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Genetic variants associated with rotator cuff tearing utilizing multiple population-based genetic resources

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Background: The etiology of rotator cuff tearing is likely multifactorial, including a potential genetic predisposition. The purpose of the study was to identify genetic variants associated with rotator cuff tearing utilizing the UK Biobank (UKB) cohort, confirm variants using a separate genetic database, and evaluate tissue expression of genes with associated variants following rotator cuff tearing using RNA sequencing.

Methods: *Genome-wide association study (GWAS)*: A GWAS was performed using data from UKB with 5701 cases of rotator cuff injury. *RNA sequencing analyses:* rotator cuff biopsies were obtained from 24 patients with full-thickness rotator cuff tears who underwent arthroscopic rotator cuff repair (cases) and 9 patients who underwent open reduction internal fixation for a proximal humerus fracture (controls). Total RNA was extracted and differential gene expression was measured by RNAseq for genes with variants associated with rotator cuff tearing.

Results: The results of the UKB GWAS identified 3 loci that reached genome-wide statistical significance: 2 loci on chromosome 7 in *GLCCI1* (rs4725069; $P = 5.0E–09$) and *THSD7A* (rs575224171; $P = 5.3E–09$), and 1 locus on chromosome 2 in *ZNF804A* (rs775583810; $P = 3.9E–09$). The association with rotator cuff injury of the *GLCCI1* single-nucleotide polymorphism (SNP; rs4725069) was confirmed in the Kaiser Permanente Research Bank cohort ($P = .008$). Twenty previously reported SNPs in 12 genes were evaluated using summary statistics from the UKB GWAS, which confirmed 3 SNPs in *TNC* with rotator cuff injury (rs1138545, rs72758637, and rs7021589; all $P < .0024$). Of 17 genes with variants associated with rotator cuff injury (14 previously from literature plus 3 new genes from current UKB GWAS), *TIMP2, Col5A1, TGFBR1,* and *TNC* were upregulated ($P < .001$ for all) and *THSD7A* was downregulated ($P = .005$) in tears vs. controls in the RNA sequencing data set.

Conclusion: The UKB GWAS has identified 3 novel loci associated with rotator cuff tearing (*ZNF804A, GLCCI1, THSD7A*). Expression of the *THSD7A* gene was significantly downregulated in rotator cuff tears vs. controls supporting a potential functional role. Three previously reported SNPs in the *TNC* gene were validated in the UKB GWAS, supporting a role for this gene in rotator cuff tearing. Finally, *TIMP2, Col5A1, TGFBR1,* and *TNC* genes were found to have significantly upregulated tissue expression in cases vs. controls supporting a biologic role in tearing for these genes.

The etiology of rotator cuff tearing is most likely multifactorial including a consequence of reduced tendon vascularity, impingement, intrinsic tendon degeneration, patient comorbidities, and genetics.[13,15,21,24,27,29] A familial predisposition for the development of rotator cuff tearing and tendinopathies in general has been identified in both sibling studies and studies using a multigenerational population database combining genealogical information and disease diagnosis data.[12,32,33] Several studies have used large genetic databases to identify genetic variants associated with rotator cuff tearing,[1,11,17,22,23,28,34] and a few studies have had sufficient power to confirm variants associated with rotator cuff tearing in external data sets.[11,22] Similarly, analysis of tissue expression changes following rotator cuff injury may also assist in confirming a potential role for genes identified as having variants associated with tearing.

Multiple genes have been previously identified as being associated with rotator cuff tearing, including genes associated with extracellular matrix, tendon maturation or modulation, cell adhesion, growth factors critical to tendon healing and function, and hormonal receptors. The method used in these studies was to identify single-nucleotide polymorphisms (SNPs) that show a genome-wide significant association between individuals with rotator cuff tears compared with a control population. SNPs are locations within the human genome where the type of nucleotide differs between individuals. Genetic variation at SNPs can affect genes located either within or near them involved in rotator cuff etiology, thereby increasing the risk of rotator cuff tearing. Genetic variants associated with rotator cuff injury have been identified in the following matrix metalloproteinases (MMPs): *MMP1* (targets type I collagen fibers), *MMP2* (targets gelatin and other collagens besides type I), *MMP3* (targets other collagens besides type I), and *MMP9* (targets gelatin and type IV and type V collagen).[1,11,23] Furthermore, *MMP1* and *MMP3* variants have been identified in 2 separate studies, strongly supporting a role in rotator cuff tearing.[1,11,23] Variants in growth factor or growth factor receptors associated with tendon modulation or maturation including fibroblast growth factor 1 (*FGF1*), *FGF3*, transforming growth factor beta 1 (*TGFB1*), and transforming growth factor beta receptor 1 (*TGFGR1*) have been identified as being associated with rotator cuff tearing.[11,23] Estrogen-related receptor beta (*ESRRB*) variants have been identified and confirmed in separate studies as being associated with rotator cuff tearing.[23,34] Variants in the extracellular matrix protein tenascin-c (*TNC*) have been identified by Kluger et al[17] to

be associated with rotator cuff tearing and confirmed by Figueiredo et al,[11] who also identified variants in collagen 5A1 (*COL5A1*) as well. Finally, an SNP in the gene encoding cadherin 8, a cell adhesion molecule, has been associated with rotator cuff tearing with genome-wide significance using a large genetic database.[28]

Large genetic databases are typically required to adequately power genome-wide association studies (GWASs) in order to provide enough statistical power to select true signals from the millions of SNPs being tested from the entire genome. The UK Biobank (UKB) is a large, population-based study, established to allow detailed investigations of the genetic and non-genetic determinants of a wide variety of traits, including risk for diseases and injuries.[30] One prior study utilized the UKB database to evaluate clinical risk factors associated with rotator cuff repair, although genetic association analyses were not performed.[36]

The purpose of this study was to identify new and confirm previously published genetic variants associated with rotator cuff tearing using 2 large genetic databases as well as identify potential biologic roles for these variant genes using rotator cuff tear tissue specimens. We planned to accomplish this goal through 3 separate experiments. First, we performed a GWAS for rotator cuff tearing in the UKB data set, with confirmation in the Kaiser Permanente Research Bank (KPRB) data set to identify new genetic variants associated with rotator cuff tearing. Second, we confirmed previously reported variants associated with rotator cuff tearing using the summary statistics from the UKB GWAS. Third, we evaluated differential gene expression of all genes harboring variants associated with rotator cuff injury using RNA sequencing data from a set of rotator cuff tear cases and control specimens from intact rotator cuff tendons in order to determine a potential biologic role for the identified variant genes in rotator cuff tearing.

