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A Controlled Case Study of the Relationship between Environmental Risk Factors and Apoptotic Gene Polymorphism and Lumbar Disc Herniation

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To explore the etiologic role of apoptosis-related genes, environmental risk factors, and their interaction in the occurrence of lumbar disk herniation (LDH), a controlled case study was performed with 128 LDH patients and 132 age- and sex-matched controls. Matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry assay was used to analyze the genotype of nine polymorphism sites in three genes, including *Fas* -1377G/A rs2234767, *Fas* -670G/A rs1800682, *Fas* rs2147420, *Fas* rs2296603, *Fas* rs7901656, *Fas* rs1571019, *Fas* ligand (*FasL*) -844C/T rs763110, caspase 9 (*CASP9*) -1263A>G rs4645978, and *CASP9* -712C>T rs4645981. The patients and controls showed similar age and sex, but had significant differences in lumbar load, bed type, amateur sports, and leisure activities ($P < 0.05$). The correlation analysis revealed that polymorphism of *FasL* -844C/T (rs763110) and *CASP9* -1263A>G (rs4645978) had a significant correlation with LDH, indicating that the genotypes of *FasL* -844C/T TT and *CASP9* -1263A>G GG are probably high-risk genotypes for LDH. The results of environment-gene interaction analysis revealed that, in LDH, the interaction of the *FasL* -844TT genotype and level III to IV lumbar load was consistent with the ultramultiplying model, and the interaction of the *CASP9* rs4645978 GG genotype and level III to IV lumbar load was consistent with the submultiplicative model. Therefore, the risk of LDH was determined by both environmental and genetic risk factors, and the mechanisms of interactions between different genotypes and environmental factors also differed. (*Am J Pathol* 2013, 182: 56–63; <http://dx.doi.org/10.1016/j.ajpath.2012.09.013>)

Lumbar disk herniation (LDH), which has a high incidence and is the major cause of low back pain, has become a major public health problem because of its derived disability and social cost.¹ LDH is induced mainly by lumbar intervertebral disk degeneration (LDD). Previous etiologic studies have focused on environmental risk factors. For instance, occupational factors (eg, heavy physical work, lifting heavy objects, bending over and twisting, whole-body vibration, and static operating position) can induce LDD, resulting in increased intradiscal pressure and weakened annulus fibrosus strength, which are the pathologic bases for LDH. Previously, we showed that the important risk factors of LDH included lumbar load, family history, average body weight index, leisure activities, amateur sports, and social psychology.² However, environmental risk factors are not

the only cause of LDH. Recently, it was suggested that the heritability rate of LDH is 52% to 68%,³ indicating that genetic factors also may play an important role in the pathogenesis of LDH. Hence, it has been accepted that LDH is a complex disease determined by the interactions of genetic and environmental factors. With the development of high-throughput sequencing technology, the location and function of susceptibility genes of complex diseases has become a hot research area. The susceptibility genes of many complex diseases have been located, including LDH.^{4–9} However, little information is available about the interaction of susceptibility genes and environmental risk factors in the

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pathogenesis of LDH. Elucidating the environment-gene interaction mechanisms of LDH will help show LDH pathogenesis and the development of effective early prevention and treatment measures.

Previous research has suggested that apoptosis plays an important role in the pathology of LDD. The apoptotic pathways involved in LDD include the Fas/Fas ligand (L)-mediated death receptor pathway (exogenous pathway) and the mitochondrial pathway (endogenous pathway).^{10,11} The typical exogenous pathway is the Fas (also known as CD95)—mediated apoptotic pathway. FasL always appears in a trimer form, in which three Fas receptors bind to one FasL. After FasL binds to its ligand, Fas recruits the intracellular Fas associated protein with death domain (FADD) by binding the C-terminal death domain of the FADD protein through its death domain. In the next step, the death domain of Fas Associated protein interacts with the death domain of caspase-8 zymogen to recruit the caspase-8 zymogen in the cytosol, and subsequently form the death-inducing signaling complex.¹² In the mitochondrial pathway, caspase accepts the death signal to initiate apoptosis, which in turn activates the downstream pathway to degrade the cytoskeleton, proteins, and nucleotides. Caspase is essential for apoptosis, and caspase-9 is the key protease to activate this pathway. The effective caspase cannot selectively bind to Apaf-1, so it is dependent on caspase-9.¹³ Therefore, we propose that FAS, FASL, and caspase-9 probably play important roles in LDH.

