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Evidence of genetic variations associated with rotator cuff disease

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Background: Rotator cuff disease (RCD) is a complex process influenced by a multitude of factors, and a number of gene pathways are altered in rotator cuff tears. Polymorphisms in these genes can lead to an extended tendon degeneration process, which explains why subsets of patients are more susceptible to RCD.

Materials and methods: Twenty-three single-nucleotide polymorphisms within 6 genes involved in repair and degenerative processes (*DEFB1, DENND2C, ESRRB, FGF3, FGF10,* and *FGFR1*) were investigated in 410 patients, 203 with a diagnosis of RCD and 207 presenting with absence of RCD. Exclusion criteria were patients older than 60 years and younger than 45 years with a history of trauma, rheumatoid arthritis, autoimmune syndrome, pregnancy, and use of corticosteroids. Genomic DNA was obtained from saliva samples. Genetic markers were genotyped with TaqMan real-time polymerase chain reaction. The χ^2 test compared genotypes and haplotype differences between groups. Multivariate logistic regression analyzed the significance of many covariates and the incidence of RCD.

Results: Statistical analysis revealed female sex ($P = .001$; odds ratio, 2.07 [1.30-3.30]) and being white ($P = .002$; odds ratio, 1.88 [1.21-2.90]) to be risk factors for RCD development. A significant association of haplotypes CCTTCCAG in *ESRRB* ($P = .05$), CGACG in *FGF3* ($P = .01$), CC in *DEFB1* ($P = .03$), and *FGFR1* rs13317 ($P = .02$) with RCD could be observed. Also, association between *FGF10* rs11750845 ($P = .03$) and rs1011814 ($P = .01$) was observed after adjustment by ethnic group and sex.

Conclusions: Our work clearly supports the role of *DEFB1, ESRRB, FGF3, FGF10,* and *FGFR1* genes in RCD. Identification of these variants can clarify causal pathways and provide a clue for therapeutic targets.

Level of evidence: Level III, Cross Sectional Study, Epidemiology Study.

Keywords: Rotator cuff disease; tendon; polymorphism; haplotype; genetic; degenerative process

This study was approved under the protocol number 0024.0.305.000-11 by the Bioethics Committee of the National Institute of Traumatology and Orthopaedics, Brazil, and it was in accordance with the Ethical Principles established by the Resolution 196/96 from the National Health Council.

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Rotator cuff disease (RCD) is a spectrum of disorders varying from reversible tendinopathy to frank tear[26]; it is a frequent cause of pain and shoulder disability, affecting 30% to 50% of the population older than 50 years.[31] However, despite advances in imaging and surgery, RCD has multiple causes,[10] including genetic, and the high failure rate after repair is still worrisome.[28] It has been suggested that rotator cuff tears or tendinopathies are not a purely mechanical phenomenon but also involve underlying biochemical changes classified as intrinsic degeneration.[22] The cellular, vascular, and extracellular matrix composition of the tendon edge as well as its metabolism and viability is altered.[22]

Despite the previously related risk factors associated with RCD, such as the aging process[25] and a history of trauma,[38] there is still the question of whether genetic variation among individuals predisposes to RCD. A study[29] correlated variants within pyrophosphate metabolism genes and rotator cuff tear arthropathy. However, whether these variants are in strong linkage disequilibrium with actual disease-causing mutations remains to be established.[6,13,14,18,21]

Fibroblast growth factors (FGFs) play a critical role in angiogenesis and mesenchymal cell mitogenesis and may also modulate rotator cuff repair. Various models have suggested improved tendon healing with the addition of basic FGF,[4,34,35] which demonstrated a significant increase in enthesis strength and tendon maturity in rats.[15] FGFs mediate their cellular responses by binding to and activating a family of 4 receptor tyrosine kinases (FGF receptors FGFR1-FGFR4) that display different biologic functions.[16] In addition, FGF expression stimulates the production of collagen in the meniscus in sheep,[9] indicating that genes encoding FGF are associated with collagen synthesis and turnover.

In a recent report,[37] the involvement of β-defensin with progressive muscle degeneration in mice was identified. This protein is encoded by *DEFB1* and is constitutively expressed by a wide variety of tissues.[42] Defensins could act on diverse immune cells through Toll-like receptor 4, regulating the entire immune response.[39] Several diseases have been associated with polymorphisms in *DEFB1*,[32] including muscular dystrophy[37] and cystic fibrosis.[7] However, the function of *DEFB1* in degenerative processes related to RCD was not previously studied.

