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RESEARCH ARTICLE

Association of FAS and FAS ligand polymorphisms with the susceptibility and severity of lumbar disc degeneration in Chinese Han population

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Abstract

Context: Apoptosis is involved in the mechanism of lumbar disc degeneration (LDD).

Objective: We aim to determine whether the polymorphisms of FAS and FASL are associated with the presence and severity of LDD.

Methods: A total of 348 patients with LDD and 215 healthy controls were genotyped.

Results: Patients with LDD showed higher frequency of−1377GA and AA, as well as −844CT and TT genotypes than normal controls. These genotypes were found to be associated with the risk of higher grades of LDD.

Conclusion: The polymorphisms of FAS and FASL may be associated with the presence and severity of LDD.

Keywords: polymorphism, FAS, FAS ligand, lumbar disc degeneration

Introduction

Lumbar disc degeneration (LDD) is the major cause of low back pain (LBP). LBP is a major source of disability and has brought a huge medical and economical burden to society (Andersson, 1999). Some environmental risk factors, such as physical loading, vehicular vibration, aging and smoking, have been suggested to play great roles in the etiology of LDD (Kawaguchi et al. 2002). A number of gene polymorphisms including genes coding for collagen IX ($P=0.043$) (Higashino et al. 2007), aggrecan ($P<0.001$) (Mashayekhi et al. 2010), vitamin D receptor ($P=0.037$) (Kawaguchi et al. 2007) and matrix metalloproteinase 2 ($P<0.001$) (Dong et al. 2007) have been demonstrated to be associated with LDD. Recently, apoptosis was shown to be involved in the mechanism of LDD (Zhao et al. 2006).

Apoptosis is programmed cell death (PCD) and occurs in both normal and pathological states. Evidence has indicated that PCD plays an important role in LDD (Martin et al. 2002). With increasing compressive stress, the inner and middle anulus of mouse tail discs became progressively more disorganized, and the percentage of cells undergoing apoptosis increased (Lotz et al. 1998). In another study, mouse models of intervertebral disk degeneration (IVDD) showed annulus fibrosus cell apoptosis and severe degeneration of the mouse disks. Mechanical overload in cultured rabbit IVD cells induced apoptosis with increased caspase-9 activity and decreased mitochondrial membrane potential. Furthermore, Z-LEHD-FMK, a caspase-9 inhibitor, attenuated the overload-induced apoptosis (Rannou et al. 2004).

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A central mechanism for upstream apoptotic signaling is the activation of the FAS receptor (TNFRSF6 gene), a member of the nerve growth factor/tumor necrosis factor (NGF/TNF) receptor super family (Feuk et al. 2000). The binding of FAS ligand (FASL) to FAS leads to the recruitment of FAS-associated death domain (FADD) forming a complex, termed death-inducing signaling complex (DISC), which can bind to initiator caspase (caspases 8 and 10), thereby triggering the activation of caspase-3, 7 and 9, finally leading to apoptotic events (Stoneman et al. 2009). FAS and FASL were indicated to be involved in the mechanism of LDD. There was a significant difference in the percentage of FAS-positive disc cells between the contained and non-contained discs (Park et al. 2001). In addition, significant differences in the percentage of FASL-positive nucleus pulposus cells were found between the normal discs and the stabbed discs in a rabbit model of IVDD (Wang et al. 2007).

Single nucleotide polymorphisms (SNP) in the promoter regions of FAS (GenBank accession no: AY450925, mapped on chromosome 10q24.1) and FASL ((GenBank accession no: Z96050, mapped on chromosome 1q23), including FAS -1377G/A (rs2234767), FAS -670A/G (rs1800682) and FASL -844C/T (rs763110) have been previously identified (Huang et al. 1997, Wu et al. 2003). These SNP have been elucidated to be associated with various diseases, such as cancer (Cao et al. 2010), idiopathic azoospermia or severe oligozoospermia (Wang et al. 2009), alopecia areata (Fan et al. 2010) and Alzheimer's disease (Erten-Lyons et al. 2010).

As we know, the investigation on the association of these polymorphisms with LDD has not been performed yet. Therefore, this study aims to determine whether the polymorphisms of FAS and FASL genes are associated with the presence and severity of LDD in Chinese Han population.