In the present study, we performed a controlled case study to explore the correlation between the polymorphism of *Fas*, *FasL*, *CASP9*, and the risk of LDH. We identified the single nucleotide polymorphism (SNP) sites that showed positive correlation, and analyzed their pathogenic interaction with environmental risk factors. Through the preliminary study (30 patients and controls), we already found that some polymorphisms (ie, *CASP9* rs2308941, rs2308950, *FasL* *INV2nt* -124A>G rs5030772) do not exist in the Chinese Han population. Therefore, in the present study, we selectively analyzed nine SNP sites, including *Fas* -1377G/A rs2234767, *Fas* -670G/A rs1800682, *Fas* rs2147420, *Fas* rs2296603, *Fas* rs7901656, *Fas* rs1571019, *FasL* -844C/T (rs763110), *CASP9* -1263A>G rs4645978, and *CASP9* -712C>T rs4645981.

Materials and Methods

Subjects

The case group consisted of 131 LDH patients admitted to the Department of Orthopaedics of the First Affiliated Hospital, Medical College of Xi'an Jiaotong University, Shaanxi Provincial People's Hospital, and Xian Tang City Hospital from January 2005 to January 2007, for reasons of back leg pain, computed tomography/magnetic resonance imaging—diagnosed lumbar disk herniation, and typical sciatica. Patients with lumbar spinal stenosis, spinal congenital

dysplasia, intraspinal tumor, and spondylolisthesis were excluded from this study. The 137 subjects in the control group were selected randomly from in-patients or participants of medical examinations who had no history of back pain at present, for more than a month, or ever; sciatic nerve pain; spinal instability from trauma; scoliosis; or spondylolisthesis. The control and case groups were matched with respect to race, sex, age, and living areas. All studied individuals were Chinese Han subjects. Information including age, sex, height, weight, and disease history were collected.

Environmental Risk Factors

The potential environmental risk factors of LDH were determined based on the preliminary data and were evaluated quantitatively through a questionnaire survey. The first risk factor was lumbar load. The lumbar load was quantified using a grading system based on a physical load assessment questionnaire developed by the Stockholm Musculoskeletal Research Center (Sweden),¹⁴ the occupational classification point standard,¹⁵ and the actual situation. Level I (slight) represents no fixed occupation and little physical labor; level II (mild) represents mainly sitting at work; level III (moderate) represents mainly bending over and twisting and whole-body vibrating at work; and level IV (severe) represents mainly heavy lifting and heavy labor work. The second risk factor was smoking, defined as smoking at least a cigarette per day for 1 year or an annual amount greater than 18 packs. The smoking index is equal to a year of smoking X number of cigarettes per day × 20 - 1. The third risk factor was drinking, defined as consuming alcohol twice per week and 100 g each time for 1 year. The fourth risk factor was leisure activities (eg, housework). Finally, the fifth risk factor was amateur sports (ie, fitness training), defined as more than 20 minutes of exercise each time until the heartbeat was accelerated and the individual was sweating.

SNP Genotyping

DNA was extracted from peripheral blood leukocytes using the proteinase K digestion and phenol/chloroform extraction methods. The quality and quantity of extracted DNA were determined using a spectrophotometer (ND-100; NanoDrop, Wilmington, DE). MassARRAY Assay Design software version 2.0 (Sequenom, Salt Lake City, UT) was used to design the amplification and allele-specific primer extension. The polymerase chain reaction (PCR) mixture consisted of 5 ng genomic DNA (5 ng/μL), 1 μL of each specific primer, 2.5 mmol/L of each dNTP, 3.25 mmol/L MgCl₂, and 0.5 U of Hot-StarTaq DNA polymerase (5 U/μL; Qiagen, Valencia, CA). The PCR was performed at 94°C for 15 minutes, 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, followed by 72°C for 3 minutes. The final products were analyzed using a modified Brucker Autoflex Matrix-Assisted Laser Desorption/Ionization Time

of Flight Mass Spectrometry (MALDI-TOF-MS) (Brucker, Billerica, MA).

Statistical Analysis

The age, sex, smoking history, and genotype distribution were analyzed using the χ^2 test with SPSS software version 17.0 (SPSS, Stanford, CA). The Hardy-Weinberg equilibrium test was performed using Excel 2003 software (Microsoft, Redmond, WA). The nonconditional logistic regression model using age, sex, height, and weight as covariates was applied to analyze the relationship between SNP polymorphism and LDH risk factors. The relative risk was represented by an odds ratio (OR) and 95% CI. All analyses were two-sided probability tests and a P value < 0.05 was used as statistically significant threshold in this study.

