

Original Article

Gene polymorphism of IL-6 and MMP-3 decreases passive range of motion after rotator cuff repair

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Abstract: Objectives: Post-operative stiffness is common after rotator cuff repair, given the difference in susceptibility and severity, the genetic factors may be involved. Interleukin 6 (IL-6) and Matrix metalloproteinases 3 (MMP-3) were previously found as key cytokines in the pathologies of adhesive capsulitis. The present study aims to investigate whether variants within the IL-6 and MMP-3 gene contributed to post-operative stiffness in a Chinese Han population. Methods: A total of 188 patients diagnosed with rotator cuff tears treated with mini-open surgery were enrolled in this study, among which 87 patients were diagnosed as post-operative stiffness and the remaining 101 patients as controls. All subjects were genotyped for IL-6 and MMP-3 SNPs. Results: The rs1800796 of IL-6 and rs679620 of MMP-3 were found significantly associated with increased susceptibility and severity of post-operative stiffness. Conclusion: The rs1800796 SNP of IL-6 and rs650108 SNP of MMP-3 were associated with increased risk of post-operative stiffness susceptibility and severity. This finding can be used in guiding the rehabilitation procedure after rotator cuff surgery, in another word, those with the genetic susceptibility factors should receive a more radical rehabilitation procedure and those without the susceptibility factors can be more conservative.

Keywords: IL-6, MMP-3, SNP, postoperative stiffness

Introduction

Rotator cuff (RC) injuries in the shoulder account for overuse injuries in sports as well as in jobs that require repetitive activity, which accounts for more than 4.5 millions physician visits per year [1-4]. Excessive mechanical loading is considered the major causation factor. When RC tears are symptomatic and non-operative management fails, they are typically repaired surgically, with over 250 000 RC repair surgeries performed annually in the United States [5]. The RC has limited ability to heal back to its insertion on the humerus [6], as a result, the retear rates after RC repair have been reported to be as high as 70% for large tears (>5 cm) and 20% for small tears (≤3 cm) [7]. Conservative rehabilitation after RC repair is important for a successful outcome, nowadays, the patients are required for a long-term immobilization to decrease the retear rate. The rehabilitation procedure like passive motion

exercise is usually delayed as the early motion may influence the tendon healing and increase the retear rate. Unfortunately, an important complication observed during long-term immobilization is the post-operative stiffness, which decreases the surgical effect and quality of life significantly [8-10].

Stiffness is affected by many factors including patient comorbidities such as diabetes mellitus, proneness to scar formation, preoperative shoulder stiffness, surgical technique, and postoperative rehabilitation [11]. For example, surgical repair techniques for RC repair can be divided into open, mini-open, and arthroscopic approaches. Because of advances in skill and technology, arthroscopic repair has emerged as similar effect and less post-operative stiffness, which has gradually replaced the open technique. Though the arthroscopy procedure is minimal invasive, post-operative stiffness is not totally avoided. Controversy still exists regard-

ing the best time to initiate the passive range of motion (ROM) exercise to balance the rate of common stiffness and retear. Importantly, the fact is not all patients face the problem of post-operative stiffness and not all patients face the same severity. It seems the genetic factors may play an important role in it, among which, the single nuclear polymorphism (SNP) may be involved.

Post-operative stiffness, also known as adhesive capsulitis (AC), involves thickened capsule because of chronic inflammatory infiltrate and moderate to extensive subsynovial fibrosis, resulting in obliteration of the normal patulous axillary fold by adhesions and fibrosis of the capsule itself [12, 13]. Previous results demonstrate that levels of inflammatory (interleukin 6, IL-6) and fibrogenic cytokines (Matrix metalloproteinases 3, MMP-3) are elevated in the synovium of patients with AC compared with controls. Given the genetic factors like SNP may be involved in the pathologies of post-operative stiffness, the gene polymorphism of IL 6 and MMP 3, which are elevated in AC, may associated with the risk of post-operative stiffness.

Accordingly, this study aims to determine whether the IL-6 SNPs (rs10499563, rs180-0796) and MMP-3 SNPs (rs679620, rs591058 and rs650108) were associated with post-operative stiffness in Chinese Han Populations.

Patients and methods

The study was approved by the ethics committee of the Qilu Hospital of Shandong University, and informed consent was obtained from patients and control participants.

Study population

A total of 188 patients diagnosed with rotator cuff tears treated with mini-open surgery in Qilu Hospital of Shandong University were recruited in this study. All subjects included in this study were Chinese Han Population. Patients were recruited among those suffered from persistently painful cuff tear and undergo a mini-open rotator cuff repair in Qilu hospital. And patients were excluded with radiographic glenohumeral arthritis, concomitant glenohumeral injuries, an irreparable tendon injury, post-operative infection, and performed revision cuff repair.