In spite of the fact that tendons are relatively avascular, degenerate shoulder tendons display evidence of hypoxia.[40] The enthesis is poorly vascularized in all tendons, as is the so-called critical zone where the majority of rotator cuff tears take place.[20] Studies have found high levels of hypoxia-inducible factor (HIF) in torn rotator cuffs.[20] *ESRRB* (estrogen-related receptor β) has been identified as an essential cofactor of HIF in mediating the adaptation to this hypoxic environment.[2] These data suggest that hypoxia is a relevant damage factor in tendon injury and that appropriate vascular response may be essential for normal repair.[20]

There is evidence that genetic factors act as intrinsic risk factors for rotator cuff tendon injury[14] and that subsets of patients have increased genetic susceptibility to RCD.[6] Greater knowledge about gene factors related to RCD may offer greater insight into the tendon disease process and help identify therapeutic targets, providing better strategies to optimize outcomes of rotator cuff therapy. On the basis of *FGF3, FGF10, FGFR1, ESRRB,* and *DEFB1* functions and their possible participation in multiple pathways involved in the tendon-muscle intrinsic degeneration, the purpose of this study was to investigate whether genetic variants within these genes are correlated with RCD.

Materials and methods

Subject selection

All consecutive patients aged 45 to 60 years from both sexes referred during 1 year to the Specialized Care Center of Shoulder and Elbow from our Institute with a clinical complaint of pain in the shoulder joint and further diagnosed as having RCD were asked to participate in the study. They underwent routine consultations in the Shoulder and Elbow Center of the National Institute of Traumatology and Orthopaedics and were included in the study after signature of an informed consent document. Patients with a history of trauma, bursitis, rheumatoid arthritis, autoimmune diseases, pregnancy, chronic use of systemic corticosteroids, and hyperlaxity were excluded. Data on medical history and smoking habits were collected. RCD was diagnosed in 203 patients; 207 volunteers (caregivers or relatives of patients hospitalized in our institution) without RCD, as diagnosed after clinical examination and anamneses, showing absence of pain in the shoulder joint or any other joint were recruited as controls. The baseline clinical parameters for the subject population are shown in Table I.

Diagnosis of rotator cuff disease

The diagnosis of RCD was established by clinical examination and imaging (radiography and magnetic resonance imaging) of the involved shoulder. Tendinosis, partial-thickness cuff tear, and full-thickness cuff tear (even in a single tendon or a massive tear) were considered for the diagnosis of RCD. The control group inclusion criteria were absence of history of shoulder pain, negative specific test result for impingement syndrome[27] in a complete physical examination of the shoulders, and absence of tendinopathy in other joints. All clinical evaluations were performed by one of the authors (M. V. A.) from the Specialized Care Center.

DNA collection and purification

Genomic DNA was obtained from saliva samples as previously described.[19] The amount and purity of the DNA were determined

Table I Baseline demographic and clinical characteristics of the study population

