VARIATION WITHIN THE *iNOS* GENE INFLUENCES RISK OF ACHILLES TENDON PATHOLOGY IN A BRITISH COHORT

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INTRODUCTION

There are three isoforms of the nitric oxide synthase (NOS) enzymes, *eNOS, iNOS,* and *nNOS* . The enzymes generate nitric oxide (NO) through L-arginine oxidation (Bokhari, and Murrell, 2012). mRNA levels of the *iNOS* isoform of NO were upregulated four-fold in the healing tendon $p < 0.01$ (Szomor *et al.,* 2006). *iNOS* levels have been associated with apoptosis in non-insertional Achilles tendinopathy (Pearce et al., 2010), and NO is suggested to be toxic in large doses, but important as a messenger molecule in small doses (Nakazawa *et al.,* 2017). In 2012, Nell *et al.,* (2012) observed a potential heterozygous advantage of the C/A genotype of the *iNOS* variant rs2779249 within their Australian cohort in their preliminary analysis. The effects of the *iNOS* gene variant rs2779249 as a risk factor for ATP are not fully understood.

AIM

We aimed to discover whether the rs2779249 C/A variant that lies -1026 base pairs upstream of the *iNOS* gene was associated with the risk of Achilles Tendon Pathology (ATP) in a British cohort. We also aimed to establish whether there were any sex specific effect of the variant.

METHODS

121 ATP cases were recruited from the County Clinic in Northampton, UK, and 129 controls were recruited from the Midlands, UK. Oragene-DNA sputum collection kits (OG-500) were used for DNA collection and prepIT-L2P DNA extraction kits were used to successfully purify the DNA. The DNA concentration's (ng/µl) and purity's (260/280 ratio) were measured using a NanoDrop 2000 spectrophotometer. Following this, the samples were diluted to a standard concentration of 10 ng/µl. Custom TaqMan® SNP Genotyping Assays were used to conduct qPCR on the StepOnePlus platform, and the subsequent StepOne software was used to automatically determine the samples genotypes. Pearson's Chi-squared ($\chi 2$) and Fisher's Exact tests were applied to analyse genotypic and allelic frequencies. The SNPStats association software was used to test for Hardy-Weinberg equilibrium (HWE), linkage disequilibrium and haplotype frequency estimations, alongside providing multiple inheritance models (Xavier et al., 2006). One-way analysis of variance (ANOVA) was carried out to analyse differences between participant characteristics. $P < 0.05$ was accepted as significant for the aforementioned tests.

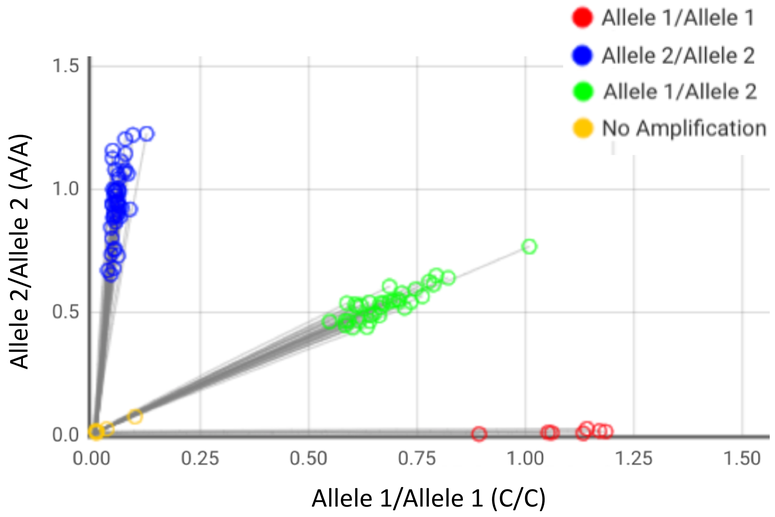
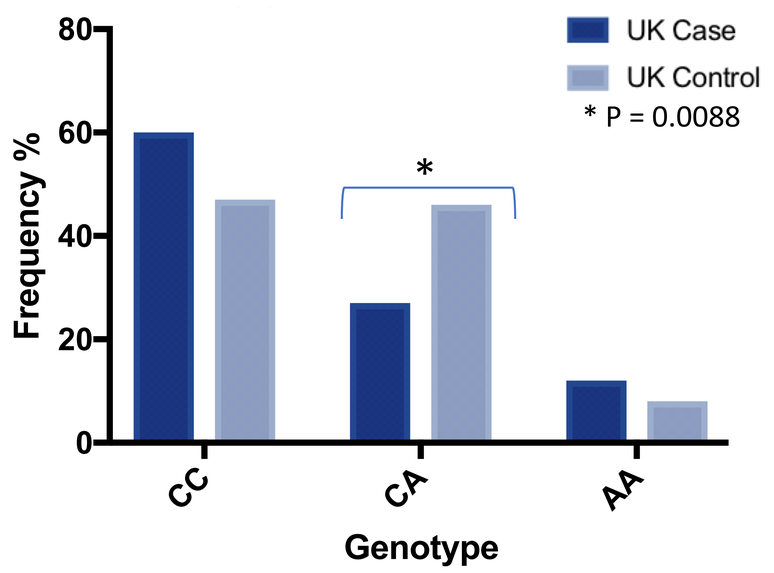
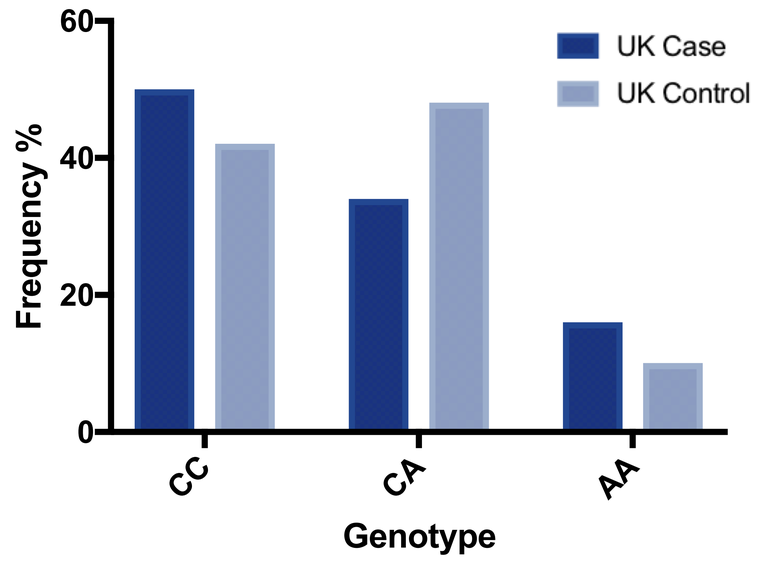


