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More than movement: the proprioceptive system as a new regulator of musculoskeletal biology

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The proprioceptive system is essential for the control of coordinated movement and posture. Thus, traditionally, the study of proprioception has focused on its role in motor control. In this review, we present more recent findings on other, non-traditional functions of this system. We focus on its involvement in musculoskeletal development, function and pathology, including the regulation of spinal alignment, bone fracture repair and joint morphogenesis. We present the hypothesis that the proprioceptive system plays a central role in musculoskeletal biology, and that understanding the underlying molecular mechanisms will promote both basic science and medical innovations. As an example, we discuss recent evidence indicating that Piezo2, a key mechanosensitive ion channel of proprioception, regulates spine alignment and joint development. The presented findings show that the proprioceptive system regulates a wide range of developmental and physiological processes and that its dysfunction may contribute to the etiology of various musculoskeletal pathologies.

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Introduction

Proprioception is the sense of the relative position and movement of one's own body parts, the sense of tension or force and of the effort exerted by acting muscles. As such, proprioception is essential for the control of coordinated movement and posture [1,2]. Proprioceptive information is produced by specialized mechanosensory organs termed proprioceptors. In the musculoskeletal system of humans and other terrestrial vertebrates, the predominant types of proprioceptors are the muscle spindle and the Golgi tendon organ (GTO). These types differ in morphology, location, measured input, effect and other traits [3–5]. However, common to both organs is the ability to sense the biomechanical environment, initiate a neural response in specialized sensory afferent fibers, often termed proprioceptive neurons, and modulate local muscle tension by forming the muscle spindle and GTO reflex arches [1]. While the sensory nerve endings of these neurons are located within skeletal muscles and tendons, their cell bodies are found in the dorsal root ganglion (DRG) and they terminate in the spinal cord, where they communicate with motoneurons and interneurons to form reflex arcs. Additionally, these proprioceptive neurons continuously inform the central nervous system (CNS) about the tension, forces and movement of our muscles [1,6,7].

The vertebrate musculoskeletal system is composed of diverse tissue types, including skeletal tissues (bone, cartilage, and joints), muscles, tendons and ligaments. Its development and function were shown to be regulated by cross-tissue communication, which can be mediated by both molecular and mechanical cues [8,9]. The importance of mechanical signals such as muscle contraction for musculoskeletal development is conserved from fish to humans (reviewed in Ref. [9]), indicating that embryonic movement is necessary for this process. In the following, we refer to this type of regulation as non-autonomous, meaning that signals from one tissue, in this case, muscles, affect another tissue, namely skeleton. However, while the non-autonomous effect of muscle activity on skeletal development is well established, it raises questions regarding the nature of the movement that is necessary. Are there certain types of movement that are essential for proper development, or would any movement do? If indeed specific patterns of movement are needed, then abnormal proprioceptive function may be

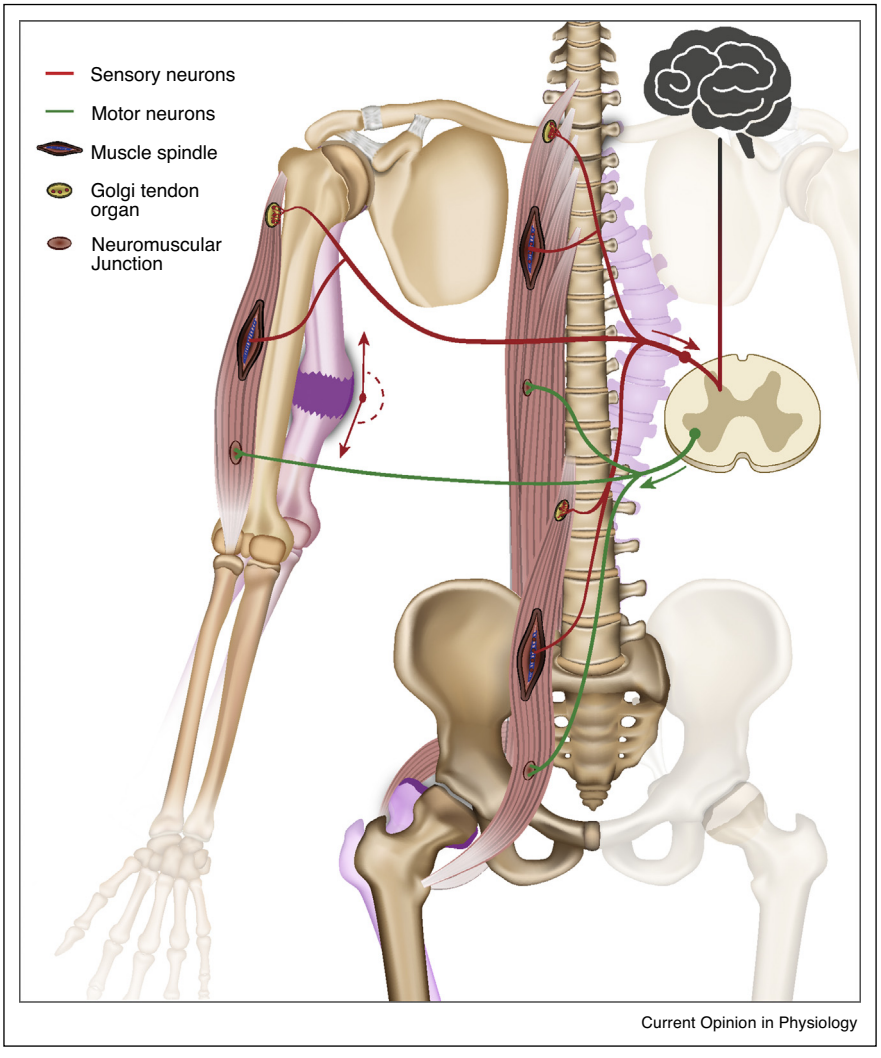
the cause for various musculoskeletal syndromes and pathologies.

