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ORIGINAL RESEARCH

Polymorphism of IL-1β rs16944(T/C) Associated with Serum Levels of IL-1β and Subsequent Stimulation of Extracellular Matrix Degradation Affects Intervertebral Disk Degeneration Susceptibility

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Purpose: To investigate the association of polymorphism of IL-1β rs16944(T/C) with intervertebral disk degeneration (IDD), explore the possible mechanism and evaluate the predictive value of IL-1β for IDD.

Patients and Methods: A total of 196 consecutive patients with IDD were recruited, and 196 healthy controls were matched to these patients based on sex and age (±3 years). The polymorphisms of IL-1β rs16944(T/C), rs1143623(G/C), rs10490571(T/C) and rs2853550(A/G) were determined, and serum IL-1β, MMP-1, MMP-3, MMP-9 and a disintegrin-like and metalloproteinase with thrombospondin motif-4 (ADAMTS-4) levels were measured. Univariate analysis was performed with Student *t*-test or one-way ANOVA followed by post hoc and Chi-square test. Variables with two-sided $P<0.10$ were included in multivariate analysis, which employed a backward stepwise logistic regression model. Receiver operating characteristic (ROC) curve was used to evaluate the predictive value.

Results: Multivariate analysis showed that the polymorphism of IL-1β rs16944(T/C) was independently associated with IDD. The risk for IDD was significantly increased in TT and TC genotype compared with CC genotype, and the OR of TT genotype was higher than that of TC genotype. ANOVA analysis showed that serum concentration of IL-1β was highest in IL-1β rs16944 TT genotype, intermediate in TC genotype, and lowest in CC genotype. Similarly, serum concentrations of MMP-3 and ADAMTS-4 demonstrated the same tendency of TT > TC > CC genotype. Serum concentrations of MMP-1 and MMP-9 were higher in TT genotype than in TC and CC genotype. The area under curve (AUC) of IL-1β levels in predicting IDD was 0.788 (SE: 0.023, $P=0.001$, 95% CI: 0.742–0.834), and the predictive value was modest with a sensitivity of 77.0% and a specificity of 75%.

Conclusion: Polymorphism of IL-1β rs16944(T/C) affected IDD susceptibility through upregulation of serum levels of IL-1β and subsequent stimulation of ECM degradation. IL-1β levels could be applied in predicting IDD.

Keywords: intervertebral disk degeneration, single-nucleotide polymorphisms, IL-1β, susceptibility, predictive value

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Introduction

Low back pain is a common and chronic medical problem around the world, leading to increased health care costs, functional impairment, loss of working ability and decreased quality of life.[1–3] Intervertebral disk degeneration (IDD) is thought to be the major cause of low back pain,[4] beginning with changes of the local cellular microenvironment and progressing to structure and function impairments of intervertebral disks.[5] The main pathological characteristics of IDD include an altered phenotype of normal disk cells, the presence of pro-inflammatory mediators, a breakdown of extracellular matrix (ECM) and a decrease in active cell numbers.[6,7] Both environmental and genetic factors are involved in occurrence and development of IDD.[8,9] However, the molecular mechanisms associated with IDD has not yet been fully clarified.

IDD is a polygenic disease, and multiple single-nucleotide polymorphisms (SNPs) have been confirmed significant associations with IDD and/or its related phenotypes.[10–12] IL-1β expression is significantly up-regulated in cells and tissues of degenerative intervertebral disks,[13] and it participates in various pathological processes of disk degeneration.[14] Studies have demonstrated that the polymorphism of IL-1β rs16944(T/C) is associated with lumbar disc disease, and increased IL-1β levels are detected in T allele carriers.[15,16] At the same time, the polymorphisms of IL-1β rs1143623(G/C), rs10490571(T/C) and rs2853550(A/G) were significantly associated with IL-1β levels.[17,18] However, the independent association of the polymorphism of IL-1β rs16944(T/C) with IDD has not been investigated with adjusting for potential confounders. In this paper, the association of the polymorphism of IL-1β rs16944 with IDD was analyzed with adjusting for potential confounders, including age, sex, IL-1β rs1143623 (G/C), rs10490571(T/C), and rs2853550(A/G), etc. Furthermore, the possible mechanism was explored and the predictive value of IL- 1β for IDD was evaluated.

