J Shoulder Elbow Surg (2021) 30, 200–215

ELSEVIER

Journal of Shoulder and Elbow Surgery

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REVIEW ARTICLES

Polymorphisms and alterations in gene expression associated with rotator cuff tear and healing following surgical repair: a systematic review

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Background: Rotator cuff tears (RCTs) are a common cause of shoulder disability, yet both conservative and surgical treatment strategies can lead to poor results in some patient populations. Enhanced understanding of the genetic processes associated with RCTs can assist in the development of more effective management options and help predict individual responses to surgical treatment. This systematic review analyzes the current literature on the genetic footprint associated with RCTs and interprets these findings to enhance the current understanding of RCT pathogenesis, potential treatment regimens, and prognostic biomarkers of outcomes after surgical repair.
Methods: A systematic search of the Embase, PubMed, and Web of Science electronic databases was performed. Medical Subject Headings (MeSH) and Emtree index terms were formulated from the concept terms ''rotator cuff tear,'' ''genetics,'' and ''human,'' and synonyms of these concepts were applied to the Web of Science search. Articles were screened against predefined inclusion and exclusion criteria. Eligible studies compared gene expression patterns and genetic polymorphisms between cases (with RCTs) and controls (without RCTs). Quality assessment was performed with studies being rated as high, moderate, or poor quality. A modified best-evidence synthesis was applied, and studies were determined to be of strong, moderate, or limited evidence.
Results: The search identified 259 articles. Of these studies, 26 were eligible for review. Two studies were considered poor quality; 15 studies, moderate quality; and 9 studies, high quality. Analysis of these articles found that RCTs were associated with alterations in genes that code for the extracellular matrix, cell apoptosis, immune and inflammatory responses, and growth factor pathways. In particular, there was strong evidence of a significant association between RCTs and the genes *MMP3*, *TNC*, and *ESRRB*. Strong evidence of an association between *BMP5* upregulation and successful healing after surgical repair was also found.
Conclusion: This review provides strong evidence of an genetic association with RCTs. The genotype and gene expression patterns detailed within this review can assist in deciphering the biological mechanisms resulting in RCTs, as well as predicting an individual's

Institutional review board approval was not required for this systematic review.

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1058-2746/$ - see front matter
https://doi.org/10.1016/j.jse.2020.07.045

response to surgical repair. Future research could investigate whether manipulating these genes—or their associated signaling pathways—could assist in RCT healing and whether genetic biomarkers could be used clinically to predict patient outcomes after surgical repair of RCTs.

Level of evidence: Basic Science Study; Molecular Biology; Systematic Review

Keywords: Rotator cuff; gene; genetic association; gene expression; transcriptome; genetic polymorphism; single nucleotide polymorphism; human

Rotator cuff tears (RCTs) are the most common cause of shoulder disability and are present in approximately 40% of the population aged $\geq$ 50 years.[6,59,66] Despite the high prevalence of this condition, the pathophysiology and healing potential are not well understood, making the condition challenging to predictably treat in some patient populations.[21,73,91]

Conservative treatment of RCTs typically consists of physiotherapy and exercises and may include the use of pharmacologic or biological agents, taken orally or directly administered to the tendon.[22,29,32,40] Unfortunately, such agents have limited efficacy in RCT healing.[9,18,19,33] To assist in the development of more conservative treatment agents, a better understanding of RCT pathophysiology is required. Studies that identify genetic associations with RCTs, as well as the molecular pathways that genes affect, play a key role in improving this understanding.[35]

Surgical repair of RCTs is common following failed conservative treatment, yet retears occur in 25%-50% of patients within 12 months.[28] Thus, we require a refined method to predict and identify the candidates who will receive the greatest benefit from surgery.[52,54,77] The development of such a method can be aided by identifying genotypes that are associated with successful or poor outcomes after surgical repair. This contributes to the understanding of biological pathways associated with RCTs and can assist in developing directed biological adjuvants for repairs to improve healing outcomes.

Three literature reviews have analyzed genetic associations with RCTs. These reviews found preliminary evidence of a genetic and familial disposition to RCTs,[21] as well as a number of genotypes[46] and gene expression changes associated with RCTs.[15] No review to date has analyzed both polymorphisms and alterations in gene expression in humans or considered the genetic association with RCT healing after surgical repair. Furthermore, recent publications of genetic association studies using more contemporary methods have not been analyzed in previous reviews. This review provides an up-to-date and comprehensive analysis of studies investigating the genetic association of RCTs in humans and successful healing following surgical repair. These findings further our understanding of the pathophysiology of RCTs, which can help identify prognostic markers of postsurgical healing and assist in the development of future treatment modalities for RCTs.

Methods

We completed a systematic review of population-based case-control studies; this review used the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines[43] and was prospectively registered with PROSPERO[60] (no. CRD42019141473).

Eligibility criteria

Included articles investigated genetic associations in persons with RCTs. An RCT was defined as a partial or complete tear of $\geq$1 rotator cuff tendon, as assessed on medical imaging (using magnetic resonance imaging or ultrasound) or directly during surgery. Eligible studies compared gene expression patterns and genetic polymorphisms between cases (with RCTs) and controls (without RCTs). We also included studies that compared gene expression patterns or polymorphisms associated with tendon healing in patients at least 1 year after surgical repair of RCTs. Only population-based, peer-reviewed case-control studies[61] that were available in full text and the English language were included. Studies on nonhuman species were excluded as the genetic profile of animals cannot be expanded to form conclusions about human disease.[74,84] Studies that selected controls based on a known diagnosis of impingement syndrome, adhesive capsulitis, or other inflammatory shoulder diseases were excluded as these conditions have been associated with RCTs, which can confound results.[1,26,31,41] Studies were included irrespective of the cause or known duration of RCTs, as many tears have a subacute-on-chronic or chronic-on-subacute pattern, making such a classification unreliable.[81] No restraints were implemented for subject age or RCT type or size.

Information sources and literature search

We identified studies by searching the Embase, PubMed, and Web of Science electronic databases from each database's inception to July 23, 2019. Medical Subject Headings (MeSH) and Emtree index terms were developed from the concepts ''rotator cuff tear,'' ''genetics,'' and ''human,'' and synonyms of these concepts were used to search the Web of Science database. No restraints were implemented for the searches of Embase and PubMed, but Web of Science database filters were applied to exclude review articles, books, book chapters, and letters. A line-by-line search was conducted of results yielded from each database searched. Records were collated and duplicates were removed both manually and with EndNote X8 software (Clarivate Analytics, Philadelphia, PA, USA). Search strategies and their results are available in Supplementary Tables S1-S3.