Methods

GWA analyses for rotator cuff injury were performed using data from the v3 release of UK Biobank.[4] The genotypes of the UK Biobank participants were assayed using either of 2 genotyping arrays, the Affymetrix UK BiLEVE Axiom or Affymetrix UK Biobank Axiom array. Genotype data were imputed centrally by UK Biobank with IMPUTE2 using the Haplotype Reference Consortium and the UK10k+ 1000GP3 reference panels.[14] Metrics for quality control were established and then used to filter

DNA variants by UK Biobank.[4] Imputed SNPs were excluded if they had an IMPUTE2 info score <0.4.

Individuals were excluded if they were outliers based on genotyping missingness rate or heterogeneity, whose sex inferred from the genotypes did not match their self-reported sex, who withdrew from participation, or who were not of European ancestry. Genetic variants were excluded that failed quality control procedures in any of the genotyping batches, that showed a departure from Hardy-Weinberg of $P < 10^{-50}$ or that had an MAF < .001. Sex was determined based on heterozygosity of the X chromosome.

Determination of genetic ancestry was performed by principal components analysis computed centrally by UK Biobank, as previously described.[4,16,28] These ancestry principal components were used in the GWAS to adjust for genetic ancestry.

Phenotype definitions

Rotator cuff injury cases were identified from electronic health records using either ICD10, Read v2, or Read v3 codes (5701 cases and 406,310 controls; 412,011 total individuals) (Table I). The electronic health records include injuries entered until June 2019 (accessed as version September 2019).

Genome-wide association study and meta-analysis

A genome-wide association analysis was conducted using PLINK v2.0a.[16] SNP associations were tested with rotator cuff injury with a logistic regression model using allele counts for typed and imputed SNPs. The model was adjusted for genetic sex, height, weight, and race/ethnicity using 15 principal components, as previously described.[16] The final number of SNPs that were analyzed was 17,136,336. To account for inflation due to population stratification, the genomic control parameter (λgc) was calculated (λgc = 1.015). λgc is defined as the median of the resulting chi-squared test statistics divided by the expected median of the chi-squared distribution.[9] Subsequently, P values were adjusted for genomic control. A P value threshold of 5×10^{-8} was used for genome-wide significance.

QQ and Manhattan plots were created using qqman.[35] Regional association plots were generated for each locus with LocusZoom (accessed January 1, 2020).[26] The genomic context of each SNP was investigated using RegulomeDB web tools (accessed February 1, 2020).[3] Whether each SNP is an expression quantitative trait locus (eQTL) was queried using the NCBI eQTL Browser (accessed February 1, 2020) and the Genotype-Tissue Expression (GTEx) Portal (accessed February 1, 2020). ChIP seq data from the ENCODE project was used to determine whether SNPs were located within transcription factor–binding sites.[8] Summary statistics for all SNPs from the fixed effects meta-analysis will be available at NIH GRASP (https://grasp.nhlbi.nih.gov/FullResults.aspx) on acceptance of this manuscript. This study analyzed stored data from UK Biobank subjects who consented to genomic testing and use of their genomic data, as well as health data from the UK Biobank electronic health records. The health and genotype data for the subjects were deidentified.

Table I Rotator cuff injury cases identified from electronic health records using either ICD10, Read v2, or Read v3 codes

	Code description	Number
ICD9 code		
727.61	Complete rupture of rotator cuff	1
840.4	Rotator cuff (capsule) sprain	1
ICD10 code		
M75.1	Rotator cuff syndrome	6663
read2		
7H530	Plastic repair of rotator cuff of shoulder	52
7H534	Revision repair of rotator cuff	9
7H535	Plastic repair of rotator cuff of shoulder	51
7H536	Revisional repair of rotator cuff	0
7H537	Plastic repair of multiple tears of rotator cuff of shoulder	0
7H538	Revisional repair of multiple tears of rotator cuff of shoulder	0
N2115	Full thickness rotator cuff tear	47
S5Q0	Rupture supraspinatus tendon	3
S5Q1	Rupture supraspinatus tendon	9
S5Q2	Rupture supraspinatus tendon	115
read3		
7H530	Plastic repair of rotator cuff of shoulder	134
N2115	Full thickness rotator cuff tear	191
X600L	Repair of rotator cuff of shoulder	451
Xa9Cd	Rupture of rotator cuff of shoulder	359
Xa9wn	Complete repair of rotator cuff	61
Xa9wo	Partial repair of rotator cuff	9
XaBDZ	Injury of tendon of the rotator cuff of shoulder	148
XaPsK	Plastic repair of rotator cuff of shoulder	0
XaPsL	Revisional repair of rotator cuff	0
XaPsM	Plastic repair of multiple tears of rotator cuff of shoulder	0
XaPsN	Revisional repair of multiple tears of rotator cuff of shoulder	31
XaYi8	Injury of muscle of rotator cuff of shoulder	33
S5Q2	Rupture supraspinatus tendon	650
S5Q1	Rupture subscapularis tendon	18
S5Q0	Rupture infraspinatus tendon	21
Total cases		5701
Total controls		406,310

Validation of UKB GWAS SNPs and previously published associated SNPs with rotator cuff tearing

SNPs with a genome-wide significant association with rotator cuff injury from the UKB GWAS were validated using summary statistics from a previous GWAS from the KPRB using only individuals with European ethnicity.[28] Previously published SNPs

associated with rotator cuff tearing from candidate gene studies were validated with summary statistics from the UKB GWAS.