Patients and Methods

Participants

Between April 2018 and April 2020, 196 consecutive patients with IDD were recruited in The Second Poverty Relief Hospital of Xinjiang Uygur Autonomous Region (The Fifth People's Hospital of Xinjiang Uygur Autonomous Region). During the same period, 196 healthy controls undergoing the health screening in the same hospital were enrolled, who were matched to the IDD patients based on sex and age (±3 years) at a ratio of 1:1. Among the 196 IDD patients, 16 patients were graded as Type I, 43 patients as Type II, and 137 patients as Type III. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of The Second Poverty Relief Hospital of Xinjiang Uygur Autonomous Region (approval reference number: 201706D019). Each participant provided written informed consent.

Inclusion and Exclusion Criteria

The inclusion criteria of IDD patients included 1) with chronic low back pain more than 3 months; 2)newly diagnosed with IDD through magnetic resonance imaging (MRI) examination demonstrating Pfirrmann grades 3, 4 or 5;[19] 3) confirmed by postoperative pathological results; 4) informed consent. The exclusion criteria of IDD patients included 1) low back pain caused by spine neoplasm, trauma, spondylolisthesis, spondylolysis, spondyloarthropathies, ankylosing spondylitis and other systemic inflammatory diseases; 2) undergoing previous surgical treatment; 3) congenital deformities of the spine.

The inclusion criteria for health controls included 1) without current or previous history of chronic low back pain; 2) without systemic inflammatory diseases; 3) without previous surgical treatment; 4) without history of disc hernia treatment; 5) family members having no disc herniation and/or clinical treatment for low back pain.

Data Collection

In order to adjust potential confounders, demographic and lifestyle data were collected in all participants, including sex, age, height, weight, education level, occupation, smoking, drinking, pain localization (low back, low back + cervical), physical activity (at least 90 min per week or not), posture at work (seated or standing) and load weight at work (yes or no).

Detection of Genotypes

Peripheral blood of 5 mL was collected in EDTA-containing falcon tubes in all participants. DNA was extracted through the Phenol–Chloroform method, as described by Loparev et al.[20] The extracted DNA was stored at −70 °C before SNP genotyping. The optical density and 260/280 ratio were measured to evaluate the quality and concentration of the extracted DNA. The polymorphisms of IL-1β rs16944(T/C),

rs1143623(G/C), rs10490571(T/C) and rs2853550(A/G) were determined using TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, USA), and the ABI 7500 Fast real-time PCR System (Applied Biosystems, USA) was used to conduct PCR amplification.

Measurement of Serum Inflammatory Cytokines

Blood samples were centrifugated for 10 min at 3000 g and then stored at −70 °C. Serum IL-1β, MMP-1, MMP-3, MMP-9 and a disintegrin-like and metalloproteinase with thrombospondin motif-4 (ADAMTS-4) levels were measured with corresponding human ELISA kits (Abcam, Shanghai, China), strictly following the manufacturer's instructions.

Statistical Analysis

For all four SNPs, Hardy–Weinberg equilibrium (HWE), allele and genotype frequencies were analyzed using Haploview software version 4.2 (http://www.broad.mit.edu/mpg/haploview; developed in Mark Daly's laboratory at the Broad Institute).[21] All statistical analysis was conducted using the SPSS version 20.0 (SPSS Inc., USA). The distribution of continuous data was determined through Kolmogorov–Smirnov test. Data with normal distribution were described with mean ± standard deviation and compared with Student *t*-test or one-way ANOVA followed by post hoc. Qualitative data were described with percentages or ratios (%) and compared with Chi-square test. After univariate analysis, variables with two-sided $P<0.10$ were included in multivariate analysis, which employed a backward stepwise logistic regression model. Receiver operating characteristic (ROC) curve was used to evaluate the predictive value of IL-1β for IDD. Significance was set at two-sided $P<0.05$.

Results

Demographic and Lifestyle Data

Demographic and lifestyle data of IDD patients and healthy controls are demonstrated in Table 1. According to univariate analysis, smoking, occupation, physical activity and load weight at work were statistically different

Table 1 Demographic and Lifestyle Data of IDD Patients and Healthy Controls

		IDD Patients (n=196)	Healthy Controls (n=196)	t/χ^2	*P*
Sex	Male Female	102 94	102 94	–	–
Age (yeas)		42.43±5.07	42.25±4.98	0.355	0.749
BMI		22.71±2.65	23.12±2.74	−1.506	0.121
Education level	Elementary school High school College	59 76 61	54 70 72	1.378	0.502
Occupation	Taxi driver Construction worker Office worker	78 59 59	70 40 86	9.106	0.011
Smoking	Yes No	75 121	52 144	6.162	0.013
Drinking	Yes No	69 127	60 136	0.936	0.333
Pain localization	Low back Low back + cervical	183 13	–	–	–
Physical activity	Yes No	51 145	76 120	7.280	0.007
Posture at work	Standing Seated	62 137	52 144	1.029	0.310
Load weight at work	Yes No	101 95	67 129	11.484	0.001

