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ASSOCIATION BETWEEN VITAMIN D RECEPTOR GENE POLYMORPHISMS, INFLAMMATORY CYTOKINES (IL-1, IL-6, AND IL-10), AND FRACTURE HEALING IN SPORTS-RELATED INJURIES

ASOCIJACIJA IZMEĐU POLIMORFIZAMA GENA RECEPTORA ZA VITAMIN D, INFLAMATORNIH CITOKINA (IL-1, IL-6 I IL-10) I ZARASTANJA PRELOMA KOD SPORTSKIH POVREDA

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Summary

Background: In this study, we analysed the correlation between polymorphisms in the Vitamin D receptors (VDR) gene at the Apa I and FoK I loci and sports-related fractures (SRF). We also examined the inflammatory cytokines IL-1, IL-6, and IL-10 to explore their potential role in fracture healing.

Methods: A retrospective analysis was conducted on 110 patients with SRF admitted to our hospital from February 2023 to October 2023. Among these, 41 patients with poor fracture healing (research group) and 69 patients with good fracture healing (control group) were included. Serum levels of Vitamin D (VD), bone mineral density (BMD), and inflammatory cytokines (IL-1, IL-6, and IL-10) were measured to assess their relationship with fracture healing. Additionally, polymorphisms in the VDR gene at the Apa I and FoK I loci were analysed to determine the differences between the two groups.

Results: The VD levels in the research group were significantly lower than in the control group ($P<0.05$). VD levels were positively correlated with BMD in both groups ($P<0.05$). Inflammatory cytokine analysis revealed that IL-1 and IL-6 levels were significantly higher, while IL-10 levels were lower in the research group compared to the control group ($P<0.05$). Regarding VDR polymorphisms, the research group had a higher frequency of the FoK I CC genotype and a lower frequency of the TT genotype ($P<0.05$), while no significant differences were found for the Apa I polymorphism ($P>0.05$).

Kratak sadržaj

Uvod: U ovoj studiji je analizirana korelacija između polimorfizama gena receptora za vitamin D (VDR) na lokusima Apa I i FoK I i sportskih preloma (SRF). Takođe su ispitani inflamatorni citokini IL-1, IL-6 i IL-10 kako bi se istražila njihova potencijalna uloga u zarastanju preloma.

Metode: Retrospektivna analiza je obuhvatila 110 pacijenata sa SRF koji su primljeni u našu bolnicu od februara 2023. do oktobra 2023. godine. Od toga, su u studiju uključeni 41 pacijent sa slabim zarastanjem preloma (grupa ispitanika) i 69 pacijenata sa dobrim zarastanjem preloma (kontrolna grupa). Mereni su serumski nivoi vitamina D (VD), mineralna gustina kostiju (BMD) i inflamatorni citokini (IL-1, IL-6 i IL-10) kako bi se procenila njihova povezanost sa zarastanjem preloma. Pored toga, analizirani su polimorfizmi gena VDR na lokusima Apa I i FoK I radi utvrđivanja razlika između ove dve grupe.

Rezultati: Nivoi VD u grupi ispitanika su značajno niži nego u kontrolnoj grupi ($P<0,05$). Nivoi VD su bili u pozitivnoj korelaciji sa BMD u obe grupe ($P<0,05$). Analiza inflamatornih citokina pokazala je da su nivoi IL-1 i IL-6 značajno viši, dok su nivoi IL-10 niži u grupi ispitanika u poređenju sa kontrolnom grupom ($P<0,05$). U pogledu polimorfizama VDR, grupa ispitanika je imala veću učestalost FoK I CC genotipa i nižu učestalost TT genotipa ($P<0,05$), a nisu pronađene značajne razlike za Apa I polimorfizam ($P>0,05$).

Zaključak: Polimorfizmi gena VDR, posebno na FoK I lokusu, usko su povezani sa napretkom rehabilitacije kod

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Conclusions: VDR gene polymorphisms, particularly at the FoK I locus, are closely associated with the rehabilitation progress of SRF. Additionally, an inflammatory imbalance, characterised by elevated IL-1 and IL-6 and reduced IL-10 levels, may contribute to poor fracture healing.

Keywords: bone mineral density, interleukins, IL-1, IL-6, IL10, rehabilitation status, SRF, vitamin D receptors, vitamin D

SRF. Pored toga, inflamatorna neravnoteža, koju karakterišu povišeni nivoi IL-1 i IL-6 i smanjeni nivoi IL-10, može doprineti slabom zarastanju preloma.

