



# Mechanisms and clinical implications of intervertebral disc calcification

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**Abstract** | Low back pain is a leading cause of disability worldwide. Intervertebral disc (IVD) degeneration is often associated with low back pain but is sometimes asymptomatic. IVD calcification is an often overlooked disc phenotype that might have considerable clinical impact. IVD calcification is not a rare finding in ageing or in degenerative and scoliotic spinal conditions, but is often ignored and under-reported. IVD calcification may lead to stiffer IVDs and altered segmental biomechanics, more severe IVD degeneration, inflammation and low back pain. Calcification is not restricted to the IVD but is also observed in the degeneration of other cartilaginous tissues, such as joint cartilage, and is involved in the tissue inflammatory process. Furthermore, IVD calcification may also affect the vertebral endplate, leading to Modic changes (non-neoplastic subchondral vertebral bone marrow lesions) and the generation of pain. Such effects in the spine might develop in similar ways to the development of subchondral marrow lesions of the knee, which are associated with osteoarthritis-related pain. We propose that IVD calcification is a phenotypic biomarker of clinically relevant disc degeneration and endplate changes. As IVD calcification has implications for the management and prognosis of degenerative spinal changes and could affect targeted therapeutics and regenerative approaches for the spine, awareness of IVD calcification should be raised in the spine community.

Intervertebral discs (IVDs) are fibrocartilaginous structures that lie between the vertebrae, enable limited movement between the vertebrae and resist spinal compression while distributing the compressive load evenly on the adjacent vertebral bodies. IVDs comprise three main components: the inner nucleus pulposus, the outer annulus fibrosus and the cartilaginous endplates (CEPs)<sup>1</sup> (FIG. 1). The nucleus pulposus is a highly hydrated, gelatinous, proteoglycan-rich tissue. The annulus fibrosus encloses the nucleus pulposus and is a highly organized fibrous structure composed of concentric lamellae of tilted collagen fibres with scattered proteoglycans. The nucleus pulposus and the annulus fibrosus are separated from adjacent vertebral bodies by the CEPs, thin layers of hyaline cartilage. Degeneration of the IVD is often involved in low back pain<sup>2</sup>, and is an intricate event involving multiple morphological, functional and biochemical changes<sup>3,4</sup>. The degenerated IVD is reduced in height compared with its healthy counterpart owing to extracellular matrix depletion, a fibrous and dehydrated nucleus pulposus, severe structural modifications of annulus fibrosus collagen fibres, extensive damage to the CEP and sclerosis of the subchondral bone (FIG. 1). Among the many degenerative features, IVD calcification can also occur<sup>5-7</sup>. IVD calcification exists either

in the form of calcium pyrophosphate dihydrate crystals, which usually deposit in the annulus fibrosus, or as basic calcium phosphate crystals, which affect the central region and are known as calcifying nucleopathy. The resorptive phase of IVD calcification is extremely symptomatic and causes pain, stiffness, paravertebral muscular contracture, and limitation of motion of the spinal segment<sup>8,9</sup>.

For the initial assessment of any spinal pathological condition associated with low back pain, radiography is usually the first diagnostic choice in a clinical setting. Plain radiographs are commonly used to evaluate bony features associated with IVD degenerative characteristics and are a good tool for assessing IVD calcification; however, subtle IVD calcification is sometimes missed owing to either small foci of deposits or rotated or malpositioned vertebrae. The fact that IVD calcification can easily be missed means that the reported prevalence of IVD calcification is lower in the clinical setting than in cadaveric radiographic studies<sup>10</sup>. MRI is considered the clinical gold standard method for the qualitative and quantitative assessment of IVDs owing to its sensitivity in assessing soft tissue<sup>11</sup>. MRIs can assess, to a degree, water content of an IVD, which is an indirect assessment of proteoglycan loss that may not be associated with pain

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**Key points**

- Intervertebral disc (IVD) calcification is associated with IVD degeneration and can lead to pain.
- IVD calcification may affect disc kinematics and degeneration severity, and can also affect adjacent vertebral endplates, which might lead to pathological non-neoplastic subchondral bone marrow lesions (Modic changes).
- Calcification in the IVD degenerative process is comparable with mineralization of the degenerative process of cartilaginous tissues in osteoarthritis.
- IVD calcification is a unique phenotype that is clinically relevant, that could influence personalized approaches to patient care and that warrants further investigation.

or predict symptoms<sup>12</sup>. Nonetheless, determining IVD calcification on traditional MRI scans is a challenge in the clinical setting. The correlation of high-intensity zones on MRI with disc calcification has suggested that further improving MRI technology might be helpful in the diagnosis of IVD calcification. High-intensity zones have high specificity and high positive predictive values in identifying a symptomatic IVD<sup>13,14</sup>; IVD calcification, if synonymous with high-intensity zones, might be more clinically relevant than is currently assumed.

The progression of IVD degeneration is associated with several changes, including functional changes in spinal motion and biomechanical behaviour<sup>15</sup>. The spinal motion segment has non-linear biomechanical behaviours that vary with loading direction owing to the complex composition and structural organization of the IVD<sup>15</sup>. Spinal stiffening is generally believed to occur with increasing IVD degeneration but is not always the case, and biomechanical changes with IVD degeneration can be difficult to detect owing to competing changes that can occur as degenerative grade progresses<sup>16,17</sup>. However, altered mechanics and biomechanical changes can result in disc degenerative changes, especially calcification, as is observed in scoliotic discs<sup>18,19</sup>. The pro-inflammatory cytokines secreted in disc tissues are known to promote osteoprogenitor differentiation and angiogenesis, which eventually activate ectopic ossification, and altered biomechanical environment might be one factor that leads to the production of these pro-inflammatory cytokines<sup>20</sup>.

The strong association of disc degeneration and calcification in the setting of low back pain<sup>10</sup> has raised awareness of the clinical importance of this degenerative feature. The inability of conventional imaging techniques, such as plain radiography and MRI, to detect IVD calcification might explain why clinically relevant spinal phenotypes have not previously been defined.

