

## REVIEW

# Structural maintenance of chromosome complexes and bone development: the beginning of a wonderful relationship?

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Bone development depends on environmental, nutritional and hormonal factors. Yet, an ordered and timed activation of genes and their associated molecular pathways are central for the growth and development of healthy bones. The correct expression of genes depends on both cis- and trans-regulatory elements. Of these, the elusive role of chromatin ultrastructure is just beginning to become appreciated. Changes in the higher-order structure of chromatin are affecting the expression of genes in response to intrinsic and environmental signals. Cohesin and condensin are members of the structural maintenance of chromosome (SMC) family of protein complexes, which mediate higher-order chromatin structure by tethering distinct regions of chromatin either inter- or intra-molecularly. In recent years, SMCs had been identified for their function in the regulation of gene expression and developmental processes, whereas malfunction of cohesin or condensin has an impact on human health. However, little is known about the specific roles of SMC complexes in bone development and their possible effect on bone health. Here, we review studies that suggest an intimate link between SMCs and bone development, as well as a plausible effect, direct or indirect, on the bone health. We describe genetic syndromes associated with SMCs with distinctive bone phenotypes and identify links between SMCs and bone-related molecular pathways. Future studies of the relationship between SMCs and bone development will reveal new understandings of both the cellular and molecular roles of SMC complexes and provide new insights into the growth and developmental processes in the bone.

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### Regulation of Bone Development

The human skeleton consists of >200 bones that serve as a mechanical scaffold, which supports and protects organs as well as anchors muscles. Early development of the skeleton in vertebrates depends on genes that are responsible for patterning cell distribution and proliferation into mesenchymal condensations at sites of future skeletal elements. Within these condensations, cells further differentiate into chondrocytes or osteoblasts and form cartilage and bone, respectively.<sup>1</sup> During childhood and adolescence, bone undergoes longitudinal and radial growth through a mechanism called bone formation (osteogenesis).<sup>2</sup> In most of the skeleton, osteogenesis involves differentiation of the mesenchymal tissue into bone and cartilage, which is later replaced by bone in a process called

enchondral ossification. During this process, chondrocytes proliferate, undergo hypertrophy and die. Cartilage extracellular matrix is then invaded by blood vessels and becomes calcified; the scaffold is invaded by hematopoietic cells and osteoprogenitor cells (which differentiate into osteoblasts), the latter depositing bone on remnants of the cartilage matrix.<sup>3</sup>

Environmental, nutritional and hormonal factors are known to affect bone growth, development and repair. Yet, a large proportion of these processes are determined by genetics. The three major signaling cascades involved in osteoblast differentiation, bone formation and repair are WNT signaling, transforming growth factor- $\beta$ /bone morphogenetic protein, and fibroblast growth factor pathways.<sup>4</sup> Genetic studies have uncovered hundreds of genes involved in these pathways and others that are related to embryonic cartilage development

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(chondrogenesis) and postnatal processes, such as osteoblasto- and osteoclastogenesis, endochondral bone formation and bone remodeling.<sup>4</sup> The genetics of bone formation and modeling is complex and involves interactions between many genes and both *cis*- and *trans*-regulatory elements. Changes in the higher-order structure of chromatin are just beginning to become appreciated as a central player in the regulation of gene expression and developmental processes.<sup>5</sup> The family of protein complexes called structural maintenance of chromosome (SMC) family facilitates higher-order chromatin organization and have key roles in chromosome stability and dynamics. Herein we present evidence for the involvement of SMC complexes in bone growth and development. In this review, we will discuss potential crossroads between SMC complexes and bone-related molecular pathways and propose possible mechanisms in which SMCs can affect these pathways.

