Metabolic and endocrine diseases, cartilage calcification and arthritis

Adam M. Taylor

Purpose of review
Osteoarthritis is the most common form of rheumatologic disease, with numerous factors increasing the risk of developing the condition; calcification of cartilage is common place in osteoarthritis. Regardless of these risk factors, certain disorders predispose individuals to developing arthritis. Pathologic mechanisms in cartilage calcification and advances in their understanding are reviewed alongside metabolic and endocrine arthritis.

Recent findings
There is growing evidence suggesting that changes in chondrocytes and the extracellular environment both contribute to the calcification. Further evidence suggests that signaling cascades that are involved in physiological mineralisation are involved in the pathological process(es); data in mouse models continue to add weight to these hypotheses and correlate with human osteoarthritis data. Recent study of rare forms of arthritis is adding useful information that may help understand joint diseases in the general population and how therapies may be targeted.

Summary
There is little doubt that calcium-containing crystals are involved in the osteoarthritis process contributing both biomechanically and biochemically. Understanding the processes involved provides important therapeutic opportunities. Furthermore, important information is often discovered in studying rare conditions in which these pathologies are inevitable.

Keywords
alkaptonuria, arthritis, cartilage calcification, crystal

INTRODUCTION

Osteoarthritis is a disease of the whole joint organ; the initiating cellular changes in the joint are still debated but the subsequent manifestations of the changes are seen across the tissues. These changes include cartilage degeneration, subchondral sclerosis, cyst formation, inflammation, changes in the soft tissues and composition of the synovial fluid. There are a multitude of factors that cause osteoarthritis and its progression including: hereditary conditions, long-term overloading or severe trauma, ageing and deposition of calcium-containing crystals in the joint [1▪▪]. The two strongest indicators of developing osteoarthritis are age and obesity – both of which will become more prevalent with an increasingly elderly population in the developed world and the rising obesity epidemic [2]. The calcification of cartilage is a widely observed feature of joint compartments affected by late stage osteoarthritis and more recently in compartments of osteoarthritis joints, which are not readily affected [3]. The process of deposition of calcium crystals among the extracellular matrix is normal in the tissues of the skeleton and dentition. However calcification in soft tissues (cartilage and synovium) is pathologic and contributes to the further deterioration of the joint organ – although there are different mechanisms by which this occurs, both mechanically and biochemically. Regardless of the risk factors for the general population, there are a number of conditions whose sufferers will develop arthritis regardless of any of these factors; these include conditions such as alkaptonuria (AKU), hemochromatosis and other metabolic and endocrine diseases. This review will

Lancaster Medical School, Lancaster University, Lancaster, UK

Correspondence to A.M. Taylor, Lancaster Medical School, Lancaster University, Lancaster, LA1 4YB, UK. Tel: +44 0 1524 592503; fax: +44 0 1524 593747; e-mail: a.m.taylor@lancs.ac.uk

Curr Opin Rheumatol 2013, 25:198–203
DOI:10.1097/BOR.0b013e32835d8ff8

KEY POINTS

- Calcium crystals and calcification are a clear part of the osteoarthritis process.
- Pathological calcification appears to be mediated by many of the same genes and pathways associated with endochondral ossification.
- Rare arthropathies, alongside knockout murine models, provide useful information in the pathological disease processes.
- A clearer understanding of the calcification process is key to development of effective therapies and targets.

focus on advances in cartilage calcification alongside the latest advances in metabolic and endocrine arthritis and general osteoarthritis.

CALCIFICATION OF ARTICULAR CARTILAGE

The nonmineralized hyaline articular cartilage (HAC) is an integral biochemical and biomechanical part of the joint system. It is usually divided into three distinct zones, which overlie the hypermineralised articular calcified cartilage (ACC). This in turn sits atop the mineralized subchondral bone plate (SCB) (Fig. 1). With ageing and osteoarthritis, the biochemical and biomechanical relationship between these tissues changes, often resulting in an increase in the thickness of the ACC and SCB and presence of calcifications in the HAC [4]. The initiation of calcification in articular cartilage (and other soft tissues) is still unclear, but a number of causal changes are known. Whether these are inter-related or are part of an incompletely understood cascade remains to be determined. The changes can be attributed to both the cellular and extracellular component of tissues. Cellular changes include ageing, altered chondrocyte phenotype and their response to cytokines and mediators of normal homeostatic processes. Similarly, alterations in the extracellular matrix, some of which are directly resultant from the cells modify the balance of pro and inhibitory mineralisation factors, dysregulation of extracellular Ca^{2+}, inorganic pyrophosphate (PPi) and inorganic phosphate [5,6]. The factors involved in initiating and mediating cartilage calcification are as follows:

