Curr Rheumatol Rep (2012) 14:155–160
DOI 10.1007/s11926-011-0230-6

CRYSTAL ARTHRITIS (MH PILLINGER, SECTION EDITOR)

Genetics and Mechanisms of Crystal Deposition in Calcium Pyrophosphate Deposition Disease

Florence W. L. Tsui

Published online: 24 December 2011

Abstract Calcium pyrophosphate deposition (CPPD) disease (common in older adults) can be asymptomatic, associated with osteoarthritis, or can present as acute/chronic inflammatory arthritis. Due to the phenotypic complexity of CPPD, the European League Against Rheumatism (EULAR) recently made recommendations on terminology, diagnosis, and management based on available research evidence and expert consensus. There are no disease-modifying treatments for CPPD disease, and therapy remains nonspecific with the use of anti-inflammatory and analgesic drugs. For years, it has been known that inorganic phosphate and pyrophosphate regulate the formation of CPP or hydroxyapatite crystals. The discovery of *ANKH* (human homologue of *progressive ankylosis*) mutations in familial CPPD disease confirmed the importance of phosphate/pyrophosphate homeostasis in CPPD, with ANKH being a regulator of inorganic pyrophosphate transport. Despite progress in our understanding of the function of ANKH, much remains to be investigated. This review summarizes the genetic basis of this disease and focuses on the challenges of research in this area.

Keywords Calcium pyrophosphate deposition (CPPD) · Chondrocalcinosis (CC) · Human homologue of progressive ankylosis (ANKH) · Familial disease · Mutations · Animal models · Tissue nonspecific alkaline phosphatase (TNAP) · Sodium/phosphate co-transporter PiT-1 · Genetics · Mechanisms · Crystal deposition · Crystal arthritis

F. W. L. Tsui (✉)
Toronto Western Hospital,
Mc14-419, 399 Bathurst Street,
Toronto, Ontario M5T 2S8, Canada
e-mail: ftsui@uhnres.utoronto.ca

Introduction

Calcium pyrophosphate (CPP) crystals were first reported in the early-1960s [1]. For 40 years, it was apparent that the nomenclature of CPP deposition (CPPD)-associated diseases was confusing and that many complex clinical phenotypes were involved. Early in 2011, the European League Against Rheumatism (EULAR) made recommendations on terminology, diagnosis, and management of CPPD disease [2••, 3••] based on expert consensus and available research evidence. The spectrum of CPPD includes asymptomatic/osteoarthritis (OA) CPPD and acute/chronic CPP crystal arthritis. Eleven and nine recommendations were made for diagnosis [2••] and management [3••], respectively. The presence of CPP crystals in synovial fluids (SF) is the gold standard for a definitive diagnosis [4•]. Radiographic chondrocalcinosis (CC) often showed discrepancies regarding the presence of CPP crystals in SF and thus lacks specificity and sensitivity for CPPD diagnosis. Management strategy is focused on relieving symptoms, including pain, and preventing acute attacks. The recommendation of a symptomatic control approach is due to the lack of pharmacologic options to modulate the formation or dissolution of CPP crystals. A better understanding of mechanisms underlying CPPD pathogenesis would lead to the development of novel and specific therapy.

CPPD can be sporadic, familial, and metabolic disease associated. Metabolic disease–associated CPPD is not discussed in this review. The prevalence of CPPD in the general population remains unclear. Estimates from several studies indicate that it is less than 10%. Age and ethnic differences were reported [5].

Rare genetic diseases with known gene mutations are extremely informative in unraveling the underlying disease mechanisms. Earlier genetic studies on multiplex CPPD

