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Research paper

Interleukin and growth factor gene variants and risk of carpal tunnel syndrome

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ABSTRACT

Recent research has identified DNA sequence variants within genes encoding structural components of the collagen fibril, the basic structural unit of tendons, to modify the risk of carpal tunnel syndrome (CTS). Since the expression of these previously associated genes are regulated by cytokine and growth factor signalling pathways, the aim of this study was to determine whether variants within these cell signalling pathway genes, namely interleukin 1β (*IL-1β*), *IL-6*, interleukin 6 receptor (*IL-6R*) and vascular endothelial growth factor A(*VEGFA*), are also associated with CTS.

One hundred and three self-reported Coloured participants, with a history of carpal tunnel release surgery (CTS) and 149 matched control participants (CON) without any reported history of CTS symptoms were genotyped for the functional *IL-1β* rs16944 (-511C/T), *IL-6* rs1800795 (−174G/C), *IL-6R* rs2228145 (C/A) and *VEGFA* rs699947 (−2578C/A) variants. Only the *IL-6R* variant was significantly associated with CTS ($p = 0.005$, OR = 0.41, 95% CI 0.22–0.75). When the previously reported associated *COL5A1* and *BGN* variants were included in the analysis, gene–gene interactions were also shown to significantly modulate the risk of CTS.

In conclusion, the AA genotype of *IL-6R* rs2228145 was independently associated with reduced risk of CTS in a South African Coloured population. The *IL-6R* variant interacted with the previously reported *COL5A1* and *BGN* variants to modulate CTS risk. This highlights that interleukin and growth factor gene variants should also be considered, in addition to the extracellular matrix proteins, for future research in determining the aetiology of CTS.

1. Introduction

The association of DNA sequence variants within genes encoding for structural components of the collagen fibril, the major building block of tendons and other connective tissue structures within the carpal tunnel structure, in modulating the risk of carpal tunnel syndrome (CTS) have previously been investigated (Burger et al., 2014a,b). Specially, variants within the *COL5A1 and BGN* genes, which encode for the structural type V collagen and biglycan proteins respectively, are associated with increased risk of CTS in a South African Coloured (Anon) population (Burger et al., 2014a,b). The expression of these genes is regulated by signalling cascades in response to stimuli such as repetitive mechanical loading, which is often mentioned as a risk factor for CTS (Aroori and Spence, 2008; Abbas et al., 1998) and other occupational, overuse injuries (Yuan et al., 2002). In support of this, previous studies have reported altered expression of several cytokines, such as interleukin-1β (IL-1β), and interleukin-6 (IL-6), and growth factors, such as vascular endothelial growth factor (VEGF) in tendon and ligament injuries (September et al., 2007; Riley, 2004; Nell et al., 2012; Archambault et al., 2002; Tsuzaki et al., 2003; Abate et al., 2009; Millar et al., 2009; Rahim et al., 2014).

Functional DNA sequence variants within the *IL-1β* (rs16944, C/T), *IL-6* (rs1800795, C/G), *VEGFA* (rs699947, C/A), as well as the *IL-6R* (rs2228145 A/C), which encodes for the IL-6 receptor, genes have all been associated with various multifactorial conditions (Genovese et al., 2013; Lu et al., 2013; Kiyohara et al., 2014). Although not independently associated, *IL-1β* rs16944 and *IL-6* rs1800795 interact with a *COL5A1* variant (rs12722) to modulate the risk of chronic Achilles tendinopathy (September et al., 2011). This highlights that a pathway-based approach may be more informative to fully elucidate the role of genetic risk factors in multifactorial conditions, such as tendinopathy

