inhibitors are capable to inhibit all the MMPs in intervertebral disc degeneration. Although they have ability to degrade all subtypes of the collagen such as MMP-1, MMP-8 and MMP-13, they are effective especially in degradation of the fibrillar collagen which provides mechanical strength to the tissues. MMP-1 is expressed by the activated macrophages (19,61). Weiler et al found that MMP-1, 2 and 3 were highly correlated with development of cleft and scar in the disc tissue[81]. In a study by Song et al. on the adult Japanese population, an association was found between the lumbar disc degeneration and the prevalence of MMP-1 promoter polymorphism[70]. In a their study conducted in order to demonstrate the associationship between lumbar intervertebral disc herniation and the gene transcription of MMP and ADAMTS, Tsarouhas et al. did not observe mRNA expression of MMP-1 in the control or herniated disc samples[74].

MMP-2 and MMP-9 are zinc-dependent proteinases and since they use gelatin, laminin and denatured collagen as substrate they also termed as gelatinases. In a study by Bergknut et al. comparing intervertebral disc degeneration in humans and dogs, increase of the severity of intervertebral disc degeneration was reported to be correlated with the increase in MMP-2 activity in the nucleus pulposus[5]. Rastogi et al. showed MMP-2 expression in annuluses of the rodents' intervertebral discs which were exposed to loading and drilling damage, and they proposed that MMP-2 played an important role in degenerative alterations in the intervertebral disc[59]. In their study with the dogs which have thoracolumbar extruded disc hernia, Karli et al. demonstrated the increase of MMP-2 and MMP-9 in the affected dogs. In addition, they suggested that increased MMP-2 might contribute to degeneration of the extruded disc material in epidural region during the intervertebral disc degeneration process[36]. In their study, Dong et al. showed that the frequency of MMP-2-1306CC genotype was significantly higher in the patients having lumbar disc disease than in the controls. The same authors also proposed that MMP-2-1306C/T polymorphism might be a genetic risk for development of lumbar disc disease in young adults[12]. In addition, correlation between the degenerative disc lesions and increased expression in the MMP-2 activity was demonstrated in a study by Crean and Roberts[10,61]. In a study by Hsieh et al., MMP-2 production was shown to increase in the disc cells exposed to an abnormal physical loading[29]. In addition, in their study on mice Lotz et al. reported that MMP-2 has an important function in disc degeneration[45]. Kozaci et al. reported that the levels of Pro-MMP-2 increased in the early periods of degenerative disc disease[42]. In their study on rabbits, Hua et al. demonstrated that, ulinastatin which is the inhibitor of urinary trypsin decreased expression of MMP-2 and MMP-3 in IL-1β-induced disc generation[30]. In a study conducted on rodents by Rostagi et al., MMP-2 was proposed to have an important function in the etiology of degenerative disc disease and might have a potential therapeutic role[59]. In a research by Rugets and Kang on humans, the relationship between rough morphological changes in the annulus fibrosus and MMP-2 was demonstrated[35,64].

MMP-3 is a proteoglycan degrading enzyme which plays an important role in intervertebral disc degeneration. MMP-3 regulation is primarily adjusted at the level of transcription. MMP-3 is a key enzyme in degradation of extracellular matrix. MMP-3 activates collagenases and MMP-7 as well as other MMPs through macrophages and indirectly affects degradation of cartilage matrix[20]. Takahashi et al. evaluated the relationship between disc degeneration and MMP-3 gene polymorphism in 54 young females and 49 adults in Japanese society. They found that, MMP-3-5A5A and -5A6A genotypes were more associated with degenerative findings in adults compared to -6A6A genotype[72]. In their study on 720 British female patients, Valdes et al. showed the association between intervertebral disc degeneration and MMP-3[76]. In their study, Zigouris et al. demonstrated the relationship between aging and MMP-3. Again in that study, the authors argued that, MMP-1 expression was more in young population and therefore MMP-1 might play a role in degradation of matrix in the young patients[85]. In a study with 174 patients and 284 control individuals in the Chinese society, Yuan et al. reported that the persons who have mutation of MMP-3 5A alleles were predisposed to disc degeneration. In addition they reported that, coupled with vibration of all the body, bending and torsional movements, -5A allele mutation increased the risk for lumbar disc degeneration[82]. In their study, Nemato et al. proposed that MMP-3 was produced especially by slightly degenerated discs[49]. Kanemoto et al., found that MMP-3 expression increased more compared to TIMPS-1 in degenerative intervertebral disc disease[34]. Haro et al. reported that MMP-3 was required for the matrix degradation in herniated intervertebral disc resorption model[25]. In a study by Roberts et al. conducted with immunohistochemical staining method on 49 disc tissue which they collected from 46 patients, MMP-3 was reported to show immunopositivity by 65%[61]. In their study on the mouse intervertebral discs, Fujita et al. reported that MMP-3 induced proteoglycan loss during the aging process [18]. In their study on the bovine intervertebral disc, Furtwangler et al. reported that MMP-3 provoked nor the visible matrix degradation neither major shift in the gene expression[19]. In a disc degeneration model which was induced by static compression loading in rodents, Yurube et al. reported that, MMP-3 played an important role in disc degeneration and was a proper indicator of the degeneration[83].