### SMC Complexes

SMC complexes mediate higher-order chromatin structures by inter- or intra-molecularly tethering distinct regions of chromatin. SMCs are involved in the formation of chromatin loops, which can connect regulatory elements that are located at remote regions on the chromosome. These chromosome structures can either activate or suppress gene expression, define insulation regions and control the accessibility of transcription factors to the DNA. The SMC complexes cohesin and condensin that are evolutionary conserved in all eukaryotic cells feature a common molecular architecture but differ in their subunits (**Figure 1** and **Table 1**).<sup>6,7</sup> The heart of the SMC complex is a heterodimer composed of rod-like SMC proteins. The two ends of SMC proteins, called the hinge and the head, dimerize and form a ring structure. The dimerization of the SMC heads also creates two active ATPase domains. A kleisin superfamily protein is bound to the SMC heads and serves as the mediator of interaction with a set of the non-SMC regulatory subunits that vary between SMCs. Some of the mitotic subunits of cohesin are replaced by special subunits during meiosis (**Table 1**). SMC complexes are regulated by a large number of factors, generally referred to as auxiliary factors. Although the architecture shared by SMC complexes suggests a common

biochemical mechanism, the molecular details of their activity remain elusive.<sup>6,7</sup> SMCs are well known for their role in maintaining genome stability. However, this classical function of SMCs has been expanded in recent years to the regulation of gene expression.<sup>8</sup> In addition, SMCs have been recognized as central players in developmental processes. Furthermore, studies have associated cohesin and condensin with several human developmental disorders and cancer.<sup>9–11</sup>

### Cohesin

Cohesin is a four-subunit SMC complex that mediates sister chromatid cohesion, the process in which the newly replicated DNA strands, assembled into distinct chromatin fibers, are held together from the time of their formation until their separation during mitosis and meiosis (**Figure 1**). Cohesion maintains the fidelity of chromosome segregation by ensuring the bipolar attachment of the sister chromatids centromeres to the spindle.<sup>6,7</sup> Cohesin was also identified as a key factor in non-mitotic processes. During the cellular response to double-strand breaks, cohesin induces cohesion between sister chromatids adjacent to the break in order to suppress non-homologous recombination events.<sup>12</sup> Most intriguingly, cohesin is also central to the regulation of gene expression.<sup>13</sup> The complex is localized to a large number of regulatory elements on the chromosome and in many cases it is colocalized with the chromatin insulator CTCF (CCCTC-binding factor).<sup>14</sup> Cohesin and CTCF mediate the formation of long-range chromatin interactions that are required for promoter choices and gene activation.<sup>14</sup> It has been suggested that cohesin is recruited to

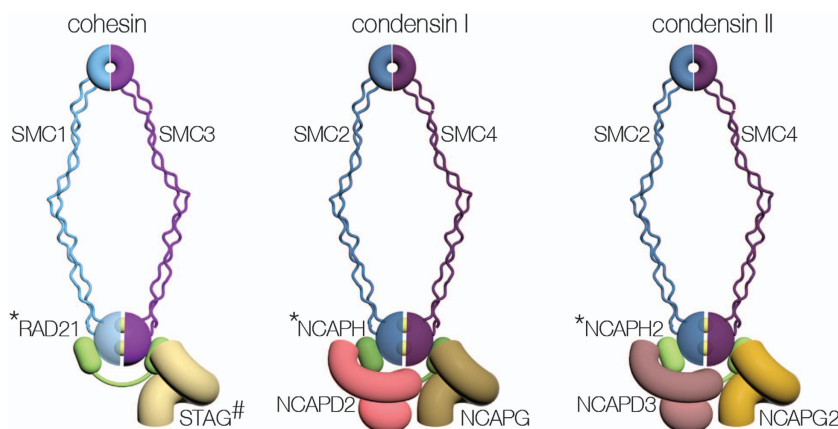
**Table 1** Subunits of SMC complexes

	Cohesin <sup>a</sup>	Condensin I	Condensin II
SMC protein	SMC1A/SMC1- $\beta^b$	SMC2	SMC2
SMC protein	SMC3	SMC4	SMC4
Kleisin	RAD21/REC8 <sup>b</sup> /RAD21L <sup>b</sup>	NCAPH	NCAPH2
Non-SMC	STAG1/STAG2/STAG3 <sup>b</sup>	NCAPG	NCAPG2
Non-SMC		NCAPD2	NCAPD3

Abbreviation: SMC, structural maintenance of chromosome.

<sup>a</sup>Isoforms are separated by a backslash.

<sup>b</sup>Meiotic isoform.



