Association between *GDF5* single nucleotide polymorphism rs143383 and lumbar disc degeneration

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Abstract. The association between growth differentiation factor 5 (*GDF5*), single nucleotide polymorphism (SNP) rs143383 and lumbar disc degeneration (LDD) was investigated. A total of 210 patients with LDD (observation group) and 320 patients without lumbar diseases (control group) diagnosed in Shanghai General Hospital of Nanjing Medical University from August 2013 to March 2017 were randomly selected. Then, deoxyribonucleic acid (DNA) was extracted from the blood of each patient, and Taq-man fluorescent quantitative polymerase chain reaction (qPCR) technique was used to detect rs143383 in *GFD5* gene. The frequency of different genotypes in observation group and control group was counted, and the associations between different SNP genotypes and the incidence of LDD were analyzed. Good genotyping results were found in both LDD patient group and control group. There were no significant differences in distribution frequency of TT and TC genotypes at site rs143383 between LDD patient group and control group (P>0.05), but the distribution frequency of CC genotype at site rs143383 in LDD patient group had a statistically significant difference from that in control group (P<0.05). In dominant models, odds ratio (OR) of (TC+CC/TT) was 1.195 (P=0.532). In recessive models, OR of (CC/TT+TC) was 4.333 (P=0.028). In co-dominant models, ORs of (TC/TT) and (CC/TT) were 0.967 and 4.43, respectively (P=0.99). The differences in 3 genotypes showed no statistical significance among different pathological grades (Grade I to V) (χ^2=1.034, P=0.998), and there was no statistically significant difference in T and C (χ^2=0.012, P=0.999). Pathological grades in dominant models, recessive models and over dominant models were analyzed, and no statistically significant difference was found (P>0.05). In conclusion, CC mutant type at rs143383 in *GDF5* gene has a strong association with the incidence of LDD, and a high prevalence risk, but it has no evident correlation with pathological grades.

Introduction

Lumbar disc degeneration (LDD) is a worldwide orthopedic disease. In particular, 80-90% of the population aged over 60 years are suffering from LDD. LDD is a common disease affecting the health of the elderly (1,2). The pathogenesis of LDD is relatively complicated, and the risk factors are not only related to the external environment and labor involved, but also closely associated with the genetic factors of the body. Recent studies have reported that multiple human genetic mutations can make lumbar disc more susceptible to disease (3). Growth differentiation factor 5 (GDF5) is a transforming growth factor, which is a key protein factor in the growth and development of bone and cartilage and plays an important role in the formation of bone, especially joint (4,5). Some findings have revealed that GDF5 can affect the expression of isotypic collagenase gene by virtue of the multiple differentiation and proliferation abilities of stem cells, thereby improving the structure of the lumbar disc in rats (6). As a single nucleotide polymorphism (SNP) site in *GDF5* gene, rs143383 is located in 5' non-coding region of *GDF5* gene, and a mutation at this site is sure to result in downregulated gene expression, decreasing *GDF5* gene expression in the body, thereby increasing the onset risk of LDD (7,8). This study investigated the correlation between SNP rs143383 in GDF5 and LDD.

Materials and methods

General data. A total of 210 patients with LDD diagnosed and treated in Shanghai General Hospital of Nanjing Medical University (Shanghai, China) from August 2013 to March 2017 were randomly selected as observation group, and 320 patients without lumbar diseases diagnosed and treated in the hospital during the same period were randomly selected as control group. In the observation group, there were 120 males and 90 females aged 39-81 years, with mean age of 64.2±19.3 years. Among them, in terms of Thompson's pathological grading, there were 21 cases of Grade I, 45 cases of Grade II,

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38 cases of Grade III, 56 cases of Grade IV and 50 cases of Grade V. Control group had 190 males and 130 females aged 37-83 years, whose mean age was 65.5±19.7 years. All patients were aware of this study and signed the informed consent, and this study was approved by the Ethics Committee of Shanghai General Hospital of Nanjing Medical University. There were no statistically significant differences in sex, age, living habits, between he two groups (P>0.05) (Table I), and the results were comparable.

