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# Association of MMP3 genotype with susceptibility to frozen shoulder: a case-control study in a Chinese Han population

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**ABSTRACT.** Genetic factors may play an important role in frozen shoulder etiology, which may involve matrix metalloproteinase-3 (MMP3) gene polymorphisms. In this study, we examined single nucleotide polymorphisms in MMP3 for their association with frozen shoulder susceptibility in a Chinese Han population. The rs591058, rs650108, and rs679620 polymorphisms in the MMP3 gene were genotyped in 112 subjects diagnosed as having frozen shoulder and in 143 healthy controls. rs650108 was found to be significantly associated with an increased risk of frozen shoulder. For other single nucleotide polymorphisms, no statistically significant associations with frozen shoulder were found. In conclusion, our data demonstrated that the MMP3 rs650108 variant was significantly associated with increased frozen shoulder susceptibility in a Chinese Han population.

**Key words:** Frozen shoulder; Matrix metalloproteinase-3; Single nucleotide polymorphism

 

## INTRODUCTION

Adhesive capsulitis ("frozen shoulder") is a common cause of shoulder disability and pain. Its prevalence is estimated to be 2-5% in the general population and increases to 10.8-29% in patients with diabetes; frozen shoulder predominantly occurs in women aged 40-70 (Balci et al., 1999; Hannafin and Chiaia, 2000; Kordella, 2002). Frozen shoulder is a protracted and insidious condition that begins with pain and progressive restriction of both active and passive motion of the shoulder joint. It is generally divided into two categories, idiopathic and acquired. The former is associated with no known intrinsic shoulder disorders and the latter is related to predisposing conditions affecting the shoulder such as surgery or trauma. Frozen shoulder is considered to be a self-limited disease; however, some patients show little or no improvement after conservative treatment including motion exercises. Thus, operative treatment (such as arthroscopic capsular release) is the treatment of choice (Holloway et al., 2001).

The etiology of adhesive capsulitis remains unclear. Histologic studies revealed a matrix of type I and type III collagen populated by fibroblasts and myofibroblasts in the capsular tissue (Bunker and Anthony, 1995). More recently, several studies revealed that the underlying pathophysiologic process in adhesive capsulitis involves synovial inflammation with subsequent fibrosis of the joint capsule (Ogilvie-Harris and Myerthall, 1997; Hannafin and Chiaia, 2000; Ryu et al., 2006; Ramage et al., 2007; Çinar et al., 2010). Additionally, previous studies demonstrated that the elasticity of the joint capsule decreased in an adhesive capsulitis model and the range of motion became normal after capsular release (Hagiwara et al., 2006; Chimoto et al., 2007). Growth factors such as transforming growth factor-beta (TGF-$\beta$), platelet-derived growth factor, and hepatocyte growth factor are also involved in frozen shoulder (Rodeo et al., 1997; Bunker et al., 2000). Matrix metalloproteinases (MMPs) may play an important role in the pathogenesis of frozen shoulder (Bunker et al., 2000). Genetic factors should may also be involved in frozen shoulder susceptibility and severity.

MMPs are zinc-dependent proteases involved not only in the breakdown of the extracellular matrix (ECM) during normal physiological processes such as cell proliferation, tissue remodeling, reproduction, differentiation, angiogenesis, and apoptosis, but also in pathologies such as arthritis, tumor invasion, cancer, and inflammation (Fu et al., 2011; Radenkovic et al., 2014; Yang et al., 2014). The MMP3 gene codes for the MMP3 protein, a key regulator of the ECM that degrades multiple collagen and other matrix proteins (Somerville et al., 2003). Given that frozen shoulder pathology involves both inflammation and fibrosis, the role of MMP3 in frozen shoulder development and the MMP3 single nucleotide polymorphism (SNP) variants associated with frozen shoulder pathologies should be examined.