The multivariate logistic regression model was used to evaluate the pathogenic interaction of environmental risk factors and candidate genes. The covariates of the model included age, sex, height, and weight, and the variables of the model included genetic and environmental risk factors, as well as their interactions. We hypothesized that gene polymorphism is correlated to LDH risk only when environmental factors are present. This has been defined as a two-type interaction by Khoury et al,^{16,17} Ottman,¹⁸ and Taioli et al.¹⁹ β_e denotes the phenotypic effects of environment risk factors. β_{eg} denotes the phenotypic effects explained by the interaction between environment risk factors and candidate genes. The interaction coefficient ($\gamma = \beta_{eg}/\beta_e$) was used to determine the interaction and its pattern and intensity as follows. First, if odds ratio $(OR)_{eg} = OR_e + OR_g$, the interaction is consistent with the multiplicative model; if $OR_{eg} > OR_e + OR_g$, the interaction is consistent with the ultra multiplying model; if $OR_{eg} < OR_e + OR_g$, the interaction is consistent with the submultiplicative model; if $OR_{eg} = OR_e + OR_g - 1$, the interaction is consistent with the additive model. Second, γ was used as the two-variable interaction coefficient, $\gamma > 1$ represents that genetic factors can amplify the effects of environmental factors, $\gamma < 1$ represents that genetic factors can reduce the effects of environmental factors, and $\gamma = 1$ represents no interaction. If an environmental increases the risk of target disease and $\beta_e > 0$ and $\gamma < 0$, the gene has a protective effect.

Results

Patient Information

A total of 131 patients and 137 controls were used in this study. The results of restriction fragment length polymorphism electrophoresis and mass spectrometry analysis of DNA revealed 8 unqualified cases (3 patients and 5 controls), which then were excluded. The information on the final 128 patients and 132 controls included is listed in Table 1. The patients had an average age of 46.2 years

(mean = 46.2; SD = 13.1), and the control group had an average age of 47.4 years (mean = 47.4; SD = 12.9). We found that the two groups showed significant differences in amateur labor ($P = 0.011$), bed type ($P = 0.003$), and spinal load index ($P < 0.001$), but were similar in age, sex, weight, smoking history, and drinking.

Correlative Analysis of the Polymorphism of Apoptosis-Related Genes and the Risk of LDH

The results of the Hardy-Weinberg equilibrium test revealed that the genotype distribution of nine SNP sites in the three genes was consistent with the Hardy-Weinberg equilibrium law ($P > 0.05$), indicating that the samples were representative. As shown in Table 2, among the nine SNP sites of the three genes, *FasL* (rs763110) and *CASP9* rs4645978 were correlated significantly with LDH risk ($P < 0.05$).

Among the 128 patients, there were 64 cases of *FasL* (rs763110) (50%), 51 cases of CT (39.8%), and 13 cases of TT (10.2%). Among the 132 controls, there were 77 cases of *FasL* -844CC (58.3%), 50 cases of CT (37.9%), and 5 cases of TT (3.8%). The LDH risk of CT patients was 1.227 times that of the CC patients ($\chi^2 = 0.615$; 95% CI, 0.736 to 2.048), the LDH risk of TT patients was 3.128 times that of the CC patients ($\chi^2 = 4.601$; 95% CI, 1.059 to 9.242), and the LDH risk of T-allele patients was 1.329 times that of the C-allele patients ($\chi^2 = 2.047$; 95% CI, 0.900 to 1.963). There was a significant difference between the TT genotype and the CC genotype ($P < 0.05$).

Among the 128 patients, there were 22 cases of *CASP9* rs4645978AA (17.2%), 69 cases of *CASP9* rs4645978AG (53.9%), and 37 cases of *CASP9* rs4645978GG (28.9%). Among the 132 controls, there were 36 cases of *CASP9* rs4645978AA (27.3%), 73 cases of *CASP9* rs4645978AG (55.3%), and 23 cases of *CASP9* rs4645978GG (17.4%). The LDH risk of AG patients was 1.570 times that of the AA patients ($\chi^2 = 2.701$; 95% CI, 0.915 to 2.692), the LDH risk of GG patients was 2.760 times that of the AA patients ($\chi^2 = 10.08$; 95% CI, 1.464 to 5.203), and the LDH risk of G-allele patients was 1.572 times that of the A-allele patients ($\chi^2 = 9.122$; 95% CI, 1.171 to 2.110). There was a significant difference between the GG genotype and AA genotype ($P < 0.05$).