(1) Cellular
  (a) Ageing
  (b) Change in phenotype
  (c) Response to cytokines and mediators of homeostasis
  (d) PPi and inorganic phosphate
(2) Extracellular
  (a) Ageing
  (b) Loss of proteoglycan
  (c) Ca^{2+} dysregulation
  (d) PPi and inorganic phosphate
  (e) Collagen type II
(3) Other factors
  (a) Trauma
  (b) Genetic predisposition

The articular cartilage matrix has a significant role in mediating calcification. Healthy HAC matrix is rich in proteoglycan, one of the most potent mineralisation inhibitors, which is lost early in osteoarthritis [7] with type II collagen, another matrix protein, which is inhibitory to the mineralisation

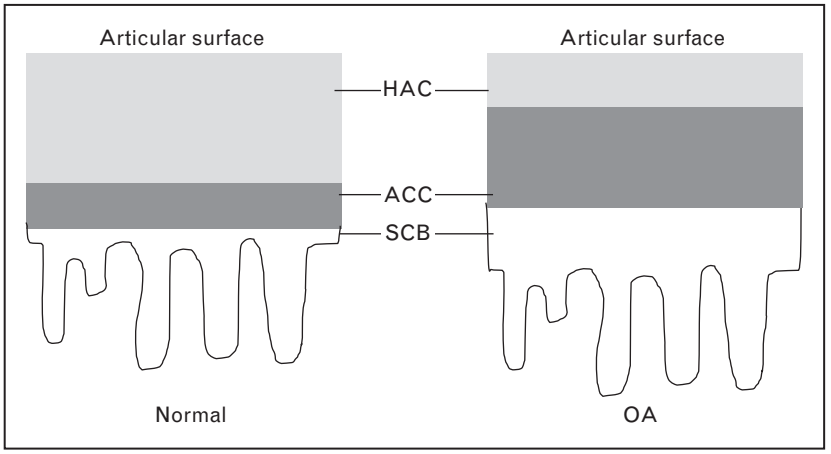

FIGURE 1. (Left) The normal overview of the relationship between cartilage and bone in joint tissues. Subchondral bone (SCB) lies beneath the articular calcified cartilage (ACC) with the hyaline articular cartilage (HAC) on top of this. (Right) Thickening of SCB, advancement of the ACC and thinning of the HAC are all characteristics of osteoarthritis.

process. Alongside loss of proteoglycans, osteoarthritis cartilage also demonstrates production of matrix containing an increase in type I collagen and altered type II collagen [8,9].

Due to the avascular and aneural nature of cartilage many matrix proteins have a long life, and take many years to turnover. The differential turnover of matrix components suggests that modifications which occur may be a novel means of monitoring disease progression [10▪▪]. The chondrocytes are responsible for maintaining the matrix, which in their mature state is straightforward. However, those located within osteoarthritis joints undergo terminal differentiation and become hypertrophic [11], which is mediated by Bone Morphogenetic Protein (BMP)-2 [12]. Hypertrophy is characterized by collagen type X production and expression of alkaline phosphatase (Alk Phos). Alongside this, mineralising cells also produce matrix vesicles [13]. These show the initial deposition of apatite crystals associated with calcification [14,15]. A number of these proteins ensure that key ions are present to promote hydroxyapatite formation, specifically annexins 2, 5 and 6 which facilitate the influx of calcium into the vesicles by forming channels and the Type III sodium/inorganic phosphate cotransporters which include PiT-1 and PiT-2. These mediate influx of inorganic phosphate into the vesicles [16–18]. The contents of the vesicles are extensive and appear to contain numerous enzymes, transport proteins and other proteins, many of which play a further role in maintaining the mineralisation state of the matrix [19▪▪]. Interestingly, recent work shows that mice which are AnxA5(−/−) AnxA6(−/−) Col10a1(−/−) exhibit an expanded growth plate, alongside normal endochondral ossification and mineralisation at 13 days and 1 month [20▪]. This suggests that although the proteins are involved in mediating the influx of calcium and pyrophosphate ions into the vesicles, they are not essential for physiological mineralisation and may be ancillary in the mineralisation process in the growth plate cartilage.

