Happy Ending? The Ankh Gene of Calcium Pyrophosphate Deposition Disease (CPDD) and Craniometaphyseal Dysplasia

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The spectrum of heterotopic calcification or ossification is expanding because of the reports of several kindred with calcium pyrophosphate deposition disease, apatite deposition disease, and others with less common syndromes associated with extracellular matrix calcification such as fibrodysplasia ossificans progressiva and related syndromes (1). Genomic DNA studies in both humans and mice provide a shortcut to understanding the genetic basis of promotion and prevention of extra-cellular matrix calcification. Mutation in the COL2A1 gene has been identified in three families with spondylo-epiphyseal dysplasia and calcium pyrophosphate and apatite crystalline deposits (1). In another kindred with precocious osteoarthritis without spondyloepiphyseal dysplasia, the phenotype was linked to markers of chromosome 8 (CCAL1) (1). In a British kindred with primary chondrocalcinosis associated with infantile recurrent febrile seizures, a linkage to chromosomal interval in the region of5p 15 (MIM 11860: gene symbol CCAL1) has been identified (1). CPPDD has also been linked to chromosome 5 markers in two unrelated kindred: one from Cordoba, Argentina of northern Italian ancestry (Cuneo, Piamonte) (1), and the other from Alsace, France (1) (Figure 1). Other large North American kindred of primary CPPDD of British and German ancestries that settled in the Ohio Valley in the USA have also been linked to chromosome 5p (1). An ideogram of the short arm of the chromosome 5, depicting the most likely location of the CPPDD locus in the British, Argentinean, and French kindred, is shown in Figure 1. On the basis of these studies, the gene for familial CPPDD (unfortunately designated with the nonspecific denomination of chondrocalcinosis, MIM 118600 gene symbol CCAL2) has been localized to a 0.8-cM interval on the short arm of chromosome 5 between the polymorphic microsatellite markers D5S 416-D5S 2114 which express the human ANK gene (pyrophosphate channel) (2). The progressive ankylosis gene (ANK) was recently identified in mice with articular apatite deposits and ankylosis (2). The ANK protein is expressed in the outer cell membrane of many cells including chondrocytes and osteoblasts and shuttles inorganic pyrophosphate, a major inhibitor of apatite crystal formation and gene is located in the ANK human chromosome 5. The ANKH gene encodes a 492 amino acids multipass transmembrane protein with 10 potential transmembrane domains which regulate pyrophosphate transport. The first mutations in the ANKH gene were described in 14 families with craniometa-physeal dysplasia (CMD) characterized by prominent osteosclerosis of the facial bones (Figure 4) (3, 4). It seems likely that the mice ANK mutation, as well as the human CMD mutations, lead to a decreased transport of pyrophosphate to the extracellular matrix, which might induce osteosclerosis in CMD and apatite crystal deposition in the joint of ANK mice (Figure 2A-B, Figure 3).

In five families with CPPDD linked to chromosome 5p, the phenotypes have been linked to mutations in the ANKH gene (Figure 4). In the British family, the mutation induced a cysteine to thymine base change and the ATG initiation codon (5). This change generates an alternative ATG initiation codon and adds four amino acids to the N terminus of the ANKH protein. Affected individuals of the French family were heterozygous for a thymidine to cytosine base change in exon 2. This missense mutation substitutes threonine for methionine (8). In the Argentinean family, a cysteine to thymidine transition at 14bp into exon 1 segregated with the disease. This sequence change is predicted to cause a proline to leucine substitution at amino acid

position 5 (6). Two other mutations have been described in two United States families, and in one other case of sporadic chondrocalcinosis from England (7, 8).

While the recessive mouse mutation causes early and widespread deposition of hydroxy-apatite mineral in articular cartilage and synovial fluid, the dominant human mutations recently described might lead to adult-onset deposition of calcium pyrophosphate dihydrate (CPPD) crystals. The different crystal types formed in the mouse and human diseases may result from different effects of the mutant alleles on pyrophosphate levels (Figure 4). The mouse nonsense mutation truncates the ANK protein, sharply reduces protein activity *in vitro*, and causes a decrease in pyrophosphate levels outside cells (5) (Figure 2 and 4). This drop in PPi levels would eliminate a normal physiological block to hydroxyapatite formation and lead to widespread deposition of hydroxyapatite mineral seen in mutant mice (5). However, in two families with apatite chondrocalcinosis, no mutations in the ANK gene have been found (5).