We hypothesized that MMP3 SNPs contribute to inflammation and subsequent fibrosis of the capsule in patients with idiopathic adhesive capsulitis. The objective of this study was to determine whether MMP3 SNPs (rs591058, rs650108, and rs679620) are associated with idiopathic frozen shoulder in a Chinese Han population.

## MATERIAL AND METHODS

The study was approved by the Ethics Committee of the Yuhuangding Hospital, and informed consent was obtained from patients and control participants.

## Study population

A total of 112 patients diagnosed with idiopathic frozen shoulder and 143 age-matched healthy controls who had no symptoms or signs of frozen shoulder, no other types of shoulder disability, no joint stiffness, and no family history of frozen shoulder were recruited in this study. All subjects included in this study were Chinese Han.

Inclusion criteria were representative of the typical features of frozen shoulder: an insidious onset of pain and stiffness with a clinical reduction in the range of motion, a >50% reduction in external rotation, and without an underlying radiologic abnormality (Miller et al., 1996). All patients were required to have had symptoms for a minimum of 3 months. The exclusion criteria were used to eliminate patients with an inappropriate diagnosis of idiopathic frozen shoulder and other medical conditions that may complicate the pathologic process.

At baseline, all patients underwent a standardized subjective and objective examination. Range of motion was measured in a standardized manner using a universal goniometer from bone landmarks. Most patients were unable to reach 90° of abduction; therefore, external rotation was measured at the maximum pain-free angle of abduction. This point of abduction was recorded as the baseline and used for subsequent measures to ensure the comparability of the results. Each measurement was recorded 3 times and the mean was determined. Routine anteroposterior and lateral radiographs were performed to exclude bone causes of stiffness, such as osteoarthritis. Primary and secondary outcome measures were evaluated.

## Genotyping

DNA samples were obtained from the peripheral blood of all participants using the Chelex-100 method (Walsh et al., 1991). The SNPs were then genotyped using the Taqman assay (7500 Real-Time PCR System, Applied Biosciences, Foster City, CA, USA) and dual-labeled probes in real-time polymerase chain reaction. The primers and probes were designed and synthesized by Sigma (St. Louis, MO, USA). Genotyping was performed by independent laboratory personnel who were blinded to the study, and 3 authors independently reviewed the genotyping results, data entry, and statistical analyses. In addition, we randomly selected 5% of case and control samples for reproducibility tests at least twice on different days and found 100% concordance.

## Statistical analysis

The SPSS version 16.0 software for Windows (SPSS, Inc., Chicago, IL, USA) and the HaploView software were used for statistical analysis. The demographic and clinical data are reported as means ± SD. Student *t*-test was used to compare groups. The genotype and allelic frequencies were evaluated for Hardy-Weinberg equilibrium and compared using the chi-square test. The association between the MMP-3 SNPs and frozen shoulder was assessed under the following genetic models, which were treated as a dichotomous variable: i) m-allele (minor) *vs* M-allele (major) for allele level comparison; ii) Mm+mm *vs* MM for a dominant model of the m allele; iii) mm *vs* mM+MM for a recessive model of the m-allele; and iv) mm *vs* MM for the extreme genotype. $P < 0.05$ was considered to indicate a statistically significant difference.

The linkage disequilibrium (LD) maps and the associations between haplotypes of selected SNPs and risk of frozen shoulder were estimated using the HaploView software. $P < 0.05$ was considered to indicate a statistically significant difference.

## RESULTS

### Patient characteristics

Demographic data of the population studied and the number of individuals in each group are shown in Table 1. There were no significant differences between groups in terms of age and gender.

**Table 1.** Summary of the basic characteristics of the groups.

| Clinical characteristics | Patients | Controls | P value |
|---|---|---|---|
| No. | 112 | 143 | |
| Age (years) | 53.2 ± 5.4 | 52.2 ± 4.2 | n.s |
| Female/Male | 59/53 | 77/66 | n.s |

### Association between MMP3 polymorphisms and frozen shoulder

As expected, the distribution of the genotypes of SNPs in the MMP3 gene conformed to Hardy-Weinberg equilibrium and the genotyping success rate was 100%. Table 2 shows the genotype and allele distributions of the 3 SNPs for the cases and controls. Only rs650108 was found to be significantly associated with an increased risk of frozen shoulder regardless of which genetic model was used. For other SNPs, no statistically significant associations with frozen shoulder were found. LD within the MMP3 gene was only found between rs591058 and rs650108, indicating that these 2 polymorphisms belong to one haploblock (Figure 1).

