Association between single-nucleotide polymorphism rs145497186 related to NDUFV2 and lumbar disc degeneration: a pilot case–control study

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Abstract

Objective: The association between the single-nucleotide polymorphisms (SNPs) rs28742109, rs12955018, rs987850, rs8093805, rs12965084 and rs145497186 related to gene named NADH dehydrogenase [ubiquinone] flavoprotein 2 (NDUFV2) and lumbar disc degeneration (LDD) was preliminary investigated in a small sample size.

Methods: A total of 46 patients with LDD and 45 controls were recruited at Qilu Hospital of Shandong University, and each participant provided 5 mL peripheral venous blood. NA was extracted from the blood of each participant for further genotyping. The frequency of different genotypes in the case group and control group was determined, and analysis of the risk of LDD associated with different SNP genotypes was performed. The visual analogue scale (VAS) scores of the patients' degree of chronic low back pain were calculated, and the relationship between VAS scores and SNPs was analysed.

Results: After excluding the influence of sex, age, height, and weight on LDD, a significant association between SNP rs145497186 related to NDUFV2 and LDD persisted \((P = 0.006)\). Simultaneously, rs145497186 was found to be associated with chronic low back pain in LDD populations.

Conclusion: NDUFV2 rs145497186 SNP could be associated with susceptibility to LDD and the degree of chronic low back pain.

Keywords: NDUFV2, Single-nucleotide polymorphism, Lumbar disc degeneration, Visual analogue scale, Case–control study

Introduction

Chronic low back pain (CLBP) is a common cause of reduced quality of life worldwide [1]. According to statistics, approximately 60–80% of people experience CLBP symptoms during their lifetime [2]. In-line with reports thus far, up to 42% of CLBP cases are related to lumbar disc disease [3]. There are two types of lumbar disc disease that lead to CLBP: secondary pain caused by lumbar disc herniation or lumbar spinal stenosis and lumbar disc...
SNPs related to musculoskeletal diseases can affect athleticism; it is interesting that some studies have shown that these SNPs have been recognized as playing a critical role in disease susceptibility [12]. Many studies have shown the association between SNPs and susceptibility to common orthopaedic diseases [13], such as muscle injuries [14], tendon and ligament injuries [15, 16]. Furthermore, it is interesting that some studies have shown that these SNPs related to musculoskeletal diseases can affect athletic performance [17, 18]. Therefore, our study aimed to assess the association between NDUFV2 rs28742109, rs12955018, rs987850, rs8093805, rs12965084 and rs145497186 SNPs and LDD [19].

Materials and methods
Study subjects
A total of 91 participants were recruited at Qilu Hospital of Shandong University from March 2019 to May 2019, including 46 patients with degenerative lumbar disc herniation and 45 controls. This study was approved by the ethics committee of Qilu Hospital of Shandong University and was conducted according to the guidelines of the Declaration of Helsinki. All participants were aware of this research and signed the informed consent form [20]. General data, including sex, age, height, weight and body mass index (BMI), were collected [21].

Inclusion and exclusion criteria
Inclusion Criteria 1. adults over 18 years old, 2. the case group was patients admitted for surgery who had symptoms of low back pain, and MRI showed that they suffered from LDD, 3. normal mental consciousness and able to actively cooperate with the researchers, and 4. voluntarily participated and agreed to sign informed consent.

Exclusion Criteria 1. a history of scoliosis, spinal deformities, metabolic diseases, cardiovascular diseases, and malignant tumours, among others, 2. a history of spinal trauma, 3. undergone operations at the same intervertebral disc, and 4. unable to cooperate with the researchers.

This study used these criteria as a reference to select participants.

Genotyping
Genomic deoxyribonucleic acid (DNA) was extracted from peripheral venous blood and stored at −80 °C until genotyping was performed [22, 23]. Capital Biotechnology Precision Medicine Research Array Kit (CBT-PMRA) (Thermo Fisher Scientific, Waltham, MA, USA), a specialized chip based on the Axiom 2.0 platform, was used for genotyping. The microarray experiments were carried out according to the official standard operating procedures (Axiom™ 2.0 assay 96-array format manual workflow user guide). After amplification, hybridization, ligation, staining, washing and array scanning, data were obtained for further research.

Chronic low back pain VAS score assessment
Forty-six patients diagnosed with LDD were evaluated with theVAS: zero points, no pain; one to three points, mild pain not affecting sleep; four to six points, moderate pain mildly affecting sleep; seven to ten points, severe pain and unable to sleep [24].