In this review, we present recent findings implicating the proprioceptive system in regulating musculoskeletal biology, as well as in musculoskeletal pathologies. In the first part, we describe the roles of proprioception in spinal alignment, restoration of fractured bones and hip joint morphogenesis (summarized in Figure 1). These findings establish the regulatory role of proprioception in skeletal biology and suggest that mutations in genes associated with proprioception might be involved in musculoskeletal pathologies. This possibility is discussed in the second part of this review, using Piezo2 as a proof of concept. We also present other molecular component that might function within proprioception to regulate musculoskeletal biology. Finally, we speculate on possible future directions, including studying the involvement of proprioception in other musculoskeletal pathologies.

Proprioception in musculoskeletal development and function

In this chapter, we provide several examples for the regulation of key aspects in musculoskeletal biology by the proprioceptive system. These examples highlight the possibility that malfunction of the proprioceptive system might be the underlying mechanism in various human musculoskeletal pathologies.

Figure 1



The proprioceptive system regulates skeletal biology.
In the presence of a functional proprioceptive circuitry, properly regulated muscle contractions maintain proper alignment and morphology of skeletal elements. However, loss of proprioceptive circuitry results in skeletal deformity, such as spinal malalignment, aberrant hip joint morphology or impairment in natural reduction of fractured bones (depicted in purple).

a. Proprioception and scoliosis

The vertebral column is the central axis of the body, serving to support weight and maintain posture while allowing movement. To perform these functions and withstand high stresses created by body weight and by muscle loads, the position and orientation of dozens of vertebrae and intervertebral discs must be tightly maintained. Yet, little is known about the mechanisms that regulate spine alignment. An example for failure of these mechanisms is a condition known as scoliosis, which is defined as a lateral spinal curvature of 10 degrees or more [10]. The most common type of the disease is adolescent idiopathic scoliosis (AIS), which appears during puberty without other skeletal anomalies in around 3% of school-age children worldwide. Although severe cases may interfere with breathing, the mechanisms underlying the pathogenesis of AIS are still largely unknown [11,12].

Since AIS patients have morphologically intact spinal elements, spinal alignment may be regulated non-autonomously. A large body of evidence supports the involvement of the neuromuscular system in the etiology of scoliosis. These include anatomical alterations of motor brain areas, such as the cerebellum, cerebral cortex and vestibular system [13–15]. AIS patients also displayed abnormal somatosensory evoked potentials [16] and asymmetric strength and calmodulin distribution in trunk muscles [17,18]. In addition, neural injuries such as stroke [19] and cerebral palsy [20] are known to be associated with the development of postural imbalance and spine deformity. Finally, in animal models, lesions in dorsal column and posterior horn of the spinal cord [21] or nerve roots [22,23] resulted in scoliosis, demonstrating evolutionary conservation of the association between neuromuscular function and spine alignment.

Interestingly, both stroke [24,25] and cerebral palsy [20] have been shown to affect proprioception, suggesting a mechanistic link between neuromuscular function and the acquired deformity. Indeed, several observations support the notion of impaired proprioceptive function in AIS patients. These include asymmetrical gait [26] and impaired postural and balance control [27,28], as well as abnormal proprioception-related neural responses, such as inability to reproduce joint angle [29], vibratory sensation [30] and size-weight response [31]. Notably, a reduced number of muscle spindles was found in paravertebral muscles of AIS patients [32]. Additionally, the onset of AIS coincides with the maturation of the proprioceptive system, which occurs during the second decade of life [33,34]. While these data indicate a correlation between scoliosis and proprioception, direct evidence for the hypothesized involvement of proprioception and, specifically, of muscle spindles in the control of spine stability has only recently started to emerge.