In contrast, the human alleles seen in the CPPDD families cause small amino acid changes instead of truncations, and each of these alleles shows high levels of activity compared to the mouse mutation *in vitro*. The combined data and the dominant inheritance of CPDD suggest that the human ANK mutations are gain of function alleles that lead to excess accumulation of pyrophosphate *in vivo* (5). Elevated pyrophosphate concentrations can trigger direct precipitation of calcium and pyrophosphate ions, leading to deposition of CPP crystals instead of hydroxyapatite. This effect is also seen in several other metabolic diseases that raise pyrophosphate levels including hypomagnesemia, hemochromatosis, and hypophosphatasia and osteoarthritis (7) (Figure 5). The current data indicates that other small mutation since the ANKH protein can lead to CPP deposition, sclerosis of long, bones and skin in patients with craniometaphyseal dysplasia.

The dual role of pyrophosphate as both an inhibitor of hydroxy apatite deposition, and a promoter of CPP deposition may help explain how different mutations in the same gene can lead to formation of different types of crystal (8). Agents that inhibit ANKH transport activity, such as the anion transport inhibitor probenecid, might prove as a useful therapeutic approach for lowering pyrophosphate levels and reducing CPP deposition in a subset of chondrocalcinosis patients (5). However, any effective therapy is likely to depend upon maintaining extra-cellular PPi within a narrow physiological range given that we now know that abnormal increase in extra-cellular PPi/ANKH function might lead to pathological CPP deposition, but excessive decreases in extracellular PPi/ANKH function may lead to pathological hydroxyapatite deposition.

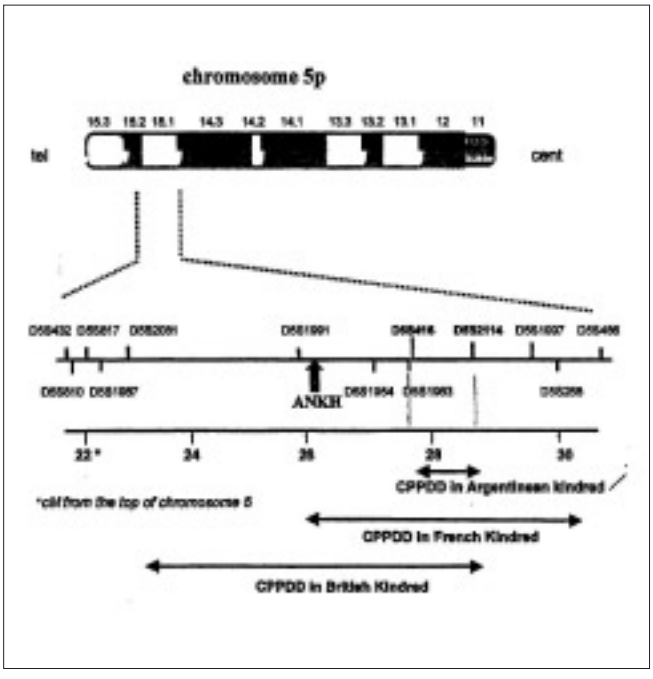

***Figure 1**. Ideogram of the short arm of chromosome 5. The calcium pyrophosphate deposition disease interval for the British, Argentinian, and French families is shown. (Published with permission)..*

