RESEARCH ARTICLE

Matrix Metalloproteases 8 Polymorphism as Risk Factor for Rotator Cuff Tear

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Abstract

Objectives: Rotator Cuff Tear (RCT) is a multifactorial disease, but an important one is the increased collagen degradation that would lead to a higher chance of tear. MMP-8 is a protein that degrades type I collagen, and it is known that MMP-8 has a polymorphism in which a T allele in the gene promoter region increases its transcription activity. This study aims to investigate the association between MMP-8 polymorphism g.-799 C>T (rs11225394) and RCT.

Methods: To do that, we collected DNA samples from buccal epithelial cells of 128 patients (separated into RCT group and control group in a proportion 1:1) and genotyped the DNA using PCR. The statistical analyses were done using the ARLEQUIN Version 2.0, and the data normality was tested with the Shapiro-Wilk test.

Results: The results showed a significantly higher frequency of T/T genotype in the test group (29% in the control group and 39% in the test group, p=0.0417), and that would represent a risk factor for increased collagen degradation.

Conclusion: The MMP-8 g.-799 C>T (rs11225394) SNP was associated with RCT. With the description of a new risk factor, future research can be done to analyze how to prevent RCT or develop new treatment strategies since the disease's failure index is currently high.

Level of evidence: II

Keywords: Genetic polymorphism, Metalloproteases, Risk factor, Rotator cuff tear

Introduction

Rotator Cuff Tear (RCT) and tendinopathy are the most common causes of chronic pain in the shoulder. Its incidence increases with aging, affect between 30 to 50% of individuals older than 50 years and more than 50% those over 80 years.[1]

The treatment of this tendinopathy has a major failure index due to scar tissue that can be formed and the changes that occur in injured tendon tissue at the histologic level, such as loss of cellularity, disorganization and thinning fibers, and incidence of granulation tissue.[2] These disorganized collagen fibers have significant importance. Because of that, the proteins involved in their production and turnover, such as the matrix metalloproteases (MMPs), have become a study target.

The MMPs are a family of zinc-dependent enzymes that degrades a lot of different substrates in the extracellular matrix. In the pathogenesis of an RCT, there are decreased syntheses and increased degradation of collagen fibers associated with an alteration of MMPs.[3-5]

In the last decade, the target of many tendinopathy etiology types of research has been identifying gene variants and highlighting the importance of single nucleotide polymorphism (SNPs) in etiopathogenesis and RCT.[6] SNPs are genetic variations in which the most frequent allele has a frequency lower than 99% and may influence a disease development mechanism in humans.[7] The genes that are often involved in tendinopathy code for structural proteins (mainly collagens), repair mechanisms (including MMPs), and apoptotic pathways.[4]

A significant relationship between SNPs and rotator cuff

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disease was report for different genes,[8-12] including MMPs (MMP-1, MMP-2, MMP-3).[13-15]

The MMP-8 is a collagenase, and fibroblasts in the connective tissue synthesize it. This MMP degrades type I collagen, the primary constitute of the rotator cuff. So, SNP in its gene may play a role in rotator cuff tearing and/or remodeling. However, no studies evaluate the association of MMP-8 polymorphism with RCT.

The MMP-8 g.-799 C>T (rs11225394) SNP is in the promoter region of its gene. The electrophoretic mobility shift assays shown modifications in nuclear protein binding to oligonucleotides representing the -799C/T genotypes, suggesting that a specific allele may increase the transcription activity of this gene.[16] A larger quantity of the protein could be related to a bigger chance of developing RCT.

Many studies discuss how to treat this injury, but only some elucidate the molecular and genetic aspects that can be crucial to understanding physiopathology deeply. The genetic influence comprehension of this disease could help to identify susceptible people to evolve with this condition and help to prevent and intervene early and in the best way.

Therefore, the purpose of this study was to investigate the MMP-8 g.-799 C>T (rs11225394) SNP with risk factor to RCT.

Materials and Methods

Study population

This is a case-control study with a ratio of one to one, which followed the guidelines of the Declaration of Helsinki and was approved by Ethical Committee in Research (n° 611.645).

Participants were recruited from the patient pool at the Department of Orthopedics and Traumatology of the University. After informed consent, the following data were collected from all participants: age, sex, ethnicity, medical data (presence of high blood pressure, smoking, hypothyroidism, tendinopathies in other joints and familiar history for RCT), and diary habit, which sports with shoulder involvement or work with repeated and sustained arm abduction.