Variables	Total (n = 410)	Controls (n = 207)	RCD cases (n = 203)	*P* value*	Odds ratio	95% CI
	n (%)	n (%)	n (%)			
Ethnic group						
Whites	270 (65.9)	123 (59.4)	147 (72.4)	.002	1.88	1.21-2.90
Nonwhites	140 (34.1)	84 (40.6)	56 (27.6)			
Age (years)†	52.6 ± 5.1	53.5 ± 5	51.8 ± 5.1	.94	1.1—	—
Sex						
Female	295 (72.0)	135 (65.2)	160 (78.8)	.001	2.07	1.30-3.30
Male	115 (28.0)	72 (34.8)	43 (21.2)			
Smoking						
Nonsmoking	366 (89.3)	183 (88.4)	183 (90.1)	.50	1.24	0.63-2.43
Smoking	44 (10.7)	24 (11.6)	20 (9.9)			
General medical condition						
Systemic disease	107 (26.1)	87 (42.0)	20 (9.8)	.000	0.14	0.08-0.26
Diabetes	19 (4.6)	11 (5.3)	8 (3.9)	.47	0.71	0.26-1.96
High blood pressure	153 (37.3)	59 (28.5)	94 (46.3)	.0004	2.06	1.34-3.17
Hypothyroidism	1 (0.2)	0	1 (0.5)	.31	—	—
Current medication						
Any medication	239 (58.3)	139 (67.1)	100 (49.2)	.000	0.44	0.29-0.67
Antihypertensive	118 (28.8)	62 (29.9)	56 (27.6)	.47	0.86	0.54-1.34
Calcium	12 (2.9)	10 (4.8)	2 (0.9)	.01	0.19	0.03-0.94
Analgesic	4 (0.9)	2 (0.9)	2 (0.9)	.99	0.99	0.10-9.92
Antimicrobials	3 (0.7)	2 (0.9)	1 (0.5)	.55	0.49	0.02-6.97
NSAIDs	17 (4.1)	12 (5.7)	5 (2.4)	.07	0.40	0.12-1.25
SAIDs	2 (0.4)	1 (0.5)	1 (0.5)	.99	0.99	0.03-36.44
Antidepressant	8 (1.9)	4 (1.9)	4 (1.9)	.98	0.99	0.21-4.77
Bisphosphonate	5 (1.2)	4 (1.9)	1 (0.5)	.17	0.24	0.01-2.33
Muscle relaxant drug	5 (1.2)	3 (1.4)	2 (0.9)	.64	0.66	0.08-4.88

RCD, rotator cuff disease; *NSAIDs,* nonsteroidal anti-inflammatory drugs; *SAIDs,* steroidal anti-inflammatory drugs.
* χ^2 test. *P* values <.05 are considered significant.
† Mann-Whitney test.

by the use of a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All DNA samples presented $A_{260\ nm}/A_{280\ nm}$ ratios greater than 1.9.

Single-nucleotide polymorphism selection and genotyping

Twenty-three single-nucleotide polymorphisms (SNPs) in *DEFB1, DENND2C, ESRRB, FGF3, FGF10,* and *FGFR1* were selected, taking into consideration linkage disequilibrium relationships and structure of the genes. These SNPs had previously been identified and reported in the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) with minor allele frequencies >0.12. Details of the studied genetic variants are shown in Table II.

Endpoint analysis of polymerase chain reactions with TaqMan chemistry (Applied Biosystems, Foster City, CA, USA) held in total 1.5 μL/reaction were used for genotyping of the selected markers in a PTC-225 tetrad thermocycler (Peltier Thermal Cycler, Bio-Rad Life Science, Hemel Hempstead, Hertfordshire, UK). Because *DEFB1, ESRRB, FGF3,* and *FGF10* are found in the same chromosome, polymorphisms in these genes were analyzed not only individually but also in combination as haplotypes.

Statistical analyses

The sample size needed for a study with 80% power was calculated with an online calculator (http://statpages.org/proppowr.html). Nominal variables were expressed as frequencies and percentages. To access the significance of nominal variables between groups, the χ^2 test was performed. Continuous variables were expressed as mean and standard deviation. Then, after the Shapiro-Wilk test showed normal and abnormal distribution among variables, analyses of variance with the Student *t* test and Mann-Whitney test were performed, respectively. Differences in the frequency of genotypes and alleles between RCD and control groups were analyzed by the Fisher exact and χ^2 tests after fitting for Hardy-Weinberg equilibrium. Values of $P < .05$ were considered statistically significant, and the risks associated to individual alleles and genotypes were calculated as the odds ratio (OR) with 95% confidence intervals (CI). Multivariate logistic regression analysis was performed to permit the exploration of many covariates simultaneously. Statistical analysis was performed with STATA 11.1 (StataCorp, College Station, TX, USA). To calculate linkage disequilibrium and haplotypes, the computer program package ARLEQUIN was used (v.20; http://anthro.unige.ch/arlequin). Bonferroni correction was used to correct multiple comparisons (http://www.quantitativeskills.com/sisa/calculations/bonfer.htm).

