J Shoulder Elbow Surg (2014) 23, 227-235

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Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

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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1058-2746/$ - see front matter © 2014 Journal of Shoulder and Elbow Surgery Board of Trustees.
http://dx.doi.org/10.1016/j.jse.2013.07.053

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