Study selection

Two independent reviewers assessed each study for eligibility by reviewing the titles and abstracts; if there was uncertainty, the full-text article was reviewed. Discrepancies were resolved by consensus agreement between the 2 reviewers.

Data collection

Data extraction from eligible studies was completed on pre-specified forms and included the following: date of publication, geographic location of the study, sample size, age and sex, selection criteria, sample tissue analyzed, transcriptome or genetic analysis technique used for data analysis, statistical methods, and genetic data found within the study to be significantly associated with RCTs or RCT healing. Data on time to follow-up were also extracted for studies that assessed the outcomes of surgical repair of RCTs. One of the 2 reviewers (J.J.M. and L.-M.H.-B.) extracted data from half the studies and reviewed the data extracted by the other reviewer for accuracy.

Quality assessment

Quality assessment was performed using a modified version of the Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control studies.[92] The NOS is recommended by The Cochrane Collaboration for analysis of case-control studies.[71,94] It is widely used in the assessment of nonrandomized studies investigating genetic variations associated with musculoskeletal disease.[34,50,76,87] A score is attributed to each study based on 3 domains: selection of cases and controls, comparability between cases and controls, and exposure. Cases and controls were considered comparable if they were matched for age and sex or if no significant difference in age and sex was established. A maximum of 2 points could be awarded, 1 point each for age and sex. "Exposure" was defined as subject exposure to a certain gene expression or genotype, and a point was awarded if the genetic analysis was deemed appropriate. The criterion regarding selection of controls was removed, as a point could only be provided if cases and controls were community patients. Because subjects were required to be hospital patients undergoing surgical repair of RCTs, this criterion was deemed inappropriate. Both investigators independently applied the NOS to all eligible studies and formulated independent quality assessment scores. The reviewers discussed discrepancies in NOS scores until consensus was reached. Studies with scores of 0-3 points were considered low-quality studies; 4-6 points, moderate-quality studies; and 7-8 points, high-quality studies.[20,44]

Table I Modified version of best-evidence synthesis criteria[85]

Level of evidence	Criteria
Strong	>2 studies with high-quality assessment scores and generally consistent findings
Moderate	1 study with a high-quality assessment score AND >1 study with a moderate-quality assessment score OR >2 studies with low-quality assessment scores and generally consistent findings
Limited	1 study with a high-quality assessment score OR >2 studies with low- or moderate-quality assessment scores and generally consistent findings
Insufficient	A finding in 1 study with a moderate- or low-quality assessment score
Conflicting	Consistent findings reported by <75% of studies

Evidence was defined as "generally consistent" if ≥75% of the studies assessing a particular genetic polymorphism or gene expression pattern reported consistent findings.

Data synthesis

Owing to the heterogeneity of study designs and data synthesis across studies, a qualitative analysis was applied. Gene expression findings associated with RCTs across studies were first stratified into the molecular pathway in which they have the greatest influence within the shoulder, per Ahn et al[3]; these included extracellular matrix (ECM)–related genes, apoptotic signaling genes, immune and inflammatory response genes, and growth factor genes. Genetic findings across studies that were associated with failed healing after surgical repair of RCTs were stratified into these same molecular pathways. Polymorphisms associated with RCTs were stratified into commonly investigated candidate genes.

Qualitative analysis was further performed with a modified version of the best-evidence synthesis described by van Tulder et al.[85] This method has been previously applied in systematic reviews analyzing genetic associations in tendon injuries.[36,83] An explanation of the modified best-evidence synthesis is provided in Table I.

Results

Study selection

The results of the literature search are documented in Figure 1. A total of 259 records were identified from all 3 databases after duplicates were removed. Screening of articles against eligibility criteria by both independent investigators yielded 26 eligible studies.

Individual study characteristics and results

Of the 26 eligible studies, 8 investigated single-nucleotide polymorphisms (SNPs) for the association with RCTs.[7,12,37,47,59,69,79,81] 13 investigated the association of altered gene expression in human shoulder tissue with RCTs,[2,4,11,13,16,39,42,49,55,56,64,70,82] and 5 investigated genetic associations with healing after surgical repair of RCTs at ≥1 year following surgery.[3,27,38,72,80] Study characteristics are described in Supplementary Tables S4-S6.

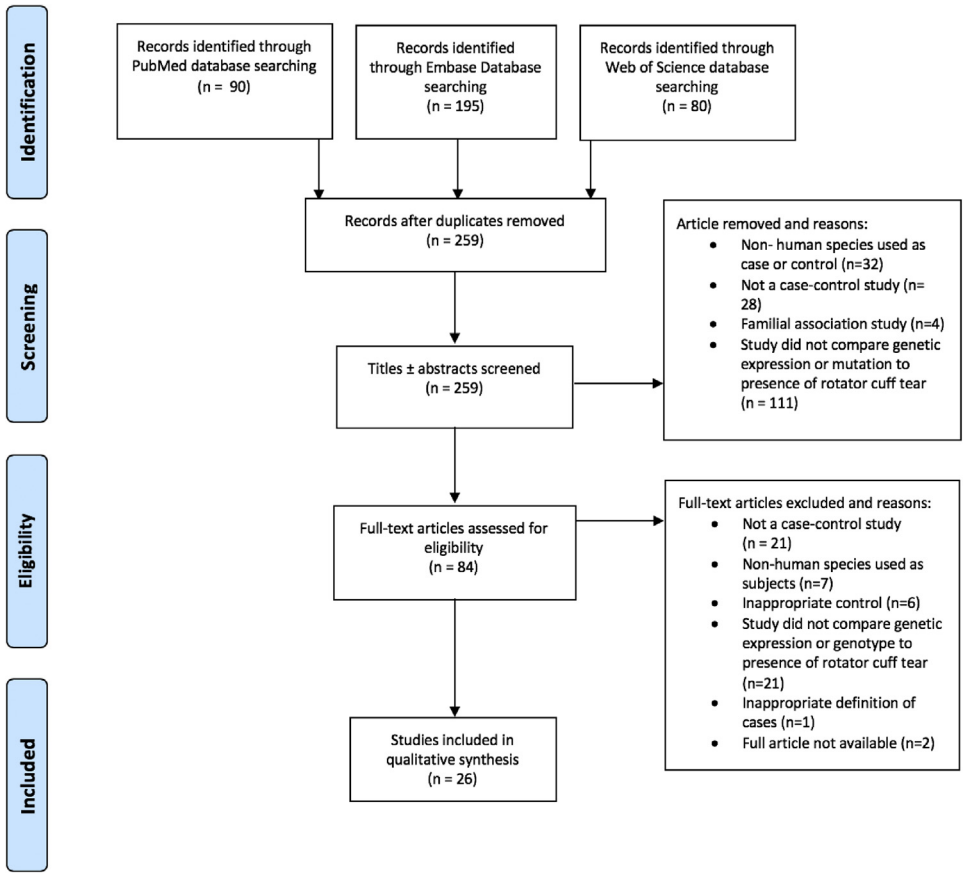

Figure 1 Flow diagram detailing systematic search process.

