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CHONDROCALCINOSIS - RISK FACTORS AND
RADIOGRAPHIC PHENOTYPE

by

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Thesis submitted to the University of Nottingham for the
degree of Doctor of Philosophy

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Declaration
This is to certify that the work submitted in this thesis is the result of original research carried out by me at the University of Nottingham, Nottingham, UK. It has not already been submitted, or accepted for any other degree. Three publications and four poster presentations at an international conference have resulted from the work undertaken during this research.

This thesis is based on the Genetics of Osteoarthritis and Lifestyle (GOAL) study, recruitment for which began in 2002, and was completed in 2006. Study design, ethical approval, study visits, data collection, and radiographic scoring were completed before I started my research. The costs of GOAL were funded primarily by AstraZeneca, Macclesfield, UK, and assisted by infrastructure support funded by Arthritis Research UK. The GOAL study is guided by an expert steering group from the University of Nottingham, UK; University of Warwick, UK; and AstraZeneca, Macclesfield UK. The steering group comprises Professor Michael Doherty, Dr Weiya Zhang, Professor Kenneth Muir, and Dr Rose Maciewicz.

Mrs Eleanor Mitchell coordinated the planning, recruitment, data collection, radiographic assessment and data entry. A team of 6 research metrologists led by Mrs Sally Doherty gathered all demographic and exposure data; collected urine and blood samples; and carried out clinical examinations. All radiographs were performed at the Radiology Department, City Hospital Nottingham, Nottingham University Hospitals NHS Trust, and the scoring of hand, knee and hip radiographs for osteoarthritis was undertaken by Mrs Sally Doherty, Senior Research Metrologist, Academic
Rheumatology, University of Nottingham, UK. DNA extraction from whole blood was performed by the Quintiles laboratory in Edinburgh, UK.

For this thesis, I used data from both the baseline, and follow-up GOAL questionnaires. Quality checks on the baseline GOAL study data have been performed by Dr Kate Holliday (nee Limer), a former PhD student at the Division of Epidemiology and Public Health, University of Nottingham, UK. Data for the follow-up GOAL questionnaire was collected by Dr Stella Muthuri, a former PhD student at the Division of Epidemiology and Public Health, University of Nottingham, UK.

This thesis comprises work which was undertaken while I was a registered PhD student at Academic Rheumatology, University of Nottingham, UK. During this period, I was supervised by Professor Michael Doherty, and Dr Weiya Zhang. In this period, I contributed to the GOAL database by measuring the frontal plane knee alignment on knee radiographs of over 3000 participants. Additionally, I selected candidate genes, and single nucleotide polymorphisms for genotyping to identify genetic associations of chondrocalcinosis. In this endeavour, I was guided by both my supervisors; Dr Ann-Marie Sims, Clinical Geneticist, AstraZeneca, Macclesfield, UK; and last but not least by Dr Ana Valdes, Clinical Geneticist, St Thomas’ Hospital, London, UK. Genotyping of these single nucleotide polymorphisms was carried out by the laboratories at AstraZeneca, Macclesfield, UK, and by Kbiosciences, Hoddesdon, Herts, UK.
**Acknowledgements**

I would like to express the most sincere thanks and appreciation to my supervisors Professor Michael Doherty and Dr Weiya Zhang for their support, expertise, guidance, and encouragement without which this work would not be complete. They have inspired me, and continue to do so. I am most thankful to both of them for guiding and supporting me through this mighty task. I also wish to thank Dr Rose Maciewicz, Dr Ann-Marie Sims, Dr Ana Valdes, and Professor Kenneth Muir for their comments and guidance.

I am indebted to colleagues and the staff in Academic Rheumatology, University of Nottingham, UK, for making this period an enriching learning experience for me. I would like to thank Mrs. Sally Doherty, and the team of metrologists who did the groundwork which went into establishing the GOAL database. I would like to acknowledge AstraZeneca, Macclesfield, UK and Arthritis Research UK for funding the GOAL study.

I owe an enormous gratitude to my family for their surpassing support and encouragement. My heartfelt thanks also go to all my friends for the support over the course of my studies. I dedicate this thesis to all who stood by me and supported me in this enormous endeavour.
Abstract

Objectives: The objectives of this study were to a) examine the distribution of chondrocalcinosis (CC), b) determine the risk factors of CC, and c) examine the radiographic phenotype of osteoarthritis (OA) associated with CC.

Methods: Data from the Genetics of Osteoarthritis and Lifestyle (GOAL) study were used to describe the radiographic distribution of CC, and to conduct a case-control study in which cases with CC were compared with controls without CC. All participants had already completed a detailed questionnaire, been examined by a research metrologist, had radiographs of knees, hands, and pelvis, and had given urine and blood samples. All radiographs had been scored for structural radiographic changes of OA, and for the presence of CC. Frontal plane knee alignment was measured on all knee radiographs. The prevalence (95% confidence interval (CI)) of CC was calculated. The odds ratio (OR) and 95% CI were calculated for risk factors of CC, and for structural changes associated with CC in joints with OA. This was adjusted for age, gender, body mass index (BMI), and OA as appropriate, using logistic regression.

Results: 3170 participants were included in this study. There were 431 cases with CC. The overall prevalence (95%CI) of CC in the GOAL population was 13.7% (12.5% - 14.9%). In the GOAL population, knee was the commonest site of CC. However, 42% of participants with CC did not have any knee involvement. There was evidence for a generalized predisposition to CC. For example, CC at one joint associated with CC at distant joints. Joints with CC clustered together more than would be expected by chance alone. At knees,
wrist and hips, bilateral CC was more likely to associate with CC at distant joints than unilateral CC – also supporting the existence of a systemic predisposition to CC.

After adjusting for confounding factors, there was an association between CC and increasing age, lower current BMI, and OA. The association between OA at one joint and CC at the same joint was present for all joints except for the hip. There was no association between CC and gender, diuretic intake, and selected single nucleotide polymorphisms in enzymes involved in pyrophosphate (PPI) metabolism. CC associated with peri-articular calcification, vascular calcification, low cortical bone mineral density (BMD) but not with low cancellous BMD. Self-reported arthroscopy, meniscectomy, knee injury, occupational knee joint loading and knee mal-alignment in the 3rd decade of life associated with knee CC. However, after adjusting for confounding factors including OA, there was no association between either self-reported or radiographically assessed current knee mal-alignment and knee CC.

In joints with OA, the additional presence of CC at the same joint associated with a different radiographic phenotype of structural arthropathy. For example, in knees with OA, knee CC associated with attrition. In hips with OA, hip CC associated negatively with osteophytes, joint space narrowing, and sclerosis at the right hip but not at the left. Similarly, in wrists with OA, wrist CC associated with sclerosis in the right but not in the left wrist; in scapho-trapæzioid joints (STJs) with OA wrist CC associated with sclerosis on both sides; in metacarpophalangeal joints with OA, wrist CC
associated with cysts in the right but not in the left hand; and in 1st carpometacarpal joint with OA, wrist CC associated with cysts in the left but not in the right hand. In knees with OA, the additional presence of CC at distant joints associated with knee attrition. Those with knee CC + OA were excluded from this analysis to remove any local effects of CC. CC at distant joints did not associate with a distinct structural OA phenotype in other joints examined.

**Conclusion:** These findings suggest that CC results form a systemic predisposition, and that it commonly occurs at other joints in the absence of knee involvement. Established risk factors of CC such as age, OA, and previous arthroscopy and/or meniscectomy were validated in this study. Several novel risk factors of CC e.g. low current BMI, low cortical BMD, and vascular calcification were identified. Several novel associations of knee CC i.e. early life knee malalignment, self-reported knee injury, and occupational knee loading were also recognised. There was convincing evidence to suggest that in joints with OA, the additional presence of CC modifies the OA phenotype, and that this varies from joint to joint.
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<td>BCP</td>
<td>basic calcium phosphate</td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>bone mineral density</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>Chondrocalcinosis</td>
<td></td>
</tr>
<tr>
<td>cDNA</td>
<td>copy deoxy ribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>CHN</td>
<td>City Hospital Nottingham</td>
<td></td>
</tr>
<tr>
<td>CILP</td>
<td>cartilage intermediate layer protein</td>
<td></td>
</tr>
<tr>
<td>CMC(J)</td>
<td>carpometacarpal (joint)</td>
<td></td>
</tr>
<tr>
<td>CPP</td>
<td>calcium pyrophosphate crystal</td>
<td></td>
</tr>
<tr>
<td>CPPD</td>
<td>calcium pyrophosphate deposition</td>
<td></td>
</tr>
<tr>
<td>DEXA</td>
<td>dual energy x-ray absorptiometry</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>deoxy ribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>ENPP1</td>
<td>ectonuclotide pyrophosphohydrolase 1 gene</td>
<td></td>
</tr>
<tr>
<td>ePPi</td>
<td>extracellular pyrophosphate</td>
<td></td>
</tr>
<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>food frequency questionnaire</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>general practitioner</td>
<td></td>
</tr>
<tr>
<td>GOA</td>
<td>generalised osteoarthritis</td>
<td></td>
</tr>
<tr>
<td>GOAL</td>
<td>Genetics of Osteoarthritis And Lifestyle</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg equilibrium</td>
<td></td>
</tr>
<tr>
<td>HFE</td>
<td>high ferritin gene</td>
<td></td>
</tr>
<tr>
<td>IGF1</td>
<td>insulin like growth factor 1</td>
<td></td>
</tr>
<tr>
<td>IL1β</td>
<td>interleukin 1 beta</td>
<td></td>
</tr>
<tr>
<td>IVU</td>
<td>intra-venous urogram</td>
<td></td>
</tr>
<tr>
<td>MCP(J)</td>
<td>metacarpophalangeal joint</td>
<td></td>
</tr>
</tbody>
</table>
MTP(J) metatarsophalangeal joint
MV matrix vesicle
NA not applicable
NTP nuclotide triphosphate
NTPPPH NTP pyrophosphohydrolase
OA Osteoarthritis
OR odds ratio
PAS patient administration system
PC1 plasma cell glycoprotein 1
PFJ patellofemoral joint
Pi Phosphate
PiT-1 sodium phosphate co-transporter
PIP (J) proximal interphalangeal joint
PPi Pyrophosphate
PTH parathyroid hormone
QMC Queen's Medical Centre
RA rheumatoid arthritis
RR relative risk
SLAC scapholunate advanced collapse
SNP single nucleotide polymorphism
SD standard deviation
SF synovial fluid
ST(J) scapho trapezium (joint)
TFJ tibio femoral joint
TFR transferrin gene
TGFβ transforming growth factor beta
THR total hip replacement
TJR total joint replacement
TNAP tissue non-specific alkaline phosphatase gene
UK United Kingdom
WOMAC western ontario mcmaster university knee & hip osteoarthritis index
Chapter 1 Introduction

1.1 Literature search strategy

In order to identify the published scientific literature on chondrocalcinosis (CC), the bibliographic database (Pubmed) was searched using the following keywords separately: ‘chondrocalcinosis’, ‘CC’, ‘calcium pyrophosphate deposition’, ‘calcium pyrophosphate dihydrate deposition disease’, ‘CPPD’, ‘CPPDDD’, ‘pseudogout’, ‘pyrophosphate arthritis’, ‘pyrophosphate metabolism’, ‘ANKH’ or ‘ANK’. Original papers were retrieved where possible and the references were reviewed to identify and retrieve other relevant publications. Publications in languages other than English were excluded except for a few early reports of chondrocalcinosis.
1.2 Historical perspective

Robert Adams, a Dublin surgeon was the first to describe articular cartilage calcification in association with chronic joint disease in the year 1854, more than 70 years before the description of radiographic articular cartilage calcification (Dieppe et al 1989). Radiographic articular cartilage calcification known as CC was first described in the late 1920s (Mandl 1927; Werwath 1928). Werwath drew attention to the association between CC and synovitis (Werwath 1928). However, Zitnan and Sitaj were the first to identify CC as a cardinal manifestation of a separate disease entity which they termed ‘chondrocalcinosis articularis’ (Figure 1) (Zitnan et al 1960). Although no formal definition exists, CC is regarded as a linear calcification or spotty punctuate calcification arranged in a linear fashion occurring in the fibro- or hyaline articular cartilage (McCarty et al 1966a).

Figure 1 Knee chondrocalcinosis

Legend: There is calcification of the meniscus, hyaline articular cartilage, and the capsule.
The aetiology of ‘chondrocalcinosis articularis’ was unknown till 1962 when calcium pyrophosphate (CPP) dihydrate crystals were demonstrated in the synovial fluid of five patients with apparently acute ‘gouty’ arthritis (McCarty et al 1962). The similarity of clinical presentation of these patients to acute gout led to the use of the term ‘pseudogout’ to describe the condition. Two of these five patients also had CC visible on plain radiographs (McCarty et al 1962). Therefore, McCarty et al. proposed that CPP crystals cause ‘pseudogout’, articular CC, and associated arthropathy. At about the same time, Zitnan and Sitaj described 27 cases with arthropathy and CC (Zitnan et al 1960; Zitnan et al 1963). Twenty-one of the 27 cases belonged to 5 families (Zitnan et al 1963). This was the first description of familial CC.

However, it was soon realised that radiographic CC can be associated with the presence of non-CPP crystal species especially hydroxyapatite crystals (McCarty, D. J., Jr 1966a, Halverson et al 1986). In a post-mortem study, meniscal knee calcification was present in 7.0% cadavers (McCarty et al 1966a). The crystal types isolated in menisci with calcification were: CPP crystals (3.3%), dicalcium phosphate dihydrate (brushite) crystals (2.3%), and hydroxyapatite crystals (1.4%) (McCarty et al 1966a). However, in this study hydroxyapatite crystals were reported to associate with solitary irregular calcification while CPP and brushite crystals associated with a spotty or linear meniscal calcification which is in keeping with CC (McCarty et al 1966a). In another synovial fluid study of knee osteoarthritis (OA) patients, radiographic knee CC was present in knees with CPP and BCP crystals and in knees with CPP crystals alone but not in knee joints with BCP crystals
alone (Halverson et al 1986). Thus, while other crystal species may be present in joints with radiographic CC, both these studies suggest the primary role of CPP crystal deposition in the occurrence of radiographic CC (McCarty, D. J., Jr 1966a, Halverson et al 1986).

Initial reports described patients with florid symptoms, widespread CC and impressive joint damage (Zitnan et al 1960; McCarty et al 1962; Zitnan et al 1963). However, it soon became apparent that CC may be asymptomatic (Phillips et al 1965). After the initial description of pseudogout, CPP deposition (CPPD) was reported to associate with several distinct clinical manifestations. This led to an expanded classification system for CPPD containing a wide range of pseudo-syndromes. However, whether these constitute distinct clinical subset is questionable. This complex system encouraged inconsistent use of terminology. Terms prefixed by pseudo- do not specify the causative crystal, are a source of confusion, and imply that CPPD is only of secondary interest compared to gout. Recently, a European League Against Rheumatism (EULAR) Task Force suggested simpler terminology and classification (Zhang W et al 2011).
1.3 Epidemiology

1.3.1 Incidence of chondrocalcinosis

There are no studies of incidence of CC in the general population. Two retrospective (Reuge et al 2001; Nalbant et al 2003), and two prospective (Hernborg et al 1977; Massardo et al 1989) hospital based studies of patients with knee OA without knee CC at baseline have examined the incidence of de-novo CPPD at the knee (Table 1). After a follow up of 8 -12 years, de-novo CPPD occurred in 6.5% - 26.2% of cases without radiographic knee CC at baseline. The estimated incidence of radiographic knee CC was 0.8% - 2.1% per year, while that of CPPD at the knee was 2.7% - 5.5% per year. The incidence of CPPD depended on the technique used for its identification (Table 1). A higher incidence of CPPD was observed in studies using synovial fluid examination in addition to radiographic examination than in those employing radiographic examination alone. This is because CPP crystals may be present in the absence of radiographically visible CC (Ledingham et al 1993a; Pattrick et al 1993).

Table 1 Incidence of calcium pyrophosphate deposition

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Mean follow-up (years)</th>
<th>Prevalence of CPPD at end of follow up</th>
<th>Estimated incidence of CPPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Radiographs (CC only)</td>
<td>Radiographs or SF(^2) analysis (CC or CPP crystal)</td>
</tr>
<tr>
<td>Nalbant et al 2003</td>
<td>26</td>
<td>3.6</td>
<td>-/-(^3)</td>
</tr>
<tr>
<td>Massardo et al 1989</td>
<td>31</td>
<td>8.0</td>
<td>6.5%</td>
</tr>
<tr>
<td>Reuge et al 2001</td>
<td>59</td>
<td>9.0</td>
<td>17.0%</td>
</tr>
<tr>
<td>Hernborg et al 1977</td>
<td>84</td>
<td>13.0</td>
<td>26.2%</td>
</tr>
</tbody>
</table>

\(^1\) Incidence = (follow up prevalence - baseline prevalence)/mean duration of follow up
\(^2\) Synovial fluid
\(^3\) No radiographs
\(^4\) No synovial fluid analysis carried out
1.3.2 Prevalence of chondrocalcinosis

1.3.2.1 Community based studies

The prevalence of CC has been estimated in relatively few community based studies (Table 2). The estimates for prevalence of CC vary depending on the number of joints surveyed, and the age of the population under study (Ellman et al 1975; Trentham et al 1975; Bergstrom et al 1986a; Felson et al 1989; Sanmarti et al 1993; Neame et al 2003; Salaffi et al 2005; Ramonda et al 2009). For example, in the 3 large radiographic studies of middle aged and older people, the prevalence of CC at the knee was 7.0% - 8.1%, rising to 10.0% if knees, wrists and hands; and to 10.4% if knees and hips were considered together (Felson et al 1989; Neame et al 2003; Ramonda et al 2009). Other studies restricted to older people reported a higher prevalence of CC (Ellman et al 1975; Bergstrom et al 1986a), whilst studies based in residential homes reported an even greater prevalence of CC (Ellman et al 1975). This may be because they are additionally affected by selection bias – people with symptomatic arthropathies are more likely to be in a residential home than in the community (Ellman et al 1975).

Only a few studies have examined the prevalence of CC in non-Caucasians. The prevalence of knee CC appears to be lower in Chinese men and women (Zhang et al 2006). In a population based study comparing the prevalence of CC in Beijing, China, and in Framingham, USA, the age-standardized prevalence ratio (95% confidence interval (CI)) of knee CC in Chinese men was only one-third of that observed in white men (0.34 (0.20–0.54). The difference was significant for bilateral CC (age-standardized prevalence ratio 0.20 (0.10–0.38)) but not for unilateral CC (age-
standardized prevalence ratio 1.21 (0.56–2.32)) (Zhang et al 2006). A similar difference was observed in women (age-standardized prevalence ratio (95%CI) 0.43 (0.31–0.59)) (Zhang et al 2006). Compared with white subjects, Chinese men and women also had a much lower prevalence of radiographic CC in the wrist, with an age-standardized prevalence ratios of 0.06 (0.01–0.18), and 0.18 (0.10–0.30) respectively (Zhang et al 2006).

As unilateral knee CC may be a result of local joint pathology (e.g. meniscectomy, OA, injury), the authors conclude that the difference in prevalence of bilateral knee CC suggests the lack of a systemic predisposition in the Chinese population. The authors reported a 15 fold higher concentration of calcium in the tap water from Beijing (67.6 mg/litre), than in Framingham (4.5 mg/litre). They suggest that high calcium levels in the tap water from Beijing possibly suppressed parathyroid hormone secretion, thereby protecting against CC (Zhang et al 2006).

The prevalence of CC also appears to be lower in Saudi Arabian people compared to that observed in Caucasians even when only those older than 60 years in age are considered (Al-Arfaj et al 2002a).
Table 2 Prevalence of chondrocalcinosis in community studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Age in years (range)</th>
<th>Joints surveyed</th>
<th>Number with CC/total number of participants</th>
<th>Prevalence of CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Community dwelling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salaffi <em>et al</em> 2005</td>
<td>18-91</td>
<td>Symptomatic joints</td>
<td>9/2155</td>
<td>0.42%</td>
</tr>
<tr>
<td>Neame <em>et al</em> 2003</td>
<td>40-86</td>
<td>Knee</td>
<td>119/1727</td>
<td>7.0%</td>
</tr>
<tr>
<td>Felson <em>et al</em> 1989</td>
<td>63-93</td>
<td>Knee</td>
<td>114/1402</td>
<td>8.1%</td>
</tr>
<tr>
<td>Ramonda <em>et al</em> 2009</td>
<td>&gt;65</td>
<td>Knee, pelvis</td>
<td>169/1629</td>
<td>10.4%</td>
</tr>
<tr>
<td>Sanmarti <em>et al</em> 1993</td>
<td>60-88</td>
<td>Wrist, hand, knee</td>
<td>27/261</td>
<td>10.0%</td>
</tr>
<tr>
<td>Bergstrom <em>et al</em> 1986b</td>
<td>70-79</td>
<td>Wrist, hand, knee</td>
<td>37/352</td>
<td>11.5%</td>
</tr>
<tr>
<td>Bergstrom <em>et al</em> 1986a</td>
<td>79</td>
<td>Wrist, hand, knee</td>
<td>13/81</td>
<td>16.0%</td>
</tr>
<tr>
<td><strong>Residential home</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trentham <em>et al</em> 1975</td>
<td>41-97</td>
<td>Wrist</td>
<td>2/100</td>
<td>2.0%</td>
</tr>
<tr>
<td>Ellman <em>et al</em> 1975</td>
<td>70-94</td>
<td>Wrist, pelvis, knee</td>
<td>16/58</td>
<td>27.6%</td>
</tr>
<tr>
<td><strong>Non-Caucasians</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhang <em>et al</em> 2006</td>
<td>&gt;60</td>
<td>Wrist</td>
<td>18/2510</td>
<td>0.7%</td>
</tr>
<tr>
<td>Zhang <em>et al</em> 2006</td>
<td>&gt;60</td>
<td>Knee</td>
<td>-/-</td>
<td>1.79%</td>
</tr>
<tr>
<td>Al-Arfaj <em>et al</em> 2002a</td>
<td>50-93</td>
<td>Wrist, hand, knee</td>
<td>6/153</td>
<td>3.9%</td>
</tr>
</tbody>
</table>

1 Participants living in residential home only  2 Male  3 Female  4 6.7% in >60 years;  *community dwelling

1.3.2.2 Hospital based studies

The prevalence of CC in hospital based studies varies from 7.0% - 34.0%. Just as in community based studies, this depends on the joints surveyed, and the age of the population under study (Bocher *et al* 1965; Ellman *et al* 1981a; Wilkins *et al* 1983; Gordon *et al* 1984) (Table 3). Hospital based studies have an inherent recruitment bias as they include patients who underwent musculoskeletal radiographs for clinical reasons (Bocher *et al* 1965; Ellman *et al* 1981a) or include hospitalized in-patients who are likely to have multiple co-morbidities and risk factors for arthritis (Wilkins *et al* 1983;
Gordon et al 1984). Just as has been observed in community based studies, hospital based studies from the Middle East also reported a lower prevalence of CC (≤2.0%) (Malaviya et al 1998; Malaviya et al 2001).

Table 3 Prevalence of chondrocalcinosis in hospital based studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Age in years (range)</th>
<th>Joints surveyed</th>
<th>Number with CC/total number of participants</th>
<th>Prevalence of CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bocher et al 1965</td>
<td>59-93</td>
<td>Knee</td>
<td>32/455</td>
<td>7.0%</td>
</tr>
<tr>
<td>Ellman et al 1981a</td>
<td>&gt;50</td>
<td>Knee</td>
<td>55/574</td>
<td>9.6%</td>
</tr>
<tr>
<td>Gordon et al 1984</td>
<td>&gt;55</td>
<td>Hand, wrist, knee, pelvis</td>
<td>20/127</td>
<td>15.7%</td>
</tr>
<tr>
<td>Wilkins et al 1983</td>
<td>65-97</td>
<td>Hand, wrist, knee, pelvis</td>
<td>34/100</td>
<td>34.0%</td>
</tr>
</tbody>
</table>

1.3.2.3 Autopsy studies.

Between 10.0% and 22.0% of post-mortem knee radiographs have evidence of CC (McCarty et al 1966a; Gordon et al 1984b; Mitrovic et al 1988; Sokoloff et al 1988). In three studies, CPP crystals were found in all CC cases where its presence was specifically sought (McCarty et al 1966a; Mitrovic et al 1988; Sokoloff et al 1988) while in another study no CPP crystals were identified in those with radiographic CC and severe knee OA (Gordon et al 1984b).