**Figure 1** Architecture of SMC protein complexes. The general architecture and subunit composition of cohesin, condensin I and condensin II are shown. The yellow balls between the SMC heads represent ATP molecules. \* indicates the kleisin subunits STAG, NCAPH and NCAPH2. The cohesin subunit STAG is mark by # to indicate there are two paralogs called STAG1 and STAG2.

gene promoters by interacting with the RNA polymerase II mediator complex and other transcriptional regulators, such as ATRX.<sup>15–16</sup> Yet, the mechanism through which cohesin localizes to regulatory elements and affects gene expression is poorly understood. During development, sets of genes are activated or suppressed according to a precise program. Changes in the higher order of chromatin can affect gene expression levels directly or through the regulation of master transcription factors. Indeed, cohesin has been identified as a key factor in the development of the immune system and nerve cells.<sup>17–18</sup> Several lines of evidence, discussed in the following sections, suggest that cohesin is also important for the proper development of bones.

### Condensin

In eukaryotes, the canonical condensin I is conserved in almost all organisms, whereas the related condensin II complex is observed only in vertebrates.<sup>11</sup> Both condensin I and II share core subunits and molecular architecture but differ in their kleisin and two non-SMC regulatory subunits (**Figure 1** and **Table 1**).<sup>19</sup> Condensins mediate the compaction of interphase chromatin before mitosis. Condensin II is localized on the chromatin throughout the cell cycle and functions in the early stages of chromatin condensation in prophase. In contrast, condensin I is cytoplasmic during interphase, and gains access to the chromatin only after nuclear membrane breakdown in pro-metaphase. Only then, condensin I and II collaborate to complete chromatin compaction.<sup>19</sup> Condensin I and II distribute along the entire length of the chromosome in an alternated pattern. Depletion of condensin I or II subunits result in distinct swollen or curly chromosome morphology, respectively. Cloud-like chromosomes without observed sister chromatids cohesion were detected when both condensins were depleted.<sup>19</sup> The spatial organization of condensin I and II, as well as the characteristic chromosome morphology obtained from their depletion suggest on a discrete function of each one of the complexes. The ratio between condensin I and II and the interplay with cohesin are essential for chromosome shaping.<sup>20</sup> It is possible that after reformation of the nuclear envelope, a small fraction of condensin I remain in the nucleus, contributing to non-mitotic processes.<sup>21</sup>

The role of condensin in the regulation of gene expression is elusive. Condensin is involved in the expression regulation of the recombinant DNA and transfer RNA genes in budding and fission yeast and in the organization of polytene chromosomes in *Drosophila*.<sup>22–24</sup> Condensin is involved in T- and B-cell development and maintenance of quiescence state by regulating the accessibility of transcription factors to chromatin.<sup>25,26</sup> Many of our knowledge of the roles of condensin in the regulation of interphase chromosome come from studies of the dosage compensation complex (DCC) present in *Caenorhabditis elegans*. The DCC is a unique form of condensin I, which regulates X chromosome expression in *C. elegans* hermaphrodites. Unlike mammalian X inactivation, both X chromosomes in *C. elegans* hermaphrodite are actively transcribed but the expression of each X chromosome is reduced by 50%. In DCC, the SMC1 core subunit of condensin I is replaced by the related DPY27 subunit and the complex has a distinct set of non-SMC regulatory elements. However, it is unclear how this change affects the function of the DCC in

comparison with condensin.<sup>27</sup> Studies aiming to generalize the special function of the DCC to vertebrates and dissection of the full role of condensin in transcription, as well as the mechanisms of action are yet to be explored.

### SMC-Related Genetic Disorders Associated with Bone Abnormalities

The link between SMC complexes and bone has not been fully established, but an increasing number of reports suggest that the two are intimately associated. More specifically, syndromes caused by mutations in genes encoding SMC complex components are associated, in part, with skeletal abnormalities. In addition, SMC complexes have been identified as regulators of key factors in bone development. In-depth analysis of this intriguing interplay remains a future challenge for both bone and SMC fields.