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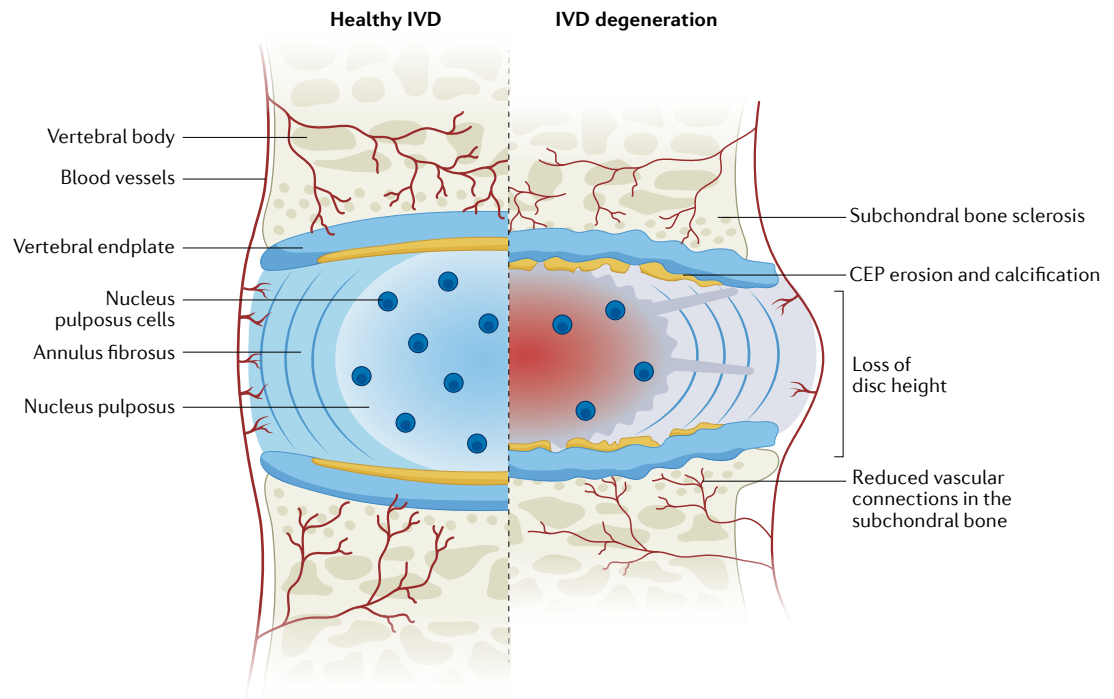
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**Epidemiology of IVD calcification**

The first description of IVD calcification dates back to 1858 in a textbook of anatomy entitled *Die Halbgelenke des menschlichen Körpers* written by German anatomist Hubert von Luschka<sup>21</sup>. Later, in 1897, Beneke demonstrated the presence of IVD calcification on cadaveric radiographs<sup>22,23</sup>. IVD calcification was first shown on human spine radiographs by Calvé and Gallandin 1922 (REFS<sup>21,22</sup>). IVD calcification can occur in association with certain systemic disorders, such as ochronosis, haemochromatosis, chondrocalcinosis and hyperparathyroidism<sup>21</sup>, and also in the setting of spinal deformities such as scoliosis, or in progressive inflammatory or congenital conditions whereby the IVD exhibits ossification<sup>18</sup>. These conditions mostly involve multiple IVDs, whereas calcification resulting from degenerative IVD disease can manifest as focal IVD involvement<sup>24</sup>. Reports of asymptomatic and symptomatic IVD calcification in children have been reported sporadically<sup>25–29</sup>. In most paediatric cases, IVD calcification predominantly occurs in the lower cervical and upper thoracic regions and tends to regress spontaneously<sup>30–32</sup>; however, IVD calcification can affect the lower lumbar spine as well<sup>33,34</sup>. In children, IVD calcification sometimes coincides with ossification of the posterior longitudinal ligament<sup>27,35</sup>. In adults, the prevalence of radiographic IVD calcification has varied from 5% up to 71% in cadaveric radiographic studies<sup>21,23,36,37</sup>. However, the sampling of individuals in these studies had methodological flaws, which meant that researchers were unable to gauge the true prevalence, risk factors and clinical relevance of IVD calcification within the different age groups and sexes. The diagnostic tool used to assess IVD calcification has usually been conventional plain radiography and occasionally CT. Some studies tried to establish the role of MRI scans in detecting IVD calcification and reported that high signal intensity on T1-weighted MRI was associated with IVD calcification<sup>38,39</sup>; however, this interpretation was later challenged, with researchers suggesting that high signal intensity of some calcified IVDs on T1-weighted MRI images was associated with fatty infiltration rather than IVD calcification<sup>40,41</sup>. Conventional plain radiography may not be the most sensitive imaging modality for assessing IVD calcification, as the rotation and/or tilting of the vertebrae can obscure proper visualization and small calcium deposits might go unnoticed<sup>42</sup>. As such, the occurrence of IVD calcification may be under-reported and its extent as well as its topography within the IVD remains relatively unknown. In adults, calcium pyrophosphate dihydrate or hydroxyapatite crystals can be deposited in the nucleus pulposus or annulus fibrosus and, unlike paediatric IVD calcification, are permanent in most cases<sup>19</sup>.

**IVD calcification and degeneration**

A number of studies have suggested that calcification is a feature of IVD degeneration that tends to increase with increasing grades of degeneration<sup>43,44</sup>. Studies have also shown that the osteogenic potential of degenerative IVDs increases in direct relation to the severity of degeneration and is related to the enhanced expression of the calcium-sensing receptor (CaSR), transcription factors



**Fig. 1 | Comparison of a healthy and a degenerated intervertebral disc in the spine.** The intervertebral disc (IVD) is a shock-absorbing structure with three main components: the inner nucleus pulposus, the outer annulus fibrosus and the cartilaginous endplates (CEPs), which anchor the disc to the adjacent vertebrae. The healthy nucleus pulposus is a highly hydrated, gelatinous, proteoglycan-rich tissue. The healthy annulus fibrosus encloses the nucleus pulposus and is a highly organized fibrous structure composed of concentric lamellae of tilted collagen fibres with scattered proteoglycans. The degenerated IVD is reduced in height compared with its healthy counterpart owing to extracellular matrix depletion, a fibrous and dehydrated nucleus pulposus, severe structural modifications of annulus fibrosus collagen fibres, extensive damage to the CEP and sclerosis of the subchondral bone. Reprinted from REF.<sup>1</sup>, Springer Nature Limited.

such as *osterix* (also known as transcription factor Sp7) and *Runx*, and proteins such as bone morphogenetic protein 2 (BMP2) and osteocalcin<sup>33,43,45</sup>, all of which are markers that have the potential to induce ossification in IVD tissues<sup>34,43,46</sup>. Active hedgehog signalling has also been shown to correlate positively with the degree of disc degeneration, and Sonic hedgehog proteins had the propensity to facilitate mineral deposition in degenerated nucleus pulposus cells<sup>47</sup>. On the basis of these insights, it is conceivable that calcification has a role in IVD degeneration (FIG. 2).

