Collagen Type V Polymorphism in Spontaneous Quadriceps Tendon Ruptures

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abstract

Spontaneous simultaneous bilateral quadriceps tendon rupture is associated with multiple medical conditions and pharmacological treatments; however, identifying prior risk factors is impossible in most cases. Achilles tendon and anterior cruciate ligament ruptures are associated with collagen, type V, alpha 1 (COL5A1) polymorphism. This genetic variant may be implicated quadriceps tendon rupture. The COL5A1 encodes the protein for pro-α1 chains of the low-abundance heterotrimeric type V collagen. In most noncartilaginous tissues, type V collagen is a quantitatively minor component of type I collagen that has been implicated in the regulation of the size and configuration of type I collagen fibrils. The functional significance of COL5A1 polymorphism in relation to type V collagen expression or activity has not been determined.

This article describes a patient with COL5A1 polymorphism and spontaneous simultaneous quadriceps tendon rupture. However, genetic and histologic studies performed on blood and tendon tissues and 3 consecutive sex- and age-matched controls showed a statistically significant reduction in collagen type V expression and an alteration in collagen structure in the tendon. These findings might explain the pathomechanisms of spontaneous tendon ruptures associated with COL5A1 polymorphism.

Figure: Intraoperative photograph showing the quadriceps tendon after tying the knots of the anchors positioned on the superior border of the patella.
Bilateral simultaneous quadriceps tendon rupture is rare. The largest case series of 5 patients was reported by MacEachern and Plewes; 105 cases have been reported between 1949 and 2004.2 Bilateral simultaneous quadriceps tendon rupture is associated with multiple medical conditions3 and pharmacological treatments.4,5 The role of risk factors has been well documented in 30% to 64% of patients,2,6,7 with most patients sustaining spontaneous quadriceps tendon ruptures with no prior risk factors.

Genetic predisposition may influence the risk of quadriceps tendon rupture. The rs12722 BstUI restriction fragment length polymorphism in the 3’ untranslated region of the collagen, type V, alpha 1 (COL5A1) gene is associated with Achilles tendon8,9 and anterior cruciate ligament (ACL) injuries.10 Longo et al11 described a man with this restriction fragment length polymorphism and bilateral and consecutive quadriceps tendon rupture. This polymorphism consists of a sequence variation involving a substitution of the ancestral DNA nucleotide cytosine (C) for another DNA nucleotide, thymine (T), at position 6166 of the COL5A1 gene on chromosome 9.

The COL5A1 gene encodes the protein for pro-α1 (V) chains of the low-abundance heterotrimeric type V collagen.12 The pro-α1 (V) chain is the rate-limiting component of type V collagen trimer assembly13 and is found in most of the isoforms detected in the tendon tissue.14 In most noncartilaginous tissues, type V collagen is a quantitatively minor component of type I collagen; it is implicated in the regulation of the size and configuration of type I collagen fibrils.15 This article describes a man with COL5A1 polymorphism and bilateral simultaneous quadriceps tendon rupture.

**Case Report**

A previously healthy 55-year-old man (body mass index, 29) presented with severe knee pain; he was unable to bear weight or ambulate. He had felt a sudden, burning ache in his knees a few days previously while walking downstairs with no preceding trauma. The patient reported no history of previous knee injuries, diseases, or continuous drug use (eg, steroids or antibiotics). Physical examination revealed severe pain and inability to actively extend his knees, although passive range of motion (ROM) was possible. The knees were swollen with diffuse tenderness over the joints, and a distinct palpable suprapatellar gap existed in both knees. Distal pulses were intact.

Clinical findings and routine laboratory screening for rheumatologic, endocrine, and renal disease were unremarkable. Knee radiographs revealed indirect signs of damage, including low-riding, forward-tilting patellae. Bilateral patellar spurs existed at the quadriceps tendon insertions. Magnetic resonance imaging (MRI) revealed hemorrhage and disruption of both quadriceps tendons next to their patellar insertions.

Intraoperatively, both quadriceps tendons were avulsed from the cranial pole of the patella, and the retinacula were ruptured (Figure 1). After debridement of the tendon ends, each tendon was refixed by 3 transosseous suture anchors on the superior surface of the patella (Figure 2) and reinforced with epitiendinous sutures. The extensor retinaculum was reaproximated medially and laterally with long-lasting absorbable sutures. Biopsies of the quadriceps tendon were tacked bilaterally. The patient’s knees were placed in plaster splints, and low-molecular-weight heparin was administered for 2 weeks postoperatively. Physical therapy was started after removal of the splints, with restriction of active extension of the knees up to 6 weeks postoperatively. Full ROM was reached bilaterally within 4 months. No ROM limitation or hypotrophy of the quadriceps muscles existed in either knee at 2-year follow-up. The patient returned to activities of daily living.

