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Prospective cohort study of the risk factors for stress fractures in Chinese male infantry recruits

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Abstract

Objective: To determine potential risk factors that could predict stress fractures over an 8-week basic military training in Chinese male infantry recruits.

Methods: Recruits from three infantry units enrolled in this prospective study. At baseline, demographic data, personal history of stress fractures, mean duration of weekly exercise and smoking history were recorded on questionnaires and blood samples taken for analysis of bone turnover biomarkers and genetic factors.

Results: Of the 1516 male recruits who volunteered to participate in the study, 1398 recruits provided data for analysis. In total, 189 stress fracture cases were observed (incidence rate: 13.5%) during the 8-week training period. Recruits with stress fractures had a significantly higher incidence of prior fracture history and lower exercise level prior to enrolment compared with those without stress fractures. A significant difference in both allelic frequency and genotypic distribution of the growth differentiation factor 5 (*GDF5*) gene rs143383 polymorphism was observed between recruits with and without stress fractures. However, no difference in serum bone turnover biomarkers was detected between groups.

Conclusion: This prospective, cohort study indicates that fracture history, lower exercise level and *GDF5* rs143383 may be predictive risk factors for stress fractures in Chinese male infantry recruits.

Keywords

Bone remodelling, military recruits, bone turnover biomarkers, growth differentiation factor 5, single nucleotide polymorphism, stress fractures

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Introduction

Stress fractures often occur with no history of a specific injury and are commonly observed in athletes, dancers and military recruits.[1] The pathophysiology of a stress fracture is usually related to repetitive loading of the bone that leads to an imbalance between the micro damaged bone and the processes of bone remodelling and repair.[2] Several risk factors, including reduced body weight, decreased body mass index, increased height (or tallness), poor physical condition, low bone mineral density and high serum parathyroid hormone levels have been suggested as being associated with the development of stress fractures.[3,4] In addition, indirect evidence supports the existence of genetic factors in the pathogenesis of stress fractures.[5,6] However, no general agreement has been reached on the major causal and contributing or susceptibility factors in the development of bone stress injury. Observational and cross-sectional investigations have been conducted but prospective cohort studies are rare.[3–6]

In the USA, the incidence of stress fractures in military recruits during training has been estimated to be in the range of 0.9%–12.3% at an estimated cost of $34 000 per soldier.[7,8] Therefore, these injuries that can occur in military training can lead to serious financial implications for military budgets. This present study was designed to help identify recruits with a high risk of stress fractures during an 8-week basic military training period with the intention of providing similarly susceptible recruits with an alternative training programme to mitigate the risk of stress fractures. To this end, a prospective study was undertaken involving infantry recruits during military basic training to estimate the possible association between stress fractures and potential risk factors, including anthropometric parameters, physical activity, personal fracture history and serum bone turnover biomarkers. In addition, research has suggested that there is a positive association between bone formation and the growth differentiation factor 5 (*GDF5*) gene.[9] Defects of this gene have been shown to correlate with abnormal joint development or skeletal disorders in humans.[10,11] A functional single nucleotide polymorphism (rs143383) of the *GDF5* gene has been reported to be associated with an increased risk of the development of musculoskeletal diseases.[12] Rs143383 is located at the 5′-untranslated region of the *GDF5* gene with a single nucleotide base change from T to C.[12] Therefore, this present study also investigated if the *GDF5* rs143383 polymorphism was associated with stress fractures in this cohort of military recruits.

Subjects and methods

Study population

This prospective study invited male and female recruits from three infantry military units in China to volunteer to participate in the study. During an 8-week basic training programme, all recruits had similar diets and training programmes that included increased physical activity (i.e. marching, running, various exercise training exercises and stationary standing procedures).

Subjects were assessed at the beginning of their basic military training. After providing written informed consent, each subject completed a questionnaire that provided demographic data, personal history of stress fractures and mean duration of weekly exercise prior to enrolment. The military training department collected information on height, body weight and results of physical fitness tests including individual scores for a 3-mile running speed, the number of push-ups in 2 min and the number of sit-ups in 2 min. The maximum possible score for physical fitness was 9. Study protocols were approved by the Institutional Ethics Committee at the Fourth Military Medical University, Xi'an, Shannxi, China

(no. SOP-SJ-00582) and the study was conducted according to the World Medical Association Declaration of Helsinki.