Table 2 Allele and Genotype Frequencies of the Case and Control Group

		Allele Frequency		Genotype Frequency			HWE *P*
rs16944		T	C	TT	TC	CC	
	Case group*			55	99	42	>0.05
	Control group			41	87	68	>0.05
rs1143623		G	C	GG	GC	CC	
	Case group*			36	103	57	>0.05
	Control group			24	93	79	>0.05
rs10490571		T	C	TT	TC	CC	
	Case group			10	61	125	>0.05
	Control group			6	62	128	>0.05
rs2853550		A	G	AA	AG	GG	
	Case group			4	31	161	>0.05
	Control group			6	25	165	>0.05

Note: *$P<0.05$, vs genotype and allele frequencies of the control group.
Abbreviation: HWE, Hardy–Weinberg equilibrium.

between IDD patients and healthy controls ($P<0.05$), and age, body mass index (BMI), education level, drinking and posture at work were not statistically different ($P>0.05$).

SNP Results

All the four SNPs were successfully genotyped in all participants. Their genotype frequencies were not significantly different from those expected through Hardy–Weinberg equilibrium (Table 2). Chi-square test demonstrated that the genotype frequencies of IL-1β rs16944(T/C) and rs1143623 (G/C) of IDD patients were significantly different from those of healthy controls (χ^2=8.541, P=0.014; χ^2=7.048, P=0.029), but rs10490571(T/C) and rs2853550(A/G) were not significantly different (χ^2=2.223, P=0.329; χ^2=1.025, P=0.599).

Multivariate Analysis

Smoking, occupation, physical activity, load weight at work, polymorphism of IL-1β rs16944(T/C) and rs1143623(G/C) were included in multivariate logistic regression analysis. The results (Table 3) showed that the polymorphism of IL-1β rs16944(T/C) was associated with IDD with adjustment for smoking, occupation, physical activity, load weight at work and rs1143623(G/C). The risk for IDD was significantly increased in TT and TC genotype compared with CC genotype, and the OR of TT genotype was higher than that of TC genotype.

Serum Inflammatory Cytokines

According to one-way ANOVA analysis, serum concentration of IL-1β was highest in IL-1β rs16944 TT genotype, intermediate in TC genotype, and lowest in CC genotype (Figure 1A). Similarly, serum concentrations of MMP-3 and ADAMTS-4 demonstrated the same tendency of TT genotype > TC genotype > CC genotype (Figure 1C and E). Serum concentrations MMP-1 and MMP-9 were higher in TT genotype than in TC and CC genotype, but their differences between TC and CC genotype were not significant (Figure 1B and D).

Predictive Value of IL-1β for IDD

Serum concentration of IL-1β was higher in IDD patients than in healthy controls (4.19±1.97 vs 2.45±1.24 pg/mL, t=10.465, $P<0.001$). Moreover, serum concentration of IL-1β was higher in Type II and III than in Type I among IDD patients (Figure 2). Both IDD patients and healthy controls were classified into 4 grades according to the quartile of serum IL-1β concentration of healthy controls (Table 4). The value of the grade of serum IL-1β concentration for predicting IDD was evaluated with ROC curve (Figure 3). The area under curve was 0.788 (SE: 0.023, P=0.001, 95% CI: 0.742–0.834), and the predictive value was modest with a sensitivity of 77.0% and a specificity of 75%.

Discussion

The polymorphism of IL-1β rs16944(T/C) is correlated with various clinical conditions, such as lumbar disc disease, rheumatoid arthritis, breast cancer, keratoconus and febrile seizure.[15,16,18,22,23] In this study, our results showed that the polymorphism of IL-1β rs16944(T/C) was associated with IDD with adjustment for smoking, occupation,