The first demonstration of the involvement of proprioception in regulating spine alignment was provided by analyzing *Runx3* knockout (KO) mice, which lack functional proprioceptive neurons [35,36]. These mice developed peripubertal scoliosis without prior vertebral dysplasia or muscle asymmetry [37••]. Runx3 was also shown to be involved in differentiation of chondrocytes [38,39] and osteoblasts [40,41]. Therefore, a conditional knockout approach was applied to identify the tissue in which Runx3 function is necessary to prevent scoliosis. Interestingly, while conditional deletion of *Runx3* in skeletal tissues did not affect spine alignment, its deletion from peripheral nervous tissue or specifically from peripheral sensory neurons produced a similar scoliotic phenotype, indicating a non-autonomous role of the nervous system in skeletal alignment. Moreover, deletion of enhancer elements driving *Runx3* expression in proprioceptive neurons induced a similar phenotype, further indicating that proprioceptive neurons are necessary to maintain spinal alignment. A similar phenotype but of reduced severity was seen in *Egr3* KO mice, which lack muscle spindles but not Golgi tendon organs [42,43]. Functional assays revealed a decrease in gait regularity, which was also more pronounced in *Runx3* KO than in *Egr3* KO mice. These findings implicate impaired proprioceptive signaling in acquired scoliosis and suggest that both receptor types are required for this regulatory mechanism.

b. Proprioception and bone fracture healing

Restoration of bone morphology is essential for successful fracture healing and functional outcome [44,45]. In the orthopedic practice, this procedure is called reduction [46]. However, several pieces of evidence suggest that in nature too, broken bones are rapidly realigned following injury. In humans, humeral birth fractures, even when severely angulated, usually heal well and with little residual deformity without intervention [47]. Studies of primate skeletons revealed high incidence of up to 30% of bone fractures, occurring mostly in early life, which also healed well with minimal residual deformity [48–50].

These findings indicate that during evolution, vertebrates have acquired the ability to actively restore the morphology of fractured bones [51]. Indeed, it was shown that fractured humeri of neonatal mice undergo realignment without any intervention by a process that involves substantial movement of the two fracture fragments, which has been termed natural reduction [52]. This suggests the existences of a mechanism that regulates the restoration of bone morphology and guides realignment [52]. Recent findings showed that natural reduction failed in fractured bones of *Runx3* KO mice, as well as upon conditional deletion of *Runx3* in the peripheral nervous system. Similar to the spine alignment experiments, *Egr3* KO

mice displayed a less severe phenotype. Interestingly, inactivation of muscles surrounding the fracture site also resulted in failed realignment. These findings suggest that both proprioceptor types, as well as muscle contraction, are necessary for the regulation of natural reduction [53••].

Both molecular and mechanical interactions among musculoskeletal tissues have been shown to regulate the development of this system [9,54–57]. For example, mechanical signals generated by skeletal muscles control the formation of the circumferential shape of developing long bones [58] and maintain joint-forming cells committed to their fate [59]. Recently, it was suggested that muscle-derived satellite cells express growth factors that contribute to bone fracture repair [60]. The findings that muscle activity and proprioceptive signaling are involved in the regulation of fracture repair further demonstrates the importance of such cross- tissue interactions.

Bones are mechanosensitive organs [51,61–64] that adjust their morphology [58,65] and composition [66,67] in response to dynamic changes in mechanical loading. Bone and cartilage cells, including, osteoblasts [68] osteocytes [69] and chondrocytes [70,71], have been shown to be mechanosensitive, as is fracture callus [72–74]. The finding of a mechanism that monitors bone integrity and instructs its restoration following trauma through proprioceptive signaling adds a level of nonautonomous mechanosensitivity to the current view of fracture repair.

Interestingly, unlike in most repair processes, we found that natural reduction becomes more effective with age, concurrently with the maturation of the proprioceptive system. In mice, the sensory endings of muscle spindles continue to develop until about postnatal day 40 [75,76], whereas in humans, the performance of proprioception-dependent tasks improves during adolescence [33,34]. These findings support the notion that the observed improvement in natural reduction was due to augmented proprioceptive function.

