Pharmacogenomics and Personalized Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

REVIEW

Genetic Influence of Fracture Nonunion (FNU): A Systematic Review

Mir Sadat-Ali[1,*], Hussain K Al-Omar[2,*], Khalid W AlTabash[1,*], Ammar K AlOmran[1,*], Dakheel A AlDakheel[1,*], Hasan N AlSayed[1,*]

[1]The Department of Orthopedic Surgery, King Fahd Hospital of the University, Imam AbdulRahman Bin Faisal University, Dammam, Saudi Arabia; [2]Department of Orthopedic Surgery, King Fahd Military Medical City, Dhahran, Saudi Arabia

*These authors contributed equally to this work

Correspondence: Mir Sadat-Ali, King Fahd Hospital of the University, POBOX 40071, AlKhobar, 31952, Saudi Arabia, Tel +966505848281, Fax +966138820887, Email drsadat@hotmail.com; smali@iau.edu.sa

Purpose: Nonunion of fractures occurs in about 15% of all fractures causing repeated surgical interference and prolonged morbidity. We performed this systematic review to assess genes and polymorphisms influencing fractures' nonunion (FNU).

Methods: We searched between 2000 and July 2022 in PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and Cochrane Database of Systematic Reviews, Genome Wide Association Studies (GWAS) Catalog, and the Science Citation Index, with the keywords nonunion of fractures, genetic influence, and GWAS. The exclusion criteria were review articles and correspondence. The data were retrieved to determine the number of studies, genes, and polymorphisms and the total number of subjects screened.

Results: A total of 79 studies were reported on nonunion of fractures and genetic influence. After the inclusion and exclusion criteria, ten studies with 4402 patients' data were analyzed. Nine studies were case-controlled, and 1 GWAS. It was identified that patients with polymorphisms in the genes *ANXA3, BMP2, CALY, CYR61, FGFR1, IL1β, NOG, NOS2, PDGF gene, and TACR1* are prone to develop a nonunion of fractures.

Conclusion: We believe that for patients who develop an early nonunion of fractures, a genetic study should be conducted for single nucleotide polymorphism (SNP) and genes so that alternative and more aggressive treatment can be performed to heal fractures without prolonged morbidity.

Keywords: nonunion, fractures, genome wide association study, genes, influence, morbidity

Introduction

According to a 2019 annual report, over 178 million fractures occur universally, with more than 450 million pending patients with acute and chronic complications related to fractures every year.[1] One of the more calcitrant complications is fracture nonunion (FNU), which occurs in about 15% of all fractures.[2]

It is estimated that about nine million FNU occurs yearly the world over and causes tremendous morbidity and clinical consequences.[3] Many causes have been implicated in FNU, including host factors, diseases, site of fractures, mechanical, infection, and surgical failures.[4–9] FNU provokes prolonged morbidity, decreased productivity, disability, impaired quality of life, and soaring healthcare costs. In Great Britain, the direct hospital cost of treating each FNU costs between £7000 and £79,000.[10,11]

It was accepted long before that individual genetic variations influence all human diseases, but it was difficult to identify specific genes and single nucleotide polymorphisms (SNP). In the past 20 years since the inception of the Human Genome Project and technological advances and analytical approaches, it can be pinpointed in the genetic variations that cause the specific disease process.[12–17]

In the last decade, few of the genes and SNPs that cause FNU have been described, which otherwise would have united. This systematic review aims to compile all the genes and SNPs associated with FNU to provide information to orthopedic surgeons, so they can confirm the genetic cause of FNU and take appropriate action.

Received: 5 February 2023
Accepted: 26 May 2023
Published: 5 June 2023

Methods

Our review protocol was designed according to the Preferred Reporting Items of Systematic Reviews and Meta-Analysis for Protocols (PRISMA-P). The authors followed the current best practices for a systematic review and the protocol was registered in PROSPERO[18] (ID395302).

Information Sources

An extensive literature search was designed and performed by a central medical librarian of the Imam Abdul Rahman Bin Faisal University, Dammam, for the concepts of nonunion of traumatic fractures and genes and SNPs. Pertinent publications were identified by searching the following databases with preset keywords: We searched between 2000 and July 2022 in PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and Cochrane Database of Systematic Reviews, Genome Wide Association Studies (GWASs) Catalog, Embase (via Elsevier, 2 to present), MEDLINE, Web of Science Core Collection (via Thomson Reuters), including the Science Citation Index. The keywords for the search were nonunion of fractures, genetic influence, SNPs, and GWASs. Searches were limited to English language literature. The data was analyzed as soon as consensus was reached to finally include the full-text articles.