As would be expected, the prevalence of CPP crystals in post-mortem knee aspirates varies according to the joint aspirated, and the presence or absence of joint effusion. For example, the prevalence of CPP crystals in post-mortem joint aspirates was 30% in knees with a detectable joint effusion (Gordon et al 1984b), 8% (11 of 138) in knees irrespective of the presence of a detectable joint effusion (Moens et al 1985), and only 0.3% (2 of 70) at the
1st metatarsophalangeal joints (MTPJs) (Wall et al 1983). Similarly, on post-mortem examination, the estimated prevalence of CPP crystals in ankle of those older than 18 years was 1.6% (Muehleman et al 2008).
1.4. Risk factors

1.4.1 Age

Early descriptions of CC suggested that it predominantly affects young or middle aged individuals with a median age of onset of symptoms in the 4th decade of life (Zitnan et al 1963). Subsequent hospital based case series suggested that CC predominantly affects older people (Moskowitz 1966; Skinner et al 1969; Moskowitz et al 1973; Bjelle et al 1974; Fam et al 1981; Gordon et al 1984; Canhao et al 2001). Since then, the relation between age and CC has been examined in several hospital (Ellman et al 1981a; Wilkins et al 1983), community (Bergstrom et al 1986b; Felson et al 1989; Sanmarti et al 1993; Neame et al 2003; Zhang et al 2006), and autopsy studies (Mitrovic et al 1988; Sokoloff et al 1988; Muehleman et al 2008).

The prevalence of knee CC increases with age (Figure 2). In a community based study in Nottinghamshire, UK, the prevalence of knee CC increased from 3.7% at age 55 - 59 years to 17.5% at age 80 - 84 years (Neame et al 2003). Similarly, in the Framingham study, there was more than a doubling in the prevalence of knee CC with each 10 years increase in age after the age of 60 years [Relative Risk (95%Confidence Interval) (RR (95%CI) of CC per 10 year increase in age 2.40 (1.97-2.91)] (Felson et al 1989). Similar findings have been reported in previous Swedish studies (Bergstrom et al 1986a; Bergstrom et al 1986b). In these studies, the prevalence of knee CC at age 70, 75, and 79 years was 6.8%, 8.0% and 12.3%, while that of CC at either knee, wrist or hand was 7.5%, 10.1% and 16.0% respectively (Bergstrom et al 1986a; Bergstrom et al 1986b).
Hospital based studies report a similar dramatic increase in prevalence of CC with increasing age. In one hospital based study, the prevalence of CC at any site (either knee, wrist, hands or pelvis) rose from 15% in the 65 - 74 year age group, to 36.4% in the 75 - 84 year age group, and to 44% in the 85 - 94 year age group (Wilkins et al 1983). There is also an age associated increase in prevalence of CPP crystal deposition at unusual sites like the ankle (Muehleman et al 2008). In a post-mortem study, the prevalence of CPPD at the ankle rose from 0.07% in the 50 - 59 years age group, to 1.01% in the 60 - 69 years age group, to 2.4% in the 70 - 79 years age group, and to 3.5% in those older than 80 years in age (Muehleman et al 2008). In a post-mortem study using Faxitron x-ray device, cartilage calcification increased with increasing age irrespective of the grade of OA, suggesting that ageing may have a greater contribution to cartilage calcification than OA (Mitsuyama et al 2007), however, other studies suggest that the association between CC and OA is independent of age (See section 1.3.4).
Figure 2 Prevalence of knee chondrocalcinosis increases with age
### 1.4.2 Gender

Many hospital based case-series suggest that CC is more common in women than in men, reporting that between 62.0% - 72.4% of patients with CC were women (Moskowitz 1966; Skinner et al 1969; Moskowitz et al 1973; Fam et al 1981; Dieppe et al 1982; Louthrenoo et al 1999; Canhao et al 2001). However, a Swedish hospital based case-series found equal numbers of men and women in 50 consecutive CC cases with arthropathy (Bjelle et al 1974). The notion that CC is more common in women than in men is supported by several other hospital (Bocher et al 1965; Wilkins et al 1983; Gordon et al 1984), community (Bergstrom et al 1986a; Sanmarti et al 1993; Zhang et al 2006), and autopsy studies (Mitrovic et al 1988) (Table 4). However, other hospital (Bocher et al 1965; Ellman et al 1981a), community (Felson et al 1989; Al-Arfaj et al 2002a; Neame et al 2003), and autopsy (Sokoloff et al 1988) studies do not find any difference in prevalence of CC according to their gender (Table 4). However, when the demographics of gender and age distribution in the community are taken into account, both the Nottinghamshire study and the Framingham study suggest an approximately equal gender distribution (Felson et al 1989; Neame et al 2003). Similarly, the age independent association between gender and CC in the ProVA study (Ramonda et al 2009) became non-significant after adjusting for OA (Musacchio et al 2011). Therefore, the association between gender and CC observed in earlier studies may be due to the fact that knee OA is more common in women, and when present is more likely to be severe than in men (van Saase et al 1989).
Table 4 Prevalence of chondrocalcinosis according to gender

<table>
<thead>
<tr>
<th>Study</th>
<th>Joints surveyed</th>
<th>Prevalence of CC</th>
<th>Difference in prevalence women – men (95%CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Community dwelling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neame et al 2003</td>
<td>Knee</td>
<td>6.1%</td>
<td>8.2%</td>
</tr>
<tr>
<td>Felson et al 1989</td>
<td>Knee</td>
<td>9.0%</td>
<td>8.1%</td>
</tr>
<tr>
<td>Ramonda et al 2009</td>
<td>Knee, Pelvis</td>
<td>12.8%</td>
<td>7.0%</td>
</tr>
<tr>
<td>Sanmarti et al 1993</td>
<td>Wrist, hand, knee</td>
<td>14.1%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Bergstrom et al 1986b</td>
<td>Wrist, hand, knee</td>
<td>14.7%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Ellman et al 1975^c</td>
<td>Wrist, pelvis, knee</td>
<td>32%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ellman et al 1981a^3</td>
<td>Knee</td>
<td>8.3%</td>
<td>12.4%</td>
</tr>
<tr>
<td>Bocher et al 1965^4</td>
<td>Knee</td>
<td>6.7%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Gordon et al 1984^3</td>
<td>Hand, wrist, knee, pelvis</td>
<td>24.3%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Wilkins et al 1983^4</td>
<td>Hand, wrist, knee, pelvis</td>
<td>40.6%</td>
<td>19.4%</td>
</tr>
<tr>
<td>Mitrovic et al 1988^6</td>
<td>Knee</td>
<td>26.1%</td>
<td>15.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhang et al 2006</td>
<td>Knee</td>
<td>2.67%</td>
<td>1.79%</td>
</tr>
<tr>
<td>Zhang et al 2006</td>
<td>Wrist</td>
<td>1.0%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Al-Arfaj et al 2002a</td>
<td>Wrist, hand, knee</td>
<td>6.7%</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

^1 Non-significant after adjusting for age - Neame et al. 2003, and Felson et al. 1989; and knee OA (Musacchio et al 2011). ^2 Residential home ^3Radiology files ^4Elderly care hospital ^5Medical inpatients ^6Autopsy. Trentham (Trentham et al 1975) only recruited men. Salaffi (Salaffi et al 2005) and Sokoloff (Sokoloff et al 1988) do not give detailed information about gender. Patients in (Bergstrom et al 1986a) were included in (Bergstrom et al 1986b).

*The difference in prevalence and 95% confidence interval (CI) were calculated as:

\[
p_1 - p_2 \pm 1.96 \sqrt{\frac{(p_2(1-p_2))}{n_1} + \frac{(p_2(1-p_2))}{n_2}}\]

px, nx, is prevalence of CC and number of participants.
1.4.3 Heredity

The occurrence of familial CC has been reported since early description of CC (Zitnan et al. 1963; McCarty 1976). In fact, 21 of the 27 CC cases described by Zitnan and Sitaj belonged to 5 families (Zitnan et al. 1963). Also, of the 12 cases initially described by McCarty et al., 3 had other family members affected by CC (McCarty 1976). In another study, 57 of the 160 CC cases in Chiloe islanders were from 12 families (Reginato 1976). A high degree of consanguinity in families with CC reported in this study further supported the hypothesis that CC may be hereditary (Reginato 1976). This hypothesis was further supported by the fact that autosomal dominant familial CC with varying degrees of associated arthropathy was reported from different parts of Europe, the Americas and North Africa at about the same time (Perry et al. 1969; Reginato et al. 1970; Reginato et al. 1974; Reginato et al. 1975; Rodriguez-Valverde et al. 1980; Bjelle 1981; Gaudreau 1981; Milazzo et al. 1981; Nunez-Roldan et al. 1981; Bjelle et al. 1982a; Bjelle et al. 1982b; Richardson et al. 1983; Hamza et al. 1990; Doherty et al. 1991). Taken together, these reports suggested a strong hereditary component to the ‘apparently sporadic CC’. Two main clinical patterns of hereditary CC were described (Reginato 1976):

a) florid early polyarticular CC with frequent ‘pseudogout’, spinal involvement, and severe symptoms; and

b) mild oligoarticular CC with a late age of onset of symptoms.

Subsequent hospital based studies showed that 11.0%-27.0% of cases with apparently sporadic CC had at least one other affected family
member, suggesting that up to a quarter of sporadic CC cases were in-fact familial (Rodriguez-Valverde et al 1980; Fernandez Dapica et al 1986; Balsa et al 1990). However, these studies have many significant limitations. They lack a control group, have hospital based recruitment, and have a high rate of non-participation among family members. Being hospital based, these studies are likely to have cases with severe polyarticular CC or those with OA and CC. The familial association could be mediated by OA, which runs in families (Sandell 2012), and is an established risk factor for CC (See chapter 1.4.4 Osteoarthritis).

Other larger studies suggest that apparently sporadic CC is not hereditary in nature. In a controlled study, the prevalence of CC was compared between 122 siblings of index cases with knee OA and CC awaiting joint replacement surgery, and 1727 community dwelling adults (Zhang et al 2004). The prevalence of CC was twice as high in siblings of index cases compared to the community controls (13% vs. 6.9%). However, after controlling for age, gender, body mass index (BMI), and knee OA the prevalence of CC was no greater in siblings of index cases with OA and CC, than in community dwelling adults (Zhang et al 2004). The adjusted Odds Ratios (aOR) (95%CI) were 1.16 (0.58 to 2.29) for CC irrespective of OA status, 1.07 (0.41 to 2.78) for isolated CC (i.e CC without OA), and 1.09 (0.44 to 2.68) for CC with OA (Zhang et al 2004).
1.4.4 Osteoarthritis

The early descriptions of CC were in patients with significant structural arthropathy (Zitnan et al 1963; Dieppe et al 1989). In early hospital based studies, 50.0% – 90.4% of joints with CC had structural changes of OA (Bocher et al 1965; Bjelle et al 1974; McCarty 1974a). OA and CC were reported to co-localise at knees, wrists, MCPJs, hips, shoulders, elbows, and ankles. Subsequent large community based studies showed a smaller but strong association between OA and CC (Felson et al 1989; Sanmarti et al 1996; Neame et al 2003; Ramonda et al 2009). The association between OA and CC was not due to confounding by age or gender as the association persisted after adjusting for these factors (Felson et al 1989; Sanmarti et al 1996; Neame et al 2003; Ramonda et al 2009) (Table 5).

Most large studies were focussed on knee CC and OA. In the only large study to examine the association between radiographic hip OA and CC (at either knee, hip or symphysis pubis), there was no association between CC and radiographic hip OA, suggesting that the association between OA and CC may be joint specific, and may predominantly exist at the knee (Ramonda et al 2009). In a later publication from the same dataset, both clinically diagnosed knee OA (p=0.05), and knee OA according to the American College of Rheumatology classification criteria (p=0.003) associated with CC at knee, hip, or symphysis pubis after adjusting for age, and gender (Musacchio et al 2011). However, there was no association between clinically diagnosed hip OA, and hip OA according to the American College of Rheumatology classification criteria and CC at knee, hip or symphysis pubis (Musacchio et al 2011). The association between OA and
CC has not been shown in some studies from the Middle East, possibly due to a very low (≤2%) prevalence of CC in these populations (Malaviya et al 1998; Malaviya 2001).

Although, the association between knee CC and prevalent knee OA is well established, findings from the Framingham study suggest that knee CC does not result in incident knee OA (aRR (95%CI) 1.20 (0.50-2.70)) (Felson et al 1997).

### Table 5 Association between chondrocalcinosis and osteoarthritis

<table>
<thead>
<tr>
<th>Study</th>
<th>Joints examined</th>
<th>age adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Community dwelling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neame et al 2003</td>
<td>Knees</td>
<td>2.45 (1.65-3.65)¹</td>
</tr>
<tr>
<td>Felson et al 1989</td>
<td>Knees</td>
<td>1.52 (1.22-1.90)¹²</td>
</tr>
<tr>
<td>Sanmarti et al 1996</td>
<td>Knee OA and CC at knees, hips or wrist</td>
<td>4.30 (1.60-11.8)</td>
</tr>
<tr>
<td>Musacchio et al 2011</td>
<td>Knee OA and CC at knees, symphysis</td>
<td>3.02 (1.95-4.69)³</td>
</tr>
<tr>
<td></td>
<td>pubis or hips</td>
<td></td>
</tr>
<tr>
<td>Musacchio et al 2011</td>
<td>Hip OA and CC at hips, symphysis</td>
<td>1.06 (0.64-1.76)³</td>
</tr>
<tr>
<td></td>
<td>pubis or knees</td>
<td></td>
</tr>
<tr>
<td><strong>Hospital based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gordon et al 1984</td>
<td>Knees</td>
<td>4.20 (1.40-12.8) right knee</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.00 (1.10-8.2) left knee</td>
</tr>
</tbody>
</table>

¹ Additionally adjusted for gender ² Relative Risk (95%CI) ³Crude.

Radiographic studies underestimate the prevalence of CPPD in those with OA (Gibilisco et al 1985; Viriyavejkul et al 2007). For example, at the time of total joint replacement for knee OA, 17% patients without radiographic knee CC had CPP crystals (Viriyavejkul et al 2007). Other studies suggest that from one quarter to one half of OA knees with CPP
crystals do not have radiographic CC (Ledingham et al 1993a; Pattrick et al 1993).

The prevalence of CPP crystals is even higher in knees with OA and joint effusion in the absence of any radiographic CC. For example, in those with symptomatic knee OA with joint effusion, and without radiographic knee CC, CPP crystals were present alone in 13 of 72 (18.1%) cases, and co-existed with hydroxyapatite in 29 of 72 (40.2%) cases (Gibilisco et al 1985).

CPP crystals, basic calcium phosphate (BCP) - principally hydroxyapatite - crystals, and OA co-associate with each other. The association could be mediated by pronucleating changes in cartilage matrix e.g. reduced type 2 collagen, excess type 1 collagen (with small amounts of type 2 collagen), few large proteoglycans; alterations in Pi/PPI levels, apoptotic chondrocytes which serve as a nidus for crystal formation, and hypertrophic chondrocytes – a pro-mineralizing chondrocyte phenotype (Jubeck et al 2008; Ryan et al 1995; Masuda et al 1991; Rosenthal et al 1999). Two studies suggest that BCP and CPP crystals coexist more often than being found alone (Gibilisco et al 1985; Halverson et al 1986). In hospital based case series, either CPP or BCP crystals were present in 28.0% – 59.0% of knees with symptomatic OA (Gibilisco et al 1985; Ledingham et al 1993a; Pattrick et al 1993; Derfus et al 2002; Nalbant et al 2003; Viriyavejkul et al 2007). In symptomatic knee OA, CPP crystals were present alone in 7.0% - 42.0% cases, and in combination with apatite or alizarin red positivity in 16.0% - 40.0% of cases (Gibilisco et al 1985; Halverson et al 1986; Ledingham et al 1993a; Pattrick et al 1993; Derfus et al
In two recent studies, BCP crystals were present in all joints with end stage OA while CPP crystals were identified in only 10.0% hips, and 18.0% knees respectively (Fuerst et al 2009a; Fuerst et al 2009b). This suggests that the deposition of BCP crystals is integral to the process of end stage OA. However, in joints with cartilage defects and cortical damage, intra-articular hydroxyapatite crystals may also originate from the subchondral bone (Dieppe et al 1984).

1.3.4.1 Association between severity of osteoarthritis and chondrocalcinosis

The prevalence of CPPD increases with severity of OA at the index joint. For example, in clinic based studies of knee OA, the prevalence of CPPD at the knee varies between 8.0% – 33.0% (Massardo et al 1989; Dougados et al 1992; Ledingham et al 1993a; Reuge et al 2001; Neogi et al 2006), increasing to 31.8% - 52.9% at the time of joint replacement surgery (Derfus et al 2002; Viriyavejkul et al 2007). While at the hip, the prevalence of CC was 1.5% in clinic based studies (Ledingham et al 1992), increasing only to 6% at the time of joint replacement surgery (Sokoloff et al 1988) (Table 6).
Table 6 Prevalence of chondrocalcinosis and osteoarthritis severity

<table>
<thead>
<tr>
<th>Study</th>
<th>Age in years (range)</th>
<th>Joints surveyed</th>
<th>Number with CC/ Total number of participants</th>
<th>Prevalence of CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Knee OA: clinic based radiographic studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Massardo et al 1989; Dougados et al 1992</td>
<td>50-79</td>
<td>Hand</td>
<td>2/31</td>
<td>6.6%</td>
</tr>
<tr>
<td>Neogi et al 2006</td>
<td>47-93</td>
<td>Knee</td>
<td>23/265</td>
<td>8.8%</td>
</tr>
<tr>
<td>Massardo et al 1989</td>
<td>50-79</td>
<td>Knee</td>
<td>3/31</td>
<td>10.0%</td>
</tr>
<tr>
<td>Dougados et al 1992</td>
<td>50-79</td>
<td>Knee</td>
<td>26/259</td>
<td>10.0%</td>
</tr>
<tr>
<td>Neogi et al 2006</td>
<td>70-79</td>
<td>Knee</td>
<td>69/373</td>
<td>18.5%</td>
</tr>
<tr>
<td>Ledingham et al 1993a</td>
<td>34-91</td>
<td>Knee, pelvis, hand, wrist, shoulder, spine</td>
<td>83/252</td>
<td></td>
</tr>
<tr>
<td><strong>Knee OA: clinic based synovial fluid studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reuge et al 2001</td>
<td>50-82</td>
<td>Knee</td>
<td>41/227</td>
<td>18.1%</td>
</tr>
<tr>
<td>Ledingham et al 1993a</td>
<td>34-91</td>
<td>Knee</td>
<td>1/23</td>
<td>28.0%</td>
</tr>
<tr>
<td>Pattrick et al 1993</td>
<td>33-96</td>
<td>Knee</td>
<td>97/300</td>
<td>32.0%</td>
</tr>
<tr>
<td><strong>Knee OA: total joint replacement studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derfus et al 2002</td>
<td>52-90</td>
<td>Knee</td>
<td>16/53^</td>
<td>30.2%</td>
</tr>
<tr>
<td>Sokoloff et al 1988</td>
<td>52-90</td>
<td>Knee</td>
<td>18/55</td>
<td>32.7%</td>
</tr>
<tr>
<td>Viriyavejkul et al 2007</td>
<td>69 (mean)</td>
<td>Knee</td>
<td>53/101^</td>
<td>52.9%</td>
</tr>
<tr>
<td><strong>Hip OA: clinic based radiographic studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ledingham et al 1992</td>
<td>29-86</td>
<td>Hip</td>
<td>2/133</td>
<td>1.5%</td>
</tr>
<tr>
<td>Ledingham et al 1992</td>
<td>29-86</td>
<td>Knee, hand, wrist, shoulder, spine</td>
<td>21/133</td>
<td>15.8%</td>
</tr>
<tr>
<td><strong>Hip OA: total joint replacement study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sokoloff et al 1988</td>
<td>53-82</td>
<td>Hip</td>
<td>5/84</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

^1 synovial fluid analysis ^25 radiographic CC, 10 both CC and CPP on synovial fluid and 18 CPP crystals only on synovial fluid
1.4.5 Other musculoskeletal conditions

Early studies reported that CC associates with hyperuricemia, gout, and rheumatoid arthritis (RA) (Moskowitz et al 1965; McCarty 1966b; Moskowitz et al 1967). Subsequent hospital based controlled studies did not confirm any association between CC and hyperuricemia (Hollingworth et al 1982), but reported an association between CC and gout (Stockman et al 1980), which may be mediated by:

- ‘epitaxy’ - deposition of one crystal type on another, or
- generalized predisposition to crystal deposition and growth.

The latter could be mediated by either an excess of pronucleating factors or a paucity of inhibitors of crystal formation and growth. Such phenomena occur in vivo. This is supported by the presence of pronucleating factors like gamma globulin and collagen fibre in the synovial fluid and serum of gout patients (McGill et al 1991a; McGill et al 1991b), and the presence of a macromolecular inhibitor of hydroxyapatite crystal growth in the serum (Garnett et al 1990). Garnett et al reported that 50% of the inhibitory action of serum on hydroxyapatite crystal growth is mediated by albumin (Garnett et al 1990). Recent studies suggest that albumin, and fetuin A have a dual inhibitory and seeding effect on hydroxyapatite crystal formation (Wu et al 2009). Other examples of physiologic inhibitors of crystal formation and growth include lithostathine in pancreatic juice (Bernard et al 1992); uropontin, citrate, PPi, osteopontin, glycoproteins, glycosaminoglycans, proteoglycans in urine (Shiraga et al 1992; Worcester 1994). On the other hand ferric ion, phospholipids, proteolipid phospholipid complex, and calcium phospholipid phosphate complex increase crystal nucleation and their
excess may predispose to the co-existence of different types of crystals (Boskey et al 1982; Raggio et al 1986; Naughton et al 2001).

The association between CPPD and RA has been examined by several groups. Several hospital based case-control studies report a negative association between radiographic CC, or synovial fluid CPP crystal, and RA (Hollingworth et al 1982; Doherty et al 1984; Brasseur et al 1987). Similarly, a large community based study did not find any association between RA and CC at either knees, hips, or symphysis pubis (Ramonda et al 2009). On the contrary, examination of defleshed skeletal remains suggested an association between CC, and both RA and spondyloarthropathy (Rothschild 2007). A retrospective hospital based case series reported that 25.8% of synovial fluid aspirates from patients with RA had CPP crystals (Gerster et al 2006). The high prevalence of CPP crystal in the latter study may be due to age (mean age 64.5 years), and long disease duration of RA (mean 12 years), which may in turn result in secondary joint degeneration (Gerster et al 2006). In summary, current evidence suggests that there is either a negative or no association between CC and RA.

There is no association between Paget’s disease of bone and CC (Boussina et al 1976). Forrestier’s disease (vertical spinal ossification with characteristic dripping wax appearance) is distinct from CPPD (Rogers et al 1985).
1.4.6 Injury
Previous joint injury is a risk factor for CC at the index joint. The joint injury may be isolated e.g. surgery or meniscectomy, or recurrent e.g. hypermobility syndrome. This association is supported by a case-control study and by several case-series.

- After a mean follow-up of 25 years, there was a five-fold increase in the risk of CC in knee joints with previous meniscectomy when compared to the opposite knee (Doherty et al 1982). A similar result was found by comparing the index knee with previous meniscectomy to an age, and gender matched control group (Doherty et al 1982).

- Of the 76 patients with knee CC seen at a hospital, 18 were young (mean age 43.1 years) and developed CC in a joint which had previously been injured or operated upon (de Lange et al 1985). Significant structural changes of OA were present in 2 young patients only, and an underlying predisposition to CC was absent in all these patients (de Lange et al 1985).

- 65.5% of 85 patients with CPPD identified in a review of 3228 arthroscopies had a past history of knee injury, meniscectomy or other knee surgery (Fisseler-Eckhoff et al 1992).

- Four of the seven cases with long standing hypermobility syndrome, and knee synovitis had CPP crystals (Bird et al 1978). However, all four also had severe knee OA in the index joint, and CPPD may be secondary to OA.
Four cases with localized joint hypermobility, and CPPD in the index joint with or without co-existing OA were reported (Settas et al 1982).

### 1.4.7 Metabolic diseases

A number of metabolic conditions were initially reported to associate with CC (Hamilton 1976). While the association between diabetes and CC may be confounded by age, other reported associations between uncommon diseases like Wilson’s disease, and ochronosis were based on case reports of florid CC in young patients (Jones et al 1992). A case control study did not show any association between hypothyroidism and CC (Chaisson et al 1996). Two subsequent detailed literature reviews suggest that haemochromatosis, hyperparathyroidism, hypomagnesemia and hypophosphatasia are risk factors for CC and their presence should be specifically sought in young (<50 years) patients, especially those with florid polyarticular CC (Jones et al 1992; Richette et al 2009).