Table II Genetic variants in each investigated gene

SNP ID	Chromosome	Base pair position	Base change*	MAF†	SNP type	Nearest gene locus
rs11362	Ch8	6735399	C > T	0.404	5′ UTR	*DEFB1*
rs1800972	Ch8	6735423	C > G	0.154	5′ UTR	*DEFB1*
rs10858049	Ch1	115196166	A > G	0.173	Intron	*DENND2C*
rs10861369	Ch12	105670337	C > T	0.240	Intergenic	Intergenic
rs4903399	Ch14	76775202	C > T	0.177	Intergenic	Intergenic
rs1077430	Ch14	76897677	C > T	0.384	Intron	*ESRRB*
rs2860216	Ch14	77006008	C > T	0.302	Intergenic	Intergenic
rs10132091	Ch14	76870818	C > T	0.484	Intron	*ESRRB*
rs1676303	Ch14	76992164	C > T	0.204	Intergenic	Intergenic
rs745011	Ch14	76917275	C > T	0.417	Intron	*ESRRB*
rs4903419	Ch14	76984655	A > G	0.150	Intergenic	Intergenic
rs6574293	Ch14	76870600	A > G	0.129	Intron	*ESRRB*
rs7932320	Ch11	69625511	C > T	0.486	Intron	*FGF3*
rs1893047	Ch11	69626472	A > G	0.428	Intron	*FGF3*
rs12574452	Ch11	69631731	A > G	0.327	Intron	*FGF3*
rs4631909	Ch11	69614847	C > T	0.478	Intergenic	Intergenic
rs4980700	Ch11	69619417	A > G	0.439	Intergenic	Intergenic
rs1448037	Ch5	44352344	C > T	0.361	Intron	*FGF10*
rs900379	Ch5	44369656	C > T	0.483	Intron	*FGF10*
rs1011814	Ch5	44335820	C > T	0.485	Intron	*FGF10*
rs593307	Ch5	44358915	A > G	0.364	Intron	*FGF10*
rs11750845	Ch5	44373060	C > T	0.379	Intron	*FGF10*
rs13317	Ch8	38269514	C > T	0.275	3′ UTR	*FGFR1*

UTR, untranslated region.
Location, SNP IDs, base change, and minor allele frequency of the 23 single-nucleotide polymorphisms (SNPs).
* Base change according to Applied Biosystems.
† MAF: minor allele frequency according to GenBank.

Results

Clinical findings

From a total of 410 subjects enrolled in the study, RCD was diagnosed in 203 (RCD group). In this group, there were 160 women and 43 men, with a mean age of 51.8 ± 5.1 years. The control group (without RCD) consisted of 207 individuals, 135 women and 72 men, with a mean age 53.5 ± 5 (Table I). No differences were found between the groups for age and smoking habits. Whites ($P = .002$) and women ($P = .001$) showed a higher prevalence of RCD. On the basis of odds ratio calculation, the risk associated with RCD in women (OR, 2.07; 95% CI, 1.30-3.30) and whites (OR, 1.88; 95% CI, 1.21-2.90) was 2 times higher than in control subjects. The control group had a higher prevalence of systemic diseases ($P < .0001$), medication use ($P = .01$), and calcium supplementation ($P = .01$). The incidence of high blood pressure was significantly higher in RCD patients ($P < .001$).

Genetic association study

Allele and genotype frequencies for all SNPs were within Hardy-Weinberg equilibrium in both groups. The most significant associations between genetic variants and RCD are summarized in Table III. *DEFB1* rs1800972 CC genotype was significantly less frequent in RCD-affected individuals ($P = .004$). The CC genotype of the *FGFR1* polymorphism was overrepresented in unaffected individuals ($P = .02$). The calculation of the odds ratio revealed that individuals with CT and TT genotypes seemed to be almost 3 times more susceptible to RCD (OR, 2.67; 95% CI, 1.02-7.21). Difference was found between rs4903399 ($P = .03$) and rs1676303 ($P < .001$) and RCD development. *FGF3* rs12574452 also exhibited an association with lower RCD frequency ($P = .01$).

Significant differences in the frequency of the rs1676303 C allele ($P < .001$) and *FGF3* (rs12574452) A allele ($P = .004$) between controls and RCD individuals were found. The minor allele frequency observed in rs4903399 ($P = .02$) was associated with absence of RCD; the *DEFB1* allele C showed significant lower frequency in the control group ($P = .01$; Table IV).

