Review

Genetic studies of disorders of calcium crystal deposition

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Disordered calcification of cartilage and ligaments occurs commonly amongst the elderly, yet the reasons for this are very poorly understood. Chondrocalcinosis affects 25% of the population aged over 85 yr and 3% of people aged between 65 and 69 yr show radiological evidence of the disease [1]. Diffuse idiopathic skeletal hyperostosis (DISH, Forestier’s disease), which causes ossification of the anterior longitudinal spinous ligament and peripheral entheses, has been reported to be as prevalent as 25% in males and 15% in females over the age of 50 yr [2]. In Asian populations, in particular amongst Japanese, a similar condition, ossification of the posterior longitudinal ligament (OPLL) has a prevalence of 1.9–4.3% [3]. OPLL and DISH commonly occur together: Resnick et al [4] have reported that 50% of cases of DISH are associated with some degree of OPLL. Although many cases of these conditions are asymptomatic, this is not universally so. Chondrocalcinosis is an important cause of joint damage and may cause acute or chronic inflammatory arthritis. OPLL is a common cause of spinal canal stenosis in Japanese. These common disorders can thus cause significant morbidity, and there is an increasing body of evidence to suggest that they share common aetiopathogenic factors. In this review we will examine the evidence that variation in genes encoding proteins involved in pyrophosphate metabolism plays an important role in articular chondrocalcinosis, and may also be involved in other common conditions of ectopic calcification.

Whilst the aetiology of all these conditions is largely unknown, for each there is some evidence that disordered pyrophosphate metabolism may play a key role. In general, conditions which favour increases in inorganic pyrophosphate (PPI) promote calcium pyrophosphate dihydrate (CPPD) crystal formation (Table 1) and inhibit calcium hydroxyapatite crystal formation. In the ank/ank mouse, which develops severe hydroxyapatite chondrocalcinosis and spinal ossification, low extracellular levels of PPI are thought to permit hydroxyapatite deposition. Whether low levels of PPI promote calcium hydroxyapatite deposition in humans is unknown. High levels of PPI in hypophosphatasia inhibit hydroxyapatite deposition, leading to defective bone and cartilage mineralization [5]. This property of PPI and related polyphosphates is exploited in toothpaste to reduce hydroxyapatite tartar accretion. In the majority of cases no cause for these conditions can be identified (Table 1). Genetic studies, however, provide some clues about their aetiopathogenesis.

Although most cases of chondrocalcinosis are non-familial, there is considerable evidence that genetic factors are involved. Many multicase families with chondrocalcinosis have been reported [6–24]. Most familial cases appear to be inherited in an autosomal dominant manner, with early onset and varying severity even within families. Only small studies of the recurrence risk ratio have been reported for the general community, and these suggest recurrence risk rates in first-degree relatives of 11–27% [6, 10], but the true heritability of the condition and likely genetic model have yet to be established.

Linkage studies have established that single loci are involved in most chondrocalcinosis families studied. Two particular regions on chromosomes 5 and 8 have been implicated. In one North American family with early-onset osteoarthritis and chondrocalcinosis, linkage was established with chromosome 8q (Mendelian Inheritance in Man: MIM 600668 [25]). The association with early onset osteoarthritis raises the possibility that the chondrocalcinosis was secondary to this rather than being the primary cause of the arthropathy. Two studies have reported linkage of a narrow region of chromosome 5p with familial chondrocalcinosis in the absence of skeletal dysplasia. Doherty et al. [8] studied five presumed unrelated English families with chronic CPPD arthropathy, including one family in which the affected individuals also suffered recurrent benign convulsions in childhood. Three of these families had late-onset arthropathy clinically indistinguishable from sporadic pyrophosphate arthropathy. Genome-wide linkage studies demonstrated strong linkage to a region on

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Increased pyrophosphate (PPi) levels due to:
(A) reduced breakdown of PPi by alkaline phosphatase (ALP) due to:
(i) reduced ALP levels, e.g. hypophosphatasia
(ii) presence of ALP inhibitors such as calcium, copper and iron, e.g. chronic hypercalcaemia, Wilson’s disease and haemochromatosis
(iii) deficiency of ALP cofactors such as magnesium, e.g. chronic hypomagnesaemia due to diet, chronic diarrhoea, Gitelman’s syndrome and Bartter’s disease
(B) increased PPi production through:
(i) chronic stimulation of adenylate cyclase, as in hyperparathyroidism
(ii) stimulation of PPi production by chondrocytes, e.g. by vitamin A and TGF-β
Increased calcium concentration
Enhanced crystal nucleation, e.g. by iron or copper
Decreased crystal solubility, such as in hypomagnesaemia