Alongside producing these matrix vesicles, the chondrocytes themselves express a number of proteins which mediate mineralisation of the surrounding matrix; the most significant of these relate to homeostasis of extracellular inorganic pyrophosphate (ePPi), extracellular inorganic phosphate (ePi) and Ca^{2+} concentrations. The balance is maintained by three proteins, which are antagonistic in regulation of ePPi. Firstly, ePPi is broken down by hydrolysis, which is performed by tissue nonspecific Alk Phos (TNAP) to produce two ePi [21]. This facilitates mineralisation by reducing ePPi. Conversely, ePPi is produced by two different factors: nucleotide pyrophosphatase phosophodiesterase 1 (NPP1 or PC-1), which breaks down nucleotide triphosphates, and by ankylosing protein (ANKH), which transports PPi to the extracellular environment from within the cell [22,23]. Both of these mechanisms increase the ePPi concentration and inhibit the mineralisation process. Cleavage of ATP by NPP1 is the major means of generating PPi by chondrocytes [24].

Due to the difficulty of studying the concentrations of these ions at the nano level associated with mineralizing fronts, the majority of information on the mineralisation process has arisen through study of knockout animals, often analysing cellular proteins which manufacture, transport or break down. Examples of this include the ank knockout mice, which show a deficiency in ePPi, which subsequently leads to hydroxyapatite deposition [23]. Similarly the ttw and PC-1 knockout mice also display a lack of ePPi and hydroxyapatite deposition. Conversely excess ePPi results in calcium pyrophosphate deposition. In humans, absence or mutation of the any of the proteins tissue nonspecific Alk Phos, ANKH and NPP1 all display pathologies of mineralisation: hypophosphatasia, chondrocalcinosis and idiopathic infantile arterial calcification, respectively [25–28]. Recent work by Bertrand *et al.* [29▪▪] has shown that NPP1 appears to be the critical protein in this pathway, inversely correlating with cartilage calcification and osteoarthritis severity in both human and murine osteoarthritis. The most striking calcification in murine models comes from knockout of a further inhibitor of mineralisation, Matrix Gla Protein (MGP). Mice with MGP deficiency exhibit arterial calcification and inappropriate calcification of the growth plate [30]. MGP is expressed by chondrocytes, preventing mineralisation of the cartilage by ensuring that any mineral crystals are bound to the MGP, rather than acting as a nucleation for deposition in the extracellular matrix. MGP has gla (y-carboxylated glutamic acid residues), which, with a posttranslational modification, give this protein high affinity for hydroxyapatite crystals. Furthermore, humans who have mutations in the MGP gene suffer a rare autosomal recessive disorder: Keutel Syndrome. Sufferers display hypermineralisation of the cartilage [31]. Recent work by O'Young *et al.* [32▪▪] demonstrates the efficiency of MGP to bind hydroxyapatite, and that the substitution of y carboxylation or phosphorylation sites reduces this efficiency, thus increasing the likelihood of matrix calcification. More interesting work has also recently demonstrated that Osterix is involved in the regulation of calcification and degradation of cartilage matrix through matrix metalloproteinase

13 (MMP13) expression associated with Runx2. The involvement of Osterix has been previously shown to inhibit both chondrocyte differentiation and conversely increase calcification of cartilage in postnatal conditional Osterix KO mice [33▪▪]. The crosstalk between genes involved in bone formation, calcification and those involved in the degradation and calcification of cartilage matrix represents a new avenue of investigation for understanding and therapeutic opportunity in targeting the calcification process.

