RESEARCH ARTICLE

A genetic variant in COL11A1 is functionally associated with lumbar disc herniation in Chinese population

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Conflict of interest

The authors have no conflict of interest to declare.

Abstract

This study aimed to explore whether the genetic variant of COL11A1 is functionally associated with the development of lumbar disc herniation (LDH) in Chinese population. SNP rs1676486 of COL11A1 was genotyped in 647 patients and 532 healthy controls. The differences of genotype and allele distributions between LDH patients and healthy controls were evaluated using the Chi-square test. One-way ANOVA test was used to compare the relationship between genotypes and clinical features including tissue expression of COL11A1 and the degree of disc degeneration. Patients were found to have a significantly higher frequency of TT than the controls

(10.2% vs. 7.3%, p = 0.004). Besides, the frequency of allele T was found to be remarkably higher in the patients than the controls (34.8% vs. 28.1%, p < 0.001), with an odds ratio of 1.36 (95% confidential interval = 1.14 - 1.63).Patients with genotype TT were found to have remarkably more severe disc degeneration (p = 0.02). Besides, the expression of COL11A1 in the lumbar disc was significantly lower in the patients with genotype TT than in those with genotype CT or CC (p < 0.001). Moreover, the expression level was inversely correlated with the severity of disc degeneration (p < 0.001). We confirmed that the rs1676486 of COL11A may be functionally associated with LDH in the Chinese population. Extracellular matrix related proteins may play an important role in the pathogenesis of LDH. Our findings shed light on a better understanding of the pathogenesis of LDH, which could be a promising target for a novel treatment modality of LDH.

Introduction

Lumbar disc herniation (LDH) is a degenerative lumbar disease characterized by morphological and biochemical changes of the lumbar disc (Park et al., 2001; Yang et al., 2015). It has been concluded as the predominant cause of low back pain (LBP), which can lead to reduced physical activity, decreased quality of life, and psychological distress (Akgun et al., 2010; Hirayama et al., 2006). Prevention of LDH seems much more important than clinical treatments, due to the high cost and disability resulted from symptomatic LDH. Therefore, to facilitate preventative and therapeutic measures for LDH, it is beneficial to understand its etiology. LDH is commonly believed to be a complex disease with both genetic and environmental factors contributing to its development and progression (Sansoni et al., 2016;

Scapinelli, 1993; Zhang et al., 2013). Although many risk factors have been investigated for LDH, however, its pathogenesis remains unknown.

Previous familial and twins studies have strongly supported the role of heredity in the development of LDH (Matsui et al., 1992; Obukhov et al., 1996). Associations between candidate genetic variants and LDH have been documented in many studies (Colombini et al., 2016; Colombini et al., 2014; Cong et al., 2014; Hasvik et al., 2014; Hirose et al., 2008; Mio et al., 2007; Olsen et al., 2012; Aparicio et al., 2011; Sansoni et al., 2016; Zhang et al., 2013). Paz et al (Paz et al., 2011) reported that genetic polymorphism of the IL-1β is remarkably associated with symptomatic LDH in the Caucasians with an odds ratio of 1.7. Karasugi et al (Karasugi et al., 2009) examined the association between the SKT and LDH in two independent Japanese populations, and found allele A of rs16924573 in the SKT can increase the risk of LDH by 1.31 folds. Hirose et al (Hirose et al., 2008) reported that an intronic SNP in the THBS2 is significantly associated with LDH in Japanese population with an odds ratio of 1.38. Collectively, it appears increasingly possible that there are many genetic risk factors of LDH with each of them conferring a small relative risk. Different genetic backgrounds and environment exposures in different ethnic population may affect the pathogenesis of LDH. Therefore, replication studies are always warranted to identify genetic variants loci that are truly associated with LDH.