Genetic and histological studies were performed on the patient’s blood and tissues to investigate possible causal mechanisms and specific biological targets associated with this injury. These findings were compared with those for 3 consecutive sex- and age-matched patients who underwent arthroplasty for knee osteoarthritis. All study procedures were approved by our local Ethics Committee, and informed consent was obtained from the patients.

**Histological Analysis**

Tendinous tissues harvested intraoperatively from the quadriceps tendons of patients were immediately fixed by immersion in 4% buffered formaldehyde for 24 hours at 4°C, washed, dehydrated, and embedded in paraffin. For light microscopy analysis, sections were cut (4 μm), mount-
ed on Polysine slides (Menzel-Glasar, Braunschweig, Germany), and stained with Mallory’s Trichrome Stain Kit (ID Lab Biotechnology, London, Ontario, Canada). For transmission electron microscopy, all specimens were fixed in 3% glutaraldehyde and prepared in 0.1 M phosphate buffer at 4°C. Samples were post-fixed in 3% osmium tetroxide for 2 hours, dehydrated in graded acetone, and embedded in Araldite (Sigma-Aldrich, Buchs, Switzerland). Ultrathin sections were prepared using a diamond knife (Electron Microscopy Sciences, Hatfield, Pennsylvania) collected on G300-Cu copper grids (Electron Microscopy Sciences), contrasted using lead citrate and uranyl acetate, and examined with a EM-900 electron microscope (Carl Zeiss, Oberkochen, Germany).

Histologic evaluation of the tendinous tissue from our patient revealed degenerative changes at the rupture site and a macroscopical proximal intact tendon compared with control patients. Histopathological features included collagen structure alterations with loss of the normal parallel bundles and the presence of myxoid-like areas of degeneration (Figure 3). Transmission electron microscopic examination of the ruptured tendon showed amorphous degenerated patches near normal collagenic areas (Figure 4) and diffuse disorientation of collagen fibers with the presence of cellular debris (Figure 4). Findings in the tendon from controls were unremarkable.

**COL5A1 Genotyping and Collagen Quantitative Analysis**

Peripheral leucocytes were isolated using Ficoll (GE Healthcare) from 4 mL of venous blood collected in ethylenediaminetetraacetic acid vacutainer tubes by forearm vein venipuncture. The buffy coat was washed twice with buffer phosphate saline. DNA was extracted from the peripheral leucocytes and the formalin-fixed, paraffin-embedded tissue was extracted using a PureLink Genomic DNA Kit (Invitrogen, Carlsbad, California) according to the manufacturer’s instructions. A 300-bp fragment containing the genetic variant rs12722 in the 3′-untranslated region of the COL5A1 gene was amplified. The polymerase chain reaction was performed in a total volume of 100 µL containing 200 ng of DNA, 10 pmol of the forward and reverse primers, 1.5 U of Taq DNA polymerase (Invitrogen), 200 µM of each dNTP, and 1 mM of magnesium chloride (Invitrogen), using the COL5A1 forward primer and COL5A1 reverse primer. Amplifications were conducted by denaturing at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 45 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 60 seconds; and a final extension at 72°C for 10 minutes. The resultant polymerase chain reaction fragment was separated with a 100-bp DNA size standard (Promega, Madison, Wisconsin) on 2% agarose gels, and the sizes of the DNA fragments were determined. The polymerase chain reaction product was cut and purified from agarose gel using a QIAquick Gel Extraction kit (Qiagen, Valencia, California). Genetic variants were analyzed through automatic sequencing using a primer.

Total RNA was extracted from the formalin-fixed paraffin-embedded tendon tissue using a GenElute Mammalian Total RNA Purification Kit (Sigma-Aldrich, St. Louis, Missouri) according to the manufacturer’s instructions. RNA was purified and reverse transcribed as previously documented.16 The purified RNA was reverse transcribed using the ImProm-II Reverse Transcription System (Promega) and the following primers: collagen I: forward, reverse; collagen III: forward, reverse; collagen V: forward, reverse. The expression level of the human collagen I, III, V genes was normalized to the GAPDH gene.