families revealed two disease loci: chromosomes 8q [6] and 5p [7]. Aside from the linkage of early-onset OA and CC with DNA markers on 8q in a large New England family [6], the identity of the gene involved remained unclear. Since the molecular cloning of *ANKH* (human homolog of progressive ankylosis, which is located on human chromosome 5p) [8], several dominant *ANKH* mutations that cosegregate with disease were identified in several multiplex CPPD disease families from different countries [9–12]. *ANKH* mutations are also found in a rare skeletal human disorder, craniometaphyseal dysplasia (CMD) [13, 14]. In at least one family, women with CMD were associated with CC [15]. The key lesson learned from these mutations relates to the structure/function of the ANKH protein and its associated protein. The general consensus is that CPPD-associated *ANKH* mutations lead to a gain of function, while CMD-associated *ANKH* mutations result in a loss of function. The recessive *ank/ank* (progressive ankylosis) mouse with a loss of Ank function is extremely informative for understanding the function of Ank. However, considering that CPPD crystals have never been detected in mice and other small rodents, such as rats and rabbits, detailed work related to this mutant mouse is not discussed here. Several recent published reviews have discussed various aspects relating to Ank/ANKH [16••, 17, 18]. The focus of this review emphasizes outstanding issues regarding the structure of the ANKH protein, its role in inorganic pyrophosphate (PPi) and phosphate (Pi) homeostasis, and (most importantly) the challenges in dissecting how CPPD disease–associated *ANKH* mutations might lead to pathogenesis of the disease.

Structure/Function of Ank/ANKH

It is well-established that Ank/ANKH is a highly conserved transmembrane protein [8]. However, the exact number of transmembrane domains in this protein remains unclear. Programs for structural prediction suggested that there are 7 to 12 transmembrane domains [10], though the favored models involve 10 or 12 transmembrane helices. Known gene mutations are extremely informative for structure/function analyses of the gene/protein involved. In familial CPPD, *ANKH* mutations are found at both the amino and carboxyl termini of the protein. The hot spot of mutation is on amino acid 5 (proline being changed to either leucine or threonine) [11]. In a British family, the *ANKH* mutation occurred in the 5′ untranslated region, resulting in four more amino acid at the amino terminal of the protein. These two types of amino acid changes are located in the cytoplasmic end of ANKH. One British patient who was initially thought to have sporadic CPPD had an ANKH protein missing amino acid 490 (glutamic acid, located at the cytoplasmic carboxyl end of the protein) [9]. Subsequently, two unaffected family members were found to have the same heterozygous mutation. In a French family, the methionine in amino acid 48 was changed to threonine. We recently showed that these mutant ANKH M48T proteins failed to interact with the sodium/phosphate cotransporter, PiT-1 [19••]. It is not clear whether ANKH binds to PiT-1 directly; if so, M48 might be a crucial contact point of the interaction. If the latter were true, M48 would be located intracellularly and thus would favor the 7-transmembrane model as predicted by the PRED-TMR program [10]. However, based on the favored models involving 10 or 12 transmembrane helices, T48 of this mutant ANKH would be located on an extracellular loop. ANKH M48T proteins are expressed on the cell surface (Tsui, unpublished data), but it is unclear whether this mutant protein has the same conformation as the wild-type protein.

Distinct from the CPPD-associated *ANKH* mutations, CMD-associated *ANKH* mutations are localized and clustered in more internal (involving exons 7–10) and presumably intracellular domains of ANKH (based on the 10- or 12-transmembrane models) [13–15]. As with CPPD-associated *ANKH* mutations, CMD-associated *ANKH* mutations (with the exception of one family [20•]) are dominant in nature. CMD is associated with aberrant bone remodeling with reduced bone resorption [21]. This is supported by the observed reduced osteoclast activities found in a knock-in murine model with a CMD *ANKH* mutation (deletion of aa377 [Phe] in exon 9) [22]. A complex CMD-associated mutation (with amino acid changes and deletion in *ANKH* exon 7) resulted in a mutant protein that was retained in the cytoplasm, though the precise mechanism of this loss-of-cell-surface expression has not been clarified [23•]. In one CMD family with *ANKH G389R* mutation in which only affected women showed symptoms of CC, it remains unclear whether this represents a true or coincidental association [15].