RNA sequencing analysis using torn rotator cuff and intact rotator cuff specimens

Thirty-three patients undergoing surgical treatment of their shoulder had rotator cuff tissue biopsies. Hospital investigational review board approval at the University of Utah was obtained prior to initiation of the study, and all patients signed informed consent. Twenty-four patients with full-thickness posterosuperior rotator cuff tears underwent arthroscopic rotator cuff repair, and 9 patients underwent open reduction internal fixation for a proximal humerus fracture. Inclusion criteria included any patient willing to consent to the biopsy who was undergoing a repair of a full-thickness posterosuperior rotator cuff tear or open reduction internal fixation of a proximal humeral fracture.

The average age of the rotator cuff tear patients and proximal humeral fracture patients was 63.4 (range, 49-77) years and 62.2 (range, 43-77) years, respectively ($P = .6$). The rotator cuff tear group had an average sagittal tear size of 2.7 ± 1.1 cm and coronal tendon retraction of 2.4 ± 1.1 cm. Supraspinatus fatty degeneration was determined by Goutallier grading as follows: 6 grade 0 patients (25%); 12 grade 1 patients (50%); 1 grade 2 patient (4%); 2 grade 3 patients (8%); and 3 grade 4 patients (13%). The proximal humerus fracture group was composed of 1 two-part, 2 three-part, and 6 four-part fractures. There were 6 female and 3 male patients in the fracture cohort. The average body mass index of the fracture cohort was 30 (range, 21-49). Three of 9 patients in the fracture cohort were currently smoking. There were 9 female and 15 male patients in the rotator cuff tear cohort. The average body mass index of the rotator cuff tear cohort was 28 (range, 19-42). Four of 24 patients in the rotator cuff tear cohort were currently smoking. Eleven of 24 rotator cuff tears had an acute traumatic onset within 6 months of the time of surgery and 13 of 24 patients were chronic tears with no history of injury and symptoms greater than 6 months durations. Six of 24 patients had a cortisone injection at some point prior to the surgical procedure. The average duration of symptoms prior to surgery was 29 months (range, 1-240 months).

All 24 patients with full-thickness rotator cuff tears were operated on by a single orthopedic surgeon fellowship trained in shoulder and elbow surgery (R.Z.T.). Each of 9 patients with a proximal humerus fracture underwent surgery by one of 3 orthopedic surgeons with fellowship training in trauma surgery or the orthopedic surgeon with fellowship training in shoulder and elbow surgery who performed the rotator cuff repairs. All patients who had samples collected at the time of rotator cuff repair had tissue removed arthroscopically. An arthroscopic tissue biter with a 3-mm width and depth was used to take tissue at the edge of the torn tendon, which was immediately placed into RNAlater (Qiagen, Valencia, CA, USA) and stored until processing. The tissue collected at the time of open reduction internal fixation was taken from the anterior edge of the supraspinatus at the rotator cuff interval as it was opened during the procedure. A scalpel was used to remove a 3×3-mm piece of supraspinatus tendon, which was placed in RNAlater and then stored until processing.

Total RNA was extracted from the samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen). Libraries were prepared using the Illumina TruSeq RNA kit (Illumina, San Diego, CA, USA), quality checked with the Agilent Bioanalyzer RNA 6000 chip (Agilent Technologies, Santa Clara, CA, USA), captured using the RiboZero method (Illumina) thereby excluding ribosomal RNAs from the RNAseq library, and sequenced by 50-cycle end-reads on an Illumina HiSeq 2000. Reference fasta files were generated by combining the chromosome sequences from hg38 with all possible splice junction sequences, which were generated with USeq (v8.8.8) MakeTranscriptome using a radius of 46 and annotated with Ensembl transcripts from the UCSC browser. Reads were aligned with Novoalign (v2.08.01), allowing up to 50 alignments per read. USeq's SamTranscriptomeParser selected the best alignment for each read and converted the coordinates of the sequence from the RNA read to the sequence coordinates from the genome by accounting for RNA splices. Differential gene expression was measured using USeq's DefinedRegionDifferentialSeq. Briefly, the number of reads aligned to each gene were calculated and normalized in DESeq2. Log2 of the fragments per kilobase per million reads (FPKM) values were centered and scaled by gene, and adjusted P values were reported for each gene comparing rotator cuff tear tissue expression to control intact rotator cuff tendon expression. The expression levels of the 14 previously published genes with variants associated with rotator cuff tearing along with new variants identified in the current UKB GWAS were evaluated for significant differential gene expression between rotator cuff tear cases and control samples.

Results

UKB GWAS

We examined sex, weight, and height to determine if there were associations with rotator cuff injury (Table II). We found that being male, shorter in height, older, and heavier were associated with increased risk of rotator cuff tears. We performed a genome-wide association analysis for rotator cuff injury with the UK Biobank cohort with 5701 cases and 406,310 controls (412,011 individuals total). Cases of rotator cuff injury were identified from the electronic medical records (Table I). Participants were restricted to those with European ancestry, based on genotype, to reduce population stratification. We performed logistic regression using sex, height, weight, and 15 principal covariates as adjustments. The genomic control parameter (λ_{gc}) was calculated to account for inflation due to population stratification. We compared the observed P values to the distribution of P values expected by chance in a Q-Q plot (Fig. 1). The black dots deviate from the red line for the lowest observed P values in the upper right-hand corner, indicating that the observed association signals are significantly stronger than the signals that would be expected by chance.