Genotyping

Blood samples were taken at the beginning of the 8-week training period. Serum samples were analysed for biomarkers of bone turnover (i.e. bone alkaline phosphatase [BALP]), cross-linked collagen telopeptide [CTX] and N-mid osteocalcin [N-mid OC]). Blood samples were centrifuged within 2 h of collection at a speed of 603 ***g*** for 15 min; the serum was aliquoted and stored at -70°C until sample analysis. BALP was assayed using an enzyme-linked immunosorbent assay (ELISA) (Quidel, San Diego, CA, USA) and its enzymatic activity was measured with intra- and inter-assay coefficients of variation (CVs) less than 4.1% and 5.4%, respectively. CTX was measured by ELISA (intra- and inter-assay CVs less than 7% and 10.4%, respectively; Nordic Bioscience Diagnostics, Herlev, Denmark). N-mid OC was also assayed by ELISA (intra- and inter-assay CVs less than 3% and 3.9%, respectively; Quidel).

Genomic DNA was isolated from peripheral venous blood leucocytes using a standard phenol-chloroform based extraction protocol containing proteinase K and analysed for the polymorphism *GDF5* rs143383.[13] Genotyping was conducted through DNA sequencing according to the manufacturer's instructions (ABI 3730 DNA analyser; Applied Biosystems, Foster City, CA, USA). All genotyping was performed blindly and 10% of all samples were repeated to ensure the concordance of the results.

Stress fracture diagnostic criteria

All recruits with complaints of bone pain, tenderness or swelling and were free of injury had an X-ray at the time of the complaint. The results were interpreted by two blinded independent radiologists. Two investigators (Q.C. and T.H.), blinded to the subject's characteristics, adjudicated the results of the orthopaedic evaluation and the X-ray. The diagnostic criteria for the presence of a stress fracture included: (i) history of pain that was aggravated by physical activity and relieved by rest; (ii) no history of injury or accident; (iii) history of a recent increase in physical activity or the beginning of a new activity or some other change in routine; (iv) palpation elicits localized tenderness over the affected bone. Most of the recruits with bone pain and normal X-rays continued to undertake some low-intensity training for 7–10 days, followed by a second X-ray. If that second X-ray was normal, then the recruit was considered to have no stress fractures.

Statistical analyses

After completing sample size calculations, an approximate total sample size of 1150 recruits was expected to provide a precision of 0.2 for 95% confidence interval for the response rate. Data from the baseline questionnaire were analysed using Student's *t*-test (for normally distributed continuous variables) and χ^2-test (for discrete variables). Analysis of covariance was used to examine differences in concentrations of serum biomarkers from recruits with and without stress fractures.

The association between *GDF5* rs143383 and stress fractures was evaluated by logistic regression analysis of three different genetic models (inheritance patterns): (i) dominant model (TT + TC versus CC); (ii) recessive model (TT versus TC + CC); (iii) codominant model (TT versus TC versus CC) with adjustments made for age, height and body weight. Odd ratios (OR) and 95% confidence intervals (CI) were calculated.

All statistical tests were two-sided and a P-value < 0.05 was considered to indicate

Table 1. Demographic and fitness characteristics of Chinese male military recruits ($n = 1398$) with and without stress fractures who were included in this study to determine the potential risk factors that could predict stress fractures.