Patients who needed concomitant procedures such as distal clavicular excision and biceps tenotomy and/or tenodesis were retained.

Mini-open repair technique

Each patient was treated with mini-open repair technique by the same operator. All patients were operated under general anesthesia in the lateral decubitus position with the arm held in a 3-point shoulder distraction device. The shoulder was prepped and draped in the usual sterile fashion. Standard posterior and anterior portals were used for the glenohumeral joint exploration for subacromial decompression. The anterolateral approach was enlarged to 3-5 cm parallel to the deltoid fibers to minimize damage to the deltoid. The tear was gain visualized, and the footprint was prepared for reinsertion of the tendon. The supraspinatus was repaired by use of 1-3 bioabsorbable suture anchors armed with 2 pairs of nonabsorbable No. 2 sutures. Side-to-side FiberWire sutures were used if necessary. The tendon was sutured to the bone via the modified Mason-Allen technique with the combination of a U-shaped mattress suture and single suture on top of the mattress suture.

Postoperative rehabilitation protocols

All patients were immobilized with the abduction brace at 30° for 6 weeks. Shrugging of shoulders, active elbow flexion/extension, active forearm supination/pronation, and active hand and wrist motion were encouraged immediately after surgery. Controlled early passive motion exercise consisting of forward flexion, abduction, and external rotation was conducted twice a day from 3 weeks after the operation. Muscle strengthening was usually initiated at 9 to 12 weeks postoperatively, and all sports activities were permitted from 6 months after the operation.

Clinical evaluations and outcomes

Passive range of motion was recorded with a goniometer for flexion, external rotation with the arm at the side, and external rotation with the shoulder abducted 90°. Internal rotation was recorded by the maximal height achieved with the thumb when attempting to reach behind the back. Subjects were followed at 3 months postoperatively for a complete clinical examination. The definition of the post-opera-

Table 1. Summary of the basic characteristics of the groups

		Post-operative stiffness	Control	P value
No. of patients		87	101	
Age (year)		62.4±5.7	60.7±5.3	0.627
Gender	male	39	49	0.661
	female	48	52	
Dominant arm	right	83	95	0.754
	left	4	6	
Comorbidities (n)	Diabetes	15	14	0.549
	Hypertension	26	38	0.544
	Thyroid disease	5	7	0.775
Smoking (n)	Yes	45	47	0.559
	No	42	54	
Tear size	Small	45	66	0.043
	Medium	25	27	
	Large	17	8	
Pre-operative stiffness	Yes	26	35	0.534
	No	61	66	

tive stiffness was the limitation of passive motion in at least 2 directions (abduction and forward flexion <100°, external rotation <20°, or internal rotation <L3) in this study.

Genotyping

DNA samples were extracted from whole-blood samples with the Chelex-100 method according to the manufacturer's instructions [14]. The alleles of the IL 6 and MMP 3 SNPs were genotyped by the 5'-nuclease TaqMan assay. The primers and probes were designed and synthesized by Sigma (Sigma-Proligo, The Woodlands, TX). A laboratory personnel blinded to the case-control status performed the assessment of genotyping results. All assays were carried out in 96-well arrays in triplicate for quality control.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows were used for statistical analysis. The demographic and clinical data were presented as Mean ± SD and compared between groups by the Student's t-tests. The genotype and allelic frequencies were evaluated by Hardy-Weinberg equilibrium and compared by the Chi-square test. The association between the SNPs and post-operative stiffness

was assessed with allele level comparison, with dominant model comparison, with recessive model comparison, and with the extreme genotype comparison. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

The patients were grouped according to the ROM evaluation at 3 months post-operative. The definition of the post-operative stiffness was the limitation of passive motion in at least 2 directions (abduction and forward flexion <100°, external rotation <20°, or internal rotation <L3)

in this study. As a result, among the 188 patients, 87 patients was diagnosed as post-operative stiffness and the remaining 101 patients defined as controls. Demographic data of the population studied and the number of individuals in each group were shown in **Table 1**. There were no significant differences between groups in terms of age, gender, dominant arm, diabetes/hypertension/thyroid disease status, smoking, and pre-operation stiffness status. However, the patients with post-operative stiffness appeared to suffer from larger cuff tear than the patients with no post-operative stiffness ($P=0.043$).