Quality assessment

NOS scores ranged from 3 to 8 points. Of the studies, 2 were deemed poor quality,[79,81] 15 were moderate quality,[2,4,13,27,37,39,47,49,55,56,59,64,69,70,82] and 9 were high quality.[3,7,11,12,16,38,42,47,55,70,72,80,82] These results are outlined in Table II. A further breakdown of these scores is shown in Supplementary Tables S7-S9.

All studies scored 1 point for ensuring an appropriate definition of cases, using surgical diagnosis or appropriate imaging. Almost all studies received 1 point for representativeness of cases, with this mark being awarded to studies that selected subjects receiving surgical RCT repair or patients attending clinic appointments for assessment of RCTs in which an adequate explanation of the exclusion criteria was provided.

Data synthesis

Application of the modified best-evidence synthesis found strong evidence that increased expression and mutations in the *MMP3* and *TNC* genes are associated with RCTs and failed healing after surgical repair of RCTs. Strong evidence of an association between RCTs and SNPs in *ESRRB*, as well as an association between smaller tears, improved healing outcomes after surgical repair of RCTs, and *BMP5* upregulation, was also found. There was moderate evidence of an association between smaller tears, improved healing outcomes after surgical repair of RCTs, and increased expression of *COL3*. We also found moderate evidence of increased expression of *COL5A1* and an association with RCTs, but no SNPs within this gene were associated with RCTs. Table III further details these results, including findings that were considered of limited and insufficient evidence.

Supplementary Table S10 stratifies polymorphisms associated with RCTs into commonly investigated candidate genes. Supplementary Tables S11 and S12 display the genetic association findings with RCTs and with healing after surgical repair of RCTs, respectively. The results are stratified into the molecular pathways in which the genetic findings have the greatest influence. Table IV presents commonly used acronyms.

Table II Newcastle-Ottawa Quality Assessment Scale scores for all included case-control studies

Study	Selection, points	Comparability, points	Exposure, points	Overall score, points	Quality
Genetic polymorphism studies					
Assunção et al[7]	3	2	3	8	High
Bonato et al[12]	3	2	2	7	High
Kluger et al[37]	3	0	2	5	Moderate
Longo et al[47]	3	0	2	5	Moderate
Motta et al[59]	3	1	2	6	Moderate
Peach et al[69]	2	0	2	4	Moderate
Tashjian et al[79]	2	0	1	3	Poor
Teerlink et al[81]	2	0	1	3	Poor
Gene expression studies					
Abrams et al[2]	3	0	3	6	Moderate
Akbar et al[4]	3	0	2	5	Moderate
Belangero et al[11]	3	2	2	7	High
Campbell et al[13]	3	0	2	5	Moderate
Chaudhury et al[16]	3	2	2	7	High
Kurdziel et al[39]	3	0	2	5	Moderate
Leal et al[42]	3	2	2	7	High
Lundgreen et al[49]	3	0	2	5	Moderate
Millar et al[56]	3	0	2	5	Moderate
Millar et al[55]	3	0	2	5	Moderate
Neuwirth et al[64]	3	0	2	5	Moderate
Plachel et al[70]	3	1	2	6	Moderate
Thakkar et al[82]	2	1	2	5	Moderate
Studies of genetic associations with healing after surgical repair					
Ahn et al[3]	3	2	2	7	High
Gotoh et al[27]	3	0	2	5	Moderate
Kluger et al[38]	3	2	3	8	High
Robertson et al[72]	3	2	2	7	High
Tashjian et al[80]	3	2	2	7	High

All scores were awarded using a modified version of the Newcastle-Ottawa Quality Assessment Scale for case-control studies.[92] Scores of 7-8 points indicate high quality; 4-6 points, moderate quality; and 0-3 points, poor quality.

Discussion

This review provides an up-to-date summary of evidence on the genetic association with human RCTs. Previous reviews have analyzed the current literature regarding genetic polymorphisms associated with RCTs [21,46] or considered alterations in gene expression associated with RCTs.[15] We present the first systematic review to analyze genetic polymorphisms and alterations in gene expression associated with RCTs and to consider the genetic alterations that are associated with failed healing following surgical repair. Of the 26 studies analyzed, 19 have not been included in previous reviews. The new studies included 2 investigations on genetic polymorphisms associated with RCTs,[12,69] 12 studies on alterations in gene expression associated with RCTs,[2,4,11,13,16,39,42,49,55,64,70,82] and 5 studies on genetic alterations associated with failed healing after surgical repair.[3,27,38,72,80] This review also presents the first best-evidence synthesis of studies investigating the genetic associations with RCTs, which summarizes the strength of evidence based on study quality.

The strongest evidence was found for an association between RCTs and genes that code proteins involved in the ECM signaling pathways, apoptotic signaling genes, and growth factor genes. Limited evidence was found for an association between inflammatory and immunity genes and RCTs.