Thus far, the research of fracture repair has largely overlooked the role of muscle force in bone realignment. Based on our findings, we suggest that the fracture changes the length and tonus of muscles that are attached to the bone, which are detected by muscle proprioceptors. Consequently, proprioceptive signals guide the correction of the position of misaligned fracture fragments rapidly and effectively by inducing asymmetric muscle contraction. Considering the contribution of this mechanism may improve the healing process and its outcome.

c. Proprioception and joint morphology

Hip dysplasia

Hip dysplasia, also known as developmental dysplasia of the hip (DDH), is the most common abnormality in human newborns. It is a spectrum of anatomical abnormalities of the hip joint in which the femoral head is malpositioned relative to its socket, the acetabulum [77]. Ranging from hip instability to dislocation, the condition affects hip biomechanics and, if left untreated, may cause osteoarthritis [78], limping and back pain. One in 100 children is born with hip subluxation or dysplasia and 1 out of 1000 children is born with a dislocated hip; 80% percent of affected children are female [79]. While the causes of hip dysplasia are not fully understood, it is approximately 12 times more likely when there is a family history [https://hipdysplasia.org/developmental-dysplasia-of-the- hip/causes-of-ddh/] [80].

The necessity of embryonic movement and muscle-generated forces for joint development has been well-established in various model organisms [37••,77–80], as in the absence of muscle contraction several joints fail to form [78,81,82]. However, this paradigm considers only two situations, namely the existence or absence of movement, disregarding everything in between. This dichotomous view overlooks the intriguing question of whether there are 'right' and 'wrong' types of movement, that is, if proper joint development requires specific patterns of embryonic movement.

Studies of hip joint morphogenesis in proprioception-deficient mutant mice, which preform uncoordinated movements [37••,43,83], might provide new insight into this question. Adult *Runx3* KO mice, which lack all proprioceptive neurons and, consequently, display severe ataxia, exhibited severe, irregular type hip dysplasia, which was manifested by a prominent cam over the femoral neck [84••]. Additionally, significantly increased acetabular index indicated a shallow acetabulum, whereas significantly reduced central edge angle (CEA) indicated lateralized center of rotation, which reduces joint stability and increases the risk for dislocations. Loss of *Runx3* in the peripheral nervous system, but not in skeletal lineages, led to similar joint abnormalities. Similarly, *Egr3* KO mice, which lack only muscle spindle and display reduced muscle coordination, presented a less severe hip dysplasia phenotype [84••].

The misshapen and incongruent joints observed in mutants for the proprioceptive system regulators *Runx3* and *Egr3* confirm the role of this system in hip joint morphogenesis. Moreover, they support the notion that specific patterns of movement are necessary for proper joint morphogenesis. However, how patterns of movement regulate joint morphogenesis is yet to be determined.

Sensory input controls joint integrity

The work reviewed in the previous sections, showing that altered neural activity after loss of proprioceptive information results in abnormal joint development, suggests that neural activity plays an important role in the

regulation of joint integrity. Although the significance of maintaining joint integrity is obvious when it fails, as seen in people with ligament ruptures or osteoarthritis [85,86], the role of the nervous system in this regulation is poorly understood [87,88].

The potential role of the CNS in regulating joint integrity has been difficult to evaluate, mainly because of the close relationship between joint mechanics and overall behavior [89]. In any purposeful behavior, the nervous system must act through joints in order to produce limb movement. Thus, any perturbation that impairs neural control of behavior will also alter joint stresses and strains. Conversely, any perturbation affecting neural control of joint integrity might result in damaged joint structures, which would then affect task performance.

A recent set of experiments were able to disentangle these effects and demonstrate that the nervous system directly regulates stresses and strains within joints [90,91,92•,93•]. These studies exploited the mechanical properties of the rat knee joint, evaluating whether neural control of quadriceps muscles reflects minimization of mediolateral forces on the patella. Examination of the patterns of correlation amongst quadriceps muscles revealed that the activity of muscles (vastus lateralis, VL; vastus medialis, VM) producing opposing mediolateral forces on the patella were highly correlated with one another, whereas the correlation of these muscles to muscles producing minimal mediolateral patellar forces (vastus intermedius, VI; rectus femoris, RF) was much weaker [91]. The low correlation of VM and VL to VI was surprising, since all three muscles are monoarticular knee extensors and thus have the same contribution to task performance. These results are consistent with a control strategy minimizing the net mediolateral force on the patella.

This possibility was further assessed by a series of experiments that examined how the CNS adapts to perturbations affecting joint integrity. First, the adaptations in quadriceps muscle activations in response to the addition of a lateral load on the patella were measured [93•]. Consistent with neural control of joint stresses, the ratio between the activation of VL and VM was reduced, thereby reducing net mediolateral patellar forces. In another experiment, adaptations were examined following paralysis of VL [92•]. If the CNS only regulated task performance, increasing activation of VM would replace the lost knee extension torque and restore limb kinematics. Instead, an increased activation of RF to compensate for paralysis of VL was observed. Although this adaptation strategy complicates restoration of task performance, as RF produces a hip flexion torque that is not produced by VL, it is consistent with regulation of joint stresses, since RF produces minimal mediolateral patellar force.

