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Original research

The association of novel polymorphisms with stress fracture injury in Elite Athletes: Further insights from the SFEA cohort

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ABSTRACT

Objectives: To determine, in conjunction with a wider investigation, whether 11 genetic variants in the vicinity of vitamin D, collagen and *Wnt* signalling pathways were associated with stress fracture injury in the Stress Fracture Elite Athlete (SFEA) cohort.
Design: Genotype-phenotype association study.
Methods: Self-reported stress fracture history and demographic data were recorded in 518 elite athletes, 449 male and 69 female (mean age 24.2 ± 5.5 years) from the SFEA cohort. Elite athletes were assigned to two groups based on history of stress fracture injury. Data were analysed for the whole cohort and sub-stratified in to male only and multiple stress fracture cases. Genotype was determined using a proprietary fluorescence-based competitive allele-specific polymerase chain reaction assay.
Results: SOST SNP rs1877632 and *VDR* SNPs rs10735810 and rs731236 were associated with stress fracture ($p < 0.05$). In the whole cohort, rs1877632 heterozygotes and homozygotes of the rare allele combined made up 59% of stress fracture sufferers in comparison to 46% in the non-stress fracture group ($p = 0.05$). In the multiple stress fracture cohort, homozygotes of the rare allele of rs10735810 and rs731236 showed an association with stress fracture when compared to those homozygotes for the common allele combined with heterozygotes ($p = 0.03$; $p = 0.01$). No significant associations were shown in the other SNPs analysed ($p > 0.05$).
Conclusions: These data suggest an important role for *SOST* SNP rs1877632 and VDR SNPs rs10735810 and rs731236 in the pathophysiology of stress fracture. This might be due to the role of the SNPs in the regulation of bone remodelling and adaptation to mechanical loading, with potential implications for the prevention and treatment of stress fracture injuries.

1. Introduction

Stress fracture injuries are caused by mechanical loading that is applied in a rhythmic, repeated, sub-threshold manner,[1] although the exact pathophysiology is not fully understood.[2] The high volume, intensity and type of training that is required to be successful in elite sport makes athletes in body weight loaded sports particularly susceptible to sustaining this type of injury.[3]

Stress fracture injuries account for 0.7%–20% of all athletic sports injuries,[4,5] cause significant discomfort, result in a prolonged loss of training time[6] and can have a significant detrimental financial effect on the athlete and/or the club/organisation. The pathophysiology of stress fracture is complex[7] including, but not limited to risk factors such as: the female athlete triad,[8] unaccustomed or excessive exercise,[9] nutritional deficiencies,[10] previous stress fracture[11] and abnormal bone mineral density (BMD).[12] To further compound the complexly related to the cause of injury, an individual's genotype has also been associated with an increased susceptibility to stress fracture injury.[13,14]

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Initial analysis of genetic associations with stress fracture prevalence in the SFEA cohort has shown that single-nucleotide polymorphisms (SNPs) located in the vicinity of components of the *RANK/RANKL/OPG* signalling pathway, and the *P2X7* receptor are associated with stress fracture injuries in elite athletes and military recruits.[15,16] Other SNPs, which are thought to control other bone regulatory pathways, in the vicinity of *VDR*,[17] *GC*,[18] *COL1A1*[19] and *Wnt*[20] have been associated with bone phenotypes, but any association with stress fracture injuries in athletes is yet to be shown. SNPs in the vicinity of genes in the *Wnt* signalling pathway, such as *SOST*, are particularly strong candidates to be associated with stress fracture injury in an athletic population, due to their suggested role in the regulation of bone formation, and mechanotransduction.[21] While *VDR* SNPs have been shown to regulate vitamin status, protein-protein interactions and mediate cell transcriptional factors.[22]

Therefore, the aim of the present study was determine whether 11 SNPs in the vicinity vitamin D, collagen and Wnt signalling pathways were associated with stress fracture injury in the SFEA cohort.

2. Method

A sample of 518 male (n = 449) and female (n = 69) elite athletes (Table 1) were recruited to form the SFEA cohort. Professional athletes were classified as elite due to their full time participation in sport; non-professional athletes were classified as elite if they regularly competed at international or national level. Each participant completed a statement of informed consent and a health status questionnaire, which was followed by an athletic status questionnaire detailing age and playing position if applicable. Participants with stress fracture injuries confirmed by medical imaging (*e.g.*, magnetic resonance imaging or computed tomography), were classified as cases, while those who had never experienced a stress fracture injury, or reported symptoms of stress fracture, were classified as controls. Athletes that reported to have suffered a stress fracture injury without imaging confirmation and those who had experienced symptoms of stress fracture injury were withdrawn from the analysis (n = 17). Participating elite athletes competed in various sports including, football (n = 218), cricket (n = 156), track and field (n = 67), running events (n = 62), rowing (n = 13), boxing (n = 2), tennis (n = 12), hockey (n = 26) and gymnastics (n = 7), with each sport having both stress fracture Cases and non-stress fracture Control participants. Elite athletes were mainly white Caucasian (83.2% in the stress fracture cases and 79.9% in the non-stress fracture controls). Ethical approval was granted by the Nottingham Trent University Ethical Review Committee, and each participant provided written informed consent prior to their involvement in the study.