Inclusion and Exclusion Criteria

The inclusion criteria were all genetic studies reporting specific variants (ie, GWASs, SNPs, whole genome sequencing, whole exome sequencing) published in English literature with case cohorts of at least 100. The exclusion criteria were review articles and correspondence.

Data Extraction and Synthesis

The authors created the data in an Excel spreadsheet. They collected the pertinent clinical and demographic data, type of fractures studied, year of publication, size of cohorts, controls, genes and SNPs, nucleotide level, candidate genes, GWASs, targeted sequencing, whole genome and exome sequencing. The data was finally put to analysis when the authors agreed with the data and methodology used for the review.

Study Outcomes

The primary study outcome was the presence of a nonunion of the long bones. Analyses will include odds ratios and relative risk assessments, and subgroup analysis (ie, males vs females, age groups) was applied. The retrieved data was analyzed using SPSS Inc Version 26.

Results

A total of 79 studies on the nonunion of fractures were reviewed, with a focus on the genetic influence. After the inclusion and exclusion criteria were implemented, ten studies with 4402 patients' data were analyzed. Nine studies were case controlled and 1GWAS. Figure 1 shows the PRISMA flow chart depicting the eliminated and the final ten in-depth studies examined. Table 1 gives the studies included in the analysis with the year of publication, SNPs identified, and the number of study subjects.

Three ethnic population groups were studied: Asians, Caucasians, and South Americans.

Table 2 gives the genes and SNPs strongly prone to developing FNUs. The table indicates that patients carrying these genes and SNPs are prone to get FNUs. Genes *ANXA3 SNPs GSE95849, GSE93213*, and *GSE93215; BMP4* gene polymorphism *rs17563, CYR61* polymorphism *rs3753793; FGFR1* gene polymorphism *rs1331* and *IL1β* gene polymorphism *rs2853550; NOS2* gene polymorphisms *rs2297514; rs2297514* and *rs2248814*; NOG gene polymorphisms *rs1372857* and *rs2053423, TLR2 rs5743708*; and *TACR1* gene with SNPs are located in Calcyon (CALY).

