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Research paper

Carpal tunnel syndrome: The role of collagen gene variants

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ABSTRACT

Introduction: The direct causes of idiopathic carpal tunnel syndrome (CTS) are still unknown. It is suggested that pathology of the tendons and other connective tissue structures within the carpal tunnel may play a role in its aetiology. Variants in genes encoding connective tissue proteins, such as type V collagen, have previously been associated with CTS. Since variants within other collagen genes, such as type I, XI and XII collagen, have previously been associated with modulating the risk of musculoskeletal soft tissue injuries, the aim of this study was to determine whether variants within *COL1A1*, *COL11A1*, *COL11A2* and *COL12A1* were associated with CTS.
Methods: Self-reported Coloured South African participants, with a history of carpal tunnel release surgery (CTS, n = 103) and matched control (CON, n = 150) participants without any reported history of CTS symptoms were genotyped for *COL1A1* rs1800012 (G/T), *COL11A1* rs3753841 (T/C), *COL11A1* rs1676486 (C/T), *COL11A2* rs1799907 (T/A) and *COL12A1* rs970547 (A/G).
Results: The TT genotype of *COL11A1* rs3753841 was significantly over-represented in the CTS group (21.4%) compared to CON group (7.9%, p = 0.004). Furthermore, a trend for the T minor allele to be over-represented in the CTS group (p = 0.055) with a significant association when only female participants (p = 0.036) were investigated was observed. Constructed inferred pseudo-haplotypes including a previously investigated *COL5A1* variant, rs71746744 (−/AGGG), suggest gene–gene interactions between *COL5A1* and *COL11A1* modulate the risk of CTS.
Discussion: These findings provide further information of the role of the genetic risk factors and the possible role of variations in collagen fibril composition in the aetiology of CTS. Genetic factors could potential be included in models developed to identify indivisuals at risk of CTS. Strategies that target modifiable risk factors to mitigate the effect of non-modifiable risk factors, such as the genetic risk, could be also developed to reduce incidence and morbidity of CTS.

1. Introduction

Pathology of the flexor tendons and the surrounding subsynovial connective tissue (SSCT) have been proposed to play a role in the aetiology of carpal tunnel syndrome (CTS)(Lluch, 1992; Shafer-Crane et al., 2005). In support of this, sequence variants within the gene that encodes for the α1 chain of type V collagen, a component of the collagen fibril, which is the basic structural unit of tendons, have recently been shown to alter risk of CTS(Burger et al., 2014a).

Although the collagen fibril consists predominantly of type I collagen, several other quantitively minor collagens, including types V, XI and XII, playing an important role in regulating the formation and maintaining the structural integrity of the collagen fibril and surrounding matrix. Variants within the genes encoding subunits of types I, V, XI and XII collagen have previously been investigated and/or implicated for their potential role in various musculoskeletal soft tissue injuries(Burger et al., 2014a; Posthumus et al., 2009a; Posthumus et al., 2009b; September et al., 2009; Posthumus et al., 2009c; Hay et al., 2013; September et al., 2008; Posthumus et al., 2010).

The most extensively investigated variant within *COL1A1*, which encodes for the α1 chain of type I collagen, is the functional Sp1 binding site polymorphism (rs1800012, G/T)(Posthumus et al., 2009b; Ficek et al., 2013; Khoschnau et al., 2008; Mann et al., 2001; Tilkeridis et al., 2005). The TT genotype was associated with reduced risk of anterior cruciate ligament (ACL) in several studies(Posthumus et al., 2009b;