Table 1. Metabolic diseases associated with CPPD chondrocalcinosis

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<tr>
<th>Metabolic Disease</th>
<th>Associated with CPPD Chondrocalcinosis</th>
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| Hyperparathyroidism | (A) increased PPi production through:
|                   | (i) reduced ALP levels, e.g. hypophosphatasia |
|                   | (ii) presence of ALP inhibitors such as calcium, copper and iron, e.g. chronic hypercalcaemia, Wilson’s disease and haemochromatosis |
|                   | (iii) deficiency of ALP cofactors such as magnesium, e.g. chronic hypomagnesaemia due to diet, chronic diarrhoea, Gitelman’s syndrome and Bartter’s disease |
|                   | (B) increased PPi production through:
|                   | (i) chronic stimulation of adenylate cyclase, as in hyperparathyroidism |
|                   | (ii) stimulation of PPi production by chondrocytes, e.g. by vitamin A and TGF-β |
|                   | Increased calcium concentration |
|                   | Enhanced crystal nucleation, e.g. by iron or copper |
|                   | Decreased crystal solubility, such as in hypomagnesaemia |

Chromosome 5p, lying between markers D5S810 and D5S416 [24.48 and 28.76 centimorgans (cM) respectively] [14]. Linkage studies in two families of Argentine and French origin defined a narrow region of 0.8 cM (0.3 megabases) on chromosome 5p as containing the causative gene (maximum lod (log of the odds) score 11.9 at marker D5S1963; region limited by markers D5S416 and D5S2114, lying at 28.76 and 29.52 cM respectively) [26].

The actual gene involved in each of these families has yet to be identified. However, the recent cloning of the ank gene from the ank/ank mouse has pointed strongly to involvement of this gene in these families. The human homologue of this mouse gene lies close to the chromosome 5 region implicated in familial CPPD chondrocalcinosis. The defective gene involved in the ank/ank mouse is thought to encode a membrane pyrophosphate transporter and dysfunction of the gene causes elevation of intracellular pyrophosphate and reduction in extracellular pyrophosphate [27]. The gene product is a 492-amino acid protein encoded by 12 exons. The protein contains 9–12 predicted transmembrane-spanning domains, each of ~20 amino acids, consistent with the expected structure of an integral multipass membrane protein. In situ hybridization analysis confirms that the gene is expressed in articular cartilage as well as a variety of other tissues, including heart, brain, liver, spleen, lung, muscle and kidney. The expression of ank mRNA in brain tissue is of relevance, given the linkage of this locus to families with chondrocalcinosis and convulsive disorders. As mentioned above, PPi is a major inhibitor of calcium hydroxyapatite crystal deposition, hence extracellular deficiency of this metabolite may promote calcification. Because the families reported to date have documented CPPD rather than hydroxyapatite disease, it seems likely that, if mutation in the ank gene is responsible, a gain of function mutation is involved.

Mutations of the ank gene have also been implicated in craniometaphyseal dysplasia (MIM 123000), a rare, autosomal inherited condition characterized by abnormal mineralization of membranous and enchondral bone, causing thickening of craniofacial bones, widened long-bone metaphyses and increased cortical thickness. In this condition, mutation of the ank gene has been demonstrated and is thought to promote enchondral mineralization, causing the phenotype [28, 29]. Interestingly, three families with definite linkage to the chromosome 5 region encoding ank were not found to have any coding region polymorphisms, suggesting the presence of promoter region mutations in these families. Clearly there is still much to be learned about the ank gene, such as its transcriptional regulation and the control of its tissue specificity.

Family studies of OPLL have suggested that it may be a monogenic trait [30, 31], although these studies were too small to exclude the involvement of a small number of genes rather than just one. Two regions on chromosome 6 have been implicated in the disease. First, strong linkage of the condition with the major histocompatibility complex (MHC) has been established (P = 0.000006 [32]), indicating that genes in this region are likely to be major determinants of susceptibility to OPLL. Association between OPLL and variants of the α1 fibril of type 11 collagen (gene COL11A2) and the human retinoic X receptor β (RXRβ) have been reported, suggesting that a gene involved is probably near these genes, which lie within the MHC [32, 33]. The associations reported for COL11A2 were weak for individual alleles, but all variants could be placed on four ancestral haplotypes, two of which showed significant association with the disease. One particular intronic variant (a T to A substitution four bases upstream from the start of exon 7) was most strongly associated with disease in males (P = 0.0003) and was shown to affect mRNA splicing [34]. Whilst there is strong suggestive evidence of the involvement of vitamin A in OPLL, DISH and chondrocalcinosis (vitamin A excess leads to ligamentous ossification and chondrocalcinosis), no coding variants of RXRβ were identified. The only associated variants in this gene were in the 3′-untranslated region, and were in strong linkage disequilibrium with the disease-associated COL11A2 haplotypes.