Participants were divided into two groups, matched by age (with a maximum difference of ± 2 years) sex, hypertension, smoking, hypothyroidism, sports with shoulder involvement, and work with repeated and sustained arm abduction. Each group with 64 volunteers younger than 64 years treated between 2014 and 2015. Test group underwent repair of full-thickness RCT, and control group consisted of patients treated for a routine health examination or traumatic disorders without symptoms of shoulder pain and clinical signs of rotator cuff disease. Two participants in the control group have posterior tibial tendon dysfunction.

All participants were subjects who underwent rotator cuff imaging (MRI or ultrasonography) by same group of musculoskeletal radiologists (APB, COK, MBR). The control group had intact rotator cuff tendons; 47% were evaluated by ultrasonography and 53% by MRI. The test group was assessed by MRI.

All subjects (test and control group) followed exclusion criteria: diabetes, rheumatologic disease, infectious or previous shoulder surgeries, traumatic RCT, or partial tears.

DNA extraction and genotyping

DNA from buccal epithelial cells was extracted by protocol Aidar and Line, 2007,[17] DNA concentration (ng/µL) was calculated by optical density measurements 260/280 nm (ratio > 1.9).

The minor allele frequencies was greater than 0.13 (http://www.ncbi.nlm.nih.gov/SNP/). The MMP-8 g.-799 C>T (rs11225394) genotypes were determined using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. A total volume of 8 µL of PCR, with 100 ng of DNA, 8 µL Taq Green (Amersham Pharmacia-Biotech, Uppsala, Sweden), and 200 nmol of each primer (Forward: 5'-CAGAGACTCAAGTGGGA-3' and Reverse: 5'-TTTCATTTGTGGAGGGGC-3'), was digested with 1 unit of *Bfm*I enzyme at 37^0C overnight, which cleaves polymorphic site contains allele C (but not T). Electrophoresed on 5% agarose at 20 mA and stained by GelRedTM (Biotium Inc, Fremont, CA, USA) was conducted.

Statistical Analysis

Statistical power was calculated for each group by the program Genetic Power calculator for a minimum power of 80% in which the primary outcome was chosen to be the differences in the frequencies of alleles and genotypes between the control and the test groups, with an alpha error level of 0.05, the effect size of 0.5 and 10% drop-out.

The Shapiro-Wilk test was using to tested the normality of the data. All continuous variables were normally distributed. The continuous variables were presented as means and SD, and the categorical variables as absolute value and percentage.

The comparison between groups was performed by chi-square or Fisher's exact tests, (categorical variables) and Student's t-test (continuous variables).

The chi-square test evaluated the difference in frequency of the alleles and genotypes, with odds ratio (OR) of 95% confidence interval.

The ARLEQUIN Version 2.0 (Laurent Excoffier CMPG, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland) was used to analyze Hardy-Weinberg equilibrium in the studied population. The SPSS® Version 21.0 (IBM Corp, Armonk, NY, USA) with a significance level of 5% was used for the data analysis.

Results

Characteristics of the study population

The test and control groups had a mean age of 54 ± 6 years and 53 ± 6 years, respectively (P = 0.586). Females were predominant in both groups (69% of test group and 61% of control group, P = 0.577). The groups did not differ regarding race (P = 0.692), the presence of high blood pressure (P = 0.831), or smoking (P > 0.999). Tendinopathies in other joints were more prevalent among patients with RCT (P = 0.016) [Table 1].

Patients with RCT reported, in a higher number, the existence of relatives who previously had treatment for RCT (19 of 64 [30%] versus 4 of 64 [6%]; OR, 6.3; 95% CI, 2.0-19.9; P = 0.001).