#### 1.4.7.1 Haemochromatosis

Schumacher was the first to report CC in a patient with haemochromatosis (Schumacher 1964). Subsequently, in a hospital based case series, structural arthropathy with CC was reported at the knees, wrists, hips, pubic symphysis, and MCPJs in 12 of 32 cases with haemochromatosis (Hamilton et al 1968). Numerous laboratory studies suggest that iron overload directly leads to CC by inducing cartilage matrix changes (Sokoloff 1963), chondrocyte toxicity (Brighton et al 1970), and free radical formation (Blake et al 1985). Other studies also suggest that the arthropathy of haemochromatosis is related to iron overload (Ross et al 2003), and that it
may be mediated by the middle fragment of parathyroid hormone (44-68 PTH) (Pawlotsky et al 1999).

Although the association between haemochromatosis and CC is proven beyond doubt, there is little evidence to support an association between single nucleotide polymorphisms (SNPs) which result in haemochromatosis and CC. For instance, a large community based cross-sectional study in Rotterdam did not find any association between the genotypes associated with greater iron load (C282Y/C282Y or C282Y/H63D) and CC. This may be due to a low penetrance of C282Y/C282Y or C282Y/H63D genotypes or due to the fact that factors other than iron overload are responsible for the development of CC (Alizadeh et al 2007). However, the findings of this study are contrary to that of a smaller hospital based study from the UK, which reported an association between SNPs leading to haemochromatosis and CC (Timms et al 2002).

1.4.7.2 Hyperparathyroidism
CC was first described in six patients with hyperparathyroidism seen over a ten year period at the Taplow and Hammersmith hospitals (Bayswater 1959). Early retrospective studies suggested a high prevalence of CPPD (18.0%-40.0%) in Caucasian hyperparathyroid patients (Dodds et al 1968; Glass et al 1976; Pritchard et al 1977; McGill et al 1984). Of interest, the prevalence of CPPD was lower (6.1%) in Japanese hyperparathyroid patients (Yashiro et al 1991).

The association between CC and hyperparathyroidism is supported by several hospital bases studies. In a case-control study, 30.7% of cases with
hyperparathyroidism had CC compared to 3.4% age-gender matched controls (Rynes et al 1978). This study suggests that CC is more common in hyperparathyroid patients than in age, and gender matched controls. Moreover, in other studies hyperparathyroid patients with CC were younger than controls with CC without any clinical hyperparathyroidism, suggesting that hyperparathyroidism contributed to CPPD (Pritchard et al 1977; Yashiro et al 1991). Finally, hyperparathyroid patients with CPPD were reported to have higher levels of parathyroid hormone, and a larger parathyroid adenoma (McGill et al 1984). However, as would be expected, the association between CC and hyperparathyroidism is influenced by age as hyperparathyroid patients with CC are reported to be older than hyperparathyroid patients without CC (Dodds et al 1968; Glass et al 1976; Pritchard et al 1977; Rynes et al 1978; McGill et al 1984; Yashiro et al 1991).

Evidence for the role of hypercalcemia as a pathogenic mechanism of CC in hyperparathyroidism is conflicting. Two hospital based studies suggest that hyperparathyroid patients with CC have serum calcium levels that are comparable to that observed in hyperparathyroid patients without CC (Pritchard et al 1977; Rynes et al 1978), while other studies suggest hyperparathyroid patients with CC have higher serum calcium than hyperparathyroid patients without CC (McGill et al 1984; Yashiro et al 1991). Recent in-vitro studies show that high extracellular calcium levels increase the expression of both ANK and ENPP1 mRNA and protein, which result in elevated ePPI levels (Cailotto et al 2011). This may explain the association between hyperparathyroidism and CC.
The role of parathyroid hormone (PTH) in the aetiopathogenesis of CPPD in the absence of clinical or biochemical hyperparathyroidism has been examined by four hospital based studies. Three previous case series reported similar levels of PTH and calcium in patients with and without CPPD (McCarty et al 1974b; Alexander et al 1982; Huaux et al 1986). However, recently patients with sporadic CPPD have been reported to have high middle fragment PTH (44-88 PTH) in their serum, suggesting a role for parathyroid hormone in the pathogenesis of sporadic CPPD in the absence of clinically overt hyperparathyroidism (Pawlotsky et al 2008). In-vitro studies show that mid-region PTH fragment may be biologically activity, and result in osteoblast proliferation, cartilage hypermineralization, and new bone formation (Pawlotsky et al 2008).

1.4.7.3 Hypomagnesemia
The association between hypomagnesemia and CC is supported by several case-series, and small case-control studies. A case of post-parathyroidectomy pseudogout with acute hypomagnesemia was first described in 1966 (Melvin 1966). Subsequently, several cases of CC in patients with ‘idiopathic hypomagnesemia’ (McCarty et al 1974b), and hypomagnesemia due to renal loss (Runeberg et al 1975; Ellman et al 1980) were described. Similar reports and the description of Gitelman’s variant of Bartters’ syndrome led to the establishment of hypomagnesemia - predominantly due to renal loss - as a cause of CC (Gitelman et al 1966; Munoz-Fernandez et al 1994; Smilde et al 1994). The reported association between diuretic use and knee CC has also been hypothesized to be mediated by diuretic induced hypomagnesemia (Neame et al 2003).
Hypomagnesemia due to gastro-intestinal losses may also lead to CPPD. In a cross sectional study of patients on total parenteral nutrition chronic hypomagnesemia associated with knee CC (OR (95%CI) 13.5 (2.76-127.3)) (Richette 2007). Three cases of chronic hypomagnesemia due to short bowel syndrome were also previously reported with symptomatic CPPD (Richette et al 2005).

While severe hypomagnesemia associates with CC in rare clinical scenarios, it is unclear if there is any role of mild hypomagnesemia in the pathogenesis of sporadic CC or CPPD. This is as previous hospital based studies show that serum magnesium levels are similar in people with and without apparently sporadic CPPD (McCarty et al 1974b; Alexander et al 1982; Huaux et al 1986; Ramonda et al 2009).

1.4.7.4 Hypophosphatasia
The association between hypophosphatasia and CC is based on several case reports of florid polyarticular CC at a young age (Birtwell et al 1967; O'Duffy 1970; Eade et al 1981; Whyte et al 1982; Macfarlane et al 1986; Chuck et al 1989). However, low alkaline phosphatase levels do not seem to play a role in the occurrence of apparently sporadic CPPD in the older age group. There was no difference in serum alkaline phosphatase levels in those with and without CPPD in the absence of clinical features suggestive of hypophosphatasia (McCarty et al 1974b; Alexander et al 1982; Huaux et al 1986). While some studies report low synovial fluid alkaline phosphatase in joints with CPP crystals (Russell et al 1970; Yaron et al 1970; Howell et al 1976; Tenenbaum et al 1981), other studies do not report any differences in synovial fluid alkaline phosphatise levels in those with and without CPPD
(McCarty et al 1971; Altman et al 1973; Jacobelli et al 1978; Rachow et al 1985). In summary, although CC associates with hypophosphatasia, these studies do not support any role for low alkaline phosphatase levels in the aetiopathogenesis of apparently sporadic CC at a population level.

### 1.4.8 Summary of risk factors of CC

As discussed above, there are many reported associations of CC. However, there are only a few established risk factors of CC.

These include:

1. Increasing age
2. OA
3. Metabolic diseases
   a. Hyperparathyroidism
   b. Haemochromatosis
   c. Hypomagnesemia
   d. Hypophosphatasia
4. Familial
   a. *ANKH* gene mutations
   b. *CCAL 1* gene mutations (secondary to non-dysplastic OA)
5. Meniscectomy (for knee CC)
1.5 Classification of CPPD

1.5.1 Introduction

In the early 1960s, patients with CC were classified into distinct sub-groups based on their dominant clinical features (McCarty 1966b; McCarty 1976). This classification was influenced by early descriptions of patients with florid CC and severe arthropathy (Figure 3) (McCarty et al 1963; Zitnan et al 1963). This classification (McCarty’s classification) although complex, and reliant on the severe and rare manifestations of CPPD effectively illustrates the heterogeneity of clinical features associated with CPPD (McCarty 1976).

Figure 3 Severe knee osteoarthritis with chondrocalcinosis

Legend: Tri-compartmental knee OA associated with knee CC. The CC is better visualised in the left knee.
1.5.2 McCarty’s classification

**Type A (Pseudogout):** acute/sub-acute self limiting mono- or oligo-articular attacks of synovitis lasting 1 day to 4 weeks.

**Type B (Pseudo-rheumatoid arthritis):** seen in 5% of cases, characterised by subacute/chronic polyarticular arthropathy lasting 4 weeks to several months, with morning stiffness, fatigue, synovial thickening, localized oedema, and limitation of joint movement.

**Types C (Pseudo-osteoarthritis with superimposed acute attacks):** seen in 25% of cases. There is chronic pain, and restriction of movement with superimposed attacks of pseudogout. (Figure 3)

**Types D (Pseudo-osteoarthritis without superimposed acute attacks):** seen in 25% of cases. There is chronic pain, and restriction of movement but no acute attacks. (Figure 3)

**Type E Lanthanic (Asymptomatic) CPPD:** probably the most common type (Figure 4)

**Type F Pseudoneurotrophic:** rarely seen, but with advanced destruction of the joint on X-ray, and marked symptoms. (Figure 5)

**Other Patterns:** stiffening and straightening of the spine with true bony ankylosis (pseudo ankylosing spondylitis), and proximal stiffness with shoulder restriction (pseudo-polymyalgia rheumatica).

The apparent complexity of this system, common evolution from one form to another, and possible concurrence of different types within the same individual led to the development of a simpler ‘European’ classification (Currey 1970; Russell *et al* 1970; Doherty *et al* 1986).
Figure 4 Asymptomatic chondrocalcinosis

Legend: wedge shaped meniscal calcification in the lateral compartment of each knee. The patient has no knee pain or radiographic knee osteoarthritis.

Figure 5 Pseudo-neuropathic arthritis

Legend: attrition, and gross deformity in both knees.
1.5.3 European classification
An important step in simplifying the nomenclature of CPPD was use of the term ‘acute pyrophosphate arthropathy’ for pseudogout, and ‘chronic pyrophosphate arthropathy’ for the chronic arthropathy associated with CPPD (Currey 1970). At about the same time, Russell et al. used ‘pyrophosphate arthropathy’ for both CC and pseudogout (Russell et al 1970). Both nomenclature systems are flawed. While there is no place for asymptomatic CC in Currey’s system, Russell et al. merged acute and chronic arthropathies into one category (Currey 1970; Russell et al 1970). Therefore, Doherty and Dieppe proposed a more inclusive ‘European’ classification (Doherty et al 1986):

- **chondrocalcinosis** - asymptomatic calcification of articular fibro- or hyaline cartilage identified by imaging or histology;

- **pyrophosphate arthropathy** - structural abnormality of cartilage and bone (cartilage loss, osteophyte, cysts – i.e changes of OA) together with any evidence of intra-articular CPP crystal deposition;

- **pseudogout** - the clinical syndrome of acute synovitis associated with the shedding of intra-articular CPP crystal deposits.
1.5.4 EULAR classification
In order to promote a uniform nomenclature, recently, a EULAR Task Force has suggested a simpler terminology and classification system (Zhang W et al 2011) (Table 7).

**Table 7 EULAR nomenclature of calcium pyrophosphate deposition**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium pyrophosphate crystal</td>
<td>simplified term for calcium pyrophosphate dihydrate crystals</td>
</tr>
<tr>
<td>CPP deposition (CPPD)</td>
<td>umbrella term for all instances of occurrence of CPP crystals</td>
</tr>
<tr>
<td>Chondrocalcinosis(^1)</td>
<td>cartilage calcification, identified by imaging or on histological examination</td>
</tr>
</tbody>
</table>

**Clinical presentations associated with CPPD:**

- **Asymptomatic CPPD**
  CPPD with no apparent clinical consequence i.e. isolated CC

- **OA with CPPD**
  CPPD in a joint that also shows changes of osteoarthritis (OA), on imaging or histological examination

- **Acute CPP crystal arthritis**
  acute onset, self-limiting synovitis with CPPD (previously pseudogout)

- **Chronic CPP crystal inflammatory arthritis**
  chronic inflammatory arthritis associated with CPPD

\(^1\) This is usually, but not always due to CPPD.
1.5.5 Diagnostic criteria
McCarty defined CPPD as definite, probable or possible depending on the clinical features, radiographic and laboratory findings (McCarty 1977) (Table 8).

<table>
<thead>
<tr>
<th>Table 8 McCarty's criteria for calcium pyrophosphate deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Demonstration of CPP crystals from joint aspirate or biopsy, by X-ray diffraction pattern</td>
</tr>
<tr>
<td>2. Identification of crystals in synovial fluid showing absent or weakly positive birefringence on compound polarized microscopy</td>
</tr>
<tr>
<td>3. Typical radiographic appearance of CC</td>
</tr>
<tr>
<td>4. Clinical features of acute or chronic synovitis</td>
</tr>
</tbody>
</table>

Definite CPPD 1, or 2 and 3; probable CPPD 2 or 3
1.6 Radiographic features

1.6.1 Radiographic chondrocalcinosis
On plain radiographs, articular CC is seen as a fine linear calcification of hyaline articular cartilage, parallel to and distinct from the underlying articular surface, or more commonly as a dense punctate calcification affecting the middle layers of the fibrocartilage (Genant 1976) (Figure 1, Figure 3, Figure 4). This is because CPP crystals deposit preferentially in the middle layer of fibro-cartilage and hyaline articular cartilage (Johnson et al 2003; Grassi et al 2006; Muehleman et al 2008). CC occurs at the articular fibrocartilage more often than the hyaline cartilage (McCarty and Haskin 1963; Twigg et al. 1964; Moskowitz and Katz 1967; Martel et al. 1970; Genant 1976).

CPPD can also occur in tendons, ligaments, synovial membrane, joint capsule bursae, and soft tissues (Genant 1976; Doherty 2003) (Figure 1). Capsular and tendinous calcifications tend to be linear (Genant 1976). On the other hand, synovial CPPD appears as irregular radiodense foci which may simulate intra-articular loose body, or synovial osteochondromata (Genant 1976). Interestingly, non-articular cartilage CPPD, and some synovial membrane CPPD occur in areas of chondroid metaplasia, emphasising the importance of a cartilage milieu for CPPD (Ishikawa et al 1989; Beutler et al 1993; Ishida et al 1995).
1.6.2 Joints affected by chondrocalcinosis

Early hospital based studies identified the joints affected by CC in both sporadic and familial disease (McCarty et al 1963; Zitnan et al 1963; Twigg et al 1964; Currey et al 1966; Moskowitz et al 1967; Reginato et al 1970; Bjelle et al 1974; Resnick et al 1974; Ellman et al 1975) (Table 9). These studies demonstrated that CC is usually bilateral and that familial CC associates with florid widespread involvement of symphysis pubis, elbow, shoulder, MCPJs, and ankle which are less commonly affected in sporadic CC (Table 9).

Table 9 Distribution of calcification in chondrocalcinosis

<table>
<thead>
<tr>
<th>Joint</th>
<th>Sporadic</th>
<th>Familial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bilateral</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Knee-meniscus</td>
<td>57-81%</td>
<td>10-36%</td>
</tr>
<tr>
<td>Knee-hyaline</td>
<td>35-48%</td>
<td>5-22%</td>
</tr>
<tr>
<td>Wrist</td>
<td>26-50%</td>
<td>5-26%</td>
</tr>
<tr>
<td>Symphysis pubis</td>
<td>24-44%</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>6-44%</td>
<td>5-21%</td>
</tr>
<tr>
<td>Shoulder</td>
<td>12-34%</td>
<td>0-24%</td>
</tr>
<tr>
<td>Elbow</td>
<td>12-37%</td>
<td>5-35%</td>
</tr>
<tr>
<td>MCP</td>
<td>0-15%</td>
<td>3-17%</td>
</tr>
<tr>
<td>Ankle</td>
<td>0-7%</td>
<td>0-7%</td>
</tr>
<tr>
<td>MTP</td>
<td>6-7%</td>
<td>3-12%</td>
</tr>
</tbody>
</table>

% of cases with chondrocalcinosis at a particular joint, adapted from Genant 1976.

Two subsequent hospital based radiographic surveys with 85 (Resnick et al 1977a), and 105 (Dieppe et al 1982) patients with symptomatic CPPD have studied the relative frequency of joints affected by CC (Figure 6). Other authors have also reported on the pattern of joints affected by CC in hospital based populations (Moskowitz et al 1973; Fam et al 1981; Wilkins et al 1983; Gordon et al 1984; Louthrenoo et al 1999; Canhao et al 2001).
Figure 6 Frequency of joints affected by chondrocalcinosis in two hospital based studies

% of cases with chondrocalcinosis who have involvement at a joint

- MTPJ
- MCPJ
- Ankle
- Shoulder
- Hip
- Elbow
- Wrist
- Symphysis pubis
- Knee

Dieppe et al.
Resnick et al.
1.6.3 Chondrocalcinosis at individual joints

1.6.3.1 Knee
The following are the key radiographic appearances of knee CC (Figure 7):

- predominantly occurs at the fibro-cartilaginous meniscus (McCarty et al 1963; Twigg et al 1964; Moskowitz et al 1967; Martel et al 1970; Genant 1976)
  - meniscal CC
    - appears as linear or punctate calcification (Genant 1976)
    - may be fine or dense, and commonly produces:
      - a wedge shaped calcification at the medial or lateral joint margins (Genant 1976), or
      - a Y shaped radiodensity within the joint, above the tibial plate (McCarty et al 1963).
    - more frequent in the lateral than in the medial meniscus (Bocher et al 1965; Skinner et al 1969).
  - hyaline articular cartilage calcification appears as a fine linear density parallel to the subchondral bone with a radiolucent band separating it from the articular cortex (Twigg et al 1964; Genant 1976).

- calcification of the synovial lining of the knee may occur and may be pronounced enough to be visible as osteochondromata, which commonly occur at the knee (McCarty et al 1963)
1.6.3.2 Wrist and hand
The following are key features of radiographic appearance of CC at the wrist and hand (Figure 8):

- appearance of fibro- and hyaline cartilage CC is similar to that described for the knee (section 1.5.3.1)
  - triangular fibrocartilage is the preferred site of CC with 70.0% - 98.0% of affected wrists having calcification at that site (Twigg et al 1964; Resnick et al 1974; Resnik et al 1983; Chen et al 1990; Yang et al 1995; Taniguchi et al 1997; Donich et al 2000);
  - the luno-triquetral ligament is the second most commonly affected site, affecting 54.0% - 77.0% wrists with CC (Yang et al 1995; Taniguchi et al 1997);
one study reported a higher prevalence of CC at the luno-triquetral ligament (77.0%) than at the triangular cartilage (74.0%) (Yang et al 1995);

other sites commonly affected by CC include the articular disc of the inferior radio-ulnar joint (McCarty et al 1963; Genant 1976) and the radio-carpal joints (Skinner et al 1969);

28.0% of inferior radio-ulnar joints (Yang et al 1995), and 20.0% - 31.9% of radiocarpal compartments are reported to show CC (Resnik et al 1983; Taniguchi et al 1997; Donich et al 2000);

CC may also occur at the midcarpal, trapezio-scaphoid, scaphoid-capitate, scapho-lunate, 1st and common carpometacarpal joints (Twigg et al 1964; Resnik et al 1983; Taniguchi et al 1997; Donich et al 2000).

Figure 8 Wrist chondrocalcinosis

Legend: calcification of the triangular fibrocartilage in both wrists

63
At the MCPJs, capsular, and synovial calcification are more common than cartilage calcification (Genant 1976)(Figure 9).

**Figure 9 MCPJ calcification**

*Legend*: linear capsular calcification (arrow-upper panel), and cloudy synovial calcification (arrow-lower panel)
1.6.3.3 Pelvis
The following are the key features of radiographic appearance of CC at the pelvis (Figure 10):

- symphysis pubis calcification appears as a thin linear calcification in the middle portion (McCarty et al 1963)
- at the hip, hyaline cartilage involvement appears as a curvilinear calcification parallel to the subchondral bone (McCarty et al 1963; Genant 1976)
- fibrocartilaginous acetabular labrum calcification appears as a mottled appearance at the joint margin (McCarty et al 1963; Genant 1976).

Figure 10 Hip chondrocalcinosisis and symphysis pubis calcification
Legend: mottled acetabular labrum calcification (left panel), thin linear calcification in the middle portion of the symphysis pubis (right panel)
1.6.3.4 Other sites

Shoulder: The appearance of CC at the shoulder resembles that at the hip (Genant 1976). There may be linear hyaline cartilage calcification (Figure 11), or spotty calcification of the glenoid labrum. The fibrocartilaginous discs of the acromioclavicular and sternoclavicular joints (Twigg et al 1964), and the acromioclavicular joint capsule (McCarty et al 1963) may also be involved.

![Figure 11 Hyaline cartilage calcification at the shoulder](image)

Figure 11 Hyaline cartilage calcification at the shoulder

Elbow: Linear calcification affecting the hyaline cartilage, and the joint capsule are common manifestations (Genant 1976). The hyaline cartilage calcification may have a triangular appearance in anteroposterior (AP) radiographs (Twigg et al 1964).

Foot and ankle: Calcification of ankle articular cartilage (McCarty et al 1963), talonavicular and cuneonavicular (Twigg et al 1964) joints have been reported.
**Spine:** Although based on small hospital based case series, it has been suggested that when present spinal CC preferentially occurs in the annulus fibrosus (McCarty *et al* 1963), and it seldom affects the nucleus pulposus except in familial CC (Genant 1976) (Figure 12).

![Figure 12 Chondrocalcinosis at the spine](image-url)
1.6.4 Structural changes in joints with chondrocalcinosis
Although a detailed discussion of OA is not in the scope of this thesis, it is necessary to briefly revisit the concept of OA before discussing the phenotype of structural changes in joints with CC.

1.6.4.1 Osteoarthritis
OA is defined as a usually progressive disease of synovial joints that represents failed repair of joint damage which results from stresses that may be initiated by an abnormality in any of the synovial joint tissues including articular cartilage, subchondral bone, ligaments, menisci, peri-articular muscles, peripheral nerves, or the synovium (Lane et al 2011). This process may be localised to a single joint, to a few joints or be generalised (Lane et al 2011).

The structural changes of OA include joint space narrowing, osteophytes, sclerosis, cysts, and attrition. Some patients have no or minimal symptoms attributed to OA in the presence of established structural changes and vice versa. This suggests that there is an imperfect overlap between the disease OA (structural changes visualised on imaging studies), and the illness OA (patients reported symptoms of OA) (Lane et al 2011). This becomes an important consideration in the definition of OA which can be based on the presence of radiographic changes, or symptoms, or both symptoms and radiographic changes (Lane et al 2011).

Any attempt to define OA is further complicated by the fact that radiographic techniques have varying sensitivity to demonstrate structural changes of OA. Therefore, the population defined as having OA may change according to the radiographic technique used. Moreover, the population
defined as having OA also depends on the structural radiographic change selected to define OA. For example, presence of either osteophytes, joint space narrowing, or both may be used to define OA, with implications for the population defined as having OA (Altman et al 1995, Kellgren et al 1957).

Whichever way OA is defined, OA at different joints may be distinct conditions rather than a single disease entity (Bierma-Zeinstra et al 2011). For example, knee OA, hand OA, and hip OA have differing risk factors, and even in one area there is difference between different joints e.g. thumb base OA and nodal interphalangeal OA; isolated patello-femoral joint OA and multi-compartment knee OA (Bierma-Zeinstra et al 2011). Similarly, OA at a joint may occur on the background of a generalised predisposition or be an isolated phenomenon, with presence of a generalised predisposition suggesting a worse prognosis (Bierma-Zeinstra et al 2011).

Apart from this, several structural OA subtypes have been described e.g. atrophic OA and hypertrophic OA; erosive OA and non-erosive OA; and OA with or without radiographic CC (Bierma-Zeinstra et al 2011). It is not yet known whether they reflect a continuum of severity or suggest different underlying risk factors. Atrophic OA is characterised by severe joint space narrowing in the absence of any osteophytosis or with a few small osteophytes. On the contrary, hypertrophic OA is characterised by florid osteophytosis with less severe joint space narrowing. However, the atrophic and hypertrophic OA phenotypes are relatively uncommon especially at the knees. In a population based study from Framingham, USA, the atrophic phenotype was present in 4.9% knees with radiographic OA, and the
hypertrophic phenotype was present in <1.0% knees with radiographic OA defined as Kellgren and Lawrence score ≥2 (Roemer et al 2012).