Extraction of genomic deoxyribonucleic acid (DNA). Whole blood (5 ml) was collected from each patient with an anti-coagulant tube containing ethylenediamine tetraacetic acid dipotassium (EDTAK2). Then, genomic DNA was extracted from the blood using an Omega Mag-Binds Forensic DNA kit (Omega Bio-Tek, Inc., Norcross, GA, USA). After that, the concentration and purity of DNA were determined by NanoDrop, and DNA was stored at -20°C.

SNP typing via polymerase chain reaction (PCR). Primer sequences and their Taqman probe sequences at SNP site (Table II) designed by Oligo6.0 were used. Primer synthesis was accomplished by Sangon Biotech Co., Ltd. (Shanghai, China). DNA solution (1 μl) and 1.2 μl prepared primer solution (including 0.4 μl upstream primer, 0.4 μl downstream primer and 0.4 μl probe primer) were added to 17.8 μl pre-prepared TransStart Probe qPCR SuperMix (Beijing TransGen Biotech Co., Ltd., Beijing, China), slightly shaken to mix, and placed into a CFX96 fluorescent quantitative PCR instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Reaction conditions: i) 94°C for 3 min, for 1 cycle; ii) 94°C for 15 sec and 60°C for 60 sec, for 42 cycles. After each cycle, the fluorescence value was read once. The experimental results were generated by built-in software of the instrument. Three replicate wells were made for each sample, diethyl pyrocarbonate (DEPC) water was used as negative control, and positive plasmid containing the sequence (synthesized by Sangon Biotech) was used as positive control. Determination of genotypes: The wild homozygous genotype was near the FAM abscissa, the mutant homozygous genotype was near the VIC ordinate, and the heterozygous genotype was near the 45° line.

Statistical analysis. Statistical Product and Service Solutions (SPSS; IBM Corp., Armonk, NY, USA) 19.0 software was used for statistical analysis. Chi-square test was employed for statistical analyses of genotype distribution differences between case group and control group. Logistic regression analysis was adopted for the associations between various genotypes and the risk of LDD. P<0.05 was considered to indicate a statistically significant difference.

Results

Distributions of genotypes at rs143383 in two groups. A total of 210 patients with LDD and 320 patients in control group obtained clear genotyping results (Fig. 1). There were no statistically significant differences in distribution frequency of two genotypes, namely, TT and TC, at site rs143383 between LDD patient group and control group (P>0.05), but the distribution frequency of CC genotype at rs143383 in LDD patient group

Table I. General clinical data.

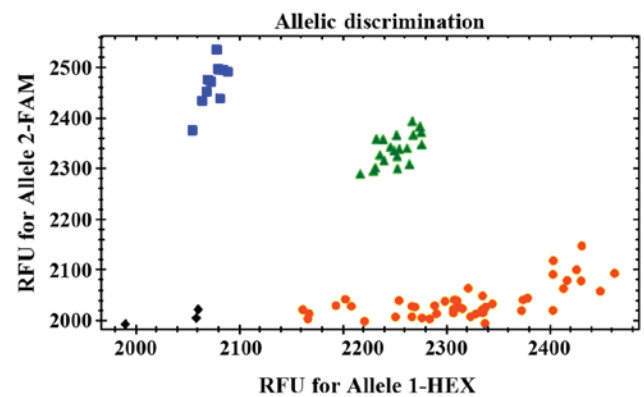| Parameters	Observation group (n=210)	Control group (n=320)	P-value
Age (years)	64.2±19.3	65.5±19.7	0.462
Sex			0.733
Male	120 (57.1%)	190 (59.4%)	
Female	90 (42.9%)	130 (40.6%)	
Body mass index (BMI) (kg/m²)	23.4±3.6	23.6±3.8	0.754
Smoking			0.415
Yes	86 (41%)	137 (42.8%)	
No	124 (59%)	183 (57.2%)	
Drinking			0.536
Yes	121 (57.6%)	191 (59.7%)	
No	89 (42.4%)	129 (40.3%)	