Table 3 Results of Multivariate Analysis Between the Case and Control Group

	Regression Coefficient	Standard Error	Wald χ^2	OR	95% *CI*	*P*
rs16944			6.985			0.031
CC				—	—	Ref=1
TC	0.537	0.186	5.889	1.584	1.122–3.058	0.040
TT	0.712	0.263	9.761	2.619	1.360–4.925	0.008
Smoking	0.449	0.135	5.496	1.497	1.113–2.939	0.042
rs1143623			2.071			0.163
CC				—	—	Ref=1
GC	0.218	0.097	1.219	1.294	0.859–1.926	0.245
GG	0.307	0.128	2.792	1.356	0.904–2.108	0.083
Occupation			2.254			0.146
Office worker				—	—	Ref=1
Taxi driver	0.239	0.106	1.287	1.301	0.871–1.985	0.239
Construction worker	0.298	0.134	3.019	1.349	0.893–2.057	0.078
Physical activity	−0.653	0.172	9.984	0.617	0.428–0.915	0.007
Load weight at work	0.735	0.204	10.526	2.472	1.236–5.047	0.004

physical activity, load weight at work and rs1143623(G/C), and the risk for IDD was significantly increased in TT and TC genotype compared with CC genotype. At the same time, serum concentrations of IL-1β, MMP-3 and ADAMTS-4 demonstrated the tendency of IL-1β rs16944 TT genotype > TC genotype > CC genotype, and serum concentrations MMP-1 and MMP-9 were higher in TT genotype than in TC and CC genotype. Therefore, The possible mechanism was associated with elevated serum levels of IL-1β and subsequent stimulation of ECM

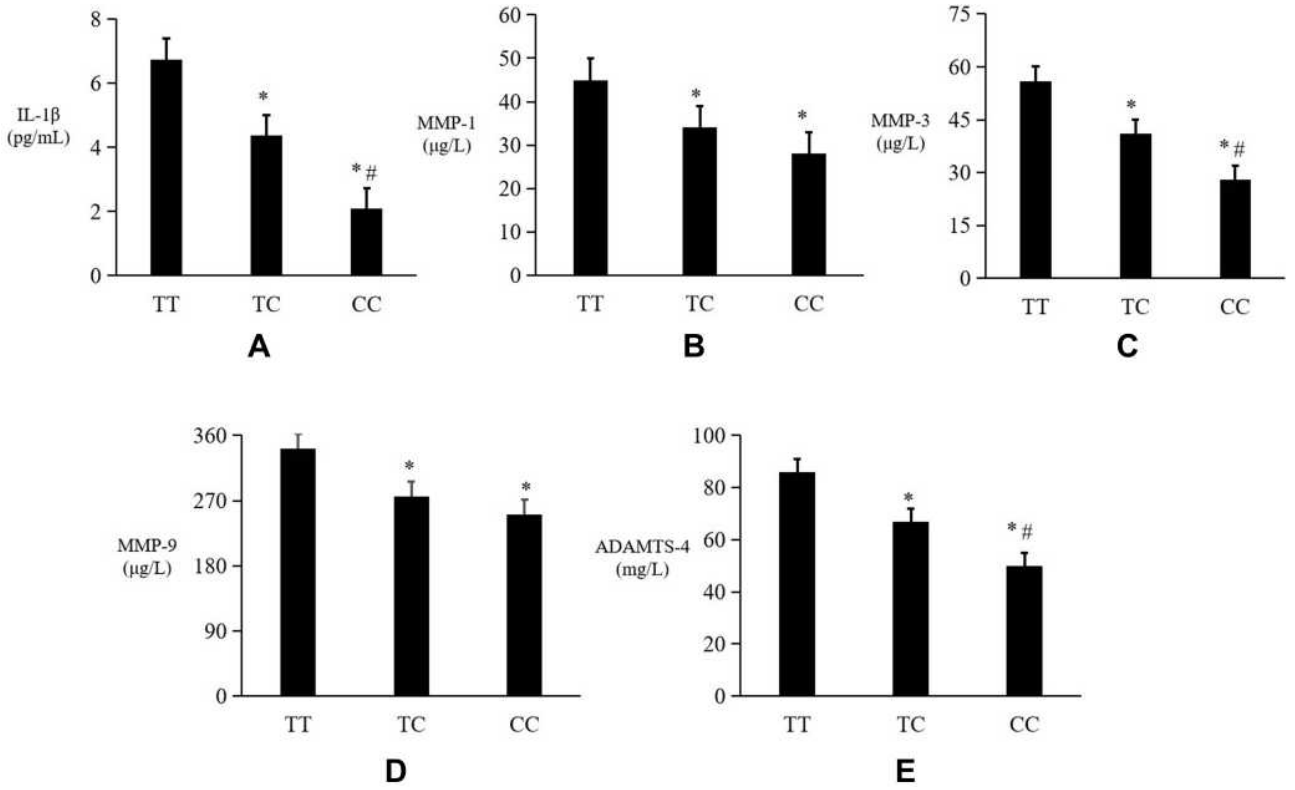

Figure 1 Concentrations of serum inflammatory cytokines. (**A**–**E**) were serum concentrations of IL-1β, MMP-1, MMP-3, MMP-9 and ADAMTS-4, respectively. *P<0.017, vs TT genotype; #P<0.017, vs TC genotype.