Inorganic Pyrophosphate and Phosphate Homeostasis

To date, the pathological events leading to CPPD crystal formation remain unclear. PPi and Pi have a central role in mineralization, the former being a potent inhibitor of mineralization, while the latter promotes the mineralization process. The Pi-to-PPi ratio dictates the type of calcium crystals to be formed: CPPD crystals are formed when the ratio is less than 3, and hydroxyapatite (HA) crystals are promoted when the ratio is greater than 100 [24, 25]. The Pi/PPi balance is controlled by a complex interplay of proteins such as Ank/ANKH, ENPP-1 (ectonucleotide pyrophosphatase/phosphodiesterase 1), PiT-1, and tissue nonspecific alkaline phosphatase (TNAP). The best-studied function of Ank/ANKH is regulating PPi transport. However, it remains unclear whether Ank/ANKH is truly a PPi transporter

or is merely a regulator of PPi transport. It has been suggested that the Ank/ANKH protein also participates in adenosine triphosphate transport [26]. In the extracellular matrix, ENPP-1 generates PPi by adenosine triphosphate hydrolysis. TNAP is regarded to be the key pyrophosphatase and is tethered externally to the cell membrane via a phosphatidylinositol linkage. PPi is tightly regulated in most tissues and serum. Formation of CPPD crystals would require PPi levels of 50 to 200 μM (10- to 40-fold higher than normal serum levels). Unlike other tissues, fibrocartilage and hyaline cartilage have a very high matrix-to-cell ratio (~20:1). It is possible that PPi can diffuse further into the cartilage matrix, where it is bound and protected from hydrolysis into Pi by pyrophosphatases (which are located closer to the cell membrane). Cartilage is capable of binding in nonmineralized form in high concentrations of calcium, magnesium, and phosphate [27]. In the chondron, CPPD nucleation is likely initiated at the pericellular matrix junction, and aggregation of CPPD crystal leads to crystal shedding into the synovial fluid. Inflammatory phagocytic cells clear the CPPD crystals via phagocytosis and pyrophosphatase enzymes are released by neutrophils/macrophages. In this model of CPPD crystal formation, aberrant PPi transport leading to high extracellular PPi (e.g., due to *ANKH* mutations) and inhibition of pyrophosphatase activities (e.g., TNAP inhibitors such as cysteine [28]) may contribute to CPPD crystal formation. Because in smaller animal species such as mice, the matrix-to-cell ratio in the fibro- and hyaline cartilage is lower (~5:1), it is possible that PPi were not sequestered far enough away from pyrophosphatase activities located near the cell membranes, and thus, CPPD crystal formation cannot be initiated. This possibility could be the reason why CPPD crystals have never been observed in mice and other smaller species, such as rats and rabbits.

Role of ANKH in Pyrophosphate and Phosphate Homeostasis

Studies on the consequences of CPPD-associated *ANKH* mutations revealed that expression of ANKH, TNAP, and PiT-1 are coordinated and linked. We took advantage of the inducible ATDC5 cells (via ITS [insulin, transferrin, selenium]) to examine the effects of overexpressing *ANKH* constructs (wild-type and mutant) on prechondrocytes (uninduced) and chondrocytes undergoing hypertrophic differentiation (induced by ITS) [19••, 29]. Alkaline phosphatase is a key player in CPPD disease, as TNAP in articular cartilage facilitates both CPPD crystal formation and dissolution [30]. In hypertrophic ATDC5 cells, overexpression of ANKH wild-type proteins led to a decrease in TNAP protein and alkaline phosphatase activity [29]. If the TNAP level in the extracellular matrix becomes limiting, this could lead to less PPi being degraded by TNAP, thus favoring CPPD crystal formation. For stable ATDC5 transfectants with mutant ANKH constructs, both endogenous Ank proteins and transfected human mutant ANKH proteins coexist and thus mimic the dominant mutations found in familial CPPD cells. Based on our results, stable ATDC5 transfectants with three different ANKH mutant proteins (ANKH P5L, ANKH ΔE490, and ANKH M48T) all have dysregulated PPi/Pi metabolism, albeit via different mechanisms. The ANKH P5L transfectants had higher alkaline phosphatase activities, as reported by others [17, 31]. The ANKH ΔE490 transfectants had low alkaline phosphatase activities throughout ITS treatment due to less TNAP protein being expressed and the presence of yet-unidentified intracellular low molecular weight inhibitors [29]. The ANKH M48T mutant protein (but not the ANKH P5L and ANKH ΔE490 proteins) failed to interact with PiT-1. Upon high phosphate treatment, the normally coordinated upregulation of endogenous *Ank* and *PiT-1* transcripts was disrupted in the *ANKH M48T* transfectants [19••]. There are limitations to this type of *in vitro* experiments. First, experiments on our stable ATDC5 transfectants were carried out under normoxic conditions. However, articular chondrocytes *in vivo* are nonproliferative and exist under hypoxic conditions. This is highly relevant, as Ank expression is repressed in hypoxic conditions, being regulated by HIF-1 [32]. Second, we have not examined the extra- and intracellular PPi and Pi levels. Earlier studies on CPPD-associated *ANKH* mutations have been focused on the effect of these mutations on extracellular PPi levels, and the results remain controversial [9, 10, 12, 31]. These are complex experiments, as the culture media and fetal bovine sera contain Pi and PPi. Furthermore, the inconsistent and contradictory results from different laboratories were probably a result of the presence of intrinsic feedback loops in cell culture systems. Many studies in chondrocytes [29, 33], cementoblasts [34], and osteoblasts [25] have shown that the expression profiles of genes regulating PPi and Pi levels (e.g., *Ank*/*ANKH*, *ENPP1*, *TNAP*, and *PiT-1*) are in turn modulated by PPi and Pi levels.