Abnormal expression of the extracellular matrix (ECM) proteins has been reported to be implicated in the etiology of disc degeneration (Antoniou et al., 1996; David et al., 2011). Furthermore, phenotypes of transgenic mice and human

mutations indicated that ECM genes can be considered as susceptible genes for LDH (Kimura et al., 1996). Recently, Mio et al (Mio et al., 2007) identified a functional polymorphism rs1676486 of the COL11A1 is associated with the susceptibility to LDH in Japanese populations. The authors found that allele T of rs1676486 was associated with decreased synthesis and stability of mRNA of collagen 11. COL11A1 plays an important role in the formation of collagen in matrix of the disc. Moreover, the expression level of the COL11A1 was found to be inversely correlated with the severity of disc degeneration (Mio et al., 2007). To the best of our knowledge, association between the genetic variant of the COL11A1 and LDH remains unexplored in Chinese population. In this study, we performed a case-control study to explore the relationship between the polymorphism of the COL11A1 and the development of LDH in Chinese population. Moreover, the relationship between the expression of the COL11A1 and the severity of LDH was also investigated.

Methods

Subjects

Patients who were diagnosed as LDH at our clinic center between June 2012 and October 2015 were retrospectively evaluated for the eligibility to be included in the current study. The total number of patients diagnosed in this period was 1137. The diagnosis of LDH was determined by the extension of the lumbar disc beyond margins of adjacent vertebral bodies on magnetic resonance imaging (MRI) obtained with a 1.5-T system (Paz et al., 2011). Patients were excluded from the study if having synovial cyst, spondylolisthesis, spinal tumor or inflammatory disease. Moreover,

those with occupational or habitual risk factors, such as heavy manual laborers, drivers, and heavy smokers were also excluded from the study. The healthy participants were recruited during their routine examinations prior to university admission. All the control subjects were verified to have normal lumbar disc through MRI examination. The protocol of our study was approved by the ethics committees of the local institution, and informed consent was obtained from the participants for the collection of blood sample. Besides, patients who underwent discectomy surgeries also gave their informed consent for the collection of intervertebral disc tissue. Baseline characteristics of the participants including gender, age, weight and height were recorded at their visit to our center. Body mass index (BMI) was calculated with the weight divided by the square of the corrected height. The severity of disc degeneration was evaluated according to Schneiderman's classification (Schneiderman et al., 1987).

Genotyping of the target SNP

A total of 845 patients qualified the inclusion criteria as mentioned in the sub-section of subjects. Peripheral blood was collected from 647 patients at their first visit to our center. The number of lost subjects was 198. Genomic DNA was extracted from the blood samples with standard DNA extraction kit (Qiagen K.K., Tokyo, Japan). SNP rs1676486 of COL11A1 was genotyped using TaqMan SNP Genotyping Assay, with the results interpreted by ABI Prism 7900HT Sequence Detection System(Applied Biosystems, Foster City, CA). Twenty percent of the samples were randomly selected and genotyped to ensure the reliability of the genotyping results.

Tissue expression of COL11A1 in LDH patients

The intervertebral disc was collected from 100 patients during the surgeries. RNA extraction was completed with a commercial kit according to the manufacturer's protocol (CWBio. Co. Ltd). Reverse transcription and real-time PCR was then carried out. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for the normalization of the qPCR value. The specific primers are as follows: forward 5'-AGGAGAGTTGAGAATTGGGAATC-3', reverse 5'- TGGTGATCAGAATCAGAA GTTCG-3' for the COL11A1, and forward 5'-CCTCTGACTTCAACAGCGACAC-3', reverse 5'-TGGTCCAGGGGTCTTACTCC-3' for GAPDH. All amplifications were completed in triplicate. A mean value of threshold cycle (Ct) scores was calculated for the determination of relative expression levels.

Statistical analysis

The SPSS software (version 17.0, Chicago, IL) was used for statistical analyses. The Hardy-Weinberg equilibrium (HWE) test was performed in both patients and controls. The differences of genotype and allele frequency between patients and healthy controls were evaluated with the Chi-square test. Odds ratio (OR) was calculated using the minor allele as a reference. One-way ANOVA test was used to compare the relationship between genotypes and clinical features including tissue expression of the COL11A1 and the severity of disc degeneration. A p value of less than 0.05 was considered to be statistically significant.