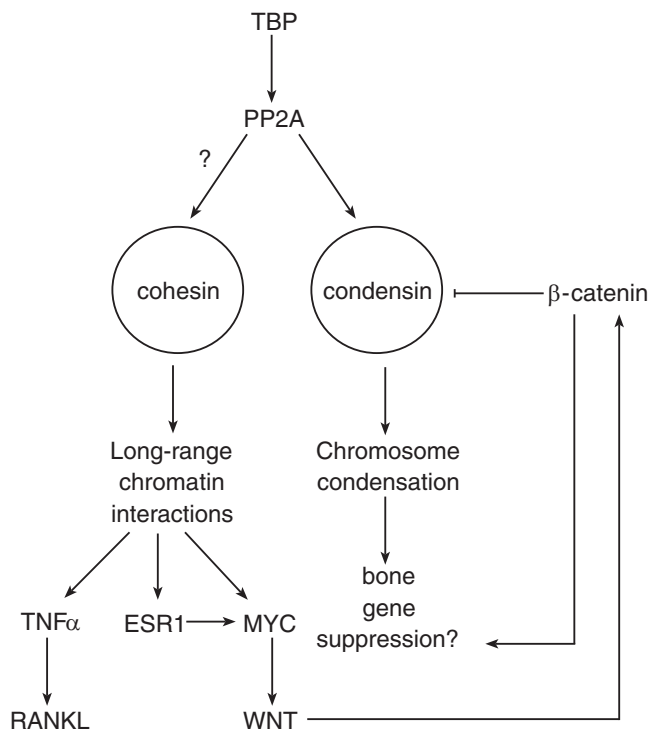
Cohesinopathy is a group of human disorders associated with mutations in cohesin subunits or auxiliary factors, all of which express a bone-related phenotype. Cohesinopathy disorders are characterized by multisystemic growth retardation and intellectual disability. Robert's syndrome (RBS, OMIM number 268300) is a rare autosomal recessive genetic disorder caused by a mutation in the cohesin auxiliary factor *ESCO2*, an acetyltransferase that modifies the SMC3 core cohesin subunit. The skeletal aberrations in RBS patients include abnormal development of all four limbs. The bones of extremities are short, particularly the forearms and the tibia. RBS patients may also have abnormal or missing fingers and toes, and joint deformities mainly at the elbows and knees.<sup>28</sup> A study in a *ESCO2* knockout zebrafish model for RBS showed normal ossification; however, the shape of ossified structures was abnormal.<sup>29</sup> Currently, the molecular pathway of cohesin in RBS is poorly understood.

Cornelia de Lange syndrome (CdLS, OMIM numbers 122470, 300590, 610759) is an additional cohesin-related genetic disorder with a prevalence of 1:10 000 live births. The disorder is classically manifested by prenatal and postnatal growth retardation and intellectual disability. CdLS patients often suffer from limb and digital anomalies, delayed skeletal maturation, abnormal thoracic configuration and flat acetabular angles in infancy. A mutation in one of five genes encoding a cohesin subunit or auxiliary factor is the cause of CdLS.<sup>30</sup> The mutation in patients with CdLS results in transcriptional misregulation of genes, including *MYC*, a multilignage transcription factor involved in appendicular skeletal development and osteogenic differentiation.<sup>31–34</sup> The combination of cohesin malfunction, misregulation of bone-related pathway and bone abnormalities implies on an unknown involvement of cohesin in bone growth and development.

Rare chromosomal aberration may reveal new information regarding the correlation between a genotype and a phenotype. A 17-Mbp chromosomal deletion in chromosome 9 that includes the *SMC2* condensin subunit locus has been reported.<sup>35</sup> The patient examined at the age of 1 year showed abnormalities in cranium, fingers, forehead and cranial sutures. However, because of the large size of the deletion, further studies are required to determine a direct link between *SMC2* haploinsufficiency in this patient and the skeletal abnormalities. Additional research will also be necessary to fully understand the effect of *SMC2* on skeletal development.

### Possible Involvement of SMCs in Molecular Pathways of Bone Development

Several links between cohesin and molecular pathways involved in bone development have been identified, and they are summarized in **Figure 2** and **Table 2**. The expression of *MYC*, which is misregulated in CdLS, is directly and positively regulated by cohesin.<sup>36</sup> *MYC* is a central component of the mitogenic signals of *WNT*, a central pathway in the bone and cartilage differentiation.<sup>37</sup> Two unanswered questions remain: is misregulation of *MYC* a primary cause for bone abnormalities in cohesinopathy? and do mutations in cohesin disrupt the *WNT* pathway? The answers to these questions would define a new pathway in bone development but remain a future challenge.