1.6.4.2 Phenotype of structural arthropathy associated with chondrocalcinosis

Early reports of CC were in patients with severe degenerative joint disease (McCarty et al 1963; Zitnan et al 1963). For example, in an early report, 14 of 15 (93%) knee joints with radiographic CC had structural arthropathy, which was severe in 4, moderate in 4, and mild in 6 cases (McCarty et al 1963). Even in later reports, 11 of 19 (58%) cases with CC had severe structural arthropathy at the affected joints (Martel et al 1970). This led to the impression that CC associates with severe degenerative arthropathy.

Also, patients with CPPD + OA were reported to have an unusual location of joint involvement compared to those with OA alone. Apart from the knee, which is commonly involved in OA with or without co-existent CPPD, structural changes were reported to be more common at the MCPJs, radio-carpal compartments, trapezio-scaphid joint, shoulder, elbow, and ankle in those with CPPD + OA (Figure 13) (Martel et al 1970; Watt 1983). In a case control study, 8 of 11 cases with CPPD and structural changes of OA had MCPJ arthropathy compared to 1 of 25 cases with OA without CPPD (Martel et al 1970). Such an arthropathy predominantly affecting the MCPJs and sparing the inter-pahalangeal joints (IPJs) had been previously reported in a patient with pseudogout (Janower 1964).

A few studies have examined the association between CC at any joint, and OA at distant joints.
• In a hospital based case-control study, patients with CPPD had significantly greater overall structural changes at the MCPJs (mean (S.D.) score 4.62 (3.53)) than primary OA (mean (S.D.) score 3.60 (3.29)), p<0.05, Mann-Whitney U test; and had significantly less overall structural changes at the trapezio-metacarpal joint (mean (S.D.) score 5.39 (5.36)) than primary OA (mean (S.D.) score 7.15 (5.55)), p<0.03, Mann-Whitney U test (Riestra et al 1985).

• In another hospital based case control study, wrist arthropathy, and MCPJ arthropathy was more common in those with generalised OA (GOA – defined as OA at ≥6 joints) and CPPD at ≥2 joints than in those with GOA alone (Bourqui et al 1983).

• ST joint OA without concomitant OA of the 1st CMCJ was reported to be common in CPPD (Peter et al 2001).

• In a community based study, CC predominatly at the knees (96%) associated with OA in the 1st - 3rd MCPJs in either hand (age adjusted OR (95%CI) 3.1 (1.1-8.8)) (Sanmarti et al 1996).

• In a primary care based case-control study, CC at the knees (with or without wrist CC) increased the risk of OA in the MCPJs and inter-carpal joints (Al-Arfaj 2002b). The age and gender adjusted ORs (95% CI) were 3.27 (1.44-8.93), 6.92 (1.99-25.54) and 5.69 (1.14-29.7) for 2nd MCPJ, 3rd MCPJ, and inter-carpal joint respectively (Al-Arfaj 2002b).
Therefore, it has been suggested that the presence of CC may alter the distribution of OA. This suggests that the occurrence of CC may be related to a ‘generalised effect’ rather than a joint specific ‘local effect’. However, the association between CC at one site on OA at distant sites has not been examined in a large study.

Figure 13 Radiocarpal and MCPJ arthropathy associated with chondrocalcionosis

*Legend:* MCPJ narrowing, osteophytes, sclerosis; and wrist sclerosis, cysts, attrition associated with MCPJ calcification, and wrist chondrocalcinosis.

Not only was structural change reported to be common in joints with CC, and to be present at different locations than in those with isolated OA, joints with CC and structural arthopathy were reported to have an unusual appearance characterised by more subchondral sclerosis (Figure 3), marginal osteophytes (Figure 3), and numerous discrete subchondral cysts (Figure 13) making them distinct from OA without CC (Martel et al 1970).
Several subsequent reports also suggest that the radiographic appearance of structural arthropathy associated with CPPD is distinct from that of OA without CC (Genant 1976; Resnick et al 1977a; Martel et al 1981). According to these reports, the key distinguishing features are:

1. **A different distribution of joint involvement:** Along with weight bearing joints (e.g. knees), non-weight bearing joints like the glenohumeral, wrist, MCP, and elbows joints also had structural changes of OA in those with CC (Genant 1976; Resnick et al 1977a; Martel et al 1981).

2. **A different distribution of involved compartments in the affected joints:** Structural changes of OA were common at the patello-femoral (Figure 3), radio-carpal (Figure 13), and MCPJ (Figure 13) in those with CPPD (Genant 1976; Resnick et al 1977a; Dieppe et al 1982). On the contrary, 1st CMCJ involvement was reported to be less common (Genant 1976). Structural changes at the patello-femoral joints (PFJs) were frequently reported to occur in the absence of any tibio-femoral joint (TFJ) involvement (Figure 14) (Dieppe et al 1982).
Figure 14 Patello-femoral joint osteoarthritis associated with knee chondrocalcinosis

Legend: Left knee CC, with isolated patella-femoral joint OA

3. Frequent subchondral cysts with CPPD: The subchondral cysts noted were multiple, large, present at many joint sites, and usually had sclerotic margins (Figure 15) (Resnick et al 1977a,b; Martel et al 1981; Watt 1983). In some instances, the subchondral cysts were arranged in a ‘string of beads pattern’ (Martel et al 1981). Subchondral sclerosis was also reported to be common (Dieppe et al 1982).
Figure 15 Subchondral cyst in hip osteoarthritis associated with chondrocalcinosis at distant joints

4. Severe joint destruction manifesting as:

   a. Rapidly evolving suchondral erosive arthropathy affecting both weight bearing, and non-weight bearing joints (Genant 1976).

   b. Fragmentation of subchondral bone (Genant 1976), with collapse of articular surfaces (Martel et al. 1981), resulting in the formation of intra-articular loose bodies which may mimic neuropathic arthropathy without any gross neurological involvement – the so called pseudo-neuropathic arthropathy (Resnick et al. 1977a; McCarty et al. 1963; Watt 1983) (Figure 16).
Pseudo-neuropathic arthropathy was first reported by McCarthy et al in 1963, and reported to affect both non-weight bearing, and weight bearing joints. It was reported to lead to pronounced joint destruction over a relatively short period of time. Several subsequent hospital based case series suggested that such an arthropathy is common in patients with CPPD + OA at the knee or the hip (Genant 1976; Fam et al 1981; Martel et al 1981). However, Dieppe et al were unable to separate pseudo-neuropathic arthropathy as a distinct person specific clinical entity in a larger case series of over 100 patients with CPPD + OA (Dieppe et al 1982).

The pathophysiology of this condition is not well understood. Local joint instability has been proposed to precede the pseudo-neuropathic arthropathy, with the instability resulting in mechanical damage and crystal deposition (Dieppe et al 1982).

5. Variable osteophytosis: In some patients, irregular large bony excrescences were present (Figure 17, Figure 18) (Dieppe et al
1982), while in others joint space narrowing, subchondral sclerosis, and bony fragmentation occurred with minimal or no osteophytosis (Figure 16) (Resnick et al. 1977a; Watt et al. 1983a). However, different radiographic phenotypes can be present in the same person (Figure 18).

Figure 17 Knee chondrocalcinosis with osteophytosis and joint space narrowing
Figure 18 Knee chondrocalcinosis with marked joint space narrowing and minimal osteophytosis

Legend: Right knee: joint space narrowing without osteophytes. Left knee: joint space narrowing, remodelling, subchondral sclerosis, and marked osteophytes

The association between CC and structural change seems to be at least in part a joint specific phenomenon. For example, in those with knee CC, the additional presence of wrist CC associates with structural arthropathy at the wrist (Taniguchi et al 1997).

CC at knees, hips or symphysis pubis may associate with more severe global structural changes of OA at knees but not at hips (Musacchio et al 2011). In a recent large case-control study of healthy community dwelling older people from Southern Italy, participants with CC at knees, hips or symphysis pubis had a greater summated global knee OA radiographic
severity score than those without CC (Musacchio et al 2011). In this study, right and left knees were analysed separately and osteophytosis, joint space narrowing, subchondral sclerosis and malalignment at the TFJs were summated to yield a global radiographic features score ranging from 0-24 for each knee (Musacchio et al 2011). The mean radiographic features score at the knees were: right knee 5.7 when CC was present, and 3.8 when CC was absent (age-adjusted p<0.0001); and left knee 5.1 when CC was present and 3.4 when CC was absent (age-adjusted p<0.0001) (Musacchio et al 2011). The differences were still statically significant when the population was stratified by gender (age-adjusted p=0.003) (Musacchio et al 2011). Interestingly, the radiographic features score was worse in the right knee than in the left knee both for participants with and without CC (p=0.001) (Musacchio et al 2011).

As shown previously, there appears to be no association between CC and the severity of radiographic features at either hip. The mean radiographic features score at the hips were: right hip 2.5 when CC was present, and 2.2 when CC was absent (p>0.05); and left hip 2.6 when CC was present, and 2.3 when CC was absent (p>0.05) (Musacchio et al 2011). An analysis of association between CC and OA at the same joint was not carried out in this study. However, the greater knee OA radiographic features score in those with CC reported in this study could be explained by the association between CC and knee OA, and the lack of association between CC and hip OA.
Nevertheless, not all studies support the view that CC associates with a different OA phenotype.

- In a hospital based case control study of 21 cases with knee OA and CC, and 21 age matched controls with knee OA alone, osteophytosis, joint space narrowing, subchondral sclerosis, and subchondral cysts were no more common in knee joints with CC + OA than in knee joints with OA alone (Hansen et al 1984). However, according to this study, joints with CC + OA were more likely to have condylar flattening, anterior femoral cortical erosion, and supra-patellar effusion (Hansen et al 1984).

- Similarly, in a hospital based study of 74 symptomatic CPPD cases, and 68 age and gender matched OA controls, CPPD did not associate with greater overall radiographic scores (Riestra et al 1985).
  - In this study, the mean (S.D) overall summated score of individual radiographic changes of OA was 4.48 (4.66) in those with CPPD, and 4.31 (3.21) in those with OA alone, p>0.05 (Riestra et al 1985).
  - Severe lower limb arthropathy was no more common in those with CPPD than in those with OA alone, occurring in 5 patients with CPPD, and in 4 patients with OA alone (Riestra et al 1985).
  - Also, there was no difference in the overall severity of joint space narrowing in those with CPPD and in those with OA.
alone except at the trapezio-metacarpal joint where participants with CPPD had less severe joint space narrowing than those with primary OA (Riestra et al 1985). The mean grade of joint space narrowing at the trapezio-metacarpal joint in those with CPPD was 2.00 (1.83), whereas the mean grade of joint space narrowing in those with primary OA alone was 2.63 (1.73), p<0.035 (Riestra et al 1985).

![Wrist chondrocalcinosis with trapezio-scaphoid arthropathy](image)

Figure 19 Wrist chondrocalcinosis with trapezio-scaphoid arthropathy

Legend: Trapezio-scaphoid arthropathy (sclerosis and joint space narrowing) with relative sparing of the trapezio-metacarpal joint associated with bilateral wrist chondrocalcinosis.

- In a prospective study, the presence of CPP crystals did not associate with progressive joint space loss, osteophytosis, sclerosis and cyst formation in those with knee OA observed for 2 years (Ledingham et al 1995).

- In a community based prospective study with follow-up of between 15 months to 3 years, the presence of knee CC was shown to protect against or to have no effect on progressive joint space loss in community dwelling adults with symptomatic radiographic knee OA
((aRR (95%CI)) 0.4 (0.2-0.7) Boston OA Knee study and 0.9 (0.6-1.5)) Health ABC Study) (Neogi et al 2006).

One study suggests that the greater osteophytosis and more severe joint space narrowing observed in joints with CPPD is limited to joints with co-existent CPP and BCP crystals (Halverson et al 1986). In this case control study of patients presenting to a rheumatology clinic with knee pain, the extent of knee OA changes correlated with the presence of BCP crystals. In joints with no crystals in the synovial fluid aspirate and in joints with CPP crystals alone, the prevalence of three compartment osteophytosis was 26.3% and 12.5% respectively, whereas joints with BCP crystals alone or joints with co-existent BCP and CPP crystals had a higher prevalence of three compartment osteophytosis – 78.6% and 72.2% respectively. In this study, none of the joints with BCP crystals alone had CC, while CC was present in 87.5% of patients with CPP alone and in 77.8% of patients with both CPP and BCP crystals (Halverson et al 1986). However, this reported association between OA and BCP crystals may be due to the fact that intra-articular BCP crystal deposition is integral to the development of end-stage OA (Fuerst et al 2009a; Fuerst et al 2009b).

In summary, the published evidence to date does not convincingly support or refute the notion that CPPD associates with a specific radiographic phenotype which is distinct from that observed in OA alone at any joint.
1.6.4.2 Chondrocalcinosis and joint destruction
Apart from the three studies discussed previously (Genant 1976; Resnick et al 1977a; Martel et al 1981), other hospital based studies also suggest that CC associates with a rapidly destructive arthropathy (Figure 3, Figure 5, Figure 20).

![Image](image_url)

**Figure 20 Destructive arthropathy associated with chondrocalcinosis**
For example, in those with GOA, CC was reported to associate with destructive arthropathy at large joints.

- In a case control study, 46.4% participants with GOA (defined as OA at ≥6 joints) and CC at 2 joints, and 7.0% participants with GOA alone had large joint destructive arthropathy (p<0.001) (Gerster et al 1975a). In another case control study, 28.8% participants with GOA (defined as OA at ≥6 joints) and CC at 2 joints, and 7.5% participants with GOA alone had large joint destructive arthropathy (p<0.01) (Gerster et al 1975b). Non weight-bearing joints like shoulders, elbows and wrists also showed changes of joint destruction (Gerster et al 1975b).
Similarly, in a hospital based study, CC was reported to associate with rapidly progressive OA.

- 28 of 86 hip OA patients with rapid destruction (>10mm upward migration of femur over 1 year) and 16 of 86 hip OA patients without rapid destruction of the hip had CC at 2 joint sites (p<0.05) (Menkes et al 1985).

Also, CPP crystals were reported to associate with attrition in knee OA (Figure 3, Figure 5, Figure 20).

- CPP crystals associate with attrition in knee OA over a 2 year period (aOR (95%CI) : 2.41 (1.33-4.39)) (Ledingham et al 1995).

Several other reports also suggest that there is a high prevalence of destructive arthropathy in those with structural arthropathy and CPPD.

- 6.3% (5/80) patients with idiopathic CPPD seen at a single hospital had destructive arthropathy at the hips, knees, spine, hands, and shoulders (Richards et al 1974) (Figure 20).

- 13.5% (15/113) patients with CC diagnosed over 3 years had destructive arthropathy (Menkes et al 1976). Destructive arthropathy was more common in women, and in the elderly (Menkes et al 1976).

- 35.6% (16/45) patients with 'pseudogout' had destructive OA changes, with large subchondral bony cysts, subchondral
collapse and fragmentation, large osteophytes, and intra-articular osteochondromata (Fam et al 1981). (Figure 20, Figure 3)

- Three cases with destructive wrist arthropathy in patients presenting with acute CPP crystal arthritis were reported (Smathers et al 1982).

A high proportion of patients with CC and destructive arthropathy have been reported to have destruction of non-weight bearing joints. For example, of the 15 patients with CC and destructive arthropathy destructive changes were seen in knees, shoulders, hips, and wrists in 12, 12, 7, and 4 participants respectively (Menkes et al 1976). Similarly, of the five patients with idiopathic CPPD and destructive arthropathy, destructive changes were present at the shoulders, and wrists in 2 patients each (Richards et al 1974).

However, other case-control studies do not show any association between CPPD and more severe arthropathy (Riestra et al 1985). Similarly, the two studies to prospectively examine the association between knee CC and radiographic progression of knee OA did not find any convincing evidence of association between CC and progressive joint space loss (Ledingham et al 1995; Neogi et al 2006). One of these studies also reported a lack of association between knee CC at baseline, and progressive osteophytosis, sclerosis, and cyst formation in knee OA (Ledingham et al 1995). These studies suggest that although a subset of OA patients with CC have destructive arthropathy, but CC in itself does not worsen the outcome of OA.
1.6.5 Radiographic phenotype of osteoarthritis in joints with chondrocalcinosis

1.6.5.1 Knee

*Number of compartments affected by structural arthropathy:*

Only a few studies have examined the number of knee compartments with structural changes in those with CPPD + knee OA compared to knee OA alone. In a hospital based cross-sectional study of symptomatic knee OA, the presence of CPP crystals associated with multi-compartment knee OA (aOR (95%CI) 3.31 (1.61-6.77)) (Ledingham *et al* 1993a), and with bi- or tri-compartmental involvement (Pattrick *et al* 1993), but not with uni-compartmental OA (Pattrick *et al* 1993).

However, not all studies report that knee CC associates with structural changes in multiple knee compartments. For instance, in a hospital based case control study, tri-compartmental structural changes were significantly less common in knees with CPPD + OA than in knees with OA alone (Resnick *et al* 1977a). Similarly, in a hospital based case series of 50 cases of knee OA + CPPD, 70.7% of knees had uni-compartmental OA, 25.9% had bi-compartmental OA, and tri-compartmental OA changes were particularly rare, occurring in only 2 (3.4%) knees (Bjelle *et al* 1974).

*Location of knee compartment affected by structural changes in CPPD*

Several studies have examined the compartmental location of structural changes in knees with OA + CPPD. Early studies suggested that structural changes are more common at the PFJs than at the TFJs.
In a hospital based study of 50 cases with knee CC plus OA, OA changes were more common in the PFJs (62.1%) than in the medial TFJ compartment (51.7%) (Bjelle et al 1974). However, isolated PFJ OA (36.2%) was only slightly more common than medial compartment TFJ OA (34.5%) (Bjelle et al 1974).

In another hospital based case control study, isolated PFJ OA was more common in joints with CPPD + OA than in joints with OA alone (Resnick et al 1977a). Isolated PFJ OA occurred in 47 of 116 knees with CPPD and knee OA, compared to 4 of 52 knees with knee OA alone (Resnick et al 1977a). Isolated PFJ OA was significantly more common than medial TFJ OA in those with CPPD plus OA at the knee (Resnick et al 1977a) (Figure 14).

Several subsequent studies have examined the compartmental localization of OA in knees with CPPD (Table 10). A predisposition to involvement of any knee compartment was not observed in two case control studies (Hansen et al 1984; Riestra et al 1985), and in one cross-sectional study (Neame et al 2003), whereas another cross sectional study reported that TFJ OA, but not PFJ OA associates with knee CC (Ledingham et al 1993a). Also, in a cross-sectional study examining the risk factors for TFJ and PFJ OA, the prevalence of CC in isolated TFJ OA, isolated PFJ OA, and combined TFJ and PFJ OA was similar i.e. 17.9%, 21.9%, and 20.0% respectively (McAlindon et al 1996).

Some studies suggested that knee CC associates with structural changes in the lateral TFJ compartment. In a cross-sectional study, knee CC
associated with osteophytosis in the lateral aspect of the knee joint, that is osteophytes in the lateral femur, lateral tibia, lateral patella and lateral trochlea (aOR (95%CI) 4.38 (1.45-13.2), 28.6 (3.69-221.9), 2.77 (1.09-7.04) and 4.71 (1.85-12.0) respectively) (Nagaosa et al 2002) but not with osteophytosis in the medial aspect of the knee joint. In a primary care based case-control study, presence of CC at the knees (with or without wrist CC) increased the risk of OA in the lateral tibio-femoral compartment of the knee (Al-Arfaj 2002b) (Figure 21). The age and gender adjusted OR (95% CI) was 10.59 (3.47-34.9) (Al-Arfaj 2002b). However, such a predisposition has not been reported in two other studies (Ledingham et al 1993a; Sanmarti et al 1996). Therefore, based on the published literature, no firm conclusion can be drawn as to whether any particular knee compartment is affected more frequently in those with CPPD + OA at the knee.

Figure 21 Atrophic lateral tibio-femoral compartment osteoarthritis associated with knee chondrocalcinosis
Table 10 Association between chondrocalcinosis and compartmental location of knee osteoarthritis

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Lateral TFJ</th>
<th>Medial TFJ</th>
<th>PFJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neame et al 2003</td>
<td>Cross sectional</td>
<td>2.00 (1.11-3.60)¹</td>
<td>2.08 (1.38-3.12)¹</td>
<td></td>
</tr>
<tr>
<td>Ledingham et al 1993a</td>
<td>Cross sectional</td>
<td>6.19 (3.03-12.6)²</td>
<td>7.07 (1.41-35.4)²</td>
<td>No association²</td>
</tr>
<tr>
<td>Sanmarti et al 1996</td>
<td>Case control</td>
<td>5.50 (2.00-14.90)¹</td>
<td>1.90 (0.70-5.00)¹</td>
<td>Not studied</td>
</tr>
<tr>
<td>Hansen et al 1984</td>
<td>Case-control</td>
<td>No association³</td>
<td>No association³</td>
<td></td>
</tr>
<tr>
<td>Riestra et al 1985</td>
<td>Case-control</td>
<td>No difference in severity of joint space narrowing between two compartments</td>
<td></td>
<td>Not studied</td>
</tr>
</tbody>
</table>

¹aOR (95%CI) adjusted for age and gender¹, age and BMI² and age³ respectively.

**Individual structural changes in knee joints with OA+ CPPD**

At the knee, CPPD has been variously associated with greater osteophytosis, sclerosis, attrition and cystic change (Ledingham et al 1993a; Pattrick et al 1993; Neame et al 2003), but not with joint space narrowing (Ellman et al 1981b; Ledingham et al 1993a; Neame et al 2003).

Other specific radiographic phenotypes reported to associate with CPPD at the knee include:

- femoral condylar flattening (Hansen et al 1984)
- anterior femoral cortical erosion (Lagier 1974; Hansen et al 1984)
- suprapatellar effusion (Hansen et al 1984)
- femoral condyle osteonecrosis with a predisposition for the medial femoral condyle (Watt et al 1983b; Kwak et al 1999)
• cystic change in the head of fibula (Ledingham et al 1993b)

• tibial stress fractures (Ross et al 1983)

However, in one study of symptomatic radiographic knee OA, anterior femoral cortical erosions were found to be no more frequent in joints with CPP crystals than in joints without CPP crystals (Pattrick et al 1993).

1.6.5.2 Wrist
In wrists with OA + CPPD, structural arthropathy occurs at the radio-carpal, mid-carpal and common carpo-metacarpal joints (between the proximal end of the 2nd to 4th metacarpal bones and the distal row of carpal bones) in a descending order of frequency (Figure 13, Figure 19) (Resnick et al 1974). In fact, mid-carpal involvement is reported not to occur in the absence of radio-carpal involvement, and common carpo-metacarpal involvement is rare (Resnick et al 1974). Similar findings were reported in a study of 102 wrists from 51 patients with probable or definite CPPD at any site (Resnik et al 1983). Structural arthropathy manifesting as joint space narrowing was present in 21% radio-carpal, 16% trapezio-scaphoid (ST), and 14% other midcarpal (except ST) joints, and was absent at the common CMCJ (Resnik et al 1983) (Figure 13, Figure 19). Structural arthropathy was most common at the radio-scaphoid, radio-lunate, and ST joints (Resnick et al 1974; Resnik et al 1983) (Figure 13, Figure 19). However, these studies are limited by the lack of a control group.

Subtle changes in location of structural arthropathy at the wrist in those with OA + CPPD has been reported when compared to OA alone. Of note, patients with CPPD are reported to have a predilection for the structural
changes to occur at the ST joints and to spare the trapezio-metacarpal joint (Figure 19). This is based on following studies:

- The mean (S.D.) OA grade at the ST joint in CPPD + OA was higher than that in OA alone (2.63 (1.83), compared to 2.63 (1.73); p<0.035, Mann-Whitney U test) (Riestra et al 1985).

- In a hospital based case control study of cases with CPPD at any site, and controls with hand OA (IPJ or 1st CMCJ OA), the presence of grade ≥2 STJ OA associated with CPPD, aOR (95%CI) 13.8 (3.4-59.8) (Stucki et al 1999).

- In a hospital based case control study of 50 patients with CC and 100 age matched controls, STJ arthropathy was observed in 28% cases compared to 5% controls (Bensasson et al 1976). Moreover, STJ arthropathy in the absence of 1st CMCJ involvement occurred in 16% of CC cases and did not occur in any controls (Bensasson et al 1976).

- Similarly, in a hospital based case control study of 160 participants with wrist CC, and 160 age and gender matched controls, STJ arthropathy was present in 43.7% of CPPD wrists, and in 14.4% of control wrists (Donich et al 2000).