Interaction of the Polymorphism of Apoptosis-Related Genes and the Risk of LDH

The interactions of *FasL* -844C/T polymorphism and environmental risk factors are shown in Table 3. Under the influence of environmental factors, the interaction between the lumbar load and *FasL* -844C/T was consistent with the ultramultiplying model. Level I and II lumbar loads were defined as slight, and level III and IV were defined as severe. After adjusting for age, sex, height, weight, family history, smoking, bed type, leisure activities, and amateur sports, the OR_e was 3.49 (95% CI, 1.58 to 7.69) for level III

Table 1 Subject Information

	Patient (%)	Control (%)	χ^2	OR (95% CI)	P value
Total	129	132			
Sex			0.667	0.812 (0.493–1.338)	0.414
Male	81 (63.3)	77 (58.3)			
Female	47 (36.7)	55 (41.7)			
Age, years			1.847	0.713 (0.438–1.162)	0.174
≤45	68 (53.1)	59 (44.7)			
>45	60 (46.9)	73 (55.3)			
Weight index			0.978	1.432 (0.706–2.870)	0.323
≤25	107 (83.6)	116 (85.8)			
>25	21 (16.4)	16 (14.2)			
Lumbar load			32.34	4.627 (2.686–7.969)	<0.001
Levels I and II	57 (44.5)	104 (78.8)			
Levels III and IV	71 (55.5)	28 (21.2)			
Bed type			9.085	2.575 (1.376–4.821)	0.003
Soft	37 (28.9)	18 (13.6)			
Hard	91 (71.1)	114 (86.4)			
Smoking			0.927	1.286 (0.770–2.145)	0.336
No	80 (62.5)	90 (68.2)			
Yes	48 (37.5)	42 (31.8)			
Drinking			0.163	0.872 (0.447–1.701)	0.687
No	109 (85.2)	110 (83.3)			
Yes	19 (14.8)	22 (16.7)			
Amateur sports			6.475	0.496 (0.288–0.855)	0.011
No	99 (77.3)	83 (62.9)			
Yes	29 (22.7)	49 (37.1)			
Leisure activities			14.788	0.281 (0.144–0.550)	<0.001
No	38 (29.7)	14 (10.6)			
Yes	90 (70.3)	118 (89.4)			

and IV lumbar loads, 3.16 (95% CI, 0.73 to 13.75) for *FasL* –844TT, and 21.1 (95% CI, 2.43 to 182.67) for both. Because $21.0 > 3.49 \times 3.26$, the interaction between *FasL* –844TT and level III/IV lumbar loads was consistent with the ultramultiplying model ($P = 0.006$), which was statistically significant.

The interactions of *CASP9* rs4645978 polymorphism and environmental risk factors are shown in Table 4. Under the influence of environmental factors, the interaction between the lumbar load and *CASP9* rs4645978 was consistent with the submultiplying model. After adjusting for age, sex, height, weight, family history, smoking, bed type, leisure activities, and amateur sports, the OR_e was 5.67 (95% CI, 2.08 to 15.45) for level III and IV lumbar loads, 1.55 (95% CI, 0.51 to 4.69) for *CASP9* rs4645978 GG, and 7.40 (95% CI, 1.77 to 30.94) in both cases. Because $7.53 < 1.55 \times 5.67$, the interaction between *CASP9* rs4645978 GG and level III/IV lumbar loads was consistent with the submultiplying model ($P = 0.006$), which was statistically significant.

Discussion

In the present study, we performed a controlled case study to explore the relationship between the risk of LDH and the nine SNP sites in three genes, including *Fas*, *FasL*, and *CASP9*.