Characteristic	Stress fracture group $n = 189$	No stress fracture group $n = 1209$	Statistical significance[a]
Age, years	18.5 ± 1.4	18.5 ± 1.8	NS
Height, cm	172.25 ± 5.67	171.78 ± 4.71	NS
Body weight, kg	62.62 ± 6.27	62.36 ± 6.12	NS
Leg length, cm[b]	88.29 ± 4.45	88.49 ± 4.38	NS
Army physical fitness test[c]	6. 33 ± 2.45	6. 40 ± 2.18	NS
Prior fracture	28 (14.8)	108 (8.9)	$P = 0.011$
Mean weekly exercise duration prior to training			
<7 h	75 (39.7)	374 (30.9)	$P = 0.0003$
≥7 h	114 (60.3)	835 (69.1)	
Smoking			
Never smoked	53 (28.0)	362 (29.9)	NS
Former smoker	91 (48.1)	616 (51.0)	
Current smoker	45 (23.8)	231 (19.1)	

Data presented as mean ± SD or *n* of patients (%).
[a]Continuous variables were analysed using Student's *t*-test and discrete variables were analysed using χ^2-test.
[b]Length measured from the anterior superior iliac spine to the medial malleolus.
[c]Maximum possible score was 9; included 3-mile run score, push-up score and sit-up score.
NS, no statistically significant between-group difference ($P \geq 0.05$).

statistical significance. Statistical analyses were performed using SPSS software (version 20 for Windows®; IBM, Somers, NY, USA).

Results

Due to the low number of female recruits, only male recruits were included in the study. Of the 1516 recruits who volunteered to participate in the study, 118 (7.8%) were excluded during the 8-week military basic training. Reasons for exclusion included: withdrew due to personal reasons ($n = 15$); did not complete training successfully ($n = 67$); did not provide accurate information ($n = 36$). Therefore, 1398 recruits provided data for analysis.

A total of 189 stress fracture cases were observed (incidence rate: 13.5%) during the 8-week training period, and of these, 50.8% (96/189) were metatarsal, 33.3% (63/189) were tibia, 4.8% (9/189) femur, 6.9% (13/189) pelvis and 4.2% (8/189) femoral neck. Baseline demographic and fitness characteristics of the recruits with and without stress fractures are shown in Table 1. Recruits with stress fractures had a higher incidence of previous fractures than those without a stress fracture ($P = 0.011$, OR 1.77, 95% CI 1.13, 2.77). Almost all fractures were peripheral. For recruits who exercised <7 h per week over the previous year, there was a higher incidence of stress fractures compared with those that exercised for ≥7 h per week ($P = 0.0003$, OR 1.84, 95% CI 1.32, 2.56). No differences between the two groups were observed in the military physical fitness tests. There were also no statistically significant differences in age, height, body weight, leg length, and smoking habits between the two groups.

The geometric means of all three bone turnover biomarkers BALP, CTX and N-mid OC from serum samples taken at the beginning of the 8-week training period

are shown in Table 2. No statistically significant differences in bone markers were observed between recruits with and without stress fractures.

The distributions of genotype frequencies for *GDF5* rs143383 were all within the Hardy–Weinberg equilibrium.[14] Statistically significant differences in both allelic frequency (i.e. T or C) and genotypic distribution in *GDF5* were observed between recruits with and without stress fractures (Tables 3 and 4). The T allele was identified as a risk factor for stress fractures because of its higher frequency in recruits with stress fractures compared with those without fractures ($P < 0.001$) (Table 3). Statistically significant findings were also observed under the conditions of dominant and recessive models, as well as the codominant model ($P < 0.05$ for all comparisons) (Table 4).

Table 2. Serum bone turnover biomarkers measured in Chinese male military recruits ($n = 1398$) with and without stress fractures.

Biomarker	Stress fracture group $n = 189$	No stress fracture group $n = 1209$
BALP, U/l	24.4 ± 5.3	24.8 ± 5.7
N-mid OC, ng/ml	12.1 ± 3.3	12.6 ± 3.4
CTX, ng/ml	0.36 ± 0.15	0.32 ± 0.16

Data presented as mean ± standard error.
BALP, bone alkaline phosphatase; CTX, cross-linked collagen telopeptide; N-mid OC; N-mid osteocalcin.
No statistically significant between-group differences ($P \geq 0.05$).

Discussion

This prospective study investigated possible risk factors associated with the development of stress fractures, such as anthropometric indices, physical fitness, serum bone turnover biomarkers and genetic factors in male military recruits undergoing an 8-week basic training course. The study showed that low levels of previous exercise, previous fracture history and the presence of the single nucleotide polymorphism, rs143383, in the *GDF5* gene were predictive factors for stress fractures.