Association of IL-6 and MMP-3 polymorphisms with post-operative stiffness susceptibility

As expected, the distribution of the genotypes of SNPs of IL-6 and MMP-3 gene conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. **Tables 2 and 3** listed the genotyped and allele distributions of the 5 SNPs for the cases and controls.

For the analysis of the IL-6 SNPs, no statistical association was found for the rs10499563 SNP no matter which genetic model was used. For the rs1800796 SNP, the increased G allele was found significantly associated with increased susceptibility of post-operative stiffness with the allele level comparison ($P < 0.0001$),

Gene polymorphism of IL-6 and MMP-3 and rotator cuff repair

Tables 2. Genotype and allele distributions of the 2 SNPs of IL-6 for the cases and controls

Group	rs10499563					
	TT	CC	TC + CC	TT + TC	CC	T (%) C (%)
Control	66	3	35	98	3	81.2 18.8
Case	53	4	34	83	4	78.2 21.8
OR (95% CI)	/	1.660 (0.356 to 7.748)	1.210 (0.668 to 2.192)	/	1.574 (0.342 to 7.239)	/ 1.204 (0.968 to 1.498)
P	/	0.700	0.547	/	0.706	/ 0.107

Group	rs1800796					
	CC	GG	CG + GG	CC + CG	GG	C (%) G (%)
Control	61	5	40	96	5	77.7 22.3
Case	38	11	49	76	11	65.5 34.5
OR (95% CI)	/	3.532 (1.138 to 10.960)	1.966 (1.099 to 3.519)	/	2.779 (0.926 to 8.343)	/ 1.835 (1.506 to 2.237)
P	/	0.030	0.028	/	0.070	/ <0.0001

Tables 3. Genotype and allele distributions of the 3 SNPs of MMP-3 for the cases and controls

Group	rs591058					
	CC	TT	CT + TT	CC + CT	TT	C (%) T (%)
Control	49	8	52	93	8	70.3 29.7
Case	49	7	38	80	7	74.1 25.9
OR (95% CI)	/	0.875 (0.294 to 2.600)	0.731 (0.411 to 1.300)	/	1.017 (0.353 to 2.929)	/ 0.827 (0.680 to 1.007)
P	/	1.000	0.308	/	1.000	/ 0.065

Group	rs650108					
	AA	GG	AG + GG	AA + AG	GG	A (%) G (%)
Control	40	15	61	86	15	62.4 37.6
Case	23	22	64	65	22	50.6 49.4
OR (95% CI)	/	2.551 (1.109 to 5.868)	1.825 (0.980 to 3.397)	/	1.941 (0.934 to 4.032)	/ 1.62 (1.356 to 1.936)
P	/	0.037	0.064	/	0.097	/ < 0.0001

Group	rs679620					
	GG	AA	GA + AA	GG + AG	AA	G (%) A (%)
Control	51	13	50	88	13	68.8 31.2
Case	30	25	57	62	25	52.9 47.1
OR (95% CI)	/	3.269 (1.457 to 7.334)	1.938 (1.075 to 3.495)	/	2.730 (1.296 to 5.750)	/ 1.963 (1.636 to 2.357)
P	/	0.005	0.038	/	0.010	/ <0.0001

with dominant model comparison ($P=0.028$), and with the extreme genotype comparison ($P=0.030$). But no statistical difference was found with the recessive model comparison ($P=0.070$), which may be a result of the relatively small population.

For the analysis of the MMP-3 SNPs, the increased A allele in rs679620 SNP was found significantly associated with increased incidence rate of post-operative stiffness, no matter with the allele level comparison ($P<0.0001$), with dominant model comparison ($P=0.038$), with recessive model comparison ($P=0.010$), or with the extreme genotype comparison ($P=0.005$). For the rs650108 SNP of MMP-3, the significant association was only found with the extreme genotype comparison (OR=2.551, 95% CI 1.109- 5.868, $P=0.037$) and the allele

level comparison (OR=1.62, 95% CI 1.356-1.936, $P<0.0001$). And no statistical association was found for the rs591058 SNP no matter which genetic model was used.

Association of IL-6 and MMP-3 Polymorphisms with post-operative stiffness severity

As only the rs1800796 SNP of IL-6 and rs679620 SNP of MMP-3 were found to be associated with the risk of post-operative stiffness, these two SNPs were chose to be analyzed in association with post-operative stiffness severity. And the forward flexion was selected as the definition of the severity of stiffness. One way ANOVA analysis found that there was a significant decreased forward flexion degree in correction with the minor allele of the SNP rs1800796 (**Figure 1A**; $P<0.0001$) and SNP rs679620 (**Figure 1B**; $P<0.0001$).