Although the pathological calcification process recapitulates, in part, developmental mineralisation, there are a number of further knockon effects. The dysregulation of PPi and Ca^{2+} homeostasis results in crystals, which themselves can be biomechanically damaging to the joint [34▪▪]. Recent work by Boyde *et al.* [35▪▪] studying equine joints has shown that cracking of ACC and SCB results in the presence of a hypermineralised matrix, which penetrates into the HAC acting as a stress riser and has the potential to alter chondrocyte phenotype which would result in cartilage damage and degradation. Alongside biomechanical changes of calcified deposits in the matrix, these deposits also induce inflammatory responses, which are outside the scope of this article, but were recently reviewed [36▪] and interesting developments show synovial fluid uric acid, the instigator of gouty arthritis, correlates with IL-1B and IL-18 and osteoarthritis disease progression [37▪]. This occurs through NLRP3/NALP3 inflammasome activation, which has also been demonstrated to be activated by the presence of CPPD [38].

METABOLIC AND ENDOCRINE ARTHRITIS

Individuals with certain metabolic and endocrine disorders are predisposed to arthritis due to the pathological progression of their disease. It is not uncommon for these disorders to be rare, or even ultra-rare in their prevalence. As has already been described, the study of hypophosphatasia and Keutal's Syndrome demonstrates the significance of their respective enzyme deficiencies in normal joint organ function [25,31]. The most notable study [39] of rare diseases in musculoskeletal biology and its implications for common disorders in the general population relates to the study of van Buchem's disease and sclerosteosis and the discovery of the sclerostin (SOST) gene and its effects on the Wnt signaling pathway which mediates bone formation.

Novel bone and cartilage phenotypes have recently been observed in a rare form of metabolic arthritis, which is often misdiagnosed as osteoarthritis. Alkaptonuria is an autosomal recessively inherited condition of tyrosine metabolism, which has a triad of clinical features, urine: which darkens on standing or alkalinisation, ochronosis (darkening) of collagenous tissues, and rapid, early onset arthropathy of load-bearing joints [40]. Calcified cartilage and subchondral bone play a key role in the initiation and progression of arthropathy in these patients, with complete resorption of both calcified joint tissues being evident following biochemical and biomechanical changes in the cartilage composition [41▪]. The rapid onset and severity of the arthritis in these patients show new insights into bone metabolism, turnover and formation. Novel bone structures have been observed which do not conform to the widely accepted Frostian model of coupled turnover; furthermore the authors have demonstrated that this occurs in generalised osteoarthritis bone samples – adding further evidence to the abnormality of tissue structure and function in pathological joint conditions [42▪▪].

Arthritis is also a common feature of hereditary hemochromatosis and usually the means by which the condition is diagnosed. It results from an iron overload due to a mutation in the hemochromatosis (HFE) gene. The condition mimics osteoarthritis in its presentation and is most commonly associated with the second and third metacarpophalangeal joint and chondrocalcinosis is observed in approximately 30% of patients [43]. Histological examination of cartilage and synovia from individuals with hereditary hemochromatosis displays the presence of iron deposits; their exact effect on the structure is not clear and how they go about inducing structural damage and their role in pain is unclear. The means of treatment for hereditary hemochromatosis is iron depletion by phlebotomy; this has provided interesting results on Col2a1 markers from patients. Serum levels of both Col2a1 and CPII (carboxyl propeptide), which represent collagen II degradation and synthesis respectively, were increased significantly after therapy – offering evidence that cartilage homeostasis is altered following this therapy [44]. Recent work by Ohno *et al.* [45▪] demonstrates that iron overload inhibits mineralisation and differentiation of ATDC5 cells in culture by inhibiting chondrocyte differentiation, which may subsequently promote arthritis.

Acromegaly is almost exclusively the result of a pituitary adenoma, which results in excess production of growth hormone (GH) and insulin-like growth factor-1 (IGF-1). Periarticular and cartilaginous thickening are commonplace, contributing to the arthritis. Chen-An *et al.* [46▪] in their investigation of chondrocyte hypertrophy demonstrate an increase in Procollagen II N-Terminal Propeptide

and a phenotypic change of chondrocytes towards a terminal stage, which would promote calcification, when treated with IGF-1.