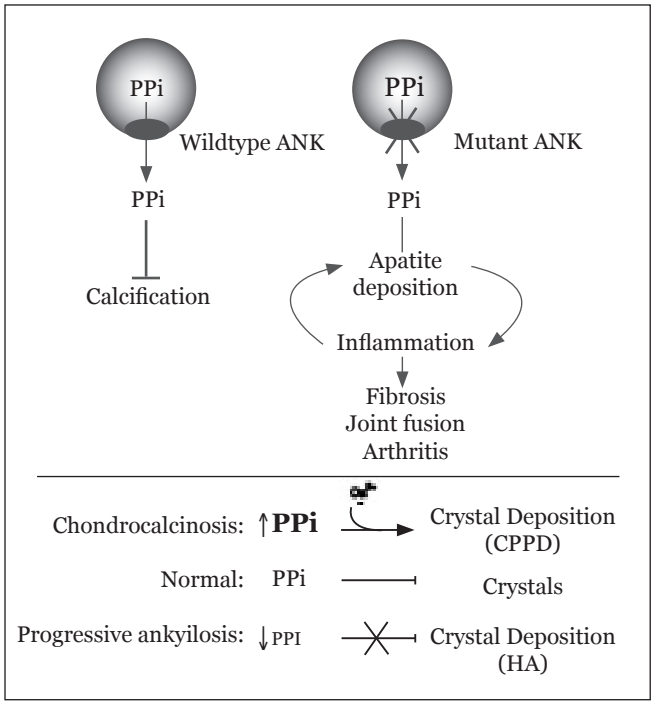

***Figure 2A.** Role of the ank gene for extracellular matrix calcification*

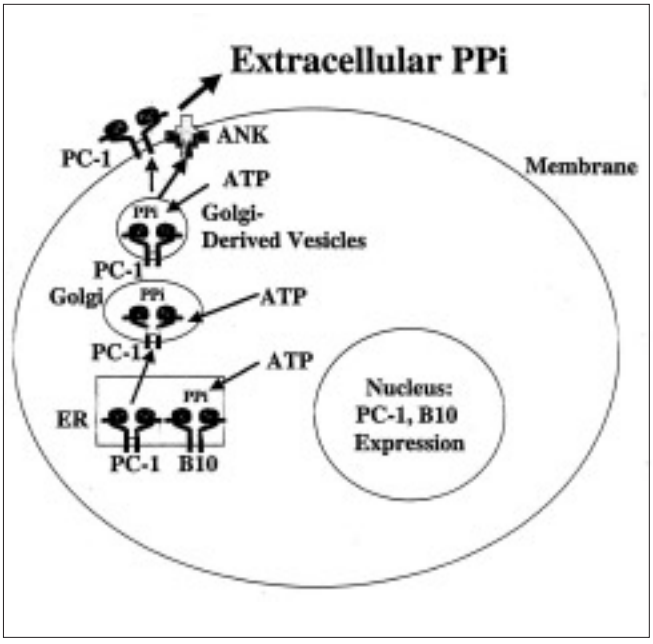

Figure 2B. *Model for extracellular PPi elevation by chondrocytes.*

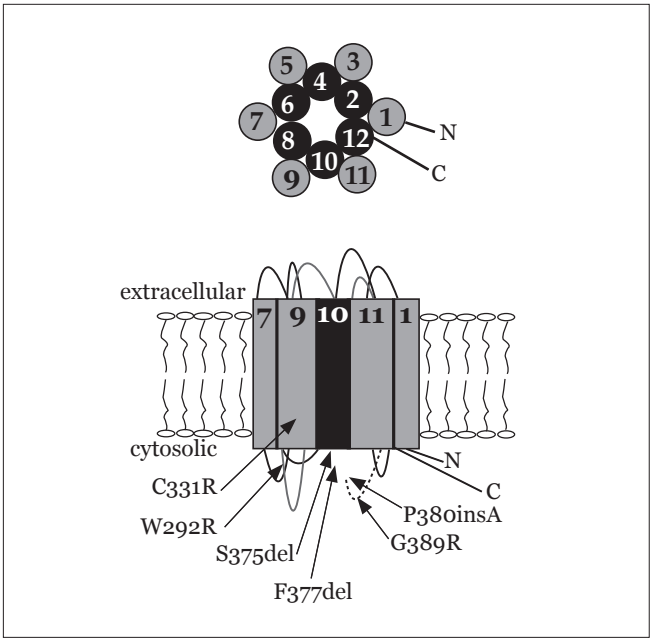

Figure 3. *Schematic representation of ANK transmembrane helix assembly with sites of mutation indicated (arrows). Views from above and from the side are shown. The six-membered helical assembly results in a central channel wide enough to permit the passage of a pyrophosphate molecule, whereas, the channel of a five-membered ring would be too narrow.*