- The association between STJ OA and CPPD has been reported in another hospital-based study (Peter et al 2001). In this study structural changes in the STJ were described as ‘atrophic’ with a predominance of joint space narrowing and subchondral sclerosis but with little or no osteophytosis (Peter et al 2001).
However, a hospital based case-control study of 46 patients with GOA (OA at ≥6 joints) and CPPD at ≥2 joints, and 46 with GOA alone did not find an association between CC and STJ OA (Bourqui et al 1983).

Radiographic structural abnormalities reported to be common in wrists with CC at the index or distant joints include joint space loss, sclerosis, and cysts (Resnick et al 1974) (Figure 13, Figure 19). The joint space narrowing is reported to occur predominantly in the radio-carpal joint, particularly at the radio-scaphoid, and to a lesser extent at the radio-lunate joints (Resnick et al 1974). Sclerosis is most prominent in the scaphoid and lunate (Resnick et al 1974). Subchondral cysts are marginal, eccentric, with a sclerotic margin, and at the wrist are most common in the proximal carpal row, notably in the scaphoid, lunate and capitate (Resnick et al 1974; Donich et al 2000). Patients with CPPD plus OA have more subchondral cysts at the wrist compared to patients with OA without CPPD (mean (S.D.) grade 1.43 (1.94) versus 0.85 (1.78) in primary OA; p<0.01, Mann-Whitney U test) (Riestra et al 1985). Subchondral cysts with or without joint space narrowing were also significantly more common in those with CPPD and GOA than in those with GOA alone (Bourqui et al 1983). These were sometimes, but not always, associated with joint space narrowing (Bourqui et al 1983).

Scapho-lunate dissociation and a radiographic appearance similar to scapho- lunate advanced collapse (SLAC) may also associate with wrist CC (Resnick et al 1974; Resnick et al 1977a; Donich et al 2000). SLAC comprises joint space narrowing at the radio-scaphoid and capito-lunate joints with relative sparing of the luno-radial joint together with scapho-lunate
dissociation (horizontal distance at midpoint of scapho-lunate joint >2mm) (Resnick 1985) (Figure 22). In a hospital based case-control study of 160 wrists with CC, and 160 age and sex matched control wrists, SLAC was present in 5.9% of the CPPD wrists, and in none of the control wrists (Donich et al 2000). SLAC was reported in 9.0 - 21.0% of CPPD wrists (Resnik et al 1983; Chen et al 1990) and was present in 12 patients presenting with acute CPP crystal arthritis (Doherty et al 1993).

![Figure 22 Scapholunate advanced collapse associated with wrist chondrocalcinosis](image)

It is possible that SLAC wrist associates with CC at any site and is not a direct association of wrist CC. For example, in a hospital based case-control study from Japan, the overall prevalence of SLAC wrist in those with CC at any site (knee or wrist) was 4.7% (Taniguchi et al 1997). In these patients, the prevalence of SLAC wrist was comparable in those with (5.0%) or without (4.3%) wrist CC (Taniguchi et al 1997).
1.6.5.3 Hand
In CPPD structural changes are common at the MCPJs (Resnick et al 1974). The structural changes are reported to be more frequent at the MCPJs than at the wrist (Adamson et al 1983; Resnik et al 1983), and in one study structural changes were rare at the wrist in the absence of MCPJ arthropathy (Adamson et al 1983) (Figure 23).

![Image of MCPJ osteoarthritis associated with wrist chondrocalcinosis](image)

**Figure 23** MCPJ osteoarthritis associated with wrist chondrocalcinosis

*Legend:* Note the relative sparing of the radio-carpal joint. The trapezio-scaphoid joint shows sclerosis and loss of joint space.

The second and third MCPJs are most frequently affected with structural arthropathy (Resnick et al 1974; Adamson et al 1983; Bourqui et al 1983; Sanmarti et al 1996) (Figure 23, Figure 24). Patients with CPPD plus MCPJ OA have been variously reported to have severe subchondral sclerosis, multiple cysts, joint space narrowing, and florid osteophytosis at the MCPJs (Resnick et al 1974; Adamson et al 1983; Resnik et al 1983). Although exuberant osteophyte formation creating hook like excrescences on
the radial aspects of the metacarpal heads is associated with haemochromatotic arthropathy, these changes have been reported to also occur in joints with idiopathic CPPD plus OA (Adamson et al 1983).

![Image](image_url)

**Figure 24 MCPJ osteoarthritis associated with chondrocalcinosis**

*Legend:* MCPJ OA associated with MCPJ calcification. Note involvement of the 2nd and 3rd MCPJs, and sparing of the 4th and 5th MCPJs.

However, these observations are based on hospital based case series, and frequently lack a control group. In a hospital based case-control study, hand x-rays of 46 patients with CPPD at ≥2 joints and GOA (defined as radiographic OA at ≥6 joints) were compared with hand x-rays of 46 patients with GOA alone (Bourqui et al 1983). In this study, the additional presence of CPPD in those with GOA associated with subchondral cysts in the metacarpal head, and with MCPJ axis deviation, but did not associate with osteophytosis or joint space narrowing (Bourqui et al 1983).
Finger IPJ OA was reported to be common in early hospital based case series of CPPD patients (Resnick et al 1974). This is not surprising, as patients with structural arthropathy and symptoms are likely to be over-represented in such hospital based case series (Figure 25). However, since then, structural changes at IPJs have been shown to either associate negatively with, or have no association with CPPD. This is supported by findings from one case series (Adamson et al 1983), and several case-control studies (Bourqui et al 1983; Ledingham et al 1993a). Similarly, in a community based cross-sectional study, there was no association between knee CC and finger nodes – which are a marker of underlying IPJ OA (Rees et al 2012) - 40.6% of those with knee CC had finger nodes, compared to 35.1% of those without CC (p=0.38) (Neame et al 2003).

![Figure 25 MCPJ osteoarthritis associated with knee chondrocalcinosis](image)

**Figure 25 MCPJ osteoarthritis associated with knee chondrocalcinosis**
Legend: Note severe MCPJ OA associated with knee CC. There are OA changes at the IPJs, and 1st CMCJs, suggesting the presence of GOA.
1.6.5.4 Hip

As mentioned before, CC is uncommon at the hips. There are only a few studies of structural changes associated with CC at the hip. In a post-mortem study of 8 hips with OA + CPPD and 42 hips with OA alone, Resnick et al reported on the universal presence of multiple ‘geodes’ in joints with OA + CPPD while joints with OA alone sometimes had single cysts (Resnick et al 1977c). This study raises the possibility that in those with hip OA + CPPD, cysts would be more common than in hips with OA alone (Figure 26). However, in a hospital based nested case-control study, there was no association between subchondral cyst and hip CC (Ledingham et al 1992).

![Image: Hip osteoarthritis with multiple cysts, subchondral sclerosis, and osteophytosis associated with knee chondrocalcinosis](image)

Figure 26 Hip osteoarthritis with multiple cysts, subchondral sclerosis, and osteophytosis associated with knee chondrocalcinosis

Also, there was no association between the pattern of migration of femoral head, attrition, osteophytes and CC at any hip (Ledingham et al 1992). In another study, hips of patients with CPPD were significantly more
likely to have axial migration compared to hips with OA alone (Resnick et al 1977a,b) (Figure 27).

![Figure 27 Atrophic hip osteoarthritis associated with hip chondrocalcinosis](image)

Legend: Atrophic hip OA (left hip), chondrocalcinosis in the acetabular labrum at the right hip.

In a hospital based case-control study, hips with OA + CPPD had a milder OA phenotype (Resnick et al 1977a). In this study, there was a negative association between joint space narrowing in those with CPPD plus hip OA compared to hip OA alone. Joint space narrowing was present in 29.0% of hips with CPPD and OA and in all hips with OA alone. However, according to this study, hip CC associates with an atrophic bone response in hips with OA (Ledingham et al 1992) (Figure 27).
1.6.6. Peri-articular calcification

1.6.6.1 Tendon calcification

Ill-defined tendinous calcifications were first described in patients with CC by McCarty et al. (McCarty et al 1963). In a hospital based case series, 9 of 19 (Martel et al 1970), and 7 of 40 (Martel et al 1981) CPPD cases had tendon calcification. This was reported to be bilateral (Martel et al 1970), and to extend far from the tendon attachments (Martel et al 1981). The common sites of involvement were the tendo achilles, gastrocnemius, triceps, and supraspinatus tendons (Martel et al 1970; Martel et al 1981). Tendinous calcifications associated with CPPD is frequently linear and extensive compared to the more discrete and focal periarticular calcification seen with BCP (mainly hydroxyapatite) deposition (Watt 1983) (Figure 28).

Figure 28 Tendon and soft-tissue calcification

In those with CC, tendon calcification associates with increasing age, female gender, widespread, and dense knee CC (Kanterewicz et al 1993). Tendon calcification in CC is generally asymptomatic. However, 3 of 10
cases with CC and Achilles tendon calcification developed acute attacks of tendinitis (Gerster et al 1980). An age matched case-control study showed tendon calcification to be more frequent in those with CC and GOA than in GOA alone (Gerster et al 1977). Thirteen percent of cases with CC and GOA had tendon calcifications compared to none of the 52 cases with GOA alone (Gerster et al 1977). In another study, 30% of those with CC and OA had tendon calcification compared to 3.3% age and gender matched controls (Gerster et al 1984). In a hospital based case control study, tendo achilles calcification on US was more common in those with chondrocalcinosis than in those with OA, or healthy controls (57.9% vs. 0%) (Falsetti et al 2004). In the same study, plantar fascia calcification was more common in those with CC, than in those with OA alone (15.8% vs. 2%) (Falsetti et al 2004). Similar prevalence of tendo achilles and plantar fascia calcification were reported in a recent study (Ellabban et al 2012).

**Table 11 Prevalence of tendon calcification in chondrocalcinosis**

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>X-ray</th>
<th>Gastrocnemius</th>
<th>Quadriceps</th>
<th>Achilles</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang <em>et al</em> 1996</td>
<td>Radiology files</td>
<td>Knee</td>
<td>31.9%</td>
<td>10.1%</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>Foldes <em>et al</em> 1996</td>
<td>Knee pain</td>
<td>Knee</td>
<td>40.5%</td>
<td>Not</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>Pereira <em>et al</em> 1998</td>
<td>Radiology files</td>
<td>All available</td>
<td>20.5%</td>
<td>21.0%</td>
<td>25.5%</td>
<td>Elbow 13.8%, rotator-cuff 12.1%</td>
</tr>
<tr>
<td>Falsetti <em>et al</em> 2004</td>
<td>Hospital based</td>
<td>Feet</td>
<td>-/-</td>
<td>-/-</td>
<td>52.6%¹</td>
<td>Plantar fascia 15.8%</td>
</tr>
<tr>
<td>Kanterewicz <em>et al</em> 1993</td>
<td>Case series</td>
<td>Pelvis</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>Hip adductor 25.3%</td>
</tr>
<tr>
<td>Gerster <em>et al</em> 1977</td>
<td>Hospital based</td>
<td>Feet and knee</td>
<td>None</td>
<td>13.5%</td>
<td>13.5%</td>
<td>Plantar fascia 1.9%</td>
</tr>
</tbody>
</table>

¹30 of 57 cases on x-ray, 3 more cases identified on heel ultrasound.
1.6.6.2 Tophaceous calcium pyrophosphate deposition

Peri-articular or intra-articular CPPD may enlarge and present as a subcutaneous mass or ‘tophus’ (Ling et al 1982). Tophaceous CPPD was first reported near an IPJ in a dog (Gibson et al 1972). Similar tumoral calcified masses have been reported at the temporomandibular joint (Pritzker et al 1976), IPJ (Leisen et al 1980), and MCPJ (Ling et al 1982) in humans. Tophaceous CPPD occurs in localised areas of chondroid metaplasia, and does not associate with CC or with destructive arthropathy at other joints (Ling et al 1982).
1. 7 Pathogenesis

1.7.1 Determinants of calcium pyrophosphate crystal formation

CPP crystals form extracellularly, either in the pericellular cartilage matrix, or in the enzyme rich membrane bound extracellular organelles - the articular cartilage vesicles (ACVs) (Pritzker et al 1988; Derfus et al 1992; Ryan et al 1995). Although calcium is a constituent of CPP crystals, high calcium concentration alone has not been shown to increase CPP crystal formation (Mandel et al 1984). However, high extracellular pyrophosphate (ePPi) concentration appears to be a key determinant of CPP crystal formation (Ryan et al 1995; Terkeltaub 2001). Synovial fluid PPi is higher in joints with CPP crystals than in those with OA without CPPD, gout or rheumatoid arthritis (Altman et al 1973; Doherty et al 1996). This is a local abnormality as the plasma and urine concentrations of PPi are not raised (Pflug et al 1969; Russell et al 1970; Altman et al 1973). CPPD is also facilitated by nucleating factors and cartilage matrix changes which promote crystal nucleation and growth (Ishikawa et al 1989; Doherty 2003).

As PPi is not absorbed from the gut, nearly all PPi is produced endogenously mainly by nucleotide tri-phosphate (NTP) hydrolysis (Russell 1976). PPi is also released during synthesis of proteins, lipids, phospholipids, nucleotides, glycogen, and polysaccharides (Russell 1976). In adults, several kilograms of PPi is estimated to be produced per day (Russell 1976). However, the majority of PPi is rapidly hydrolyzed to phosphate (Pi) releasing 6.6 kcal/mmol (Russell 1976; Murray RK 1996).
1.7.2 Pyrophosphate concentration and type of crystal formed

PPI concentration regulates whether CPP or BCP crystals are formed. High PPI concentration promotes CPP crystal formation, and inhibits hydroxyapatite and other BCP crystal nucleation and growth, while lower PPI levels promote hydroxyapatite crystal formation (Cheng et al 1983; Thouverey et al 2009). In fact it is the Pi/PPI ratio that is critical to the type of crystal formed. In a chicken embryo growth plate matrix vesicle (MV) model, CPP crystals formed exclusively when the Pi/PPI ratio was <6, and hydroxyapatite crystals were produced optimally when the Pi/PPI ratio was >140 (Thouverey et al 2009). The formation of hydroxyapatite crystals was completely inhibited when the Pi/PPI ratio was <70, and the formation of CPP crystals was inhibited when this ratio was >28.4 (Thouverey et al 2009).

1.7.3 Pyrophosphate metabolism

Synovial fluid PPI is produced by both fibro- and hyaline articular cartilage chondrocytes (Ryan et al 1981). Some periarticular structures like tendon and ligaments release smaller amounts of PPI (Rosenthal et al 1993). Since PPI cannot diffuse across plasma membranes, synovial fluid PPI mainly originates from:

[1] pyrophosphohydrolysis of the phosphodiesterase I bond in the synovial fluid (extracellular) nucleotide tri-phosphate (NTP) by NTP pyrophosphohydrolase (NTPPPH) enzyme plasma cell glycoprotein 1 (PC1 – also called ENPP1) situated on the plasma membrane or in extracellular ACVs (Derfus et al 1992; Johnson et al 2001), or
transport of intracellular PPi across the plasma membrane by ANKH (ankylosis human) a multipass transmembrane protein (Pendleton et al 2002; Gurley et al 2006) (Figure 29).

The dominant source of synovial fluid PPi has not been directly studied in man. However, it is likely that ePPi levels depend more on the PPi generating activity of PC1 than on the PPi transport function of ANKH. This is supported by the fact that ePPi levels in TNAP knockout mice is completely corrected in TNAP/PC1 double knockout mice but only partially corrected in TNAP/ANK double knockout mice (Hessle et al 2002; Harmey et al 2004). Moreover while PC1 is present in MVs, ANKH is not expressed there (Harmey et al 2004).

**Figure 29 Extracellular pyrophosphate metabolism and calcium pyrophosphate crystal formation**
ePPI synthesis by NTPPPH.
Of the three NTPPPH enzymes expressed by chondrocytes, PC1 (also termed ENPP1) appears to be the only significant contributor to ePPI level (Lotz M 1995; Johnson et al 1999; Johnson 2001a; Johnson et al 2001b). Resting chondrocytes release ATP (adenosine tri-phosphate) which may be hydrolysed by PC1 producing AMP and PPI (Ryan et al 1992; Graff et al 2000). Mechanically loaded chondrocytes secrete more ATP potentially increasing ePPI levels (Graff et al 2000) (Figure 30). Cartilage intermediate layer protein (CILP) was initially thought to have NTPPPH like activity (Hirose et al 2000). However, this was not confirmed in subsequent studies (Johnson et al 2003).

Ankh in trans-cellular PPI transport
The progressive ankylosis (ANK) gene in the mouse and its human homologue ANKH encodes a multipass transmembrane protein ANK
(ANKH), which acts as a PPI transporter, allowing the elaboration of ePPI from intracellular sources (Ho et al 2000; Gurley et al 2006). ANKH has 492 amino acids, weighs 54.3 kDa and is highly conserved in vertebrates (Netter et al 2004).

The central role of ANKH and PC1 in CPPD is illustrated by a recent in-vitro study using human meniscal chondrocytes from knee OA and from non-OA knee chondrocytes (Sun et al 2010). In tissue cultures, meniscal chondrocytes from OA knees formed significantly more calcium crystals than non-OA chondrocytes, and this was associated with increased expression PC1, and ANKH genes (Sun et al 2010). Both hyaline articular cartilage and meniscal cells formed calcium crystals, but as would be expected, meniscal fibrocartilage chondrocytes formed more calcium crystals than the former (Sun et al 2010).

**PPI destruction**
ePPI is rapidly complexed to magnesium and then hydrolysed to orthophosphate (Pi) by TNAP - the main extracellular pyrophosphatase (Caswell et al 1983; Xu et al 1991; Xu et al 1994). Intracellular PPI is hydrolysed by biosynthetic enzymes like glucose-6-phosphatase and other acid phosphatases (Terkeltaub 2001). It is therefore expected that patients with hypophosphatasia have high systemic PPI levels and exhibit articular CPPD (Hamilton 1976).

**Source of elevated ePPI**
High ePPI concentration may result from increased PC-1, reduced TNAP, or increased ANKH activity (Figure 29). The situation with respect to TNAP and ANKH, however, is complex. For example, contrary to previous opinion, MV
bound TNAP generates PPi in the same way as ENPP-1 (Zhang 2005). To further complicate matters, a gain in activity of ANKH increases expression of sodium phosphate co-transporter (PiT-1) resulting in high intracellular Pi (Wang et al 2005) (Figure 31). This stimulates TNAP expression thus lowering ePPi (Wang et al 2005). Therefore, any increase in ePPi caused by increased PPi transport activity of ANKH may be reduced by this mechanism. However, these activities demonstrated in chick embryo growth plate cartilage have yet to be confirmed in adult mammalian chondrocytes (Wang et al 2005; Zhang 2005).

**Figure 31 Sodium phosphate co-transporter in pyrophosphate metabolism**

Growth factors also affect the ePPi levels (Table 12). Transforming growth factor β (TGFβ) appears to exert a greater effect in cartilage from older animals, potentially explaining why the prevalence of CPPD increases with age (Rosenthal et al 1994; Rosen F 1997; Hirose et al 2000). TGFβ also has a greater effect on fibro- than hyaline cartilage, and on deep meniscal chondrocytes than on superficial ones, potentially explaining why
fibrocartilage is affected more often than hyaline cartilage, and in fibrocartilage, the deeper layers are more likely to be affected than superficial layers (Rosenthal et al 1993).

**Table 12 Regulation of pyrophosphate metabolism**

(Abhishek et al 2010)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>J↑/↓</th>
<th>Regulatory</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1 (ENPP1)</td>
<td>↑</td>
<td>TGFβ, Ageing, Thyroid hormone, Retinoic acid*</td>
<td>IL1β, IGF1 antagonize effect of TGFβ on PC1&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>IL1β, IGF1</td>
<td>CILP-1 antagonizes effect of IGF1 on PC1</td>
</tr>
<tr>
<td>TNAP</td>
<td>↑</td>
<td>IL1β, Thyroid hormone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>TGFβ, Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td>ANKH</td>
<td>↑</td>
<td>TGFβ</td>
<td>Effect on ankh is responsible for 60% of TGFβ induced ePPi secretion</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>IGF1</td>
<td>IGF1 antagonizes effect of TGFβ</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>↑</td>
<td>TGFβ, Ageing</td>
<td>Transglutaminase also activates latent TGFβ</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>IGF1</td>
<td></td>
</tr>
<tr>
<td>CILP</td>
<td>↑</td>
<td>TGFβ, Ageing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>IGF1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>3</sup>TGFβ, *May not inhibit effect of TGFβ in chondrocytes from patients with CPPD. Glossary: IL1β (interleukin 1 beta), IGF1 (Insulin like growth factor 1), TGFβ (transforming growth factor beta)
1.7.4 **ANK(H) mutations in mice and man**

The gene responsible for progressive ankylosis in mice, described in the 1980s (Sweet 1981; Hakim 1984) was identified on the mouse chromosome 15 (Ho *et al* 2000). In mice, the autosomal recessive (*ank/ank*) progressive ankylosis phenotype shows spontaneous bony ankylosis of peripheral, and axial joints, destructive arthropathy, and osteophytosis due to hydroxyapatite deposition (Hakim 1984; Ho *et al* 2000). This results from a nonsense mutation (G to T) in an integral multipass portion of *ANK* (Ho *et al* 2000). Consequently, the ePPi concentration is low (Figure 32), leading to extensive calcification by hydroxyapatite crystals (Sweet 1981; Hakim 1984; Ho *et al* 2000).

*ANKH* mutations have different effects in men. Unlike mice, mutations in *ANKH* lead to CPPD and are inherited in an autosomal dominant manner (Gaucher *et al* 1977; Doherty *et al* 1991; Hughes 1995; Andrew *et al* 1999; Williams *et al* 2003). More importantly, these mutations are believed to confer a gain in PPi transport function leading to increased ePPi levels (Figure 32) (Pendleton *et al* 2002). It is hypothesized that chronically elevated levels of ePPi result in CPPD (Pendleton *et al* 2002). However, not all *ANKH* mutations have been shown to result in high ePPi *in vitro* and it is possible that there may be other yet undefined mechanisms (Pendleton *et al* 2002; Zhang *et al* 2005).
1.7.5 Genetics of chondrocalcinosis

Mutations in *ANKH* cause familial CPPD (Gaucher *et al* 1977; Doherty *et al* 1991; Hughes 1995; Andrew *et al* 1999; Williams *et al* 2003) (Table 14). Such familial CPPD may manifest as CC, acute CPP crystal arthritis, or as chronic CPPD arthropathy (Gaucher *et al* 1977; Doherty *et al* 1991; Andrew *et al* 1999; Williams *et al* 2003). In one UK kindred characterized by polyarticular CC without structural arthropathy, a mutation in *ANKH* also associated with recurrent infantile seizures from the age of 6 months to 6 years without subsequent mental impairment (Doherty *et al* 1991). However, there are no reports of seizures in other families with mutations in *ANKH* (Gaucher *et al* 1977; Andrew *et al* 1999; Williams *et al* 2003).

Familial CC has been reported due to mutations in other genes as well. A large US pedigree with early onset OA and CPPD with genetic defect localized to chromosome 8q (CCAL1) was reported (Baldwin *et al* 1995), but
the responsible gene has not been identified to date. In this family, CPPD appears to be secondary to severe non-dysplastic OA. Similarly, mutation in the procollagen type 2 gene (COL2A) leads to severe early OA, spondyloepiphysial dysplasia, and secondary CPPD (Netter et al. 2004).

In a study of 95 British Caucasians with apparently sporadic CC, one instance of familial CC and premature OA was identified (Pendleton et al. 2002). Affected members of this family had the E490del mutation in ANKH (Pendleton et al. 2002). In another UK based case-control study, a -4bp G to A single nucleotide polymorphism (SNP) in the 5'-UTR of ANKH associated with CPPD (OR (95%CI) for development of CPPD in homozygous state = 6.00 (2.2-16.5), p=0.0006) (Zhang et al. 2005). The mechanisms by which these mutations and the SNP may induce CPPD are described in Table 13. To date, other studies examining the association between SNP in genes of enzymes involved in PPi metabolism have shown that genetic polymorphisms in PC1 and TNAP do not associate with sporadic CC (Zhang et al. 2007).

The effects of ANKH mutations on PPi levels are conflicting. This may be because the gain-of-function mutations in ANKH are subjected to a feedback transcriptional down regulation. Moreover, the expression profiles of genes that regulate Pi and PPi are themselves changed by the Pi and PPi levels (Wang et al. 2005). Similarly, over-expression of ANKH stimulates PiT-1 leading to high intracellular Pi which in turn stimulates TNAP expression, resulting in low intracellular and extracellular PPi (Zaka 2006; Wang et al. 2009). Finally, most in vitro studies measure bulk PPi level days after
transfection with mutant ANKH. During this time other reactions may affect PPI levels, making it difficult to compare results across experiments (Zhang et al 2005; Gurley et al 2006; Zaka 2006).

