CHRNA5/CHRNA3 gene cluster is a risk factor for lumbar disc herniation: a case-control study

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Abstract

Background: Lumbar disc herniation, a type of chronic low back pain syndrome, is caused by the lumbar intervertebral disk degeneration. Genetic variation in the CHRNA5/CHRNA3 has shown strong associations with smoking-related diseases. This study’s aim is to test whether single-nucleotide polymorphisms in the CHRNA5/CHRNA3 gene are associated with lumbar disc herniation risk.

Methods: The genotype frequency distributions of the polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism in 380 lumbar disc herniation patients (case group) and 400 healthy individuals (control group). Allelic, genotypic, and haplotype analyses were performed.

Results: We found that the individuals with rs8040868 CT genotype had a 0.46-fold higher risk of lumbar disc herniation than those with rs8040868 TT genotype, in men group (OR = 0.46, 95% CI 0.25–0.84, p = 0.012). Also among women, rs8040868 CT + CC genotype still reduced the risk of lumbar disc herniation under the dominant model (OR = 0.50, 95% CI 0.28–0.89, p = 0.019). Haplotype analysis showed that compared with the CHRNA5 “TACAACCG” wild-type, the “TACACCCG” haplotype was found to be associated with a decreased risk of lumbar disc herniation (LDH) (OR = 0.79, 95% CI 0.63–1.00, p = 0.047), while, in the less than 50-year-old group, CHRNA5 “TACACCCG” increased the risk of LDH (OR = 1.46, 95% CI 1.01–2.13, p = 0.047).

Conclusions: Our data suggest that gene variance in the CHRNA5/CHRNA3 is associated with risk of lumbar disc herniation in the case-control study.

Keywords: Lumbar disc herniation, CHRNA5/CHRNA3, Susceptibility, Single-nucleotide polymorphism

Introduction

Lumbar disc herniation (LDH) is one of the more common spinal diseases caused by the degeneration and the displacement of nucleus pulposus or annulus fibrosis beyond the intervertebral disc space [1]. LDH is characterized with low back and leg pain resulting from the degenerated lumbar disc compressing the spinal nerve root [2]. Many studies have demonstrated that 70–85% of all people will suffer from low back pain at some time in life [3]. As LDH is considered a significant health care problem involving multifactorial interactions, numerous studies have been performed to identify the risk factors. Previous etiologic studies have focused on environmental risk factors, such as sex, age, height, smoking habits, and occupational factors [4–6].

As we all know, smoking is associated with increased morbidity, mortality, and personal and public cost. An early study demonstrated increased vertebral bone porosity and reduced trabecular bone thickness in mice chronically exposed to tobacco smoke [7]. Likewise, according to Nasto LA et al. [8], short-term exposure to high levels of primary tobacco smoke inhalation promotes...
degeneration of vertebral bone and discs, and then clearly established a direct cause and effect relationship between smoking and spine degeneration in mice. Moreover, the findings also suggested that smoking adversely affects spine health only in part through DNA damage. However, not all smokers develop LDH, a fact that might indicate that genetic variability also may play a significant role in the pathogenesis of LDH [9–11]. Genetic predisposition has been widely acknowledged in LDH. Several genes such as COL1A1 [12], MMPs [13], COL9A2 [14], and ADAMTS-5 [15] have been reported to be associated with LDH. Cigarette smoking is a genetically influenced addictive behavior [16, 17], and nicotine is the main component of cigarette smoke responsible for smoking dependence [18]. Several studies have indicated that the cluster of cholinergic receptor nicotinic α genes on chromosome 15q25, encoding the alpha5 and alpha3 subunits of the nicotinic acetylcholine receptors (CHRNA5 and CHRNA3), are significantly related to smoking behavior [19, 20]. However, there is no investigation that found any association between genetic variations in the nicotinic acetylcholine receptor (nAChR) gene cluster CHRNA5/CHRNA3 and LDH. This is, to our knowledge, the first case-control study that investigated the association between CHRNA5/CHRNA3 gene polymorphisms and LDH in a Chinese population with LDH.