IVD calcification has been observed in all disc locations among patients with IVD degeneration and patients with scoliosis, but the endplate was most frequently affected in patients with scoliosis<sup>19,48</sup>; calcification is more common in patients with higher degrees of scoliotic deformity. Although scoliosis and IVD degeneration are distinct processes, scoliosis is known to accelerate IVD degenerative changes<sup>19,49–51</sup>. Hristova et al.<sup>18</sup> showed the presence of calcium deposits and collagen type X in IVDs of patients with degenerative disc diseases and patients with scoliosis, but not in control IVDs, and the level of the indicators of calcification potential (for example, alkaline phosphatase (ALP) activity and calcium and inorganic phosphate concentrations) were consistently higher in degenerative and scoliotic IVDs than in control IVDs. These results suggested that IVD degeneration in adults might be associated with ongoing mineral deposition and that the mineralization or

calcification process in IVDs from patients with adolescent idiopathic scoliosis (AIS) might simply reflect a premature degenerative process.

Illien-Junger et al.<sup>52</sup> performed histological analyses of human IVDs from several different stages of degeneration and identified areas of calcification within the nucleus pulposus and CEP; these areas of calcification were commonly located close to fissures in degenerated tissue areas (FIG. 3b) and were associated with increased IVD degeneration. The close proximity of calcifications to these IVD defects suggested that calcifications might be nucleation points for these defects. Hard inclusions in soft materials are known to create stress concentrations (areas where the stress is significantly higher than in the surrounding area), and the proximity of the observed calcifications in the relatively soft cartilaginous IVD tissues might have been fracture nucleation sites that initiated cracks and fissures<sup>53</sup> (FIG. 3), although CEP defects and/or ruptures are not always associated with IVD degeneration. Excessive calcification is thought to have a role in generalized endplate sclerosis that is assumed to be associated with reduced nutritional supply to the IVD; the occlusion of nutritional channels leads to increased anaerobic metabolism and intradiscal acidity along with reduced metabolic rates and reduced cellularity and could contribute to IVD degeneration<sup>54,55</sup>. However, a few studies have reported contrary findings and suggested that nutritional diffusion is not interrupted by endplate sclerosis but instead that endplate

porosity increases with disc degeneration<sup>56,57</sup>. These findings suggest that disc degeneration is more mechanically driven rather than being attributed to reduced metabolic transport<sup>56,57</sup>. IVDs with advanced degeneration, such as Thompson grade V IVDs, involve IVD collapse with extensive ossifications including osteophyte formation, extensive calcification and eventual fusion. Excessive loading and instability can result in IVD collapse and osteophyte formation so that these degenerative changes are mechanically modulated<sup>58</sup>. As a result, calcifications are likely to contribute to altered biomechanical behaviours (for example, fissure initiation altering IVD stiffness) while also being mechanically modulated (for example, IVD compaction and osteophyte formation from overloading)<sup>58</sup>.

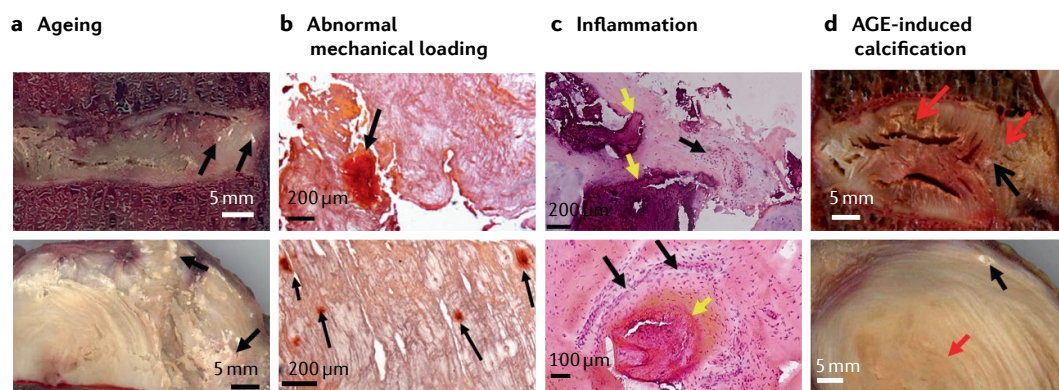
### IVD calcification and genetics

Limited evidence in various species suggests that genetic background is involved in IVD calcification. A study in three different mouse strains (C57BL/6, LG/J and SM/J) described different ageing spinal phenotypes<sup>59</sup>. Notably, the LG/J mouse strain seemed to develop disc calcification at the age of 2 years, equivalent to 70 human years<sup>59</sup>. This study described in detail the transcriptomic landscape involved in the different ageing and degeneration phenotypes<sup>59</sup>, but as yet the underlying genes involved in disc calcification in mice remain elusive. In humans, no large-scale, robust study has been performed investigating the genetic underpinnings related to the development of IVD calcification. The -66T>G gene polymorphism in osteopontin has been shown to be associated with susceptibility to cervical spondylotic myelopathy<sup>60</sup>. Although the underlying mechanism is unknown, it is tempting to hypothesize that this association might be related to the role of osteopontin in the growth plate and in cartilaginous degenerative diseases such as osteoarthritis (OA)<sup>61</sup> and IVD degeneration<sup>52</sup>. In the dachshund, a chondrodystrophic canine breed