Table 13 Genetic variation in ANKH associated with calcium pyrophosphate deposition

<table>
<thead>
<tr>
<th>Location</th>
<th>cDNA position</th>
<th>Nucleotide variation</th>
<th>Amino acid change (position)</th>
<th>Type of mutation</th>
<th>Role in sporadic CPPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'UTR</td>
<td>-11bp</td>
<td>C to T</td>
<td>+4 amino acid (1-4)</td>
<td>Base substitution</td>
<td>No</td>
</tr>
<tr>
<td>5'UTR</td>
<td>-4bp</td>
<td>G to A</td>
<td>NA²</td>
<td>Transition</td>
<td>Yes</td>
</tr>
<tr>
<td>Exon 1</td>
<td>+13bp</td>
<td>C to A</td>
<td>Pro to Thr (5)</td>
<td>Missense</td>
<td>Not known</td>
</tr>
<tr>
<td>Exon 1</td>
<td>+14bp</td>
<td>C to T</td>
<td>Pro to Leu (5)</td>
<td>Missense</td>
<td>No</td>
</tr>
<tr>
<td>Exon 2</td>
<td>+143bp</td>
<td>T to C</td>
<td>Meth to Thr (48)</td>
<td>Missense</td>
<td>No</td>
</tr>
<tr>
<td>Exon 12</td>
<td>+490bp</td>
<td>GAG del</td>
<td>Glu deletion (490)</td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>

¹c DNA posn.1: ATG initiation codon, ²not applicable

Table 14 Mechanisms underlying calcium pyrophosphate deposition due to genetic variations in *ANKH*

<table>
<thead>
<tr>
<th>cDNA nucleotide change</th>
<th>Ankh gene expression</th>
<th>e or i PPi level in rare allele vs. wild type</th>
<th>effects on other proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′UTR-4bp G to A</td>
<td>&gt; wild type</td>
<td>ePPi &gt; wild type</td>
<td>no effect on PC1, TNAP</td>
</tr>
<tr>
<td>5′UTR-11bp C to T</td>
<td>not known</td>
<td>iPP &lt; wild type</td>
<td>not reported</td>
</tr>
<tr>
<td>Exon 1+13bp C to A</td>
<td>= wild type</td>
<td>ePPi =, iPPi &gt; wild type</td>
<td>no effect on PC1, TNAP</td>
</tr>
<tr>
<td>Exon 1+14bp C to T</td>
<td>&gt;/= wild type</td>
<td>ePPi &gt;/= wild type</td>
<td>no effect or increase PC1, no effect on TNAP</td>
</tr>
<tr>
<td>Exon 2+143bp T to C</td>
<td>= wild type</td>
<td>ePPi =, i PPi =/&gt; wild type</td>
<td>No effect or high PC1, TNAP. Mutant <em>ankh</em> does not interact with PiT-1.</td>
</tr>
<tr>
<td>Exon 12+490bpGAG del</td>
<td>&gt; wild type</td>
<td>ePPi, i PPi = wild type</td>
<td>no effect on PC1, TNAP no effect or low</td>
</tr>
</tbody>
</table>

*generates an alternative initiation codon putatively adding 4 amino acids to the N-terminus. This may lead to a larger pore size, increasing PPI leakiness. *  
\(^2\) skin fibroblasts \(^3\) skin fibroblasts and lymphoblast

1.7.6 Cartilage matrix changes promoting chondrocalcinosis

Changes in the cartilage matrix may encourage CPPD. CPP crystals form in areas of abnormal pericellular matrix, and are most commonly observed in areas showing reduced total collagen content, elevated type 1 collagen, damaged type 2 collagen, increased calcium binding matricellular proteins, few large proteoglycans, numerous small proteoglycans, and abundant phospholipids (Ishikawa 1985; Ishikawa et al 1989; Kalya et al 2005; Jubeck et al 2008). Transglutaminase enzymes (type 2 and factor XIIIA) induce cross links in cartilage matrix and activate osteopontin induced CPP crystal nucleation (Rosenthal et al 2000; Rosenthal et al 2001; Heinkel et al 2004; Rosenthal et al 2007). Interestingly osteopontin itself stimulates transglutaminase activity providing a positive feedback loop (Rosenthal et al 2007).

Histologically, CPP crystals also co-localise with hypertrophic chondrocytes which associate with adjacent matrix changes and with high ePPI levels (Masuda et al 1991; Rosenthal et al 1999). Furthermore, both the hypertrophic chondrocyte phenotype and the cartilage matrix changes that encourage CPP crystal formation are common features of OA, which may explain the association between CPPD and OA.
1.8 Hypothesis and rationale for the study
Although there is a strong association between OA, ageing and CC; it is not well established as why only some patients with OA, and some elderly people develop CC. The risk factors for CC remain poorly defined, and much of the information is derived from clinical observation, case series, and hospital based case-control studies. For example, mechanical loading of chondrocytes leads to high synovial fluid P Pi. It is unknown if other factors leading to increased mechanical loading of the joint such as body weight, body shape, knee mal-alignment, and occupational joint use associate with CC. Early small studies reported an association between knee malalignment and knee CC (Ellman et al 1975; Hernborg et al 1977). However, this has not been examined in a large study. Similarly, the association between joint injury, joint surgery e.g. meniscectomy, and CC have not been examined in a systematic manner. Also the association between diuretic use and CC, hypothesized to be mediated by diuretic-induced hypomagnesemia, has not been replicated. It is hypothesized that as P Pi is complexed to magnesium before being hydrolysed to Pi by TNAP, low magnesium levels will lead to higher P Pi and predispose to CPP crystal formation.

Although P Pi metabolism is reasonably well understood, the precise change leading to excess eP Pi in those with CPPD is unknown. The studies of association between SNPs in TNAP, PC1 and high ferritin (HFE) gene and sporadic CC, are limited by the fact that the control group has not been screened for absence of CC, and the analysis has not been adjusted for the presence of OA.
As excess ePPi favours CPP crystal formation, and inhibits BCP crystal formation and growth; it is likely that patients with CC will have a low bone mineral density (BMD). However, CC also associates with OA which itself associates with high BMD. The association between CC and BMD is yet to be examined. The radiographic distribution of CC at the knees, wrists, MCPJs, and hips has only been studied in hospital based case series and in a few small case-control studies. Similarly, the association between hip OA, MCPJ OA or wrist OA and CC at these sites has been examined in small hospital based studies only. There are numerous small and often conflicting reports of a distinct radiographic phenotype of structural changes in joints with OA + CPPD, compared to joints with OA alone. A large systematic survey examining whether CC at a joint modifies the radiographic appearance of OA at that joint is required to clarify the matter.

In this study, we will examine the radiographic distribution of CC at knees, hips, symphysis pubis, wrists, and of MCPJ calcification. We hypothesize that the following will increase the risk of CC:

1. increased joint load and adverse mechanical factors due to high BMI, large body shape, knee malalignment, prior joint injury or surgery, and occupational joint use

2. diuretic intake

3. SNPs in genes encoding enzymes involved in P Pi metabolism.
We also hypothesize that individuals with CC will have a low BMD; and that joints with OA + CC will have a different radiographic phenotype of structural arthropathy compared to joints with OA alone.
1.9 Introduction to the GOAL study
Genetics of Osteoarthritis and Lifestyle (GOAL) is a case-control study of 45-80 year old Caucasians that recruited 1042 knee OA cases, 1007 hip OA cases, and 1121 controls. The GOAL study was approved by the Nottingham City Hospital Local Research Ethics Committee (reference EC02/06) in February 2002, and was funded primarily by AstraZeneca, UK as a collaborative project with the University of Nottingham, UK.

Cases with hip OA were Nottingham residents, awaiting or having undergone total hip replacement (THR) for primary hip OA. They were recruited from the orthopaedic waiting lists of the City Hospital Nottingham (CHN) or Queens Medical Centre (QMC). Cases with knee OA were recruited in the same manner except for a small number of severe symptomatic knee OA cases who were recruited from a specialist knee OA rheumatology clinic. Where possible, knee and/or pelvis radiographs of potential cases were examined prior to recruitment to ensure that the total joint replacement was performed for OA. The controls were all Nottingham residents recruited from the intravenous urogram (IVU) lists at CHN or QMC. This identified patients who have had an x-ray of the pelvis. The pelvis x-ray was used to ascertain that they did not have hip OA.

Participants were classified as they were recruited. For cases, the ascertainment was severe symptomatic large joint OA sufficient to warrant referral to hospital for consideration of surgery or having already undergone total joint replacement (TJR) for severe symptomatic OA at the hip or knee in the last 5 years. Controls were ascertained as individuals with no symptoms
or clinical signs of OA, and no history of treatment for OA. Cases and controls were matched for age and gender.

Detailed information about the demographics, lifestyle, and social history was collected by a metrologist during a home visit. The metrologist examined the participant to assess their OA status. A comprehensive food frequency questionnaire (FFQ) about diet in their 4\textsuperscript{th} decade was given to the participant to complete and bring to the hospital visit. At the hospital visit, participants underwent a clinical questionnaire; had a joint examination, mobility assessment, and anthropometric measurements; gave blood and urine samples; and had x-rays of the hips, knees and hands (if not undertaken within the past 12 months). Genomic DNA was isolated from whole blood and extracted using Gentra PureGene extraction methods.
1.10 Aims and Objectives

The aims of this study were to:

[1] describe the distribution of CC,

[2] explore the known and putative risk factors for CC, and

[3] determine whether CC at a joint associates with a specific radiographic OA phenotype.

The specific objectives were to:

[1] describe the distribution of CC at the knees, pelvis and hands.

[2] investigate the putative constitutional and environmental risk factors for the development of CC including: age, gender, OA, BMI, self-reported body shape, frontal plane knee malalignment (current and previous), BMD, soft-tissue calcification, diuretic use, occupational joint use, and knee injury, or surgery.

[3] study the SNPs in candidate genes in cases with sporadic CC and controls.

[4] compare the radiographic phenotype of cases with CC plus OA at a joint with that of controls with OA alone at the same joint, and in the presence of local effects, to examine for the presence of distant effects by comparing radiographic phenotype of cases with CC at distant joints and OA at the index joint, to that of controls without CC at distant joints and with OA at the index joint.
Chapter 2 Methods

2.1 The GOAL study
The GOAL study was established primarily for case-control studies to examine gene-environment interaction, and specific genetic risk factors associated with hip or knee OA.

The 3170 participants in the GOAL study were recruited into 3 groups:

a) Knee OA (case): 1042 participants (536 male) with symptomatic and radiographic knee OA, sufficiently severe clinically to warrant referral to hospital and/or consideration for joint replacement

b) Hip OA (case): 1007 participants (499 male) with symptomatic and radiographic hip OA, sufficiently severe clinically to warrant referral to hospital for consideration of joint replacement

c) Control: 1121 participants (600 male) without symptoms, signs or radiographic evidence of knee or hip OA.

2.1.1 Case recruitment
Patient identification: Participants who had undergone, or were on the waiting list for total joint replacement (TJR) were considered for recruitment (Figure 33). Orthopaedic waiting lists obtained from the Information Bureaux at the CHN and QMC were used to recruit participants. Patients referred with clinically severe symptomatic knee OA to the Nottingham Knee OA Clinic were also considered for recruitment.

Pre-contact patient checks: The pre-operative radiographs (for TJR) or current radiographs (if on waiting list for surgery) were screened by the
Senior Research Metrologist to check for (1) presence of radiographic OA, and (2) absence of other joint or bone disease as the indication for TJR (eg rheumatoid arthritis, osteonecrosis, fracture) which would exclude the patient from GOAL. Prior to contact with participants, their current status and address were checked on the Nottingham Hospitals Patient Administration System (PAS). No checks were made with the participants’ general practitioner (GP).

Method of contact: A letter of invitation signed by the operating surgeon, a participant information sheet, and a GOAL leaflet were sent to each person. If they responded positively they were then contacted by telephone within the following two weeks to discuss the study in more detail, to answer any questions, and to screen for any exclusion criteria (See section 2.1.3).

2.1.2 Recruitment of controls
Identification of controls: All controls were recruited from IVU lists from CHN and QMC (Figure 33). These lists were obtained from the hospital Information Bureaux. In the first part of the study, no age matching was undertaken in order to facilitate recruitment of controls. However, in the last study year age matching was undertaken to ensure an appropriate age mix in the control group to both the knee and hip OA groups. Age matching was by date of birth plus or minus 2 years.

Pre-contact checks: Prior to contact with participants their current status and address were checked on the Nottingham University Hospitals Patient Administration System (PAS). No checks were made with the participants’ GP.
Method of contact: For confidentiality reasons, the first letter to the potential control was sent with the address of the Radiology Department where their IVU was undertaken and signed by the Radiology Directorate representative. This first letter asked (1) for their permission to view their x-rays to check for presence of OA or other joint disease, and (2) whether they might wish to participate in the GOAL study should their pelvis x-ray show no hip OA. If after two weeks no reply was received, a single postal reminder was sent.

If the participant answered in the affirmative, and gave consent for their radiograph to be examined, their IVU film was obtained and screened for hip OA status by the Senior Research Metrologist. If the hip joints showed evidence of OA, inflammatory arthritis or partial or total hip joint replacement, they were excluded from the study.

If the control had a normal pelvic radiograph, they were sent a further letter of invitation to participate in GOAL study with a participant information sheet and GOAL leaflet. This letter was followed up by a telephone call to inform the participant about the study in more detail, to answer any questions, and to screen for any exclusion (see below).
Figure 33 Recruitment of participants in the GOAL study

Genetics of Osteoarthritis and lifestyle (GOAL) – a case control study

**Case**
- Total number mailed and telephoned: 3475
- Replied: 3303
- Eligible: 3180
- Agreed to participate: 2168
- Completed: 2049

**Control**
- Total number mailed: 3441
- Replied: 3117
- Agreed to IVU being reviewed: 2327
- Eligible: 2022
- Agreed to participate: 1312
- Completed: 1121

Knee OA 1042  Hip OA 1007
2.1.3 Exclusion criteria
The following were exclusion criteria for this study: known diagnosis of ankylosing spondylitis, Paget's disease of bone, Perthe's disease, slipped femoral epiphysis, trauma directly before a joint replacement, hip dysplasia, avascular necrosis of femoral head or distal femoral condyle, congenital deformities and polio. Any patient suffering with long-term serious illnesses, such as carcinoma, myeloma, severe dementia, severe respiratory/renal problems (as confirmed on their x-ray report) or with inability to give fully informed consent were excluded.

2.1.4 Data collection
Patients with knee or hip OA were seen twice; at home for their initial appointment, and at CHN to complete the assessment. At the home visit, the metrologist performed an initial assessment using a standardised protocol to check the participant's OA status.

*Home visit:* An extensive questionnaire was completed by the metrologist at the home appointment. This included information about general educational background, ethnic origin, siblings, occupational history, activity history, footwear, diet, dietary supplements, medical history and male and female hormonal history. At the end of the home visit, an extensive diet questionnaire was left with the subject for them to complete and return when they attended for their hospital appointment. The questionnaire asked about the diet during their 30’s and 40’s.

*Hospital visit:* At the hospital, information about knee and hip pain; significant injury to knees, hips and other joints; surgery to knees, hips and other joints;
pain in other joints; fractures; recent pain and stiffness in the knees, hips or hands (within the last year) was collected. Participants also filled in the AUSCAN and WOMAC questionnaires (Bellamy N et al 1988).

The patient’s height, weight, waist circumference, hip circumference and arm span were measured in centimetres. The metrologist performed a targeted joint examination of the knees, hips, hands and feet. At this point, a blood sample was taken, a urine sample was given (second or third void, morning specimen) and the patient underwent a calcaneal dual energy x-ray absorptiometry (DEXA) scan. Participants had new x-rays of their hands, knees and pelvis, unless these had been performed within the last 2 years on joints that had not been replaced. All pre-surgical films of the knees and/or pelvis were sought prior to arranging first contact with the participant. For pre-surgical knee x-rays, lateral patello-femoral and non-weight-bearing tibio-femoral films were acceptable. For patients having new radiographs, a standardised protocol was used. The Rosin template (standing semi-flexed) was used for tibio-femoral compartments and a skyline view was taken of the patello-femoral compartments. Supine pelvis and AP hand views were taken.

Controls underwent an identical questionnaire, examination, and investigational procedure to provide equivalent data as for cases for the GOAL database.

Follow up questionnaire: In January 2008, a follow up questionnaire was mailed to all live GOAL participants who were still resident in the UK (n=3022). Of these, 89 declined participation, 43 were lost to follow-up, and 32 died during or after follow-up questionnaire. Therefore, of the 3022
questionnaires sent out, 2172 completed response were returned, yielding a response rate of 71.9%. The additional questionnaire enquired about the relative length of index and ring fingers from both hands (2D:4D ratio), hallux valgus (using a line-drawing instrument), physical activity (occupational, recreational and domestic) including climbing stairs, body shape, knee and foot alignment (using a line-drawing instrument): currently and in their 20s, history of cardiovascular diseases and osteoporosis, family history of OA, and footwear use (using a line drawing instrument). This questionnaire also included a food group table, to establish the test re-test intra-rater reliability for the FFQ.

*Storage of recorded data, samples and quality control:* All information was entered onto a structured query language server database and linked to the serially numbered DNA and other samples for each participant. The master database is held in Academic Rheumatology, University of Nottingham, UK. Biological samples are stored as blood, plasma, serum and urine.

The questionnaires were checked manually by designated personnel. 10% of all data entered into the database was checked against the questionnaire. The minimum accuracy was 98% for each section of the questionnaire. The accuracy under this threshold required full data check and re-entry for the section. To ensure all metrologists were gathering high quality data, they were assessed once every 2-3 months. Feedback was given by the assessor shortly after the interview, in order to aid improvement for future interviews.
2.1.5 Available radiographic measurements
As mentioned before, all participants in the GOAL study underwent knee, pelvis and hand radiographs. These radiographs were scored by a single trained Senior Research Metrologist for the presence or absence of CC at the knees, hips, symphysis pubis, wrists and for synovial calcification at the MCPJs. As there is no radiographic atlas of CC, the senior research metrologist was trained and guided by Professor Doherty in the interpretation of CC, and MCPJ calcification. CC was regarded as present if there was a:

a) linear calcification in fibro- or hyaline articular cartilage, or

b) spotty cartilage calcification, arranged in a linear manner, predominantly seen in the fibrocartilage, or

c) ‘cloudy’ synovial calcification, especially at the MCPJs.

All knee radiographs with CC were scored for its compartmental (lateral, or medial TFJ compartment), and cartilaginous (hyaline- vs. fibro cartilage) location. Knee, hand, and pelvis radiographs of twenty GOAL participants for each of the three joint regions were randomly selected (approximately 25% - 35% with CC) and re-scored for CC by the Senior Research Metrologist, and another independent observer (AA) for determining the intra-rater and inter-rater agreement. The observers were blinded to each other, and to the previous CC scores. The overall intra- and inter rater agreement (k statistic (95%CI) for CC at any site was 0.96 (0.93-0.98), and 0.96 (0.93-0.98) respectively. The intra-rater agreement (k statistic (95%CI) for CC at the knee, pelvis, and hand was 1, 1, and 0.90 (0.75-0.96) respectively. The inter-
rater agreement (k statistic (95%CI) for CC at the knee, pelvis, and hand was 1, 0.94 (0.84-0.98), and 0.94 (0.84-0.98) respectively.

There are potential methodological problems with using plain radiographs for detecting CC at different sites. This is as the sensitivity of plain radiographs for the detection of CC may vary from site to site. This may be a particular problem in the case of hip CC. Hip CC may be more difficult to detect than knee CC, especially in the presence of OA. However, other imaging techniques like ultrasound have not been validated for CC at joints other than the knee, and it would be difficult to do an ultrasound study of CC in such a large number of people at so many joints.

These radiographs were also scored for structural radiographic changes of OA by the same single observer. At the knee, osteophytes at each of the eight sites (medial and lateral tibial, femoral, patellar, and trochlear) were graded according to their size (grade 0 - 5) (Nagaosa et al 2002). Joint space narrowing was graded for each (medial and lateral) TFJ and PFJ compartment using an ordinal line diagram atlas, with negative scores indicating joint space widening (grade -1 to 5) (Wilkinson et al 2005). Knee radiographs were also scored for the presence or absence of attrition, and subluxation at the TFJs, and PFJs.

Hip radiographs were scored for osteophytosis, sclerosis, cysts, and the minimum joint space width was measured in millimetres correct to two decimal places. Osteophytes at the femoral neck, femoral head, and acetabulum were scored on a 0-3 ordinal scale (Altman et al 1995). Sclerosis
and cysts were scored as present or absent at the femoral head and at the acetabulum.

The radio-carpal, mid-carpal, common carpo-metacarpal, first carpo-metacarpal, scapho-trapezoid, individual MCP, and IP joints were scored for the severity of osteophytosis (0-3), joint space narrowing (0-3), and for presence of cysts (0,1), sclerosis (0,1) and erosions (0,1) using the OARSI radiographic atlas (Altman et al 1995).

TFJs, PFJs, hips, 1st CMCJs, MCPJs, and IPJs were allocated a 0-4 Kellgren & Lawrence (K&L) score for global OA severity (Altman et al 1995). Knee and pelvis radiographs were scored for the presence of peri-articular soft-tissue calcifications, and pelvis radiographs were scored for the presence of vascular calcifications. Intra-rater reliability was calculated for the scoring of structural radiographic changes of OA as part of the GOAL study, and is enclosed in appendix iv.

2.1.6 New radiographic measurements
Frontal plane knee alignment was measured on all knee radiographs. The mechanical axis is the gold standard measure of knee alignment and is the angle formed by a line from the centre of the head of the femur to the centre of the tibial spines and from the centre of the tibial spines to the midpoint of the talus at the ankle joint (Moreland et al 1987). This requires a weight-bearing AP radiograph of both lower extremities from the pelvis to the ankle (Moreland et al 1987; Sharma et al 2001). These full-limb radiographs are expensive, entail radiation exposure to the pelvis, require skilled radiographers and special equipment (Kraus et al 2005). The radiation from
standard knee x ray is 3 millirems while that from standard and digital full length x ray is 51 and 15 millirems respectively (McDaniel et al 2010). This makes the full limb radiograph unsuitable for use in large epidemiologic study.

The anatomic axis measured on standard posterior anterior (PA) and AP knee radiographs on a 14x17 inch cassette is a valid surrogate of the mechanical axis (Kraus et al 2005). Techniques for the measurement of anatomic axis are based on two broad principles:

(1) Join a line from the midpoint of the femoral shaft 10 cm from the knee joint to the centre of the tibial spine with a line from the midpoint of the tibial shaft 10 cm from the knee joint to the centre of the tibial spines. There is some variation between studies as to how the centre of the tibial spines is defined (McDaniel et al ; Kraus et al 2005; Hinman et al 2006; Colebatch et al 2009; Wong et al 2009). Some studies recommend using a goniometer to identify the midpoint of the femoral and tibial shafts 18 cm away from the centre of the tibial spines (Issa et al 2007).

(2) Join the femoral and tibial anatomic axis. The femoral anatomic axis is represented by a line joining two points in the mid-femoral shaft, 10 cm and at least 15 cm from the femoral condyle. Similarly, the tibial anatomic axis is represented by the line joining two points in the mid-tibial shaft, 10cm and at least 15 cm from the tibial plateau. (Prakash et al 2001; Takahashi et al 2004)

The former approach is most extensively studied, and has good inter- and intra-observer reliability with an intra-class coefficient ICC >0.92 (Kraus...

The anatomic axis has a good to excellent correlation with the mechanical axis \((r=0.54-0.88)\). In a recent study, the anatomic axis measured using the midpoint of the tibial spine tips, projected downwards on to the tibial plateau as the centre of the angle had the best correlation with the hip-knee angle \((r=0.65)\) (McDaniel et al 2010).

Knee alignment assessed by the anatomic axis is offset from the mechanical axis angle in the valgus direction. The offset is mainly due to the femoral neck which is not included in the 14 x 17 inch knee radiograph. It may also be explained by femoral and tibial bowing in individuals with OA, which again is not measured on a standard 14x17 inch knee radiograph (Chang et al 2010). The offset on PA radiographs is 3.27° for women and 5.87° for men, and on AP radiographs is 3.10° for women and 7.40° for men (Kraus et al 2005). Recent studies suggest that the degree of offset may vary according to the severity and direction of knee mal-alignment (Sheehy et al 2011). However, unfortunately these studies do not provide offset values that can be applied to short axis films of varying degrees of knee OA severity.