**Material and methods**

**Subjects**

For this case-control association analysis of LDH, a total of 380 LDH patients and 400 unrelated healthy controls were recruited from Xi’an Honghui hospital. All participants lived in Xi’an area, and all participants were Han people. So, there are no regional and ethnic differences. The diagnosis of LDH required the following criteria: (1) patients who had a history of lumbar sprain and/or a history of chronic strain, (2) patients who had pain in the inferior lumbar part of the spine and regional sciatic nerve pain in the leg caused by bed rest, (3) patients with tenderness beside the lumbar spine that affects the leg or foot, (4) patients whose lumbar flexion range was obviously limited, (5) patients with positive results in the straight-leg raising test and augmentation test (Bragard’s sign), and (6) patients who had the following nerve injury symptoms: muscular atrophy, motor weakness, decreased sensation, and hyporeflexia. Meeting any one of (1)–(6) and then combined with patients with clinical manifestations of LDH in accordance with imaging findings, including computed radiography, computed tomography, and/or magnetic resonance imaging, is considered positive for LDH. Primary exclusion criteria included spinal and joint diseases such as trauma, spinal tumor, synovial cyst, inflammatory disease, scoliosis, osteoarthritis, spondylosis, and spondylolisthesis. Moreover, patients who have history of labor work or heavy smoking were also excluded. According to the Labor Protection Measures Standard for Heavy Labor Work (No. 1030079791) and the National Standard “Classification of Physical Labor Intensity” (No. BG386983), we exclude labor worker by inquiring about the nature of their work and the intensity of their physical consumption. By referring to the literature [21], according to the smoking index is equal to the number of cigarettes per day multiplied by the number of years of smoking (365 day/year), the smoking index ≥ 60 pack years is defined as a heavy smoker, using this criterion to distinguish whether the patient is a severe smoker. Four hundred unrelated healthy controls were recruited from Xi’an Honghui hospital. Inclusion criteria of the control group were (1) good health as confirmed by physical examination, (2) no recent infections, (3) no history of tumors, and (4) history of lumbar sprain and/or chronic strain.

Ahsan et al. adopted a retrospective case-control study found that physical exertion, work stress, and daily work time of more than 8 h were highly correlated with the occurrence of LDH [22]. An HS and other studies found that smokers had a 50% increased risk of disc herniation compared with non-smokers [23]. It shows that heavy labor or smoking has a greater impact on lumbar disc herniation. So, we exclude labor worker or heavy smoker.

**Genotyping of SNPs**

Fifteen tag single-nucleotide polymorphisms (SNPs) of CHRNA5 and CHRNA3 were selected from the NCBI SNP database (www.ncbi.nlm.nih.gov/SNP) and the HapMap database (www.hapmap.org). All the SNPs selected had a minor allele frequency (MAF) of more than 0.05. And primers for 15 tag SNP typing were designed by Agena on-line design software (https://agenacx.com/online-tools/).

Genomic DNA was extracted from peripheral blood leukocytes of affected individuals and controls using standard protocols. The DNA quantity was evaluated by spectrometry (DU530UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). The SNP genotyping was performed on the Agena MassARRAY SNP genotyping platform (Agena Bioscience, San Diego, CA, USA) according to the manufacturer’s protocol.

**Statistical analyses**

Statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Hardy–Weinberg equilibrium was assessed by using a chi-square test. Measurement data were expressed as the mean ± standard deviation (SD). The difference between the two groups was compared using a t test. In a multivariate logistic regression model, we assessed the independent
association between CHRNA3/CHRNA5 gene polymorphism, smoking, drinking, and risk of LDH. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the relative risk of LDH. Haplotype blocks and the linkage disequilibrium (LD) patterns were estimated by using the Haploview program (version 4.2, Broad Institute of MIT, and Harvard, Cambridge, MA, USA). Linkage disequilibrium coefficients ($D'$ and $r^2$) were calculated as described previously [24]. All tests were two-sided, and $p < 0.05$ indicated a significant difference.

**Results**

**Study population**

The general characteristics of both groups are summarized in Table 1. A total of 780 Northwest Chinese subjects with Han ethnicity were recruited in this case-control study, comprising 380 patients with LDH (152 females and 228 males; mean age 50.43 ± 12.27 years) and 400 healthy controls (166 females and 134 males; mean age 50.79 ± 8.13 years). No significant differences in gender and age were found between the two groups. Of the 380 patients, 43 were smokers and 337 were non-smokers; 12 were drinking and 368 were non-drinking.