Abbreviations: ACL, Anterior cruciate ligament; **ANOVA**, Analysis of variance; **BMI**, Body mass index; ***COL1A1***, The gene encoding the α1 chain of type I collagen; ***COL5A1***, The gene encoding for the α1 chain of type V collagen; ***COL11A1***, The gene encoding for the α1 chain of type XI collagen; ***COL11A2***, The gene encoding for the α2 chain of type XI collagen; ***COL12A1***, The gene encoding for the α1 chain of type V collagen; **CON**, Control group; **CTS**, Carpal tunnel syndrome; **FACITs**, Fibril Associated Collagens with Interrupted Triple Helices; **HWE**, Hardy–Weinberg Equilibrium; **OA**, Osteoarthritis; **OR**, Odds Ratio; **PCR**, Polymerase chain reaction; **RA**, Rheumatoid arthritis; **RFLP**, Restriction fragment length polymorphism; **SNPs**, Single Nucleotide Polymorphism(s); **SSCT**, Subsynovial connective tissue; **UTR**, Untranslated region; **VEGFA**, vascular endothelial growth factor A; ***VEGFA***, The gene encoding VEGFA.

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Ficek et al., 2013; Khoschnau et al., 2008) and shoulder dislocations in a single study(Khoschnau et al., 2008). The TT genotype has however been reported to be associated with increased risk of lumbar disc disease (Mann et al., 2001; Tilkeridis et al., 2005), but not associated with chronic Achilles tendinopathy(Posthumus et al., 2009a) or tennis elbow(Erduran et al., 2014). These findings highlight the possible association of *COL1A1* rs1800012 with altered risk of other injuries, such as CTS.

It has been proposed that types XI and V collagen interact to regulate fibrillogenesis during tendon development(Wenstrup et al., 2011). This suggests that variants within the genes encoding for type XI collagen may, similar to *COL5A1* variants, also modulate the risk of tendon injuries. In support of this, several variants within *COL11A1* (rs3753841, T/C, and rs1676486, C/T) and *COL11A2* (rs1799907, T/A) was associated chronic Achilles tendinopathy in South African and Australian cohorts (Hay et al., 2013). In addition, the *COL11A1* variants have also been reported to interact with the *COL5A1* rs71746744 (−AGGG) variant to modulate the risk of Achilles tendinopathy (Hay et al., 2013).

Similar to the types V and XI fibrillar collagen, the type XII non-fibrillar collagen, which belongs to the family of Fibril Associated Collagens with Interrupted Triple Helices (FACITs), is also involved in the regulation of fibrillogenesis (Young et al., 2002). Variants within the *COL12A1* gene have been investigated in both Achilles tendinopathy(September et al., 2008) and ACL injuries(Posthumus et al., 2010). The AA genotype of *COL12A1* rs975047 (A/G) was reported to be associated with increased risk of ACL injury in females(Posthumus et al., 2010).

Therefore the aim of this study was to investigate whether the *COL1A1* rs1800012 (G/T), *COL11A1* rs3753841 (T/C), *COL11A1* rs1676486 (C/T), *COL11A2* rs1799907 (T/A) and *COL12A1* rs975047 (A/G) variants, previously associated with altered risk of tendon and/or ligament injuries, were also associated with altered risk of CTS.

2. Methods

A case–control genetic association study, following a candidate gene approach, was conducted and reported using recommendations outlined in the genetic association study specific STREGA initiative (Little et al., 2009).

2.1. Participants

A total of 103 self-reported Coloured individuals (94 female and 9 male), who had undergone bilateral (n = 50, 50.4%) or unilateral (dominant hand n = 34, 36.6%; non-dominant hand n = 9, 9.7%; right hand of ambidextrous individuals n = 2, 2.1%) carpal tunnel release surgery, (CTS group) were previously recruited (Burger et al., 2014a). Nerve conduction studies were performed in some cases, however since it is not a requirement for the commissioner of worker's compensation in South Africa, the results were not recorded. Furthermore, 150 (133 female and 17 male) apparently healthy, self-reported Coloured individuals with no history of carpal tunnel syndrome-related symptoms (CON group) were also previously recruited (Burger et al., 2014a). The CTS and CON groups had been matched for type of occupation and years of exposure. The majority of participants (CTS n = 29, 28.4%; CON n = 76, 50.7%) were general poultry processing workers or general workers in other industries requiring repetitive movement of the upper limbs. The other major self-reported occupations included administration (CTS n = 18, 17.6%; CON n = 18, 12.0%) and nursing (CTS n = 11, 10.8%; CON n = 12, 8.0%). The remaining participants were from several occupations where a high percentage of the work day required use of the wrists(Burger et al., 2014a).