Taken together, these findings support the hypothesis that the nervous system chooses muscle activation patterns to minimize joint stresses and strains while achieving desired task performance. However, how the nervous system monitors these stresses and strains remains unclear [87,88,94]. Although it is possible that joint afferents provide direct information about the state of internal joint structures [95,96], the experiments on proprioception-deprived mice raise an intriguing possibility that muscle proprioceptors may also play an important role. For instance, the pattern of stretch reflexes amongst quadriceps muscles in the cat is consistent with the minimization of mediolateral patellar forces described in our previous experiments [97]. Deep muscles in the shoulder and hip are rich in proprioceptors, suggesting that they might act as sensory structures that signal the position of the femoral or humeral head relative to their sockets [98,99]. Joint loading might be monitored by GTO activity, with the nervous system using 'internal models' of how muscle forces affect joint structures to estimate joint stresses and contact forces.

Although the role of muscle proprioceptors in the production of behavior and task performance has been examined extensively [1,2], there are relatively few studies examining the potential role of muscle proprioceptors in regulating joint integrity [94,100]. Future studies in animals lacking muscle proprioceptors are needed to establish the connection between proprioception and joint stability.

In summary, the above examples provide strong evidence for the involvement of the proprioceptive system in regulation of skeletal development and pathology. Therefore, understanding the molecular components of the proprioception system might provide new insight into the mechanisms underlying some of these pathologies.

Molecular components mediating proprioception

While many of the molecular components regulating different aspects of neurobiology have been extensively studied and identified, the molecular mechanisms that regulate the development and function of proprioceptors have attracted less attention. In order to translate the findings that the proprioceptive system regulates musculoskeletal biology into therapeutic applications, it is necessary to decipher these mechanisms. Indeed, several groups have recently started to provide this information [101–103]. In this chapter, we discuss several molecular components within proprioceptors that might function to regulate musculoskeletal biology.

a. Mechanotransduction ion channels in proprioceptive neurons

Proprioceptive neurons transduce mechanical strain, experienced by muscles and joints, into electrical signals

[1]. While the molecular mechanisms underlying proprioception transduction are largely unknown, it was shown that the major mechanotransducer of mammalian proprioceptors is the ion channel Piezo2 [83].

Piezo2: the key mechanosensitive ion channel of proprioception

Piezo1 and Piezo2 are calcium-permeable mechanosensitive ion channels. They are larger in size than other ion channels and their unique structure includes a series of four transmembrane helical bundles termed Piezo repeats, which together compose flexible propeller blades [104–107]. In recent years, this family of ion channels has been implicated in a variety of developmental and physiological processes. Piezo2 was shown to be expressed in lung [108], where it is important for the sensation of airway dilatation [109], in the gastrointestinal tract [110] and in skin, where this channel is involved in sensation of fine touch [111]. Another tissue where both Piezo1 and Piezo2 were found to be expressed is articular cartilage. It was shown that synergy between the two channels provides articular chondrocytes with mechanotransduction ability under high-strain mechanical stress, which is potentially deleterious to these cells [112].

In addition, Piezo2 was shown to be expressed by dorsal root ganglia (DRG) neurons, including proprioceptive mechanosensing neurons ending in muscle spindles and GTOs [83]. Loss of Piezo2 in proprioceptive neurons of mice resulted in severely uncoordinated body movements and abnormal limb positions, leading the authors to suggest that Piezo2 is the main mechanotransducer of mammalian proprioceptors [83].

In humans, mutations in the *PIEZO2* gene result in a variety of pathologies such as proprioception defects, skeletal abnormalities including scoliosis, hip dysplasia and arthrogryposis, a congenital contracture of multiple joints, neonatal respiratory insufficiency and muscle weakness [113–116]. Considering the wide range of tissues that express this gene, it is difficult to associate between phenotype and affected tissue in humans with *PIEZO2* mutations. In mice, however, this question can be addressed directly by creating tissue-specific knockout of *Piezo2*.

Given the observation of skeletal phenotypes in humans, the autonomous role of *Piezo2* was evaluated in mice by blocking its expression in chondrogenic or osteogenic lineages. However this deletion did not lead to alterations in skeletal morphology [84••,117]. By contrast, loss of *Piezo2* from proprioceptive neurons led to spinal malalignment and hip dysplasia [84••]. *In vivo* CT scans revealed that by P60, these mice developed scoliosis as well as kyphosis. Histological examination and morphometric analysis showed that the spinal deformities were not caused by aberrant development or morphogenesis of vertebrae or surrounding tissue. Morphological abnormalities in the hip joint included elevated acetabular index and flattened type dysplasia, loss of joint congruency and a femoral cam deformity. These findings establish the proprioceptive system as a regulator of spine alignment and joint development and identify Piezo2 as a key molecular component in this regulatory mechanism. Moreover, they also reinforce the need to better understand the molecular mechanism of proprioception and focus the attention on PIEZO2 regulation and signaling.