Results

Clinical characteristics of the patients

Overall, 647 patients with LDH and 532 normal controls were included in this study. As shown in Table 1, the mean age was 46.5 ± 8.7 years for the patients and 17.9 ± 2.2 years for the controls, respectively. The mean BMI was 23.2 ± 5.6 kg/m^2 for the patients and 22.7 ± 6.2 kg/m^2 for the controls. The two groups were matched in terms of the ratio of male to female. 235 patients were prescribed with conservative treatment, and the other 412 patients underwent surgical intervention. According to the Schneiderman's classification of disc degeneration as mentioned above, there were 72 patients with grade 1, 178 patients with grade 2, 276 patients with grade 3 and 121 with grade 4.

Association of COL11A1 with the development of LDH

The SNP rs1676486 was successfully genotyped for all the subjects. HWE test showed no significant difference regarding the genotype frequency of the patients or of the controls. As shown in Table 2, there were significant differences in the genotype and allele frequency between the patients and the controls. Patients were found to have a significantly higher frequency of TT than the controls (10.2% vs. 7.3%, $p = 0.004$). Besides, the frequency of allele T was found to be remarkably higher in the patients than the controls (34.8% vs. 28.1%, $p < 0.001$), with an odds ratio of 1.36 (95% confidential interval = 1.14 - 1.63).

Relationship between genotypes of rs1676486 and clinical characteristics of LDH patients

Results of the comparison among patients with different genotypes were summarized in Table 3. Patients with genotype TT were found to have remarkably

more severe disc degeneration as indicated by higher Schneiderman's grade ($p = 0.02$). Besides, the expression of COL11A1 in the lumbar disc was significantly lower in the patients with genotype TT than in those with genotype CT or CC ($p < 0.001$). Moreover, the expression level was inversely correlated with the severity of disc degeneration. Patient with Schneiderman's grade 4 was found to have remarkably lower expression of the COL11A1 than the other patients ($p < 0.001$) (Table 4).

Discussion

It has been well accepted that the interplay between multiple genetic factors and environmental events may contribute to the early stage of symptomatic disc herniation (Sansoni et al., 2016; Scapinelli, 1993; Zhang et al., 2013). To date, numerous studies have been performed to investigate the genetic background of LDH (Sedighi & Haghnegahdar, 2014; Sun et al., 2013; Tsarouhas et al., 2011). As concluded by the recent systematic review of the genetic research of LDH (Eskola et al., 2012), there were over 50 candidate gene association studies in this field. Several genes were found to have a moderate level of biologically plausible evidence in the development of LDH, including asporin (ASPN) (Song et al., 2008), COL11A1 (Mio et al., 2007), growth differentiation factor 5 (GDF5) (Williams et al., 2011), SKT (Karasugi et al., 2009), THBS2 (Hirose et al., 2008) and matrix metalloproteinase 9 (MMP9) (Hirose et al., 2008). To be noted, however, many of these studies had relatively small number of patients, and were lack in validating the reported association signal in different populations. These limitations can potentially lead to a weak level of association evidence in general population, thus justifying a replication

study in different population with sufficient sample size.

In this study, we replicated a functional polymorphism of the COL11A1 in Chinese LDH patients. For the first time, we confirmed that the genetic variant of the COL11A1 contributes to the development of LDH in Chinese population. Allele T of rs1676486 in COL11A1 can increase the risk of LDH by 1.36 folds. Our findings were consistent with those of Mio et al (Mio et al., 2007), who had also observed significant association between rs1676486and LDH in Japanese population. And the odds ratio of risky allele T was 1.42 in Japanese population (Mio et al., 2007), which was comparable with that in the Chinese population. To clarify the functional impact of rs1676486, we investigated the allelic difference of the mRNA expression of COL11A1 in the intervertebral disc. Comparably with the finding of Mio et al (Mio et al., 2007), we observed that the expression level of the COL11A1 was significantly lower in patients with the risk allele T than in those with allele C. Collectively it is obvious that the functional variant rs1676486 of the COL11A1 could be involved in the etiology of LDH but with a limited contribution to the disease. Due to the complex inheritance mode, the genetic pathogenesis of LDH awaits to be further illustrated with more susceptible genes.