The results indicated that *FasL* –844C/T (rs763110) and *CASP9* –1263A>G rs4645978 were correlated with LDH risk, and the genotypes of *FasL* –844C/T TT and *CASP9* –1263A>G GG were probably the risk factors for LDH. Further analysis of the interaction between the two genotypes and environmental risk factors revealed that the interaction of the *FasL* –844TT genotype and level III/IV lumbar loads was consistent with the ultramultiplying model, and the interaction of the *CASP9* rs4645978 GG genotype and level III/IV lumbar loads was consistent with the submultiplying model. Quantitative analysis of environmental factors is most important for evaluating the interaction between the environment and genes. Although it has been realized that lumbar load is the highest risk factor for LDH, the specific *in vivo* biological marker is still lacking for quantitative evaluation of the lumbar load. At present, there is still no consensus about the grading of the lumbar load, and the grading system used in this study needs to be tested in future research. LDH is a complex disease involving multilevel interactions, such as gene-gene, gene-environment, and gene-age. Because of the small sample size in the present study, we only analyzed the interaction of the gene-environment. In future research, large-scale investigation is required to fully show the relevant mechanism.

The patients and controls did not show significant differences in age or sex, which makes it possible to perform

Table 2 Relationship Between LDH Risk and Apoptotic Gene Polymorphism

Gene polymorphism	Genotype	Control (%)	Patient (%)	χ^2	OR (95% CI)	P value
<i>Fas</i> rs2234767	AA	11 (8.3)	14 (10.9)		1.00 (reference)	
	GA	65 (49.2)	55 (43.0)	0.851	1.504 (0.632–3.581)	0.356
	GG	56 (42.4)	59 (46.1)	0.181	1.208 (0.506–2.884)	0.670
	A frequency	87 (33.0)	83 (32.4)	0.017	1.025 (0.710–1.478)	0.897
	G frequency	177 (67.0)	173 (67.6)			
<i>Fas</i> rs1800682	AA	50 (37.9)	49 (38.3)		1.00 (reference)	
	GA	68 (51.5)	59 (46.1)	0.206	0.885 (0.523–1.498)	0.650
	GG	14 (10.6)	20 (15.6)	0.878	1.458 (0.663–3.207)	0.348
	A frequency	168 (63.6)	157 (61.3)	0.295	1.104 (0.774–1.574)	0.587
	G frequency	96 (36.4)	99 (38.7)			
<i>Fas</i> rs2147420	CC	51 (38.6)	49 (38.3)		1.00 (reference)	
	CT	64 (48.5)	58 (45.3)	0.047	0.943 (0.556–1.601)	0.829
	TT	17 (12.9)	21 (16.4)	0.432	1.286 (0.607–2.722)	0.511
	C frequency	166 (62.9)	156 (60.9)	0.208	1.086 (0.762–1.547)	0.649
	T frequency	98 (37.1)	100 (39.1)			
<i>Fas</i> rs2296603	AA	38 (28.8)	43 (33.6)		1.00 (reference)	
	AG	75 (56.8)	63 (49.2)	1.130	0.742 (0.428–1.287)	0.288
	GG	19 (14.4)	22 (17.2)	0.004	1.023 (0.482–2.173)	0.952
	A frequency	151 (57.2)	149 (58.2)	0.054	0.960 (0.678–1.359)	0.816
	G frequency	113 (42.8)	107 (41.8)			
<i>Fas</i> rs7901656	CC	39 (29.5)	40 (31.3)		1.00 (reference)	
	CT	71 (53.8)	62 (48.4)	0.320	0.851 (0.488–1.487)	0.571
	TT	22 (16.7)	26 (20.3)	0.149	1.152 (0.561–2.365)	0.699
	C frequency	149 (56.4)	142 (55.5)	0.050	1.040 (0.736–1.471)	0.824
	T frequency	115 (43.6)	114 (11.5)			
<i>Fas</i> rs1571019	CC	43 (32.6)	41 (32.0)		1.00 (reference)	
	CT	69 (52.3)	63 (49.2)	0.024	0.958 (0.554–1.655)	0.877
	TT	20 (15.2)	24 (18.8)	0.380	1.259 (0.606–2.616)	0.538
	C frequency	155 (58.7)	145 (56.6)	0.228	1.040 (0.769–1.542)	0.633
	T frequency	109 (41.3)	111 (43.4)			
<i>FasL</i> rs763110	CC	77 (58.3)	64 (50.0)		1.00 (reference)	
	CT	50 (37.9)	51 (39.8)	0.615	1.227 (0.736–2.048)	0.433
	TT	5 (3.8)	13 (10.2)	4.601	3.128 (1.059–9.242)	0.039
	C frequency	204 (77.3)	179 (69.9)	2.047	1.329 (0.900–1.963)	0.153
	T frequency	60 (22.7)	77 (30.1)			
<i>CASP9</i> 4645978	AA	36 (27.3)	22 (17.2)		1.00 (reference)	
	AG	73 (55.3)	69 (53.9)	2.701	1.570 (0.915–2.692)	0.100
	GG	23 (17.4)	37 (28.9)	10.08	2.760 (1.464–5.203)	0.002
	A frequency	145 (54.9)	113 (44.1)		1.0	
	G frequency	119 (45.1)	143 (55.9)	9.122	1.572 (1.171–2.110)	0.030
<i>CASP9</i> 4645981	CC	70 (53.0)	81 (63.3)		1.0	
	CT	54 (40.9)	42 (32.8)	2.828	0.687 (0.443–1.065)	0.093
	TT	8 (6.1)	5 (3.9)	1.418	0.547 (0.200–1.494)	0.234
	C frequency	194 (73.5)	204 (79.7)		1.0	
	T frequency	70 (26.5)	52 (20.3)	3.480	0.717 (0.506–1.018)	0.062