Table 1. Baseline demographic and clinical characteristics

Characteristic	Test Group	Control Group	p Value
Age (years)	54 ± 6	53 ± 6	0.586
Sex			
Male	20 (31%)	24 (37%)	0.577
Female	44 (69%)	40 (63%)	
Smoking			
Yes	9 (14%)	9 (14%)	> 0.999
No	55 (86%)	55 (86%)	
Hypertension			
Yes	13 (20%)	15 (23%)	0.831
No	51 (80%)	49 (77%)	
Hypothyroidism			
Yes	1 (2%)	2 (3%)	> 0.999
No	63 (98%)	62 (97%)	
Work with repeated and sustained arm abduction			
Yes	26 (41%)	23 (36%)	0.716
No	38 (59%)	41 (64%)	
Sports with shoulder involvement			
Yes	5 (8%)	8 (12%)	0.560
No	59 (92%)	56 (88%)	
Other tendinopathies			
Yes	10 (16%)	2 (3%)	0.016
No	54 (84%)	62 (97%)	

Continuous data = means ± SD; categorical data = number (%)

Relationship between the MMP-8 g.-799 C>T (rs11225394) SNP and RCT risk

All genotype distributions were in Hardy–Weinberg equilibrium. In the present study, MMP-8 g.-799 C>T (rs11225394) SNP was associated with RCT [Table 2]. The C allele was found in 53.1% and 72.7% of the test and control group, respectively (P = 0.0019, OR 95% 2.34 (1.39-3.94)); and the genotype more frequent was C/C (P = 0.0417, OR 95% 2.28 (1.05-4.98)).

Table 2. SNPs frequencies of MMP-8 g.-799 C>T (rs11225394) SNP in the control and test groups

SNPs	Test Group	Control Group	p- value	OR * (95%CI)
Allele	*n = 128*	*n = 128*		
C	68 (53.1%)	93 (72.7%)	p < 0.0019	2.34 (1.39-3.94)
T	60 (46.9%)	35 (27.3%)		
Genotype	*n = 64*	*n = 64*		
C/C	29 (45.3%)	43 (67.2%)	p < 0.0417	2.28 (1.05-4.98)
C/T	10 (15.6%)	7 (10.9%)		
T/T	25 (39.1%)	14 (21.9%)		

Values are expressed number (%)

Discussion

Although MMPs are important for the tendon's degradation and remodeling, there is only limited data suggesting the association of MMP-8 polymorphism with tendinopathy.[18,19] and when it comes to MMP-8 and RCT, any study has been made.

As an example of the lack of information, literature described the association of many genes with RCT. Among the positively correlated ones are MMP-1, 2, and 3, reinforcing the increased susceptibility to develop RCT when having a more considerable quantity of metalloproteinases.13,14 However, the group didn't test the association between RCT and MMP-8. On the other hand, our group also showed that the MMP-1 and -3 SNPs are associated with RCT in the Brazilian population.[15]

In the present case-control cross-sectional study, we identified an association between MMP-8 g.-799 C>T (rs11225394) SNP and RCT. The C/C genotype was observed in most of both groups (67% in the control group and 45% in the test group); however, a significantly higher frequency of the T/T genotype was observed in the test group (29% in the control group and 39% in the test group, p=0.0417). The C allele was found in 73% of the control group, while a much lower frequency of 53% was found in the test group (p=0.0019). We suggest that a superexpression of the protein caused by the T allele may provide the molecular basis for more intense collagen degradation in RCT. Considering the higher frequency of the C allele found in the test group, we hypothesize that it may be a risk factor for RCT.

Known risk factors for RCT studies were described, such as age, diabetes, and overhead activities.[20] A positive point to validate in this study is that the risk factors studied did not differ from the two groups we studied, showing that the criteria for obtaining the sample were satisfactory. This database results from strict criteria to get the sample to reduce the influence of systemic factors that may mask or increase the fundamental role of genetic polymorphisms in RCT.

Some potential limitations might influence our results. First, two types of exams were used to confirm the integrity of the rotator cuff in asymptomatic individuals, MRI or ultrasonography. However, we also can affirm that ultrasonography had similar sensitivity and specificity to MRI in various studies.[21,22] Second, risk factors for RCT, such as BMI and dominant arm,[23] were not evaluated and could act as influencing factors. Third, we have a limited sample size and massive interethnic admixture in a Brazilian population. Bigger and in different population studies should be conducted to confirm this association between SNP and RCT.

And more, since RCT is a multifactorial disease, SNP has a limited impact. So, it is important to consider that this SNP may have its effects disguised by other SNPs. However, it is essential to analyze the relative contribution of each SNP to the disease phenotype to understand genetic influence in RCT. Thus, higher-powered studies are needed to confirm the findings of the present study.

Conclusion

In conclusion, MMP-8 g.-799 C>T (rs11225394) SNP is a genetic risk factor of RCT and a biomarker for early screening and treatment of RCT.

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