Athletes were recruited from a range of sports (as above) and with their competitive level ranging from elite national class to Olympic medallists. Males, and individuals with multiple stress fractures were analysed in separate sub-classifications due to the size of the cohort (males n = 449) and the greater genetic component that may be present in cases of multiple stress fracture injury.

Saliva samples were collected, and genomic DNA was extracted with Norgen saliva collection and extraction kits (Norgen Biotek Corp., Saliva DNA Collection Kit, Thorold, Canada). All procedures were conducted in accordance with manufacturers guidelines.

SNPs were selected on the basis of their association with BMD and fragility fracture[17–20,22–26] and samples were genotyped by LGC genomics (Herts, UK), who were blinded to the clinical status (Cases or Controls) of the genotyped individuals, using proprietary fluorescence-based competitive allele-specific polymerase chain reaction assay.

Statistical analyses were performed using statistical package SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Student's t test was used for analysis of descriptive variables. Pearson's chi-squared (χ^2) test was used to assess associations in genotype frequencies and to assess the observed frequency of each genotype with what would be expected in accordance with Hardy–Weinberg equilibrium. The Benjamini and Hochberg false discovery rate test was applied in order to account for multiple comparisons. Acceptable level of significance was classified as $p < 0.05$.

3. Results

All SNPs were in accordance with Hardy Weinberg equilibrium (HWE; Supplementary Table 1) and none of the SNPs were in linkage disequilibrium.

Stress fracture injuries were recorded in 125 of the athletes that participated: breakdown of stress fracture injuries by sport: Football n = 34, Cricket n = 42, Running events n = 27, Hockey n = 4, Rowing n = 5, Tennis n = 6, Boxing n = 1, Gymnastics n = 4, Field events n = 2. Therefore the breakdown of Control participants is as follows: Football n = 184, Cricket n = 114, Running events n = 35, Hockey n = 22, Rowing n = 8, Tennis n = 6, Gymnastics n = 3, Field events n = 3.

Of the 11 SNPs investigated, three were significantly associated with stress fracture injury in one or more of the classifications (Table 2). Significant associations ($p < 0.05$) with stress fracture injury were shown with *SOST* and *VDR* alleles. No differences were seen in the other SNPs investigated ($p > 0.05$).

SOST SNP rs1877632 heterozygotes were associated with stress fracture risk in the whole cohort, and cases of multiple stress fracture, when compared to homozygotes of the common allele ($p < 0.05$; Table 3). Associations were also shown when heterozygotes were combined with homozygotes of the rare allele and compared to homozygotes of the common allele in the same classification ($p < 0.05$). The frequency of the rare allele was greater in stress fracture sufferers in the whole cohort and cases of multiple stress fracture in comparison to non-stress fracture Control groups ($p < 0.05$). No associations were shown when stress fracture occurrence was assessed with either *LRP5* SNP rs3736228 or *Wnt16* SNP rs3801387 genotypes ($p > 0.05$).

An association between *VDR* SNP rs10735810 and increased occurrence of multiple stress fracture injury was shown in homozygotes of the rare f allele, when compared to homozygotes of the common allele combined with heterozygotes ($p < 0.05$; Table 3). Stress fracture occurrence was associated with the frequency of the rare allele in cases of multiple stress fracture and in male athletes ($p < 0.05$). Those with at least one copy of the rare allele of rs731236 had a greater stress fracture occurrence in cases of multiple stress fractures ($p < 0.05$). No significant associations were shown between *VDR* SNPs rs1544410 and rs79752321, GC SNPs rs7041and rs4588, CTR SNP rs1801197 and COL1A1 SNP rs1800012 and stress fracture occurrence ($p > 0.05$).

After correcting for multiple comparisons, using the Benjamini and Hochberg false discovery rate test, none of the associations above remained significant.

4. Discussion

The present study shows that three novel SNPs, in close proximity to *SOST* and *VDR* genes, were associated with stress fracture injury in elite athletes. The sclerostin encoding *SOST* SNP rs1877632, suggested to have a role in the functioning of the *Wnt* signalling pathway,[20] was associated with stress fracture injury in elite athletes, and in cases of multiple stress fracture. The *Wnt* signalling pathway is a predominant regulator of bone metabolism,

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