commonly affected by clinical IVD disease and predisposed to early-onset IVD degeneration and calcification, a major locus has been identified that affects IVD calcification<sup>62</sup>. With the aid of targeted resequencing and focusing only on protein-coding regions, two synonymous variants were identified in *MB21D1* and one in the 5'-untranslated region of *KCNQ5* (REF.<sup>63</sup>) (which encodes a potassium channel protein) that were associated with IVD calcification. The working mechanism remains unexplored; *MB21D1* is a cytosolic DNA sensor and responsible for immune activation, whereas *KCNQ5* channels control resting properties of mammalian nerve terminals (synapses)<sup>64</sup>. In Scandinavian countries, dachshunds are screened for IVD calcification at approximately 2 years old, and preliminary work has shown that IVD calcification at 2 years of age increases the risk of future clinical IVD disease by 42%<sup>65</sup>. However, dogs treated surgically for IVD herniation at a median age of 74 months are equally likely to present with mineralized IVDs as they are with non-mineralized IVDs, indicating that herniation is not necessarily related to the calcification process itself or perhaps that a different sub-phenotype of IVD changes might exist in the context of calcification<sup>66</sup>.

### Proposed pathogenesis

Physiological and pathological mineralization are complex processes. Although physiological mineralization is regulated within the context of appropriate cellular signalling, pathological calcification encompasses a broad range of processes, some of which involve cells and some of which do not. In adults, IVD calcification is usually perceived as a sign of ageing, although it can also occur in younger adult IVDs<sup>22,67</sup>. IVD calcification is a frequent finding in degenerated IVDs, but whether it is a cause or a consequence of disc degeneration is not well understood<sup>18</sup>. IVD calcification might be involved in the accumulation of advanced glycation



**Fig. 2 | Calcification in degenerated discs.** **a** | Sagittal (top image) and transverse (bottom image) sections of aged intervertebral discs (IVDs) showing several calcified spots (black arrows); scale bar = 5 mm. **b** | Histological sections of scoliotic discs stained with Alizarin red stain showing that abnormal mechanical loading can induce mineral deposition in the IVDs (black arrows); scale bars = 200  $\mu$ m. **c** | Inflammation and calcification are highly associated with each other. H&E stained disc sections of nucleus pulposus (top image) and inner annulus (bottom image) showing the coexistence of inflammation and calcification, calcified deposits (yellow arrows) and adjacent inflammation (black arrows); scale bars upper 200  $\mu$ m and lower 100  $\mu$ m. **d** | Advanced glycation endproduct (AGE)-induced calcification can be seen in the sagittal (top image) and transverse sections (bottom image) of the disc showing the yellowing effect of AGE (red arrows) and calcification (black arrows), suggesting a role of AGEs in calcification; scale bars = 5 mm.

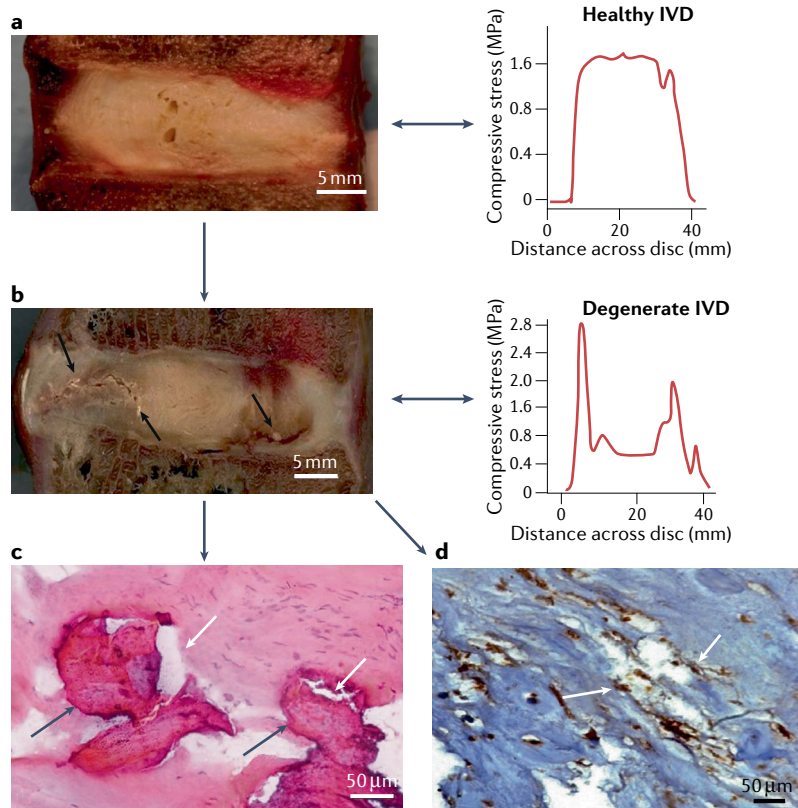
end products (AGEs). AGEs are associated with calcification in multiple conditions including OA and vascular calcification<sup>68,69</sup>, and researchers have established a role for AGEs in the formation of IVD calcification<sup>52</sup>. Immunohistochemical assessment of human IVDs demonstrated calcified structures in pericellular regions surrounding cells positive for AGEs, which were often co-localized with collagen type X and osteopontin<sup>52</sup>. Together with experimental evidence from cell culture studies that demonstrate the role of oxidative stress and consequent degeneration and calcification of both nucleus pulposus<sup>52</sup> and CEP cells<sup>70</sup>, these observations suggest that AGEs induce calcification by increasing the expression of osteogenic markers<sup>52,71</sup>.