The P value for every SNP is shown in a Manhattan plot (Fig. 2). There were 36 SNPs with a genome-significant association with rotator cuff injury using $P = 5 \times 10^{-8}$ as a cut-off. These SNPs are located in 3 loci: rs775583810 on chromosome 2, a set of 34 SNPs in a small region on chromosome 7, and rs575224171 in a different
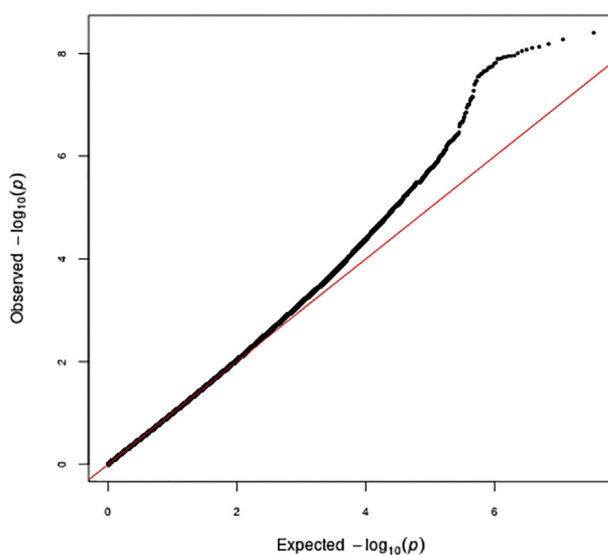
Table II   Demographics of cases and controls for UKB GWAS

	Case	Control	P value
Male, n	2989	184,091	
Female, n	2722	222,951	<.001[*]
Height, cm	167.9 (9.3)	168.5 (9.2)	<.001
Weight, kg	81.1 (15.9)	78.2 (15.9)	<.001
Age, y	59.9 (6.9)	56.9 (8.0)	<.001

*UKB*, UK Biobank; *GWAS*, genome-wide association study.
Unless otherwise noted, values are mean (standard deviation).
[*] *P* value vs. male.

Figure 1   Quantile-quantile plot for genome-wide association analysis of rotator cuff injury. The expected vs. observed log transformed values for 17,136,336 *P* values from the GWAS are graphed. The *y* axis shows the observed *P* values and the *x* axis shows the *P* values expected by chance. The black dots represent the SNPs arranged by their observed *P* values and the red line shows the expected trajectory if the SNPs had *P* values expected by chance. *GWAS*, genome-wide association study; *SNPs*, single-nucleotide polymorphisms.

region on chromosome 7 (Table III; Supplementary Table S1). For the cluster of 34 SNPs on chromosome 7, one possibility is that some or all of them are in the same linkage disequilibrium block, with rs4725069 showing the lowest *P* value in this group (6.59×10^{-9}). To determine whether these SNPs cosegregate as 1 linkage disequilibrium block, a conditional analysis was performed using rs4725069 as the sentinel SNP. The association with rotator cuff injury for all of the 33 SNPs in this region of chromosome 7 was dependent on the genotype of rs6943211; that is, each had *P* >.05 if the genotype of rs4725069 was included in the regression (Supplementary Table S1). This result shows that the 34 SNPs in this region of chromosome 7 are in a single linkage disequilibrium block, with rs4725069 showing the strongest association. Hereafter, we
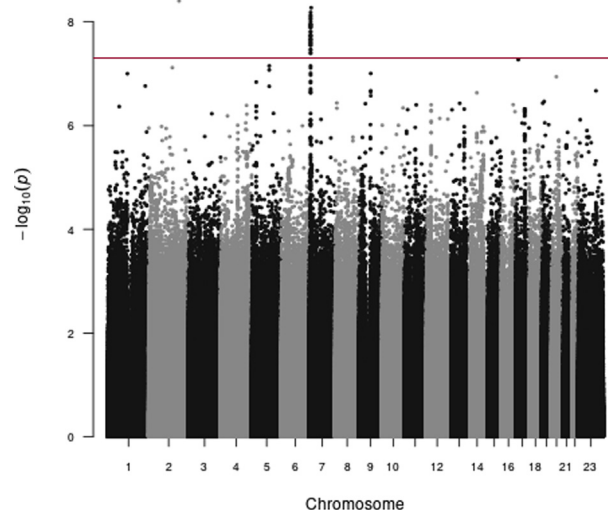
Figure 2   Manhattan plot for genome-wide association analysis of rotator cuff injury. The $-\log_{10} P$ values for association with rotator cuff injurys for SNPs from the GWAS are plotted by genomic position with chromosome number listed across the bottom. The *y* axis shows the $-\log_{10} P$ value for association with rotator cuff injury. *SNPs*, single-nucleotide polymorphisms; *GWAS*, genome-wide association study.

refer to the locus defined by this linkage disequilibrium block using the sentinel SNP rs4725069.

None of the 3 SNPs (rs775583810, rs4725069, or rs575224171) were directly genotyped on the Affymetrix chips, but rather their genotype was imputed. The info scores from IMPUTE2 were 0.55, 0.99, and 0.79 for rs775583810, rs4725069, or rs575224171, respectively. The info score for rs4725069 indicates that the imputed genotype is fairly accurate. The info scores for rs775583810 and rs575224171 are lower, indicating that there may be some error in the imputed vs. the true genotype. Errors in imputation would be expected to increase noise in the genetic associations, and hence the true associations for rs775583810 and rs575224171 may be stronger than the associations revealed by imputation. But even so, care should be taken until the true genotypes for these SNPs can be ascertained directly.