**Figure 2** Possible pathways linking cohesin, condensin and bone development. See text for details. The '?' symbol next to the arrow connecting protein phosphatase 2A (PP2A) and cohesin indicates a link between PP2A and cohesin. However, this interaction has been characterized only on mitotic chromosomes. Similar interaction in the context of cohesin role in transcription has not been described.

The estrogen-signaling pathway is also tightly involved in skeletal development, bone maturation and closure of epiphyseal plates in growing bones in both females and males.<sup>38</sup> The activation of *MYC* in response to changes of estradiol levels is regulated by cohesin.<sup>39</sup> *ESR1*, which encodes the estrogen receptor, is associated with changes in the bone mineral density and has a major role in osteoporosis and bone's osteogenic response.<sup>40–42</sup> Cohesin is enriched in promoters of estrogen-responsive genes, pointing out a possible collaboration between it and *ESR1*. Furthermore, depletion of the cohesin subunit *SMC3* significantly impaired the estrogen-regulated transcriptome.<sup>43</sup> Taken together, these findings suggest that cohesin modifies the higher-order structure of chromatin in estrogen pathway-related genes and serves toward establishment of the correct pattern of gene expression. Although most of these effects of estrogen were observed in oncogenic models,<sup>39</sup> similar processes may affect normal bone growth and development and are awaiting to be characterized.

The cohesin subunit *STAG2* has been identified as a transcriptional coactivator of the tumor necrosis factor- $\alpha$  (*TNF $\alpha$* ), an inflammatory cytokine involved in immunity and inflammation.<sup>44</sup> *TNF- $\alpha$*  promotes osteolysis, or pathological bone resorption, by activation of osteoblasts and tissue stromal cells to express receptor activator of nuclear factor- $\kappa$ B ligand (*RANKL*), which induces osteoclast differentiation and activity function.<sup>45</sup> In addition, *TNF- $\alpha$*  can act directly on osteoclast precursors, often via parallel signaling pathways and in synergy with *RANKL*, to promote osteoclastogenesis. Remarkably, while *TNF- $\alpha$*  stimulated waves of transcription of target genes in human umbilical vein endothelial cells, cohesin bound at the boundaries of the target genes stalls or slows down RNA polymerase, suggesting that cohesin antagonizes the *TNF- $\alpha$* -dependent activation of genes.<sup>46</sup> To this end, it is unclear how does the stalling of RNA polymerase by cohesin reflect on the developmental program of the cell.

To make the picture even more tantalizing, a genome-wide analysis identified an association between the height of German warmblood horses and the non-SMC condensin I complex subunit *NCAPG*.<sup>47</sup> Such correlation was also found in cattle, suggesting an evolutionarily conserved effect of condensin I on the growth of bones.<sup>48</sup> A recent study revealed a new link between the *WNT* pathway and condensin. The  $\beta$ -catenin/*TCF4* transcription factor binds to the *SMC2* promoter, suppressing the gene expression and inhibiting cell proliferation.<sup>49</sup>

**Table 2** Suggested bone genetic-related mechanisms involving SMC complexes

Gene	Gene function	Role in bone development	Relationship with SMC complexes	Reference
<i>MYC</i>	Transcription factor, central regulator of cell growth, proliferation, and apoptosis	Involved in appendicular skeletal development and osteogenic differentiation	Expression is regulated by cohesin	31,34,36,39
<i>Wnt</i>	<i>Wnt</i> genes encode secreted glycoproteins, which mediate intercellular signaling	Has a central role in bone and cartilage differentiation	Inhibits cell proliferation by suppressing the expression of the condensin subunit <i>SMC2</i>	4,33,37,49
<i>ESR1</i>	Receptor for estrogen	Changes in bone mineral density; A suggested role in osteoporosis	Cohesin binds chromatin and regulates gene expression in response to estrogen	40–42,43
<i>TNF-<math>\alpha</math></i>	An inflammatory cytokine involved in immunity and inflammation	Promotes osteolysis by activation of osteoblasts	The cohesin subunit <i>STAG2</i> is as a transcriptional coactivator of <i>TNF-<math>\alpha</math></i> ;	44,45
<i>NCAPG</i>	Condensin complex subunit 3	Associated with height	Non-SMC subunit of condensin I	47,48

Abbreviations: SMC, structural maintenance of chromosome; *TNF- $\alpha$* , tumor necrosis factor- $\alpha$ .