A role for AGE accumulation in IVD calcification has also been suggested in a mouse model in which dietary AGE ingestion was associated with AGE accumulation and calcification in CEPs<sup>71</sup>. In a mouse model of diabetes mellitus, the same researchers found that fissures and deposition of granulated structures in IVDs correlated with AGE accumulation and the expression of

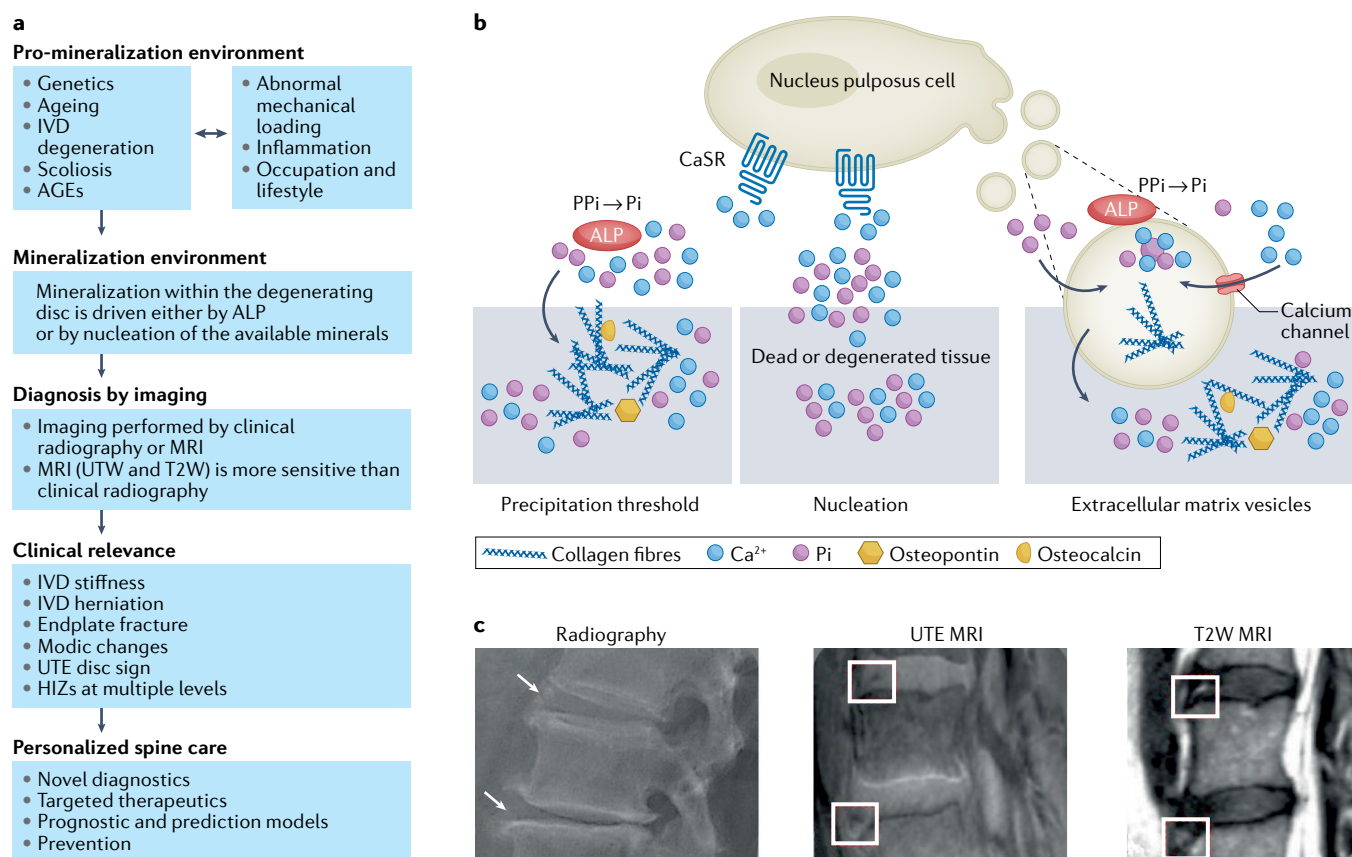
the pro-inflammatory cytokine TNF<sup>72</sup>. AGE formation therefore seems to be associated with pro-inflammatory conditions. The potential association of calcification with inflammation has been seen in various vascular studies<sup>73,74</sup>, but the causal pathway of events is difficult to ascertain<sup>75</sup>. Microcalcifications are triggered by various cytokines during inflammatory processes; TNF seems to have a particularly important role and might exert its effects by stimulating the release of BMP2, a potent bone anabolic factor<sup>73,76</sup>. Studies have shown calcification in degenerative IVDs, especially near fissures and tears<sup>6,52</sup> (FIG. 3), which supports, at least in part, previous hypotheses that annular fissures and tears are linked with the formation of vascular inflammatory granulation tissue<sup>77,78</sup>. Investigations have noted increased expression of CaSR in degenerated IVDs, most probably induced by pro-inflammatory cytokines such as IL-1 $\beta$  and TNF<sup>79,80</sup>. The common findings of local inflammation and angiogenesis in herniated and ossified discs<sup>77–80</sup> indicate that these two processes might be involved in disc calcification. Altered Wnt signalling and the overexpression of some inflammation-related factors are two elements believed to be involved in disc ossification<sup>81</sup>.

Another mechanism that might induce IVD calcification is abnormal mechanical loading, as is seen in disorders affecting spinal alignment, such as scoliosis<sup>18,19</sup> (FIG. 2). Parameters of spinopelvic alignment — in particular low pelvic incidence — can predict disc calcification, which shows that altered biomechanics might induce disc calcification that can affect disc kinematics<sup>82</sup>. Abnormal mechanical loading has been shown to increase levels of collagen type X<sup>83,84</sup>, which is a known marker of endochondral ossification<sup>84,85</sup>. Therefore, altered mechanical loading and uneven distribution of stress in IVDs is a possible factor associated with IVD calcification. Runt-related transcription factor 2 (Runx2) is highly expressed by annulus fibrosus cells during altered mechanical loading in degenerative discs<sup>6</sup>. Annulus fibrosus cells have characteristics of progenitor cells and, under appropriate stimuli, are capable of differentiating into chondrocytes and osteoblasts *in vitro* as well as *in vivo*<sup>86,87</sup>.