To identify proteins that interact with Piezo2, a mass spectrometry analysis was performed on Piezo2 in murine DRG somatosensory neurons [118]. This analysis revealed several candidates for Piezo2 regulation, including myotubularin-related protein 2 (Mtmr2) [118]. Subsequent work has shown that Mtmr2 regulates Piezo2 signaling by decreasing Piezo2-mediated rapidly adapting mechanically activated (RA-MA) currents [119]. Interestingly, Mtmr2 has been implicated in Charcot-Marie-Tooth type 4B1 disease (CMT4B1), a peripheral neuropathy characterized by neuronal demyelination, muscle weakness and sensory loss [120]. In severe cases, various degrees of skeletal deformities such as scoliosis, pectus carinatum, hand clawing, femoral anteversion, internal tibial torsion, in-toeing, and severe equinovarus were also observed [121].

It was recently shown that the regulatory channel TMEM150C/Tentonin3 can regulate Piezo1/2 and TREK-1 ion channels [122,123]. Moreover, TMEM150C/Tentonin3 was shown to be co-expressed with Piezo2, suggesting that it can regulate Piezo2 activity in proprioceptive neurons. Correspondingly, TMEM150C/Tentonin3 mutant shows impaired motor coordination [123]. It remains to be seen whether this fine regulation of proprioceptive sensing, which still affects motor behavior, also affects skeletal alignment.

Are there other mechanosensitive ion channels?

Notwithstanding the central role of Piezo2 in mediating proprioceptive signals, it is more than likely that other mechanosensitive channels also contribute to or modulate the stretch-activated response of proprioceptive neurons. Indeed, proprioceptive sensory neurons express other stretch-sensitive channels, such as epithelial sodium channels (ENaCs) including the acid-sensing ion channels (ASICs) [3,124–126]. Knockout of *Asic3* in mice impairs mechanotransduction of proprioceptive DRG neurons and leads to deficits in motor tasks that rely specifically on proprioception, such as grid and balance beam walk [126]. This suggests that, at least in mouse, ASIC3 also contributes to proprioception, albeit to a lesser degree than Piezo2.

Whether other ASIC family members also contribute to proprioceptive mechanotransduction is still unknown.

Functional ASIC channels are assembled from three homo/heterotrimeric subunits, and different combinations of ASIC subtypes display different electrophysiological properties [125,127]. However, the exact ASIC combinations in proprioceptive neurons remain unclear and need further investigation. Nonetheless, since ASIC3 was shown to be important in proprioceptive sensing, it would be interesting to examine whether it also plays a role in regulating skeletal integrity and alignment.

While the above studies have identified several ion channels that are required for proprioceptive neuron activity, the molecular signaling within sensory neurons remains elusive. To identify some of these molecules, a transcriptome analysis of proprioceptors was recently performed, revealing molecular markers for proprioceptive neurons, including specific markers for muscle spindles and GTOs [101]. Interestingly, some of these putative markers encode for ion channels and regulatory channel molecules, which are potential candidates for regulating proprioceptive signaling. We wonder whether different, perhaps more subtle, proprioceptive impairments would result in similar spine alignment and joint phenotypes, or in other musculoskeletal defects.

b. Muscular components of the proprioception sensory organs

Major components of muscle spindle are specialized muscle fibers termed intrafusal fibers. The intrafusal muscle fibers are innervated by proprioceptive sensory afferents and are morphologically distinct form their surrounding extrafusal fibers. The three types of intrafusal fibers, termed nuclear bag1, bag2 and chain fibers, differ in their myonuclear arrangement [128,129].

In muscle spindle, differentiation of intrafusal fibers begins with the connection between sensory afferents expressing neuregulin 1 (NRG1) and primary myotubes expressing its receptor Erb-B2 receptor tyrosine kinase 2 (ErbB2, also known as HER2) [130–132]. NGR1–ErbB2 signaling activates downstream targets such as early growth response 3 (Egr3), which mediates the development and maturation of muscle spindle in mice [42,43,133]. Analyses of *Egr3* KO mice, which have nonfunctional muscle spindles, revealed skeletal malalignment [37••,53••,84••], indicating that proprioceptive input from muscle spindles is required not only for motor coordination but also for skeletal alignment. However, the muscular components within intrafusal muscle fibers that are important for their function are still mainly unknown.