Type XI collagen is a cartilage-specific ECM protein expressed both in the annulus fibrosus and nucleus pulposus of the disc (Keene, Oxford, & Morris, 1995). It plays an important role in the formation of cartilage collagen fibril as well as in the interplay of collagens and proteoglycans. Besides, type XI collagen can regulate the diameter of cartilage collagen fibrils with its N-terminal noncollagenous region

blocking further accretion of type II collagen (Blaschke, Eikenberry, Hulmes, Galla, & Bruckner, 2000). Mutations in COL11A1 may cause various types of chondrodysplasias complicated by abnormalities of the spine including narrowing of the intervertebral disc (Spranger, 1998). All these evidence supported that the type XI collagen genes are good candidates for the genetic research of LDH. In the current study, we further evaluated the expression of the COL11A1 in intervertebral disc tissues to provide insight into the role of type XI collagen in LDH. We observed that patients with less COL11A1 expression could have more severe disc degeneration as evaluated by MRI. Similarly, Mio et al(Mio et al., 2007) also reported that COL11A1 expression level was inversely correlated with the severity of disc degeneration in patients with LDH. And they observed weak immunostaining of type XI collagen around the nucleus pulposus cells of LDH patients (Mio et al., 2007). Collectively, these findings suggested that a decrease of COL11A1 expression could be implicated in the pathogenesis of LDH.

Although our findings indicated that rs1676486 could be functionally associated with LDH through the abnormal expression of COL11A1, the underlying regulatory mechanism was not investigated in the current study. Mio et al (Mio et al., 2007) reported that the rs1676486 T allele can result in decreased synthesis and stability of COL11A1 mRNA. Therefore, it is possible that rs1676486 or other SNPs in the linkage disequilibrium block of rs1676486 can affect the expression of COL11A1 by altering the binding affinity of certain transcriptional factors. More in vivo and vitro experiments are warranted to further investigate the regulatory mechanism of

functional variants in COL11A1. Another limitation lies in that the cases and controls were not matched in terms of age. Although the controls were excluded to have LDH as confirmed by MRI, some of them could have the disease at their 40s or older. Selection bias may be introduced, and in future study age-matched controls should be recruited for a more valid conclusion.

Conclusions

Based on the sufficient sample size and strict inclusion criteria for the case-only analysis, our study confirmed that the rs1676486 of the COL11A may be functionally associated with LDH in the Chinese population. ECM-related proteins may play an important role in the pathogenesis of LDH. Our findings shed light on a better understanding of the development of LDH, which could be a promising target for a novel treatment modality of LDH.

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Table 1 Baseline characteristics of the subjects

	Patients (n = 647)	Controls (n = 532)	P
Age (years)	46.5 ± 8.7	17.9 ± 2.2	<0.001
BMI (kg/m^2)	23.2 ± 5.6	22.7 ± 6.2	0.14
Sex			0.97
Males	358	294	
Females	289	238	

BMI indicates body mass index

Table 2 Association of the rs1676486 with development of LDH

	Patients (n = 647)	Controls (n = 532)	P	Odds Ratio (95%CI)
Genotype			0.004	N/A
CC	263	272		
CT	316	221		
TT	68	39		
Alleles			<0.001	1.36 (1.14 - 1.63)
C	842	765		
T	452	299		

CI indicates confidential interval

Table 3 Relationship between genotypes of rs1676486 and clinical characteristics of LDH

Genotype	Severity of disc degeneration [a]	mRNA expression of COL11A1[b]
CC (n = 32)	2.4 ± 0.8	0.0447 ± 0.0238
CT (n = 47)	2.5 ± 0.7	0.0359 ± 0.0217
TT (n = 21)	3.2 ± 0.9†	0.0218 ± 0.0184††

† p <0.05

†† p <0.001

[a] Classified into 4 grade according to Schneiderman's grade

[b] Normalized with the value of GAPDH as reference

Table 4 Relationship between expression of COL11A1 and severity of disc degeneration

Severity of Disc degeneration	mRNA expression of COL11A1	p
Grade 1 (n = 16)	0.0427 ± 0.0217	<0.001
Grade 2 (n = 25)	0.0372 ± 0.0185	
Grade 3 (n = 40)	0.0269 ± 0.0156	
Grade 4 (n = 19)	0.0227 ± 0.0193	