Materials and methods

Patients

The study consisted of 348 patients with LDD and 215 healthy controls. The cases, aged 30–68 years, were diagnosed by magnetic resonance imaging (MRI) of LDD with clinical symptoms and signs. Patients had a continuous period of moderate to severe LBP that had failed a minimum of 6 months of supervised non-operative treatment, including activity modification, medication, physical therapy and/or corticosteroid injections. The inclusion criteria allowed for the presence of referred pain in the buttock/hip and proximal thigh area. However patients with significant sciatica, Bechterew's disease, previous fracture of the spine, malignancies involving the spine, lumbar spinal stenosis, and poliomyelitis were excluded from this study. Control participants were recruited from healthy subjects with medical check-up and matched to the cases by age, sex, occupation and smoking. Control subjects were evaluated by CT or MRI, and had no medical history of LBP, sciatica or LDD. All subjects were Han population from South China.

The lumbar spines of all patients were imaged using a 1.5 Tesla Magnetom unit (Siemens AG, Erlangen, Germany). We applied a sagittal T2-weighted image with a slice thickness of 5 mm, a repetition of 2500 ms, and an echo time of 90 ms. The grade of disc degeneration was determined according to Schneiderman's classification for MRI (Schneiderman et al. 1987): Grade 1 (normal), normal signal height and intensity; Grade 2 (intermediate), a speckled pattern or heterogeneous decreased signal intensity; Grade 3 (marked), a diffuse loss of signal; and Grade 4 (absent), a signal void (Figure 1). The grade of the disc degeneration was determined when the evaluation of the disc degeneration was agreed upon by at least three observers with no information about the genetic analysis and the others' evaluation results. When there was less agreement, the lower grade of disc degeneration was assigned. The score of ≥2 was used to indicate the presence of LDD. In addition, the subjects with score 1 were considered as normal controls.

This study was approved by the ethics committee of The First Affiliated Hospital of Guangzhou Medical College, and informed consent was obtained from all participants.

DNA genotyping

Genomic DNA from all the subjects was extracted from peripheral blood leukocytes using a DNA isolation kit following the manufacturer's instructions (Qiagen). The polymorphisms of FAS–1377G/A, –670A/G and FASL–844C/T were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. The primer sequences for these polymorphisms were as follows: forward for FAS–1377G/A, 5′-TGTGTGCACAAGGCTGGCGC-3′;

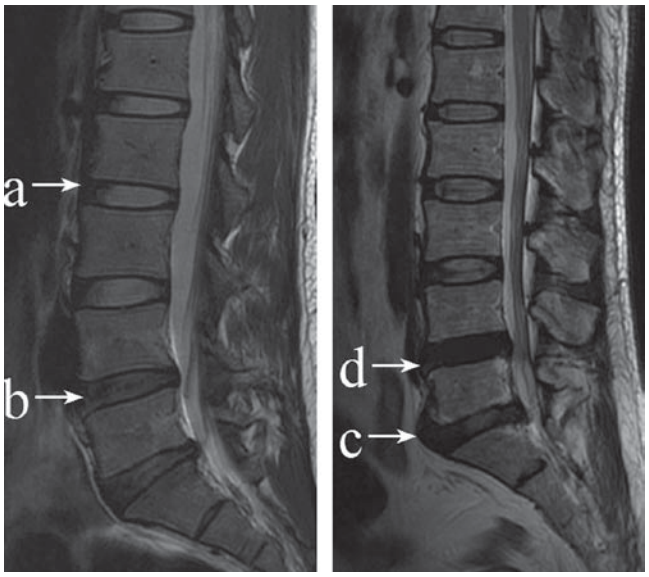

Figure 1. Presentation of sagittal MRI of LDD with different grades. (a) Grade 1, normal signal height and intensity; (b) Grade 2, a speckled pattern or heterogeneous decreased signal intensity; (c) Grade 3, a diffuse loss of signal; and (d) Grade 4, a signal void.

reverse for FAS–1377G/A, 5′-TGCATCTGTCACTGC ACTTACCACCA-3′; and forward for FAS–670A/G, 5′-ATAGCTGGGGCTATGCGATT-3′; reverse for FAS–670A/G, 5′ - CATTTGACTGG GCTGTCCAT -3′; and forward for FASL–844C/T, 5′-CAATGAAAATGAACACA TTG-3′; reverse for FASL–844C/T, 5′-CCCACTTTAGAAATTAGATC-3′ (Wang et al. 2009). PCR was performed using Taq DNA polymerase (Life Technologies, Inc-Invitrogen, Carlsbad, CA) with supplied buffer. The amplified PCR products for the FAS–1377G/A, –670A/G and FASL–844 C/T polymorphisms were 122 base pairs (bp), 193 bp and 85 bp respectively. PCR products were digested using restriction enzyme BstUI, ScrFI, and DraIII (Takara, Otsu, Japan) respectively to distinguish the three polymorphisms, which resulted in 104-bp and 18-bp fragments in the presence of the FAS–1377G allele; 136-bp and 57-bp fragments in the presence of the FAS–670G allele; and 66-bp and 19-bp in the presence of the FASL–844T allele. A 10.00% random sample of these study populations was genotyped again in a blinded fashion. The results were found to be 100% consistent with the primary results of genotyping.