Statistical and bioinformatic analyses
SNPs related to NDUFV2 were detected via CBT-PMRA chips. A chi-square test was used to assess allele deviation from Hardy–Weinberg equilibrium (HWE) [25]. Then, the chi-square test was used to determine statistically significant SNPs. Analysis of a genetic association was estimated under five different models (allelic, dominant, recessive, heterozygous and homozygous) using SPSS 21.0 statistical software (SPSS Inc., Chicago, USA) [26]. In screening for SNPs with statistically significant differences, P values < 0.01 in two-tailed test were considered statistically significant, for other statistical analyses, P values < 0.05 were considered statistically significant. Linkage disequilibrium (LD) analysis was performed with Haplovie and LD blocks [27], where the number shown in each block is one hundred times the D’ value. The statistical analysis was performed using the statistical computing software R [28].
To determine the functional consequences of target SNPs, RegulomeDB and 3D SNP databases were used. RegulomeDB annotates SNPs with known and predicted regulatory elements in the intergenic regions of the human genome [29]. 3D SNP is a database for linking human noncoding SNPs to three-dimensional interacting genes [30]. It comprehensively evaluates the role of SNPs based on six different functional categories, including 3D interacting genes, enhancer state, promoter state, transcription factor-binding sites, sequence motifs altered and conservation; the higher the score is, the stronger the role of the SNP in this function is [31].

Results
Characteristics of the study subjects
Statistical tests were conducted by collecting the basic information of the participants. As sex, age, height, weight, and BMI were not significantly different between the case and control groups (P > 0.05) (Additional file 1: Table S1), these factors had no significant relationship with LDD.

The P values of the HWE test for the investigated SNPs were all greater than 0.05 (Additional file 2: Table S2). Therefore, it was believed that these SNPs in the research population satisfied the HWE and that the samples reflected the population [32].

There were six SNPs detected from the SNP chip, including rs145497186, rs28742109, rs12955018, rs987850, rs8093805 and rs12965084. The results of the relationship of these six SNPs between the case and control groups showed significant differences for rs8093805, rs12965084 and rs145497186 (Table 1). The relationship between the genotypes of these SNPs and LDD was analysed by the chi-square test (Table 2), with rs145497186 having a significant relationship with LDD.

Distribution of the SNP rs145497186 genotype and its relationship with LDD
The genotypes of rs145497186 for the LDD patients and controls are listed in Table 2. The distribution of rs145497186 between the LDD patients and controls showed a statistically significant difference (P = 0.006), and no deviation from HWE was observed in the control group (P = 0.930) (Additional file 2: Table S2).

The genotype frequencies in the control group were 11 (100%), 12 (0%), and 22 (0%), and those in the case group were 11 (84.8%), 12 (15.2%), and 22 (0%). The results of the chi-square test revealed distribution differences in rs145497186 between the case and control groups in the allelic model (P = 0.008), dominant model (P = 0.006) and heterozygous model (P = 0.006) (Table 2).

Relationship between single-nucleotide polymorphisms related to NDUFV2 and chronic low back pain
The 46 participants in the case group were divided into two groups based on VAS score: VAS < 4 and VAS ≥ 4 [33]. The groups did not differ significantly in terms of sex, age, height, weight, body mass index (BMI), spinal canal occupation ratio (SCOR), or decrease spinal canal ratio (DSCR) (Additional file 3: Table S3). Among all SNPs, rs145497186 was significantly different in the VAS score groups (group 1 included patients whose VAS scores were < 4; group 2 included patients whose VAS scores were ≥ 4) (Table 3).

Linkage disequilibrium of the screened SNPs related to NDUFV2 in LDD
LD among the six SNPs was low in general (Fig. 1). For rs987850 and rs12965084, the D’ value was 0.89, the r² value was 0.381, and a significant association signal was identified. However, the D’ value of rs145497186 and rs987850 was 0.355, and their r² value was 0.001, which were both the lowest in the LD plot.

Table 1  Chi-square Test of rs28742109, rs12955018, rs987850, rs8093805, rs12965084 and rs145497186 related to NDUFV2

| SNP            | Control group (n) | Case group (n) | P value |
|----------------|-------------------|---------------|---------|
|                | 11    | 12    | 22    | 11   | 12   | 22   |        |
| rs28742109     | 28    | 13    | 4     | 35   | 11   | 0    | 0.085  |
| rs12955018     | 41    | 4     | 0     | 36   | 8    | 2    | 0.162  |
| rs987850       | 26    | 18    | 1     | 36   | 10   | 0    | 0.086  |
| rs8093805      | 18    | 20    | 7     | 31   | 11   | 4    | 0.032  |
| rs12965084     | 18    | 20    | 7     | 29   | 15   | 2    | 0.048  |
| rs145497186    | 45    | 0     | 0     | 39   | 7    | 0    | 0.006  |