Analysis was performed on the ruptured tendon of our patient and the quadriceps tendons of the healthy age- and sex-matched patients without COL5A1 polymorphism. Each experiment was performed in triplicate. Student’s t test was used to assess

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**Figure 3**: Light microscopic histology of the ruptured quadriceps tendon. Tissues are characterized by disorganized collagen fibers and large mucoid deposits (reddish tissue) (trichromic Mallory staining, ×100). Inset shows high magnification of the mucoid areas.

**Figure 4**: Electron micrographs of the tissue close to the tendon rupture showing areas of degeneration (×3150 magnification) (A) and breakdown of collagen fibers with cellular debris (×6300 magnification). Inset shows higher magnification of the cellular debris (B).
the significance of the differences between values. For all analyses, \( P < 0.05 \) was considered significant. Statistical analysis was performed using SPSS software version 17.0 (IBM, Chicago, Illinois).

The variant rs12722 of the COL5A1 gene existed in our patient with bilateral simultaneous quadriceps tendon rupture. A nucleotide mutation of cytosine 6166 (C to T) existed in the DNA purified from peripheral leucocytes and from tendon tissue. The patient was homozygous at this locus. Real-time polymerase chain reaction analysis showed a statistically significant difference (\( P = .0005 \)) in collagen V expression between the tendon tissue and the control tissues (\( n = 3 \)). No statistically significant differences existed in the expression of the collagen I and III genes (\( P = .6044 \) and \( P = .1936 \), respectively).

**DISCUSSION**

This article describes COL5A1 polymorphism in a patient with bilateral simultaneous quadriceps tendon rupture. Spontaneous quadriceps tendon rupture has been described in patients with chronic diseases, such as diabetes mellitus, obesity, gout, osteogenesis imperfecta, hyperparathyroidism, alkaptonuria, amyloidosis, and systemic lupus erythematosus. Our patient’s medical history, clinical examination, and radiological and laboratory analyses excluded these risk factors. The mechanism of tendon rupture in our patient was a combination of contraction of the quadriceps muscle with a slightly flexed knee and a fixed foot. Therefore, the presence of a minor trauma in a healthy patient suggested that the causal mechanism of this injury should be investigated, evaluating the genetic background as a possible factor.

Genetic evaluation revealed TT polymorphism of the COL5A1 gene in our patient but not in the healthy individuals. Our findings agree with reports that the CC, CT, and TT published genotype frequencies are 11.3%, 64.2%, and 24.5%, respectively, for European populations. The TT genotype of the COL5A1 gene is associated with an increased musculotendinous flexibility, which has traditionally been cited as a predisposing factor for musculotendinous injuries.

Previous studies demonstrated that COL5A1 polymorphism is associated with Achilles tendon and ACL injuries. Moreover, a possible correlation exists between quadriceps tendon rupture and COL5A1 polymorphism. The functional significance of marker rs12722 in relation to type V collagen expression or activity has not been determined. We investigated the collagen of the ruptured tendon and compared it with healthy individuals with no COL5A1 gene polymorphism. To our knowledge, we report the first statistically significant difference in collagen V expression between the variant rs12722 of the COL5A1 gene and the controls.

We hypothesized that TT COL5A1 gene polymorphism could be implicated in bilateral simultaneous quadriceps tendon rupture in the absence of triggering trauma, probably altering the amount of type V collagen fibrils of the tendons. This hypothesis agrees with the ultrastructural findings in our patient. Histological examination revealed scattered cellular debris in the disorganized collagen matrix of the ruptured tendon, which suggests cell death. The extracellular matrix may play an important role in sustaining cell survival and in promoting cell death pathways; a reduction in the proportion of type V collagen severely disrupts matrix organization. Detachment of cells from their natural substrate and inappropriate adhesion may result in anoikis, a special form of cell death. The reduced collagen V expression in the diseased tendon might have affected the cell and matrix interactions and resulted in apoptotic processes.

**CONCLUSION**

Our findings might explain the pathomechanisms of spontaneous tendon ruptures associated with COL5A1 polymorphism. This hypothesis should be confirmed in future studies. Some rare diseases present with atypical clinical findings, making diagnosis relatively challenging without molecular genetics. The diagnosis for tendon ruptures is clinical. However, the significance of genetic laboratory tests to investigate COL5A1 polymorphism in patients with atypical tendon injuries needs to be clarified to elaborate on preventive measures and minimize injury to patients with risk factors. Future genetic testing could show subtypes of individuals and explain the clinical features of patients with tendon diseases.

**REFERENCES**