Statistical analyses

Statistical analyses were performed using SPSS version 16.0 software. The characteristics of LDD patients and controls were compared with chi square or Student's *t* test according to the variable types. For each SNP, a chi-square test was performed to assess Hardy–Weinberg equilibrium. Arlequin software was used to analyze whether–1377G/A and–670A/G polymorphisms in the FAS gene are in linkage disequilibrium (LD) with each other in the studied sample. The genotypic and allelic frequencies between the two groups were compared using the chi-square test. For the purpose of statistical analysis, patients with homozygous and heterozygous mutation alleles were combined into a single group, respectively. The severity of disc degeneration of different alleles and genotypes among LDD patients were analyzed using chi square. Unconditional logistic regression was used to estimate the association between polymorphisms and risk of LDD development. Two-tailed $P < 0.05$ was considered statistically significant.

Results

The characteristics between patients with LDD and normal controls

No significant differences in sex, age, occupational character and smoking status were found between the two groups. The incidence of positive family history of LDD was significantly higher in the LDD group compared with that in controls (21.8% vs. 5.6%, $P<0.001$). The characteristics of patients with LDD and healthy controls are displayed in Table 1.

Table 1. The characteristics between patients with LDD and normal controls.

Characteristics	Patients with LDD	Normal controls	*P* value
Age (years)	50.17 ± 10.30	49.37 ± 9.64	0.363
Gender (male/female)	225/123 (64.7%/35.3%)	133/82 (61.9%/38.1%)	0.503
Occupational character (Non-manual/Half manual/Manual)	41/184/123 (11.8%/52.9%/35.3%)	29/123/63 (13.5%/57.2%/29.3%)	0.327
Smoking (%)	111 (31.9%)	62 (28.8%)	0.445
Family history of LDD (%)	76 (21.8%)	12 (5.6%)	<0.001

Occupational character
Non-manual job presents mainly mental job or white-collar work
Manual job presents mainly physical job or blue-collar work
Half manual presents partly mental job and physical job.
Smoking
A smoker was defined as a current smoker or as a smoker who had stopped smoking less than 2 years, and a nonsmoker as an individual who had never smoked or had stopped smoking for more than 2 years.

Three polymorphisms in patients with LDD and normal controls

Table 2 shows the genotype distribution of the FAS–1377G/A, –670A/G and FASL–844C/T polymorphisms in LDD group and control group. The genotype frequencies of these three polymorphisms among the controls were all in agreement with Hardy-Weinberg equilibrium ($P=0.707$, $P=0.616$ and $P=0.455$ respectively). Linkage disequilibrium (LD) analysis showed that–1377G/A and–670A/G polymorphisms in the FAS gene are in LD with each other in the studied sample. Higher frequencies of FAS–1377GA and AA genotypes (predisposing genotypes) were found in patients with LDD than in normal controls (49.4% vs. 42.8% and 15.8% vs. 11.2% respectively) ($P=0.022$). There were higher frequencies of A allele (predisposing allele) in LDD group than that in normal group (40.5% vs. 32.6%) ($P=0.007$). In addition, LDD group showed higher genotype frequencies of FASL–844CT and TT genotypes (predisposing genotypes) compared with healthy subjects (42.5% vs. 35.3% and 7.2% vs. 3.7% respectively) ($P=0.027$). There were higher frequencies of T allele (predisposing allele) in LDD group than that in normal group (28.4% vs. 21.4%) ($P<0.001$). Unconditional logistic regression analysis revealed that FAS–1377GA and AA genotypes (predisposing genotypes) were significantly associated with the presence of LDD compared with GG genotype (protecting genotypes) ($P=0.023$; OR 1.530; 95.00% CI 1.060–2.208 and $P=0.025$; OR 1.875; 95.00% CI 1.084–3.244 respectively). The A allele (predisposing allele) was significantly associated with the risk of LDD compared with G allele (protecting allele) ($P=0.007$; OR 1.411; 95.00% CI 1.096–1.816). Similarly, FASL–844CT and TT genotypes were significantly associated with the presence of LDD compared with CC genotype (protecting genotypes) ($P=0.039$; OR 1.188; 95.00% CI 1.020–2.084 and $P=0.044$; OR 2.339; 95.00% CI 1.022–5.352 respectively). T allele (predisposing allele) was significantly associated with the risk of LDD compared with C allele (protecting allele)

Table 2. The genotype distribution of FAS–1377G/A,–670A/G and FASL–844C/T polymorphisms in LDD group and control group.

