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ORIGINAL ARTICLE

Associations of *Caspase-3* gene polymorphism with lumbar disc herniation

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Abstract To investigate *Caspase-3* gene polymorphisms (rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C) and susceptibility to lumbar intervertebral disc herniation (LDH). The genotype frequency distributions of the polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism in 107 LDH patients (case group) and 121 healthy individuals (control group). SHEsis software was used to conduct gene linkage disequilibrium and haplotype analysis. Regression analysis was used to analyze possible risk factors for LDH. Statistically significant differences in family history of LDH, amateur sports, leisure activities, bed types, and spine load grade were found between the case and control groups. The distribution of allele and genotype frequencies of rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C polymorphisms of *Caspase-3* were significantly different between the case and control groups. Haplotype analysis showed that the G-G-C (rs4647693-rs4647610-rs12108497) haplotype might be a risk factor for LDH, whereas the A-A-T haplotype might be a protective factor ($p < 0.05$). Binary logistic regression analysis showed that the GA+AA genotype of rs4647693 was negatively associated with the risk of LDH, whereas high spine load grade was positively associated with the risk of LDH. These findings revealed that rs4647693 G/A, rs4647610 A/G, and rs12108497 T/C polymorphisms of *Caspase-3* may be associated with susceptibility to LDH and that interaction and modification effects may exist between *Caspase-3* polymorphisms.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Lumbar disc herniation (LDH), a type of chronic low back pain syndrome, is caused by the degeneration and herniation of the nucleus pulposus of the intervertebral disc [1]. LDH remains a considerable clinical problem that can affect up to 80% of individuals at any time in their lives, resulting in important social and economic consequences [2]. Specifically, LDH has an estimated worldwide incidence rate of 7.62% per year, with people aged 25—55 years at a higher risk of developing LDH [3]. Generally, LDH is considered a complex disease involving multifactorial interactions, but the precise etiology and pathogenesis underlying LDH is complex and still poorly understood. In the past few years, various factors such as sex, age, height, smoking habits, and physical activity have been reported in the literature as being associated with LDH [4—6]. In addition, recent molecular epidemiological studies have highlighted the potential and significant role of genetic variability in LDH [7—9]. To date, with the completion of high-quality sequencing of the human genome and a deeper understanding of disease mechanisms at the cellular and molecular level, the genes responsible for susceptibility to many complex diseases, including LDH, have been identified [4,10].

Caspase-3, also known as CPP32/Yama/apopain, is one of the most extensively studied apoptotic proteins in the cysteine—aspartic acid protease (caspase) family and is encoded by the *Caspase-3* gene [11]. Caspase-3 acts as the main executor of apoptosis and mediates both extrinsic and intrinsic cell death signaling pathways [12]. Apoptosis is considered an essential biological event, which is modulated by the activation of multiple caspases, including caspase-3, and plays a crucial role in cellular and tissue homeostasis. Thus, the inappropriate regulation of apoptosis might be one of the most important factors in influencing the development and progression of many diseases [13,14]. Therefore, it seemed biologically plausible that genetic alterations of *Caspase-3* might be involved in the failure of apoptosis, which might lead to human diseases such as LDH [15—17]. More importantly, there is evidence suggesting that the failure of apoptosis could reduce disc cell numbers and destroy normal disk function during disc aging and degeneration, thus resulting in diminished generation and organization, or ultimately leading to lumbar disc disease [18,19]. Thus, the *Caspase-3* gene, as an important apoptosis-related gene located at 4q35.1, is naturally a good candidate gene for LDH.

To test this hypothesis, the present study was designed to search for an association between *Caspase-3* single-nucleotide polymorphisms (SNPs) and haplotypes in LDH patients and to identify the mechanism of action of Caspase-3 in LDH.

Methods

Ethical statement

This study was approved by the ethics committee of Changsha Central Hospital, Changsha, China, 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.

Study participants

From January 2011 to January 2015, 107 LDH patients in the spinal surgery department of Changsha Central Hospital and First Affiliated Hospital of Guangxi Medical University were enrolled in our experiment as the case group. All patients were from the Chinese Han population and included 68 men and 39 women, aged 19—58 years with a mean age of 38.75 $\pm$ 12.31 years. The diagnostic criteria were: (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); (6) patients who had the following nerve injury symptoms: muscular atrophy, motor weakness, decreased sensation and hyporeflexia; and (7) patients with clinical manifestations of LDH in accordance with imaging findings, including computed radiography, computed tomography, and/or magnetic resonance imaging. Exclusion criteria were: (1) patients with mental illness or severe dysfunction of the heart, lung, liver, or kidney; (2) patients with blood disease, diabetes, autoimmune disease, or tumors; and (3) patients identified as underweight/malnourished with a body mass index (BMI) < 18.5 kg/m^2 or overweight/obese with a BMI ≥ 28 kg/m^2. A total of 121 healthy individuals who received a physical examination in our hospital during the same period were allocated to the control group, including 71 men and 50 women, with an average age of 38.25 $\pm$ 11.73 years. Inclusion criteria of the control group were: (1) people of the Chinese Han ethnicity whose age and sex were matched to the patients in the case group; (2) good health as confirmed by physical examination; (3) no recent infections; (4) no history of tumors; and (5) history of lumbar sprain and/or chronic strain.

General information collected

(1) Spine load grade was based on the Swedish translated version of the Short Musculoskeletal Function Assessment Questionnaire (SMFA) [20], which is referred to as the core and occupational classification system [21]. The classifications are as follows: Grade I: freelance and less manual; Grade II: sedentary jobs; Grade III: mainly whole body vibration, bending over and twisting work; and Grade IV: lifting and heavy work. (2) Smoking was defined as having one or more cigarettes per day for 1 year or longer, or as smoking $>$ 18 packs of cigarettes per year. (3) Drinking was defined as having two or more drinks (of at least 100 mL) every week for 1 year or longer. (4) Amateur sports were defined as activities performed after-working hours such as household chores. (5) Leisure activities were defined as exercise performed three times a week for at least 20 minutes that led to an increased heart rate and sweating.