CHONDROCALCINOSIS - RISK FACTORS AND RADIOGRAPHIC PHENOTYPE

by

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Chapter 4 Risk factors for chondrocalcinosis

4.1 Age, gender, body mass index

Aim: The overall aim of this study was to investigate the demographic risk factors for CC. The specific objectives were to examine the association between age, gender, BMI and CC.

Result: Patients with CC at any site were older than those without CC. The mean (S.D.) age of individuals with and without CC was 69.61 (7.90) and 66.15 (6.99) years respectively. The mean (95%CI) age difference was 3.45 (2.67-4.26) years, $p<0.001$. This association between age and CC was independent of knee or hip OA status (Table 1). There was no association between gender and CC (Table 1).

Current BMI was negatively associated with CC at any site (Table 1). However, there was only a small difference in BMI between the two groups. The mean (S.D.) current BMI of individuals with and without CC was 28.73 (4.70) and 29.37 (5.35) kg/m^2 respectively, a mean (95%CI) difference of 0.64 (0.10-1.17) kg/m^2, $p=0.019$. As the negative association between BMI and CC at any site is not widely recognized, we examined current weight and height in those with and without CC. Participants with CC were on an average lighter and shorter than those without CC. The mean (S.D) weight and height of those with CC was 78.53 (14.99) kg, and 1.65 (9.32) m respectively, whereas those without CC weighed 81.48 (16.42) kg, and were 1.67 (9.21) m in height. The mean (95%) difference in weight, and height was 2.95 (1.30-4.60) kg $p<0.001$, and 1.30 (0.36-2.23) cm, $p=0.007$ respectively.

Table 1 Association between age, gender, current body mass index and chondrocalcinosis

	CC +	CC -	OR(95%CI)	aOR (95%CI)[1]
Age (years)				
1st tertile (≤63)	70	954	1.00	1.00
2nd tertile (64-70)	150	840	2.43 (1.81-3.28)	2.06 (1.52-2.80)
3rd tertile (≥71)	211	914	3.15 (2.37-4.18)	2.49 (1.86-3.34)
Gender				
Male	233	1397	1.00	1.00
Female	208	1311	0.99(0.81-1.22)	1.03(0.84-1.27)
BMI (kg/m^2)				
1st tertile (≤26.50)	162	882	1.00	1.00
2nd tertile (26.51-30.82)	133	913	0.79(0.62-1.02)	0.66(0.52-0.86)
3rd tertile (≥30.83)	136	913	0.81(0.63-1.04)	0.67(0.52-0.87)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI(tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip.

Next, we examined the association between current BMI and CC at individual sites, i.e at the knee, hip, symphysis pubis and wrist separately. After adjusting for age, gender, and OA at the index joint there was a negative association between current BMI and CC at the knees and symphysis pubis (Table 2). However, there was no association between current BMI and CC at the wrist or hip.

Table 2 Association between current body mass index and chondrocalcinosis at individual sites

Current BMI	aOR (95%CI)[1]			
	Knee	Hip	Symphysis pub	Wrist
1st tertile (≤26.50)	1.00	1.00	1.00	1.00
2nd tertile (26.51-30.82)	0.71 (0.51-0.97)	0.75 (0.51-1.09)	0.44 (0.28-0.71)	0.84 (0.59-1.19)
3rd tertile (≥30.83)	0.61 (0.44-0.86)	0.71 (0.48-1.05)	0.46 (0.29-0.73)	0.97 (0.69-1.36)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present at the index joint if K&L score was ≥3 at either knee, or ≥2 at either hip, or there was definite joint space narrowing at the wrist.

Key results:

- CC associates with increasing age, and a lower current height, weight, and BMI
- There is no association between CC and gender

Discussion: In this study, patients with CC were older than patients without CC. The mean (S.D.) age difference was 3.45 (2.67-4.26) years. The association between age and CC was independent of OA (knee or hip). This is in keeping with previous studies (Felson *et al* 1989). The OR for associations between age and CC were comparable with that reported in the Framingham study.

In this study, there was no association between gender and CC. There are several conflicting reports about the association between gender and CC. One small primary care based study and one large community based survey suggest that CC occurs more frequently in women than in men after adjusting for age (Sanmarti *et al* 1993; Ramonda *et al* 2009). The association between gender and CC was lost when additional adjustement was carried out for knee OA in the large community based study (Musacchio *et al* 2011). However, two large community based studies suggest that CC is no more common in women than in men after adjusting for age (Felson *et al* 1989; Neame *et al* 2003). None of these studies adjusted for radiographic knee OA. This is the largest radiographic survey of CC to examine the association between gender and CC and to have adjusted for confounding factors such as age, BMI and OA status. Thus, when taken in conjunction with previous large community based studies these findings suggest that there is no convincing evidence that CC associates with any particular gender. However, the findings regarding gender are limited by the fact that cases with OA were gender matched with controls without OA in the GOAL study.

