SAP30BP gene is associated with the susceptibility of rotator cuff tear: a case-control study based on Han Chinese population

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Abstract

Background: Multiple studies have indicated that genetic components contribute significantly to the risk of rotator cuff tears. Previous studies have suggested that the SAP30BP gene may play an essential role in the development of rotator cuff tears. The aim of this study was to evaluate the potential association of the SAP30BP gene with the susceptibility to rotator cuff tears in a Han Chinese population.

Methods: A total of 394 patients with rotator cuff tears and 998 healthy controls were included in the study. Twelve tag single nucleotide polymorphisms (SNPs) located in the region of the SAP30BP gene were selected for genotyping. Genetic association analyses were performed using χ² tests for each SNP. Significant associations were searched in the GTEx database for their functional consequences.

Results: SNP rs820218 was significantly associated with rotator cuff tears (χ² = 9.49, P = 0.0021, OR [95% CI] = 0.67 [0.52–0.87]). In addition, SNP rs820218 was found to be significantly associated with the gene expression level of SAP30BP in whole blood (NES = 0.12, P = 1.00 × 10⁻⁶).

Conclusion: Our study has shown that the genetic polymorphism of SAP30BP contributes to the risk of rotator cuff tears in Chinese Han people. Individuals with the A allele for SNP rs820218 were less susceptible to developing rotator cuff tears.

Keywords: SAP30BP, Single nucleotide polymorphism, Genetic susceptibility, Rotator cuff tear

Background

With the aging of society and the overuse injuries of shoulders occurring in sports as well as in jobs, the incidence of rotator cuff tears has increased from 5 to 39%, seriously harming human health and quality of life [1]. Recently, some genetic studies have shown that genetic factors might contribute to the development of rotator cuff tears [2, 3]. It has been reported that the prevalence rates of rotator cuff tears in first- and second-degree relatives of patients were significantly higher than that in the general population [4]. Moreover, researchers have also found that rotator cuff tears in siblings are more likely to progress over a period of 5 years [5]. In addition, a related study reported that the second- and third-degree relatives of younger rotator cuff tear patients (younger than 40 years old) have a significantly increased relative risk for developing tears than those of older patients [6]. However, the understanding of the etiology and pathogenesis of rotator cuff tears remains unclear [7]. Given that genetics has been investigated as a factor involved in the pathogenesis of rotator cuff...
pathology [1], it is urgent that the susceptibility gene to rotator cuff tears and the molecular mechanism of this disease are clearly identified.

Previously, Maffulli et al. have focused their attention on the study of genetic susceptibility of rotator cuff tears [1, 7–9], and many susceptibility genes associated with rotator cuff tears have been identified through linkage analyses in candidate gene association studies and genome-wide association studies (GWAS) [10, 11]. Among them, a recently published study documented the results of the GWASs on rotator cuff tears conducted to date and identified significant evidence of an association of rs820218 in the SAP30-binding protein (SAP30BP) gene with rotator cuff tears in the American population [11]. It has been reported that SAP30BP inhibits transcription and induces apoptosis by interacting with the SAP30-associated mSin3 complex [12]. By assessing the edges of torn supraspinatus rotator cuff tendons collected during surgery on patients with rotator cuff tears, the authors have found excessive apoptosis in rotator cuff tear patients, and this was a primary cause of tendinopathy [13]. Furthermore, researchers have also found that upregulated p53 may induce apoptosis in patients with torn supraspinatus tendons compared with controls using immunohistochemistry [14]. Hence, it is reasonable to suppose that the SAP30BP gene may be involved in the occurrence and development of rotator cuff tears by inducing apoptosis in tendons.

However, the most recent GWAS did not show a significant association between the SAP30BP gene and rotator cuff tears [15]. Given that different ethnic populations may exhibit genetic heterogeneity related to rotator cuff tears, replications of the study using more samples from different populations are needed to confirm these results. Currently, no information is available on the Han Chinese population regarding the SAP30BP gene and rotator cuff tears. Therefore, in the present study, we conducted a case-control association study to evaluate the relationship between the SAP30BP gene and rotator cuff tears in a Han Chinese population.