Regardless of the underlying cause, three mechanisms are believed to be involved in the initiation of calcification in IVDs<sup>88</sup> (FIG. 4). One mechanism suggests that in order for calcification to occur, the calcium and inorganic monophosphate (Pi) ion product must reach the threshold of precipitation<sup>88</sup>, which requires Pi to accumulate by ALP activity. A second mechanism (nucleation) suggests that the calcium and Pi ion product can remain at physiological levels and nucleating agents are required to induce precipitation<sup>88</sup>. A third mechanism involves extracellular matrix vesicles, produced by budding from the cellular membrane of chondrocytes<sup>89</sup> and found in the growth plate cartilage. Initially, it was thought that matrix vesicles initiate calcification<sup>90,91</sup> because they are enriched with ALP, calcium and Pi<sup>92,93</sup> and associated with collagen type X<sup>94</sup>, all of which are associated with calcification<sup>95</sup>. More recently, matrix vesicles have been shown to contain enriched populations of microRNA related to bone formation signalling pathways<sup>96</sup> and have been suggested to share homology



**Fig. 3 | Images showing presence of fissures near calcified spots. a** | Sagittal image of a cadaveric healthy disc with a representative graph of intradiscal stress; scale bar = 5 mm. **b** | Sagittal cadaveric section of a degenerated intervertebral disc with fissures (black arrows) near calcified spots with representative images of intradiscal stress; scale bar = 5 mm. The representative images of intradiscal stress show the stress profile of discs: the stress profile of the healthy disc shows an even distribution of stress throughout the disc whereas the stress profile of the degenerated disc shows multiple stress peaks. **c** | Haematoxylin and eosin stained section of a degenerated intervertebral disc showing coexistence of calcification (dark grey arrows) and fissures (white arrows); scale bar = 50  $\mu$ m. **d** | Bone morphogenetic protein 2 immuno-stained sections of a degenerated intervertebral disc, showing association of both calcification and fissures (white arrows); scale bar = 50  $\mu$ m.



**Fig. 4 | Disc calcification: mechanisms, diagnosis and clinical relevance.**

**a** | Overview of disc calcification, diagnosis and clinical relevance. Mineralization within the degenerating disc has received increasing recognition as being important as the spinal phenotypes identified are associated with pain and disability. Disc mineralization deserves further attention in order to improve patient stratification by novel diagnostics to aid targeted therapeutics and even generate prognostic models to enable prevention of disc disease. **b** | The initiating factors and mechanisms of disc mineralization. Mineralization within the degenerating disc may involve three mechanisms. In one mechanism, calcium (Ca<sup>2+</sup>) and inorganic monophosphate (Pi) ions accumulate above physiological levels, reaching the threshold of precipitation; in this process alkaline phosphatase (ALP) activity drives accumulation of Pi. In degenerated IVDs there is enhanced expression of calcium-sensing receptor (CaSR), which is involved in Ca<sup>2+</sup>

sensing and downstream signalling and as such may contribute to the process of intervertebral disc (IVD) calcification. In another mechanism, Ca<sup>2+</sup> and Pi present even within physiological levels may precipitate owing to the presence of nucleating agents in dead or degenerated tissue. In a third mechanism, extracellular vesicles secreted by cells are enriched with Ca<sup>2+</sup>, Pi, and ALP and also contain biological messages (such as microRNAs) involved in bone-formation signalling pathways. Extracellular vesicle biology in the IVD is only beginning to emerge. **c** | Imaging methods for diagnosing disc mineralization. Mineralization within the intervertebral disc (shown by arrows and boxes) imaged with the aid of multiple imaging modalities, such as plain radiographs, ultra-short time-to-echo (UTE) MRI and T2-weighted MRI (T2W). AGEs, advanced glycation end products; HIZ, high-intensity zone on T2-weighted MRI; UDS, ultra-short time-to-echo disc sign.

with exosomes<sup>97</sup>, which, according to the International Society of Extracellular Vesicles, should be termed 'extracellular vesicles'. Extracellular vesicles mediate cell-to-cell communication, influence signalling pathways and as such might regulate endochondral bone formation and mineralization or calcification<sup>98</sup>. Many signalling pathways are implicated in mineralization<sup>99</sup>, but only a few of these have thus far demonstrated to also be related to IVD calcification.

The field of extracellular vesicles is only beginning to emerge within the spine community; proteomic analysis has indicated the presence of extracellular vesicles in healthy nucleus pulposus tissue<sup>100</sup>, and both notochordal cells<sup>101</sup> and human degenerated nucleus pulposus cells<sup>102</sup> have been shown to secrete extracellular vesicles. What the cargo of these extracellular vesicles is, how the cargo changes with degeneration of the IVD and how the cargo influences IVD calcification remain to be determined.

Thus far, the prevailing thought is that IVD calcification is an accumulation of calcium salts<sup>103</sup>, initiated by ALP<sup>104</sup>. The substrates of ALP are not all fully known<sup>92,105</sup>, but among the myriad phosphate substrate suspects is inorganic pyrophosphate<sup>106,107</sup>, which is an inhibitor of apatite formation<sup>92</sup>. ALP can hydrolyse inorganic pyrophosphate to generate Pi ions, which are incorporated into the mineral crystals<sup>108</sup> in the presence of calcium (Ca<sup>2+</sup>). Increasing the amount of Ca<sup>2+</sup> is known to not only promote calcification in culture but also promote the terminal differentiation of chondrocytes and osteoblasts undergoing maturation<sup>109–111</sup>. Accumulation of calcium salts occurs normally in bone and growth plate but calcium can be deposited abnormally in blood vessels, articular cartilage, damaged tissue and IVD (cartilage endplate, nucleus pulposus and annulus fibrosus) using similar mechanisms<sup>103,109–112</sup>. However, whereas calcium transport and calcium signalling have

been widely studied in bone cells, no studies have investigated calcium channels and pathways that regulate intracellular calcium in disc cells. IVD degeneration might provide appropriate calcification conditions through increased calcium and phosphate levels. In the course of IVD degeneration, the pH decreases further owing to raised lactic acid concentrations in the disc<sup>113</sup>. Once this pH decrease is sensed by the disc cells it can result in increased intracellular calcium concentrations. Furthermore, the increase in phosphate levels is thought to be a result of ALP activity, as discussed above<sup>92,104–107</sup>.