Bold text indicates $P < 0.05$.

further comparisons. The two groups showed significant differences in lumbar load, bed type, and amateur sports, but not in smoking or drinking, which is consistent with our previous observations² and makes it possible for us to analyze the interaction between the environmental factors and apoptosis genes. The weight index is a risk factor for lumbar disk herniation. Our results indicated that the weight index of the case group was only slightly higher than that of the control group, without significant difference. This is probably because the weight index of the subjects were

recorded from the cross section but not the onset of the disease or accumulative data.

Our results indicated that *FasL* –844C/T (rs763110) and *CASP9* –1263A>G (rs4645978) were correlated to the LDH risk. It has been found that there were two SNP sites in the promoter of *Fas* –1377G/A polymorphism and *Fas* –670A/G polymorphism,^{20,21} which were functionally very important. The –1377G/A polymorphism occurs in the specificity protein 1 (SP-1) binding site, and the –1377A allele can destroy the binding site of SP-1 to reduce the

Table 3 Interaction of *FasL* –844C/T Polymorphism and Environmental Risk Factor

Environmental factor	Classification	Genotype	Control	Patient	χ^2	OR (95% CI)	P value
Lumbar load*	Slight	CC	60	33		1.00 (reference)	
	Slight	CT	40	19	0.15	0.86 (0.41–1.82)	0.695
	Slight	TT	4	5	2.36	3.16 (0.73–13.75)	0.125
	Severe	CC	17	31	9.62	3.49 (1.58–7.69)	0.002
	Severe	CT	10	32	10.82	4.56 (1.85–11.27)	0.001
	Severe	TT	1	8	7.63	21.0 (2.42–182.64)	0.006
Smoking†	No	CC	50	43		1.00 (reference)	
	No	CT	36	28	0.13	0.87 (0.42–1.83)	0.719
	No	TT	4	9	1.29	2.25 (0.56–9.15)	0.273
	Yes	CC	27	21	0.35	0.78 (0.35–1.76)	0.662
	Yes	CT	14	23	0.01	1.03 (0.41–2.58)	0.955
	Yes	TT	1	4	3.71	9.58 (0.96–95.39)	0.054
Bed type‡	Hard bed	CC	67	44		1.00 (reference)	
	Hard bed	CT	42	35	0.07	1.09 (0.56–2.14)	0.786
	Hard bed	TT	5	12	4.20	3.62 (1.06–12.39)	0.037
	Soft bed	CC	10	20	7.43	3.66 (1.44–9.32)	0.006
	Soft bed	CT	8	16	3.46	2.70 (0.95–7.70)	0.063
	Soft bed	TT	0	1	0.00	7.896×10^{-9}	1.000
Leisure activities§	No	CC	8	17		1.00 (reference)	
	No	CT	6	19	0.05	0.86 (0.22–3.41)	0.824
	No	TT	0	2	0.00	5.014×10^{-8}	0.999
	Yes	CC	69	47	6.06	0.28 (0.10–0.77)	0.014
	Yes	CT	44	32	5.19	0.29 (0.10–0.84)	0.023
	Yes	TT	5	11	0.004	1.05 (0.24–4.53)	0.943
Amateur sports¶	No	CC	49	52		1.00 (reference)	
	No	CT	31	39	0.14	0.87 (0.43–1.76)	0.700
	No	TT	3	8	1.27	2.39 (0.53–10.89)	0.260
	Yes	CC	28	12	2.39	0.50 (0.20–1.21)	0.122
	Yes	CT	19	12	0.44	0.72 (0.28–1.89)	0.509
	Yes	TT	2	5	2.35	4.00 (0.68–23.53)	0.126

*Amateur sports, bed type, smoking, and leisure activities were adjusted as covariates for χ^2 analysis, OR, 95% CI, and P value.