Although ANKH mutant proteins appear to have a dominant role in familial CPPD disease, they are rarely found in sporadic CPPD patients. A recent study suggested that *ENPP-1* and *TNAP* are not major determinants of sporadic CPPD disease susceptibility [35]. However, ANKH, TNAP, and PiT-1 are regulated by various cytokines, such as transforming growth factor-β [36, 37], tumor necrosis factor-α [38], and interleukin-1β [38]. *ANKH* transcripts were upregulated in articular hyaline cartilage from sporadic CPPD disease patients [39]. It is possible that cytokines could be responsible for dysregulation of players involved in Pi/PPi homeostasis, leading to sporadic CPPD development.

It has long been observed that both hydroxyapatite and CPPD crystals are found in patients with CPPD disease or OA. It is likely that in these patients, the dynamic interplay of regulators of Pi/PPi homeostasis (e.g., Ank/ANKH, TNAP, and PiT-1) led to a fluctuating local Pi/PPi ratio, resulting in the formation in the joints of either hydroxyapatite (when the ratio is ~100) or CPPD crystals (when the ratio is <3). CPPD patients usually have flare-up episodes that likely represent situations in which the intrinsic feedback loops are suboptimal.

Outstanding Questions

How Do *ANKH* Mutations Lead to Calcium Pyrophosphate Deposition Disease Pathogenesis?

To date, the role of ANKH in CPPD disease pathogenesis remains incompletely understood. Based on what we learned from the biochemical defects of known CPPD-associated *ANKH* mutations, there appears to be some correlation between the degree of Pi/PPi homeostasis dysregulation and the severity of the CPPD disease. For example, the uncoupling of the ANKH and PiT-1 interaction due to the *ANKH M48T* mutation resulted in earlier disease onset (prior to 35 years of age) with acute articular attacks [40].

It is generally thought that CPPD-associated *ANKH* mutations resulted in gain of function of ANKH. However, similar to the finding in Ank-deficient mice with an ANKH M48T transgene [41], results from our stable transfectants did not show any evidence that this CPPD-associated *ANKH* mutation is an activating mutation.

Is *ANKH* Mutation Sufficient for the Development of Calcium Pyrophosphate Deposition Disease?

In some familial CPPD disease, autosomal dominant transmission has 100% penetrance (e.g., the English kindred with a mutant ANKH protein that has four additional amino terminal amino acids [9]). However, in other multiplex families, the autosomal dominant transmission has incomplete penetrance. For example, the CPPD patient from which the *ANKH ΔE490* mutation was identified has two family members with this heterozygous mutation, but with no clinical CPPD symptoms [9]. It is possible that because of the modest effect of this mutation (on Pi/PPi dysregulation), additional contributing genetic or nongenetic factor(s) might be required before CPPD symptoms develop.