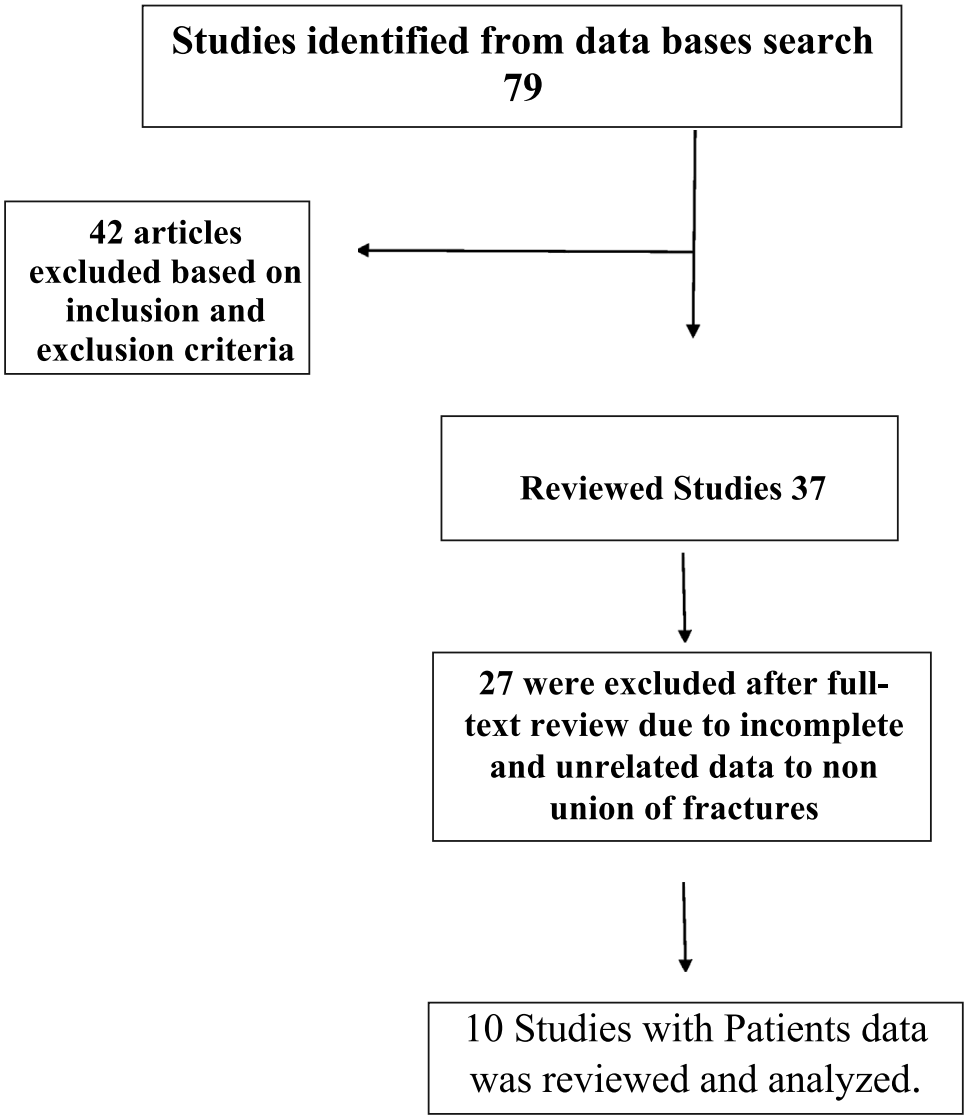

Figure 1 PRISMA flow chart of the review.
Notes: PRISMA figure adapted from Moher D, Shamseer L, Clarke M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4(1):1. Creative Commons.[28]

Discussion

Our analysis covered ten publications and 4402 patients. It showed that specific genes and SNPs contribute to a higher risk of FNU. Three studies reported similar genes and SNPs.

Three genes located on chromosome 4: *ANXA3 GSE95849, TACR1 rs229812* and *TLR2 rs5743708*; on chromosome 17, *NOG rs2053423*; and *2248814* and *NOS2 rs2297514*.

These SNPs have shown that in fracture or nonunion, not one but many genes and SNPs are involved. This should help surgeons dealing with nonunion that prove resistant to commonly used treatments consider genetic analysis to rule out the presence of genes and SNPs that influence nonunion.

Several factors have been found to contribute to the nonunion of fractures. These are due to the patient's general health and secondly due to the type of fracture, locations, and biological and mechanical environment at the site.[29–32] Recently, Mills et al (2016)[4] reported that in their series of patients with proven nonunion, 75% of patients had multiple causes that could cause a nonunion. These results suggest that, in addition to genetic causes, there are other contributing factors to nonunion of fractures. There is no definitive treatment for nonunion fractures except to investigate and find the real cause or causes of contributing factors and to treat them. In many instances, despite all the efforts in implementing