CONCLUSION

Deposition of calcium-containing crystals is normal in development; many of the genes and signaling pathways involved in this pathway are also involved in pathological calcification of cartilage. It is clear that the crystals deposited as part of the process exert both a biomechanical and a biochemical pressure on the cells and their matrix. These pathways represent a therapeutic target in the treatment of osteoarthritis and other joint disorders. Study of rare forms of arthritis in which joint involvement is a certainty has the potential to elucidate information on the pathogenesis of osteoarthritis in the general population.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

A.M.T. has no conflicts of interest. The work done by A.M.T. is kindly supported by the AKU society, the Rosetrees Trust and University Hospitals of Morecambe Bay NHS Foundation Trust.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 277).

1. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: ■■ a disease of the joint as an organ. Arthritis Rheum 2012; 64:1697–1707.

An up-to-date and significant review on the latest pathogenesis of osteoarthritis, comprehensive evidence demonstrating that osteoarthritis is a disease of the whole joint organ.

2. Loeser RF. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clin Geriatr Med 2010; 26:371–386.
3. Ea HK, Nguyen C, Bazin D, *et al.* Articular cartilage calcification in osteoarthritis: insights into crystal-induced stress. Arthritis Rheum 2011; 63:10–18.
4. Ferguson VL, Bushby AJ, Boyde A. Nanomechanical properties and mineral concentration in articular calcified cartilage and subchondral bone. J Anat 2003; 203:191–202.
5. Ea HK, Liote F. Advances in understanding calcium-containing crystal disease. Curr Opin Rheumatol 2009; 21:150–157.
6. Terkeltaub RA. What does cartilage calcification tell us about osteoarthritis? J Rheumatol 2002; 29:411–415.
7. Chen CC, Boskey AL, Rosenberg LC. The inhibitory effect of cartilage proteoglycans on hydroxyapatite growth. Calcif Tissue Int 1984; 36:285–290.
8. Heinegard D, Bayliss B, Lorenzo P. Articular cartilage: biochemistry and metabolism of normal and osteoarthritic cartilage. In: Brandt K, Doherty M, Lohmander L, editors. Osteoarthritis, 2nd ed. Oxford: Oxford University Press; 2003. pp. 73–82.
9. Shakabei M, Abour-Rebyeh H, Merker HJ. Integrins in aging cartilage tissue in vitro. Histol Histopathol 1993; 8:715–723.
10. Catterall JB, Hsueh MF, Stabler TV, *et al.* Protein modification by deamidation ■■ indicates variations in joint extracellular matrix turnover. J Biol Chem 2012; 287:4640–4651.

The authors show an exciting association of systemic biomarkers with osteoarthritis severity and site, demonstrating that posttranslational modification of extracellular matrix proteins can be used to observe osteoarthritis progression.

11. Von der Mark K, Kirsch T, Nerlich A, *et al.* Type X collagen synthesis in human osteoarthritic cartilage. Indication of chondrocyte hypertrophy. Arthritis Rheum 1992; 35:806–811.
12. De Luca F, Barnes KM, Uyeda JA, *et al.* Regulation of growth plate chondrogenesis by bone morphogenetic protein-2. Endocrinology 2001; 142: 430–436.
13. Anderson HC. Matrix vesicles and calcification. Curr Rheumatol Rep 2003; 5:222–226.
14. Anderson HC. Electron microscopic studies of induced cartilage development and calcification. J Cell Biol 1967; 35:81–101.
15. Bonucci E. Fine structure of early cartilage calcification. J Ultrastruct Res 1967; 20:33–50.
16. Kirsch T, Harrison G, Golub EE, Nah HD. The roles of annexins and types II and X collagen in matrix vesicle-mediated mineralization of the growth plate cartilage. J Biol Chem 2000; 275:35577–35583.
17. Kirsch T, Swoboda B, Nah H. Activation of annexin II and V expression, terminal differentiation, mineralization and apoptosis in human osteoarthritic cartilage. Osteoarthritis Cartilage 2000; 8:294–302.
18. Montessuit C, Caverzasio J, Bonjour JP. Characterization of a Pi transport system in cartilage matrix vesicles. Potential role in the calcification process. J Biol Chem 1991; 266:17791–17797.
19. Golub EE. Biomineralization and matrix vesicles in biology and pathology. ■■ Semin Immunopathol 2011; 33:409–417.

A fascinating review on the role of matrix vesicles in physiological function and pathology, covering their formation, composition and the influential role they play in the extracellular environment.