**Table 2.** Genotype and allele distributions of the 4 SNPs for the cases and controls.

| Group | Genotype (frequency) rs679620 | | | | | | Allele (%) | |
|---|---|---|---|---|---|---|---|---|
| | GG | AG | AA | AG+AA | GG+AG | AA | G | A |
| Control | 36 | 74 | 33 | 107 | 110 | 33 | 51.0 | 49.0 |
| Case | 30 | 61 | 21 | 82 | 91 | 21 | 54.0 | 46.0 |
| OR (95%CI) | - | - | 0.76 (0.37 to 1.59) | 0.92 (0.52 to 1.62) | - | 0.77 (0.42 to 1.42) | - | 0.89 (0.74 to 1.06) |
| P | - | - | n.s | n.s | - | n.s | - | n.s |
| Group | Genotype (frequency) rs591058 | | | | | | Allele (%) | |
| | TT | TC | CC | TC+CC | TT+TC | CC | T | C |
| Control | 44 | 71 | 28 | 99 | 115 | 28 | 55.6 | 44.4 |
| Case | 33 | 61 | 18 | 79 | 94 | 18 | 56.7 | 43.3 |
| OR (95%CI) | - | - | 0.86 (0.41 to 1.81) | 1.06 (0.62 to 1.83) | - | 0.79 (0.41 to 1.51) | - | 0.96 (0.80 to 1.14) |
| P | - | - | n.s | n.s | - | | - | n.s |
| Group | Genotype (frequency) rs650108 | | | | | | Allele (%) | |
| | GG | AG | AA | GA+AA | GG+AG | AA | G | A |
| Control | 68 | 58 | 17 | 75 | 126 | 17 | 67.8 | 32.2 |
| Case | 35 | 38 | 39 | 77 | 73 | 39 | 48.2 | 51.8 |
| OR (95%CI) | - | - | 4.46 (2.21 to 8.98) | 2.00 (1.19 to 3.35) | - | 3.96 (2.09 to 7.50) | - | 2.26 (1.89 to 2.71) |
| P | - | - | < 0.0001 | 0.0101 | - | < 0.0001 | - | < 0.0001 |

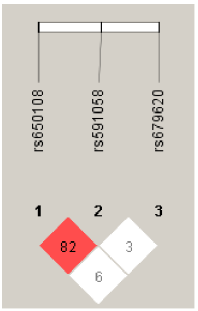


**Figure 1.** Linkage disequilibrium (LD) across the MMP3 gene. The results of LD mapping were generated using the Haploview software. The values for D' between each SNP are presented in each box. Red/pink boxes (D' < 1, LOD#2), white boxes (D' < 1, LOD < 2), blue boxes (D' = 1, LOD < 2), and bright red (D' = 1, LOD#2).

## Association between MMP3 haplotypes and frozen shoulder

Haplotype analysis revealed that 2 haplotypes, ACA (P = 0.02) and ACG (P = 0.013), were associated with an increased risk of frozen shoulder.

## DISCUSSION

The most important finding of this study was that the MMP3 rs650108 variant was significantly associated with increased frozen shoulder susceptibility in our Chinese Han population.