Figure 1. Results of genotyping at rs143383. Symbols near X-axis: TT, symbols near Y-axis: CC, and green symbols in the middle: TC (Table III).

showed a statistically significant difference compared with that in control group (P<0.05) (Table III). The distributions in two groups were in line with Hardy-Weinberg equilibrium [P(control)=0.31 and P(observation)=0.35].

Onset risks of LDD analyzed in different models. In dominant models, odds ratio (OR) of (TC+CC/TT) was 1.195 (P=0.532). In recessive models, OR of (CC/TT+TC) was 4.333 (P=0.028). In co-dominant models, ORs of (TC/TT) and (CC/TT) were 0.967 and 4.43, respectively (P=0.09) (Table IV).

Association between genotypes at rs143383 and clinicopathologic grade of LDD. There were no statistically significant differences in three genotypes (TT, TC and CC) among different pathological grades (Grade I-V) (χ^2=1.034, P=0.998), and the differences in T and C also showed no statistical significance (χ^2=0.012, P=0.999) (Table V).

Associations between different models and clinicopathologic grades of LDD using logistic regression analysis. Pathological

Table II. Primer sequences.

SNP	Primer sequence	Probe sequence
rs143383	Upstream: 5'-CAGGCAGCATTACGCCATTCTTC-3'	FAM: 5'-CGGTCGGCTTTCTCCTTTCAAG-3'
	Downstream: 5'-CACCGTCTCCAGTCAGCAGCTG-3'	VIC: 5'-CGGTTGGCTTTCTCCTTTCAAG-3'

Table III. Distribution frequency at rs143383 (n, %).

Genotype	Observation group (n=210)		Control group (n=320)		χ^2 value	P-value
	n	%	n	%		
TT	125	59.5	204	63.8	0.391	0.532
TC	64	30.5	108	33.7	0.235	0.628
CC	21	10	8	2.5	4.8	0.028
T	314	74.8	516	80.6	0.971	0.324
C	106	25.2	124	19.4		

grades in dominant models, recessive models and co-dominant models were analyzed, and the results showed that there were no statistically significant differences among pathological grades in dominant models, as well as in recessive models and co-dominant models. (P>0.05) (Table VI).

Discussion

The expression function of genes may be influenced by many factors including the effects of non-coding regions and various regulators and the changes of gene structure caused by SNP in gene, affecting the action of translation function, indirectly influencing health, and leading to a variety of diseases (9,10). Therefore, predicting the risks of associated diseases via statistical analyses of SNPs in the body becomes very meaningful. rs143383 is located in 5' non-coding region of *GDF5* gene, where the promoter of gene manipulation system is located, so the regulation of *GDF5* gene expression will inevitably be affected (11). Moreover, the main function

of *GDF5* gene is to code transforming factors that are closely related to the regeneration and formation of bone. Therefore, rs143383 polymorphism is certainly associated with bone-related diseases (12,13). The incidence of LDD is mainly correlated with following factors: age is increased, the rate of bone formation is lower than that of bone loss, osteoporosis occurs, the elasticity of bone deteriorates, the ability to withstand load declines, and fracture and dislocation occur easily due to external pressure (14). Recent studies have found that mutations in *GDF5* gene are highly correlated with osteoarthritis, developmental dysplasia of hip and lumbar disc-related diseases (15).

