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Original Article

Genetic association of the polymorphisms in apoptosis-related genes with osteoarthritis susceptibility in Chinese Han population

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Abstract: Background: Apoptosis is a normal physiological process in organs development, but excessive apoptosis is pathological and harmful. In previous studies, apoptosis-related genes are considered to be involved in the onset of osteoarthritis, but the mechanism is unclear. Therefore, we selected two common polymorphisms of apoptosis-related genes (*BAX* -248G>A, *BCL2* -717C>A) to explore the relationship with osteoarthritis. Methods: The two polymorphisms were genotyped by polymerse chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 134 cases with osteoarthritis and 142 controls. These genotypes distributions in controls were checked whether conformed to Hardy-Weinberg equilibrium (HWE). χ^2 test was used to calculate odds ratio (OR) with corresponding 95% confidence interval (95% CI) which evaluated the strength of association between gene polymorphism and osteoarthritis susceptibility. Results: The χ^2 test showed that genotype frequencies of *BAX* -248G>A (rs4645878), *BCL2* -717C>A (rs2279115) polymorphisms in control group were consistent with HWE. In *BAX* -248G>A polymorphism, compared with mutant genotype GA+AA, the common genotype GG increased the susceptibility to osteoarthritis significantly (OR=1.84, 95% CI=1.13-3.00), so G allele was (OR=1.76, 95% CI=1.16-2.68). The homozygous mutant genotype AA in *BCL2* -717C>A carriers easily suffered from osteoarthritis in some condition, compared with CC genotype (*P*=0.03). A allele also increased 0.43 times risk of osteoarthritis development than C allele (OR=1.43, 95% CI=1.02-2.00). Conclusions: *BAX* -248G>A and *BCL2* -717C>A polymorphisms may be the independent risk factors for the development of osteoarthritis.

Keywords: *BAX*, *BCL2*, apoptosis, polymorphism, osteoarthritis

Introduction

Osteoarthritis is one of the common joint diseases which are a leading cause of disability in mid-aged population, so it lays heavy burdens on the society [1-3]. Its symptoms are joint pain and stiffness, hindering the daily life of patients [4]. So far, there is no effective way to cure osteoarthritis and all treatments only focus on relieving the pain and recovering the part function of the damaged joints [5, 6]. Recently, the pathologic basis and the first manifestation of osteoarthritis are considered as the damage of articular cartilage [7]. Its pathological process is complicated and multiple factors involve, including biological, genetic and environmental factors [8-10]. In addition, several publications report that cartilage apoptosis, metabolism imbalance of extracellular matrix and autoimmune response may participate in the onset of osteoarthritis.

Apoptosis is a normal program under the control of genes to eliminate the needless and damaged cells, but excessive apoptosis leads to the generation and development of various diseases. In previous studies, the excessive apoptosis of cartilage has been proved to play an important role in the generation of osteoarthritis [11-13]. Therefore, apoptosis-related genes are also supposed to be associated with osteoarthritis. Among of them, *BCL2* gene family is a major factor in regulating apoptosis, and *BAX*, *BCL2* are two members of this family [14].

BAX gene can encode apoptosis regulator BAX protein, also known as BCL2-like protein 4, which forms a heterodimer with BCL2 encoded by

BCL2 gene as an apoptosis activator [15, 16]. The functional genetic variant in promoter region of *BAX* and *BCL2* has been showed to alter the function and/or expression of the protein, which has an influence on regulating apoptosis. A common polymorphism *BAX* -248G>A involved in 5'-untranslated region of *BAX* is a substitution from G to A allele at -248 nucleotide position [17], and another polymorphism *BCL2* -717C>A is a mutation from C allele to A at -717 nucleotide position in promoter region [18]. They are found in development of many diseases, but no report refers to the effect of these two polymorphisms on the generation of osteoarthritis.

In present study, we explored the relationship between *BAX* -248G>A and *BCL2* -717C>A polymorphisms and osteoarthritis in 134 cases and 142 controls. The objective aimed at verifying the role of apoptosis-related genes polymorphisms on osteoarthritis and proving some evidences for the mechanism of osteoarthritis.

Materials and methods

Selection of study population

A total of 276 subjects were enrolled in this study, including 134 patients with primary osteoarthritis as cases and 142 healthy persons as controls. The cases were from the orthopedics department of First Affiliated Hospital, Chongqing Medical University, diagnosed by X-ray, computed tomography (CT) magnetic resonance imaging (MRI) and routine laboratory tests. Among of them, males accounted for 63.5% and the patients who suffered from tumors were excluded. Their age was 52-78 years with the average age of 64±10.52. The healthy persons who experienced the physical examination of the same hospital with the cases in the same period were as the controls, including 89 males and 53 females with the mean age of 66±9.23 years. There were no significant differences between the case and control groups in gender and age. The design of this article was supported by the Research Ethics Committee of First Affiliated Hospital, Chongqing Medical University, Chongqing and written consents were required from every participant.