Recent advances in RNA sequencing have indorsed the analysis of isolated nuclei from muscle fibers. Recently, single-nucleus RNA sequencing of muscle spindle identified six different nuclear subtypes inside intrafusal fibers that refer to different compartments [134]. A comparison between neuromuscular junction (NMJ) and myotendinous junction (MTJ) clusters of extrafusal and intrafusal fibers revealed high similarity between intrafusal and extrafusal muscle fibers in these zones [134]. However, it would be interesting to identify the elements in the transcriptome that separate between the two fiber types. In the context of regulation of skeletal integrity by proprioception, the association between mutations in genes identified in each cluster and skeletal abnormalities should be investigated. Notably, the bag and intrafusal-NMJ clusters were shown to express the transcript of *Piezo2*, raising the possibility that it might also function inside intrafusal fibers. However, this potential role of Piezo2 is yet to be elucidated.

This snRNA-seq analysis has set the ground for future studies to compare the transcriptomes of intrafusal and extrafusal fibers, with aim to identify genes that are responsible for the specialized functions of each type of fibers.

Myosin composition and skeletal alignment

A major difference between intrafusal and extrafusal muscle fibers is the identity of their heavy-chain subunits of myosin (MyHCs), which include MyHC-emb (*MYH3*), MyHC-neo (*MYH8*), MyHC-slow (*MYH7*), MyHC-α cardiac (MYH6) and ancient myosins, MYH7b and MYH15 [135–139]. Moreover, it was shown that bag and chain fibers also differ in MyHC composition, as bag fibers express MyHC-slow and MyHC-α cardiac, and chain fibers express MyHC-emb and MyHC-neo isoforms [140]. This suggests that myosin composition may play an important role in muscle spindle function. Indeed, bag and chain fibers also differ in their functions, as bag1 fibers are solely responsible for the dynamic stretch response, whereas both bag2 and chain fibers contribute to the static stretch response.

In humans, mutations in the *MYH3* gene coding for MyHC-emb have been associated with two major distal arthrogryposis (DA) syndromes, namely Freeman–Sheldon syndrome (FSS) and Sheldon–Hall syndrome (SHS) [141]. FSS is characterized by congenital facial and limb contractures in addition to congenital scoliosis. How can embryonic myosin cause contractures of adult muscle and scoliosis? A recent study in *Myh3* mutant mice has shown the effects of MyHC-emb on myogenesis, regulating MyHC composition, fiber type, number and size [142]. These findings suggest that the contracture phenotype might be due to altered muscle size, type and MyHC composition. Additionally, adult *Myh3*-null mice exhibit scoliosis, similarly to the human FSS phenotype. Given the restricted expression of MyHC-emb to intrafusal muscle in adults, we wonder whether the contractile and scoliotic phenotypes are partly due to a nonautonomous effect of muscle spindle and proprioception on extrafusal muscles and spine. Additional studies, such

as analyzing the muscle spindle structure and function in *Myh3*-null mice and blocking *Myh3* expression specifically in muscle spindles, may reveal the involvement of proprioception in the FSS and SHS phenotypes.

Myh3 mutation was also implicated in spondylocarpotarsal synostosis (SCT), a skeletal disorder characterized by progressive vertebral, carpal and tarsal fusions [143]. This study identified a regulatory role for Myh3 in the TGFβ signaling pathway, and proposed that altered signal transduction in spinal muscles may lead to the development of vertebral fusions [143]. These findings suggest a nonautonomous role for Myh3 in skeletal regulation.

Muscle spindle in muscular dystrophy and skeletal alignment

Insight into other muscular components that may affect proprioception can come from muscular diseases. Many neuromuscular diseases are accompanied by impaired function of muscle spindles, resulting in a decline of motor performance and coordination. However, in many cases, such as in Parkinson's disease and amyotrophic lateral sclerosis [144–146], proprioceptive impairment results from degeneration of neurons. Conversely, muscle diseases such as muscular dystrophy (MD), where the muscles are primarily affected, might also affect intrafusal fibers. Indeed, MD patients often suffer from balance and posture problems, suggesting that their proprioceptive system might be impaired. The most common form of MD is Duchenne muscular dystrophy (DMD), an X-linked recessive disorder caused by a mutation in the gene encoding the protein dystrophin [147]. DMD patients also develop scoliosis; however, the reason for this is poorly understood [148]. A recent study has analyzed muscle spindles from wild type and dystrophic mice, revealing a concentration of dystrophin and β-dystroglycan in intrafusal fibers outside the region of contact with the sensory neuron [149]. While the morphology and number of muscle spindles were similar in dystrophic and wild-type mice, an increased dynamic sensitivity of muscle spindle afferent responses was seen in dystrophic mice [149]. This altered proprioceptive response might contribute to the unstable gait and scoliosis observed in DMD patients. Again, this raises the possibility that some of the DMD skeletal phenotypes are the consequence of abnormal regulation of skeletal muscles by muscle spindle and proprioception.