**Hardy–Weinberg equilibrium and SNP alleles**

The basic information about all the SNPs including gene, band, position, alleles, and Hardy–Weinberg equilibrium (HWE) results are presented in Table 2. The results of the HWE showed that the genotype frequency distributions of CHRNA5/CHRNA3 in the case and control groups were in line with genetic balance (all $p > 0.05$), which showed that all of the 15 tag SNPs were at equilibrium and were representative. In the allele model, we found that there was no site affected by the genetic risk of lumbar disc herniation. Genetic models (genotype, dominant, recessive, and additive) and the genotype frequencies were used to

**Table 1** The general characteristics of study subjects

| Independent variables | LDH patients (n = 380) | Healthy controls (n = 400) | p value |
|-----------------------|------------------------|---------------------------|---------|
| **Age**               |                        |                           |         |
| $\leq$ 50 [N (%)]     | 177 (46.6%)            | 203 (51.0%)               |         |
| > 50 [N (%)]          | 203 (53.4%)            | 197 (49.0%)               |         |
| **Gender**            |                        |                           | 0.610   |
| Female [N (%)]        | 152 (40%)              | 166 (41.0%)               |         |
| Male [N (%)]          | 228 (60%)              | 234 (59.0%)               |         |
| **Mean age (mean ± SD, years)** | 50.43 ± 12.27         | 50.79 ± 8.13              | 0.629   |
| **Smoking**           |                        |                           |         |
| Yes                   | 43 (11.3%)             | 186 (46.5%)               |         |
| No                    | 337 (88.7%)            | 214 (53.5%)               |         |
| **Drinking**          |                        |                           |         |
| Yes                   | 12 (3.2%)              | 168 (42.0%)               |         |
| No                    | 368 (96.8%)            | 232 (58.0%)               |         |

LDH lumbar disc herniation, $p < 0.05$ indicates statistical significance

**Table 2** Allele frequencies in cases and controls and OR estimates for LDH

| SNP ID | Gene | Alleles A/B | Case | Control | MAF | HWE, $p$ | Allele model | OR (95% CI) | p $^\dagger$ |
|--------|------|-------------|------|---------|-----|---------|--------------|-------------|-------------|
| rs667282 | CHRNA5 | C/T | 90 | 91 | 0.484 | 0.764 | 1.05 (0.86–1.28) | 0.645 |
| rs16969948 | CHRNA5 | G/A | 1 | 2 | 0.054 | 0.377 | 0.94 (0.61–1.45) | 0.770 |
| rs588765 | CHRNA5 | T/C | 15 | 17 | 0.214 | 0.523 | 1.14 (0.89–1.45) | 0.310 |
| rs6495306 | CHRNA5 | G/A | 15 | 17 | 0.214 | 0.523 | 1.13 (0.88–1.45) | 0.328 |
| rs17486278 | CHRNA5 | C/A | 27 | 29 | 0.247 | 0.803 | 0.86 (0.68–1.08) | 0.185 |
| rs680244 | CHRNA5 | T/C | 27 | 26 | 0.270 | 0.894 | 1.10 (0.87–1.38) | 0.422 |
| rs569207 | CHRNA5 | T/C | 94 | 91 | 0.492 | 0.764 | 1.08 (0.89–1.32) | 0.439 |
| rs692780 | CHRNA5 | C/G | 18 | 19 | 0.220 | 0.438 | 1.11 (0.87–1.42) | 0.404 |
| rs374077 | CHRNA3 | T/C | 21 | 21 | 0.239 | 0.772 | 1.10 (0.87–1.39) | 0.426 |
| rs1317286 | CHRNA3 | G/A | 5 | 6 | 0.093 | 0.150 | 0.98 (0.70–1.38) | 0.915 |
| rs938682 | CHRNA3 | G/A | 87 | 78 | 0.464 | 1.000 | 1.10 (0.90–1.34) | 0.354 |
| rs12914385 | CHRNA3 | T/C | 25 | 26 | 0.245 | 0.448 | 0.87 (0.69–1.09) | 0.232 |
| rs2869546 | CHRNA3 | C/T | 19 | 20 | 0.233 | 0.884 | 1.07 (0.85–1.36) | 0.557 |
| rs374075 | CHRNA3 | T/C | 71 | 72 | 0.438 | 0.107 | 0.91 (0.75–1.12) | 0.369 |
| rs8040868 | CHRNA3 | C/T | 36 | 40 | 0.302 | 0.369 | 0.87 (0.70–1.08) | 0.198 |