The anatomic axis measured on knee radiographs is sensitive and specific for the diagnosis of varus or valgus knee malalignment. Using the mechanical axis measured on full limb radiographs as the gold standard, the sensitivity and specificity for varus and valgus knee mal-alignment (defined as: any deviation from 0° (the neutral)) measured on knee radiographs was 0.84, 0.84; and 0.98, 0.73 respectively. When a deviation of >2° from neutral
was used for defining varus or valgus knee mal-alignment the sensitivity and specificity were 0.82, 0.90; and 0.91, 0.84 respectively (Issa et al 2007).

Varus or valgus knee mal-alignment measured by the method of Krause et al. has been shown to have predictive validity for progression of knee OA. The OR (95%CI) for progression of medial compartment knee OA in varus knee mal-alignment was 4.82 (1.93-12.00) using full limb radiographs and 4.25 (2.08-8.72) using short knee radiographs (Felson et al 2009).

The mechanical axis can be calculated from the anatomic axis, either by subtracting the offset, or by using one of the several proposed formulae. Different formulae have been proposed for AP and PA knee radiographs.

**AP view:**

mechanical axis = (anatomic axis)*0.67 + 55.86, r=0.65 (Kraus et al 2005)
mechanical axis = (anatomic axis)*0.915 + 13.895, r=0.88 (Hinman et al 2006)

**PA view:**

mechanical axis = (anatomic axis)*0.69 + 53.69, r=0.75 (Kraus et al 2005)

However, the anatomic axis may only provide an imperfect estimate of the mechanical axis. For example, in another study the correlation between categorical knee mal-alignment assessed on knee radiographs and that on full length lower limb radiographs was only moderate to good (r =0.43-0.74) (Colebatch et al 2009; Felson et al 2009). Other investigators have reported only a moderate correlation (r=0.34) between the anatomic and mechanical axis at the knee (van Raaij et al 2009).
In summary, we have selected the method developed by Krause et al. to measure knee mal-alignment in our study as it has been shown to have predictive validity for progression of knee OA (Felson et al 2009), and has the best correlation with the hip-knee angle (r=0.65) in a direct comparison of five methods for measurement of knee mal-alignment using short axis knee radiographs (McDaniel et al 2010).
2.2 Epidemiological analysis

2.2.1 Study design, and case definition
This is a case-control study embedded in the GOAL study (n=3170). For the current analysis, the original groups of GOAL participants recruited according to their knee or hip OA status were merged together. Participants were then reclassified according to the presence or absence of CC at any joint. Cases with CC were compared with controls without CC.

Case definition for analysis of risk factors of CC:
All GOAL participants were included in the analysis of risk factors of CC. Cases were participants with CC at any knee, hip, symphysis pubis, wrist, or with calcification at any MCPJ. Controls were participants without CC at all the above mentioned sites, and without MCPJ calcification. For examining the constitutional, genetic, and environmental risk factors of CC, cases with CC at any site were compared with controls without CC at all sites (person based analysis).

Case definition for analysis of radiographic phenotypes associated with CC plus OA:
Only participants with radiographic OA at the index joint were included in this analysis. Participants were considered to have OA at the index joint if there was definite joint space narrowing in that joint i.e. K&L grade ≥3 at the knee, MCPJs or thumb-base, or ≥2 at the hip; definite JSN (score of 2, on a 0-3 scale) at the radio-carpal, mid-carpal, common carpo-metacarpal joint or STJs (as above). In order to examine if CC plus OA associates with a distinctive radiographic phenotype at the index joint compared to OA alone,
cases were participants with CC plus OA at the index joint and controls were participants with OA alone at the same joint. Any association with a specific radiographic phenotype would suggest a local effect of CC on that particular OA phenotype at that joint. Right and left sides were compared separately (joint based analysis). If a local effect was present further analyses were carried out to examine if CC at distant joints associated with that particular structural radiographic change at the index joint. For this analysis, participants with CC at the index joint were excluded to reduce any confounding by local effects.

2.2.2 Scoring of exposure variables, and epidemiological analysis

2.2.2.1 Age, gender
Age at hospital visit (years) was considered the participant’s age. This was converted into tertiles for all analyses. Risks were computed for age in tertiles with the 1st tertile being referent. Information about gender was collected at the home visit. For the purpose of this study, female gender was 1, and male 0. Risks were computed for female gender with male gender being referent.

2.2.2.2 Osteoarthritis
For the analysis of association between OA as a risk factor for CC participants were considered to have OA if they had a K&L score of ≥2 at either hip, or a K&L score of ≥3 at either TFJs, or PFJs. Nodal OA was present if there was at least one node (either Heberden, or Bouchard) on two rays of each hand. Similarly nodal GOA was present if there was nodal OA and OA at either hip or TFJs or PFJs as defined above. Risks were computed for OA, nodal OA, and nodal GOA.
Risks were computed for the association between OA and CC at the index joint and for the association between OA at the index joint and CC at distant joints. Risks were also computed for the association between OA on one side and CC on the opposite side for each joint area.

Risks were computed for number of knee compartments with OA changes and CC at the same knee. For this, a knee was regarded to have three compartments: the medial and lateral TFJ and the PFJ. A knee compartment was considered to have OA if there was definite joint space narrowing in that compartment i.e a joint space narrowing score of ≥2 using the ordinal Nottingham line diagram atlas for knee joint space width measurements (Wilkinson et al 2005). The number of compartments with knee OA was calculated for each side and risks of knee CC for increasing number of knee compartments with OA (0-3) were calculated, with no knee compartment with OA being the reference category.

2.2.2.3 Body Mass Index
Participants’ weight (kilogram) and height (meter) were measured to the second decimal point at the hospital visit. This was used to calculate their current body mass index (BMI) as kg/m². Participants self-reported their weight in each decade of life starting from their third decade (20s). This and the current height were used to calculate the BMI in each decade of their life. Current BMI and BMI in their 20s, 30s etc were used as indicators of obesity at the relevant age (i.e prior to, and at the time of development of CC or OA). BMI was converted to tertiles, and used for all analyses. Risks were computed for BMI in tertiles, with the 1st tertile being the reference category.
2.2.2.4 Body shape
Men and women self-reported their body shape in each decade of their life beginning from the 20s, as well as their current shape. A separate ordinal line diagram instrument was used for each gender, with nine drawings labelled from 1-9 (Figure 34). This line diagram instrument requires participants to select one of the nine drawings which best represents their body shape in their 20s, as well as current body shape. For this study, participants selecting body shape 1-4 were regarded as referent, and participants selecting body shape ≥5 were regarded as exposed to the risk factor at that age. Risks were computed for the body shape ≥5.

Figure 34 Line diagram instrument for self-reported body shape
2.2.2.5 Self-reported knee mal-alignment (current and previous)
Information about self-reported current knee malalignment and knee malalignment in their 20s was obtained from the responses to the follow-up questionnaire mailed to the GOAL participants in January 2008 (Figure 35). Self-reported knee alignment was assessed using a validated line diagram instrument (Ingham et al 2010). This line diagram instrument has good reproducibility ($\kappa=0.73$), and good inter-observer agreement ($\kappa=0.72$) (Ingham et al 2010).

Figure 35 Line diagram instrument for self-reported knee alignment

For the purpose of this analysis, participants who were very bow legged (A) or bow legged (B) were considered to have varus knee malalignment, and participants who had very knock-knees (E) or knock-knees (D) were considered to have valgus knee malalignment. Risks were computed for the presence of varus, valgus, and any knee mal-alignment in their 20s, and currently.
2.2.2.6 Radiographic knee malalignment
The anatomic axis measured on knee radiographs was used to calculate the mechanical axis using the formula described by Krause et al. (2005) The absolute value for deviation from the neutral (180°) was computed for each knee. Knees with up to 2° mal-alignment from neutral (180°) were considered to have neutral alignment. Knees with >2° varus, and >2° valgus were considered to have varus, and valgus knee mal-alignment. Risks were computed separately for each side. Absolute degree of mal-alignment was compared between knees with CC, and knees without CC, separately for right and left sides. Similarly risks were computed for valgus, varus, and any knee mal-alignment and knee CC on the same side.

2.2.2.7 Occupational joint use
Comprehensive information on tasks performed during all occupations to the date of recruitment was recorded for each participant. The longest held job was selected as the index job. For TJR cases, the occupational exposure was truncated at the time of TJR. Results from published literature on occupational risk factors for knee OA were used to select tasks that would be treated as risk factors in the analysis (Blagojevic et al 2010). Individuals were scored for risk factors in their longest held job. The following were coded as present or absent:

- kneeling for ≥1 hours per day
- squatting for ≥1 hours per day
- heavy work standing for ≥1 hours per day
- lifting 25kg ≥10 times per week
• lifting 50kg or ≥100kg times per week

Risks were computed for the presence of each occupational risk separately and for the presence of any occupational risk factor.

2.2.2.8 Knee injury, or surgery
All participants self-reported information about previous significant joint injuries. As the knee is a target site for CC only knee joint injury was considered for this analysis. Participants were considered to be exposed if they had a history of knee injury. In those with TJR this had to be prior to the joint replacement. Risks were computed for those with previous significant knee injury, with those without previous significant knee injury being the referent. Participants self-reported previous arthroscopy and/or meniscectomy. This was used to classify previous knee surgery as arthroscopy (+/- meniscectomy), and meniscectomy.

2.2.2.9 Diuretic use
Information about diuretic use, including name of the diuretic, and month, year of starting and stopping treatment was self-reported by GOAL participants. Diuretic intake was validated by asking the patients the name of the diuretic, and also by examining what proportion of patients self-reporting diuretics self-reported hypertension. Of those self-reporting diuretic intake, 95% knew the name of the diuretic, and 87% also self-reported hypertension. This was used to calculate the number of years on thiazide or loop diuretics. As potassium sparing diuretics do not lead to hypomagnesemia, exposures to potassium sparing diuretics alone were disregarded. Similarly, diuretic exposures for <3 months was disregarded. Risks were computed for exposure to either loop or thiazide diuretic. The duration of exposure to
thiazide and loop diuretics was summated, and converted to tertiles. This was used to examine the dose-response relation between number of years on thiazide or loop diuretic and risk of CC.

2.2.2.10 Bone mineral density
Cortical BMD was estimated from metacarpal index calculated from the hand radiographs. Metacarpal index was calculated as \((W-w)/W\) where \(W\) is the total external diameter and \(w\) is the endosteal diameter of the mid-shaft of the 2\(^{nd}\) metacarpal (Nielsen 2001). The trabecular BMD was measured directly using calcaneal ultrasound (Apollo). The trabecular BMD measures were normalised to yield z scores in the GOAL control population, which was then categorised as \(<-1SD, -1 to +1SD, and >1SD. Metacarpal index was converted to tertiles. Risks for CC were computed for higher trabecular and cortical BMD with the lowest tertile of trabecular and cortical BMD being the reference.

2.2.2.11 Soft-tissue and vascular calcification
Participants with peri-articular soft tissue calcification at the knee were considered to have soft-tissue calcification. Vascular calcification was only ascertained on the pelvic radiograph. Risks were computed for the associations between peri-articular soft tissue calcification at the knee, and CC at any site. Similarly risks were computed for the association between vascular calcification and CC at any site.

2.2.3. Genetic risk factors
The genetic study focused on examining associations between SNPs in genes involved in PPi metabolism and CC. This is a genetic replication study
exceeding previously published associations and does not seek to identify novel SNPs.

A detailed literature search was carried out to identify SNPs in genes involved in P Pi metabolism. SNPs were selected for genotyping if they met any one of the following criteria:

1. previous reports of association with CC
2. association with metabolic abnormalities and diseases associated with CPPD
3. amino-acid changing SNP, and
4. exonic location.

SNPs were selected if they were known to occur in Caucasians, and had a minor allele frequency of > 0.05. Seventeen SNPs were selected for genotyping for their association with CC, P Pi levels, OA, and iron overload states (Table 15).

Four SNPs in ANKH were selected for genotyping. One of this, the -4bp G to A transformation in the 5'-UTR region has previously been associated with sporadic CPPD in a hospital based case-control study from Oxford, UK. In this study, cases with CC were selected from rheumatology clinics, and controls were healthy blood donors (Zhang et al 2005). The minor allele of this SNP associated with CPPD with a genotype relative risk of 6.0 (p=0.0002). Another SNP in ANKH, rs3045 associated with rotator cuff tear in a hospital based case-control study, and the minor allele resulted in low
intracellular PPi levels - a marker of high extracellular PPi levels – in *in vitro* studies (Peach *et al* 2007). The other two SNPs in ANKH selected for genotyping associated with serum parathyroid levels in a study of 244 nuclear families (840 individuals) after correcting for age, gender, and BMI (Vistoropsky *et al* 2007). The AC haplotype of these two SNPs in ANKH (rs39968, and rs875525) associated with low parathyroid hormone levels (Vistoropsky *et al* 2007).

Three TNAP SNPs were selected for genotyping. Of these, homozygosity of the amino acid changing SNP rs3200254 associated with both high BMD, and high TNAP enzyme activity in a study of over 500 postmenopausal women from Japan (Goseki-Sone *et al* 2005). The higher TNAP activity was explained by a lower Michelis constant (Kₘ) which reflects increased substrate affinity for the enzyme (Goseki-Sone *et al* 2005). rs 4654760 associated with rotator – cuff tear in a hospital based case-control study, and TNAP571 was the commonest cause of adult onset hypophosphatasia in a study of 361 unrelated samples sent to a tertiary reference laboratory (Peach *et al* 2007; Fauvert *et al* 2009). The latter SNP was selected as hypophosphatasia associates with CPPD.

Five SNPs in *ENPP1/PC1* gene were selected. Three of these (rs858342, rs1044498, rs1800949) have been associated with hand OA in a population based study of over 570 community dwelling Caucasians between age 18 and 90 (Suk *et al* 2005). Another SNP in this gene (rs943003) was selected for its association with obesity in a Finnish case control study of over 240 morbidly obese cases, and over 480 lean controls (Valli-Jaakola *et al*...
The SNP rs28933977 was selected for its association with hand OA (Suk et al 2005), and generalised arterial calcification of infancy – a condition caused by inactivating mutations in ENPP1, which leads to low PPi, and deposition of hydroxyapatite crystals in the vasculature (Cheng et al 2005). The other candidate SNPs were selected for their established associations with haemochromatosis (Alizadeh et al 2007), and iron overload (Benyamin et al 2009). Genotyping was carried out by geneticists at AstraZeneca’s laboratories using the TaqMan method. If they were not able to develop an assay for the SNP, the genotyping was outsourced to Kbiosciences UK.
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Disease or biochemical association</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANKH</td>
<td>-4bpGtoA</td>
<td>5'-UTR</td>
<td>G to A</td>
<td>No</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>rs3045</td>
<td>3'-UTR</td>
<td>A to G/ T to C</td>
<td>No</td>
<td>Rotator cuff tear</td>
</tr>
<tr>
<td></td>
<td>rs39968</td>
<td>Intron</td>
<td>A to G/ C to T</td>
<td>No</td>
<td>Low PTH</td>
</tr>
<tr>
<td></td>
<td>rs875525</td>
<td>Intron</td>
<td>C to T/ A to G</td>
<td>No</td>
<td>Low PTH</td>
</tr>
<tr>
<td>TNAP</td>
<td>rs3200254</td>
<td>Exon</td>
<td>T to C</td>
<td>Tyr to His</td>
<td>Increased enzyme activity, increased BMD</td>
</tr>
<tr>
<td></td>
<td>rs4654760</td>
<td>Intron</td>
<td>C to T</td>
<td>No</td>
<td>Rotator cuff tear</td>
</tr>
<tr>
<td></td>
<td>TNAP571</td>
<td>Exon</td>
<td>G to A</td>
<td>Glu to Lys</td>
<td>25% adult onset hypophosphatasia</td>
</tr>
<tr>
<td>ENPP1/PC1</td>
<td>rs858342</td>
<td>Intron</td>
<td>A to G</td>
<td>No</td>
<td>Hand OA</td>
</tr>
<tr>
<td></td>
<td>rs1044498</td>
<td>Exon</td>
<td>A to C</td>
<td>Lys Gln</td>
<td>Hand OA</td>
</tr>
<tr>
<td></td>
<td>rs1800949</td>
<td>Intron</td>
<td>C to T</td>
<td>No</td>
<td>Hand OA</td>
</tr>
<tr>
<td></td>
<td>rs28933977</td>
<td>Exon</td>
<td>C to T</td>
<td>Arg to Cys</td>
<td>Hand OA, generalized arterial calcification</td>
</tr>
<tr>
<td></td>
<td>rs943003</td>
<td>Intron</td>
<td>A to G</td>
<td>No</td>
<td>Obesity</td>
</tr>
<tr>
<td>HFE</td>
<td>rs1800562</td>
<td>Exon</td>
<td>G to A</td>
<td>Cys to Tyr</td>
<td>Haemochromatosis</td>
</tr>
<tr>
<td></td>
<td>rs1799945</td>
<td>Exon</td>
<td>C to G</td>
<td>His to Asp</td>
<td>Haemochromatosis</td>
</tr>
<tr>
<td>TFR</td>
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<td>C to T</td>
<td>No</td>
<td>High transferrin</td>
</tr>
<tr>
<td></td>
<td>rs2280673</td>
<td>Intron</td>
<td>A to C</td>
<td>No</td>
<td>High transferrin</td>
</tr>
<tr>
<td></td>
<td>rs3811647</td>
<td>Intron</td>
<td>A to G</td>
<td>No</td>
<td>High transferrin</td>
</tr>
</tbody>
</table>

All SNPs were checked for Hardy-Weinberg equilibrium (HWE) prior to analyzing genetic risk. According to the binary distribution of allele frequencies in the general population, sum of proportion of common homozygotes, rare homozygotes, and compound heterozygotes should be 1 in the presence of a stable population i.e. $p^2 + q^2 + 2pq = 1$, where $p$ is the frequency of one of the tested allele, and $q$, the frequency of the other allele is $1 - p$. The distribution of expected and observed genotype frequencies were compared using a chi-square distribution (Fishers’ exact test) (Table 16). If there was a significant difference in the observed and expected genotype frequencies ($p < 0.05$), the alleles at that SNP were not in HWE suggesting genetic drift.

**Table 16 Chi square test for Hardy-Weinberg equilibrium**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common homozygote</td>
<td>A</td>
<td>$p^2$</td>
</tr>
<tr>
<td>Rare homozygote</td>
<td>B</td>
<td>$q^2$</td>
</tr>
<tr>
<td>Heterozygote</td>
<td>C</td>
<td>$2pq$</td>
</tr>
</tbody>
</table>

Overall genotype risks ($OR_{GENOTYPE}$) were computed for associations between different genotypes and CC. $OR_{GENOTYPE}$ is the OR for association between increasing number of tested alleles at the particular SNP, and risk of CC i.e. the risk of CC for each additional rare allele of that SNP. The $p$ value of genotype OR was corrected for multiple tests using Bonferroni corrections.
Since we examined the association between 17 candidate SNPs, and CC, the OR\textsubscript{GENOTYPE} was considered significant if the p value was <0.003.

2.2.4. Summation of radiographic structural changes
Scores allocated to individual radiographic structural changes at different sites or compartments within a joint were summated to yield a global radiographic score. Due to their additive nature, global scores reflect the severity of structural changes at a joint. The strategy for summation varied according to the joint.

\textit{At the knee}: Osteophytes were scored from 0 - 5 at eight sites in the knee – 4 each in the TFJ, and the PFJ. The osteophyte scores were added to yield a global score reflecting the amount of osteophytosis in the index knee (maximum score 40). Joint space narrowing was graded as -1 to 5 for the two TFJ, and PFJ compartment on each side. For calculating global JSN score for each knee, negative scores were transformed to 0 and JSN scores for each compartment were summated. Separate scores were calculated for each side. Attrition was scored as present or absent on each side and was analysed as a dichotomous variable.

\textit{At the hip}: Osteophytes at the hip were scored on a 0 - 3 scale at three sites in each hip. The osteophyte scores were summated using the same approach as at the knee (maximum score 9). Minimum joint space width measured at each hip in millimeteres correct to two decimal points was converted to tertiles. Other radiographic phenotypes like sclerosis, cysts, and attrition were scored as present or absent on each side, and were analysed as dichotomous variables.
At the wrist: Osteophytes were scored from 0 - 3, in the radio-carpal, mid-carpal, and common carpo-metacarpal joints. These were added to yield a global score reflecting osteophytosis in the index wrist (maximum score 9). Joint space narrowing was graded as 0 - 3 for each of the radio-carpal, mid-carpal, and common carpo-metacarpal joints. These were added to yield a global score reflecting joint space narrowing at the index wrist (maximum score 9). Separate scores were calculated for each side. Other radiographic phenotypes like sclerosis, and cysts, have been scored as present or absent on each side, and were treated as dichotomous variables.

At the 1st carpo-metacarpal, and trapezio-scaphoid joint: Osteophytes and joint space narrowing were allocated a single global score on a 0-3 scale at these joints. Other radiographic phenotypes like sclerosis, and cysts, have been scored as present or absent on each side, and were treated as dichotomous variables.

At the MCPJs: Osteophytes were scored from 0-3 at each MCPJ. The osteophyte scores from the 2nd to 5th MCPJ in each hand were added to yield a global score reflecting the amount of osteophytosis at the MCPJs in one hand (maximum score 12). Joint space narrowing on a 0-3 scale at each MCPJ was summated to yield a global joint space narrowing score (maximum score 12) for the MCPJs in each hand. Other radiographic phenotypes like sclerosis, and cysts were scored as present or absent on each side, and were regarded as dichotomous variables.
2.2.5. Risk estimation for summated radiographic scores

Osteophyte scores at each joint were converted to tertiles. Risks were computed for association between increasing tertiles of osteophytes score and CC at that joint with the 1st tertile being referent. A similar approach was adopted for joint space narrowing. Risks were computed for association between sclerosis, cysts, and attrition in the index joint, and CC at the same joint. All associations were examined to identify if this was a local effect. If local effects were present, further analysis was carried out to look for distant effects – i.e. whether CC at distant joints affected the phenotype of OA at the index site. Joints with CC at the index site were excluded from this analysis.

2.2.5 Statistical analysis

Mean (S.D.) and n (%) were used for descriptive studies. The prevalence of CC and its 95% confidence interval (CI) was calculated as follows.

\[ p = \frac{c}{n} \]

\[ 95\% \text{ CI} = p \pm 1.96 \times \sqrt{\frac{p(1-p)}{n}}. \]

\[ p - \text{prevalence of CC} \]

\[ c - \text{number of participants with CC} \]

\[ n - \text{total number of participants} \]

The overall prevalence (95% (CI)) of CC was calculated. The prevalence of CC was calculated at each joint area for unilateral CC, for bilateral CC, and for isolated CC i.e. CC at a target joint in the absence of CC at any of the distant joints. Similarly, the prevalence of CC was calculated for medial and lateral compartment TFJ and for fibro- and hyaline cartilage involvement at the knee.

Cluster analysis was used to test whether CC is likely to occur in multiple joints in the same individual i.e. to identify if there is a systemic
predisposition to CC. For this, we calculated the number of subjects who would have 0, 1, 2, 3, 4 or 5 joint areas with CC, assuming that the presence of this CC in different regions is independent. We then compared the observed frequency of subjects with number of joint areas affected with the expected frequency using a chi-square distribution.

Independent sample T-test and chi-square test were used to compare cases and controls. Kappa statistic \((k)\), and its’ 95% CI were calculated to assess the intra-observer agreement for consistency, and inter-observer agreement for absolute agreement using a two way mixed model. OR (95% CI) were calculated to examine associations. Binary logistic regression was used to adjust for confounders.

a) the associations between CC at the index joint and CC at distant joints were adjusted for age at hospital visit (tertiles), gender (male 0, female 1), BMI (tertiles), and definite OA at distant joints (absent 0, present 1). OA was considered to be present at the distant joints if the K&L score was ≥2 at either hip, ≥3 at either knee, and there was definite joint space narrowing at either wrist i.e at either radio-carpal, mid-carpal, or common carpometacarpal joints.

b) the associations between risk factors and CC were adjusted for age at hospital visit (tertiles), gender (female 1, male 0), BMI (tertiles), knee OA (K&L score ≥3), and hip OA (K&L score ≥2). Current BMI was used when adjusting BMI for all risk factors except for self-reported knee mal-alignment in 20s when BMI in their 20s was used.
For all SNPs in HWE overall genotype risks were calculated and adjusted as above.

c) In those with OA at the index joint, the association between CC and structural radiographic changes was adjusted for age (tertiles), gender, and BMI (tertiles).