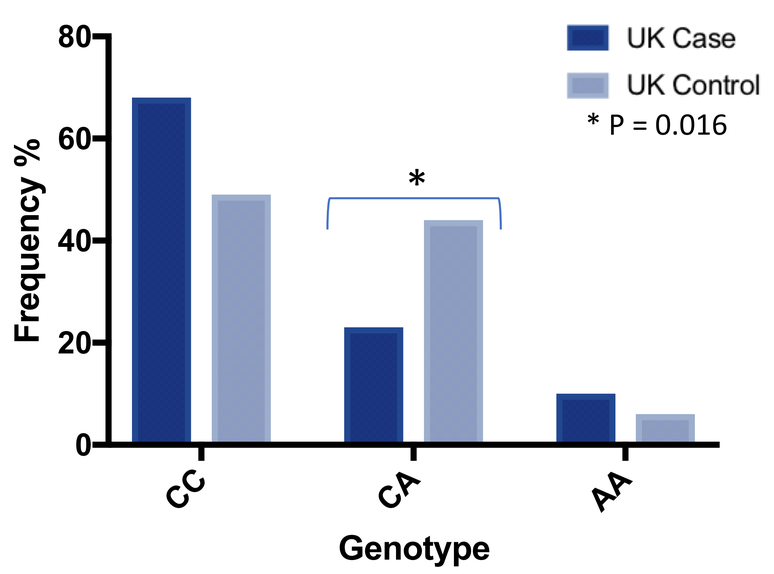
Figure 1: Allelic Discrimination Plot



Graph 1: Genotypes for the *iNOS* variant rs2779249 within the whole cohort.



Graph 2: Genotypes for the *iNOS* variant rs2779249 within the female subset.



Graph 3: Genotypes for the *iNOS* variant rs2779249 within the male subset.

DISCUSSION

A significant difference in genotype distribution was observed between ATP cases (C/C, 60.3%; C/A, 27.3%; A/A, 12.4%) and controls (C/C, 46.5%; C/A, 45.7%; A/A, 7.8%). An association was observed between the *iNOS* rs2779249 variant and ATP in the British cohort, highlighting the heterozygous C/A genotype as under-represented in the ATP population ($P = 0.0088$). This under-representation suggests a heterozygous advantage model for Achilles tendinopathy, this is consistent with preliminary research previously reported by Nell, *et al*., (2012). This under-representation remained in the tendinopathy sub-set ($P = 0.0047$), but did not remain in the rupture subset. The under-representation also remained in the male subset ($P = 0.016$), but did not remain significant in the female subset. We observed no direct effect of age, weight, height or BMI.

Fu et al, (2009) observed that allele A of the *iNOS* rs2779249 variant increased transcriptional activity of the *iNOS* promoter fivefold compared to allele C. This suggests an expressional difference of the *iNOS* enzyme, and a potential concentration difference of the inducible NO. Taking into account NO's ability to be both toxic and serve as a messenger molecule, it can be hypothesised that NO has a dual role in cellular processes and this is an area that requires further elucidation in regard to tendinopathy.

CONCLUSION

- The *iNOS* rs2779249 variant shows a heterozygous advantage in a British ATP case control cohort, with a specific effect identified in males.
- This research could be used to further improve risk determination for individuals susceptible to tendinopathy.

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