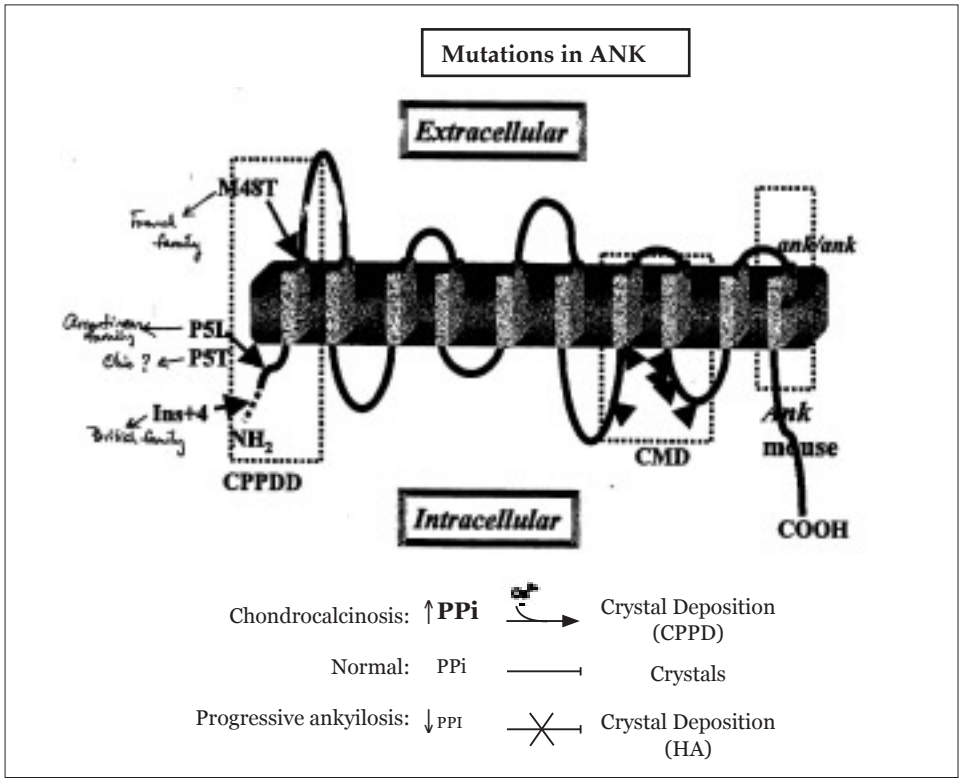

Figure 4. *Summary of known ANKH mutations and model of differents effects on Hydroxyapatite and CPPD mineral deposition.*

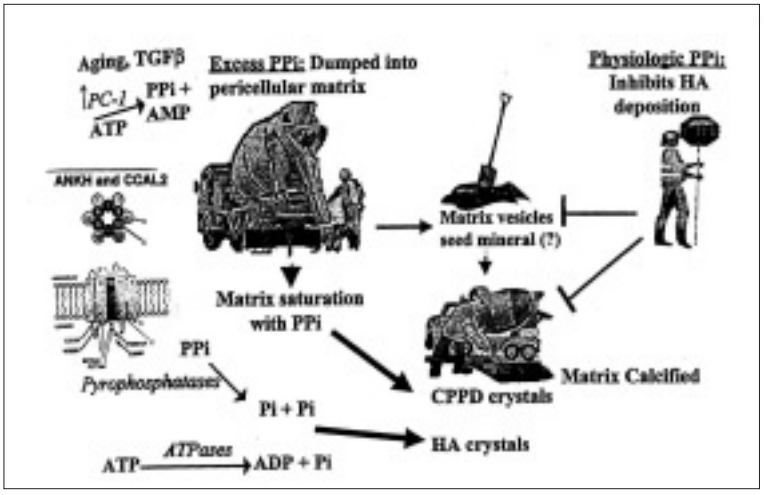

Figure 5. *How chondrocytes build CPPD and HA crystal deposits: roles of PC-1, Ppi metabolism and inorganic phosphate (Pi) generation. This paradigm and the additional potential of Pi to stimulate calcification by effects on expression of mineralization regulatory genes are discussed in detail in the text (Modified from Terkeltaub.)*

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