Sample collecting and DNA extraction

We collected 2 ml peripheral venous blood from every subject with an empty stomach and put in the anticoagulative tube with EDTA. The blood genome DNA was extracted by the method of the chloroform/isoamyl alcohol extraction and then DNA samples were stored at -20°C.

Genotyping of polymorphisms in apoptosis-related genes

In this article, the polymerse chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to conduct the genotyping. The PCR primers sequences of *BAX* -248G>A polymorphism: 5'-TTAGAGACAAGCCTGGGCGT-3' (forward) and 5'-CAATGAGCATCTCCCGATAA-3' (reverse) [19]. A total of 35 μl solution was used to perform PCR reaction, including 2 μl genome DNA template, each 0.5 μl of forward and reverse primers, 17.5 μl PCR Master Mix and 14.5 μl deionized water. The PCR program was as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 1 min, and finally extension at 72°C, 7 min. The length of PCR products was 280 bp. They were digested by *Tau I* restriction enzyme and then separated by 3% agarose gel electrophoresis (AGE).

The *BCL2* -717C>A polymorphism was genotyped according to the method of Hirata et al. [20]. The PCR products were digested by restriction enzyme *Bcc I* and finally separated by 2% AGE.

Statistical analysis

The genotypes distributions of *BAX* -248G>A and *BCL2* -717C>A polymorphisms was checked by χ^2 test whether were consistent with Hardy-Weinberg equilibrium (HWE). All data were represented with $\bar{x}\pm s$ or %. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated using the χ^2 test to represent the associated intensity between apoptosis-related genes polymorphisms and osteoarthritis risk.

Results

The HWE test

HWE test was conducted to evaluate the genotypes distributions of *BAX* -248G>A and *BCL2* -717C>A polymorphisms in the control group. The results demonstrated that both of two polymorphisms in controls conformed to HWE

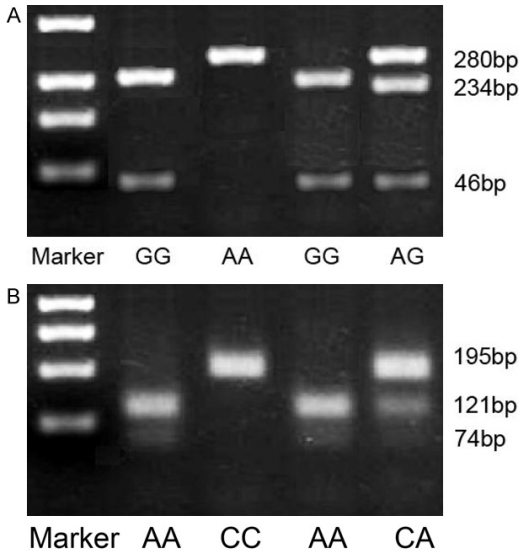

Figure 1. PCR-RFLP results. A. For BAX -248G>A (rs4645878); B. For BCL2 -717C>A (rs2279115) polymorphisms.

(*P*=0.30, 0.15) and our study population possessed of a representativeness to support our conclusions.

Effect of BAX -248G>A polymorphism on osteoarthritis

Genotyping results for BAX -248G>A polymorphism was shown in **Figure 1A**. As was shown in **Table 1**, the frequency of the common genotype GG in *BAX* -248G>A polymorphism was obviously higher than that of the mutant genotype GA+AA (OR=1.84, 95% CI=1.13-3.00, *P*=0.02) and the percentage of GG genotype in cases was also higher than the controls (67.91% & 53.53%). Similarly, G allele also showed a relationship with osteoarthritis development (OR=1.76, 95% CI=1.16-2.68). In a word, *BAX* -248G>A polymorphism was associated with the development and progression of osteoarthritis and might be an independent risk factor.

Association between BCL2 -717C>A polymorphism and osteoarthritis

Genotyping results for BCL2 -717C>A polymorphism was shown in **Figure 1B**. In the analysis of *BCL2* -717C>A polymorphism and osteoarthritis susceptibility, AA genotype was considered to be related to osteoarthritis occurrence remarkably, compared with the common genotype CC (OR=2.28, 95% CI=1.09-4.74) and the results were showed in **Table 2**. In addition, A allele frequency in cases was significantly higher than in controls (48.13% & 39.14%) and it increased 0.43 times risk for osteoarthritis development, compared with the wild allele C (OR=1.43, 95% CI=1.02-2.00). Therefore, *BCL2* -717C>A polymorphism might be involved in the pathogenesis of osteoarthritis.

Discussion

Osteoarthritis is a clinical common disease with high morbidity and progressively causes loss of joint function, which leads to the disability of mid-aged people [21]. Meanwhile, it is difficult for osteoarthritis to cure, therefore the daily life of patients are influenced. Due to the generation of osteoarthritis characterized by long-term, chronic, and advance gradually, the pathogenesis is still unclear. Previous studies have showed that all of biological, environmental and genetic factors participate in the development of osteoarthritis [22]. So far, more and more scholars devote themselves to discovering the mechanism of osteoarthritis.