To assess factors concurrently, we performed a multivariate logistic regression of individual parameters. The initial univariate analysis demonstrated that ethnic group ($P = .002$), sex ($P = .001$), *DEFB1* (rs1800972) genotypes ($P = .02$), and *FGFR1* rs13317 genotypes ($P = .03$) are potential predictive factors for RCD. *ESRRB* rs4903399 and rs1676303 ($P = .04$) as well as *FGF10* rs1011814 and rs900379 frequencies were significantly different between

Table III Analysis of genotypic distribution of 23 SNPs in patients with rotator cuff disease and control individuals

Gene	SNP	Genotypes	Frequency		*P* value*	Odds ratio	95% CI
			Control (n = 207)	RCD (n = 203)			
DEFB1	rs11362	CC-CT-TT/CT+TT	86-93-28/121	78-94-31/125	.78/.51	1.14	0.75-1.73
	rs1800972	CC-CG-GG/CG+GG	8-57-142/199	0-46-157/203	.004/.005	1.98	1.79-2.18
DENND2C	rs10858049	AA-AG-GG/AG+GG	131-69-7/76	138-54-8/62	.17/.18	0.76	0.49-1.17
	rs10861369	CC-CT-TT/CT+TT	127-66-14/80	134-60-9/69	.46/.32	0.82	0.54-1.25
ESRRB	rs4903399	CC-CT-TT/CT+TT	127-69-11/80	145-51-7/58	.09/.03	0.64	0.41-0.98
	rs1077430	CC-CT-TT/CT+TT	35-96-76/172	30-100-73/173	.91/.55	1.17	0.67-2.06
	rs2860216	CC-CT-TT/CT+TT	24-99-84/183	27-86-90/176	.53/.60	0.85	0.46-1.60
	rs10132091	CC-CT-TT/CT+TT	34-106-67/173	36-93-74/167	.54/.72	0.91	0.53-1.57
	rs1676303	CC-CT-TT/CT+TT	6-53-148/201	23-68-112/180	.0002/.0008	0.23	0.08-0.62
	rs745011	CC-CT-TT/CT+TT	54-88-65/153	45-100-58/158	.37/.35	1.24	0.77-2.00
	rs4903419	AA-AG-GG/AG+GG	132-59-16/75	136-60-7/67	.16/.49	0.87	0.57-1.33
	rs6574293	AA-AG-GG/AG+GG	3-40-164/204	1-43-158/201	.56/.66	1.48	0.20-12.76
FGF3	rs7932320	CC-CT-TT/CT+TT	33-113-61/174	39-94-70/164	.24/.38	0.80	0.46-1.37
	rs1893047	AA-AG-GG/AG+GG	46-111-50/161	48-100-55/155	.66/.98	1.00	0.61-1.62
	rs12574452	AA-AG-GG/AG+GG	6-37-164/201	18-41-144/185	.02/.01	0.31	0.11-0.84
	rs4631909	CC-CT-TT/CT+TT	45-100-62/162	43-98-62/160	.98/.89	1.03	0.63-1.70
	rs4980700	AA-AG-GG/AG+GG	53-107-47/154	54-97-52/149	.70/.81	0.95	0.60-1.51
FGF10	rs1448037	CC-CT-TT/CT+TT	80-95-32/127	69-99-35/134	.60/.32	1.22	0.80-1.87
	rs900379	CC-CT-TT/CT+TT	51-103-53/156	63-93-44/137	.28/.07	0.68	0.43-1.07
	rs1011814	CC-CT-TT/CT+TT	54-108-45/153	46-93-64/157	.08/.41	1.20	0.75-1.94
	rs593307	AA-AG-GG/AG+GG	34-96-77/173	34-105-64/169	.45/.92	0.98	0.56-1.70
	rs11750845	CC-CT-TT/CT+TT	87-99-20/119	104-81-18/99	.18/.06	0.70	0.46-1.05
FGFR1	rs13317	CC-CT-TT/CT+TT	18-71-118/189	7-74-122/196	.08/.02	2.67	1.02-7.21

SNP, single-nucleotide polymorphism; *RCD,* rotator cuff disease.

RCD and controls. Table V summarizes the multivariate logistic regression analysis. Being white ($P < .001$) and female sex ($P = .001$) are potential predictors of RCD. *ESRRB* rs1676303 and *FGF3* rs12574452 polymorphisms are associated with RCD. *FGFR1* rs13317 remained associated with RCD despite the multivariate analysis.