Challenges

Approach to Unraveling the Basis Underlying Calcium Pyrophosphate Deposition Disease Pathogenesis

As CPPD arthropathy is seen in aged primates and canines, but not in smaller animal species such as mice, canines might be a good candidate to set up *in vivo* models and *in vitro* systems to study mechanisms underlying the development of CPPD disease.

Therapeutic Approaches to Calcium Pyrophosphate Deposition

CPPD crystal deposition is restricted to articular tissues such as fibro- and hyaline cartilage, synovium, and intervertebral disc. Specific therapy for crystal dissolution or prevention of crystal formation would require appropriate tissue targeting. TNAP has the ability to modulate both formation and dissolution of CPPD crystals in the articular cartilage [30], and endogenous amino acids (e.g., cysteine [28]) could serve as TNAP inhibitors. Thus, this represents an attractive area for therapeutic manipulations. There are some indications that low-Pi diet could be beneficial in some cases. For example, avoiding a high-Pi diet might be beneficial to patients with the *ANKH M48T* mutations [19••]. The notion that low-Pi diet could modulate the balance in Pi/PPi homeostasis is exemplified in *ank/ank* (with a loss of Ank function) and *ENPP1*$^{-/-}$ mice. On a high-Pi diet, both types of mutant mice showed increased bone hydroxyapatite mineralization and ectopic mineralization in the extracellular matrix of arteries and skin [42].

Conclusions

Molecular studies on the consequences of CPPD-associated *ANKH* mutations have confirmed that these ANKH mutant proteins led to a dysregulation of modulators of Pi/PPi homeostasis such as TNAP and PiT-1. The dynamic interplay of these Pi/PPi modulators would result in fluctuating local Pi/PPi ratio, leading to the formation of CPPD/hydroxyapatite crystals in the joints under pathological conditions.