In their study on rat tails which were dynamically loaded, MacLean et al. reported important mRNA alterations of MMP-3 [46]. In a study by Bachmeier et al. in the degenerated and herniated discs, an important elevation was found in mRNA levels of MMP-3 and MMP-8[3]. It was reported in a study by Tsarouhas et al. that MMPs and ADAMTS-4 showed a synergistic effect in intervertebral disc herniation. Furthermore, they demonstrated that smoking descreased the expression of MMP-3 [74]. In their study on 48 intervertebral disc materials collected from 42 patients, Canbay et al. found a significant correlation between the histopathological grade of intervertebral disc degeneration and MMP-3 using magnetic resonance images and, this correlation became more prominent with aging[8].

MMP-7 plays an important role in degradation of the diseased articular cartilage. MMP-7 is activated by MMP-3. Stromelysin (MMP-3, -10) and matrilysins (MMP-7, -26) are broad spectrum proteinases that have important regulatory functions in activation of the other MMPs [80]. MMP-7 secreted from macrophages is important for the secretion of soluble TNF-α [26]. In a study by Haro et al., MMP-7 was reported to be important for the disc resorption. In their different study, Haro et al. demonstrated that recombinant human MMP-7 (rhMMP-7) degraded the disc tissue in the surgical samples, depending on concentration. Again Haro et al. reported in their study conducted on dogs that intradiscal application of rhMMP-7 caused a decrease in the water content and proteoglycan component of the disc. Again in the study by Haro et al. MMP-7 production from macrophages was demonstrated to be required in the secretion of TNF-α which is needed for macrophage migration to the disc tissue[27].

MMP-8, known as neutrophil collagenase, is synthesized and stored in the polymorphonuclear leukocytes. MMP-8 plays a role rather in the degradation of Type 1 collagen[41]. In a study by Roberts et al., MMP-8 activity was reported to increase by 4 folds in prolapsed disc hernias[61]. Tsaoruhas et al. reported similar results[74].

MMP-9 is a zinc-dependent proteinase associated with matrix degradation in the disc tissue. It is also classified as gelatinase B and uses gelatin, laminin and denatured collagen as substrate. MMP-9 is synthesized in proenzyme form and stored in the neutrophil granules. In fact, MMP-9 is associated with M2-polarized macrophages and involved in wound healing and tissue remodeling[80]. In their study with 43 patients, Zigouris et al. showed the association between degree of herniation and MMP-9 expression in the patients under 30 years of age. Again in that study, correlation was reported between histological degeneration score and MMP-9 expression in all age groups[85]. In their study on dogs, Karli et al. reported that MMP-9 expression decreased in the acute and increased in the subacute and chronic processes of disc disease[36]. In a study by Roberts and Weiler, MMP-9 expression was reported to increase in the degenerated disc tissue[61,81]. Similarly, in a study by Crean et al., MMP-9 level was shown to increase in the degenerated discs[10]. In tehir study conducted in order to demonstrate catabolic and anabolic gene expression in intervertebral disc degeneration using rat tail compression model, Yurube et al. found a significant increase in mRNA expression of MMP-3, 7, 9 and 13 enzymes[83]. In their study on intervertebral disc samples collected from the posterior open discectomy in 63 patients, Tsarouhas et al. demonstrated that levels of MMP-9 and -13 mRNA significantly increased in the patients with chronical pain as a result of neovascularization and chronic inflammation. In addition, in that study the authors reported that mRNA levels of MMP-9 were lower in the sequestred discs than in the protruded ones[74].

MMP-13 is a member of collagenases and degrades triple helical region of the fibrillary collagen. MMP-13 degrades type 2 collagen rather which is dominant collagen in the nucleus pulposus and it is the most potent peptidolytic enzyme in this group[41,80]. In a study by Klawitter et al., MMP-13 expression was reported to increase in Thompson 5 degree degenerated disc samples[40]. Similar results were obtained in a study by Le Maitre and Anderson[1,43]. In their study on 49 disc materials collected from 46 patients after anterior surgeries, Roberts et al. reported that MMP-13 was positively stained by 88% in the immunohistochemical staining of the degenerated disc tissue samples and this rate reached to 100% in the protruded disc materials[61]. In their study on the bovine intervertebral discs, Furtwangler et al. reported that MMP-13 expression increased by ten thousand folds in the nucleus pulposus in the degenerated disc samples[19].

## CONCLUSION:

Intervertebral disc degeneration is a multifactorial condition having a complex etiopathogenesis. Today disc degeneration process can not be avoided with still used clasical treatment methods and mostly semptomatic treatment for degenerative disc disease are applied with conservative and surgical approaches whic are implemented after disc degeneration. In disc degeneration course, besides the genes that are above mentioned which effects were demonstrated in numerous studies, there is evidence showing also effects of the cartilage intermediate layer protein, osteonectin gene (SPARC), polymorphisms of tissue metalloproteinases inhibitor 1 (TIMP1) and cyclooxygenase 2 (COX-2) and ADAMTS-5 polymorphisms[22,23,66,76,84,86]. However, number of the studies regarding the role of genetic factors which are active in disc degeneration process for the treatment is limited. We hope that, degenerative disc disease will be transformed from a treatable process into the preventable condition as a

result of better understanding of genetic factors involved in intervertebral disc degeneration and the relationship between them in the future.

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