Gene polymorphism of IL-6 and MMP-3 and rotator cuff repair

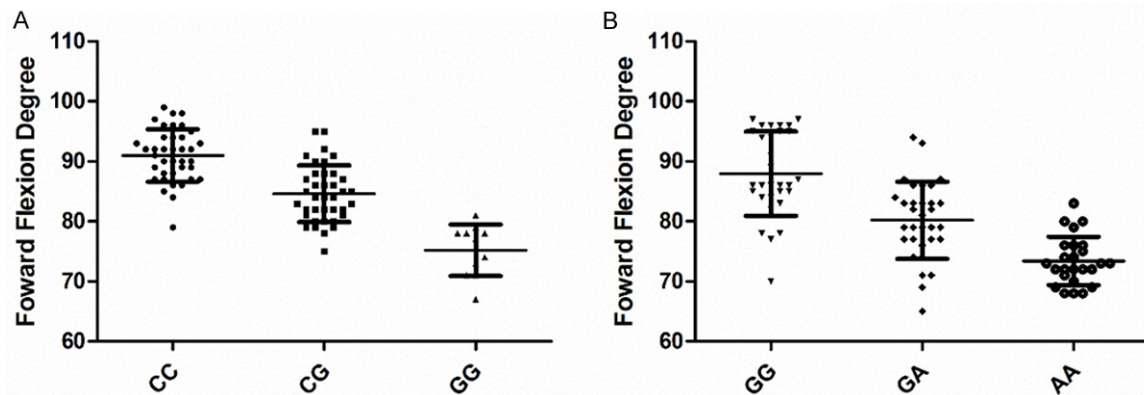


Figure 1. Association of IL-6 and MMP-3 Polymorphisms with post-operative stiffness severity. (A: rs1800796 SNP of IL-6; B: rs679620 SNP of MMP-3).

Discussion

Nowadays, genetic factors have been related to various disease pathologies. But no study has been performed with regard to the genetic factors of post-operative stiffness, while the fact of the difference in risk of stiffness susceptibility and severity. With the present study, we demonstrated the rs1800796 of IL-6 and rs679620 of MMP-3 were associated with increased risk of post-operative stiffness susceptibility and severity.

Post-operative stiffness, also known as adhesive capsulitis, is a quite common disease happened after all surgeries, not only after rotator cuff repair. Previous studies demonstrated the pathogenesis of adhesive capsulitis is caused by the evolution of a controlled inflammatory response to an unknown injurious stimulus into an aberrant fibrotic process.

Recently, histological studies demonstrated a matrix of type I and type III collagen populated by fibroblasts and myofibroblasts in the capsular tissue [15, 16]. High levels of MMP and even higher levels of TIMP have been found in AC patients [17]. Another study also showed the serum levels of MMP-1, MMP-2, MMP-3, TIMP-1, and TIMP-2, which are the key cytokines in remodeling of the extracellular matrix (ECM), may be associated with the pathologies of adhesive capsulitis [18]. On the other hand, studies have found increased levels of growth factor and cytokines [17, 19] and the presence of chronic inflammatory cells [20] in biopsy specimens of capsular tissue from patients with adhesive capsulitis. A consistent finding is

that of vascular hyperplasia in the capsular tissue [17, 20, 21]. Ryu et al have also demonstrated an increased expression of vascular endothelial growth factor and angiogenesis in diabetic patients with frozen shoulder [21]. The altered levels of inflammatory cytokines may act as a persistent fibrogenic stimulus, causing capsular fibrosis and the development of AC [22]. Importantly, the IL-6 and MMP-3 genes, which are known as the key cytokines in inflammatory and fibrogenic response, were found elevated expressed in the synovium of patients with adhesive capsulitis.

As far as we know, this is the first study investigating the genetic factors of the post-operative stiffness. And we demonstrated here that the rs1800796 SNP of IL-6 and rs650108 SNP of MMP-3 were associated with increased risk of post-operative stiffness susceptibility and severity. This finding can be used in guiding the rehabilitation procedure after rotator cuff surgery, in another word, those with the genetic susceptibility factors should receive a more radical rehabilitation procedure and those without the susceptibility factors can be more conservative. However, there was some limitation in this study. Firstly is the relative small population number. And a case control study is not of high level of evidence for supporting the conclusion. Second, no functional analysis of the rs1800796 SNP of IL-6 and rs650108 SNP of MMP-3 were performed to understand the mechanism of the association with post-operative stiffness. Moreover, besides the SNPs explored in this study, there may have more other polymorphisms in developing stiffness, a genome-wide association study with a large sample size is a better choice for it.

Disclosure of conflict of interest

None

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