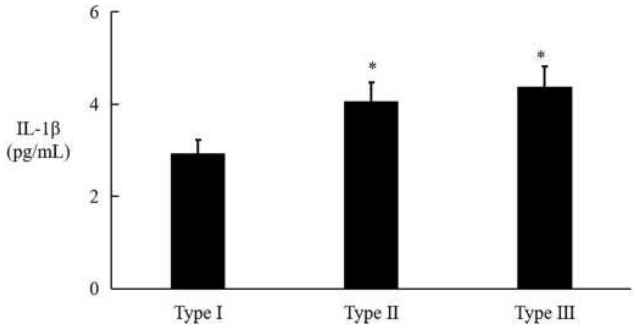

Figure 2 Concentrations of serum IL-1β in IDD patients of Type I, Type II and Type III. *P<0.017, vs Type I.

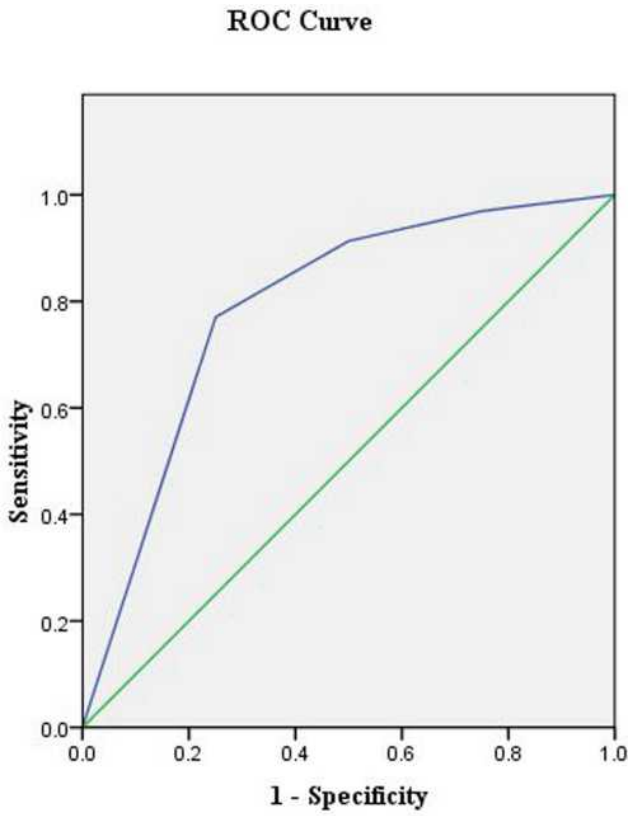

Figure 3 ROC curve of the grade of serum IL-1β concentrations for predicting IDD.

degradation. In this study, the sample size was calculated according to the method for "Tests for Two Proportions (Odds Ratios)": 1-β=0.80, α=0.05, sample allocation ratio=1.0. Expected OR was set at 2.0 and control group proportion of rs16944 TT+TC genotype was set at 0.65 according to the pilot investigation. The sample size was determined as 150 per group. A total of 392 participants (196 IDD patients and 196 controls) were included in this study, and the statistical power was 0.8317. Additionally, serum concentrations of inflammatory cytokines between different genotypes of rs16944 were compared with one-way ANOVA followed by post hoc. The corrected significance was set at P<0.017 (0.05/3) to avoid Type I error. Univariate analysis of demographic data, lifestyle data and SNP results included many variables with different properties, up to 12, and moreover the objective was just screening the variables entering logistic regression model. Therefore, correction of P-value was not applied.