Ključne reči: mineralna gustina kostiju, interleukini, IL-1, IL-6, IL-10, status rehabilitacije, SRF, receptori za vitamin D, vitamin D

Introduction

Sports-related fractures (SRF) are among the most common injuries seen in athletes and individuals who engage in physical activity. These fractures typically occur due to trauma, direct impact, or excessive muscle contractions during exercise (1). While SRF can affect individuals of all ages, the risk tends to increase with age (2). The incidence of fractures is particularly high in competitive sports, where the intensity of physical activity elevates the likelihood of injury (3). Epidemiological data reveals that professional athletes account for approximately 50% of all SRF, while older people contribute to around 30% of this population (4). Although bones are regenerative, the recovery process following a fracture generally extends beyond three months. Common sites of SRF include the femur, ankle joint, lumbar spine, and other regions, often leading to a significant loss of mobility and considerable disruption to daily life (5). Consequently, enhancing the rehabilitation process for these fractures has become a critical area of clinical research.

Vitamin D (VD) is a crucial trace element that plays a fundamental role in regulating calcium-phosphorus metabolism and maintaining bone health. A study by Wen Y et al. (6) confirmed that VD status is associated with skeletal muscle loss after ACL reconstruction, whereas Yoon S et al. (7) stated that VD is a potential nutritional factor that can significantly affect physical function and musculoskeletal injury in athletes. The Vitamin D receptor (VDR), a member of the steroid hormone/thyroid hormone receptor superfamily, functions as a nuclear transcription factor that regulates the differentiation and proliferation of various cell types (8). VDR help increase the production of osteocalcin, promoting the secretion of cytokines by bone cells, which are essential for bone tissue formation and mineralisation (9). Additionally, VDR mediates the active form of VD, 1,25-dihydroxy vitamin D3, to maintain calcium homeostasis and regulate immune function (10). We conjecture that VDR gene polymorphisms may determine the state of skeletal rehabilitation in patients with SRF, but there is a lack of studies to confirm our view.

Emerging evidence suggests that cytokines such as Interleukin (IL)-1, IL-6, and IL-10 play a critical role in the inflammatory and healing processes associated with bone fractures. IL-1 and IL-6 are pro-inflammatory cytokines that are involved in bone resorption

and the inflammatory response following injury, while IL-10 is an anti-inflammatory cytokine that helps modulate the immune response and promote tissue repair (11). Understanding the interactions between VD, VDR, and these cytokines may provide valuable insights into improving fracture healing and rehabilitation outcomes.

This study aims to explore the relationship between VDR gene polymorphisms, inflammatory cytokines (IL-1, IL-6, IL-10), and the rehabilitation progress of SRF. These results will provide new objective references for future rehabilitation of SRF, thus optimising the patient's prognosis.

Materials and Methods

Subjects

First, we estimated the sample size of the study using the G-Power software: effect size=0.5, alpha err prob=0.05, power=0.9, and the estimation was that at least 34 study subjects were needed in each group. A retrospective analysis was conducted on 110 patients with SRF admitted to our hospital from February 2023 to October 2023. Among them, 41 patients with poor fracture healing were regarded as the research group, and 69 patients with good fracture healing were regarded as the control group. This study, approved by the Ethics Committee of the Sports Education Center, Xijing University, was conducted strictly following *the Helsinki Declaration*. Because the study was a retrospective analysis, the requirement for informed consent was waived.

Eligibility and exclusion criteria

Inclusion criteria: Patients included were confirmed with SRF by imaging examinations (X-ray, CT or MRI to clarify the fracture, ask the patient about the aetiology of the fracture due to sports injury) (2), with age>18 years old and complete medical records. Exclusion criteria: Patients with autoimmune deficiency, administration of sex hormones, VD, glucocorticoids, or anti-osteoporosis drugs in recent 6 months, primary or metastatic vertebral tumours, organic diseases of vital organs such as liver and kidneys, or abnormal mental behaviours, were excluded. In addition, patients with comorbid acute or chronic inflammatory conditions were excluded.

Criteria for poor healing

Poor healing includes delayed union and non-union (12). Delayed union refers to the fact that the fracture has not healed at the return visit 4 months after discharge, with mild decalcification of the fracture end, less callus, and obvious fracture line. Non-union is defined as the separation of the fracture end, separation of the broken ends of the fractured bone, cherry blossom at the bone end, and atrophy and smoothness of both fractured ends.

Sample collection

Fasting venous blood was collected from patients within 30 minutes of admission, collected in procoagulant tubes, and divided into three portions after standing for 30 minutes at room temperature.