ECM genes

ECM compounds are particularly important in the synthesis, maintenance, repair, and remodeling of tendons, and genetic alterations that affect these processes may influence RCTs and healing. Matrix metalloproteinases (MMPs) degrade ECM components such as collagen and have been shown to influence wound healing.[62] Across studies in this review, there was strong evidence that alterations in the *MMP3* gene are implicated in the pathogenesis of RCTs,[2,7,16,27] *MMP3* was upregulated in patients with RCTs[2,16] and patients with failure to heal following surgery.[27] These findings were further strengthened by a high-quality study by Assunção et al,[7] who found an SNP

Table III Results of modified best-evidence synthesis

Level of evidence	Key findings	Evidence
Strong	Increased expression and a mutation in the *MMP3* gene were associated with RCTs and failed healing after surgical repair of RCTs	• One moderate-quality study found *MMP3* to be upregulated in the synovium in patients with RCTs compared with those without RCTs[2] • One high-quality study found *MMP3* to be upregulated in the supraspinatus tendons in patients with RCTs compared with the supraspinatus and subscapularis tendons in patients without RCTs[16] • One moderate-quality study found *MMP3* upregulation in rotator cuff tendon tissue (unspecified) in patients with failure to heal after RCT repair compared with those with successful healing after 1 yr[27] • One high-quality study found a polymorphism in *MMP3* (rs3025058) significantly associated with RCTs[7] • One moderate-quality study found no polymorphisms within *MMP3* associated with RCTs[37]
	Increased expression and mutations in the *TNC* gene were associated with RCTs and failed healing after surgical repair of RCTs	• One moderate-quality study found 6 SNPs to be associated with RCTs[37] • One high-quality study found 8 SNPs to be associated with poor healing after repair, as well as a haplotype associated with an increased risk of a large recurrent tear after repair[38] (3 SNPs were also identified in the previous study[37]) • One high-quality study found *TNC* expression to be increased in the supraspinatus tissue in patients with RCTs compared with expression in the subscapularis tissue in those without RCTs[11]
	SNPs within the *ESRRB* gene were associated with RCTs	• One study of high quality,[12] one study of moderate quality,[59] and one study of poor quality[81] found SNPs in the *ESRRB* gene to be positively associated with the presence of RCTs • One high-quality study found certain SNPs in the *ESRRB* gene to be associated with failure to heal after repair of lateral RCTs compared with patients with successful healing after 1 yr[80]
	Upregulation of *BMP5* was associated with smaller RCTs and improved healing outcomes after surgical repair of RCTs	• One high-quality study found *BMP5* expression to be upregulated in the supraspinatus tissue in patients who presented with small RCTs compared with the supraspinatus and subscapularis tissue in those without RCTs[16] • One high-quality study found *BMP5* expression to be upregulated in the supraspinatus or infraspinatus tendons in patients with successful healing after RCT repair compared with those with failure to heal after 1 yr[3]
Moderate	Increased expression of *COL3* was associated with smaller tears and improved healing outcomes after surgical repair of RCTs	• One moderate-quality study found *COL3* expression in the supraspinatus tendons to be significantly increased in patients with RCTs compared with expression from the subscapularis tendon in patients without RCTs and significantly more increased in patients with small or moderate tears compared with patients with large or massive tears[82] • One high-quality study found increased expression of the *COL1-COL3* ratio in the supraspinatus tendon to be significantly associated with failed healing of the rotator

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Table III Results of modified best-evidence synthesis (*continued*)

Level of evidence	Key findings	Evidence
		cuff after surgical repair compared with patients with successful healing after 1 yr[72]
	Increased expression of *COL5A1* was associated with RCTs, but no SNPs within this gene were associated with RCTs	• One high-quality study found an increase in the expression of *COL5A1* in the supraspinatus tissue in patients with RCTs compared with expression in the subscapularis tissue in those without RCTs[11] • Two moderate-quality studies found no association of the presence of RCTs and polymorphisms within the *COL5A1* gene[37,47]
Limited	Three high-quality studies found upregulation of certain genes associated with RCTs	• Increased expression of *COL1A2*, *FN1*, and *TGFBR1* in the supraspinatus tissue in patients with RCTs compared with expression in the subscapularis tissue in those without RCTs[11] • Increased expression of *MMP12*, *MMP15*, *MMP21*, *MMP25*, *ADAMT12*, *ADAMT15*, *ADAMT22*, *IL3*, *IL10*, *IL13*, and *IL15* in the supraspinatus tissue in patients with small and larger RCTs compared with expression in the supraspinatus and subscapularis tissue in patients without RCTs[16] • Increased expression of aggrecan, FOX-F2, type XXIV collagen, and type XXVII collagen in the supraspinatus tissue in patients with large RCTs compared with expression in the supraspinatus and subscapularis tissue in those without RCTs[16] • Increased expression of *TIMP2* and *TIMP3* in the supraspinatus tissue in patients with RCTs compared with those without RCTs[42]
	One high-quality study found alterations in gene expression in supraspinatus or infraspinatus tissue associated with successful healing (controls) compared with failed healing (cases) after surgical repair of RCTs at 1-yr follow-up	• Increased expression of *LPL*, *CIDEC*, *MGST1*, *PPARGC1A*, *NTRK3*, *C6*, *HP*, and *LTB4R2* in controls[3] • Decreased expression of *COL5A2*, *HMCN1*, *LOXL1*, *ADAMTS2*, *SELPLG*, *ARRB2*, *SLC7A8*, *LY86*, *NAIP*, *ARRB2*, *LY96*, *STAT1*, *DSG2*, *FLNC*, *EPHB2*, *EFNA4*, *CTSB*, *SDK1*, *SECTM1*, *LY86*, *SERINC5*, *NAIP*, *C1QB*, *APOBEC3C*, *CD276*, *VSIG4*, *PDCD1LG2*, *IRF8*, *C1QC*, *TLR8*, *IL32*, *FCER1G*, *LY96*, *CYBB*, *STAT1*, *IGSF6*, *PPAPDC1A*, *CTSS*, *HLADQA2*, *NMI*, and *NAIP* in controls[3]
	One high-quality study found alterations in the expression of certain genes in subjects with failed healing compared with those with successful healing at 1 yr after surgical repair of RCTs	• Decreased expression of biglycan in the supraspinatus tendons[72] • Increased expression of *COX2* in the subacromial bursa[72]
	Increased expression of *TIMP1* was associated with failed healing after surgical repair of RCTs, but no SNPs within this gene were associated with RCTs	• One moderate-quality study found increased expression of *TIMP1* from rotator cuff tendon tissue (unspecified) in patients with failed healing after surgical repair of RCTs compared with those with successful healing after 1 yr[27] • One moderate-quality study found no SNPs in the *TIMP1* gene to be associated with RCTs[37]
	Decreased expression of *COL9A3*, *IL1*, *IL8*, *IL11*, *IL18*, *IL27*, and *SAA* was associated with RCTs	• One high-quality study found downregulation of *COL9A3*, *IL1*, *IL8*, *IL11*, *IL18*, *IL27*, and *SAA* in the supraspinatus tissue in patients with RCTs compared with expression in the supraspinatus and subscapularis tissue in those without RCTs[16]
Insufficient	Four moderate-quality studies found upregulation of certain genes in patients with RCTs compared with those without RCTs	• Increased expression of *IL16* from the synovial tissue[2]