The cumulative incidence of stress fractures in the present study was 13.5%, which is higher than the published data from the basic combat training units of the US Army (6.9%) and the infantry units of the Finnish Army (8%),[3,4] but less than the incidence of stress fractures among Israeli elite infantry recruits with basic training over 14 weeks (16%–25%).[15] The explanation for this discrepancy may be due to differences in training (contents and methods), diagnosis and ethnicity.

Although one study found a high incidence of stress fractures in tall males during basic training,[4] two others demonstrated a

Table 3. The allelic frequency of the single nucleotide polymorphism, rs143383, of the growth differentiation factor 5 (*GDF5*) gene in Chinese male military recruits ($n = 1398$) with and without stress fractures.

GDF5 rs143383 allele	Stress fracture group $n = 189$	No stress fracture group $n = 1209$	Statistical significance[a]	Risk of T allele OR (95% CI)
T	299 (79.1)	1653 (68.4)	$P < 0.001$	1.75 (1.35, 2.28)
C	79 (20.9)	765 (31.6)		

Data presented *n* of alleles (%).
[a] χ^2-test.
OR, odds ratio, CI, confidence interval.

Table 4. The genotypic frequency of single nucleotide polymorphism, rs143383, of the growth differentiation factor 5 (*GDF5*) gene in Chinese male military recruits ($n = 1398$) with and without stress fractures.

Genotype[a]	Stress fracture group $n = 189$	No stress fracture group $n = 1209$	OR (95% CI)	Statistical significance[b]
Codominant				
CC	7	124		
TC	65	517	1.76 (1.29, 2.38)	$P = 0.0003$
TT	117	568		
Dominant				
TT + TC	182	1085	2.91 (1.25, 6.74)	$P = 0.013$
CC	7	124		
Recessive				
TT	117	568	1.83 (1.33, 2.52)	$P = 0.0002$
CC + TC	72	641		

Data presented as n of patients.

[a]In a model with a codominant effect of the T allele assumed, the genotypes including TT, TC, and CC were coded as 2, 1, and 0, respectively. When a dominant effect was assumed, the genotype CC was coded as 0, and the TC and TC combination was coded as 1. A score of 0 for the CC and TC combination and a score of 1 for TT were used in a model for evaluating a recessive effect.

[b]Logistic regression analysis.

OR, odds ratio; CI, confidence interval.

poor association between height and the incidence of stress fractures in female military recruits.[16,17] Therefore, the association between bone length and gender may affect susceptibility to stress fractures. Perhaps individuals with a slender body constitution and long bones experience more bending and stress on their skeleton during physical exertion that can lead to stress fractures compared with shorter individuals. However, large scale investigations are required to investigate this hypothesis. There was no difference in height between the two groups in this present study.

Although previous studies have reported that a lack of physical fitness was a major risk factor for stress fractures,[18,19] this association was not observed in this present study as no difference was detected in fitness levels between recruits with and without stress fractures. However, there is generally a high level of physical fitness in all recruits involved in military training, which may make it difficult to detect an effect of fitness on stress fracture susceptibility in this population. Nevertheless, an association between low levels of exercise prior to enrolment and stress fractures was observed in the present study. This result was not unexpected since regular physical activity can improve the capacity of the musculoskeletal system by 5%–20% and is important in preserving the stabilizing role of the connective tissue for the overall function of the musculoskeletal system.[20] In contrast, a lack of mechanical loading due to physical inactivity or immobilization has been shown to result in a dramatic loss of connective tissue content, structure, and tolerable loading within weeks.[20] In one study in marine recruits, the stress fracture rate was shown to be 2.4-times higher in an inactive group compared with an active group.[21]

A family history of stress fractures has been reported to be significantly associated with stress fractures among American and Israeli soldiers.[22,23] Unfortunately, in this present study, most subjects did not provide information on family history of stress fractures. However, when subjects were asked

about their personal history of frequent fracture or 'brittle bones' (to identify potentially unmeasured and therefore undiagnosed low bone mass) without the specific date of diagnosis, the significant association with stress fracture was observed. To the best of our knowledge, only one other study has previously addressed family history of fractures as a potential risk factor for stress fractures in military recruits and it showed a negative result.[8] A possible explanation for inconsistences in study outcomes may be recall bias that could affect the magnitude of the potential association between stress fractures and fracture history.