South African populations who self-identify as Coloured have complex genetic inheritance, with racial intermixing over approximately 350 years. This ethnic group within the Western Cape region of South Africa is ancestrally derived from admixtures of immigrants from Western Europe, slave labourers from West Africa, Indonesia, Madagascar, Java, India and Malaysia and one or more of the indigenous African populations (Khoe- and San-speaking or Bantu-speaking). The term "Coloured" in South Africa is therefore a name that encompasses a wide range of people who are unique to this country (SASHG, 2013).

All participants provided written informed consent according to the Helsinki Declaration. Personal and family medical history was ascertained by means of a questionnaire (Burger et al., 2014a; Burger et al., 2015; Burger et al., 2014b). This study has been approved by the Human Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (HREC 158/2011).

2.2. Genotyping

Blood collection and DNA extraction had been conducted, as previously described, at the Division of Exercise Science & Sports Medicine, University of Cape Town, South Africa (Burger et al., 2014b). All DNA samples were genotyped for the *COL1A1* rs1800012 (G/T, Assay ID: C_7477170_30), *COL11A1* rs3753841 (T/C, Assay ID: C_2947954_10), *COL11A1* rs1676486 (C/T, Assay ID: C_8400671_10) and *COL11A2* rs1799907 (T/A, Assay ID: C_25474257_10) gene variants by means of a custom-designed fluorescence-based Taqman® PCR assays (Applied Biosystems, Foster City, California, USA). Gene-specific primers and allele-specific probes were used in conjunction with a pre-made PCR master mix containing AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) in a final reaction volume of 8 μl. PCR reactions were carried out in a StepOnePlus Real-Time PCR system following the manufacturers recommended cycling conditions (Applied Biosystems, Foster City, California, USA). *COL12A1* rs970547 (A/G) was genotyped by means of a restriction fragment length polymorphism (RFLP) assay as previously described (September et al., 2008).

Positive controls DNA-free controls were randomly included on each PCR plate and a subset of samples was genotyped twice for quality control purposes. Genotyping calls were made by two different researchers with no discrepancies observed. Furthermore, samples that failed to genotype twice were excluded. For quality control, subsets of known genotype controls were added to each plate.

A total of 87.3% (n = 89) and 96.1% (n = 98) of the cases and 94.6% (n = 139) and 99.3% (n = 146) of the controls were successfully genotyped for *COL11A1* rs3753841 and *COL11A1* rs1676486, respectively. Ninety-two percent (n = 94) of the cases and 99.3% (n = 146) of the controls were successfully genotyped for *COL11A2* rs1799907. For *COL1A1* rs1800012, 97.1% (n = 99) of the cases and 96.6% (n = 142) of the controls were successfully genotyped whereas 89.3% (n = 92) of the cases and the controls (n = 134), respectively, were successfully genotyped for *COL12A1* rs970547.

2.3. Statistical analysis

Quanto (V.1.2.4, USC, CA, USA) was used to determine the statistical power for a given sample size, based on the expected minor allele frequencies for the individual variants. Non-missing data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA). A Pearson's chi-squared test or Fisher's exact test were used to determine significant differences in the genotype and allele distributions as well as other categorical data, namely sex and country of birth, between the CTS and CON groups. An analysis of variance (ANOVA) was used to determine significant differences in continuous data. A significant statistical difference was set at $p < 0.05$. The Hardy–Weinberg equilibrium (HWE) of the groups was established using the program Genepop web version 4.0.10 (http://genepop.curtin.edu.au/). Inferred haplotypes and pseudo-haplotypes were constructed using Chaplin version 1.2.2 (Emory University School of Medicine, Atlanta, Georgia, USA) and