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Table III Results of modified best-evidence synthesis (*continued*)

Level of evidence	Key findings	Evidence
		• Increased expression of type II collagen, type X collagen, *BMP7*, *BMP2*, and *BFGF* from the subacromial bursal tissue[64]
		• Increased expression of *MMP2*, *RUNX2*, and *P2RX7* from the subscapularis tissue[70]
		• Increased expression of *HSP27*, *HSP70*, *CFLIP*, caspase 3, and caspase 8 in the torn supraspinatus and matched subscapularis tissue in patients with RCTs compared with the intact subscapularis tissue in those without RCTs[56]
	Two moderate-quality studies found downregulation of certain genes in patients with RCTs compared with those without RCTs	• Decreased expression of *ADAMTSA*, *SCGE*, *ITGAV*, and *HAS1* in the long head of the biceps tissue[39]
		• Decreased expression of *HDAC1*, *MDM4*, *PPM1D*, and *NFKB* in the torn supraspinatus tissue of tears compared with the intact subscapularis tissue in patients without RCTs[49]
	Three moderate-quality studies compared the gene expression between torn supraspinatus tissue (torn tissue), intact subscapularis tissue from the same patient (early tendinopathy model), and intact subscapularis tissue from patients without RCTs (control); upregulation of certain genes was found in the model of early tendinopathy compared with both the torn tendon and control	• Increased expression of *HMGB1* in the early tendinopathy model compared with torn tendon and control[2]
		• Increased expression of *IL21R* in the early tendinopathy model compared with torn tendon and control[11]
		• Increased expression of *IL17A* in the early tendinopathy model compared with torn tendon and control[55]
	SNPs in the *ANKH* and *TNAP* genes were associated with the risk of RCT arthropathy[69]	• Findings of 1 moderate-quality study[69]
	No SNPs in the *MMP2* gene were associated with RCTs[37]	• Findings of 1 moderate-quality study[37]
	SNPs within *SASH1* and *SAP30BP* were associated with RCTs[79]	• Findings of 1 poor-quality study[79]
Conflicting	Upregulation or downregulation of *MMP10* and *MMP13* may be associated with RCTs, but no SNPs within the *MMP13* gene were associated with RCTs	• One high-quality study demonstrated *MMP10* and *MMP13* to be upregulated in the supraspinatus tissue in patients with RCTs compared with expression in the supraspinatus and subscapularis tissue of those without RCTs[16]
		• One high-quality study showed *MMP13* to be downregulated in the supraspinatus tissue in patients with RCTs compared with expression in the supraspinatus tissue of those without RCTs[42]
		• One moderate-quality study showed *MMP10* to be downregulated in the long head of the biceps tissue in patients with RCTs compared with those without RCTs[39]
		• One moderate-quality study found no SNPs in the *MMP13* gene to be associated with RCTs[37]
	Upregulation of *MMP1* and *MMP9* may either be associated with RCTs or assist with RCT healing, but no SNPs in the *MMP9* gene were associated with RCTs	• One high-quality study found expression of *MMP1* and *MMP9* was decreased in supraspinatus tendon samples from patients with current RCTs compared with those without RCTs[42]
		• One high-quality study found expression of *MMP1* and *MMP9* was increased in the supraspinatus tendons in patients with failure to heal after repair compared with those with successful healing[72]
		• One moderate-quality study found no SNPs in the *MMP9* gene to be associated with RCTs[37]

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Table III Results of modified best-evidence synthesis (*continued*)

Level of evidence	Key findings	Evidence
	Increased expression of *TGFB1* from the subacromial bursa was associated with RCTs, but no association was found between *TGFB1* expression from an intact subscapularis tendon and supraspinatus RCTs	• One moderate-quality study showed *TGFB1* to be upregulated in the subacromial bursa in patients with RCTs compared with those without RCTs[64] • One moderate-quality study showed no significant difference in *TGFB1* expression from intact subscapularis tendons between patients with and without RCTs of the supraspinatus tendon[70]
	SNPs within the *DEFB1*, *FGF3*, *FGF10*, and *FGFR1* genes may or may not be associated with RCTs	• One moderate-quality study found SNPs within these genes to be associated with RCTs[59] • One poor-quality study found no SNPs within these genes to be associated with RCTs[81]

The results were formulated following application of the modified best-evidence synthesis criteria (Table I). The level of evidence of key findings was determined based on the quality assessment scores of individual studies (Table II).
RCT, rotator cuff tear; *SNP*, single-nucleotide polymorphism.

in *MMP3* (rs3025058) significantly associated with RCTs. In contrast, 1 moderate-quality study found no significant association between any SNPs in *MMP3* and RCTs.[37] Notably, the average age in this study was 71 ± 7.1 years for cases and 87 ± 5.7 years for controls. These patients are much older than the patients analyzed by Assunção et al (54 ± 6 years for cases and 53 ± 6 years for controls) and much older than those usually considered for surgical repair.[25] Therefore, these findings may not translate to a typical clinical setting but could indicate that the role of *MMP3* in RCTs may dissipate with age. In other conditions such as arthritis and cancer, dysregulated MMPs have been shown to contribute to disease development.[45] The inhibition of MMPs is currently being investigated for cancer treatment,[14,93] which might have future implications for treating RCTs.

Tenascin C is an ECM glycoprotein that modulates cell adhesion.[37] It was shown to be upregulated in torn supraspinatus tissue in 1 high-quality study.[11] As tenascin C promotes cell attachment and adhesions in newly formed tissue, a defect in its action could play a role in both the formation and healing status of RCTs.[37] This review demonstrates strong evidence that SNPs within *TNC*, the gene that encodes this protein, are associated with—and may play a role in the pathogenesis of—RCTs. However, it cannot be concluded that these SNPs are the cause of alterations in tenascin C expression, as studies were completed in different patient cohorts. One moderate-quality study found 6 SNPs (rs1138545, rs3789870, rs10759753, rs72758637, rs7021589, and rs7035322) in the *TNC* gene to be associated with the presence of RCTs.[37] Another high-quality study found that 3 of the previously recognized SNPs (rs2104772, rs11793430, and rs953288), as well as 5 other SNPs (rs1138545, rs72758637, rs7021589, rs10759752, and rs72758634) in the *TNC* gene, were also associated with failed RCT healing after surgical repair.[38] Moreover, the combination of the C allele at rs1138545, the A allele at rs2104772, and the G allele at rs10759752 was found to be associated with an increased risk of large recurrent tendon tearing after RCT repair. These SNPs could act as potential biomarkers to predict both the risk of tear and the risk of failure to heal after surgical treatment. As it is not known how these SNPs alter *TNC* expression or function, future studies should assess for expression of tenascin C in RCT patients, assess for associated SNPs in the *TNC* gene, and determine any alterations in tenascin C function within a single cohort of patients.