Abbreviations: ACL, Anterior cruciate ligament; ANOVA, Analysis of variance; BGN, Biglycan; *BGN*, The gene encoding BGN; BMI, Body mass index; bp, Base pairs; *COL5A1*, The gene encoding for the α1 chain of type V collagen; CON, Control group; CTS, Carpal tunnel syndrome; ECM, Extracellular matrix; HWE, Hardy–Weinberg Equilibrium; IL-1β, Interleukin-1β; *IL-1β*, The gene encoding interleukin-1β; IL-6, Interleukin-6; *IL-6*, The gene encoding IL-6; IL-6R, Interleukin-6 receptor; *IL-6R*, The gene encoding interleukin-6 receptor; OR, Odds Ratio; PCR, Polymerase chain reaction; SNPs, Single Nucleotide Polymorphism(s); VEGFA, vascular endothelial growth factor A; *VEGFA*, The gene encoding VEGFA.

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(September et al., 2011). In addition, the CC genotype of *VEGFA* rs699947 was independently associated with increased risk of anterior cruciate ligament (ACL) ruptures (Rahim et al., 2014).

The aim of this study was therefore to determine whether functional variants within several genes involved in the signal transduction and angiogenesis pathways, namely *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (C/G), *IL-6R* rs2228145 (A/C) and *VEGFA* rs699947 (C/A), are associated with altered risk of CTS.

2. Methods

This case–control genetic association study was reported using the recommendations outlined in the genetic association study specific STREGA initiative (Little et al., 2009).

2.1. Participants

A total of 103 self-reported Coloured participants (94 female and 9 male) with a history of carpal tunnel release surgery (CTS) were recruited from various occupational health clinics in the Western Cape region of South Africa, as previously described (Burger et al., 2014a). South African populations who self-identify as Coloured have a complex history of ancestrally derived admixture dating back approximately 350 years (Quintana-Murci et al., 2010). This ethnic group within the Western Cape region of South Africa is ancestrally derived from admixtures of immigrants from Western Europe, or 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 (Anon). In addition, 132 female and 17 male apparently healthy Coloured participants, matched for type of occupation and years of exposure for wrist activity, with no reported history of CTS symptoms or surgery were recruited as control (CON) participants from appropriate industries in the Western Cape region (Burger et al., 2014a).

Prior to participation, all participants were informed about the procedures of the study and gave written informed consent. In addition, a questionnaire containing personal details as well as personal and family medical history questionnaires was completed by each participant. This study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences within the University of Cape Town (HREC 158/2011).

2.2. DNA extraction & genotyping

DNA was extracted from approximately 5 ml of venous blood, that was collected via venepuncture of the forearm, as previously described by Lahiri and Nurnberger (1991) with some modifications as described by Mokone et al. (2006). DNA extraction and genotyping, was performed at the Division of Exercise Science & Sports Medicine, University of Cape Town, Sports Science Institute of South Africa, Cape Town, South Africa. At least six positive controls of known genotype and four DNA-free controls were randomly included on each PCR plate for quality control purposes. In addition, a subset of samples (approximately 10%) was genotyped twice using the same methodology to ensure that genotyping was consistent. In order to avoid genotyping errors, samples that failed twice to amplify during PCR for a particular variant were considered to be unsuccessfully genotyped and no further attempts were made to genotype them at that specific locus.

The *IL-1β* rs16944 (C/T), *IL-6* rs1800795 (G/C) and *VEGFA* rs699947 (C/A) variants were genotyped using restriction fragment length polymorphism (RFLP) (Rahim et al., 2014; September et al., 2011) as previously described. Ninety-seven % (n = 100) of the cases and 86% (n = 128) of the controls were successfully genotyped for *IL-1β* rs16944; 96% (n = 99) of the cases and 97% (n = 145) of the controls were successfully genotyped for *IL-6* rs1800795; while 82% (n = 84) of the cases and 88% (n = 131) of the controls were successfully genotyped for *VEGFA* rs699947.