Statistically significant differences P values < 0.01 are in bold
a SNP, single-nucleotide polymorphism
b 11 presents common homozygote, 22 presents rare homozygote, 12 presents heterozygote, 1 presents common allele, 2 presents rare allele
Functional consequences of SNP rs145497186

The SNP rs145497186 is located on chromosome 18, p13, with a rank score of 2b. RegulomeDB uses a rank scoring strategy to assess the functional significance of SNPs, whereby each SNP is assigned a rank score ranging from one to six, with a lower score indicating more functional significance [34]. Therefore, rs145497186 has obvious functional significance. As a noncoding SNP, the function of rs145497186 is completely shown in the 3D SNP in Fig. 2. The enhancer score was 25.33, which was the second highest of the six categories of scores. The score of transcription factor-binding site (TFBS) was 100, which was the highest score. The results showed SNP rs145497186 to be located in a region containing 205 transcription factor-binding sites.

Table 2  The relationship between SNPs related to NDUFV2 and LDD

| Genotype* | Control group (n) | Case group (n) | $\chi^2$ value | P value |
|-----------|------------------|---------------|----------------|---------|
| rs145497186 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 90 | 0 | 85 | 7 | 7.122 | 0.008 |
| Dominant Model (11 vs. 12 + 22) | 45 | 0 | 39 | 7 | 7.418 | 0.006 |
| recessive Model (11 + 12 vs. 22) | 45 | 0 | 46 | 0 | – | 1.000 |
| Heterozygous Model (11 vs. 12) | 45 | 0 | 39 | 7 | 7.418 | 0.006 |
| Homozygous Model (11 vs. 22) | 45 | 0 | 39 | 0 | – | 0.944 |
| rs28742109 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 69 | 21 | 81 | 11 | 4.064 | 0.004 |
| Dominant Model (11 vs. 12 + 22) | 28 | 17 | 35 | 11 | 2.053 | 0.152 |
| recessive Model (11 + 12 vs. 22) | 41 | 4 | 46 | 0 | 4.277 | 0.039 |
| Heterozygous Model (11 vs. 12) | 41 | 4 | 36 | 8 | 1.647 | 0.199 |
| Homozygous Model (11 vs. 22) | 41 | 0 | 36 | 2 | 2.214 | 0.137 |
| rs12955018 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 86 | 4 | 80 | 12 | 4.195 | 0.041 |
| Dominant Model (11 vs. 12 + 22) | 41 | 4 | 36 | 10 | 2.885 | 0.089 |
| recessive Model (11 + 12 vs. 22) | 45 | 0 | 44 | 2 | 2.000 | 0.157 |
| Heterozygous Model (11 vs. 12) | 41 | 4 | 36 | 8 | 1.647 | 0.199 |
| Homozygous Model (11 vs. 22) | 41 | 0 | 36 | 2 | 2.214 | 0.137 |
| rs987850 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 70 | 20 | 82 | 10 | 4.259 | 0.039 |
| Dominant Model (11 vs. 12 + 22) | 26 | 19 | 36 | 10 | 4.396 | 0.036 |
| recessive Model (11 + 12 vs. 22) | 44 | 1 | 46 | 0 | 1.034 | 0.309 |
| Heterozygous Model (11 vs. 12) | 26 | 18 | 36 | 10 | 1.501 | 0.221 |
| Homozygous Model (11 vs. 22) | 26 | 1 | 36 | 2 | 0.355 | 0.244 |
| rs8093805 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 56 | 34 | 73 | 19 | 6.464 | 0.011 |
| Dominant Model (11 vs. 12 + 22) | 18 | 27 | 31 | 15 | 6.867 | 0.009 |
| recessive Model (11 + 12 vs. 22) | 38 | 7 | 42 | 4 | 1.007 | 0.316 |
| Heterozygous Model (11 vs. 12) | 18 | 20 | 31 | 11 | 5.877 | 0.015 |
| Homozygous Model (11 vs. 22) | 18 | 7 | 31 | 4 | 2.675 | 0.102 |
| rs12965084 |                 |               |                |         |
| Allelic Model (1 vs. 2) | 56 | 34 | 73 | 19 | 6.464 | 0.011 |
| Dominant Model (11 vs. 12 + 22) | 18 | 27 | 31 | 15 | 6.867 | 0.009 |
| recessive Model (11 + 12 vs. 22) | 38 | 7 | 42 | 4 | 1.007 | 0.316 |
| Heterozygous Model (11 vs. 12) | 18 | 20 | 31 | 11 | 5.877 | 0.015 |
| Homozygous Model (11 vs. 22) | 18 | 7 | 31 | 4 | 2.675 | 0.102 |

Statistically significant differences $P$ values < 0.05 are in bold

*1 presents common homozygote, 2 presents rare homozygote, 12 presents heterozygote, 1 presents common allele, 2 presents rare allele
Gene expression may be affected by various factors, including mutations caused by SNPs, which may alter the processes of transcription and translation [35]. Accordingly, changes in the function of the proteins produced may occur. These changes may directly or indirectly affect normal physiological activities and contribute to disease [36]. Therefore, it is very meaningful to explore the relationship between disease and SNPs.