VD detection

VD levels were detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Take 100–200 µL of serum, add methanol containing an isotopic internal standard (d3-25(OH)D$_3$), and vortex to mix. Centrifuge (10,000 × g, 10 min) to remove precipitated proteins and take the supernatant. Using a VD solid-phase extraction column, interfering substances such as phospholipids were removed. The eluate was dried and re-solubilised with a mobile phase for online analysis. LC: reversed-phase column (C18 column, 2.1×50 mm, 1.7–2.6 µm particle size), 40–50 °C. Phase A: water (containing 0.1% formic acid or 5 mmol/L ammonium formate). Phase B: organic phase (methanol, comprising 0.1% formic acid). Gradient elution, 0.2–0.4 mL/min, injection volume 5–20 µL. MS/MS: Ion source was atmospheric pressure chemical ionisation (APCI), positive ion MS/MS: Ion source was atmospheric pressure chemical ionisation (APCI), positive ion mode. MS parameters: 25(OH)D$_3$: m/z 401.3 → product ion m/z 383.3 (dehydration), 365.3 (secondary dehydration). 50–150 ms/channel. Stable isotope internal standards (e.g., d3-25(OH)D$_3$) were added to correct for matrix effects and recovery differences. Intra/interday reproducibility (CV<15%) with recoveries of 85–115%.

VDR polymorphism detection

Serum DNA was extracted and amplified using phenol-chloroform extraction. Shanghai Dynegene Technologies Co., Ltd., commissioned the primer sequence for design and construction (upstream sequence: 5'-CAACCAAGACAAGTACCGTACCGCG-TCAGTGA-3'; downstream sequence: 5'-TGGCG-GCAGCGGATGTACGTCTGC-3'), and the specific amplification product fragment was 1850 bp. VDR genotypes were analysed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). Reaction system: Taq DNA poly-merase: 1 U (0.5 µL, 2 U/µL), dNTPs: 0.2 mmol/L (1 µL, 5 mmol/L), upstream and downstream primers: 0.5 µmol/L each (1 µL of each, 10 µmol/L), 10× PCR buffer: 2.5 µL (containing Mg^{2+} 15 mmol/L), template DNA: 1 µL (50–100 ng/µL), ddH$_2$O: make up to 25 µL. The reaction conditions were 94 °C for 5 minutes, 94 °C for 30s, 61 °C for 40s, and 72 °C for 90s, for 35 cycles, followed by extension at 72 °C for 10 minutes. Reaction system: Taq DNA polymerase: 1 U (0.5 µL, 2 U/µL), dNTPs: 0.2 mmol/L (1 µL, 5 mmol/L), upstream and down-stream primers: 0.5 µmol/L each (1 µL of each, 10 µmol/L), 10× PCR buffer: 2.5 µL (containing Mg^{2+} 15 mmol/L), template DNA: 1 µL (50–100 ng/µL), ddH$_2$O: make up to 25 µL. Validation of agarose gel electrophoresis: Gel preparation: 1.5% agarose (1.5 g agarose + 100 mL 1×TAE buffer), add 0.5 µg/mL ethidium bromide. Sampling volume: take 5 µL of PCR product and mix it with 1 µL of 6×sampling buffer. Electrophoresis conditions: 100 V, 20 min, 1× TAE buffer. Results observation: 1850 bp specific bands were detected by a gel imaging system. Enzyme digestion system: PCR product: 10 µL (about 200–500 ng DNA), BsmI endonuclease: 10 U (1 µL, 10 U/µL), 10 × buffer: 2 µL ddH$_2$O: replenish to 20 µL, reaction conditions: incubation at 55 °C for 4 h. Termination: inactivate the enzyme by heating at 80 °C for 10 min.

After the reaction was terminated, the product was analysed using 1.5% agarose gel electrophoresis followed by ethidium bromide staining. The results were visualised with a gel imaging system based on DNA fragment length.

In the interpretation of results, the presence of the BsmI cleavage site (allele B) produced 1050 bp and 800 bp fragments, while the absence of the cleavage site (allele b) resulted in an uncut 1850 bp fragment. In heterozygous samples (Bb), bands corresponding to 1850 bp, 1050 bp, and 800 bp were observed.

IL detection

IL-1 and IL-6 levels were assessed using double antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific, Catalog No. 88-7013-88 for IL-1, Catalog No. 88-7066-88 for IL-6), following the manufacturer's instructions. IL-10 levels were also measured using ELISA (R&D Systems, Catalog No. D1000B).

Bone mineral density (BMD) detection

BMD around the fracture site (more than 5 cm away) was measured using a BMD instrument (9000C, Huai'an Xinyu Hong Medical Technology Co., Ltd.). Measurements were taken at three different sites, and the mean value was recorded. The same orthopaedic surgeon conducted all assessments.

Endpoints

Differences in VD and BMD between the research and control groups were analysed, along with the diagnostic value of VD for poor fracture healing and its correlation with BMD. Additionally, differences in VDR gene polymorphisms at the Apa I and FoK I loci were examined. Cytokine levels of IL-1, IL-6, and IL-10 were also compared between patients with poor and successful fracture healing.