The different studies and their findings clearly demonstrate a relationship between SMCs and key factors in bone-related pathways. Therefore, the next challenge is to dissect the molecular mechanisms, which are affecting bone development. The first mechanism involving direct regulation of differentiation genes such as *MYC* and *ESR1*, is described above. In such mechanism, SMCs mediate changes in the chromatin structure that in turn control the expression levels of the master regulators themselves. In the second possible mechanism, inspired by the functional cooperation of STAG2 and TNF- $\alpha$ , SMCs collaborate with master regulators of transcription and modulate the chromatin structure of target genes, allowing their activation or repression.

An alternative and exciting mechanism in which SMCs can affect bone development is mitotic bookmarking. At the end of each cell cycle, chromosomes are condensed in order to allow their proper segregation to the two daughter cells. Bookmarking of genes, mediated by binding of specific factors to the promoter before the onset of mitosis or by epigenetic signals, allows cells to maintain a certain differentiation pathway by reactivating the same set of genes when chromosomes decondense and re-enter the cell cycle. DNA methylation and histone modifications are involved in bookmarking.<sup>50</sup> It is possible that cohesin is a key factor in mitotic bookmarking. Evidence comes from the altered DNA methylation pattern of chromosomes derived from CdLS patients. It was suggested that cohesin marks CpG islands for methylation. Furthermore, cohesin preferably binds hypomethylated regions and in turn alters the higher-order structure of the chromatin. Cohesin-mediated structure may protect regions of the chromosomes from condensation and allow their rapid activation after mitosis.

Interestingly, condensin may indicate another mechanism of mitotic bookmarking in differentiated bone cells. The expression of lineage-specific genes is mediated by maintaining the TATA-binding protein (TBP) in their promoters in condensed chromosomes.<sup>51,52</sup> TBP has been shown to antagonize condensin binding through the dephosphorylation of its NCAPG subunit by recruiting protein phosphatase 2A (PP2A) in HeLa cells, inactivating condensin at these regions, and create a mitotic bookmark.<sup>52</sup>

### Future Perspective

An increasing number of observations and circumstantial evidence suggest a link between the SMC complexes, bone growth and development. Up to this point, two basic facts have been established experimentally. First, cohesin and condensin regulate the expression of key factors in bone development (**Figure 2**). Second, human disorders associated with mutations in genes encoding these complexes show a clear bone development phenotype. We are currently looking up in the genome-wide association study results of bone phenotype of children in order to find genetic association between SMC-encoding genes and bone development,<sup>53</sup> (MCZ, DEK and IO; unpublished).

The next fundamental enigmas that need to be explored are the specific effects of cohesin in bone development and the mechanism of its regulation. Studies showed that the expression of a gene is correlated to the amount of cohesin bound to its promoter. An intensive genomic effort is now required in order to gain new insight into the process of

osteoblast differentiation. Chromatin immunoprecipitation-sequence platform (or similar technology) can be used to analyze the cohesin occupancy in promoters by experiments in order to identify the specific genes that are being regulated by cohesin in bone cells. Chromosome conformation capture is a useful tool to study long-range interactions in chromatin, as had been shown in the ENCODE project.<sup>14</sup> A future goal is to use this technique to define the specific interactions between *cis*-regulatory modules and promoters in osteoblasts. Other new technologies such as ChIA-PET will allow combining the knowledge regarding the chromosomal localization of the SMC complexes and the higher-order structure of the chromosome in osteoblasts. These data will allow integrating the biology of the bone with the nuclear architecture of the nucleus.

On the other hand, experts in the SMC field need to explore how and when SMCs are recruited to promoters in osteoblasts and, specifically, how they gain their specificity. This is likely to involve interaction with tissue-specific transcription factors and chromatin remodelers. Identifying such bone-related factors and characterizing the temporal and spatial relationship between SMCs and bone pathways is a necessary step for understanding the functions of SMCs in the bone. The ongoing effort to dissect the biochemical details of cohesin and condensin activities will also elucidate their bone-related functions. An interesting future goal is to test whether environmental signals affect bones through the modulation of SMCs activity. These intriguing questions are to be explored. Finally, this multidisciplinary effort will provide new diagnostic techniques and therapeutic targets for osteoporosis and other bone health-related diseases.

### Conflict of Interest

The authors declare no conflict of interest.

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