The association of rs775583810, rs4725069, or rs575224171 with rotator cuff injury was evaluated in a previous GWAS based on the KPRB cohort.[28] For each of these SNPs, we looked up the summary statistics generated from GWA analysis from a cohort of 102,979 patients that included 8357 cases of rotator cuff injury. Neither rs775583810 nor rs575224171 was present in the KPRB GWAS. rs4725069 showed an association with rotator cuff injury that was Bonferroni significant ($P = .008$) and with an odds ratio (0.90, 95% CI 0.84-0.97) similar to the odds ratio from UK Biobank GWAS (0.88, 95% CI 0.84-0.92).

Zoom plots were created for each of the significant SNPs (rs775583810, rs4725069, or rs575224171). SNPs were

Table III Significant SNPs identified in UKB GWAS

SNP	Chr.	BP	EA	EAF	UK Biobank				Kaiser			
					OR (95% CI)	SE	Z statistic	P value	SE	OR (95% CI)	Z statistic	P
rs775583810	2	185831014	T	0.001082	3.68654 (2.39528, 5.67392)	0.219999	5.93042	3.96E-09	NA	NA	NA	NA
rs4725069	7	8132252	T	0.299661	0.881643 (0.845183, 0.919676)	0.0215485	−5.84582	6.56E-09	0.03754	0.9043 (0.8401, 0.9733)	−2.681	.008193
rs575224171	7	11433422	T	0.001149	3.14523 (2.1467, 4.60824)	0.19488	5.87996	5.36E-09	NA	NA	NA	NA

SNPs, single-nucleotide polymorphisms; *UKB*, UK Biobank; *GWAS*, genome-wide association study; *Chr.*, chromosome; *BP*, base pair; *EA*, effect allele; *EAF*, effect allele frequency; *SE*, standard error; *OR*, odds ratio; *CI*, confidence interval; *NA*, not applicable.

identified as being associated with a gene if it was within the gene or near to it. rs4725069 resides within an intron near the 3' end of the gene glucocorticoid-induced transcript 1 gene (*GLCCI1*) (Fig. 3, a), rs575224171 resides within an intron of the thrombospondin type 1 domain containing protein 7A gene (*THSD7A*) (Fig. 3, b) and rs775583810 is in the region 3' to the zinc finger protein 804A gene (*ZNF804A*)(Fig. 3, c). *GLCCI1* is a marker of glucocorticoid apoptosis of inflammatory cells, suggesting a role in inflammation. Variants in this gene may lead to higher levels of chronic inflammation, a condition that might increase risk of rotator cuff tears. *THSD7A* encodes an endothelial protein that promotes angiogenesis. Therefore, a variant in this gene may result in impaired vascularity increasing the risk for tendon tearing. Finally, *ZNF804A* encodes a transcriptional regulator that regulates inflammatory cytokines. Therefore, a variant in this gene may lead to chronic inflammation, a condition that could result in an increased risk for tearing. All 3 genes have known biologic roles suggesting plausible mechanisms whereby genetic variation could predispose patients toward rotator cuff tearing.

We searched for a mechanism whereby rs775583810, rs4725069, or rs575224171 might affect the activity of nearby genes to account for their effects on concussion. None of these SNPs is in a protein-coding region (Fig. 3). We next asked whether these SNPs might alter the expression of nearby genes, and thereby have an effect on risk for rotator cuff injury. SNPs that are associated with expression of a nearby gene are referred to as expression quantitative trait loci (eQTL) and can be identified by using the Genotype-Tissue Exchange (GTeX) Portal. There were no data for rs775583810 or rs575224171. For rs4725069, the risk allele (A) leads to a decrease in expression of GLCCl1 in whole blood (normalized effect size = –0.090; $P = 3.6 \times 10^{-5}$) (Fig 4).

Another approach to determine whether an SNP is associated with variation in expression of nearby genes is to determine if it is located within a transcription factor–binding site, as determined from ChIP seq experiments from the ENCODE database. However, neither rs775583810, rs4725069, nor rs575224171 were in a known transcription factor–binding region defined by ChIP seq experiments or in a known transcription factor–binding motif in the ENCODE database (Consortium 2012).

Validation of SNPs previously reported to show an association with rotator cuff tearing