In this study, current BMI associated negatively with CC after adjusting for confounding factors such as age, gender and hip or knee OA. The differences in BMI were of only small magnitude. The mean (95%CI) difference in BMI was only 0.64 (0.10-1.17) kg/m^2. Participants with CC were on an average lighter with a mean (95%CI) difference in weight of 2.95 (1.30-4.60) kg, and shorter with a mean (95%CI) difference in height of 1.30 (0.36-2.23) cm, respectively.

Similar findings have been reported previously. In a community based study from Nottingham, UK, participants with knee CC had a lower BMI than participants without knee CC. However, this was not statistically significant (mean difference (95%CI) 0.5 (-0.3 to 1.3) kg/m^2, p=0.23), and information about weight, and height was not provided (Neame *et al* 2003). In a recent community based study from Italy, participants with CC at knee, hip and symphysis pubis had similar BMI to participants without CC (mean (S.D.) BMI in people with CC was 27.6 (4.7) and in those without CC, 27.8 (4.6) kg/m^2; p non-significant) (Ramonda *et al* 2009). However, in this study participants with CC had a significantly lower weight (mean (S.D.) 67.4 (12.6) kg vs. 69.7 (12.8) kg, p=0.0003), and were significantly shorter in height (mean (S.D.) 156.2 (8.9) cm vs. 158.4 (9.2) cm, p=0.0004) than participants without CC.

Contrary to these reports, in the Framingham study, participants with knee CC had a higher BMI (mean (S.D.) 27(4)) than participants without knee CC (mean (S.D.) 26(4)) (Chaisson *et al* 1996). No statistical tests of significance were carried out, and information about height and weight was

not provided. However, it is important to remember that analyses of association between BMI and CC status in the three studies discussed above were not adjusted for age, gender or for the participants' OA status.

After reviewing findings from previous studies, it seems likely that our finding of a negative association between BMI, weight and CC is valid, and not a chance association. We have found a statistically significant difference in these parameters due to a greater number of cases, which gives this study a higher power.

There is no single explanation for the negative association between BMI and CC. It seems likely that the systemic effects of obesity more than compensates for any increase in synovial fluid PPi levels that would be expected due to mechanical loading in lower limb joints (Graff *et al* 2000). SNPs in *TNAP*, *ANKH*, and *ENPP1*, are associated with BMI, and waist-hip ratio (Korostishevsky *et al* 2010). The authors suggest that increased phosphate levels increases osteocalcin expression, which increases insulin secretion, and sensitivity, thus reducing the fat mass. Moreover, several key cytokines regulating enzymes involved in PPi metabolism are up or down-regulated in obesity (Cottam *et al* 2004). For example obesity associates with elevated TNF-α, IL-1, and IGF1 (Nam *et al* 1997; Cottam *et al* 2004), levels of all of which reduce ePPi concentration. Some of this effect may be compensated by the fact that TGFβ which increases ePPi levels is up-regulated in obesity (Raju *et al* 2006). It is also possible that people with low BMI are more active, and are more prone to suffering knee injury, which may explain the association between CC and low BMI. Moreover, radiographs of

patients with high BMI may be more difficult to score for the presence of CC due to the large amount of soft-tissue which may mask the CC. However, this negative association between BMI and CC has been suggested in other studies, and it is possible that this is a true association the mechanism underlying which is not entirely clear.

We found a negative association between current BMI and CC at the knees and symphysis pubis after adjusting for confounding factors. Although there was no association between current BMI and CC at the wrist or hip, the aORs were in the same direction. The lack of association may be due to a lower power in this group of patients. Taken together these findings support our observation that there is a negative association between current BMI, and CC at any site. However, this finding needs to be replicated in other community based studies.

4.2 Osteoarthritis

Aim: The overall aim of this analysis was to validate the association between CC and OA, and to examine if OA at one joint associates with CC at that joint, at the contra-lateral joint, and at distant joints. The association between nodal GOA and CC was also examined. The specific objectives were to examine the association between:

1) OA at either hip or knee and CC at any site,
2) OA and CC at the same joint,
3) OA at one joint and CC at the opposite joint, regardless of, or independent of OA in the opposite joints,
4) OA at one joint area and CC at a distant joint area regardless of, or independent of OA at the distant joints,
5) nodal GOA and CC at any site,
6) number of compartments with knee OA and knee CC.

Result: There was a strong association between OA at any knee or hip and CC at any site (OR (95%CI) 2.96 (2.24-3.20)). This association persisted after adjusting for age, gender, and current BMI (aOR (95%CI) 2.74 (2.05-3.67)).

On unadjusted analysis, there was an association between CC and OA at the same joint for knees and wrists (Table 3). The association was present when TFJs and PFJs were considered separately. However, there was no association between CC and OA at the hips (Table 3). There was an

association between OA at any MCPJ in one hand and any MCPJ calcification in the same hand on unadjusted analysis (Table 3). These associations persisted after adjusting for age, gender, and current BMI (Table 3).