A genetic link between chondrocalcinosis and forms of spinal ossification is suggested by the reported co-occurrence of the conditions both in humans [35–37] and in the tip-toe walking (ttw) mouse (a model of the human condition OPLL), which develops spinal ossification and hydroxyapatite arthropathy [38]. The mutant gene causing this mouse phenotype has been demonstrated to encode nucleotide pyrophosphatase
(NPPS; gene NPPS), an enzyme that produces PPi from nucleotide pyrophosphate [39]. A nonsense mutation of the gene in the ttw mouse causes dysfunction of the gene, in turn causing the ectopic calcification seen in this mouse. NPPS is expressed in a variety of tissues, including bone and cartilage, where it occurs in osteoblasts and chondrocytes respectively [40, 41]. The human homologue of this gene is encoded at chromosome 6q22–23 and variants of the gene have recently been associated with development of OPLL [42]. To date, 13 polymorphisms have been identified and two of these, IVS20-11delT and IVS15-14C→T, are associated with OPLL susceptibility [42, 43]. NPPS belongs to a class of phosphodiesterase 1 nucleotide pyrophosphatases, of which there are two other known members, PDPN2 and PDPN3 [44]. PDPN3 is located at 6q22, close to NPPS, and is thought to have arisen from PDPN1 by gene duplication. PDPN2 is located at 8q24.1. The role of variation in these other nucleotide pyrophosphatases in hydroxyapatite or pyrophosphate deposition remains poorly studied. Further support for the role of NPPS variants in human hydroxyapatite deposition disease comes from the recent report of a child affected by severe calcium hydroxyapatite arthropathy due to markedly reduced extracellular PPi levels, caused by a promoter region polymorphism of NPPS [45]. Transforming growth factor β (TGF-β) is a potent stimulator of chondrocyte PPi production, at least partially by induction of NPPS activity. A small study of Japanese OPLL cases (48 cases) has recently reported strong association (odds ratio 3.1–2.5, \( P = 0.015–0.0002 \) depending on the genetic model) of a coding polymorphism in the TGF-β1 gene (T869→C) [46]. This finding clearly requires replication in a larger study, but suggests that genetic variation in TGF-β1 may influence the risk of spinal ligament ossification, possibly by influencing PPi production by NPPS.

We therefore propose that polymorphisms which affect PPi levels may be a cause of OPLL, DISH and ‘sporadic’ hydroxyapatite chondrocalcinosis. In patients with CPPD chondrocalcinosis, levels of both PPi and NPPS are increased, raising the possibility that increased PPi production due to variation in NPPS may be a cause of CPPD chondrocalcinosis [47].

There are clearly great similarities in the spinal ossification occurring in DISH, OPLL and ankylosing spondylitis (AS), and reports of the clinical association of AS with OPLL [48] suggest that there may be shared susceptibility factors for AS and DISH/OPLL. There is an increasing body of evidence that the factors involved in susceptibility to AS are different from those determining acute disease activity and long-term functional consequences [49, 50], and it is possible that clues from the aetopathogenesis of DISH/OPLL and chondrocalcinosis may prove relevant to the causes of ossification in AS. Similarly, reports of familial chondrocalcinosis and shoulder arthropathy suggest that disordered pyrophosphate metabolism may play a role in calcific rotator cuff diseases [17].

Studies on mouse models have pointed to several other genes affecting cartilage and soft-tissue calcification, including those encoding matrix γ-carboxyglutamic acid protein [51], osteoprotegerin [52] and klotho [53], and indicate that calcification can be the end result of several different pathways. It is particularly interesting that these models, whilst causing profound osteoporosis and extracellular matrix (and particularly vascular) calcification, do not appear to cause articular chondrocalcinosis.

In summary, although many factors are likely to be involved in regulating calcification and ossification processes, studies of the causation of articular chondrocalcinosis and disorders of spinal ossification, such as DISH and OPLL, implicate control over inorganic pyrophosphate levels as being one of the most important factors in their aetopathogenesis. The findings of these studies may prove relevant to other rheumatic diseases in which ectopic ossification occurs, such as AS.

References