Twenty-three SNPs in a total of 14 different genes have been previously reported to be associated with rotator cuff tearing.[11,17,23,28,34] rs1799750 and rs1799750 in the *MMP1* gene, and rs3025058 in the *MMP3* gene could not be evaluated as they were not available in the UKB GWAS data set.[1,22] The remaining 20 SNPs were located in 12
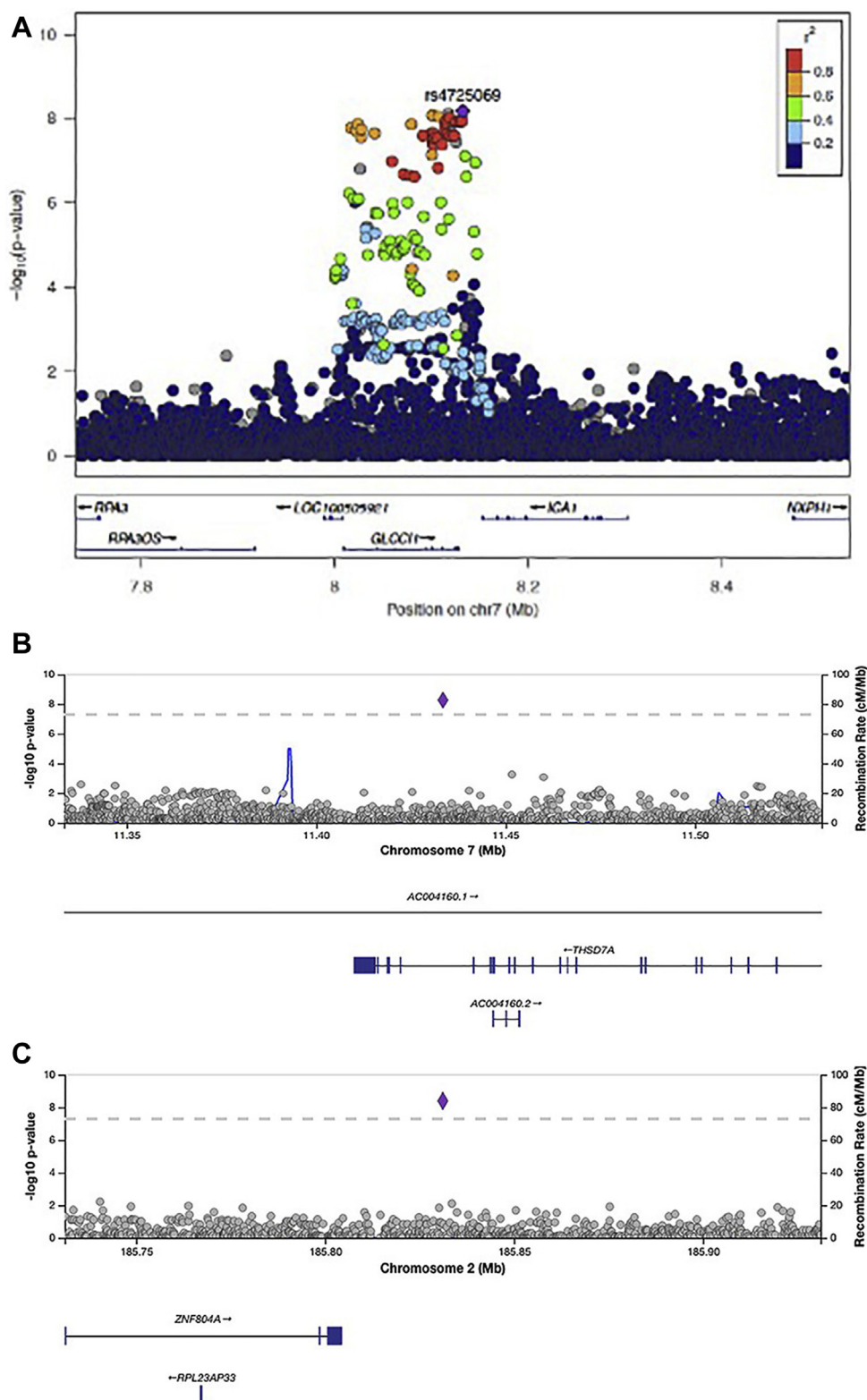
Figure 3 (**A**, **B**, **C**) Regional-association plots for significant SNPs in the GWAS. Tested SNPs are arranged by genomic position in a given interval around the lead SNP from each region (purple diamond). The *y* axis indicates –log$_{10}$ *P* values for association with rotator cuff injury for each SNP. The color of dots of the flanking SNP in (**A**) indicates the linkage disequilibrium (R^2) with the lead SNP as indicated by the heat map color key for regions with multiple significant SNPs. *SNPs*, single-nucleotide polymorphisms; *GWAS*, genome-wide association study.
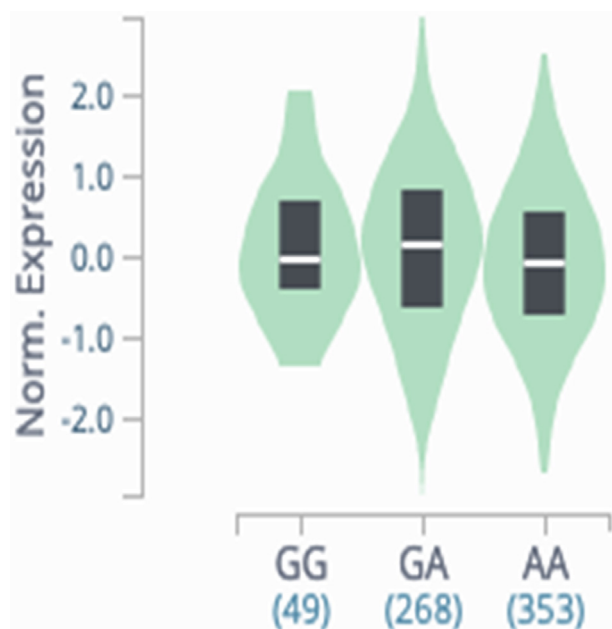
Figure 4 Violin plot for *GLCCI1* gene in whole blood. A violin plot showing expression change in whole blood of *GLCCI1* for different genotypes at rs4725069 (*x* axis). Numbers in parentheses indicate numbers of samples with each genotype. White bars indicate mean expression level, and gray boxes indicate quartile levels. The *y* axis shows the normalized effect size for changes in expression. The violin plot indicates the probability density of the data at different values, smoothed by a kernel density estimator. Data are downloaded from the Genotype-Tissue Exchange (GTeX) Portal.

genes and could be evaluated (Table IV). Kluger et al had previously reported that 3 SNPs in the TNC gene (rs1138545, rs72758637, rs7021589) showed an association with rotator cuff injury, and these showed an association with rotator cuff injuries with *P* values that were significant after Bonferroni correction (all *P* < .0024).[17] Curiously, 4 other SNPs in the TNC gene (rs7035322, rs10759753, rs2104772, rs3789870) did not show an association with rotator cuff injury (*P* > .05 for each).[17] None of the 13 SNPs in the remaining 11 genes showed significant associations using a Bonferroni-corrected threshold of $P < .25 \times 10^{-3}$.