c. Capsule and extracellular matrix components

Both muscle spindle and GTOs are surrounded by two capsular structures. It has been suggested that the muscle spindle outer and inner capsules are similar to the perineurium and the endoneurial connective-tissue cells of the peripheral nerves [150–152], suggesting that they act as a selective permeable filter to control the extracellular content. The capsule is composed of connective tissue and extracellular matrix (ECM) components such as collagens, laminins, heparan sulfate and fibronectins [153,154]. However, the development and function of the capsule is still largely unknown. To our knowledge, the question of whether the capsule plays a role in muscle spindle and GTO development and function has not been addressed.

Since the capsule is enriched with ECM molecules, we wonder whether ECM-related myopathy phenotypes are related to impaired proprioception. For example, the outer capsule, containing a basement membrane, was shown to express collagen type VI (colVI) [154]. ColVI is associated with two main types of muscle disorders: Bethlem myopathy (BM) and the more severe syndrome Ullrich congenital muscular dystrophy (UCMD) [155,156]. Typically, UCMD patients present muscle weakness and hypotonia, congenital hip dysplasia, and contractures including torticollis, kyphoscoliosis and rigid spine [156]. A mouse model of colVI deficiency has been generated by deletion of the *Col6a1* gene [157]. These mice exhibit histological features of myopathy, such as fiber necrosis and variation in fiber width, but without obvious weakness. Subsequent work using these mice linked colVI functionally also to the peripheral nervous system, demonstrating that lack of colVI leads to increased myelin thickness, impaired nerve conduction, impaired motor coordination and delayed response to acute pain stimuli [158]. This raises the hypothesis that some of the UCMD phenotypes arise from a nonautonomous effect of ECM component in glia and perhaps also in proprioception tissues on the musculoskeletal system. To examine this hypothesis, a capsule-specific deletion of coIV should be studied.

Conclusions and future directions

In this review, we focused on the contribution of the proprioceptive system to musculoskeletal biology. We have described the non-traditional roles of proprioception in spinal alignment, realignment of fractured bones and joint morphogenesis (summarized in Figure 1). Interestingly, in three different genetic mouse models, impaired proprioceptive function leads to the same skeletal deformations, suggesting non-autonomous role for the proprioceptive system in skeletal biology. Furthermore, conditional deletion of *Piezo2* in proprioceptive neurons, but not in skeletal tissues, resulted in these skeletal deformation, further supporting this non-autonomous role. However, we believe that these skeletal phenotypes are only the tip of the iceberg and that future work will identify additional aspects of musculoskeletal biology that are regulated by the proprioceptive system.

A key question that remains open relates to the mechanisms underlying the regulation of musculoskeletal development by proprioceptive signaling. We speculate that different but nonexclusive mechanisms may be involved. For example, in the case of spine alignment, it is possible that the regulatory mechanism involves muscle tensions from both sides of the spine. Thus, loss of proprioception may lead to abnormal muscle tensions, resulting in spine misalignment. In joints, loss of

proprioception leads to uncoordinated movement and muscle activation patterns, which can lead to abnormal stresses on the joint. Such abnormal stresses can lead to abnormal mechanical signals in joint cells, affecting joint integrity and resulting in aberrant joint morphology. However, further studies are needed to verify these hypotheses and to explain the regulatory role of proprioception in skeletal biology.

We argue that the proprioceptive system regulates numerous aspects of musculoskeletal development. However, the effect of the proprioception system on the development of non-skeletal tissues has yet to be established. It remains to be explored how the proprioceptive system affects muscle function, diseases and repair. Additionally, recent studies have shown that in different myopathies, the intrafusal fibers are also impaired, raising the possibility that aberrant proprioceptive function is involved in the pathogenesis of these diseases.

Finally, there is still a need to identify the molecular players that mediate proprioceptive sensing. While the molecular apparatus of proprioceptive neurons is beginning to emerge, the identification of muscular and capsular components of proprioception has lagged behind. This gap in molecular knowledge prevents a comprehensive understanding of proprioceptor development and function. Moreover, it prevents associating molecular information on human musculoskeletal pathologies with proprioceptive function. Thus, an effort should be made to reveal the complete molecular identity of proprioceptors.

The involvement of the proprioceptive system in development, maintenance and repair of the skeleton increases substantially the scope of known functions of this system. Moreover, it raises the possibility that the proprioceptive system is involved in regulating other processes and that its dysfunction may contribute to the etiology of various musculoskeletal pathologies. Thus, further research of this fascinating system may have important medical implications.

Conflict of interest statement

Nothing declared.

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