Statistical processing

Statistical analysis was performed using SPSS24.0. Data were tested for normal distribution using the Shapiro-Wilk test. Categorical variables were compared using the chi-square test. Comparisons of continuous variables were performed using independent-sample t-tests. The correlation was analysed using Pearson correlation coefficient analysis. Diagnostic value was analysed by receiver operating characteristic (ROC) curves. A significance level of $P<0.05$ was used in all analyses.

Results

Comparison of clinical data

As shown in *Table I*, there was no statistically significant difference between the two groups in terms of age, sex, body mass index, fracture site, fixation method, systolic and diastolic blood pressure, history of fractures, smoking, drinking, or convalescent train-

ing ($P>0.05$). This confirms the comparability of the groups and supports the reliability of the results.

Comparison of VD quantitative results

The quantitative analysis of VD, as shown in *Figure 1*, revealed a VD concentration of 17.54 ± 5.84 ng/mL in the research group, which was significantly lower than that in the control group ($P<0.001$). According to the ROC curve analysis, with a cut-off value of VD<22.71 ng/mL, the sensitivity and specificity for predicting poor fracture healing were 80.49% and 52.17%, respectively (AUC$=0.668$, $P<0.001$).

Relationship between VD and BMD

As shown in *Figure 2*, the BMD levels of the research and control groups were (0.65 ± 0.11) g/cm^3 and (0.67 ± 0.16) g/cm^3, respectively, without a statistical difference ($P>0.05$). Pearson correlation coefficients showed a positive correlation between VD and BMD in both groups ($P<0.05$).

Comparison of VDR FoK I polymorphisms

The results of VDR FoK I locus polymorphism detection are shown in *Table II*; the genotypes in the research group were predominantly CC-type, while the control group was predominantly TT-type. Comparing the two groups, the number of CC-type gene carriers in the research group was more than

Table I Comparison of clinical data.

| Details		Control group (n=69)	Research group (n=41)	t (or χ^2)	P
Age		33 (18, 57)	41 (19, 56)	1.587	0.116
Sex	Male/female	42/27	22/19	0.550	0.459
Body mass index (kg/m^2)		21.32±2.11	21.84±1.11	1.745	0.084
Fracture site	Femur/ankle/radius/ phalanges/lumbar spine/ thoracic spine/shoulder/other	9/21/14/9/6/7/2/1	7/12/8/5/5/4/0/0	0.589	0.997
Fixing method	Internal fixation/ external fixation	24/45	16/25	0.200	0.655
Systolic blood pressure (mmHg)		108.04±7.91	106.59±7.62	0.947	0.346
Diastolic blood pressure (mmHg)		73.35±6.45	75.07±6.66	1.340	0.183
History of fractures	Yes/no	5/64	6/39	0.242	0.623
Smoking	Yes/no	18/51	14/27	0.810	0.368
Drinking	Yes/no	12/57	12/29	2.127	0.145
Convalescent training	Yes/no	16/53	7/34	0.582	0.446
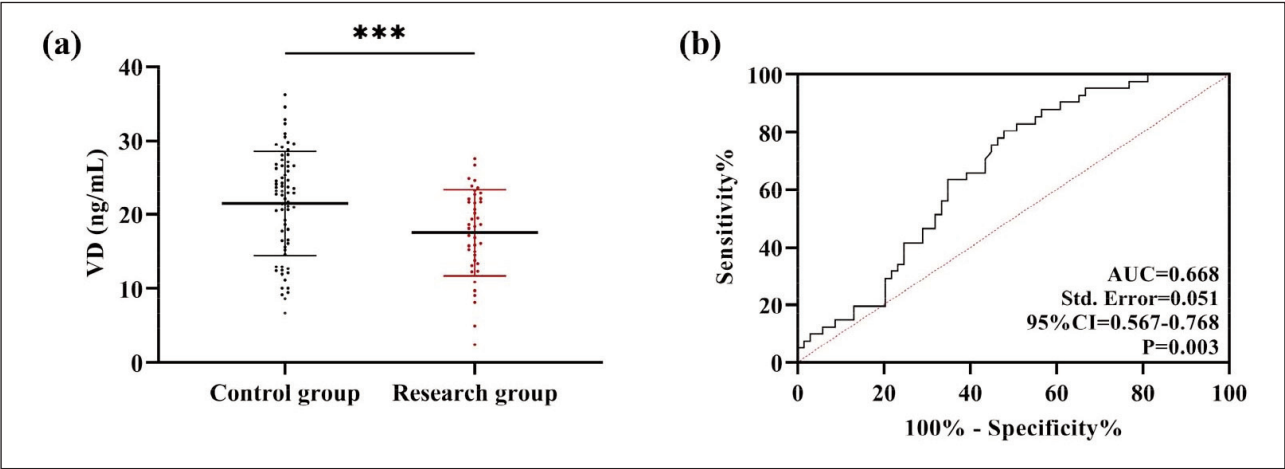
Figure 1 Comparison of VD quantitative results.