†Amateur sports, bed type, lumbar load, and leisure activities were adjusted as covariates for χ^2 analysis, OR, 95% CI, and P value.

‡Amateur sports, smoking, lumbar load, and leisure activities were adjusted as covariates for χ^2 analysis, OR, 95% CI, and P value.

§Amateur sports, smoking, lumbar load, and bed type were adjusted as covariates for χ^2 analysis, OR, 95% CI, and P value.

¶Bed type, smoking, lumbar load, and leisure activities were adjusted as covariates for χ^2 analysis, OR, 95% CI, and P value.

activity of the promoter.²¹ The –670A/G polymorphism occurs in the interferon (IFN)- γ activation signal. The IFN- γ activation signal plays an important role in the IFN- γ –STAT-1 pathway. STAT-1 binds to the IFN- γ activation signal to activate the promoter.²² The *Fas* –670G allele can destroy the IFN- γ activation signal to affect the binding of STAT-1, and thus reduce the promoter activity and *Fas* gene expression.²³ However, we did not find that these two sites were related to LDH. This might be because the changes in *Fas* on the surface of the lumbar cell were too subtle to obviously change the LDH risk. To this end, studies with a large sample size and a more powerful analysis tool will be needed. *FasL* –844T/C has the *FasL* promoter region, which is located in the CAAT-enhancer-binding protein β binding motif. The *FasL* –844C allele can cause a higher expression level of *FasL* than the T allele does, and thus has a higher risk of tumor.²⁴ In the present study, our results indicated that the *FasL* –844C/T TT genotype was associated with an LDH risk, which was 3.128 times that of the *FasL* –844CC genotype. We propose that for

individuals carrying *FasL* –844TT, the *FasL* expression level in the nucleus pulposus was lower than in those carrying *FasL* –844CC. Extremely low levels of *FasL* can reduce the binding of *Fas* to the surface of activated lymphocytes in the lumbar disk, resulting in the apoptosis of activated lymphocytes. Namely, the lumbar disk would be destroyed by immune reactions, which in turn results in LDD.

Park et al²⁵ conducted a study on *CASP9* polymorphisms and the risk of primary lung cancer in a Korean population. The findings revealed that the *CASP9* –1263 GG genotype is associated with a significantly decreased risk of lung cancer compared with the –1263 AA genotype or the combined –1263 AA and AG genotypes, but individuals with at least one T allele of –712C/T are at a significantly increased risk of lung cancer compared with those harboring the CC genotype. Inactivation of apoptosis is one of the hallmarks of cancer because it allows cells prone to genetic damage to survive.²⁶ In contrast, the activation of apoptosis results in cell decreases in the degenerated disk of LDD. We presumed that the expression and/or activity of *CASP9*

Table 4 Interaction of *CASP9* rs4645978 Polymorphism and Environmental Risk Factors