The protein produced by the NDUFV2 gene is one of the core components of Complex I, and Complex I (Cx I) (NADH-ubiquinone oxidoreductase; EC 1.6.5.3) is an electron entry point in the mitochondrial respiratory electron transport chain (ETC) [37]. Relevant studies have shown that a low abundance of the protein NDUFV2 can reduce electron transport in the mitochondrial electron transport chain, thereby decreasing the production of reactive oxygen species and free radicals, which can prolong the lifespan of a cell [38]. The NDUFV2 gene has been mainly studied in mental diseases, brain diseases, prostate cancer and hypertrophic cardiomyopathy [39–42].

To the best of our knowledge, there have been no relevant studies about variation in NDUFV2 and its impact on disease in orthopaedics, especially in the field of lumbar disc degeneration. Therefore, the main goal of this study is to detect the relationship between SNPs related

Table 3 The relationship between SNP that related to NDUFV2 and CLBP VAS Score in case group

| SNP          | VAS < 4 (n)* | VAS ≥ 4 (n)* | P value |
|--------------|-------------|--------------|---------|
|              | 11  | 12  | 22 | 11  | 12  | 22 |       |
| rs28742109   | 17  | 2   | 0  | 18  | 9   | 0  | 0.074 |
| rs12955018   | 17  | 2   | 0  | 19  | 6   | 2  | 0.246 |
| rs987850     | 16  | 3   | 0  | 24  | 3   | 0  | 0.929 |
| rs8093805    | 15  | 3   | 1  | 16  | 8   | 3  | 0.373 |
| rs12965084   | 15  | 4   | 0  | 14  | 11  | 2  | 0.133 |
| rs145497186  | 13  | 6   | 0  | 26  | 1   | 0  | 0.010 |

VAS Visual Analogue Scale
Statistically significant differences P values < 0.05 are in bold
* SNP, single-nucleotide polymorphism
* 11 presents common homozygote, 22 presents rare homozygote, 12 presents heterozygote, 1 presents common allele, 2 presents rare allele

The protein produced by the NDUFV2 gene is one of the core components of Complex I, and Complex I (Cx I) (NADH-ubiquinone oxidoreductase; EC 1.6.5.3) is an electron entry point in the mitochondrial respiratory electron transport chain (ETC) [37]. Relevant studies have shown that a low abundance of the protein NDUFV2 can reduce electron transport in the mitochondrial electron transport chain, thereby decreasing the production of reactive oxygen species and free radicals, which can prolong the lifespan of a cell [38]. The NDUFV2 gene has been mainly studied in mental diseases, brain diseases, prostate cancer and hypertrophic cardiomyopathy [39–42].

To the best of our knowledge, there have been no relevant studies about variation in NDUFV2 and its impact on disease in orthopaedics, especially in the field of lumbar disc degeneration. Therefore, the main goal of this study is to detect the relationship between SNPs related
to NDUFV2 and LDD and to explore the relationship between SNPs of NDUFV2 and the genetic susceptibility of LDD disease, then provide a new potential target for the precision medicine of LDD. A total of 112 SNPs associated with NDUFV2 were detected using a chip, six of which were significantly different and selected for follow-up analysis. Through data analysis, six related SNPs were identified: rs28742109, rs12955018, rs987850, rs8093805, rs12965084 and rs145497186. The genotype of these six SNPs in each study subject was detected. Then, five genetic models were assessed for each SNP, including the allele model, dominant model, recessive model, heterozygous model and homozygous model. The Hardy–Weinberg equilibrium test was used to identify whether the subjects were representative of the population (Additional file 2: Table S2). Furthermore, statistical analysis showed no difference in sex, age, height, weight or BMI. After excluding the influence of these factors, a chi-square test was applied to examine the relationship between each SNP and LDD (Table 1).