LDH lumbar disc herniation, SNP single-nucleotide polymorphism, MAF minor allele frequency, HWE Hardy–Weinberg equilibrium, OR odds ratio, 95% CI 95% confidence interval

$^\dagger$ p was calculated by exact test

$^\ddagger$ p was calculated by Pearson chi-squared test
further identify the associations between the SNPs and the risk of LDH. The results without stratification showed no association between SNPs and the risk of LDH (data were not shown).

**Association between CHRNA5/CHRNA3 and the risk of LDH**

In a multivariate logistic regression model, we assessed the independent association between CHRNA3/CHRNA5 gene polymorphism, smoking, drinking, and risk of LDH. We found that the individuals with rs8040868 CT genotype had a 0.46-fold higher risk of lumbar disc herniation than those with rs8040868 TT genotype, in the men group (OR = 0.46, 95% CI 0.25–0.84, p = 0.012) (Table 3). Also among women, rs8040868 CT + CC genotype still reduced the risk of lumbar disc herniation under the dominant model (OR = 0.50, 95% CI 0.28–0.89, p = 0.019) (Table 3).

**Association of CHRNA5 haplotypes with the risk of steroid-induced ONFH**

Haplotypes were constructed on the basis of the genotype data from 15 SNPs using Haploview software (version 4.2). Linkage disequilibrium $D'$ value between SNPs and the reconstructed LD plots of the 15 SNPs are shown in Fig. 1. Two LD blocks were observed according to the confidence interval method [25] ($D' > 0.9$ and $r^2 > 0.8$). The block...
contained rs667282, rs16969948, rs588765, rs6495306, rs17486278, rs680244, rs569207, and rs6927800; another includes rs3743077, rs1317286, and rs938682. Another block includes rs3743077, rs1317286, and rs938682. Compared with the CHRNA5 “TACAACCG” wild-type, the “TACACCCG” haplotype was found to be associated with a decreased risk of LDH (OR = 0.79, 95% CI 0.63–1.00, p = 0.047; Table 4), while, in the less than 50-year-old group, CHRNA5 “TACACCCG” increased the risk of LDH (OR = 1.46, 95% CI 1.01–2.13, p = 0.047; Table 4).

**Discussion**

In the current study, we examined the genetic associations and interactions between variations in the CHRNA5/CHRNA3 gene cluster and LDH in northern Chinese. Association analyses revealed that CHRNA3 rs8040868 TC-CC decreased the risk of LDH in the

| Table 4 Association between haplotype of CHRNA5 regions and LDH in individuals |
|---------------------------------|-----------------|-----------------|-----------------|
| Analysis | Gene | SNP | Haplotype | OR (95% CI) | p value † |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TACAACCG | 1 |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | CACAACTG | 1.02 (0.83–1.24) | 0.857 |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TACACCG | 0.79 (0.63–1.00) | 0.047 |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TGCAATCG | 1.02 (0.60–1.72) | 0.943 |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TATGATCC | 0.90 (0.70–1.15) | 0.387 |
| Overall | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TGCAATCC | 0.94 (0.39–2.29) | 0.892 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TACAACCG | 1 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | CACAACTG | 1.15 (0.84–1.59) | 0.390 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TGCAATCG | 0.56 (0.22–1.44) | 0.231 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TACACCG | 1.46 (1.01–2.13) | 0.047 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TATGATCC | 0.84 (0.56–1.25) | 0.383 |
| ≤ 50 | CHRNA5 | rs667282|rs16969948|rs588765|rs6495306|rs17486278|rs680244|rs569207|rs6927800 | TGCAATCC | 0.9 (0.27–3.02) | 0.860 |

LDH lumbar disc herniation

† p values were calculated by unconditional logistic regression adjusted for age and gender
female group and male group. Haplotype analysis revealed that “TACACCCG” haplotype was associated with a decreased risk of LDH, while in the less than 50-year-olds, CHRNA5 “TACACCCG” increased the risk of LDH. These results suggested that CHRNA5/CHRNA3 gene polymorphisms might be used as genetic determinants for LDH susceptibility.