There was moderate evidence that changes in collagen expression are associated with RCTs, particularly type III collagen (*COL3*) and the α1 chain of type V collagen (*COL5A1*). *COL5A1* expression from the torn supraspinatus edge was increased in 1 high-quality study.[11] *COL5A1* forms part of type V collagen and is known to assemble and form fibrils with type I collagen.[78] We hypothesize that the upregulation of *COL5A1* forms a normal, physiological element of the repair response at the torn edge of the tendon.[11] Future studies should carefully record the part of a tendon undergoing biopsy, as expression may differ within the intact part of a torn tendon. Certain mutations within *COL5A1* are associated with Achilles tendinopathy[57] and Ehlers-Danlos syndrome and contribute to poor wound healing.[23] However, 2 moderate-quality studies found no association with any SNPs in the *COL5A1* gene and RCTs, suggesting mutations within this gene do not contribute to RCTs.[37,47]

The upregulation of *COL3* was greatest in patients with smaller tears,[82] and downregulation of *COL3* (in relation to *COL1*) was noted to be associated with failed healing after surgical repair.[72] These findings support the hypothesis that *COL3* is required in the initial healing

Table IV Commonly used acronyms

Acronym	Expansion
ADAMT12	A disintegrin and metalloproteinase with thrombospondin motifs 12
ADAMT15	A disintegrin and metalloproteinase with thrombospondin motifs 15
ADAMT22	A disintegrin and metalloproteinase with thrombospondin motifs 22
ADAMTS1	A disintegrin and metalloproteinase with thrombospondin motifs 1
ADAMTS2	A disintegrin and metalloproteinase with thrombospondin motifs 2
ANKH	ANKH inorganic pyrophosphate transport regulator
APOBEC3C	Apolipoprotein B mRNA editing enzyme catalytic subunit 3C
ARRB2	Arrestin β2
ARRB2	Arrestin β2
bFGF	Basic fibroblast growth factor, fibroblast growth factor 2
BMP2	Bone morphogenetic protein 2
BMP5	Bone morphogenetic protein 5
BMP7	Bone morphogenetic protein 7
C1QB	Complement component 1, Q subcomponent, β polypeptide
C1QC	Complement C1q C chain
C6	Complement 6
cFLIP	CASP8 and FADD–like apoptosis regulator
CIDEC	Cell death–inducing DFFA-like effector C
COL1	Type I collagen
COL1A2	Type II collagen α2 chain
COL3	Type III collagen
COL5A1	Type V collagen α1 chain
COL5A2	Type V collagen α2 chain
COL6	Type VI collagen
COL9A3	Type IX collagen α3 chain
COX2	Cytochrome C oxidase subunit II
CTSB	Cathepsin B
CTSS	Cathepsin S
CYBB	Cytochrome B-245 β chain
DEFB1	Defensin β1
DSG2	Desmoglein 2
EPHB2	EPH receptor B2
ESRRB	Estrogen-related receptor β
FCER1G	Fc receptor, IgE, high affinity I, gamma polypeptide
FGF10	Fibroblast growth factor 10
FGF3	Fibroblast growth factor 3
FGFR1	Fibroblast growth factor receptor 1
FLNC	Filamin C, gamma
FN1	Fibronectin 1
FOXF2	Forkhead box F2
HAS1	Hyaluronan synthase 1
HDAC1	Histone deacetylase 1
HLADQA2	Major histocompatibility complex, class II, DQ α2
HMCN1	Hemicentin 1
HMGB1	High mobility group box 1
HP	Haptoglobin

(*continued on next column*)

Table IV Commonly used acronyms (*continued*)

Acronym	Expansion
HSP27	Heat shock protein 27
HSP70	Heat shock protein 70
IGSF6	Immunoglobulin superfamily member 6
IL1	Interleukin 1
IL10	Interleukin 10
IL13	Interleukin 13
IL15	Interleukin 15
IL17A	Interleukin 17A
IL21R	Interleukin 21 receptor
IL3	Interleukin 3
IL32	Interleukin 32
IL6	Interleukin 6
IL8	Interleukin 8
IRF8	Interferon regulatory factor 8
ITGAV	Integrin subunit α V
LOXL1	Lysyl oxidase–like 1
LPL	Lipoprotein lipase
LTB4R2	Leukotriene B4 receptor 2
LY86	Lymphocyte antigen 86
LY96	Lymphocyte antigen 96
MDM4	MDM4 regulator of p53
MGST1	Microsomal glutathione S-transferase 1
MKX	Mohawk homeobox
MMP1	Matrix metallopeptidase 1
MMP10	Matrix metallopeptidase 10
MMP12	Matrix metallopeptidase 12
MMP13	Matrix metallopeptidase 13
MMP15	Matrix metallopeptidase 15
MMP2	Matrix metallopeptidase 2
MMP21	Matrix metallopeptidase 21
MMP25	Matrix metallopeptidase 25
MMP3	Matrix metallopeptidase 3
MMP9	Matrix metallopeptidase 9
MRC1	Mannose receptor C–type 1
NAIP	NLR family apoptosis inhibitory protein
NFkB	Nuclear factor κB subunit 1
NMI	N-Myc and STAT interactor
NTRK3	Neurotrophic tyrosine kinase, receptor, type 3
P2RX7	Purinergic receptor P2X 7
PDCD1LG2	Programmed cell death 1 ligand 2
PPAPDC1A	Phosphatidic acid phosphatase type 2 domain containing 1A
PPARγ	Peroxisome proliferator-activated receptor γ
PPARGC1A	Peroxisome proliferative activated receptor, γ, coactivator 1 α
PPM1D	Protein phosphatase, $Mg2^{+}$/$Mn2^{+}$ dependent 1D
RCT	Rotator cuff tear
RUNX2	RUNX family transcription factor 2
SAA	Serum amyloid A cluster
SAP30BP	Sap30 binding protein
SASH1	SAM and SH3 domain containing 1
SCGE	Sarcoglycan ε
SDK1	Sidekick cell adhesion molecule 1
SECTM1	Secreted and transmembrane 1
SELPLG	Selectin P ligand
SERINC5	Serine incorporator 5