	LDD group		Control group			
Genotype	*n*	%	*n*	%	*P* value	95% CI
FAS –1377						
GG	121	34.8	99	46	0.022	1.00 (reference)
GA	172	49.4	92	42.8		1.530 (1.060–2.208)
AA	55	15.8	24	11.2		1.875 (1.084–3.244)
G	414	59.5	290	67.4	0.007	1.00 (reference)
A	282	40.5	140	32.6		1.411 (1.096–1.816)
FAS –670						
AA	129	37.1	89	41.4	0.534	1.00 (reference)
AG	162	46.6	96	44.7		1.164 (0.804–1.685)
GG	57	16.4	30	14		1.311 (0.781–2.201)
A	420	60.3	274	63.7	0.258	1.00 (reference)
G	276	39.7	156	36.3		1.154 (0.900–1.480)
FASL –844						
CC	175	50.3	131	60.9	0.027	1.00 (reference)
CT	148	42.5	76	35.3		1.188 (1.020–2.084)
TT	25	7.2	8	3.7		2.339 (1.022–5.352)
C	498	71.6	338	78.6	<0.001	1.00 (reference)
T	198	28.4	92	21.4		2.773 (2.104–3.654)

Table 3. Frequency distributions of the combined genotypes of the FAS and FASL polymorphisms between patients with LDD and normal controls.

No. of variant alleles of the combined genotypes	LDD group		Control group			
	n	%	*n*	%	*P* value	95% CI
0	125	35.9	89	41.4	0.211	1.00 (reference)
1	8	2.3	10	4.7		0.570 (0.216–1.500)
2	46	13.2	32	14.9		1.023 (0.604–1.733)
3	110	31.6	54	25.1		1.450 (0.949–2.217)
4	8	2.3	6	2.8		0.949 (0.318–2.832)
5	26	7.5	16	7.4		1.157 (0.586–2.283)
6	25	7.2	8	3.7		2.225 (0.959–5.160)

(*P*<0.001; OR 2.773; 95.00% CI 2.104–3.654). However, no significant differences in genotype and allele distributions of FAS–670A/G polymorphism were found between the two groups (*P* = 0.534 and *P* = 0.258 respectively).

Association between the combined genotype frequencies of FAS and FASL polymorphisms and the risk of LDD

Considering the potential interactions of the FAS and FASL genes involved in the same apoptosis pathway on the risk of LDD, the FAS and FASL polymorphisms were combined based on the numbers of variant alleles (i.e.–1377A,–670G, and–844T). As shown in Table 3, the subjects had either none or up to six at-risk alleles when the three polymorphisms of FAS or FASL were combined. However, the genotype distribution between LDD patients and normal controls was not statistically different (*P* = 0.211).

The association of three polymorphisms with the severity of LDD

The association between three polymorphisms and the severity of LDD are shown in Table 4. LDD patients with FAS–1377GA and AA genotypes had higher grades of disc degeneration than of those with FAS–1377GG genotype (*P* = 0.016). Higher grades of disc degeneration were also found in LDD patients with FASL–844CT and TT genotypes than in those with FASL–844CC genotype (*P* = 0.013). However, no significant differences in degenerative disc grades between different genotypes of FAS–670A/G polymorphism were found.

Discussion

Our study investigated whether the polymorphisms of FAS and FASL genes are associated with the presence and severity of LDD in Chinese Han population. To the best of our knowledge, this is the first study to demonstrate the association of rs2234767 and rs763110 polymorphisms with the presence and severity of LDD.

Genetic factors contribute to the presence of LDD. Many gene polymorphisms have been suggested to be associated with the susceptibility to LDD in different ethnics and populations. The polymorphisms of vitamin D receptor and aggrecan were indicated to be associated with disc degeneration and herniation in Turkey (Eser et al. 2010). Genetic variations in the interleukin-1 and the matrix metalloproteinase-3 genes together were

Table 4. The association of FAS–1377G/A,–670A/G and FASL–844C/T polymorphisms with the severity of LDD