Acknowledgment Dr. Tsui's research is funded by the Canadian Institute of Health Research and the Arthritis Society, Canada.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. McCarty D, Kohn N, Faires J. The significance of calcium pyrophosphate crystals in the synovial fluid of arthritic patients. 1. Clinical aspects. Ann Intern Med. 1962;56:711–37.
2. •• Zhang W, Doherty M, Bardin T et al. European League Against Rheumatism recommendations for calcium pyrophosphate deposition. Part I: terminology and diagnosis. Ann Rheum Dis. 2011; 70:563–70. *Based on expert consensus and research evidence, this is the first attempt to streamline the terminology for CPPD and recommend criteria for its diagnosis. A research agenda was also suggested for guiding and improving the evidence base to facilitate diagnosis.*
3. •• Zhang W, Doherty M, Pascual E, et al. EULAR recommendations for calcium pyrophosphate deposition. Part II: Management. Ann Rheum Dis. 2011; 70:571–5. *Due to the lack of specific treatment to modulate CPPD formation/dissolution, recommendations for CPPD management were restricted to symptomatic control.*
4. • Pascual E, Sivera F, Andrés M. Synovial fluid analysis for crystals. Curr Opin Rheumatol. 2011; 23:161–9. *This is a recent and critical review on acquiring/perfecting the technique to identify crystals (both CPP and monosodium urate) in SF.*
5. Richette P, Bardin T, Doherty M. An update on the epidemiology of calcium pyrophosphate dehydrate crystal deposition disease. Rheumatol. 2009;48:711–5.
6. Baldwin CT, Farrer LA, Adair R, et al. Linkage of early-onset osteoarthritis and chondrocalcinosis to human chromosome 8q. Am J Hum Genet. 1995;56:692–7.
7. Andrew LJ, Brancolini V. Serrano de la Pena L et al.: Refinement of the chromosome 5p locus for familial calcium pyrophosphate dehydrate deposition disease. Am J Hum Genet. 1999;64:136–45.
8. Ho AM, Johnson MD, Kingsley DM. Role of the mouse ank gene in control of tissue calcification and arthritis. Science. 2000;289:265–70.
9. Pendleton A, Johnson MD, Hughes A, et al. Mutations in ANKH caused chondrocalcinosis. Am J Hum Genet. 2001;71:933–40.
10. Williams CJ, Zhang Y, Timms A, et al. Autosomal dominant familial calcium pyrophosphate dihydrate deposition disease is caused by mutation in the transmembrane protein ANKH. Am J Hum Genet. 2002;71:985–91.
11. Williams CJ, Pendleton A, Bonavita G, et al. Mutations in the amino terminus of ANKH in two US families with calcium pyrophosphate dehydrate crystal deposition disease. Arthritis Rheum. 2003;48:2627–31.
12. Zhang Y, Johnson K, Russell FF, et al. Association of sporadic chondrocalcinosis with a 4-basepair G- to A- transition in the 5′-untranslated region of ANKH that promotes enhanced expression of ANKH protein and excess generation of extracellular inorganic pyrophosphate. Arthritis Rheum. 2005;52:1110–7.
13. Reichenberger E, Triziani V, Satanabe S, et al. Autosomal dominant craniometaphyseal dysplasia is caused by mutations in the transmembrane protein ANK. Am J Hum Genet. 2001;68: 1321–6.
14. Nürnberg P, Thiele H, Chandler D, et al. Heterozygous mutations in ANKH, the human ortholog of the mouse progressive ankylosis gene, result in craniometaphyseal dysplasia. Nat Genet. 2001;28: 37–41.
15. Baynam G, Goldblatt J, Schofield L. Craniometaphyseal dysplasia and chondrocalcinosis cosegregating in a family with an *ANKH* mutation. Am J Med Genet. 2009; PartA 149A:1331–1333.
16. •• Abhishek A, Doherty M. Pathophysiology of articular chondrocalcinosis—role of *ANKH*. Nat Rev Rheumatol. 2011; 7:96–104. *This is the most updated and comprehensive review on the role of ANKH in the pathogenesis of CPPD.*
17. Tsui FWL, Las Heras F, Inman RD, et al. Functional roles of ANKH/Ank: insights from CPPDD-associated *ANKH* mutations and the *ank/ank* mouse. JCRMM. 2010;Mar.
18. Collins MT, Boehm M. It ANKH necessarily so. J Clin Endocrinol Metab. 2011;95:72–4.
19. •• Wang J, Tsui HW, Beier F, et al. The CPPDD-associated *ANKH M48T* mutation interrupts the interaction of ANKH with the sodium/phosphate cotransporter PiT-1. J Rheumatol. 2009; 36:1265–72. *This is the first report of a protein, PiT-1, that normally interacts with ANKH, but the mutant ANKH M48T protein could no longer interact with PiT-1. This result suggested a coordinated interrelationship between two key proteins involved in Pi and PPi metabolism. Whether the disruption of the ANKH–PiT-1 interaction is sufficient for the development of CPPD in patients with this ANKH mutation is not known.*
20. • Morava E, Kühnisch J, Drijvers JM, et al. Autosomal recessive mental retardation, deafness, ankylosis and mild hypophophatemia associated with a novel *ANKH* mutation in a consanguineous family. J Clin Endocrinol Metab. 2011; 96:E189-98. *This is the first report of a recessive ANKH mutation (L244S) in humans and the first report of mental retardation associated with an ANKH mutation.*
21. Yamamoto T, Kurihara N, Yamaoka K, et al. Bone marrow-derived osteoclast-like cells from a patient with craniometaphyseal dysplasia lack expression of osteoclast-reactive vacuolar proton pump. J Clin Invest. 1993;91:362–7.
22. Chen IP, Wang CJ, Strecker S, et al. Introduction of a Phe377del mutation in ANK creates a mouse model for craniometaphyseal dysplasia. J Bone Miner Res. 2009;24:1206–15.
23. • Zajac A, Baek S-H, Salhab I, et al. Novel *ANKH* mutation in a patient with sporadic caraniometaphyseal dysplasia. Am J Med Genet. 2010; Part A 152A:770–6. *The complex ANKH exon 7 mutations resulted in the retention of the mutant ANKH protein in the cytoplasm and were unable to be expressed on the cell surface, indicating that this represents a loss-of-function mutation.*
24. Cheng P-T, Pritzker KPH. Pyrophosphate, phosphate ion interaction: effects on calcium pyrophosphate and calcium hydroxyapatite crystal formation in aqueous solutions. J Rheumatol. 1983;10: 769–77.
25. Thouverey C, Bechkoff G, Pikula S, et al. Inorganic pyrophosphate as a regulator of hydroxyapatite or calcium pyrophosphate dihydrate mineral deposition by matrix vesicles. Osteoarthritis Cartilage. 2009;17:64–72.
26. Costello JC, Rosenthal AK, Kurup IV, et al. Parallel regulation of extracellular ATP and inorganic pyrophosphate: roles of growth factors, transduction modulators and ANK. Connect Tissue Res. 2011;52:139–46.
27. Pritzker KPH, Chateauvert JMD, Grynpas MD. Osteoarthritic cartilage contains increased calcium, magnesium and phosphorus. J Rheumatol. 1987;14:806–10.
28. So PP, Tsui FW, Vieth R, et al. Inhibition of alkaline phosphatase by cysteine: implications for calcium pyrophosphate dihydrate crystal deposition disease. J Rheumatol. 2007;34:1313–22.
29. Wang J, Tsui HW, Beier F, et al. The *ANKH ΔE490* mutation in calcium pyrophosphate dihydrate crystal deposition disease (CPPDD) affects tissue non-specific alkaline phosphatase (TNAP) activities. Open Rheumatol J. 2008;2:25–32.

