FEBS EMBO
Paris 2014 hosted by SFBBM
30 August – 4 September

Variation within the *CASP3* gene and the risk of Achilles tendinopathy in a British case-control cohort

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1. Introduction

Achilles tendon pathology (ATP) is a degenerative condition with known genetic risk factors[1]. Excessive tenocyte apoptosis has been observed in tendinopathy and components of the apoptosis pathway have previously been implicated in the aetiology of ATP[2]. Caspases are a large family of cysteine proteases that play a key role in the execution and regulation of apoptosis[3]. Caspase-3 is one of three known effector caspases, which can selectively cleave target proteins, such as Bcl-2, after aspartate residues in their primary sequence[3].

The rs1049253 single nucleotide polymorphism (SNP) lies within a microRNA (miRNA) binding site in the 3′ untranslated region (UTR) of *CASP3*[4] (**Figure 1**). This SNP is associated with the risk of certain cancers and the CC genotype is associated with lower levels of *CASP3* mRNA[4]. MiRNAs can bind mRNAs and have an important role in regulating apoptosis associated with carcinogenesis[4]. Our aim was to determine whether *CASP3* rs1049253 was associated with ATP in a British cohort.

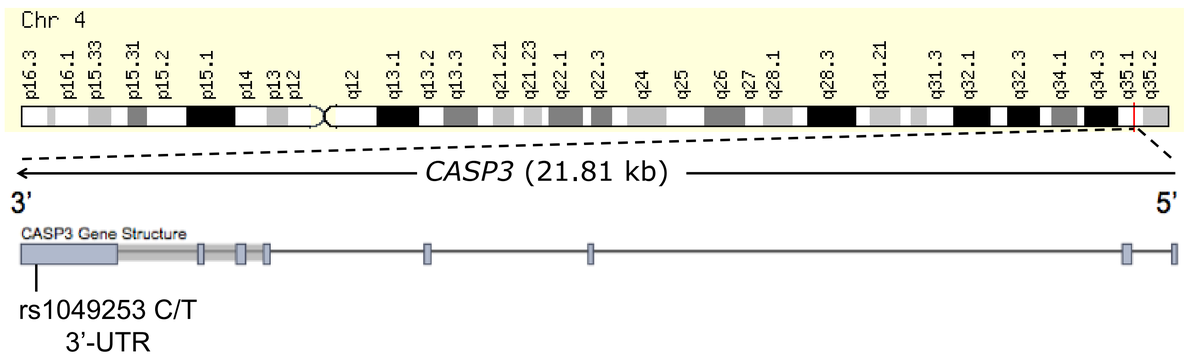

Figure 1. Schematic image of Chromosome 4 showing the location, size and exonic structure of the *CASP3* gene, along with the location of *CASP3* rs1049253 C/T in the 3′-UTR of the gene (Chr.4:185548951).

2. Methods

We recruited 261 (130 ATP cases and 131 asymptomatic controls) British Caucasian participants for this genetic association study. ATP cases were clinically diagnosed with insertional tendinopathy (INS), noninsertional tendinopathy (NON), Achilles tendon rupture or mixed pathology. Written informed consent was obtained and all participants completed a physical activity/medical history/injury questionnaire. The study was approved by the Research Ethics Committee of the University of Northampton, UK. DNA was extracted from 2 mL of saliva collected using ORAGENE-DNA collection kits (OG-500) and DNA purification was carried out using the prepIT-L2P DNA extraction kit (DNA Genotek Inc., Ontario, Canada).

TaqMan assay technology was used to genotype all participants using real-time PCR, with 10 ng of DNA and positive and negative controls included in each PCR run. The TaqMan Genotyping Assay contained FAM and VIC reporter dye labelled probes, with ROX dye as the passive reference. Genotypes were automatically called using StepOne Software, version 2.1 (Applied Biosystems, Foster City, California, USA) (**Figure 2**).

Data were analysed using IBM SPSS Statistics, version 20 (IBM Corp. Armonk, NY). A Pearson's chi-squared (X^2) or Fisher's exact test was used to analyse differences in genotype and allele frequencies for the rs1049253 variant. We compared the collective ATP group against controls. We also conducted sub-analyses for the different types of tendinopathy. Rupture and mixed pathology cases were excluded from sub-analyses. Hardy-Weinberg equilibrium (HWE) was established and $p < 0.05$ was considered to be a deviation from HWE.