Methods
Study subjects
In this study, we recruited 394 patients with rotator cuff tears and 998 controls from Honghui Hospital of Xi’an Jiaotong University from May 2016 to April 2019. All patients who had rotator cuff tears were under 70 years of age. Patients with rheumatism, diabetes, previous shoulder surgery, traumatic rotator cuff tears, and infection in the shoulder joint were excluded. The patient group and the control group were age-matched so that the maximum age difference was not more than 2 years. The controls were patients who underwent trauma treatment at the same hospital, but they did not have clinical symptoms associated with shoulder pain or other rotator cuff disorders. All samples were examined by imaging of the bilateral rotator cuff (MRI or ultrasonography). The rotator cuff tendons were intact in all subjects in the control group. Demographic information on all study subjects was collected using interviews. Informed consent forms were signed by all subjects. This study was reviewed and approved by the ethics committee of Honghui Hospital of Xi’an Jiaotong University.

SNP selection and genotyping
SNPs located within the region of the SAP30BP gene with minor allele frequencies (MAF) greater than 0.05 were chosen from the Chinese Han Beijing (CHB) 1000 genome dataset. Then, tag SNPs with $r^2 \geq 0.7$ were selected from the selected SNP set. A total of 12 tag SNPs were included for genotyping (Supplemental Table S1). In accordance with the manufacturer’s protocol (Genomic DNA kit, Axygen Scientific, Inc., CA, USA), we extracted genomic DNA from peripheral blood leukocytes. A high-throughput Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) was utilized for SNP genotyping. Briefly, the signals from the platform were automatically analyzed using Sequenom Typer 4.0 software, and genotype data were generated from the processed results [16]. To estimate the genotyping quality, 5% of the random samples were repeated for genotyping [17]. The concordance rate was 100%, so the quality of the genotyping data was confirmed.

Statistical analyses
Hardy-Weinberg equilibrium (HWE) tests were conducted within the control group for each SNP. Genetic association analyses were performed for each SNP by using $\chi^2$ tests and Plink [18]. The effect size of the association between the minor allele of each SNP and the disease status of the rotator cuff tear was presented as an odds ratio. Bonferroni corrections were applied for multiple comparisons. The statistical significance threshold for the $P$ values was $0.05/12 \approx 0.004$ for single marker-based association analyses. A linkage disequilibrium (LD) plot was made using Haploview [19] to virtualize the LD structure of the 12 selected SNPs in our samples. To further investigate the functional consequences of significant SNPs, we examined these SNPs in the GTEx database [20] to establish the link between genotypes of these SNPs and gene expression levels in multiple human tissues. Significant expression quantitative trait loci (eQTLs) from various human tissues were recorded.
Results
A total of 394 patients with rotator cuff tears and 998 healthy controls were included in the study (Table 1). No significant differences were observed in terms of the patient’s age, sex, the presence of other tendinopathies, smoking habits, drinking habits, work with repeated and sustained arm abduction or sports with shoulder involvement between the patients and controls. SNP rs820218 was significantly associated with rotator cuff tears ($\chi^2 = 9.49$, $P = 0.0021$, OR [95% CI] = 0.67 [0.52–0.87], Table 2). The MAF of this SNP was significantly lower in the patients than in the controls (0.11 vs. 0.15), and the study subjects with its minor allele, A allele, had a lower chance of having a rotator cuff tear. No other SNPs were found to be associated with rotator cuff tears. The LD plot showed that SNP rs820218 was modest to moderate in LD with the other genotyped SNPs (Fig. 1). We explored the SNP rs820218 in the GTEx database.

Table 1 Demographic and clinical characteristic information on the study subjects

|                      | Patients (N = 394) | Controls (N = 998) | Statistics | $P$ value |
|----------------------|-------------------|-------------------|------------|-----------|
| Age, mean±sd         | 53.7 ± 6.6        | 54.1 ± 6.5        | $T = -1.02$| 0.31      |
| Gender (%)           |                   |                   |            |           |
| Male                 | 181 (46)          | 456 (46)          |            |           |
| Female               | 213 (54)          | 542 (54)          |            |           |
| Other tendinopathies |                   |                   |            |           |
| Yes                  | 21 (5)            | 52 (5)            | $\chi^2 = 0.0006$ | 0.98     |
| No                   | 373 (95)          | 946 (95)          | $\chi^2 = 6.66 \times 10^{-31}$ | 1.00     |
| Smoke (%)            |                   |                   |            |           |
| Yes                  | 77 (20)           | 193 (19)          |            |           |
| No                   | 317 (80)          | 805 (81)          | $\chi^2 = 0.0001$ | 0.99     |
| Drink alcohol (%)    |                   |                   |            |           |
| Yes                  | 87 (22)           | 224 (22)          |            |           |
| No                   | 307 (78)          | 774 (78)          | $\chi^2 = 0.0057$ | 0.94     |
| Work with repeated and sustained arm abduction (%) | | | | |
| Yes                  | 161 (41)          | 401 (40)          |            |           |
| No                   | 233 (59)          | 597 (60)          | $\chi^2 = 0.0300$ | 0.86     |
| Perform sports with shoulder involvement (%) | | | | |
| Yes                  | 40 (10)           | 107 (11)          | $\chi^2 = 0.0460$ | 0.83     |
| No                   | 354 (90)          | 891 (89)          |            |           |

