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Investigating the *ELN* rs2071307 gene variant as a risk factor for Achilles Tendon Pathologies in a British Cohort

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Background

Injuries to the Achilles tendon (tendinopathies or ruptures) are considered as ones of the most severe musculoskeletal traumas in sports with an incidence rate of 50% in athletes and 10% in the general population. A number of gene variants coding for tendon structural proteins such as COL5A1[1] and FBN2[2] have previously been associated with Achilles tendon pathologies (ATP). These protein along with others maintain a harmonious interaction with elastin to allow tendons to respond to tensile load by stretching and returning to their original lengths. The *ELN* rs2071307 variant has been associated with soft tissue pathologies and is believed to be a good candidate gene as it results in the substitution of the hydrophobic amino acid glycine with the hydrophilic serine. However, in a previous study this variant was not associated with either Achilles tendinopathy or ACL rupture in populations from Australia and South Africa[2]. As recent evidence suggests that genetic risk factors for tendinopathy may depend, to some extent, on geographic location[3], the aim of this study was to determine whether the *ELN* rs2071307 variant was associated with the risk of ATP in a British cohort.

Methods

Participants

A British Caucasian cohort consisting of 108 ATP cases (TEN n=84 and RUP n=24) and 131 asymptomatic controls was recruited as described in our earlier study[4]. Participants signed a consent form and completed a medical and injury history questionnaire. This study was approved by the Research Ethics Committee at the University of Northampton, United Kingdom.

DNA Extraction and Genotyping

Saliva (2 ml) was collected from each participant using the OG-500 tubes (DNA Genotek, Ottawa, Canada) and DNA was extracted according to the manufacturer's recommendations. All participants were genotyped using custom-designed TaqMan assays technology (Applied Biosystems, Foster City, USA) for the *ELN* G/A rs2071307. Genotype calls were automatically made using the Applied Biosystem StepOnePlus real-time PCR software (Applied Biosystems, Foster City, USA).

Statistical Analyses

Population data such as genotype and allele frequencies in addition to the Hardy-Weinberg Equilibrium were calculated using the R *Genetics* package. Differences in genotypic and allelic distributions were tested using chi-squared or a Fisher's exact test. Statistical significance was accepted at $p<0.05$.

RNA Secondary Structure

The secondary RNA structures of exon 20 where the rs2071307 resides were determined using the SFold statistical algorithm (http://sfold.wadsworth.org) . The structures were folded in the absence of divalent ions at 37 C and at a concentration of 1 M.

Results

There was no significant genotypic or allelic association between the *ELN* rs2071307 and the risk of TEN (p=0.086, p=0.119), RUP (p=0.501, p=0.243), or when both pathologies were combined into the ATP group (p=0.413, p=0.399) respectively (table 1). Furthermore, no significant genotypic or allelic were reported when the dataset was split according to gender (data not shown).

Table 1. Genotype and allele frequency distribution of the *ELN* rs2071307 variant within the UK control (CON) and Achilles tendon pathology (ATP), as well as the pathological sub-groups Achilles tendinopathy (TEN) and Achilles tendon rupture (RUP).

ELN rs2071307	CON (n=131)	ATP (n=108)	TEN (n=84)	RUP (n=24)
GG	38.2 (50)	39.8 (43)	42.8 (36)	29.2 (7)
GA	45.8 (60)	50.0 (54)	51.2 (43)	45.8 (11)
AA	16.0 (21)	10.2 (11)	5.9 (5)	45.0 (6)
P Value		0.413[a]	0.086[b]	0.501[c]
HWE	0.722	0.400	0.074	0.676
A allele	38.9 (102)	35.2 (76)	31.5 (53)	47.9 (23)
P Value		0.399[a]	0.119[b]	0.243[c]

The values are expressed as a frequency with the number of participants (n) in parenthesis.
a CON vs ATP; b CON vs TEN; c CON vs RUP

We report a structural difference between the RNA secondary structure obtained from a G and an A allele at the rs2071307 locus. This structural difference results in altering the free energy of the molecule: G allele, $\Delta G°$=-70.6 kcal/mol; A allele, $\Delta G°$=-71.6 kcal/mol.

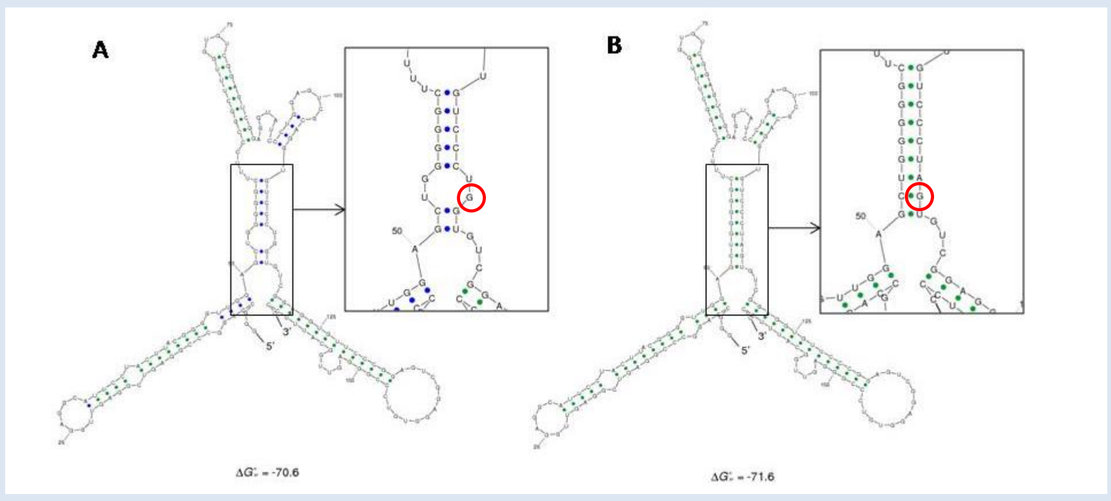

Figure 3. Comparison of the secondary structure of the RNA transcribed from exon 20 of the *ELN* gene. A) Secondary RNA structure when the G allele is present at the rs2071307 variant. B) Secondary structure when the A allele is present at the rs2071307 variant. The structural differences are magnified in the box, and the alleles are highlighted by a red circle.

Discussion

Elastin is known to display a remarkable durability following enormous stretch and recoil cycles. However, the substitution of the amino acid glycine to serine induced by the rs2071307 variant is described to alter the mechanical properties of ELN[4]. We report a difference in free energy change ($\Delta G°$) which suggests a potentially greater force driving the spontaneous forward reaction of the A allele RNA than that of the G allele RNA[5]. These thermodynamic properties could explain an over-expression of ELN mRNA as observed in post-injury Achilles tendons of mice[6]. Although the association of the *ELN* rs2071307 gene variant with soft tissue pathologies is documented in aortic stenosis and aneurysms, it appears not to be associated with the risk of ATPs in a British Caucasian cohort. This data is consistent with the early study in Australian and South African cohorts. It should be noted however, that the sample number is small and that these findings require replication in other ethnicities.

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