In this study tertiles were used to categorize continuous variables for examining associations using binary logistic regression. This approach to categorizing continuous variables gives meaningful ORs for each unit increase in the category of the exposure variable. This can then be easily communicated to the reader. If continuous variables are used on their own, the OR for each unit increase in the exposure variable is usually very small and difficult to communicate to the reader. Also, categorizing continuous variables allows for the examination of dose-response between increasing levels of exposure of interest and the outcome. This is not possible if only continuous variable are used as an exposure of interest.

Statistical significance was set at p<0.05 (two sided analysis), except for OR\textsubscript{GENOTYPE} for which statistical significance was set at <0.003. All analyses were carried out using SPSS v14.
Chapter 3 Radiographic distribution of chondrocalcinosis

**Aims:** The overall aim of this study was to examine the distribution of radiographic CC in participants of GOAL study. The specific objectives were to:

1) describe the prevalence of CC at the knees, hips, symphysis pubis, wrists and that of MCPJ calcification with an emphasis on laterality and sidedness

2) compare the prevalence of CC at the hips, symphysis pubis, wrists, and MCPJ calcification in the presence and absence of knee CC

3) compare the prevalence of CC at the medial and lateral TFJ compartment, and in the fibro- and hyaline cartilage of the TFJ

4) examine if there is a constitutional predisposition to CC

5) examine if there is an association between CC at referent and distant joint

6) examine if bilateral CC at referent joint has a higher risk of CC at distant joints than unilateral CC at referent joint.
**Results:** Of the 3170 participants, information about CC at any pelvis, knee, or hand radiographs were available for 3139 participants. Out of these 3139 participants, 431 participants had CC at any one joint. The overall prevalence (95% CI) of CC was 13.7 (12.5-14.9) %. The knee was the commonest site of CC, being affected in 8.0% of GOAL participants. Other joints involved in descending order of frequency were: wrists (6.9%), hips (5.0%), and symphysis pubis (3.6%). Isolated CC (i.e. CC at any one joint area only) occurred most frequently at the knee (2.6%), and at the wrist (2.3%) (Table 17). There was no predilection for MCPJ calcification, or CC to occur on any side (Table 17). CC was significantly more likely to be bilateral at the knees, and wrists, while it was more likely to be unilateral at the hips (Table 17). MCPJ calcification was least frequent and commonly bilateral (Table 17).

**Table 17 Prevalence of chondrocalcinosis at individual joints**

<table>
<thead>
<tr>
<th>Index joint</th>
<th>N</th>
<th>Overall (%)</th>
<th>Right (%)</th>
<th>Left (%)</th>
<th>Unilateral (%)</th>
<th>Bilateral (%)</th>
<th>Isolated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee</td>
<td>3134</td>
<td>8.0 (7.0-8.9)</td>
<td>6.7 (5.8-7.5)</td>
<td>6.0 (5.2-6.8)</td>
<td>4.0 (4.7-5.4)</td>
<td>3.3 (2.7-3.9)</td>
<td>2.6 (2.2-3.3)</td>
</tr>
<tr>
<td>Wrist</td>
<td>3130</td>
<td>6.9 (6.0-7.8)</td>
<td>5.4 (4.6-6.2)</td>
<td>5.8 (5.0-6.6)</td>
<td>2.7 (2.1-3.2)</td>
<td>4.3 (3.6-4.9)</td>
<td>2.3 (1.8-2.9)</td>
</tr>
<tr>
<td>Hip</td>
<td>3144</td>
<td>5.0 (4.3-5.9)</td>
<td>4.0 (3.3-4.7)</td>
<td>3.0 (2.4-3.6)</td>
<td>3.1 (2.5-3.7)</td>
<td>2.0 (1.5-2.5)</td>
<td>1.4 (1.0-1.8)</td>
</tr>
<tr>
<td>Symphysis</td>
<td>3168</td>
<td>3.6 (2.9-4.2)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.8 (0.5-1.8)</td>
</tr>
<tr>
<td>Any MCPJ</td>
<td>3130</td>
<td>1.5 (1.1-2.0)</td>
<td>0.9 (0.6-1.2)</td>
<td>1.1 (0.7-1.4)</td>
<td>0.4 (0.2-0.6)</td>
<td>1.1 (0.8-1.5)</td>
<td>0.1 (0.0-0.2)</td>
</tr>
</tbody>
</table>

N/A not applicable
Among the MCPJs, the 2\textsuperscript{nd} and 3\textsuperscript{rd} MCPJs were most likely to have calcification (Figure 36).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{calcification_mcpjs.png}
\caption{Calcification at MCPJs}
\end{figure}

\( n \) = number of participants with MCPJ calcification at that joint
When present, CC occurred at more than one joint in approximately two-thirds of the GOAL participants. CC was present at one joint in 35.3%, at two joints in 23.4%, and at more than two joints in 41.3% of GOAL participants (Figure 37).

Figure 37 Number of joints with chondrocalcinosis
CC was common at other joints without any knee involvement. For example, 44.4% of wrist CC, 45.9% of hip CC, 45.5% of symphysis pubis CC, and 31.3% of MCPJ calcification occurred without knee CC (Table 18).

**Table 18 Chondrocalcinosis at other joints in the presence or absence of knee involvement**

<table>
<thead>
<tr>
<th>Joint</th>
<th>Total</th>
<th>Knee CC +ve n=250</th>
<th>Knee CC –ve n=181</th>
<th>Percent with CC at index joint without knee CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrist</td>
<td>216</td>
<td>120</td>
<td>96</td>
<td>44.4%</td>
</tr>
<tr>
<td>Hip</td>
<td>157</td>
<td>85</td>
<td>72</td>
<td>45.9%</td>
</tr>
<tr>
<td>Symphysis pubis</td>
<td>112</td>
<td>61</td>
<td>51</td>
<td>45.5%</td>
</tr>
<tr>
<td>Any MCPJ</td>
<td>48</td>
<td>33</td>
<td>15</td>
<td>31.3%</td>
</tr>
</tbody>
</table>

Therefore, only 58.4% of GOAL participants with CC at any site (knees, wrists, hips, symphysis pubis), or with MCPJ calcification could be identified on radiographs of both knees only (Figure 38). This proportion increased to 82.4% if the pelvis radiograph, and to 81.5% if both wrist and hand radiographs, were also obtained.

**Figure 38**

Articular distribution of chondrocalcinosis
In those with CC at two or more joint areas, the common patterns of location of CC were: knee and wrist/hands (14.3%); knee, wrist/hands, hips, symphysis pubis (6.8%); knee, wrist/hands, hips (6.1%). The number of participants with CC in these locations is detailed in Figure 39.

Figure 39 Number of patients with chondrocalcinosis at ≥2 areas

![Bar chart showing the number of patients with chondrocalcinosis at various joint areas.](chart)
When present, knee CC commonly affected both medial and lateral TFJ compartments (Figure 40). 67% participants with right knee CC, and 59% participants with left knee CC had involvement of both TFJ compartments. CC was more common in the lateral TFJ compartment than in the medial TFJ compartment at both knees (Figure 40). The absolute difference in prevalence (95%CI) of CC between the lateral and medial compartment of TFJ was 15.2% (8.1-22.1) for the right, and 15.4% (7.1-23.0) for the left knee (Figure 40).

![Figure 40 Compartmental distribution of chondrocalcinosis at TFJs](image)

**Figure 40** Compartmental distribution of chondrocalcinosis at TFJs
At the knee, fibro- and hyaline cartilage CC co-existed in 48.6% of right TFJs and in 40.0% of left TFJs (Figure 41). However, at the TFJs CC affected the fibro cartilaginous meniscus more frequently than the intra-articular hyaline cartilage. The difference in prevalence (95% CI) between fibrocartilage and hyaline cartilage CC was 34.6% (26.9-42.3) for right and 32.6% (23.3-40.4) for left TFJ. Similarly, isolated fibrocartilage involvement was more common than isolated hyaline cartilage involvement at both TFJs – 42.8% vs. 8.2% at the right, and 46.3% vs. 13.7% at the left TFJs.

Figure 41 Cartilaginous distribution of chondrocalcinosis at TFJs
Constitutional predisposition to CC: 3118 patients had information about CC recorded at all joints. Of these 428 patients had CC at any joint. 931 patients did not have any radiographic evidence of OA at any knees, hips, or wrists. On cluster analysis, joints with CC clustered together more than would be expected by chance alone (Table 19). This was present when patients with knee, hip, and wrist OA were excluded.

### Table 19 Cluster analysis: systemic risk for chondrocalcinosis

<table>
<thead>
<tr>
<th>Number of regions with CC</th>
<th>All participants</th>
<th>Without OA at knee, hips or wrist</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2690</td>
<td>2257.13</td>
<td>872</td>
<td>850.56</td>
</tr>
<tr>
<td>1</td>
<td>219</td>
<td>782.93</td>
<td>37</td>
<td>78.00</td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td>74.55</td>
<td>12</td>
<td>2.41</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>3.32</td>
<td>5</td>
<td>0.03</td>
</tr>
<tr>
<td>≥4</td>
<td>41</td>
<td>0.07&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5</td>
<td>0&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Chi square(d.f.), p = 2974.96 (4), <0.001

92.25 (4), p<0.001

<sup>1</sup>considered as 1 for calculating chi-square statistic
On binary logistic regression, CC at any one joint area associated with CC at distant joints. The association was present for all joint pairs, and persisted after adjusting for age, gender, BMI, and OA at the distant joints (Table 20).

**Table 20 Association between chondrocalcinosis at distant joints**

<table>
<thead>
<tr>
<th>Referent joint</th>
<th>aOR (95%CI)(^1) for CC at distant joints</th>
<th>(\text{Knee})</th>
<th>(\text{Wrist})</th>
<th>(\text{Hip})</th>
<th>(\text{Symphysis pubis})</th>
<th>(\text{MCPJs})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee</td>
<td>-/-</td>
<td>27.23 (19.14-38.74)</td>
<td>19.63 (13.67-28.19)</td>
<td>17.60 (11.60-22.60)</td>
<td>23.97 (12.57-45.70)</td>
<td></td>
</tr>
<tr>
<td>Wrist</td>
<td>22.35 (15.89-31.43)</td>
<td>-/- (11.59-24.57)</td>
<td>16.88 (10.18-23.79)</td>
<td>15.56 (10.05-34.85)</td>
<td>66.95 (30.05-149.14)</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>18.87 (13.04-27.30)</td>
<td>19.67 (13.29-29.10)</td>
<td>-/- (24.34-57.42)</td>
<td>37.39 (10.05-34.85)</td>
<td>18.72 (10.05-34.85)</td>
<td></td>
</tr>
<tr>
<td>Symphysis Pubis</td>
<td>16.82 (11.01-25.69)</td>
<td>17.03 (11.00-26.36)</td>
<td>37.29 (24.29-57.25)</td>
<td>-/- (2.39-12.21)</td>
<td>5.40 (2.39-12.21)</td>
<td></td>
</tr>
<tr>
<td>Any MCPJ</td>
<td>21.04 (11.04-40.11)</td>
<td>74.51 (33.10-167.74)</td>
<td>19.42 (10.56-35.72)</td>
<td>5.17 (2.33-11.47)</td>
<td>-/- (2.33-11.47)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA at distant site except for symphysis pubis where adjustment for OA was not carried out.
Next, we examined the association between bilateral CC at one joint and CC at distant joints, with unilateral CC at the index joint being the referent. Compared to unilateral knee CC, bilateral knee CC associated with CC at wrists, hips, symphysis pubis and MCPJs. Similarly, compared to unilateral wrist CC, bilateral wrist CC associated with CC at the knee, hips and MCPJs; compared to unilateral hip CC, bilateral hip CC associated with CC at the knee, wrist and MCPJs; and compared to unilateral MCPJ calcification, bilateral MCPJ calcification associated with CC at the hip (Table 21).

**Table 21 Association between bilateral chondrocalcinosis at one joint area and distant chondrocalcinosis**

<table>
<thead>
<tr>
<th>Referent joint</th>
<th>Knee</th>
<th>Wrist</th>
<th>Hip</th>
<th>Symphysis pubis</th>
<th>MCPJs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee</td>
<td>-/-</td>
<td>2.23</td>
<td>3.79</td>
<td>2.21</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.27-3.93)</td>
<td>(2.06-6.98)</td>
<td>(1.17-4.17)</td>
<td>(1.13-6.86)</td>
</tr>
<tr>
<td>Wrist</td>
<td>3.38</td>
<td>2.15</td>
<td>1.86</td>
<td>2.21</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>(1.82-6.24)</td>
<td>(1.44-4.04)</td>
<td>(0.93-3.72)</td>
<td>(1.11-5.99)</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>3.15</td>
<td>2.69</td>
<td>1.52</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.51-6.56)</td>
<td>(1.27-5.73)</td>
<td>(0.77-2.97)</td>
<td>(1.53-11.68)</td>
<td></td>
</tr>
<tr>
<td>Symphysis pubis</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td></td>
</tr>
<tr>
<td>Any MCPJ</td>
<td>2.37</td>
<td>6.91</td>
<td>2.39</td>
<td>-/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.43-12.96)</td>
<td>(1.40-34.26)</td>
<td>(0.39-14.82)</td>
<td>-/-</td>
<td></td>
</tr>
</tbody>
</table>

1. Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles) and definite OA at distant site. 2. No cases.
Key results:

- 13.7% GOAL participants had CC at any site.
- Knee was the commonest location of CC.
- There was no predilection for MCPJ calcification, or CC to occur on any side.
- MCPJ calcification and CC were more likely to be bilateral except at the hips.
- 2nd and 3rd MCPJs were the commonest site for MCPJ calcification.
- CC commonly occurred at other joints without any knee involvement.
- Knee CC was more frequently in the lateral TFJ compartment than in the medial TFJ compartment, and in fibro- than in the hyaline articular cartilage.
- There was evidence for a systemic predisposition to CC.

Discussion: In this study, the overall prevalence of CC in knee, hand, and pelvis radiographs was 13.7%. The prevalence of CC at knees (8.0%), knees and pelvis (10.6%), and knee and wrists (10.4%) reported in this study are similar to those reported by previous community based surveys. According to these surveys, the prevalence of knee CC is 8.1% (Felson et al 1989), that of knees and pelvis is 10.4% (Ramonda et al 2009), and that of knees and hands is 10% (Sanmarti et al 1993).
Despite a higher prevalence of OA, the prevalence of CC at knees, knees and pelvis, and knee and wrists reported in this study are similar to those reported in community based surveys (Felson et al 1989; Sanmarti et al 1993; Ramonda et al 2009). This apparently surprising finding may be explained by the relationship between age, OA, and CC. GOAL participants have a high prevalence of large joint OA so they would be expected to have at least a two-fold higher prevalence of CC (Felson et al 1989). However, GOAL participants were also approximately ten years younger than those in community based surveys of CC (Felson et al 1989). Since the prevalence of CC halves for each 10 year fall in age, this probably compensates for the increase in prevalence of CC resulting from a high prevalence of OA (Felson et al 1989).

Although limited by it’s study design, this is the first large radiographic study of CC to include radiographs of all three regions commonly affected by CC i.e. the knees, pelvis, and hands. The other two radiographic surveys to systematically examine the prevalence of CC in radiographs of hands, knees, and pelvis were small, and included in-patients (Wilkins et al 1983), or symptomatic out-patients only (Gordon et al 1984).

The overall prevalence of CC in this study is lower than that observed in radiographic surveys of acute geriatric inpatients (34.0%) (Wilkins et al 1983). Similarly, the prevalence of knee CC is lower than that observed in elderly residential home residents (9.6%) (Ellman et al 1975). This may be because participants in GOAL are younger (mean age 67 years) than those in these two studies. The mean age of participants in the previous two
studies were 79.4 years (Wilkins et al 1983), and 82.6 years (Ellman et al 1975) respectively. However, the prevalence of CC reported in the current study derived from an out-patient hospital based population with a high prevalence of large joint OA should be interpreted with caution, and may have limited generalisability to the general population.

We report that the knee is the commonest site of CC. This is in keeping with most previous reports examining the prevalence of CC (Ellman et al 1975; Bergstrom et al 1986b; Sanmarti et al 1993; Ramonda et al 2009). In our study, the prevalence of CC at other sites in descending order of frequency was wrists, hips, and symphysis pubis.

While the published literature is unanimous about the joint most commonly affected by CC, there is significant disagreement about the second most frequent site of CC. Some studies report that the wrist is the second most frequently involved joint (McCarty et al 1963; Ellman et al 1975; Resnick et al 1977a; Balsa et al 1990; Louthrenoo et al 1999) while others report that the symphysis pubis is the second most commonly affected site (Twigg et al 1964; Fam et al 1981; Wilkins et al 1983; Gordon et al 1984). One early report suggested that the hip is the second most frequently involved joint (Zitnan et al 1963) but this has not been supported by any subsequent study. In a hospital based study of 69 patients with CPPD, hip CC was less common than knee CC, wrist CC, and MCPJ calcification (Riestra et al 1985). A detailed review highlights the uncertainty around the second most frequently affected joint (Genant 1976). However, since the present study is the largest study to date to examine the relative frequency of
CC at commonly affected sites, within the study limitations we can say with reasonable confidence that wrist is the second most common site for CC.

In this study, CC was more prevalent at the hips than at the symphysis pubis. This is in keeping with previous observations (Twigg et al 1964; Fam et al 1981; Wilkins et al 1983; Gordon et al 1984). However, in a large community based survey of older adults symphysis pubis calcification was more common than hip CC (Ramonda et al 2009). 33.1% of participants with CC at knees, hips, or symphysis pubis had symphysis pubis calcification while only 3.6% of these participants had hip CC. This difference from our study may be because approximately one-third of GOAL participants were selected because of severe symptomatic hip OA and that participants in GOAL were younger – older age may have a greater influence on the prevalence of CC in the symphysis pubis as this joint is not affected by OA.

As in previous reports, MCPJ calcification occurred in just over 11% of participants with CC (Sanmarti et al 1993). When present, MCPJ calcification most commonly affected the 2<sup>nd</sup> and 3<sup>rd</sup> MCPJs. This is in keeping with previous observations (Resnick et al 1977a).

We did not find a predilection for knee, wrist, or hip CC and MCPJ calcification to occur preferentially on either the right or left side. This is in keeping with most previous observations (Felson et al 1989; Neame et al 2003). There is a single previous report of CC preferentially affecting one side over the other. In a community based survey, unilateral CC was reported to be more common in the right than in the left knee (Ramonda et al 2009).
In the current study, CC was more likely to be bilateral at the knee and wrist. This concurs with previous reports (McCarty et al 1963; Zitnan et al 1963; Bocher et al 1965; Ellman et al 1975; Genant 1976; Dieppe et al 1982; Wilkins et al 1983; Felson et al 1989; Louthrenoo et al 1999; Ramonda et al 2009). However, we found that CC was significantly more likely to be unilateral at the hip – a finding which is not in keeping with previous reports (McCarty et al 1963; Zitnan et al 1963; Ellman et al 1975; Genant 1976; Wilkins et al 1983). This finding suggests that hip CC may be a result of joint specific predisposition unlike CC at other joints. A similar pattern is observed for hip OA which is more frequently unilateral (Tepper et al 1993), and related to joint specific risk factors e.g. injury rather than generalized risk factors e.g. obesity (Cooper et al 1998).

We report that 42% of patients with CC have involvement of hips, symphysis pubis, wrists, and MCPJ calcification in the absence of knee CC. Similar findings have been reported before. Two hospital based studies reported that CC can occur in the absence of knee involvement in 25.0 to 33.3% of cases (McCarty et al 1963; Wilkins et al 1983). However, there are several reports which contradict this observation, and suggest that it is rare to get CC at other joints in the absence of knee involvement. While one report suggests that CC does not occur in the absence of knee involvement (Fam et al 1981), other studies suggest that CC in the absence of knee involvement is rare, and occurs in between 3.4% - 11.0% patients only (Zitnan et al 1963; Ellman et al 1975; Gordon et al 1984; Sanmarti et al 1993; Louthrenoo et al 1999; Ramonda et al 2009).
Moreover, two hospital based studies of symptomatic CPPD patients have examined the relative distribution of CC at knees, wrists, and symphysis pubis (Gordon et al 1984; Canhao et al 2001) (Figure 42). They suggest that CC is extremely unlikely to occur at the wrists and symphysis pubis in the absence of knee CC. However, this observation may be because patients with symptomatic CPPD at the knee are over-represented in these hospital based studies since these patients are more likely to seek medical attention.

![Figure 42 Distribution of chondrocalcinosis at knee, wrist, and symphysis pubis in two hospital based studies](image)

Only 58.4% of GOAL participants with CC at any site (knees, wrists, hips, symphysis pubis or MCPJ calcification) could be identified using radiographs of both knees. Moreover, only 82.4% of participants could be identified if the pelvic radiograph was also included, and 81.5% of participants could be identified if both wrists and hand radiographs were also included. This observation has significant implications for case ascertainment for epidemiological and genetic studies of CC, i.e. controls should have no
CC at any of these three joint areas, and a large proportion of cases may have CC at wrist, hips or symphysis pubis and be missed if knee radiographs alone are used to screen for CC cases.

As previously reported, we found that knee CC is more common in the lateral TFJ compartment than in the medial TFJ compartment (Skinner et al 1969; Wilkins et al 1983; Riestra et al 1985; Felson et al 1989; Ramonda et al 2009). The cause underlying this well recognised phenomenon is not well understood.

We also found that CC occurs more frequently in fibrocartilage than in articular hyaline cartilage. This is in keeping with previous observations (McCarty et al 1963; Zitnan et al 1963; Twigg et al 1964; Genant 1976; Fam et al 1981; Wilkins et al 1983), and may be due, at least in part, to the fact that fibrocartilage chondrocytes secrete more PPi than chondrocytes from articular hyaline cartilage (Ryan et al 1981). Other factors such as cartilage matrix changes may also play a role in promoting CC.

We found evidence for systemic predisposition to CC, both on cluster analysis, and on examination of association between CC at distant sites. This persisted after adjusting for confounding factors such as age, gender, BMI and OA at the distant site. This association was observed at all joint pairs and supports the hypothesis that CC, at least in part, results from a systemic predisposition and is not only a consequence of local joint abnormality or structural arthropathy alone. Although patients with poly-articular CC are well recognised (McCarty et al 1963; Twigg et al 1964;
Bocher et al 1965), this is the first systematic study examining the systemic predisposition to CC.

We also found that bilateral CC at the knee was more likely than unilateral CC to associate with CC at distant joints. This association persisted after adjusting for age, gender, BMI and OA at the distant joints. This is a novel observation that suggests the presence of a dose-response relationship between extent of crystal deposition at one site and risk of CC at distant sites, and further supports the view that CC, at least in part, results from a systemic constitutional predisposition rather than being a consequence of local joint pathology alone. Similar associations were observed for bilateral CC at wrists, hips and distant joint CC. However, the adjusted OR for these associations was not statistically significant at the symphysis pubis.

This is the largest systematic radiographic study to examine the overall prevalence of CC using radiographs of knees, pelvis and hands in well characterised participants; and to examine the prevalence of CC at individual joints both in the presence and absence of knee CC. This is also the first study to examine the association between CC at distant joints, and to elicit a dose-response relationship between CC at referent and distant joint. It validates previous observations on the prevalence of CC and on compartmental and cartilaginous localization of knee CC. The findings suggest that CC, at least in part, results from a generalised predisposition and is not a result of local joint disease alone.
However, there are significant caveats to the findings. Firstly, this is a hospital based study carried out by reconstituting case and control group in a cohort assembled to examine risk factors of large joint OA. Thus, the study sample does not resemble a community based population. Two-thirds of participants had severe symptomatic hip or knee OA and the remaining had no radiographic or clinical features of hip or knee OA. The study design therefore limits the generalisability of the overall and joint-specific prevalence of CC. However, the face validity of the results is supported by the fact that the prevalence of CC at the knee (8.0%), knee and pelvis (11.9%), and knee and wrist (11.1%) reported here is in keeping with that observed in large radiographic surveys (Felson et al 1989; Sanmarti et al 1993; Ramonda et al 2009). Secondly, the association between CC at distant joints may be confounded by a systemic predisposition to OA. However, we have tried to minimise this bias by adjusting for OA at the index joint. The dose-response relationship between CC at the referent, and index joints further supports the existence of a systemic predisposition to CC. The results about the intra-articular distribution of CC should also be interpreted with caution as a large proportion of patients had knee OA requiring joint replacement surgery, and CC may not be easily visualised in the affected compartments. However, our findings agree with the other studies providing face validity. Finally, we only ascertained the presence of CC on plain radiographs which are insensitive for the detection of CC (Ellabban et al 2011). The sensitivity of plain radiographs for the detection of CC may be further impaired in those with OA requiring joint replacement. Therefore, the findings should be replicated in a community based study, preferably using ultrasound.