Table 2 Results of single marker-based genetic association analyses

| CHR | SNP       | POS     | A1/F_A | F_U     | A2 | $\chi^2$ | $P$    | OR [95% CI] |
|-----|-----------|---------|--------|---------|----|----------|--------|-------------|
| 17  | rs4453563 | 75674697| T      | 0.28    | 0.27| G        | 0.05   | 0.82        |
| 17  | rs8076675 | 75682774| T      | 0.16    | 0.16| C        | 0.10   | 0.75        |
| 17  | rs62090774| 75682779| C      | 0.12    | 0.11| T        | 0.07   | 0.79        |
| 17  | rs2898569 | 75685840| T      | 0.41    | 0.41| A        | 0.01   | 0.91        |
| 17  | rs1661652 | 75687957| A      | 0.25    | 0.25| T        | 0.01   | 0.91        |
| 17  | rs4999137 | 75687959| A      | 0.38    | 0.38| T        | 0.03   | 0.86        |
| 17  | rs1661651 | 75687961| A      | 0.31    | 0.31| T        | 0.01   | 0.91        |
| 17  | rs2053508 | 75689579| G      | 0.43    | 0.42| A        | 0.07   | 0.79        |
| 17  | rs820218  | 75691415| A      | 0.11    | 0.15| G        | 9.49   | 0.0021      |
| 17  | rs62090776| 75692348| T      | 0.07    | 0.06| G        | 0.47   | 0.50        |
| 17  | rs7208873 | 75696377| C      | 0.26    | 0.25| A        | 0.05   | 0.82        |
| 17  | rs3743999 | 75703466| C      | 0.16    | 0.15| G        | 0.13   | 0.71        |

CHR chromosome, SNP single nucleotide polymorphism, POS position; A1/A2: minor/major allele; F_A/F_U: minor allele frequency of the patients/controls. A significant result is indicated in bold font. The statistical significance threshold of the $P$ values was 0.05/12 = 0.004.
and identified that this SNP was significantly associated with the expression levels of five genes in various human tissues (Table 3). SNP rs820218 was found to be significantly associated with the expression level of the SAP30BP gene in whole blood. In addition to SAP30BP, SNP rs820218 was also identified as a significant eQTL hit for four other genes, including RECQL5, SMIM6, ITGB4, and SMIM5, in multiple human tissues.

Discussion

Our study showed that a genetic polymorphism located within the region of the SAP30BP gene was significantly associated with the disease status of rotator cuff tears. To the best of our knowledge, this significant hit was the first to be observed for rotator cuff tears in the Chinese Han population. The A allele of SNP rs820218 had a protective effect for rotator cuff tears, and this finding

Table 3: Significant eQTL signals for SNP rs820218 from multiple human tissues based on the GTEx data