(a) Comparison of VD in the research and control groups. (b) ROC curves for VD diagnosis of poor fracture prognosis. ***P<0.001. Receiver operating characteristic, ROC; Area under curve, AUC; 95% Confidence Interval, 95%CI.
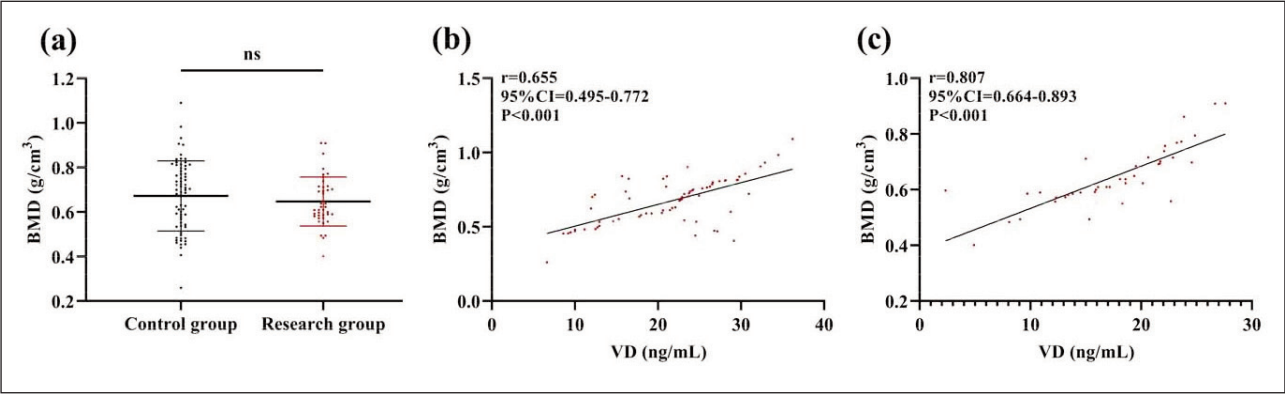

Figure 2 Relationship between VD and BMD.

(a) Comparison of BMD between research and control groups. (b) Correlation between VD and BMD in the control group. (c) Correlation between VD and BMD in the research group. nsP>0.05. Vitamin D, VD; Bone mineral density, BMD. ns: P>0.05.

Table II Comparison of VDR FoK I polymorphisms.

Groups	n	Genotype	Frequency of alleles
		CC/CT/TT	C/T
Control group	69	20/21/28	61/77
Research group	41	24/9/8	57/25
χ^2		9.781	13.250
P		0.008	<0.001

that in the control group, while the number of TT-type gene carriers was less than that in the control group (P<0.05). A comparison of allele frequencies showed that the frequency of C-type genes was significantly higher in the research group than in the control group (P<0.05).

Comparison of VDR Apa I polymorphisms

The results of VDR Apa I locus polymorphism detection are shown in *Table III* and the genotypes of the two groups of patients were predominantly of the AC type, and there was no statistically significant difference in the comparison between the groups

Table III Comparison of VDR Apa I polymorphisms.

Groups	n	Genotype	Frequency of alleles
		CC/AC/AA	C/A
Control group	69	24/42/3	90/48
Research group	41	12/26/3	50/32
χ^2		0.682	0.400
P		0.712	0.527

Table IV Comparison of IL-1, IL-6, and IL-10 levels.

		IL-1 (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control group	69	23.57±8.43	15.23±5.74	42.12±15.28
Research group	41	38.72±12.15	27.48±9.61	28.31±10.77
		5.326	4.073	4.642
		0.001	0.001	0.001

(P>0.05). As for allele frequency, the comparison of the frequency of C-type and A-type genes between the two groups was likewise not different (P>0.05).

Comparison of IL-1, IL-6, and IL-10 levels

As shown in *Table IV*, serum levels of IL-1 and IL-6 were elevated in the study group compared with the control group (P<0.05), suggesting that the inflammatory response was stronger in patients with poor fracture healing. In contrast, the anti-inflammatory cytokine IL-10 was decreased in the study group compared to the control group (P<0.05), suggesting that an imbalance in the inflammatory response may contribute to delayed healing.

Discussion

This study provides novel insights into the interplay between VDR polymorphisms (particularly Fok I-CC genotype), vitamin D (VD) deficiency, and inflammatory dysregulation in patients with sports-related fractures (SRF). To our knowledge, this is the first investigation to demonstrate that the Fok I-CC genotype puts Chinese SRF patients at increased risk of poor healing. Combined VD deficiency and pro-inflammatory cytokine elevation synergistically delay fracture healing, proposing a clinically actionable biomarker panel. The lack of association between Apa I polymorphisms and SRF outcomes.