Interestingly, CaSR expressed on the cellular surface and on free calcium has also been implicated in the calcification process. This family C G protein coupled receptor is expressed in calcitropic tissues (such as the parathyroid glands and the kidneys) and non-calcitropic tissues and has multiple biological roles, alongside its main regulatory role in bone and mineral metabolism as a sensor of free calcium<sup>114</sup>. Patients with activating mutations of the extracellular CaSR often develop nephrocalcinosis and renal insufficiency with a high degree of ectopic calcifications early in life<sup>115</sup>. The development of OA has been observed in humans with such mutations, suggesting a link between CaSR activation and disease<sup>116</sup>. Similarly, in a guinea pig model of OA, CaSR was upregulated in articular cartilage and its activation accelerated degeneration and modulated the function of the pro-inflammatory cytokine IL-1 $\beta$ <sup>117</sup>. A gain-of-function CaSR mutation in mice led to ectopic calcification<sup>118</sup>.

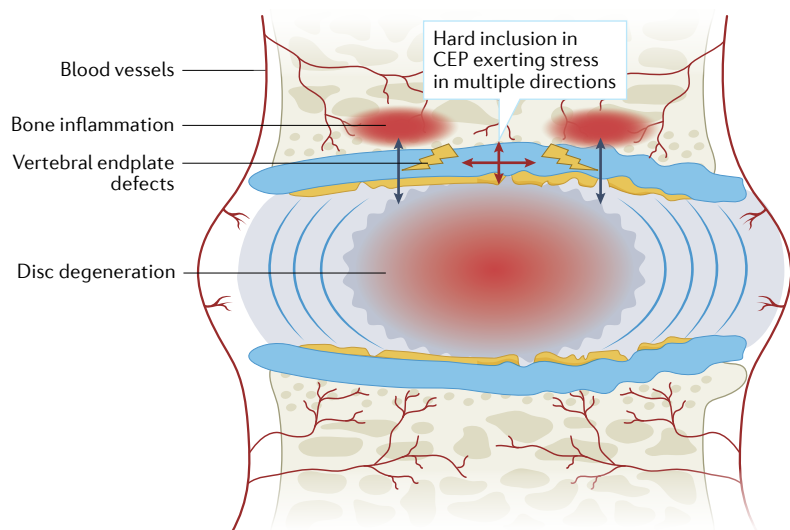
No role for CaSR in the development or maintenance of other cartilaginous tissues, such as the IVD, has yet been shown, but investigators have hypothesized that an increase in local extracellular Ca<sup>2+</sup> in the IVD microenvironment and activation of CaSR can promote degeneration of the cartilaginous endplate<sup>79</sup>. Ca<sup>2+</sup> content was shown to increase in human CEP tissue whereas proteoglycan content decreased with the grade of IVD degeneration<sup>79</sup>, a finding in agreement with previous reports showing decreased proteoglycan and collagen content in CEP tissue from degenerated IVDs<sup>119,120</sup>. Increasing the levels of Ca<sup>2+</sup> resulted in decreased secretion and accumulation of matrix molecules, such as collagens and proteoglycans, in cultured human CEP cells, through activation of CaSR<sup>79</sup>. Interestingly, aggrecan content, a major proteoglycan deposited in the CEP, was also reduced independently of CaSR activation as an increase in Ca<sup>2+</sup> directly enhanced the activity of aggrecanases<sup>79</sup>. Finally, supplementing Ca<sup>2+</sup> in IVD organ cultures was found to induce degeneration and enhance calcification of the CEP as well as decreasing glucose diffusion into the IVD<sup>79</sup>. Together, these findings indicate that extracellular CaSR and local levels of Ca<sup>2+</sup> have key roles in pathological IVD calcification.

### Clinical implications

Despite its close association with IVD degeneration, the clinical significance of IVD calcification remains unclear. Calcified IVDs are more likely to rupture and herniate than non-calcified IVDs either because calcification results in the production of an abnormal stress concentration that leads to fracture and fissures<sup>53</sup> or because of spontaneous liquification (where the consistency of

the calcified deposit changes and becomes more caseous) and damage to the annulus fibrosus or surrounding soft tissues, with inflammatory responses that can further weaken tissues, and eventually result in IVD herniation<sup>121</sup>. Patients with calcified IVD herniations, or calcified IVD tissues identified on pathological examination following herniations, are usually more symptomatic and have larger herniations than those without calcification, often presenting a challenge to resect surgically, particularly when herniations are located in regions with narrowed spinal canals and/or when herniations are in the thoracic region<sup>122,123</sup>. Calcified herniated discs are associated with myelopathy and intradural extension and are often associated with poor surgical outcomes and postoperative complications such as bleeding, neurological deterioration, cerebrospinal fluid fistula and slow recovery<sup>124,125</sup>.

A few case reports have documented the possible role of IVD calcification in discogenic low back pain in adults<sup>126–128</sup>. Some of these cases also showed migration of calcified deposits inside the vertebral body suggesting the role of such deposits in disrupting weaker areas of the vertebral endplate causing marrow oedema<sup>126,127</sup>. The formation of a calcified hard-tissue inclusion in the bone disrupts the microarchitecture of the trabecular bone and leads to endplate fracture<sup>129</sup>. Endplate fracture is identifiable on MRI in the form of mostly type I and type II Modic changes (pathological subchondral bone marrow lesions); biomechanical studies have suggested that vertebral endplate microtrauma with the resultant marrow oedema correlates with type I Modic changes<sup>129</sup> (FIG. 5). IVD calcification is clearly associated with the development of endplate fractures and potential Modic changes (types I and II), which are highly clinically relevant spinal phenotypes associated with pain and disability, but additional research is required. Using ultra-short time-to-echo (UTE) MRI, Zehra et al.<sup>10</sup> found that the UTE disc sign (that is, a hyper-intense or hypo-intense IVD band) correlated highly with radiographic IVD calcification. Their earlier study reporting the presence of the UTE disc sign noted that the UTE disc sign showed a significant positive correlation with worse disability scores, suggesting that IVD calcification might have a role in discogenic low back pain<sup>130</sup>. Furthermore, the UTE disc sign was also significantly associated with Modic changes<sup>130</sup>. This finding further underlines the importance of IVD calcification or IVD stiffness (for example, fibrosis), which can affect stress and loading elements as well as IVD kinematics, and might have an implication for the development of Modic changes and perhaps with the further crosstalk that is noted between such lesions and the degenerating IVD (FIG. 5). The location of such IVD changes might be useful for predicting the development of endplate and vertebral marrow lesions. As with the role of calcification in IVD degeneration, these clinical findings support the concept that IVD calcification is likely to have a role in mechanical back pain (for example, a role in initiating and propagating herniation and/or instability) while also being mechanically modulated (for example, a role of mechanical overloading and IVD height loss inducing osteophyte formation and fusion in advanced degeneration)<sup>10</sup>.