Genotype	*n*	Grade 2	Grade 3	Grade 4	*P* value
FAS -1377					
GG	121	80 (66.1%)	23 (19.0%)	18 (14.9%)	0.016
GA	172	88 (51.2%)	41 (23.8%)	43 (25.0%)	
AA	55	24 (43.6%)	12 (21.8%)	19 (34.5%)	
G	414	248 (59.9%)	87 (21.0%)	79 (19.1%)	0.004
A	282	136 (48.2%)	65 (23.0%)	81 (28.7%)	
FAS -670	110				
AA	129	69 (53.5%)	33 (25.6%)	27 (20.9%)	0.599
AG	162	94 (58.0%)	31 (19.1%)	37 (22.8%)	
GG	57	29 (50.9%)	12 (21.1%)	16 (28.1%)	
A	420	232 (55.2%)	97 (23.1%)	91 (21.7%)	0.456
G	276	152 (55.1%)	55 (19.9%)	69 (25.0%)	
FASL -844					
CC	175	108 (61.7%)	39 (22.3%)	28 (16.0%)	0.013
CT	148	77 (52.0%)	31 (20.9%)	40 (27.0%)	
TT	25	7 (28.0%)	6 (24.0%)	12 (48.0%)	
C	498	293 (58.8%)	109 (21.9%)	96 (19.3%)	0.001
T	198	91 (46.0%)	43 (21.7%)	64 (32.3%)	

found to be significantly associated with modic changes in endplates of lumbar vertebral bodies in Finnish population (Karppinen et al. 2008). Polymorphism of cartilage intermediate layer was shown to be associated with the susceptibility to LDD in Japan (Seki et al. 2005). In addition, polymorphisms of D14 allele of asporin, aggrecan and matrix metalloproteinase-9 were demonstrated to contribute to the presence of LDD in Chinese Han population (Song et al. 2008, Cong et al. 2010, Sun et al. 2009). LBP is one of the most common disorders seen in general and orthopedic practices. It is a major cause of morbidity and sick leaving to the detriment of all industrialized societies. LDD caused by degeneration of intervertebral discs of the lumbar spine and concurrent disc herniation is a primary cause of LBP (Lefevre-Colau et al. 2009). So it is important to identify more susceptibility genes for LDD and then to assess the genetic susceptibility to LDD at an early stage. Subjects with susceptibility genes should establish primary prevention strategies for LDD including avoiding or stopping the environmental and occupational risk factors of LDD.

The FAS and FASL system plays a key role in the apoptotic signaling. FAS (also known as TNFRSF6/CD95/APO-1) which encode a cell surface factor plays a crucial role in the apoptotic signaling in many cell types. Its natural ligand FASL, also known as TNFSF6/CD95L, a transmembrane protein belonging to the tumor necrosis factor (TNF) super family, can trigger cell death signal cascade by cross-linking with FAS (Houston et al. 2004). Recently, FAS and FASL were suggested to be involved in the pathophysiological mechanism of LDD. A higher degree of FAS receptor (FASR) expression and apoptosis in endplate cells was observed in degenerated discs than in nondegenerated discs. And the level of FASR expression and apoptosis in cells from the degenerative endplates were higher than those in unchanged endplates (Wang et al. 2011). Traumatic IVD alone demonstrated a significant increase of FASR and caspase-3/7 activity (Tschoeke et al. 2008). The expression of FAS gene was significantly up-regulated in degenerated disc tissue than that in normal disc tissues (Anderson et al. 2002). A higher degree of FASL expression in disc cells was found in non-contained discs than in contained discs (Park et al. 2001).

Our results indicated that FAS–1377GA and AA genotypes, as well as FASL–844CT and TT were associated with the presence and severity of LDD. This suggested a possible contribution of genetic variations in the FAS and FASL genes to the interindividual variability of LDD. The FAS–1377A allele disrupted the binding of Sp1 and STAT1 transcription factor, and the FASL–844T allele created a binding site for the CAAT/enhancer-binding protein B transcription factor, which may have an effect on the expression of FAS and FASL. It is hypothesized that the variation in the FAS and FASL genes may cause increased expression or activity of FAS and FASL, and then activated apoptosis pathway. This could result in elevated apoptosis of lumbar disc cells and finally the presence of LDD. Similar results were found in another study. Polymorphism of caspase 9 Ex5 + 32 G/A was indicated to be associated with LDD and disc degeneration in the Han population of northern China (Sun et al. 2010). These results suggested the key role of apoptosis in the mechanism of LDD.

The limitations of these findings merit consideration. First, this study had a relatively small population size. Second, as our study was only conducted in a sample of Chinese Han population, the result is lack of replication in other population. Last, we did not perform population stratification in our study. All these limitations may lead to false positive findings. Therefore, our data should be validated in prospective studies with a larger population size in other ethnic populations.

Conclusions

In conclusion, this study showed that the polymorphisms of FAS–1377G/A and FASL–844C/T may be associated with the presence and severity of LDD in Chinese Han population. These two polymorphisms could serve as an independent predictive genetic marker of the presence of LDD.

Declaration of interest

We certify that all authors have no financial or other conflict of interests in connection with the submitted article.

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