Expression analysis in torn rotator cuff tissue

In total, there are 17 genes with SNPs reported to show an association with rotator cuff tearing; specifically, these are the 3 genes identified in the current UKB GWAS, 12 previously identified genes listed in Table IV plus MMP1 and MMP3. These 17 genes were evaluated for changes in gene expression in rotator cuff tendons from injured vs. control samples. TIMP2, Col5A1, TGFBR1, and TNC were upregulated (*P* < .001 for all), and THSD7A was downregulated (*P* = .005) in tears vs. controls in the RNAseq data set

(Table V). Col5A1 had the greatest increase in expression (3.01-fold) in injured tendons vs. controls, supporting the role of extracellular matrix proteins such as collagen in healing injured tendon tissue. Similarly, tenascin c, another important extracellular matrix protein, was upregulated 2.20-fold in injured rotator cuff tendon tissue. Expression of the angiogenesis gene *THSD7A* was downregulated 2.6-fold in injured rotator cuff tissue compared with controls.

Discussion

The current study provides new genetic and biological insights into genetic mechanisms underlying the development of rotator cuff tearing. The UKB GWAS identified SNPs associated with rotator cuff injury at a genome-wide significant level. The association with rotator cuff injury for the *GLCCI1* SNP was validated in the KPRB data set, whereas the other 2 SNPs were not present. These SNPs are located in or near to 3 genes whose role in rotator cuff tearing was previously unknown: glucocorticoid-induced transcript 1 gene (*GLCCI1*), thrombospondin type 1 domain–containing protein 7A (*THSD7A*), and zinc finger protein 804A (*ZNF804A*). Of the previously reported SNPs in the literature, 3 SNPs in the *TNC* gene were validated for showing an association with rotator cuff tearing in the UKB GWAS data set. RNAseq data showed significant downregulation of *THSD7A* expression in rotator cuff tears vs. controls, supporting a functional role for this gene in rotator cuff injuries. Finally, 4 genes previously reported to contain SNPs associated with rotator cuff tearing (*TIMP2*, *Col5A1*, *TGFBR1*, and *TNC*) were found to have significantly upregulated tissue expression in injured tendons vs. controls, supporting a biologic role in tearing.[11] The expression data showing increased expression of *TIMP2*, *Col5A1*, *TGFBR1*, and *TNC* in rotator cuff tears vs. controls are consistent with previous expression data (Table V).[2]

Glucocorticoid-induced transcript 1 gene (*GLCCI1*) plays an important role in glucocorticoid signaling. The gene was originally found upregulated as a result of treatment of thymoma-derived cell lines with dexamethasone.[5] *GLCCI1* genetic variants are associated with reductions in the response to inhaled glucocorticoids for asthma patients.[31] *GLCCI1* may be an early marker for glucocorticoid-induced apoptosis of inflammatory cells, which is a key mechanism used by glucocorticoids to reduce or eliminate inflammation.[5,6] Reduced or altered expression of *GLCCI1* in individuals harboring the risk allele (T) of rs4725069 may lead to chronic inflammatory changes that weakens or inflames their rotator cuff and predisposes them to rotator cuff injury.

Thrombospondin type 1 domain–containing protein 7A (encoded by *THSD7A*) is an endothelial protein that promotes endothelial cell migration by regulating focal adhesions during angiogenic cell migration.[18] Soluble

Table IV Published SNPs in genes associated with rotator cuff tearing

SNP	Gene	Chr.	BP	EA	OR (95% CI)	P UKB	Study
rs13317	FGF1	8	38269514	C	0.99 (0.94 to 1.03)	6.63E−01	Motta Gda et al 2014[23]
rs1590	TGFBR1	9	101916165	G	0.98 (0.94 to 1.03)	4.47E−01	Figueiredo et al 2020[11]
rs7035322	TNC	9	117787173	A	0.99 (0.95 to 1.04)	7.32E−01	Kluger et al 2017[17]
rs7021589	TNC	9	117804667	C	1.08 (1.03 to 1.14)	2.14E−03	Kluger et al 2017[17]
rs72758637	TNC	9	117805201	G	1.08 (1.03 to 1.14)	2.43E−03	Kluger et al 2017[17]
rs10759753	TNC	9	117805294	G	0.99 (0.95 to 1.03)	6.51E−01	Kluger et al 2017[17]
rs2104772	TNC	9	117808785	A	1.03 (0.99 to 1.07)	1.60E−01	Figueiredo et al 2020[11]
rs3789870	TNC	9	117835276	A	0.99 (0.95 to 1.03)	6.37E−01	Kluger et al 2017[17]
rs1138545	TNC	9	117835899	T	1.08 (1.03 to 1.14)	2.16E−03	Kluger et al 2017[17]
rs3196378	Col5A1	9	137734882	C	1 (0.96 to 1.04)	9.86E−01	Figueiredo et al 2020[11]
rs12574452	FGF3	11	69631731	A	1.04 (1 to 1.08)	5.05E−02	Motta Gda et al 2014[23]
rs679620	MMP3	11	102713620	C	1 (0.97 to 1.04)	8.73E−01	Figueiredo et al 2020[11]
rs7157192	ESRRB	14	76866960	A	0.98 (0.94 to 1.02)	3.59E−01	Teerlink et al 2015[34]
rs1676303	ESRRB	14	76992164	C	0.96 (0.91 to 1.02)	2.30E−01	Motta Gda et al 2014[23]
rs2285053	MMP2	16	55512377	T	1 (0.94 to 1.07)	9.69E−01	Figueiredo et al 2020[11]
rs71404070	Cadherin8	16	61069815	A	1.02 (0.93 to 1.12)	6.71E−01	Roos et al 2017[28]
rs2277698	TIMP2	17	76867017	T	0.92 (0.87 to 0.98)	9.70E−03	Figueiredo et al 2020[11]
rs1800470	TFGB1	19	41858921	G	1.02 (0.98 to 1.06)	2.54E−01	Figueiredo et al 2020[11]
rs1800469	TFGB1	19	41860296	A	1.01 (0.97 to 1.05)	5.67E−01	Figueiredo et al 2020[11]
rs17576	MMP9	20	44640225	G	1 (0.96 to 1.04)	8.98E−01	Figueiredo et al 2020[11]

SNPs, single-nucleotide polymorphisms; *Chr.*, chromosome; *BP*, base pair; *EA*, effect allele; *OR*, odds ratio; *CI*, confidence interval; *UKB*, UK Biobank.