https://doi.org/10.2147/PGPM.S407308 DovePress

Table 1 List of All Published Data Analyzed

No	Authors	Title	Journal	Gene and SNP	No of Patients
1	Szczesny G, Olszewski WL, Swoboda-Kopeć E, Zagozda M, Czapnik Z, Interewicz B et al[18]	Genetic factors predisposing to bone fracture non-union. A role of single point mutation Asp299Gly TLR4 on pathogen-evoked healing	Chir Narzadow Ruchu Ortop Pol. 2010 Jan-Feb;75(1):57–63.	The Toll-like receptor 4 (TLR4) polymorphism Asp299Gly.	151
2.	Szczesny G, Olszewski WL, Zagozda M, Rutkowska J, Czapnik Z, Swoboda-Kopec E, Gorecki A.[19]	Genetic factors responsible for long bone fractures non-union.	Arch. Orthop. Trauma Surg. 2011; 131, 275–281.	TLR 4 (mutated 1/W) and TGF-β gen (mutated homozygote T and heterozygote C/T).	295
3.	Zeckey C, Hildebrand F, Glaubitz LM, Jurgens S, Ludwig T, Andruszkow H wt al.[20]	Are polymorphisms of molecules involved in bone healing correlated to aseptic femoral and tibial shaft non-unions?	J.Orthop. Res. 2011; 29, 1724–1731	Polymorphisms within the PDGF gene.	94
4.	Dimitriou R, Carr IM, West RM, Markham AF, Giannoudis PV.[21]	Genetic predisposition to fracture non-union: a case control study of a preliminary single nucleotide polymorphisms analysis of the BMP pathway.	BMC Musculoskelet. Disord. 2011; 12, 44	BMP-2: rs1005464, rs235768, rs235764. NOG rs1372857 SNP) and NOG the rs2053423 SNP)	109
5.	Guimaraes JM, Guimaraes, IC, Duarte ME, Vieira T, Vianna VF, Fernandes MB et al[22]	Polymorphisms in BMP4 and FGFR1 genes are associated with fracture non-union.	J. Orthop. Res. 2013; 31, 1971–1979.	BMP4 and FGFR1	167
6.	Sathyendra V, Donahue HJ, Vrana KE, Berg A, Fryzel D, Gandhi J, Reid JS.[23]	Single nucleotide polymorphisms in osteogenic genes in atrophic delayed fracture-healing: a preliminary investigation.	J. Bone Joint Surg. Am. 2014;96, 1242–1248	IL1β: rs2853550 NOS2: rs2297514, rs2248814 MMP-13-rs3819089 BMP6:rs270393 T allele of rs2853550, T allele of rs2297514, G allele of rs2248814. G allele of rs3819089 and G allele of rs270393	62
7.	Ali S, Hussain SR, Singh A, Kumar V, Walliullah S, Rizvi N et al[24]	Study of cysteine-rich protein 61 genetic polymorphism in predisposition to fracture nonunion: a case control.	Genet. Res. Int. 2015, 754872.	CYR61: rs3753793.	500
8.	Huang W, Zhang K, Zhu Y, Wang Z, Li Z, Zhang J.[25]	Genetic polymorphisms of NOS2 and predisposition to fracture non-union: a case control study based on Han Chinese population.	PLoS ONE 2018;13, e0193673.	NOS2 gene T allele of rs2297514	1229
9.	Liu C, Liu Y, Yu Y, Zhao Y, Zhang D, Yu A.[26]	Identification of Up-Regulated ANXA3 Resulting in Fracture Non-Union in Patients With T2DM.	Front Endocrinol 2022; 24;13:890941	SNPs GSE95849, GSE93213 and GSE93215 Gene ANXA3.	35
10.	McCoy TH, Fragomen AT, Hart KL, Pellegrini AM, Raskin KA, Perlis RH.[27]	Genomewide association study of fracture nonunion using electronic health records.	JBMR plus. 2019;3(1):23–28.	(TACR1) gene, The most strongly-associated SNP is rs2298122	1760

Table 2 Influencing Genes, SNPs and Ethnicities in the Review

Chromosome	Gene	SNPs	Ethnicity
4	ANXA3	GSE95849	Asian
1	CYR61	rs3753793	Asian
14	BMP4	rs17563	Caucasian
8	FGFR1	rs13317	South America
2	IL1β	rs2853550	Caucasian
17	NOG	rs2053423;2248814	Caucasian
17	NOS2	rs2297514	Caucasian
8	PDGF		Caucasian
4	TACR1	rs229812	Caucasian
4	TLR2	rs5743708	Caucasian

the available treatments, fractures do not heal even after treating the host factors. In some other patients without any known risk factors, fractures do not unite and develop into full-fledged nonunions.[33]

This review covers work published on how genes and their mutations and SNPs could cause or increase the risk of developing nonunion. There is ample evidence from the reported studies in the literature that reveals in FNU that genetics play a significant role. As per our review, different genes and SNPs influence different known factors that cause FNU. Szczesny et al (2011)[20] were the first to highlight that subjects with a mutant TLR 4 gene are unable or have difficulty recognizing the infecting pathogens and eradicating them from the fracture site, causing nonunion. Dimitriou et al (2011)[22] after a case–control study, showed that genes and SNPSs could cause nonunion by interfering with the BMP cascade, which is essential in the molecular and cellular regulation of fracture union. Other studies have shown how genes and SNPs can interfere with the various steps of normal fracture healing. The only GWAS in this review reported is that McCoy et al (2018)[34] found that the TACR1 gene SNP *rs2298122* is strongly associated with DNU, and the mode of the effect is related to pain sensitivity, which may interfere with fracture healing. In a recent review, Yan et al (2020)[35] concluded that there are a number of genes and SNPs that cause nonunion of fractures and better understanding of this concept will allow to intervene early to heal fractures. Panteli et al (2022)[36] further emphasized that there is ample evidence that patients with nonunion of fractures have genetic vulnerability and additional focused research is needed to identify biomarkers which can be utilized in the prediction of nonunion of fractures which also help in the development of innovative gene therapies.