Environmental factors	Classification	Genotype	Control	Patient	χ^2	OR (95% CI)	P value
Lumbar load*	Slight	AA	30	10		1.00 (reference)	
	Slight	GA	54	33	0.01	0.99 (0.46–2.12)	0.967
	Slight	GG	17	16	0.59	1.55 (0.51–4.69)	0.445
	Severe	AA	6	12	11.55	5.67 (2.08–15.45)	0.001
	Severe	GA	19	37	7.63	3.42 (1.42–8.21)	0.006
	Severe	GG	5	31	7.55	7.40 (1.77–30.94)	0.006
Smoking†	No	AA	22	14		1.00 (reference)	
	No	GA	52	40	0.82	0.71 (0.34–1.49)	0.360
	No	GG	13	14	0.34	1.41 (0.45–4.47)	0.563
	Yes	AA	14	25	0.21	0.80 (0.30–2.09)	0.644
	Yes	GA	21	29	0.06	0.89 (0.36–2.20)	0.792
	Yes	GG	10	12	0.16	1.31 (0.35–4.84)	0.693
Bed type‡	Hard bed	AA	31	16		1.00 (reference)	
	Hard bed	GA	65	50	0.34	0.82 (0.42–1.61)	0.554
	Hard bed	GG	18	26	1.35	1.83 (0.66–5.04)	0.246
	Soft bed	AA	5	6	4.59	3.26 (1.11–9.60)	0.032
	Soft bed	GA	8	19	3.53	2.91 (0.95–8.89)	0.062
	Soft bed	GG	6	11	1.02	2.30 (0.46–11.58)	0.316
Leisure activities§	No	AA	5	5		1.00 (reference)	
	No	GA	6	26	2.19	3.31 (0.22–3.41)	0.138
	No	GG	4	11	1.02	2.93 (0.36–23.66)	0.317
	Yes	AA	31	17	0.18	0.77 (0.23–2.56)	0.667
	Yes	GA	67	43	1.38	0.49 (0.15–1.61)	0.239
	Yes	GG	19	26	0.01	0.98 (0.25–3.92)	0.977
Amateur sports¶	No	AA	21	17		1.00 (reference)	
	No	GA	50	56	0.79	0.73 (0.36–1.47)	0.375
	No	GG	13	24	0.14	1.24 (0.40–3.79)	0.710
	Yes	AA	15	5	1.60	0.53 (0.19–1.42)	0.206
	Yes	GA	23	13	0.98	0.61 (0.23–1.62)	0.322
	Yes	GG	10	13	0.01	1.08 (0.28–4.19)	0.907

*Amateur sports, bed type, smoking, and leisure activities were adjusted for χ^2 analysis as covariates, OR, 95% CI, and *P* value.

†Amateur sports, bed type, lumbar load, and leisure activities were adjusted for χ^2 analysis as covariates, OR, 95% CI, and *P* value.

‡Amateur sports, smoking, lumbar load, and leisure activities were adjusted for χ^2 analysis as covariates, OR, 95% CI, and *P* value.

§Amateur sports, smoking, lumbar load, and bed type were adjusted for χ^2 analysis as covariates, OR, 95% CI, and *P* value.

¶Bed type, smoking, lumbar load, and leisure activities were adjusted for χ^2 analysis as covariates, OR, 95% CI, and *P* value.

might be higher in individuals carrying the –1263GG genotype and then the apoptosis of disk cells could be remarkably enhanced. Disk cells have a large extracellular matrix to maintain, but with highly decreased cells it is not surprising that the intervertebral disk is prone to degeneration.²⁷ Under accelerated degeneration, the radial tear of the disk finally may occur.

Lumbar load has been confirmed to be involved in LDH because LDH mainly occurs in people engaged in heavy lifting and heavy labor work.^{28,29} Animal research has revealed that apoptosis of lumbar disk cells increased with load.^{30–32} In the present study, we classified lumbar load into two types: slight load, representing levels I/II, and severe load, representing levels III/IV. Then, after adjusting for age, sex, height, weight, bed type, leisure activities, amateur sports, and smoking history, we found that the interaction of the *FasL* –844TT genotype and level III to IV lumbar load was consistent with the ultramultiplying model, and the interaction of the *CASP9* rs4645978 GG genotype and level III to IV lumbar load was consistent with the

submultiplicative model ($P < 0.05$). These results indicated that to prevent LDH, people carrying *FasL* –844C/T (rs763110) TT or *CASP9* –1263A>G (rs4645978) GG should stay away from a heavy lumbar load. The analysis of the interaction between the two genotypes and factors (smoking, bed type, amateur sports, and leisure activities) revealed that some interactions were consistent with the ultramultiplying model, although there was no statistical significance.

The majority of human diseases are caused by the interaction of genetic and environmental factors. The injury response of the body is related not only to the extent of exposure to harmful environmental factors, but also is related closely to genetic susceptibility or tolerance; namely, it is determined by the interaction of genetic and environmental factors. Although such interaction has been studied extensively in cardiovascular diseases and cancer,^{33,34} little information is available about LDD. Hence, we aimed to elucidate the role of such interaction in the pathogenesis of LDH. We found that *FasL* –844C/T (rs763110) TT and

CASP9 -1263A>G (rs4645978) had an interaction with lumbar load. Our results indicated that LDH probably is caused by the synergies of environmental risk factors and susceptibility genes, and the interaction mechanism between different environmental and genetic factors might differ. Therefore, to take preventive measures, it is necessary to consider both environmental and genetic factors. In future research, we will focus on the direct verification of susceptibility gene polymorphism. For instance, we will investigate the apoptosis of cells that contain apoptosis polymorphism, under different stresses.

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