Table V Tissue expression comparing rotator cuff tear cases vs. controls of published genes with variant SNPs associated with rotator cuff tearing

Gene	Protein	This work		Belangero 2018	
		Fold change	P value	Fold change	P value
COL5A1	Collagen type V alpha 1 chain	3.01	3.30E−04	1.73	0.002
TNC	Tenascin C	2.21	5.53E−04	1.68	0.005
TIMP2	TIMP metallopeptidase inhibitor 2	1.66	1.12E−03	ND	ND
TGFBR1	Transforming growth factor beta receptor 1	1.49	1.44E−03	2.17	0.022
THSD7A	Thrombospondin type 1 domain containing 7A	0.38	5.44E−03	ND	ND

SNPs, single-nucleotide polymorphisms; *ND*, not determined.

THSD7A binds to a receptor on the endothelial cell and triggers filopodia formation.[18] A genetic variant in the THSD7A gene may increase the risk for poor angiogenesis in the rotator cuff, thereby predisposing patients toward tearing their rotator cuffs. This view of the etiology of rotator cuff tearing is supported by decreased expression of THSD7A in rotator cuff injuries compared with normal intact tendons, which would further limit angiogenesis during the healing process.[20] Therefore, reduced levels of proangiogenic factors both before and after rotator cuff injury are potential insights about the role of THSD7A in rotator cuff tears.

ZNF804A encodes a zinc finger protein that functions as a transcriptional regulatory protein. ZNF804A variants have been previously reported to be associated with schizophrenia.[25] ZNF804A has been shown to activate expression of inflammatory cytokine TNF-alpha, which is upregulated in rotator cuff tears.[10] Therefore, the risk allele of rs575224171 (T) in ZNF804A may impair its role in mediating chronic inflammation leading to a condition predisposing one to tendon injury.[7,10] In summary, all 3 genes identified in the UKB GWAS have plausible biological roles in tendon metabolism, maturation, and repair where impairment may result in a higher predilection toward rotator cuff injury.

RNA sequencing data supports a potential role for TIMP2, Col5a1, TGFBR1, and TNC in the development of rotator cuff tearing. The increased tissue expression of these genes following rotator cuff injury is consistent with prior data showing increased expression of extracellular matrix proteins as well as inhibitors of MMPs in rotator cuff tears compared to control tissue.[2,19] The increased expression of these genes following rotator cuff injury supports their importance in repair of injured rotator cuff tissue and the attempts of injured tendon to heal. Genetic alterations in these proteins could potentially predispose

toward even further tearing or impairment of healing. Finally, data from the UKB GWAS confirmed prior work showing that variants of *TNC* are associated with rotator cuff tearing. The results from genetic association studies supports the role of *TNC* in risk for getting rotator cuff tears, and the results from expression changes following injury suggests a further role in the subsequent healing of the tear.[17]

Genetic predisposition studies are useful in several ways, although the direct clinical impact on the treatment of patients often requires further work. With the ability for individuals to obtain genotypic information about their own DNA from multiple companies, identifying a set of variants associated with rotator cuff tearing may be a method to define patients at risk for development of rotator cuff tearing. These individuals would then be able to modify either their occupation or lifestyle including preventative exercises to avoid rotator cuff injury in the future. Currently, industry is working to identify these sets of genetic variants to allow patients to assess their own relative risk for development of the disease. Second, genetic variant information allows directed research on the pathophysiology of rotator cuff tearing. Biologically directed augmentations to counteract one's genetic risk for rotator cuff tearing may be a method to improve healing after rotator cuff repair. Our lab is currently performing experiments utilizing this methodology using a mouse model to improve tendon healing using mutations in the ESRRB gene. Knowledge gained from the experiments with mice could be then translated to human use to improve outcomes after rotator cuff repair.

There are limitations to this research that are inherent to all GWA studies using the UK Biobank data set.[16] One limitation is that the phenotypes were defined from codes contained in electronic health records, and thus we have no information regarding the clinical scenarios surrounding the event. A problem with the coding is there is limited ability to differentiate partial from full-thickness tears. This may have an impact on the results as more severe injuries may be associated with a different set of genetic variants. Second, this study only evaluated individuals from the European ancestry group, and the effect in other ethnicities is unknown. Third, we have only identified associations and not biologic mechanisms of how variants in these genes may influence rotator cuff tearing. Despite this limitation, we can make inferences from prior work regarding the function of these genes in terms of how they may predispose toward rotator cuff tearing. Fourth, the SNPs in neither *THSD7A* nor *ZNF804A* could be confirmed in the KPRB cohort. Fifth, expression analysis following rotator cuff injury provides information about the response to the injury as opposed to effects that predispose towards injury.

Conclusions

Three new SNPs have been identified to be associated with rotator cuff tearing in a GWAS based on the UKB cohort, and these SNPs target genes (*GLCCI1*, *THSD7A*, *ZNF804*) with known roles in inflammation and angiogenesis suggesting biological mechanisms that could predispose one to increased injury. The *GLCCI1* SNP was replicated and validated in the KPRB data set. The *THSD7A* gene was identified to have significantly downregulated tissue expression in rotator cuff tears vs. controls, supporting a potential functional role. Previous findings reported an association of *TNC* variants with tearing, and this finding was validated using data from the UKB GWAS. Finally, *TIMP2*, *Col5A1*, *TGFBR1*, and *TNC* genes were found to have significantly upregulated expression in rotator cuff tears compared with normal controls, supporting a biologic role in the etiology tearing.

Disclaimer

Acknowledgments

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jse.2020.06.036.

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