The study has limitations due to the small number of studies and the simultaneous number of subjects in the analysis. Secondly, only three ethnic populations have been studied. Conversely, this review sheds light on the target genes with the study population of over 4000 patients screened. In conclusion, our review has implications in that it accentuates the importance of further studies with robust numbers, and the results of such studies will give definitive conclusions for the best clinical practice. To achieve this perspective, GWAS, with a large sample size, will shed light on which aspects of factors cause FNU to encourage caution in avoiding such complications.

Funding

No funding to report.

Disclosure

The authors report no conflicts of interest in this work.

References

1. I-Min W, Bisignano C, James SL, et al. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev*. 2021;2:e580–92. doi:10.1016/S2666-7568(21)00172-0
2. Schlundt C, Bucher CH, Tsitsilonis S, Schell H, Duda GN, Schmidt-Bleek K. Clinical and research approaches to treat non-union fracture. *Curr Osteoporos Rep*. 2001;16:155–168. doi:10.1007/s11914-018-0432-1

3. Stewart SK. Fracture Non-Union: a Review of Clinical Challenges and Future Research Needs. *Malaysian Orthopaedic J*. 2019;13(2):1–10. doi:10.5704/MOJ.1907.001
4. Mills L, Tsang J, Hopper G, Keenan G, Simpson AHRW. The multifactorial aetiology of fracture nonunion and the importance of searching for latent infection. *Bone Joint Res*. 2016;5:512–519. doi:10.1302/2046-3758.510.BJR-2016-0138
5. Mills LA, Aitken SA, Simpson AHRW. The risk of non-union per fracture: current myths and revised figures from a population of over 4 million adults. *Acta Orthop*. 2017;88(4):434–439. doi:10.1080/17453674.2017.1321351
6. Zura R, Xiong Z, Einhorn T, et al. Epidemiology of Fracture Nonunion in 18 Human Bones. *JAMA Surg*. 2016;151(11):e162775. doi:10.1001/jamasurg.2016.2775
7. Megas P. Classification of non-union. *Injury*. 2005;36(Suppl 4):S30–7. doi:10.1016/j.injury.2005.10.008
8. Tzioupis C, Giannoudis PV. Prevalence of long-bone non-union. *Injury*. 2007;38(Suppl 2):S3–9. doi:10.1016/S0020-1383(07)80003-9
9. Hofman M, Andruszkow H, Heyer FL, Kobbe P, Hildebrand F, Poeze M. Risk factors of non-union in intramedullary stabilized diaphyseal long bone fractures: identifying the role of fracture stabilization strategies and concomitant injuries.Eur. *J Trauma Emerg Surg*. 2021;47(6):1903–1910. doi:10.1007/s00068-020-01335-y
10. Mills L, Simpson A. The relative incidence of fracture non-union in the Scottish population (5.17 million): a 5-year epidemiological study. *BMJ Open*. 2014;3:e002276. doi:10.1136/bmjopen-2012-002276
11. Kanakaris N, Giannoudis P. The health economics of long-bone non unions. *Injury*. 2007;38:S77–S84. doi:10.1016/S0020-1383(07)80012-X
12. Lander ES, Linton LM, Birren B; International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature*. 2001;409:860–921. doi:10.1038/35057062
13. International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426:789–796. doi:10.1038/nature02168
14. 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526:68–74. doi:10.1038/nature15393
15. The Haplotype Reference Consortium. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. 2016;48:1279–1283. doi:10.1038/ng.3643
16. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285–291. doi:10.1038/nature19057
17. Karczewski KJ, Francioli LC, Tiao G, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. *Preprint at bioRxiv*. 2019;1:64. doi:10.1101/531210