Yin *et al* (16) used a variety of SNP typing methods and reported that Taqman probe method has an irreplaceable advantage in terms of the accuracy of typing. In this study, this method was used, and good genotyping results were obtained in patients with LDD and patients in control group. Tsezou (17) demonstrated that C/T at rs143383 is highly associated with LDD in White Europeans. This study found that CC genotype at rs143383 had very high association and risk with LDD in Chinese Han population with LDD. Among them, OR of (CC/TT+TC) was 4.333 (P=0.028) in recessive models, and OR of (CC/TT) was 4.43 (P=0.021) in co-dominant models. A study by Huétink *et al* (18) suggested that there is a certain association between *GDF5* gene and the severity of osteoarthritis in White Europeans. However, no significant differences were found in CC, CT and TT genotypes among different pathological grades (Grade I-V) in Chinese Han population (χ^2=1.034, P=0.998), and there were also no statistically significant differences in T and C (χ^2=0.012, P=0.999). In addition, no significant differences were found in dominant models, recessive models and over dominant models among different pathological grades (P>0.05). It is possible that SNP mutations in *GDF5* gene have various effects on different bone diseases in different ethnic groups. Therefore, it is necessary

Table IV. Onset risks of LDD analyzed in different models.

Model type	Genotype	Observation group (n=210)	Control group (n=320)	OR value [95% confidence interval (CI)]	P-value
Dominant model	TT	125	204	1	0.532
	TC+CC	85	116	1.195 (0.732-1.532)	
Recessive model	TT+TC	189	312	1	0.028
	CC	21	8	4.333 (2.321-7.786)	
Co-dominant model	TT	125	204	1	0.09
	TC	64	108	0.967 (0.657-1.214)	
	CC	21	8	4.43 (2.451-7.698)	

Table V. Genotypes at rs143383 and distributions of clinicopathologic grades in LDD patients (n, %).

Grade	n (210)	rs143383				
		TT (n=125)	TC (n=64)	CC (n=21)	T (n=314)	C (n=106)
I	36	21 (16.8)	12 (18.8)	3 (14.3)	54 (17.2)	18 (17)
II	48	29 (23.2)	14 (21.9)	5 (23.8)	72 (22.9)	24 (22.6)
III	41	24 (19.2)	13 (20.3)	4 (19)	61 (19.4)	21 (19.8)
IV	45	27 (21.6)	13 (20.3)	5 (23.8)	67 (21.3)	23 (21.7)
V	40	24 (19.2)	12 (18.8)	4 (19)	60 (19.1)	20 (18.9)
χ^2		1.034			1.012	
P-value		0.998			0.999	

Table VI. Associations between different models and clinicopathologic grades of LDD (n, %).

Model type	Genotype	Grade I (36)	Grade II (48)	Grade III (41)	Grade IV (45)	Grade V (40)	P-value
Dominant model	TT	21 (16.8)	29 (23.2)	24 (19.2)	27 (21.6)	24 (19.2)	0.999
	TC+CC	15 (17.6)	19 (22.4)	17 (20)	18 (21.2)	16 (18.8)	
Recessive model	TT+TC	33 (17.5)	43 (22.8)	37 (19.6)	40 (21.2)	36 (19)	0.975
	CC	3 (14.3)	5 (23.8)	4 (19)	5 (23.8)	4 (19)	
Co-dominant model	TC	12 (18.8)	14 (21.9)	13 (20.3)	13 (20.3)	12 (18.8)	0.990
	TT+CC	24 (16.4)	34 (23.3)	28 (19.2)	32 (21.9)	28 (19.2)	

to analyze the correlations between base mutations and LDD in different populations. Furthermore, there may be some differences in the results of the associations between SNP and diseases due to diverse sample sizes, so a larger sample size is more meaningful (19,20).

In conclusion, CC mutant type at rs143383 in *GDF5* gene is strongly associated with the incidence of LDD and has a higher prevalence risk, but it is not significantly correlated with pathological grade.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZW and YL collected and analyzed the general data of patients. YW and XW extracted genomic deoxyribonucleic acid. JZ and JT performed PCR. All authors read and approved the final version of the manuscript

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shanghai General Hospital of Nanjing Medical University (Shanghai, China). All patients were aware of this study and signed the informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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