20. Grskovic I, Kutsch A, Frie C, *et al.* Depletion of annexin A5, annexin A6 and ■ collagen X causes no gross changes in matrix vesicle mediated mineralization, but lack of collagen X affects hematopoiesis and the Th1/Th2 response. J Bone Miner Res 2012; 27:2399–2412,.

An interesting study demonstrating that the absence of annexins A5, A6 and Col X does not result in absence or reduction of mineralisation in the growth plate of deficient mice. The role of other genes in the mineralisation process at the growth plate in these animals is of further interest.

21. Hessle L, Johnson KA, Anderson HC, *et al.* Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc Natl Acad Sci USA 2002; 99:9445–9449.
22. Terkeltaub R, Rosenbach M, Fong F, Goding J. Causal link between nucleotide pyrophosphohydrolase overactivity and increased intracellular inorganic pyrophosphate generation demonstrated by transfection of cultured fibroblasts and osteoblasts with plasma cell membrane glycoprotein-1: relevance to calcium pyrophosphate dihydrate deposition disease. Arthritis Rheum 1994; 37:934–941.
23. Ho AM, Johnson MD, Kingsley DM. Role of the mouse ank gene in control of tissue calcification and arthritis. Science 2000; 289:265–270.
24. Johnson K, Hashimoto S, Lotz M, *et al.* Up-regulated expression of the phosphodiesterase nucleotide pyrophosphatase family member PC-1 is a marker and pathogenic factor for knee meniscal cartilage matrix calcification. Arthritis Rheum 2001; 44:1071–1081.
25. Reibel A, Manière MC, Clauss F, *et al.* Orodental phenotype and genotype findings in all subtypes of hypophosphatasia. Orphanet J Rare Dis 2009; 4:6.
26. Pendleton A, Johnson MD, Hughes A, *et al.* Mutations in ANKH cause chondrocalcinosis. Am J Hum Genet 2002; 71:933–940.
27. Rutsch F, Ruf N, Vaingankar S, *et al.* Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification. Nat Genet 2003; 34:379–381.
28. Rutsch F, Vaingankar S, Johnson K, *et al.* PC-1 nucleoside triphosphate pyrophosphohydrolase deficiency in idiopathic infantile arterial calcification. Am J Pathol 2001; 158:543–554.
29. Bertrand J, Nitschke Y, Fuerst M, *et al.* Decreased levels of nucleotide ■■ pyrophosphatase phosphodiesterase 1 are associated with cartilage calcification in osteoarthritis and trigger osteoarthritic changes in mice. Ann Rheum Dis 2012; 71:1249–1253.

This study shows that NPP1 KO mice demonstrate spontaneous osteoarthritis-like changes. This is translated into human osteoarthritis cartilage studies showing that NPP1 (not ANKH or TNAP) correlates inversely with calcification and osteoarthritis severity. Expression of NPP1 was also downregulated in these samples by proinflammatory cytokines; together this suggests that this calcification is a metabolically active process, making it a viable therapeutic target.

30. Luo G, Ducy P, McKee MD, *et al.* Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997; 386:78–81.
31. Munroe PB, Olgunturk RO, Fryns JP, *et al.* Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. Nat Genet 1999; 21:142–144.

32. O'Young J, Liao Y, Xiao Y, *et al.* Matrix Gla protein inhibits ectopic calcification
■■ by a direct interaction with hydroxyapatite crystals. Am Chem Soc. 2011; 133:18406–18412.

An interesting approach to understanding the calcification process using biological and computational methods to demonstrate the significance of alterations in the sites of posttranslational modifications of proteins. The techniques will also be of great use in designing therapies in the future.

33. Nishimura R, Wakabayashi M, Hata K, *et al.* Osterix regulates calcification and
■■ degradation of chondrogenic matrices through Matrix Metalloproteinase (MMP13) expression in association with transcription factor Runx2 during endochondral ossification. J Biol Chem 2012; 287:33179–33190.

An exciting study looking at endochondral ossification and the involvement of Runx2 and Osterix and the effects of MMP-13. This study illustrates the importance of MMP-13 to the calcification process and further evidence that pathological calcification involves developmental pathways.