IL-1β, one of the most important pro-inflammatory mediators, has a strong pro-inflammatory activity through stimulating the production of many pro-inflammatory mediators including matrix metalloproteinases (MMPs), chemokines and cytokines.[24–26] IL-1β is mainly secreted by stimulated macrophages and monocytes, and to a lesser extent by several other cell types including neutrophils, lymphocytes, endothelial cells and fibroblasts.[27] Besides immune cells, intervertebral disk cells themselves can secret IL-1β. Under normal circumstances, IL-1β is involved in repair and remodeling of intervertebral disks.[28] However, its level is significantly elevated in the case of IDD occurrence,[28] and moreover its level in IDD tissues demonstrated a significantly positive association with degeneration severity.[29] Immunohistochemistry results also confirmed that the IL-1β level is higher in NP of degenerate intervertebral disks than in non-degenerate intervertebral disks, and the IL-1β level is positively associated with degeneration scores.[30] In summary, the IL-1β expression is markedly upregulated in IDD, and elevated IL-1β expression may be a pathogenic factor for IDD.

Table 4 Classification Results of IDD Patients and Healthy Controls

	Classification Criteria	IDD Patients (196)	Healthy Controls (196)
Grade 1	<Q1 (1.02 pg/mL)	6	49
Grade 2	≥Q1 and <Q2 (2.11 pg/mL)	11	49
Grade 3	≥Q2 and <Q3 (4.25 pg/mL)	28	49
Grade 4	≥Q3 (5.32 pg/mL)	151	49

Proteoglycans and collagens are the main ECM components in intervertebral disks. In normal intervertebral disks, breakdown and synthesis of ECM are in equilibrium under the intricate regulation of catabolic cytokines and growth factors. IDD usually happens when catabolism of ECM is dominant over its anabolism.[31] A disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs) and matrixmetalloproteinases (MMPs) are important enzymes catabolizing ECM components. Plenty of studies have indicated that multiple members of ADAMTSs and MMPs are significantly upregulated in IDD, and they are tightly involved in catabolism of ECM and subsequent occurrence of IDD.[32–35] Meanwhile, knockdown or inactivation of ADAMTSs and MMPs has demonstrated potential in mitigating disk regeneration and promoting ECM repair.

ADAMTSs mainly participate in cleaving proteoglycans. IL-1β has an important role in promoting the production of ADAMTSs in intervertebral disks. Co-culture of human NP cells and IL-1β can lead to a marked increase in mRNA and protein expressions of ADAMTS-4 mRNA through activating MAPK and NF-κB signaling pathways.[36] Additionally, stimulation of bovine disk cells cultured in a monolayer with IL-1β can significantly elevate mRNA levels of ADAMTS-4.[37] MMPs are primarily involved in degradation of collagens.[38,39] Many studies have demonstrated that IL-1β may stimulate the generation of MMPs in intervertebral disks. Both normal and degenerate human NP cells treated with IL-1β have significantly elevated MMP-3 and MMP-9 mRNA levels.[40] Human AF cells display a significantly increased MMP-1 and MMP-3 concentrations after stimulation with IL-1β.[41] Conversely, expression of MMP-3 can be inhibited by hyperbaric oxygen through suppressing expression of IL-1β in degenerated human NP cells.[42] In addition, U0126, a specific inhibitor of extracellular signal-regulated kinase (ERK), can significantly block upregulation of MMP-3 and MMP-13 expression associated with IL-1β in rat AF cells.[43] These studies suggest that IL-1β contributes to ECM degradation and subsequent IDD occurrence through upregulating the expression of ADAMTS-4, MMP-1, MMP-3 and MMP-9. Our results were consistent with previous findings.

We further evaluated the predictive value of IL-1β for IDD. The results showed that the predictive value of IL-1β was moderate with a sensitivity of 77.0% and a specificity of 75%. Therefore, IL-1β had the potential of application to prediction of IDD. In summary, these findings provided novel tools for the prediction of IDD susceptibility and potential therapeutic targets for IDD.

The limitations of this study mainly included two aspects. On the one hand, there was no functional confirmation to support the association between the polymorphism of IL-1β rs16944(T/C) and elevated IL-1β levels. This association could be the reverse, too. On the other hand, the concentrations of ADAMTS-4, MMP-1, MMP-3 and MMP-9 in serum were detected instead of intervertebral disc tissue. The further research directions included two aspects. Firstly, we plan to confirm the association between polymorphism of IL-1β rs16944(T/C), IL-1β levels and IDD using animal models for point mutation. Secondly, we plan to study the therapeutic effect of regulating the expression of IL-1β and other therapeutic targets on IDD.

Conclusion

The polymorphism of IL-1β rs16944(T/C) was associated with IDD, and the risk for IDD was significantly increased in TT and TC genotype compared with CC genotype. The possible mechanism was associated with elevated serum levels of IL-1β and subsequent stimulation of ECM degradation.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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