| GENE   | SNP     | P-Value   | Ref | Alt | NES  | Tissue                        |
|--------|---------|-----------|-----|-----|------|-------------------------------|
| RECQL5 | rs820218| $5.60 \times 10^{-13}$ | G   | A   | 0.27 | Artery—Tibial                 |
| RECQL5 | rs820218| $1.70 \times 10^{-8}$  | G   | A   | -0.29| Testis                        |
| RECQL5 | rs820218| $1.80 \times 10^{-7}$  | G   | A   | 0.17 | Muscle—Skeletal               |
| SMIM6  | rs820218| $2.10 \times 10^{-7}$  | G   | A   | -0.40| Adipose—Visceral (omentum)    |
| RECQL5 | rs820218| $3.60 \times 10^{-7}$  | G   | A   | -0.24| Pituitary                     |
| RECQL5 | rs820218| $4.40 \times 10^{-7}$  | G   | A   | 0.20 | Adipose—Subcutaneous          |
| SAP30BP| rs820218| $1.00 \times 10^{-6}$  | G   | A   | 0.12 | Whole Blood                   |
| RECQL5 | rs820218| $1.60 \times 10^{-6}$  | G   | A   | 0.17 | Esophagus—Muscularis          |
| ITGB4  | rs820218| $1.80 \times 10^{-6}$  | G   | A   | -0.14| Skin—Sun Exposed (Lower leg) |
| ITGB4  | rs820218| $4.40 \times 10^{-6}$  | G   | A   | -0.29| Pancreas                      |
| SMIM5  | rs820218| $4.50 \times 10^{-6}$  | G   | A   | -0.28| Adipose—Visceral (Omentum)    |
| SMIM5  | rs820218| $8.10 \times 10^{-6}$  | G   | A   | -0.17| Heart—left ventricle          |

SNP single nucleotide polymorphism, Ref Reference allele, Alt alternative allele, NES normalized effect size
Interestingly, the gene expression levels of several genes neighboring SAP30BP may strengthen the evidence. Additional eQTL tests and analyses conducted in the European American population are needed for the targeted disease. Nevertheless, we need to interpret our findings from the GTEx data with caution, as the data were obtained mainly from healthy individuals. Additional eQTL tests and analyses conducted in rotator cuff tear patients may strengthen the evidence. Interestingly, the gene expression levels of several genes neighboring SAP30BP were also significantly associated with the genotypes of SNP rs820218. However, since there is no biological evidence that connects these genes with bone metabolism or bone-related diseases, SNP rs820218 should not be functionally mapped to these genes.

With the continuous development of sequencing technology and its rapid decline in cost, more and more susceptibility variants of complex diseases have been reported, such as schizophrenia [25–27]. Because it is difficult to draw reliable conclusions only based on SNP analyses [28–32], our study has several limitations. We did not assess population stratification in our sample, which is considered to be the most important confounder for genetic association mapping. As this study was an association mapping study of candidate genes, only a few SNPs were genotyped, and it would be difficult for us to implement common statistical procedures (such as principal component analysis or genomic control) to account for this confounder. Nevertheless, in the sample recruitment process, we restricted our study subjects to be local people without a history of immigration within three generations. We believe that this procedure at least partly controlled for the potential population stratifications by reducing the genetic heterogeneity of the study subjects. In addition, in this study, we only selected SNPs located within the region of the SAP30BP gene. Potential functional SNPs located in the ±20 kb region of the SAP30BP gene were not considered in the present study. Therefore, the present study might have failed to cover some important genetic regions with high functional significance. Several recent studies have indicated that rare and low-frequency variants might play an important role in the pathology of complex disorders [33, 34]. Sequencing-based studies on genetic regions and the functional significance of SAP30BP are needed in the future to identify the genetic architecture of rotator cuff tears.

In summary, our study has shown that the genetic polymorphism of SAP30BP contributes to the risk of rotator cuff tears in Chinese Han populations. Individuals with A allele for SNP rs820218 are less susceptible to developing rotator cuff tears.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13018-020-01888-z.

Additional file 1: Table S1. Basic information of the selected SNPs.

Abbreviations
GWAS: Genome-wide association study; SAP30BP: SAP30-binding protein; CHB: Chinese Han Beijing; HWE: Hardy-Weinberg equilibrium; LD: Linkage disequilibrium; eQTL: Expression quantitative trait loci; MAF: Minor allele frequency; ORs: Odds ratios; SNP: Single nucleotide polymorphism; NES: Normalized effect size

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Authors' contributions

ZDZ conceived and designed the study. BT was involved in the data search and selection of data, analyzed the data, and wrote the manuscript. BT and XK contributed to the subject screening and the collection and preparation of control DNA samples. All authors recruited, diagnosed, and gathered patients. All authors read and approved the final manuscript.

Availability of data and materials

Please contact the authors for reasonable requests.

Ethics approval and consent to participate

Written informed consent was obtained from all participants prior to their participation. The research protocol was approved by the Ethics Committee of Honghui Hospital of Xi’an Jiaotong University. The ethical approval was consistent with the standards of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

None.

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