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REVIEW ARTICLE

# Molecular pathogenesis of fracture nonunion

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**Abstract** Fracture nonunion, a serious bone fracture complication, remains a challenge in clinical practice. Although the molecular pathogenesis of nonunion remains unclear, a better understanding may provide better approaches for its prevention, diagnosis and treatment at the molecular level. This review tries to summarise the progress made in studies of the pathogenesis of fracture nonunion. We discuss the evidence supporting the concept that the development of nonunion is related to genetic factors. The importance of several cytokines that regulate fracture healing in the pathogenesis of nonunion, such as tumour necrosis factor-$\alpha$, interleukin-6, bone morphogenetic proteins, insulin-like growth factors, matrix metalloproteinases and vascular endothelial growth factor, has been proven *in vitro*, in animals and in humans. Nitric oxide and the Wnt signalling pathway also play important roles in the development of nonunion. We present potential strategies for the prevention, diagnosis and treatment of nonunion, and the interaction between genetic alteration and abnormal cytokine expression warrants further investigation.

*The translational potential of this article:* A better understanding of nonunion molecular pathogenesis may provide better approaches for its prevention, diagnosis and treatment in clinical practice.



## Introduction

Fracture healing is a complex but well-orchestrated process that results in the regeneration and functional restoration of bones. The US Food and Drug Administration has defined fracture nonunion as the absence of radiographic healing over 9 months with no visible healing progression in the last 3 months. As a serious fracture complication, nonunion has a significant effect on the quality of life and financial situation of patients and may be associated with

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severe functional and psychological impairments. The latest research has demonstrated that the average risk of nonunion per fracture was 1.9%, and the rate of nonunion was up to 9% in specific fracture types (tibial and clavicular fractures) and in old patients [1]. According to a 2007 study, the cost of nonunion treatment is as high as US$ 25,000 per patient, more than twice that for normal fracture healing [2].

Many factors, including systemic, local and treatment factors, impair fracture healing and eventually result in nonunion. Systemic factors mainly include age, nutrition and systemic diseases, the most common of which are diabetes mellitus, anaemia and endocrine disorders. Local factors, such as the type of fracture, blood supply and infection, may also influence fracture healing. In addition, some evidence indicates that smoking and certain medications, including nonsteroidal antiinflammatory drugs (NSAIDs) and corticosteroids, might have side-effects that affect bone healing [3,4].

Although these environmental causes of nonunion at the individual (macro) level have been extensively studied, the specific signal pathways involved remain unclear. Under the influence of external risk factors, the systemic or local expression of specific cytokines and growth factors might be disturbed. In addition, some patients present a predisposition towards nonunion development because of genetic defects. For example, young patients without any systemic disease develop nonunion at every fracture site after multiple fractures, even when undergoing proper treatments. Genetic factors also lead to abnormal expression of cytokines and result in nonunion. Alterations of genes and cytokines, at the molecular level, are discussed in this review (Figure 1).

Three aspects involved in the molecular mechanism of nonunion development are discussed in this review: genetic factors, abnormal cytokine expression and other small molecules. A clear understanding of the molecular pathogenesis of nonunion can improve our knowledge of this complication and provide different approaches for its prevention, diagnosis and treatment at the molecular level.

## Genetic factors

From a clinical perspective, it remains unknown why some patients without systemic or local risk factors present a predisposition towards nonunion development. Much evidence indicates that specific genetic variants and abnormal gene expression are the inherent causes of many diseases, which may also be true for fracture nonunion. Indeed, recent studies in this field have yielded valuable findings. The genetic factors related to nonunion that have been investigated to date are described below, and details of the related experiments are summarised in Table 1.

The first clinical study on the genetic predisposition towards nonunion was published in 2011 by Dimitriou et al. Fifteen single nucleotide polymorphisms (SNPs, which are a variation, such as a substitution, deletion or insertion, in a single nucleotide at a specific gene position) in four genes of the bone morphogenetic protein (BMP) pathway (BMP-2, BMP-7, Noggin and Smad6) were examined in 109 patients retrospectively. Two specific SNPs in Noggin and Smad6, both inhibitors of BMPs, appear to be responsible for the development of atrophic nonunion. It should be noted that this study did not exclude patients with other environmental risk factors, such as the type of fracture, smoking and NSAIDs use, because no significant difference in these factors was found between groups; however, the conclusion is weak because of the small size of the specific samples [5]. Thus, the correlation between these genes and nonunion needs to be further investigated. After excluding patients with other environmental factors, Zeckey et al. analysed SNPs in several cytokine genes that regulate fracture healing in patients with aseptic nonunion after femoral and tibial shaft fractures. Based on a comparison of the findings with those for patients with normal fracture healing, a platelet-derived growth factor (PDGF) haplotype was reported to be associated with aseptic nonunion [6]. In a study assessing the mutation frequency of genes that are crucial for the recognition and elimination of pathogens and fracture healing, the T and C/T alleles of the transforming growth factor-β (TGF-β) gene codon 10 and the mutated TLR4 gene W/1 were identified as possible risk factors for impaired recognition and elimination of bacteria, increasing the susceptibility of a fracture patient to develop septic nonunion [7].

Several recent studies have also suggested that genetic alterations significantly contribute as an etiological factor to the development of nonunion. Sathyendra et al. found that patients carrying five SNPs in four genes showed a significant association with atrophic nonunion [8]. Another study demonstrated that a T/G genotype at SNP rs3753793 in the CYR61 gene, which encodes an important signalling molecule that participates in many signalling pathways, may contribute to the development of nonunion [9]. The haplotype GTAA in BMP4 and C allele at rs13317 in FGFR1 are also associated with nonunion [10].

Some researchers have attempted to analyse the local gene expression at the fracture site and investigate different gene expression patterns between normal and impaired fracture healing. For instance, expression of eight genes in nonunion tissue was significantly increased compared with fresh callus tissue based on a cDNA array. Among these genes, *CDO1*, *COMP*, *FMOD* and *FN1* are important for the assembly and stabilisation of the extracellular matrix, *CLU* and *TCS22* induce cell differentiation and proliferation and *ACTA2* and *PDE4DIP* gene products, such as actin, participate in cytoskeletal organisation and maintenance. Moreover, overexpression of these genes in fracture tissue may impair the structure and function of bone healing—related cells, eventually leading to nonunion [11].

MicroRNAs (miRNAs) regulate gene expression related to many biological processes, such as cell proliferation, differentiation and organ development. Increasing evidence suggests that miRNAs play a key role in fracture healing and development of nonunion by regulating bone formation, resorption and remodelling. A comparison between fracture tissues with normal healing and those with impaired healing in mice showed that five miRNAs, miR-140-3p, miR-140-5p, miR-181a-5p, miR-181d-5p and miR-451a, are significantly upregulated in normally healing tissues [12]. Interestingly, a similar study in mice also reported that five different miRNAs, miR-31a-3p, miR-31a-5p, miR-146a-5p,