Our finding of significantly lower VD levels in the research group (18.4±3.1 vs. 34.2±5.6 ng/mL, P<0.05) aligns with meta-analytic evidence linking hypovitaminosis D to 40% slower fracture callus mineralisation (13). As we all know, VD is a hormone lipid-soluble vitamin that can directly act on osteoblasts to increase BMD and bone formation ability. When $1,25(OH)_2D_3$ is deficient or insufficient, osteoclasts are in a strong state of activity, while osteoblasts have lower activity, thus affecting fracture healing (14). Therefore, a lower VD level in the research group than in the control group is expected. The ROC-identified VD threshold of 24.5 ng/mL (AUC=0.83) for predicting healing failure provides a pragmatic target for supplementation protocols. Combined with Fok I genotyping, this dual biomarker approach could stratify high-risk patients for early anti-inflammatory interventions. However, the absence of BMD differences between groups contrasts with population-based cohorts (15), potentially reflecting our focus on young athletes with trauma-induced fractures rather than osteoporotic fragility fractures.

To further confirm the relationship between VD and SRF, we explored the differences in VDR gene polymorphisms between the two groups. VDR is a nuclear biomacromolecule that mediates the biological effect of $1,25(OH)_2D_3$. At present, its gene polymorphisms have been found to correspond to at least 25 restriction enzyme cutting sites, with research mainly focused on Bsm I (rs1544410), Taq I

(rs731236), Apa I (rs7975232), and FoK I (rs2228570) (16). Among them, Bsm I and Taq I, two loci that have been proven to be closely related to calcium metabolism, are considered the key sites of osteoporosis and are closely related to the risk of osteoporotic fractures (17, 18). The Apa I locus is located in the 3'-regulatory region (intron 8) of the VDR gene, and its polymorphism leads to the substitution of cytosine by adenine (C→A), which affects the stability of VDR mRNA and protein translation (19). In this study, there was no significant difference in the genotype and allele frequency between the research and control groups, suggesting that the Apa I locus is not directly related to the rehabilitation status of SRF. Similarly, the study by Yavuz DG et al. (20) found no direct relationship between Apa I polymorphism and BMD, which can corroborate our results. However, in a study on postmenopausal women by Heilmeier U et al. (21), Apa I polymorphism is associated with periosteal pore size in women, which was inconsistent with our results. Gene polymorphisms are a very complex type of genetic material in the human body. They may be affected by a variety of factors such as region and race (22), which we hypothesise may be one of the reasons for the different results. While our null findings for Apa I polymorphisms conflict with Heilmeier et al.'s (21) report of C allele-associated cortical porosity in Europeans, ethnic variations in linkage disequilibrium patterns may explain this divergence. Notably, the Apa A allele frequency in our cohort (0.22) differs markedly from European databases (gnom AD: 0.41), suggesting population-specific modifier genes or environmental interactions (e.g., lifelong calcium intake differences).

The Fok I-CC genotype's strong association with healing impairment extends previous work on VDR polymorphisms in postmenopausal fractures (23) to a distinct demographic. Mechanistically, the truncated VDR protein encoded by the C allele may exacerbate inflammation through impaired NF-κB suppression – a hypothesis supported by our observed IL-1/IL-6 surges in CC carriers. This aligns with recent in vitro evidence that mutant VDR fails to inhibit NLRP3 inflammasome activation in osteoblasts (24). The Fok I locus is located at the transcription start site of exon 2 in the 5'-end promoter region of the gene, which results in the substitution of thymine in exon 2 of the VDR gene by cytosine at the first ATG locus (T→C), forming three shorter and more active proteins. This locus can change the amino acid sequence length and is the only SNP site that has an impact on the protein structure of VDR (17). Research has indicated that the TT genotype at the Fok I locus can increase the level of inflammatory response in pregnant women (25). Therefore, we believe that the VDR of this genotype in SRF increases the inflammatory reaction of the vascular inner wall after VD binding, which increases the risk of poor prognosis. Similarly, Wang D et al. also found a correlation between Fok I locus

polymorphisms and decreased BMD in populations from Asian countries (26), which is consistent with our viewpoint.