The *IL-6R* rs2228145 (A/C) was also genotyped using RFLP analysis. Briefly, a 259 bp fragment containing the *Hind*III (SNP rs2228145, A/C) was amplified by means of PCR. The PCR reaction was performed in a final volume of 40 μl containing at least 100ng DNA, 20 pmol of the forward (5′-GCT TGT CAA ATG GCC TGT TG-3′) and reverse (5′-GCA ATG CAG AGG AGC GTT C-3′) primers. 2.0 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 200 μmol of dNTPs (dATP, dTTP, dCTP and dGTP) and 1 unit of DNA Taq polymerase (New England Biolabs, Ipswich, Massachusetts, USA). The amplification was performed with an initial denaturation step of 92 °C for 3 min, followed by 25 cycles of a denaturation step at 92 °C for 30 s, annealing step at 55.5 °C for 30 s and an extension step at 72 °C for 45 s followed by a final extension step of 5 min at 72 °C on a thermal cycler (Hybrid, PCR Express, Middle sex, UK). The 259 bp PCR product was digested with the restriction endonuclease, *Hind*III to produce 259 bp for the A allele and 181 bp and 78 bp for the C allele. The resultant fragments were separated together with a 100 bp molecular weight marker and SYBER® *Gold* nucleic acid gel stain (Invitrogen Molecular Probes™, Oregon, USA) on a 2% agarose gel. Genotypes were determined based the DNA fragment sizes. Eighty-three % (n = 85) of the cases and 82% (n = 122) of the controls were successfully genotyped for *IL-6R* rs2228145.

2.3. Statistical analysis

No allele frequency data was available for the Coloured, South African population in the public databases (http://www.ncbi.nlm.nih.gov/snp/). For this reason, the sample size for this study was calculated based on the range of the reported minor allele frequencies, 33.3 to 50.0% for *IL-1β* rs16944, 0.0 to 35.2% for *IL-6* rs1800795, 6.2 to 38.6% for *IL-6R* rs2228145 and 11.9 to 46.0% for *VEGFA* rs699947 previously described for populations in this public database. Quanto V.1.2.4 was used to determine the statistical power for a given sample size and minor allele frequency (Gaunt et al., 2007). A sample size of approximately 100 cases and 150 controls was found to be adequate to detect a genetic effect size ranging from 2.05 to 2.80 at a power of 80% and a significance level of 5%, assuming a minor allele frequencies ranging from 0.5 to 50.0%.

Data was analysed using STATISTICA (version 11, StatSoft Inc., Tulsa, Oklahoma, USA) and Graphpad Prism (version 5, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). A Pearson's chi-squared test or a Fisher's exact test was used to determine any significant differences between the genotype distributions or any other categorical data of the groups. An analysis of variance (ANOVA) was used to detect any significant differences between CTS and CON groups for continuous data. Where appropriate, values were adjusted for the effect of age at recruitment. A least squares difference (LSD) post-hoc test was used to identify specific differences when the overall F-value was found to be significant. Statistical significance was accepted at $p < 0.05$. Hardy–Weinberg equilibrium (HWE) was established using the program Genepop web version 4.0.10 (http://genepop.curtin.edu.au/) (Raymond and Rousset, 1995; Rousset, 2008). Linkage disequilibrium (LD) was calculated using CubeX: cubic exact solution (www.oege.org/software/cubex/) (Gaunt et al., 2007). Inferred pseudo-haplotypes were constructed from the *IL-1β* rs16944, *IL-6* rs1800795 and *IL-6R* rs2228145 variants using Chaplin (version 1.2.2, Emory University School of Medicine, Atlanta, Georgia, USA) (Epstein and Satten, 2003; Duncan et al.) and Hapstat software (version 3.0, University of North Carolina at Chapel Hill, North Carolina, USA) (Lin and Zeng, 2006). No adjustments were made for multiple testing considering no obvious appropriate method currently exists (Perneger, 1998; Nyholt, 2004). The Bonferroni adjustment was considered too conservative since the statistical tests in this and following studies are all performed on the same group of participants (Nyholt, 2004). Adjustment for multiple testing was also considered inappropriate