Gee et al. have analyzed the allelic expression of the osteoarthritis susceptibility locus mapped to chromosome 3p21, *GNL3* and *SPCS1* show obvious allelic expression imbalance in osteoarthritis and cis-acting regulator polymorphisms at *GNL3* and *SPCS1* are associated with osteoarthritis [23]. Liu et al. find that estrogen receptor alpha gene polymorphisms independently participate in the generation of osteoarthritis in Chinese Han women, this conclusion is also certified in other population [24]. Panoutsopoulou et al. report a genetic evidence that body mass index can effect osteoarthritis susceptibility through mediating fat mass and obesity-associated (FTO) gene variation [25]. In addition, the genetic variants of *BMPs*, *IGF*, IL-related, asporin genes are also found to exist in patients with osteoarthritis.

The development of apoptosis includes two signal pathways: death receptor-mediated signal transduction pathways and mitochondrial apoptotic pathway. The later can be activated in intracellular and release apoptosis-related proteins. BCL2 family genes take part in apoptosis program by regulating the mitochondrial signal pathway. In BCL2 family, there are two kinds of apoptosis proteins with reverse function, namely inhibitor of apoptosis protein, such

Table 1. Comparison of genotype and allele frequencies in *BAX* rs4645878 polymorphism between the cases and controls

Genotype/allele	Case, n (%)	Control, n (%)	χ^2 (*P*)	OR	95% CI
BAX -248G>A					
GG	91 (67.91)	76 (53.52)	5.97 (0.02)	1.84	1.13-3.00
GA+AA	43 (32.09)	66 (46.48)	-	1.00	Ref.
G	224 (83.58)	211 (74.30)	7.12 (0.01)	1.76	1.16-2.68
A	44 (16.42)	73 (22.18)	-	1.00	Ref.

Table 2. Association intensity of *BCL2* rs2279115 polymorphism with osteoarthritis risk

Genotype/allele	Cases, n (%)	Control, n (%)	χ^2 (*P*)	OR	95% CI
BCL2 -717C>A					
CC	34 (25.37)	48 (33.80)	-	1.00	Ref.
CA	71 (52.99)	76 (53.52)	0.99 (0.32)	1.32	0.76-2.28
AA	29 (21.64)	18 (12.68)	4.90 (0.03)	2.28	1.09-4.74
CA+CC	100 (74.63)	94 (66.20)	2.35 (0.13)	1.50	0.89-2.53
C	139 (51.87)	172 (60.56)	-	1.00	Ref.
A	129 (48.13)	112 (39.44)	4.24 (0.04)	1.43	1.02-2.00

as BCL2, BCL-w and promotor of apoptosis protein, such as BAX, BAK. In general, the ratio of thess two kinds of proteins determines the occurrence of apoptosis when cells are stimulated by apoptosis signal. BCL2 protein family contains four differently conserved regions, that is BH1-BH4, at least one region exists in every member [26].

In normal, BAX protein exists in cytoplasm, but it can transfer to mitochondrion to regulate cell apoptosis when cells are stimulated by apoptosis signal through the change of conformation and exposing N-terminal to form dimers and then inserting mitochondrial membrane to regulate the release of cytochrome C and activate caspase. In this article, we found that the common genotype GG of *BAX* -248G>A significantly increased the susceptibility to osteoarthritis, compared with the mutant genotype GA+AA and its frequency in cases was higher than controls. The results suggested that carrying genotype GG might lead to the excessive expression of BAX to cause the abnormal apoptosis of articular cartilage which was a leading cause of osteoarthritis development.

BCL2 is a proto-oncogene which can inhibit apoptosis and extend life-span of cells. BCL2 protein can inhibit the release of cytochrome C through regulating the apoptosis protein in mitochondrion and then effect apoptosis. In addition, it also protects the cells by extending the sleep time because cells are easily stimulated by apoptosis signal in proliferation [27]. What's more, evidence has proved that the overexpression of *BCL2* can suppress FAS-mediated apoptosis. In the study of Erlacher et al. about the expression of *BCL2* in patients with osteoarthritis and healthy persons, the outcomes was displayed that up-regulated expression of *BCL2* prevented cartilage cells from apoptosis in body [28]. SOX-mediated pathway may involve in the effect of *BCL2* on the generation of osteoarthritis. Our study on the association between *BCL2* -717C>A polymorphism and osteoarthritis showed that AA genotype or A allele carriers easily suffered from osteoarthritis. The conclusion implied that the mutation genotype and allele of this loci increased the risk of osteoarthritis development by down-regulating the expression of *BCL2* to promote apoptosis.

So far, the publications about the roles of apoptosis-related genes in osteoarthritis are few, especially in genetic variant. Although we obtained some outcomes, these conclusions still need to be verified owing to small sample size and ignoring the interactions of gene-gene, gene-environment.

Disclosure of conflict of interest

None.

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