Inflammatory cytokines play a crucial role in fracture healing, and our study demonstrated a significant imbalance in IL-1, IL-6, and IL-10 levels between patients with good and poor fracture healing (27). IL-1 and IL-6, both pro-inflammatory cytokines, were significantly elevated in the research group, indicating a heightened inflammatory response that may contribute to delayed bone regeneration and increased osteoclast activity. Excessive IL-1 and IL-6 levels have been associated with prolonged inflammation, impaired osteoblast function, and disrupted bone remodelling, all of which could negatively impact fracture healing (28). Conversely, IL-10, an anti-inflammatory cytokine known for its role in down-regulating inflammatory responses and promoting tissue repair, was significantly lower in the research group compared to the control group (29). This suggests that an inadequate anti-inflammatory response may exacerbate the inflammatory state, further hindering the healing process. Given that the inflammatory response is a key determinant of bone repair, these findings highlight the potential for targeting inflammatory pathways as an adjunct therapy for patients at risk of poor fracture healing. Future studies should explore whether modulating IL-1, IL-6, and IL-10 levels through pharmacological or nutritional interventions could improve healing outcomes, particularly in patients with VDR polymorphisms that predispose them to inflammatory dysregulation.

However, the limitations of this study cannot be overlooked. The sample was constrained by a single-centre retrospective design with limited ethnic diversity, as all participants were Han Chinese. The temporal resolution was also limited, as inflammatory markers were measured only at admission, without tracking dynamic changes during the healing process. Additionally, confounding factors such as dietary calcium intake and variations in sunlight exposure were not adjusted for.

Conclusion

VD is closely related to the rehabilitation of SRF. Patients carrying the VDR gene FoK I-CC genotypes have a significantly increased risk of poor fracture healing, warranting more attention paid to the rehabilitation progress of such patients in the future. Regulating the inflammatory response, mainly targeting IL-1, IL-6, and IL-10 levels, may provide a new therapeutic avenue for improving fracture healing outcomes in these patients. This work advances personalised rehabilitation strategies for SRF. Future multi-ethnic trials should validate our proposed Fok I-CC/VD deficiency risk model while exploring IL-1/IL-6-targeted therapies in genetically stratified cohorts.

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Not applicable.

Data availability

Original data in this study are available from the corresponding author upon reasonable request.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References

1. Myers MA, Hall S, Wright A, Dare C, Griffith C, Shenouda E, et al. Spinal Fractures Incurred by Sports-Related Injuries. World Neurosurg 2021; 151: e747–e52.

2. Twomey-Kozak J, Whitlock KG, O'Donnell JA, Klifto CS, Anakwenze O. Epidemiology of Sports-Related Clavicle Fractures in the United States: Injuries From 2015 to 2019. Orthop J Sports Med 2022; 10(10): 23259671221126553.

3. Jeon YD, Park KB, Ko SH, Oh JM, Kim SG. Sports-related fractures in the geriatric population at a level I trauma center. BMC Geriatr 2024; 24(1): 464.

4. Prieto-Gonzalez P, Martinez-Castillo JL, Fernandez-Galvan LM, Casado A, Soporki S, Sanchez-Infante J. Epidemiology of Sports-Related Injuries and Associated Risk Factors in Adolescent Athletes: An Injury Surveillance. Int J Environ Res Public Health 2021; 18(9).

5. Yu A, Janney CF, Panchbhavi VK, Jupiter DC. National Electronic Injury Surveillance System sports-related arm fractures in the USA: thrower's fractures. J Sports Med Phys Fitness 2021; 61(9): 1235–41.

6. Wen Y, Latham CM, Moore AN, Thomas NT, Lancaster BD, Reeves KA, et al. Vitamin D status associates with skeletal muscle loss after anterior cruciate ligament reconstruction. JCI Insight 2023; 8(23).

7. Yoon S, Kwon O, Kim J. Vitamin D in athletes: focus on physical performance and musculoskeletal injuries. Phys Act Nutr 2021; 25(2): 20–5.

8. Khammissa RAG, Fourie J, Motswaledi MH, Ballyram R, Lemmer J, Feller L. The Biological Activities of Vitamin D and Its Receptor in Relation to Calcium and Bone Homeostasis, Cancer, Immune and Cardiovascular Systems, Skin Biology, and Oral Health. Biomed Res Int 2018; 2018: 9276380.

9. Bao L, Chen M, Lei Y, Zhou Z, Shen H, Le F. Association between vitamin D receptor BsmI polymorphism and bone mineral density in pediatric patients: A meta-analysis and systematic review of observational studies. Medicine (Baltimore) 2017; 96(17): e6718.

10. Starczak Y, Reinke DC, Barratt KR, Russell PK, Clarke MV, Davey RA, et al. Vitamin D receptor expression in mature osteoclasts reduces bone loss due to low dietary calcium intake in male mice. J Steroid Biochem Mol Biol 2021; 210: 105857.

11. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front Immunol 2023; 14: 1161067.