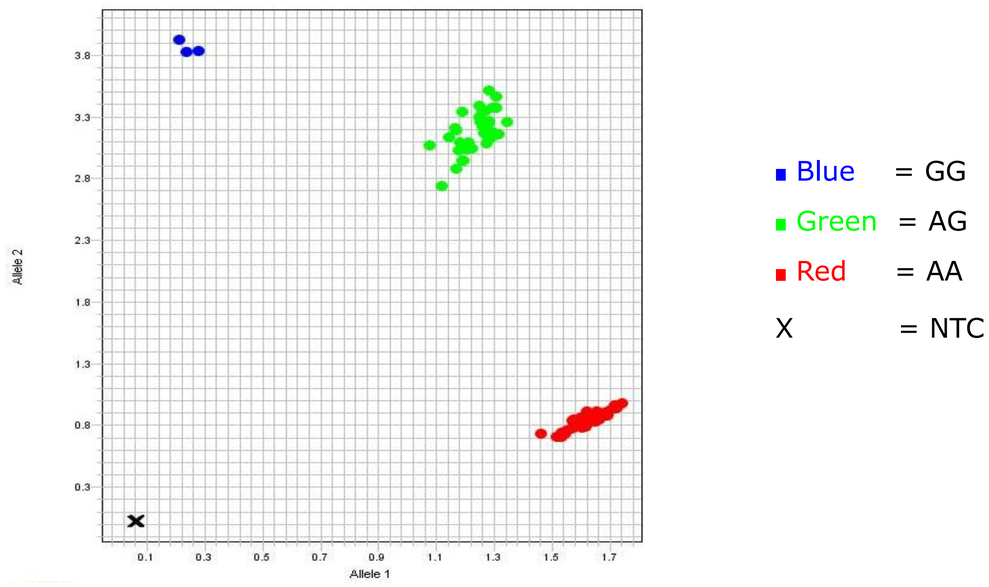

Figure 2. Typical allelic discrimination plot for *CASP3* rs1049253.

3. Results

Table 1a. Genotype and allele frequency distribution of *CASP3* rs1049253 within cases (ATP/INS/NON) and controls (CON).

CASP3 **rs1049253**	**CON** n=131	**ATP** n=130	**INS** n=29	**NON** n=47
TT	62.6 (82)	61.5 (80)	72.4 (21)	55.3 (26)
CT	34.4 (45)	33.1 (43)	20.7 (6)	38.3 (18)
CC	3.1 (4)	5.4 (7)	6.9 (2)	6.4 (3)
p-value		0.643	0.218	0.456
MAF	20.2 (53)	21.9 (57)	17.2 (10)	25.5 (24)
p-value		0.635	0.605	0.284
HWE	0.461	0.700	0.139	0.961

Table 1b. Genotype and allele frequency distribution of *CASP3* rs1049253 within male cases (ATP/INS/NON) and male controls (CON).

CASP3 **rs1049253**	**Male CON** n=82	**Male ATP** n=80	**Male INS** n=15	**Male NON** n=27
TT	68.3 (56)	58.8 (47)	73.3 (11)	55.6 (15)
CT	30.5 (25)	32.5 (26)	13.3 (2)	33.3 (9)
CC	1.2 (1)	8.8 (7)	13.3 (2)	11.1 (3)
p-value		0.072	**0.036**	0.064
MAF	16.5 (27)	25.0 (40)	20.0 (6)	27.8 (15)
p-value		0.058	0.976	0.219
HWE	0.326	0.233	**0.024**	0.379

Values are expressed as a frequency (%) with number of participants (n) in parenthesis. Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) are shown.

4. Discussion

We found no significant difference in genotype ($p = 0.643$) or allele ($p = 0.635$) frequencies between the ATP group and controls (**Table 1a**). However, we did find a genotypic association ($p = 0.036$) between male insertional tendinopathy cases (INS) and male controls (CON) (**Table 1b**). Specifically, the CC genotype appeared to increase the risk of insertional tendinopathy in males ($p = 0.045$, OR = 12.46, 95% CI 1.05-147.46). Furthermore, *CASP3* rs1049253 was not in HWE in the male INS group ($p = 0.024$). There were no significant differences between male ATP/NON and CON, nor with female ATP/INS/NON and female CON (data not shown). It it important that these data are viewed with caution due to the relatively small sample size, which might also explain the deviation from HWE observed in the male INS group. Therefore replication in a larger cohort would be necessary to increase confidence.

Our preliminary data infer a possible role for the rs1049253 variant as a risk factor for insertional tendinopathy in British males. Although additional research is needed, these results could further implicate the involvement of the apoptosis pathway in the development of ATP and could justify the inclusion of this variant in a risk assessment model for ATP. Such models alone will not predict or diagnose ATP, due to the multifactorial nature of this condition. Nevertheless, in combination with the alteration of modifiable risk factors such as training, could hold potential in lowering the risk of injury[5].

5. Acknowledgements

This research was supported by a grant to Dr Stuart M. Raleigh from the Rosetrees Trust and The University of Northampton. The authors would like to thank Professor Malcolm Collins and Dr Mike Posthumus at the University of Cape Town's UCT/MRC Research Unit for Exercise Science and Sport Medicine for discussions concerning the questionnaire design. Authors S.M.R. and W.J.R. have filed patents on the application of specific sequence variations related to risk assessment of Achilles tendinopathy and anterior cruciate ligament injuries. Attendance at FEBS EMBP Paris 2014 was supported by a general travel grant from the Biochemical Society to R.R.

6. References

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