(*continued on next page*)

Table IV Commonly used acronyms (*continued*)

Acronym	Expansion
SLC7A8	Solute carrier family 7 (amino acid transporter light chain, l system), member 8
SNP	Single-nucleotide polymorphism
STAT1	Signal transducer and activator of transcription 1
TGFB1	Transforming growth factor β1
TGFBR1	Transforming growth factor β receptor 1
TIMP1	Tissue inhibitor of metalloproteinase 1
TIMP2	Tissue inhibitor of metalloproteinase 2
TIMP3	Tissue inhibitor of metalloproteinase 3
TLR3	Toll-like receptor 3
TLR8	Toll-like receptor 8
TNAP	Alkaline phosphatase, biomineralization associated
TNC	Tenascin C
VSIG4	V-set and immunoglobulin domain containing 4

mRNA, messenger RNA; *DFFA*, DNA fragmentation factor α; *ANKH*, Progressive ankylosis protein homolog; *CASP8*, Caspase 8; *FADD*, Fas-associated protein with death domain; *EPH*, Ephrin; *Fc*, Fragment crystallizable, *IgE*, Immunoglobulin E; *MDM4*, Mouse double minute 4 human homolog; *NLR*, Nucleotide oligomerization domain (NOD)-like receptor; *STAT*, Signal transducer and activator of transcription; *RUNX*, Runt domain-containing; *SAM*, s-adenosylmethionine synthase; *SH3*, Src homology 3.

process, providing extensive cross-links and forming the scaffold for repair, before gradually being replaced by *COL1*.[72] Given the potential healing benefits, a mechanism to stimulate *COL3* expression in patients with small tears or in patients early after surgical RCT repair could be a useful target for investigation. In vitro studies have found upregulation of *COL1* and *COL3* within human RCT models following exposure to IL-17A[55] and within human skin fibroblasts following exposure to a tocotrienol-rich fraction.[53] In vivo studies are required to determine whether such agents impact RCT clinical outcomes.

Apoptotic signaling genes

Apoptosis is the process of programmed cell death. If this process is pathologically upregulated within tenocytes of rotator cuff tissue, the tendon will weaken and become more prone to tear.[67] The *ESRRB* (estrogen-related receptor β) gene transcribes a nuclear receptor that upregulates transcription of *HIF* (hypoxia-inducible factor) and contributes to apoptosis in hypoxic environments, such as degenerated tendon tissue.[5,81] Dysregulation of *ESRRB* may result in increased apoptosis of musculoskeletal tissue, particularly in the rotator cuff tendons.[80] It is interesting to note that we observed strong evidence that polymorphisms within the *ESRRB* gene are associated with RCTs [12,59,81] and a poorer prognosis following surgical repair.[80] The SNPs associated with RCTs included rs17583842,[81] rs4903399, and rs1676303[59] (specifically the genotype TT at rs1676303[12]). The SNP rs17583842 was also found to be associated with a failure to heal after surgical repair of RCTs.[80] These SNPs could form potential biomarkers to predict the risk of RCTs and surgical outcomes after repair.

One high-quality study investigated alterations in gene expression that were associated with healing vs. failure to heal after surgical repair of RCTs.[3] A significant decrease in the expression of 5 apoptotic genes was seen in the healed controls compared with unhealed cases: *LY86*, *NAIP*, *ARRB2*, *LY96*, and *STAT1*. However, one ''apoptotic'' gene, *CIDEC* (cell death–inducing DFFA [DNA fragmentation factor α]–like effector C), was upregulated in healed patients. Despite being postulated to induce apoptosis in preadipocytes, the function of *CIDEC* in other cells is unknown.[63] It is interesting to note that mutations in this gene have been linked to insulin resistance.[63] As there is an established association between RCTs and diabetes mellitus, a complication of insulin resistance,[30] future studies could investigate associations between gene expression and SNPs within *CIDEC*, RCTs, and subjects with insulin resistance or diabetes mellitus.

Immune and inflammatory response genes

Dysregulated inflammation and the associated immune response within the rotator cuff tendons and surrounding tissue can perpetuate tendon damage and apoptosis and sensitize nociceptors.[8] Five moderate-quality studies[2,4,13,55,70] and 3 high-quality studies[3,16,72] demonstrated significant associations between gene expression and RCTs or RCT healing after surgical repair. All studies identified new genetic associations, with no commonality in findings regarding specific genes across any studies. Thus, these findings were considered of limited evidence after the modified best-evidence synthesis was performed.

The heterogeneity of results can be attributed to a number of potential causes. This includes samples being taken from patients with RCTs of varying chronicity. As the inflammatory response will vary depending on the time course of disease,[51] different inflammatory and immunity genes will be expressed at different time points. Furthermore, biopsy specimens were taken from various tissue types and locations across studies, including the supraspinatus,[2-4,13,16,55,72] subscapularis,[2,4,13,16,55,70,72] synoviu m,[72] infraspinatus,[3] and subacromial bursa.[72] Expression of inflammatory and immune genes is likely to differ depending on the location and proximity of the biopsy tissue to the tear. Smoking,[11,42] active infection, autoimmune conditions,[12,59] long-term use of anti-inflammatory medication,[89] steroid medication use,[10] and statin use[24,90] all alter the expression of genes influencing the inflammatory and immune pathways. However, only 2 studies

investigating gene expression associated with healing outcomes of surgical RCT repair[38,72] and 9 studies investigating gene expression associated with RCTs[2,4,11,13,39,42,49,55,70] identified 1 or more of these variables within the cohort, stratified subjects for analysis, or excluded subjects on the basis of such variables.

One high-quality study found increased *COX2* expression in the subacromial bursa of patients with failure to heal at 1 year after surgical repair of RCTs.[72] No other studies assessed the same biopsy tissue for the same outcome or found associations between *COX2* expression and RCTs, rendering this finding of limited evidence. Despite the limitations to this evidence, these results suggest that bursal inflammation may impair RCT healing. In contrast, previous studies found that early use of nonsteroidal anti-inflammatory drugs and cyclooxygenase (COX) 2 inhibitors in RCT management after surgery resulted in reduced tendon-to-bone healing in humans, rats, and rabbits.[17,48,65] Increased COX-2 activity, induced with atorvastatin treatment, was also shown to improve tendon healing in RCTs in rats.[24] Further investigation is required to determine the role of *COX2* expression and inhibition in healing following surgical repair of RCTs. It is important that all future studies document patients who are receiving COX-inhibiting and statin medications.