Association of *FGF10* rs11750845 ($P = .03$; OR, 1.52; CI, 1.02-2.26) and rs1011814 ($P = .01$; OR, 1.23; CI, 1.10-2.71) with RCD was observed after adjustment by ethnic group and sex. The other genotyping results remained unchanged after adjustment for covariates (data not shown).

Haplotype association with rotator cuff disease

The summary of the haplotype analysis is shown in Table VI. There was a significant association of the haplotype CCTTCCAG in chromosome 14 ($P = .05$) and haplotype CGACG in chromosome 11 ($P = .01$) with the occurrence of RCD. The *DEFB1* CC diplotype was significantly associated with subjects without RCD ($P = .03$), showing a 1.58-fold increase in protecting against RCD.

Discussion

Sequence variations (polymorphisms) in human DNA contribute to the visible and measurable biologic variation observed in individuals[6] and affect how humans develop diseases, although the sequences of the 3.2 billion bases of human DNA are more than 99.9% identical. Genetic factors have been suggested as intrinsic risk factors for rotator cuff tendon injury[14] and shoulder dislocation.[18] However, there is no previous study associating RCD with polymorphic alterations. Therefore, we hypothesized that polymorphisms within specific genes involved in intrinsic tendon-muscle degeneration are partially responsible for the observed interindividual susceptibility to RCD. In this work, 23 SNPs in patients presenting with RCD were investigated. Our results showed that genes with different functions are strictly associated with RCD, suggesting that this disease can have its origin in different phases of the tendon healing and degenerative processes.

RCD begins with repeated tendon strain and progression to partial- or full-thickness tears.[11] In this study, we did not stratify the patients by the stage of their disease. However, patients with traumatic cuff failure or with calcified rotator cuff tendinitis were excluded from the study to minimize the role of external causes influencing RCD. In addition, factors known to predispose to RCD, such as diabetes[1,5] and smoking habit,[30] did not reveal any association except for the covariate of female sex. In contrast, our findings demonstrated that being white and high blood pressure are risk factors associated with RCD.

The exact mechanisms leading to the degeneration of the rotator cuff are still under debate. To elucidate the

Table IV Allele distribution analysis showing minor allele frequencies, *P* value, and odds ratio in rotator cuff disease and control individuals

SNP ID	A1	MAF_C	MAF_T	*P* value*	Odds ratio	95% CI
rs11362	T	0.36	0.38	.93	0.99	0.74-1.32
rs1800972	C	0.18	0.11	.01	1.68	1.11-2.54
rs10858049	G	0.80	0.82	.39	1.16	0.81-1.68
rs10861369	T	0.20	0.18	.21	1.24	0.87-1.76
rs4903399	T	0.22	0.16	.02	1.48	1.02-2.14
rs1077430	C	0.40	0.39	.84	1.03	0.77-1.37
rs2860216	C	0.36	0.34	.75	1.05	0.78-1.41
rs10132091	C	0.42	0.41	.68	1.06	0.79-1.41
rs1676303	C	0.16	0.28	.00001	0.48	0.33-0.68
rs745011	C	0.47	0.47	.87	1.02	0.77-1.36
rs4903419	G	0.22	0.18	.18	1.26	0.88-1.81
rs6574293	A	0.11	0.12	.83	0.95	0.61-1.50
rs7932320	C	0.43	0.42	.80	1.04	0.78-1.38
rs1893047	A	0.49	0.48	.82	1.03	0.78-1.37
rs12574452	A	0.12	0.19	.004	0.57	0.38-0.86
rs4631909	C	0.46	0.45	.86	1.02	0.77-1.36
rs4980700	G	0.49	0.50	.78	0.96	0.72-1.28
rs1448037	T	0.38	0.42	.34	0.87	0.65-1.17
rs900379	T	0.50	0.45	.09	1.27	0.95-1.68
rs1011814	C	0.52	0.46	.05	1.30	0.98-1.73
rs593307	A	0.40	0.43	.38	0.88	0.66-1.18
rs11750845	T	0.34	0.29	.12	1.26	0.92-1.71
rs13317	C	0.26	0.22	.16	1.26	0.90-1.76

SNP, single-nucleotide polymorphism; *A1,* minor allele (based on whole sample); *MAF_C,* minor allele frequency in control; *MAF_T,* minor allele frequency in test.