18. National Institute for Health and Care Research. Welcome to PROSPERO. Available from: https://www.crd.york.ac.uk/. Accessed May 29, 2023.
19. Szczesny G, Olszewski WL, Swoboda-Kopeć E, et al. Genetic factors predisposing to bone fracture non-union. A role of single point mutation Asp299Gly TLR4 on pathogen-evoked healing. *Chir Narzadow Ruchu Ortop Pol*. 2010;75(1):57–63.
20. Szczesny G, Olszewski WL, Zagozda M, et al. Genetic factors responsible for long bone fractures non-union. *Arch Orthop Trauma Surg*. 2011;131:275–281. doi:10.1007/s00402-010-1171-7
21. Zeckey C, Hildebrand F, Glaubitz LM, et al. Are polymorphisms of molecules involved in bone healing correlated to aseptic femoral and tibial shaft non-unions? *J Orthop Res*. 2011;29:1724–1731. doi:10.1002/jor.21443
22. Dimitriou R, Carr IM, West RM, Markham AF, Giannoudis PV. Genetic predisposition to fracture non-union: a case control study of a preliminary single nucleotide polymorphisms analysis of the BMP pathway. *BMC Musculoskelet Disord*. 2011;12:44. doi:10.1186/1471-2474-12-44
23. Guimaraes JM, Guimaraes IC, Duarte ME, et al. Polymorphisms in BMP4 and FGFR1 genes are associated with fracture non-union. *J Orthop Res*. 2013;31:1971–1979. doi:10.1002/jor.22455
24. Sathyendra V, Donahue HJ, Vrana KE, et al. Single nucleotide polymorphisms in osteogenic genes in atrophic delayed fracture-healing: a preliminary investigation. *J Bone Joint Surg Am*. 2014;96:1242–1248. doi:10.2106/JBJS.M.00453
25. Ali S, Hussain SR, Singh A, et al. Study of cysteine-rich protein 61 genetic polymorphism in predisposition to fracture nonunion: a case control. *Genet Res Int*;2015. 754872. doi: 10.1155/2015/754872
26. Huang W, Zhang K, Zhu Y, Wang Z, Li Z, Zhang J. Genetic polymorphisms of NOS2 and predisposition to fracture non-union: a case control study based on Han Chinese population. *PLoS One*. 2018;13:e0193673. doi:10.1371/journal.pone.0193673
27. Liu C, Liu Y, Yu Y, Zhao Y, Zhang D, Yu A. Identification of Up-Regulated ANXA3 Resulting in Fracture Non-Union in Patients with T2DM. *Front Endocrinol*. 2022;13:890941. doi:10.3389/fendo.2022.890941
28. Moher D, Shamseer L, Clarke M et al Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4(1):1.
29. Friedrich B, Klaue P. Mechanical stability and post-traumatic osteitis: an experimental evaluation of the relation between infection of bone and internal fixation. *Injury*. 1977;9:23–29. doi:10.1016/0020-1383(77)90045-6
30. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. *Injury*. 2007;38(Suppl 4):S3–S6. doi:10.1016/S0020-1383(08)70003-2
31. Perren SM. Evolution of the internal fixation of long bone fractures. The scientific basis of biological internal fixation: choosing a new balance between stability and biology. *J Bone Joint Surg*. 2002;84-B:1093–1110. doi:10.1302/0301-620X.84B8.0841093
32. Yamaji T, Ando K, Wolf S, Augat P, Claes L. The effect of micromovement on callus formation. *J Orthop Sci*. 2001;6:571–575. doi:10.1007/s007760100014
33. Gershuni DH. Fracture nonunion. *Western J Med*. 1989;150(6):689–690.
34. McCoy TH, Fragomen AT, Hart KL, Pellegrini AM, Raskin KA, Perlis RH. Genomewide association study of fracture nonunion using electronic health records. *JBMR Plus*. 2019;3(1):23. doi:10.1002/jbm4.10063
35. Yan T, Li J, Zhou X, et al. Genetic determinants of fracture non-union: a systematic review from the literature. *Gene*. 2020;751:144766. doi:10.1016/j.gene.2020.144766
36. Panteli M, Vun JSH, Pountos I. Biological and molecular profile of fracture non-union tissue: a systematic review and an update on current insights. *J Cell Mol Med*. 2022;26(3):601–623. doi:10.1111/jcmm.17096