Adhesive capsulitis (frozen shoulder) is a common disease that leads to significant morbidity and impairment of activities of daily living. It consists of four stages that can be identified using arthroscopy, starting with capsular inflammation and ending with fibrosis, which are the most important pathologies in the progression of frozen shoulder (Neviaser and Hannafin, 2010; Neviaser and Neviaser, 2011). The treatment of this condition typically focuses on controlling the inflammation and fibrosis processes. Given the inflammatory nature of frozen shoulder, steroids may be an excellent treatment modality; however, studies have demonstrated that steroids only transiently reduce the pain without always improving the range of motion and do not improve the recovery time frame (Sheridan and Hannafin, 2006; Neviaser and Hannafin, 2010; Neviaser and Neviaser, 2011). Thus, synovial fibrosis may play a more important role in frozen shoulder pathologies with regard to the range of motion and recovery time frame.

Synovial fibrosis is characterized by fibroblast proliferation and an imbalance between collagen synthesis and collagen degradation (Krieg et al., 2007). This imbalance results in excessive deposition of collagen into the ECM, leading to thickening and stiffening of the synovial membrane, which is thought to be a major contributor to both joint pain and joint stiffness (Hill et al., 2007; Solimeno et al., 2010). A key event in the onset of fibrosis is the response of fibroblasts to TGF-β. A previous study demonstrated that intraarticular injection of adenoviral TGF-β into murine knee joints resulted in persistent synovial fibrosis and, more importantly, blocking of TGF-β strongly reduced synovial fibrosis (Scharstuhl et al., 2003; Blaney Davidson et al., 2006; Remst et al., 2013). However, inhibition of TGF-β to prevent fibrosis is not a feasible treatment option, as this protein has an essential role in joint functions. Therefore, identifying genes downstream of TGF-β that are elevated in synovial fibrosis may reveal targets for treating frozen shoulder, including MMPs, which can affect collagen remodeling.

MMPs degrade the connective tissue matrix and can be inhibited by specific tissue metalloproteinase inhibitors (TIMPs), other cytokines, and growth factors. A previous study showed that frozen shoulder can be induced by administration of synthetic TIMPs, suggesting that a decrease in the MMP/TIMP ratio affects collagen turnover (Hutchinson et al., 1998). Additionally, capsular tissues from frozen shoulder patients were found to show upregulated levels of MMP and natural TIMP mRNA (Bunker et al., 2000). Thus, limiting the amount and the function of MMPs may reduce connective tissue matrix degradation and thus limit synovial fibrosis progression.

MMPs are involved in maintaining ECM homeostasis. They are known to consist of over 20 distinct endopeptidases that can catalyze a broad spectrum of both ECM and non-ECM substrates (Somerville et al., 2003). MMP3 can catalytically degrade multiple substrates, including types III, IV, V, IX, and X collagens, laminin, fibronectin, proteoglycan, decorin, and aggrecan, most of which are involved in fibrosis pathologies (Birkedal-Hansen et al., 1993; Somerville et al., 2003). MMP3 is also known to activate, via propeptide removal, several other MMPs (Somerville et al., 2003;

Visse and Nagase, 2003). Expression of the MMP3 gene can be substantially altered by the 5A/6A promoter polymorphism; this variant has been associated with a number of pathological states (Eriksson et al., 1996; Beyzade et al., 2003; Ye et al., 2007). In this study, we demonstrated that the MMP3 rs650108 variant was associated with increased frozen shoulder susceptibility.

The LD analysis of MMP SNPs in this study demonstrated that 2 SNPs, rs650108 and rs591058, belong to the same haploblock. Rather weak LD among the other SNP indicates that in genetic risk assessment, rs679620 and one of the variants belonging to the haploblock must be evaluated separately.

The most important limitation of the present study was the relatively small sample size. We examined 255 subjects, which is a relatively small number for detecting weak genetic associations. Additionally, a single center case-control study is not sufficient for conclusively determining the relationship between MMP1 polymorphisms and the susceptibility to aseptic loosening. Further prospective studies including multiple populations and larger sample sizes are needed to improve the understanding of the potential contribution of this gene to frozen shoulder risk. In conclusion, our data demonstrated the MMP3 rs650108 variant was associated with increased frozen shoulder susceptibility in a Chinese Han population.

## Conflicts of interest

The authors declare no conflict of interest.

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