The results showed the genotype of SNP rs145497186 in the control and case groups to be GG and AG, respectively. In this study, the minimum allele frequency (MAF) of A was 0.038. The data obtained from the ALFA project showed that the MAF of A in Europeans is 0.0001 and that in Africans is zero. However, in Asians, especially East Asians, the MAF of A is significantly higher, at 0.03. Therefore, rs145497186 associated with A may be a risk factor for LDD in the East Asian population.

According to previous studies, most diseases follow allelic and dominant/recessive model inheritance patterns [43]. The results also showed rs145497186 to be significantly associated with the risk of lumbar disc degeneration in the allelic model, dominant model and heterozygous model (Table 2). In addition, the patients were divided into two groups, VAS < 4 and VAS ≥ 4. The results showed that SNP rs145497186, which is related to NDUFV2, was significantly associated with the degree of chronic low back pain, excluding the influence of sex, age, height, weight, BMI, SCOR and DSCR.

Based on LD analysis, the D' value of rs987850 and rs12965084 was 0.89, and the r^2 value was 0.381. Either a D' value > 0.8 or a r^2 value > 0.33 indicates that two SNPs are in LD [44]. Therefore, rs987850 and rs12965084 fit well with LD. However, the D' value of rs145497186 and rs987850 was 0.355 and the r^2 value 0.001. Thus, rs145497186 barely fit with LD, and linkage between them was weak. Overall, the SNP rs145497186 is more likely to act alone.

Using data obtained from NCBI, RegulomeDB, and 3D SNP, the position of rs145497186 is at chr18:9,073,758 (GRCh38.p13), upstream of the NDUFV2 gene; this SNP does not directly affect the function of NDUFV2. The SNP rs145497186 is a noncoding SNP and interacts with NDUFV2 in three-dimensional space through the chromatin loop structure to exert its function. The score for the enhancer was 25.33, suggesting that SNP rs145497186 might increase expression of NDUFV2 by affecting the promoter state, resulting in increased NDUFV2 protein abundance and electron transport chain efficiency. In turn, the generation of free radicals and reactive oxygen species would increase, which might impact the cells of the intervertebral disc and ultimately lead to LDD [45]. In addition, the TFBS score was 100, and the results showed that it is located in a region of 205 transcription factor-binding sites. We speculate that the SNP rs145497186 might affect expression of NDUFV2 via transcription factors. These results all verify that the SNP rs145497186 indirectly, rather than directly, affects the function of NDUFV2.

In summary, we conclude that the SNP rs145497186 related to NDUFV2 could be associated with susceptibility to lumbar disc degeneration and the degree of chronic low back pain in Asian people.

There are still some limitations in this study. For instance, the small sample size is the major limitation of this study, so the discoveries of this study need to be validated on a larger scale in the future study. Although the sample scale is small, the study remains valuable as NDUFV2 rs145497186 has not been studied in LDD and significant conclusion is obtained in this study. In the future large-scale study, we will verify whether this finding can meet the genetic susceptibility of large populations. If the conclusions are consistent, NDUFV2 rs145497186 will be a sensitive SNP site to predict the susceptibility of East Asian populations to LDD. In addition, the specific mechanism by which rs145497186 affects the function of NDUFV2 has not been elucidated. Although a relationship between rs145497186 and LDD has been established, further research will be required to explore the role of the SNP rs145497186 in the pathogenesis of LDD.

**Abbreviations**
- SNPs: Single-nucleotide polymorphisms; LDD: Lumbar disc degeneration; CLBP: Chronic low back pain; NDUFV2: NADH dehydrogenase (ubiquinone) flavoprotein 2; VAS: Visual analogue scale; SCOR: Spinal canal occupation ratio; DSCR: Decrease of spinal canal ratio; BMI: Body mass index; HWE: Hardy–Weinberg equilibrium; LD: Linkage disequilibrium; TFBS: Transcription factor binding sites.

**Supplementary Information**
- The online version contains supplementary material available at https://doi.org/10.1186/s13018-022-03368-y.

**Additional file 1: Table S1.** General data of case and control group.
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Author contributions
ZW contributed to data processing and analysis, writing manuscript. LC contributed to revising the manuscript and providing methodology. QL contributed to samples and data collection. HZ contributed to data curation. YS contributed to data curation. LQ contributed to software operation. HW contributed to writing informed consent. YC contributed to manuscript revision and patient communication. All authors have read and approved the final version of the manuscript.

Availability of data and materials
All data generated or analysed during this study were available via contacting the corresponding author.

Declarations
Ethics approval and consent of research subjects
The study was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China). The ethics approval number was KYLL-2018 (KQ-135). All participants were aware of this study and signed the informed consents. All participants agreed to take part and provided a biological sample. And all participants agreed to use the research data obtained from them for publication.

Competing interests
The authors declare that they have no competing interest.

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