Biochemical markers of bone turnover can be easily measured in serum and are useful for assessing the dynamics of metabolic bone imbalance in various pathological bone disorders.[24] Several studies have assessed the levels of bone turnover biomarkers as a function of physical activity in athletes and soldiers and they have shown varied results.[2,25] In the present study, neither the levels of bone formation (BALP and N-mid OC) nor bone reabsorption (CTX) biomarkers showed a significant difference between recruits with and without stress fractures. This finding is consistent with results from a 12-month prospective study.[26] However, it should be noted that in this study the assays for bone turnover markers were not standardized.[26] Furthermore, a number of factors (e.g. circadian rhythm, fasting history, lifestyle, and health status) can affect the variability of bone turnover biomarkers.[27] The results of the present study suggest that an imbalance in bone remodelling processes may not be the primary event in the pathogenesis of stress fractures and that serum bone turnover biomarkers cannot be used as predictive tools for the evaluation of stress fractures in soldiers during basic training.

As a member of the transforming growth factor-β superfamily, the *GDF5* gene plays an important role in both intramembrane and endochondral bone formation during fracture healing.[28] According to previous studies, *GDF5* is correlated with a susceptibility to osteoarthritis due to reduced transcriptional activity in chondrogenic cells.[10,29] The cellular and molecular processes involved in bone regeneration after stress fractures display many similar characteristics with chondrogenesis in osteoarthritis. Thus, *GDF5* alleles could be attractive candidate genetic determinants of stress fractures. Rs143383 is the most common single nucleotide polymorphism of the *GDF5* gene and it has been reported to be associated with an increase in the risk for the development of musculoskeletal diseases.[30] One study showed that *GDF5* rs143383 regulates transcriptional activity and the encoded protein can induce articular cartilage and bone formation both in *in vitro* and *in vivo* studies.[31] Rs143383 can also influence *GDF5* allelic expression *in vivo* and the T allele when compared with the C allele can lead to an average reduction in *GDF5* allelic expression by 12%.[32] Therefore, it is reasonable to hypothesize that reduced expression of *GDF5* may weaken its capability of eliciting skeletal morphogenesis and osteogenic differentiation, thus correspondingly impairing the self-repairing capacity of bones that subsequently contributes to stress fracture repair. The results of the present study have confirmed a significant difference in both allelic frequency and genotypic distribution of *GDF5* rs143383 between subjects with and without stress fractures. Although *GDF5* rs143383 is not a functional variant, it may be directly involved in protein function via several regulatory elements, such as intron splice enhancers and silencers that regulate alternative splicing.[33] Alternatively, it may simply be involved in linkage disequilibrium with other functional single nucleotide polymorphisms or mutations in the *GDF5* gene.[34]

This present study had several limitations. First, the statistical power was limited due to the relatively small number of stress fractures. Therefore, although data on

genetic predisposition of fractures were provided by a large population, the study was underpowered to detect a genetic difference tagged by a single nucleotide polymorphism. Secondly, bone scintigraphy, widely regarded as the gold standard for a diagnostic assessment of stress fractures, was not used because of time constraints and cost. However, high diagnostic rates might be obtained using stress fracture history, typical signs, X-rays and a thorough physical examination. Thirdly, the analysis of bone turnover biomarkers was based on a single time-point collection of blood samples and so may not fully reflect bone remodelling. Since the serum samples were collected before the stress fractures, such misclassification is generally nondifferential in nature, and may result in an underestimation of the true effect size of bone turnover on stress fracture risk.

In conclusion, this prospective, cohort study indicated that previous fractures, low exercise level and the *GDF5* gene polymorphism rs143383 were associated with stress factors in Chinese male infantry recruits. These results may be beneficial in the design of future studies that intend to further elucidate the genetics of stress fracture susceptibility.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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