* *P* value calculated by χ^2. *P* values $<.05$ are considered significant.

relationship between genetic background and RCD, we evaluated the distribution of genotypes using SNPs selected from the International HapMap Project data.[36] The main results of the single-marker and haplotype analyses can be summarized as follows: (1) *DEFB1* rs1800972, *FGFR1* rs13317, and *FGF10* rs11750845 and rs1011814 were highly associated with RCD; (2) *FGF3* CGACG and *ESRRB* CCTTCCAG haplotypes in chromosomes 11 and 14, respectively, were associated with RCD; (3) there seems to be no major influence of the other loci encoding mediators involved in tendon intrinsic degeneration in RCD.

The role of β-defensin and the defensin family in muscle degeneration has been described as a novel effector of immune-mediated tissue injury.[37] The *DEFB1* gene, encoding human β-defensin 1 (8p23.1-23.2), covers approximately 8 kilobases and spans along 2 exons. *DEFB1* is constitutively expressed. Three SNPs, namely, rs1799946 (G>A), rs1800972 (C>G), and rs11362 (G>A) at the 5′ untranslated region, have been reported to cause functional variations in the expression of this gene and are correlated with susceptibility to diseases.[23,24] Our results showed a positive association between rs1800972 and increased susceptibility to RCD. The presence of the G allele, clearly associated with RCD in our study, increases constitutive expression of both human β-defensins 1 and 3.[17] We suggest that this genetic polymorphism, associated with high levels of β-defensin, can play a significant role in the establishment of RCD. However, future studies will be necessary to further demonstrate the relationship between RCD, muscle structural changes, and increased levels of β-defensin.

The tissue-specific physiology of tendon is adjusted to extreme mechanical loading, which results in acute and repetitive reductions in blood perfusion and therefore a likely ability to tolerate transient hypoxia. However, tendon itself is not deprived of blood vessels. Blood vessel amounts in early stages of rotator cuff tears increase by up to 4-fold in stark contrast with end-stage RCD.[22]

After injury, tenocytes become metabolically more active with a marked increase in oxidative phosphorylation during the repair process.[33] Torn rotator cuffs express high levels of *HIF1*, which is a master regulator in the control of the adaptive response to hypoxia and oxygen homeostasis.[20] *ESRRB*, a member of the superfamily of orphan nuclear receptors, encodes a protein with similarities to the receptor for estrogen.[12] *ESRRB* is associated with transcriptional response to hypoxia by interacting with HIF and stimulating HIF-induced transcription and is essential for the function of HIF.[2] Our findings clearly showed significant association between RCD development and *ESRRB* CCTTCCAG haplotype in chromosome 14. Transcriptional activation of hypoxic genes in cells cultured under hypoxia is largely blocked by suppression of estrogen-related

Table V Multivariate logistic regression analysis

Parameter	*z*	*P* value	Odds ratio	95% CI
Age	−1.31	.19	0.98	0.95-1.00
Ethnic group				
White	—			
Nonwhite	−3.64	.000	0.42	0.26-0.67
Sex				
Female	—			
Male	−3.31	.001	0.43	0.26-0.71
DEFB1 rs1800972				
CC	—	1.0	—	—
CG	−0.73	.46	0.83	0.51-1.35
GG	—			
ESRRB rs4903399				
CC	—	1.0	—	—
CT	−1.46	.14	0.70	0.43-1.12
TT	−0.63	.52	0.70	0.24-2.05
ESRRB rs1676303				
CC	—	1.0	—	—
CT	−1.87	.06	0.37	0.13-1.04
TT	−2.91	.004	0.22	0.08-0.61
FGF3 rs12574452				
AA	—	1.0	—	—
AG	−2.08	.03	0.31	0.10-0.93
GG	−2.13	.03	0.33	0.12-0.91
FGF10 rs1011814				
CC	—	1.0	—	—
CT	0.51	.61	1.47	0.32-6.72
TT	−0.05	.96	0.94	0.11-7.67
FGF10 rs900379				
CC	—	1.0	—	—
CT	−0.31	.75	0.77	0.14-4.03
TT	0.44	.66	1.59	0.19-12.94
FGFR1 rs13317				
CC	—	1.0	—	—
CT	1.89	.05	2.73	0.96-7.75
TT	1.67	.09	2.38	0.86-6.63

receptor or treatment with diethylstilbestrol, a pharmacologic estrogen-related receptor inhibitor. Systemic administration of diethylstilbestrol severely diminished angiogenesis in vivo.[2] Considering that nuclear receptors are outstanding targets for drug discovery, our findings not only may offer mechanistic insights into HIF-mediated transcription but also may open new avenues for targeting the HIF-*ESRRB* pathway for RCD therapy.