CHRNA3 and CHRNA5, coding for α3 and α5 subunits of the neuronal nicotinic acetylcholine receptor, have been reported to partially overlap in a tail-to-tail configuration, sharing their 3′ ends in human [26] and bovine genomes [27]. Many studies have demonstrated that CHRNA3 and CHRNA5 are functionally linked not only because they code for different subunits of nAChR but also because their protein products can oligomerize to form functional channels, thus suggesting multiple possibilities of reciprocal regulation [28]. Both the CHRNA3 and CHRNA5 genes are expressed in human brain regions relevant to nicotine addiction, such as the nucleus accumbens, amygdala, and entorhinal cortex [29]. While many CHRNA5/CHRNA3 gene polymorphisms and linkage studies have been published for numerous smoking-related diseases [30–32], no studies have been performed to indicate relationships between CHRNA5/CHRNA3 and LDH. However, it has been reported that smoking is a causal environmental risk factor for LDH [33], and α5/α3 nicotinic receptor subunit alleles increase risk for heavy smoking [29, 34]. It may be hypothesized that genetic changes in CHRNA5/CHRNA3 could affect the risk of LDH in smokers. We found that the individuals with rs8040868 CT genotype had a 0.46-fold higher risk of lumbar disc herniation than those with rs8040868 TT genotype, in the men group (OR = 0.46). Also among women, rs8040868 CT + CC genotype still reduced the risk of lumbar disc herniation under the dominant model (OR = 0.50). Haplotype analysis revealed that CHRNA5 “TACACCCG” haplotype was associated with a decreased risk of LDH in overall analysis, while in the less than 50-year-olds, CHRNA5 “TACACCCG” increased the risk of LDH. The stratified analysis uses a small sample size, which may cause false positive results. Then whether the haplotype reduced or increased the risk, we still need a large sample to verify, while eliminating as much as possible the impact of other confounding factors on the disease.

These results suggested that CHRNA5/CHRNA3 gene polymorphisms might be used as genetic determinants for LDH susceptibility. The studies have reported associations between rs8040868 and lung cancer risk [35] and schizophrenia risk [36]. As predicted by HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), rs8040868 can bind to these proteins NRSF, P300, USF1, and YY1 and also lead to Motifs changed. So we suspect that mutations in the rs8040868 (synonymous, Val53=) site would affect the targeted binding of the CHRNA3 gene to these proteins, which in turn affected the occurrence of LDH. The GTeX database has shown that rs8040868 mutation influences the expression of the CHRNA3 in blood samples (https://gtexportal.org/home/). As briefly mentioned above, we cannot draw a firm conclusion on the biological effects of the SNPs on CHRNA5/CHRNA3 cluster and future precise functional studies are worth considering.

Conclusion

In conclusion, this study reported the potential association of genetic polymorphisms of CHRNA5/CHRNA3 gene with LDH for the first time. Our results revealed a significant association of rs8040868 in CHRNA3 with LDH, and CHRNA5 haplotypes “TACACCCG” and “TACACCCG” are greatly related to the risk of LDH. Therefore, these findings may contribute to a better understanding of the pathogenic mechanisms of LDH and provide possible targets for treatment. Future studies should focus on the functional analysis and make the conclusion solid by replication in a similar study.

Abbreviations

CIs: Confidence intervals; HWE: Hardy–Weinberg equilibrium; LD: Linkage disequilibrium; LDH: Lumbar disc herniation; MAF: Minor allele frequency; ORs: Odds ratios; SNPs: Single-nucleotide polymorphisms

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Authors’ contributions

XJY, XDG, and ZH conceived and designed the experiments; YFD, WHX, and FL performed the experiments; MLL and KS analyzed the data; HYJ wrote the paper; YZ supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available.

Ethics approval and consent to participate

This study was approved by the ethics committee of Xi’an Honghui hospital, and all participants signed informed consent forms before participating in the research. The ethical approval for this study conformed to the ethical principles for medical research involving humans of the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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