30. Shinozaki T, Pritzker KPH. Regulation of alkaline phosphatase: implications for calcium pyrophosphate dehydrate crystal dissolution and other alkaline phosphatase functions. J Rheumatol. 1996;23: 677–83.
31. Zaka R, Stokes D, Dion AS, et al. P5L mutation in Ank results in an increase in extracellular inorganic pyrophosphate during proliferation and nonmineralizing hypertrophy in stably transduced ATDC5 cells. Arthritis Res Ther. 2006;8:R164.
32. Zaka R, Dion A, Kusnierz A, et al. Oxygen tension regulates the expression of ANK (*Progressive Ankylosis*) in an HIF-1-dependent manner in growth plate chondrocytes. J Bone Miner Res. 2009;24:1869–78.
33. Kim HJ, Delaney JD, Kirsch T. The role of pyrophosphate/phosphate homeostasis in terminal differentiation and apoptosis of growth plate chondrocytes. Bone. 2010;47:657–65.
34. Foster BL, Hociti Jr FH, Swanson EC, et al. Regulation of cementoblast gene expression by inorganic phosphate in vitro. Calcif Tissue Int. 2006;78:103–12.
35. Zhang Y, Brown MA, Peach C, et al. Investigation of the role of *ENPP1* and *TNAP* genes in chondrocalcinosis. Rheumatology. 2006;13:1–4.
36. Guo Y, Hsu DKW, Feng S-L, et al. Polypeptide growth factors and phorbol ester induce progressive ankylosis (Ank) gene expression in murine and human fibroblasts. C Cell Biochem. 2002;84:27–38.
37. Palmer G, Guicheux J, Bonhour JP, et al. Transforming growth factor beta stimulates inorganic phosphate transport and expression of the type III phosphate transporter Glvr-1 in chondrogenic ATDC5 cells. Endocrinology. 2000;141:2236–43.
38. Ding J, Ghali O, Lencel P, et al. TNF-alpha and IL1-beta inhibits RUNX2 and collagen expression but increase alkaline phosphatase activity and mineralization in human mesenchymal stem cells. Life Sci. 2009;84:499–504.
39. Hirose J, Ryan LM, Masuda I. Up-regulated expression of cartilage intermediate-layer protein and ANK in articular hyaline cartilage from patients with calcium pyrophosphate dehydrate crystal deposition disease. Arthritis Rheum. 2002;46: 3218–29.
40. Gaucher A, Faure G, Netter P, et al. Hereditary diffuse articular chondrocalcinosis. Dominant manifestation without close linkage with the HLA system in a large pedigree. Scand J Rheumatol. 1977;6:217–21.
41. Gurley KA, Reimer RJ, Kingsley DM. Biochemical and genetic analysis of ANK in arthritis and bone disease. Am J Hum Genet. 2006;79:1017–29.
42. Murshed M, Harmey D, Millan JL, et al. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. Genes Dev. 2005;19:1093–104.