Cytokines of the FGF family may modulate healing after rotator cuff repair[3] and stimulate the production of collagen.[9] Structural changes in collagen synthesis and turnover are observed after tendon rupture and may represent a slow repair response during the injury process.[8] FGF3 and FGF10 interact with FGFR1[41] and display various biologic functions both in vivo and in vitro, including roles in mitogenesis, cellular migration, differentiation, angiogenesis, and wound healing.[41] Therefore, FGFs have been used for the regeneration of damaged tissues such as muscle, tendon and ligament, cartilage, and bone.[41] In our study, it was evident that the *FGFR1* rs13317 polymorphism displays susceptibility to RCD. Carrying the T allele increases the risk of RCD 3 times. In addition, after adjustments for sex and ethnic background, the relationship between *FGF10* polymorphism and RCD was evident. Furthermore, patients with *FGF3* CGACG haplotype showed higher susceptibility to RCD. It is possible that mutations in FGF favor an inadequate tendon repair after biochemical injury in the rotator cuff in contrast to the repair observed in individuals without mutations.

This is the first study correlating genetic polymorphism in genes possibly associated with rotator cuff injury in a powerful sample size. We obtained supporting evidence for an association between the occurrence of RCD and *DEFB1, ESRRB, FGF3, FGF10,* and *FGFR1* genes. It can therefore be speculated that the ‘‘inert or deficient local biology’’ at the tendon-muscle site may represent a genetically predisposed environment with a reduced potential for regeneration. Consequently, a greater knowledge of the

Table VI Summary of haplotype analysis

Gene	Haplotype	Frequency estimation		*P* value	Odds ratio	95% CI
		Control (n = 207)	RCD (n = 203)			
DEFB1						
Ch8 (rs11362, rs1800972)	CG	0.47	0.50	—	1.00	—
	TG	0.35	0.38	.83	0.97	0.72-1.30
	CC	0.16	0.11	.03	1.58	1.03-2.43
ESRRB						
Ch14 (rs4903399; rs1077430; rs2860216;	CTTTTTAG	0.10	0.09	—	1.00	—
rs10132091; rs1676303; rs745011;	CTTCTTAG	0.02	0.09	.09	0.34	0.10-1.18
rs4903419; rs6574293)	CCTTCCAG	0.01	0.06	.05	0.30	0.09-1.02
	CTTTTTGG	0.02	0.05	.95	0.96	0.26-3.60
	CCTTTCGG	0.03	0.02	.13	3.21	0.71-14.54
	CCTCTCAG	0.03	0.02	.13	2.68	0.74-9.74
FGF3						
Ch11 (rs7932320; rs1893047; rs12574452;	TAGTA	0.46	0.45	—	1.00	—
rs4631909; rs4980700)	CGGCG	0.28	0.21	.2	1.26	0.89-1.78
	CGACG	0.06	0.13	.01	0.52	0.31-0.86
	TGGCG	0.06	0.06	.72	0.90	0.51-1.59
	CGATG	0.02	0.03	.77	0.89	0.39-2.00
FGF10						
Ch5 (rs1448037; rs900379; rs1011814;	TCTAC	0.36	0.39	—	1.00	—
rs593307; rs11750845)	CTCGT	0.31	0.27	.17	1.27	0.90-1.79
	CTCGC	0.16	0.16	.52	1.14	0.76-1.72
	CCTGC	0.08	0.11	.39	0.80	0.49-1.32

genes involved in RCD may provide new insights at a molecular level for new therapeutic approaches and the on-time intervention for tendon healing.

Conclusions

A genetic study of 410 patients was conducted to correlate RCD with the presence of SNPs. This research showed evident association between the occurrence of RCD and *DEFB1, ESRRB, FGF3, FGF10,* and *FGFR1* genes. In addition, the risk factors for this disease included being white and female sex.

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