34. Nguyen C, Lieberherr M, Bordat C, *et al.* Intracellular calcium oscillations in
■■ articular chondrocytes induced by basic calcium phosphate crystals lead to cartilage degradation. Osteoarthritis Cartilage 2012; 20:1399–1408.

These authors continue to publish fascinating work on the effect of crystals on cartilage degradation. This study demonstrates the significance of basic calcium phosphate crystals and their effect on intracellular Ca^{2+}, a key factor in the pathogenesis of cartilage calcification. They also shed light on the different modulators of Ca^{2+} movement and the effects of antagonists. They allude to interesting involvement of mitochondria and the endoplasmic reticulum.

35. Boyde A, Riggs CM, Bushby AJ, *et al.* Cartilage damage involving extrusion of
■■ mineralisable matrix from the articular calcified cartilage and subchondral bone. Eur Cell Mater 2011; 21:470–478.

This article shows a novel observation of hyperminalised matrix in the HAC in a model of repetitive overload arthrosis and raises questions regarding the oversight of calcified pathologies in the joints which are observed following preparation of joint samples with standard decalcification techniques.

36. Rosenthal AK. Crystals, inflammation, and osteoarthritis. Curr Opin Rheuma-
■ tol 2011; 23:170–173.

An excellent review of the intricate crosstalk between crystals and inflammation in osteoarthritis.

37. Denoble AE, Huffman KM, Stabler TV, *et al.* Uric acid is a danger signal of
■ increasing risk for osteoarthritis through inflammasome activation. Proc Natl Acad Sci U S A 2011; 108:2088–2093.

The authors offer further evidence for the involvement of the innate immune system in osteoarthritis and uric acid in synovial fluid as a risk factor for development of osteoarthritis.

38. Martinon F, Pétrilli V, Mayor A, *et al.* Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006; 440:237–241.

39. Moester MJC, Papapoulos SE, Löwik CWGM, van Bezooijen RL. Sclerostin: current knowledge and future perspectives. Calcif Tissue Int 2010; 87:99–107.

40. Taylor AM, Wlodarski B, Prior IA, *et al.* Ultrastructural examination of tissue in a patient with alkaptonuric arthropathy reveals a distinct pattern of binding of ochronotic pigment. Rheumatology (Oxford) 2010; 49:1412–1414.

41. Taylor AM, Boyde A, Wilson PJ, *et al.* The role of calcified cartilage and
■ subchondral bone in the initiation and progression of ochronotic arthropathy in alkaptonuria. Arthritis Rheum 2011; 63:3887–3896.

Our research shows novel bone and cartilage phenotypes in patients with advanced arthropathy and that prevention of catabolism of the cartilage extracellular matrix does not prevent development of arthritis.

42. Taylor AM, Boyde A, Davidson JS, *et al.* Identification of trabecular excres-
■■ cences, novel microanatomical structures, present in bone in osteoarthropathies. Eur Cell Mater 2012; 23:300–309.

Our study of arthropathy samples demonstrate novel bone structures which arise by means that do not conform to the Frostian hypothesis of bone remodeling. These structures are hypermineralised and randomly distributed around the trabecular network; the mechanism by which they are formed is unknown but may provide a useful therapeutic target.

43. Sahinbegovic E, Dallos T, Aigner E, *et al.* Musculoskeletal disease burden of hereditary hemochromatosis. Arthritis Rheum 2010; 62:3792–3798.

44. Richette P, Ottaviani S, Vicaut E, Bardin T. Musculoskeletal complications of hereditary hemochromatosis: a case-control study. J Rheumatol 2010; 37:2145–2150.

45. Ohno T, Hashimoto N, Mitsui K, *et al.* Iron overload inhibits calcification and
■ differentiation of ATDC5 cells. J Biochem 2012; 151:109–114.

A useful insight into the effect of iron on a cartilage cell model and the extracellular matrix, including the effect of iron on calcification.

46. Chen-An P, Andreassen KV, Henriksen K, *et al.* Investigation of chondrocyte
■ hypertrophy and cartilage calcification in a full-depth articular cartilage explants model. Rheumatol Int 2012. [Epub ahead of print]

An interesting model of hypertrophy in which gene expression and hypertrophic markers are investigated, demonstrating the intricacy and difficulty of investigating the calcification process. It may prove useful for future investigations.