Growth factor genes

Across studies, 18 genes the modulate growth factors were found to be associated with RCTs, with strong evidence of an association between RCT healing and *BMP5*. *BMP5* is a bone morphogenetic protein known to promote chondrocyte differentiation, and mutations within this gene have been associated with osteoarthritis.[75] The upregulation of *BMP5* appears to be necessary in the physiological repair pathway required for RCT healing. One high-quality study found *BMP5* to be upregulated in torn supraspinatus or infraspinatus tendon tissue that successfully healed after surgical repair of RCTs at 1 year, as compared with tendons with failed healing.[3] Another high-quality study found upregulation of *BMP5* in the supraspinatus tissue in patients with small RCTs (<3 cm) compared with supraspinatus and subscapularis tissue in those without RCTs.[16] Future research could consider evaluating the effects of the local delivery of isolated BMP5 to RCTs, as well as attempt to further determine the molecular impacts of this growth factor on tendons following an RCT.

Transforming growth factor (TGF) β1 stimulates wound healing by inducing the expression of ECM proteins and inhibiting ECM degradation. It also plays a role in the control and suppression of the immune system through the inhibition of T-lymphocyte proliferation.[58,88] One moderate-quality study found increased expression of *TGFB1* in the subacromial bursal tissue of cases with RCTs compared with those without RCTs,[64] but another moderate-quality study found no difference in the expression of *TGFB1* in intact subscapularis tendon tissue between cases (with RCTs of the supraspinatus tendon) and controls (with no RCTs).[70] Association is not causation, so it is unclear whether subacromial bursa expression is affecting RCTs or healing. It is also not known whether *TGFB1* expression is altered within torn tendons. However, 1 high-quality study did find increased expression of *TGFBR1*, the gene that encodes the TGF-β1 receptor, within torn supraspinatus tendons compared with intact subscapularis tendons.[11] Prior to considering TGF-β1 for use within new treatments, it must be determined whether *TGFB1* expression from the subacromial bursa is altered in patients with successful healing after RCT repair, compared with those with failed healing. Further studies could also determine whether alterations in *TGFB1* expression occur within damaged tendons.

Study quality

Confounding factors, errors, and sources of bias within included studies may have affected individual study results. The incidence of RCTs increases with age, as do substantial changes in genetic instability, gene expression, and epigenetics.[59,86] However, only 4 of the studies assessing genetic polymorphisms,[7,12,47,59] 4 of the studies assessing changes in gene expression,[11,16,42,82] and 3 of the studies assessing healing outcomes of RCTs [3,72,80] established no significant age difference between groups, or age-matched cases and controls. The variable of age could also be analyzed separately, which may identify key genes that are implicated in different age groups with RCTs. This principle can also be applied to the variable of sex. Only 3 of the studies assessing genetic polymorphisms,[7,12,47] 4 of the studies assessing changes in gene expression,[11,16,42,70] and 4 of the studies assessing healing outcomes of RCTs [3,38,72,80] matched, or corrected for, sex. As RCTs are more common in male patients, the failure to recognize this confounder could have influenced results.[42]

There were also limitations regarding the method of sampling within studies, with 4 studies failing to define the size of the RCTs being investigated.[12,47,64,69] This fails to recognize RCTs as a disease spectrum with potential differences in pathogenesis.[16] Furthermore, 7 studies that analyzed the gene expression profile associated with RCTs[4,11,13,16,55,56,82] and 3 studies that analyzed gene expression associations with healing after repair[3,27,72] compared different biopsy tissue between cases (often supraspinatus) and controls (often subscapularis). The loading and structure of these 2 tendons differ, which could result in differences in gene expression to the same environmental trigger, confounding findings.[56] The modality by which an RCT was diagnosed varied, including magnetic resonance imaging, ultrasonography, and findings at the time of surgery. Controls also varied from individuals undergoing

surgery for another reason (in whom the absence of an RCT was confirmed),[4,13,16,39,49,55,56,64,82] controls with imaging evidence of the absence of an RCT,[3,7,27,37,38,47,72,80] and controls who were both surgically and radiographically confirmed to have no RCT.[2,7,11,42,70] However, some controls were defined only by the absence of RCT symptoms,[12,59] and 3 studies used community controls, failing to record any attempt to screen for RCTs.[69,79,81] The latter controls are particularly problematic, as up to 40% of individuals aged $\geq$ 50 years have asymptomatic RCTs.[21]

A bias common to all almost all included studies, aside from 4 genome-wide association studies,[3,16,27,79,81] was the selection of the candidate genes or SNPs prior to the studies.[68,95] This narrows the scope of the findings and is likely to bias statistical analyses toward finding significance. It also creates publication bias, as genes or SNPs that do not reach a level of significant association are unlikely to be further studied.[61,95] With the exception of 3 studies,[2,7,38] the nonresponse rate was either not stated or was not equivalent for cases and controls. This introduces a risk of bias, particularly for failed follow-up in subjects after surgical repair.

Limitations

This systematic review has limitations. First, in using the modified NOS for case-control studies to assign individual study quality scores, we failed to consider some elements that may impact the quality of a study. For example, NOS points for comparability were only provided if the study matched or established no difference between cases and controls for age (1 point) and/or sex (1 point). Thus, a range of other confounders that could deem cases and controls less comparable were not considered when applying a study score. Second, the best-evidence synthesis model formed conclusions from studies that were heterogeneous in nature. To combat these limitations, earlier in the ''Discussion'' section, we have described the differences between the designs of studies that formed the key findings.

Conclusion

Genetic alterations in the ECM, cell apoptosis, immune and inflammatory responses, and growth factor pathways are correlated with RCTs. In particular, there is strong evidence of an association between RCTs and the genes *MMP3*, *TNC*, *ESRRB*, and *BMP5*. Identifying genetic alterations in patients may assist in predicting individual healing potential after surgical repair. These alterations also present potential pharmaceutical targets for treating RCTs. Future studies could consider the recommendations and hypotheses made within this review and prioritize thorough documentation and analysis of participant characteristics.

Disclaimer

The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jse.2020.07.045.

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