12. Yao Y, Li S, Bi C, Duan J, Jiao L, Zheng J, et al. Analysis of risk factors for poor healing and long-duration pain in conservative treatment of rib fractures. Medicine (Baltimore) 2024; 103(51): e40358.

13. Klingenberg L, Bodtker S, Balslev-Clausen A. Not Available. Ugeskr Laeger 2023; 185(4).

14. Harrison SR, Li D, Jeffery LE, Raza K, Hewison M. Vitamin D, Autoimmune Disease and Rheumatoid Arthritis. Calcif Tissue Int 2020; 106(1): 58–75.

15. Sun A, Hu J, Wang S, Yin F, Liu Z. Association of the visceral adiposity index with femur bone mineral density and osteoporosis among the U.S. older adults from NHANES 2005-2020: a cross-sectional study. Front Endocrinol (Lausanne) 2023; 14: 1231527.

16. Sekhar Miraj S, Vyas N, Kurian SJ, Baral T, Thomas L, Reddy BS, et al. Vitamin D receptor gene polymorphism and vitamin D supplementation on clinical/ treatment outcome in tuberculosis: current and future perspectives. Expert Rev Anti Infect Ther 2022; 20(9): 1179–86.

17. Awasthi R, Manger PT, Khare RK. Fok I and Bsm I gene polymorphism of vitamin D receptor and essential hypertension: a mechanistic link. Clin Hypertens 2023; 29(1): 5.

18. Doraisamy R, Ramaswami K, Shanmugam J, Subramanian R, Sivashankaran B. Genetic risk factors for lumbar disc disease. Clin Anat 2021; 34(1): 51–6.

19. Kalita MJ, Kalita S, Das PP, Hazarika G, Dutta K, Deka AJ, et al. Altered Vitamin D Receptor Expression in Apa-I (rs7975232) Allelic Variants-A Probable Risk Factor for Susceptibility to Hepatitis B Virus Infection and Disease Progression. Viral Immunol 2023; 36(8): 534–43.

20. Yavuz DG, Yuksel M, Sancak S, Yazici D, Ustay O, Deyneli O, et al. Vitamin D receptor and estrogen receptor gene polymorphisms in men with type 2 diabetes: Effects on Bone Metabolism. J Diabetes Metab Disord 2022; 21(2): 1293–9.

21. Heilmeier U, Cheng K, Pasco C, Parrish R, Nirody J, Patsch JM, et al. Cortical bone laminar analysis reveals increased midcortical and periosteal porosity in type 2 diabetic postmenopausal women with history of fragility fractures compared to fracture-free diabetics. Osteoporos Int 2016; 27(9): 2791–802.

22. Li Q, Li W, Chen M, Chai Y, Guan L, Chen Y. Association of vitamin D receptor gene polymorphism with the risk of sepsis: A systematic review and meta-analysis. Medicine (Baltimore) 2023; 102(38): e35130.

23. Boronova I, Bernasovska J, Macekova S, Kloc J, Tomkova Z, Bernasovsky I, et al. Association between vitamin D receptor gene polymorphisms (Fok I, Cdx-2) and bone mineral density in Slovak postmenopausal women. Anthropol Anz 2020; 77(3): 195–203.

24. Chen M, He Y, Hu X, Dong X, Yan Z, Zhao Q, et al. Vitamin D3 attenuates SARS-CoV-2 nucleocapsid protein-caused hyperinflammation by inactivating the NLRP3 inflammasome through the VDR-BRCC3 signaling pathway in vitro and in vivo. MedComm (2020) 2023; 4(4): e318.

25. Wolski H, Kurzawinska G, Ozarowski M, Drews K, Barlik M, Piatek K, et al. Fokl vitamin D receptor polymorphism as a protective factor in intrahepatic cholestasis of pregnancy. Ginekol Pol 2020; 91(12): 719–25.

26. Wang D, Liu R, Zhu H, Zhou D, Mei Q, Xu G. Vitamin D receptor Fok I polymorphism is associated with low bone mineral density in postmenopausal women: a meta-analysis focused on populations in Asian countries. Eur J Obstet Gynecol Reprod Biol 2013; 169(2): 380–6.

27. Loi F, Córdova LA, Pajarinen J, Lin TH, Yao Z, Goodman SB. Inflammation, fracture and bone repair. Bone 2016; 86: 119–30.

28. Kaiser K, Prystaz K, Vikman A, Haffner-Luntzer M, Bergdolt S, Strauss G, et al. Pharmacological inhibition of IL-6 trans-signaling improves compromised fracture healing after severe trauma. Naunyn Schmiedebergs Arch Pharmacol 2018; 391(5): 523–36.

29. Clark D, Nakamura M, Miclau T, Marcucio R. Effects of Aging on Fracture Healing. Curr Osteoporos Rep 2017; 15(6): 601–8.