**Fig. 5 | A conceptual overview of the initiation of Modic changes and disc degeneration.** Hard inclusions in the cartilaginous endplate (CEP) can initiate cracks in multiple directions that can lead to vertebral endplate defects. A break in the endplate barrier would facilitate the escape of water, free movement of various cytokines and inflammatory cells and constant crosstalk between the disc and vertebral body, predisposing the disc to decompression, degeneration and Modic changes.

IVD calcification might have a direct role in contributing to the flexibility of the curve in spinal deformities such as AIS<sup>131</sup>. Curve flexibility is a factor that can predict the response to conservative treatment for curve correction (for example bracing) and surgical management of the curve in patients with AIS. For example, surgical intervention might be able to achieve a greater degree of curve correction in a more flexible spine curve versus a more rigid curve, with less instrumentation and shorter fusion levels. A more rigid curve might require more instrumentation and a greater number of levels fused to obtain a suitable degree of curve correction, increasing the risk of neurological complications and increasing health care costs associated with the addition of more screws as well as resulting in reduced preservation of motion segments<sup>132</sup>. Furthermore, IVD calcification might indeed be a predictor of an increased rate of curve progression in patients with AIS and of its secondary effects (for example, decreased lung function, truncal shift, back pain and gait abnormalities)<sup>133</sup>; however, future studies are needed to research these speculative ideas.

### Emerging therapies

Studies using stem cells to treat degenerated IVDs in humans have failed to fully and consistently accomplish IVD rehydration or regeneration<sup>134,135</sup>. This lack of consistent findings might be because unique subphenotypes or endo-phenotypes of IVD calcification (stiffness and poor diffusion of nutrients) may affect the efficacy of such approaches. Furthermore, chondrogenic differentiation of mesenchymal stem cells (MSCs) results in growth plate-like chondrocytes rather than articular cartilage-like chondrocytes<sup>136</sup>. As such, MSC transplantation within a degenerated IVD that has active calcification processes might provide the ideal environment for hypertrophic

differentiation and subsequent mineralization<sup>137</sup>. In line with this idea, cell leakage of MSCs implanted into degenerated rabbit IVDs was thought to be a cause of excess extradiscal calcifications<sup>138</sup>. However, these calcifications could even occur within the disc if the MSCs are injected into disc tissue<sup>137</sup>. As such, combinatory strategies that have the ability to modulate the MSC phenotype upon differentiation might help to minimize the risk of disc calcification caused by hypertrophic differentiation of the MSCs within the chondrogenic lineage. In a rabbit lumbar degeneration model, researchers injected nanofibrous spongy microspheres carrying MSCs that released anti-microRNA-199a; this system promoted the nucleus pulposus phenotype and resisted calcification in vitro and in a subcutaneous environment<sup>5</sup>. More attention to the topic of IVD calcification in the design of targeted therapeutic approaches and improved patient selection could result in a more personalized approach to spinal care.

### Conclusions

Further work is needed to improve our understanding of the pathogenesis, manifestation and clinical relevance of IVD calcification and IVD 'stiffness'. Advanced techniques, such as UTE and susceptibility-weighted MRI, should be explored in order to detect IVD calcification in routine clinical examinations and to map such changes in the IVD as well as to determine the capacity of such changes to predict specific phenotypes (for example, Modic changes and high-intensity zones). Susceptibility-weighted MRI has shown potential for imaging iron deposition, haemorrhages, microbleeds and calcification in the brain, liver, prostate and spinal cord; therefore, it is a potential tool for evaluating calcification in IVD<sup>139</sup>. As various spinal phenotypes (including IVD degeneration, IVD herniations, Modic changes, endplate abnormalities and facet joint changes) are known to result in pain, disability and altered spinal alignment, we must improve our understanding of the role of IVD calcification within this context. The possible role of IVD calcification on discogenic low back pain should also be evaluated to improve clinical and surgical outcomes. The clinical implications of IVD calcification inpatient selection for potential therapeutics for IVD regeneration or halting the degenerative process need to be explored. Furthermore, profiling IVDs that are susceptible to adjacent segment degeneration or disease in relation to a fusion or prosthetic IVD replacement has been a challenge with respect to risk stratification and outcome prediction<sup>140</sup>. The implications of IVD calcification at the adjacent IVD in this setting needs further exploration. Past studies addressing the genetics of IVD degeneration failed to find robust genes that can be replicated<sup>141</sup>. A confounding factor associated with the unique IVD phenotype of calcification might need to be identified and accounted for in such and any omics analyses. An extensive understanding of IVD calcification might further facilitate blood or refined imaging biomarker discovery. In addition, in the age of artificial intelligence, big data and precision spine care, detailed phenotyping of patients has taken centre stage<sup>142,143</sup>. In an effort to establish more robust clinical decision trees



and algorithms, having a robust understanding of the disc calcification phenotype is imperative. Many funding agencies, such as the NIH in the USA, have initiated multi-centric, extremely well-funded and high-profile programmes (for example, BACPAC and Bridge2AI) that aim to provide high-quality datasets of extensively phenotyped patients in order to test algorithms and provide deep analytics to better understand the mechanisms of pain; these programmes are also aimed at developing more precise management protocols, predictive modelling and improved patient outcomes.

In summary, improved understanding of IVD calcification in both children and adults would enable more personalized and precise approaches to spinal conditions with respect to diagnosis, therapeutics and prognostic measures (FIG. 4a). Such understanding would also help to establish the role of calcification in other important bone-related and joint-related conditions, such as OA, in which the role of calcium crystals in the development and progression of disease might be clinically relevant.

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