Table 3 Association between osteoarthritis and chondrocalcinosis at the same joint

	Right		Left	
	OR (95%CI)	aOR (95%CI)[1]	OR (95%CI)	aOR (95%CI)[1]
Knee	2.39 (1.79-3.20)	2.20 (1.62-2.99)	2.78 (2.04-3.79)	2.59 (1.87-3.60)
TFJ	2.01 (1.52-2.67)	1.83 (1.36-2.46)	2.33 (1.73-3.13)	2.22 (1.62-3.03)
PFJ	1.75 (1.29-2.37)	1.59 (1.16-2.19)	1.84 (1.32-2.54)	1.65 (1.17-2.32)
Hip	1.08 (0.73-1.59)	1.05 (0.72-1.55)	0.72 (0.44-1.20)	0.68 (0.41-1.13)
Wrist	4.46 (3.24-6.13)	3.26 (2.33-4.55)	4.42 (3.26-6.00)	3.28 (2.36-4.48)
Any MCPJ	3.37 (2.97-7.38)	2.42 (1.09-5.41)	7.76 (3.85-15.64)	5.58 (2.72-11.47)

[1]Adjusted for age (tertiles), gender (female 1, male 0), and BMI (tertiles).

On unadjusted analysis, there was an association between OA in one joint and CC in the contralateral joint for knees and wrists (Table 4). This persisted after adjusting for age, gender and BMI. After additional adjustment for OA in the contralateral joint, the association between left knee OA and right knee CC, and between OA in one wrist and CC in the contralateral wrist remained significant (Table 4). However, after additional adjustment for left knee OA the association between right knee OA and left knee CC became insignificant (Table 4). When TFJs and PFJs were considered separately there was an association between OA in one joint and CC in the contralateral joint on unadjusted analysis. However, after adjusting for age, gender, BMI and OA in the contra-lateral joint, the association between left knee TFJ OA

and right knee CC was the only one to persist. There was no association between OA in one hip and CC in the contralateral hip (Table 4). The association between OA at any MCPJ in one hand and calcification at any MCPJ in the opposite hand persisted after adjusting for age, gender, current BMI and OA at any MCPJ in the opposite hand (Table 4).

Table 4 Association between osteoarthritis at one joint and chondrocalcinosis at contralateral joint

	Right OA – Left CC[1]			Left OA – Right CC[1]		
	OR (95%CI)	aOR (95%CI)[2]	aOR (95%CI)[3]	OR (95%CI)	aOR (95%CI)[2]	aOR (95%CI)[3]
Knee	2.28 (1.68-3.09)	2.03 (1.47-2.80)	1.22 (0.82-1.83)	2.26 (1.70-3.02)	2.12 (1.56-2.87)	1.53 (1.04-2.24)
TFJ	2.10 (1.56-2.82)	1.88 (1.37-2.56)	1.32 (0.91-1.92)	2.27 (1.71-3.07)	1.71 (1.07-2.92)	1.88 (1.31-2.69)
PFJ	1.46 (1.05-2.03)	1.27 (0.90-1.78)	0.94 (0.61-1.54)	1.61 (1.17-2.21)	1.48 (1.06-2.07)	1.13 (0.74-1.72)
Hip	1.13 (0.73-1.76)	1.07 (0.69-1.66)	1.39 (0.84-2.31)	1.05 (0.70-1.58)	1.01 (0.67-1.51)	0.98 (0.61-1.59)
Wrist	5.02 (3.68-6.83)	3.85 (2.78-5.32)	2.67 (1.81-3.95)	4.00 (2.92-5.48)	2.84 (2.04-3.96)	1.78 (1.19-2.66)
MCPJ	7.41 (3.71-14.82)	5.18 (2.56-10.47)	3.18 (1.31-7.46)	3.42 (1.44-8.12)	2.36 (1.47-5.72)	1.54 (1.25-4.53)

[1]Index joint and opposite side i.e. right knee OA and left knee CC, and vice versa. [2] Adjusted for age(tertiles), gender (female 1, male 0), and BMI(tertiles), and [3] also adjusted for OA on the opposite side.

Next, we examined if OA at one joint associates with CC at distant joints. On unadjusted analysis knee OA associated with CC at wrist, hips, symphysis pubis, and with MCPJ calcification (Table 5). This persisted after adjusting for age, gender, BMI and OA at the distant site. There was no association between hip OA and CC at distant joints (Table 5). Wrist OA associated with knee CC, and with MCPJ calcification, while MCPJ OA associated with wrist

CC after adjusting for age, gender, current BMI and OA at distant joints (Table 5).

Table 5 Association between osteoarthritis at one joint and chondrocalcinosis at distant joints

Osteoarthritis	Chondrocalcinosis	OR (95%CI)	aOR (95%CI)[1]
Knee	Hip	1.99 (1.35-1.67)	1.99 (1.38-2.85)
	Wrist	4.45 (3.14-6.30)	3.27 (2.25-4.74)
	Symphysis pubis	1.83 (1.27-2.63)	2.06 (1.40-6.50)
	MCP	4.73 (2.20-10.17)	2.91(1.40-6.50)
Hip	Knee	1.34(1.04-1.74)	1.30 (0.99-1.70)
	Wrist	1.47 (1.11-1.94)	1.25 (0.92-1.64)
	Symphysis pubis	1.10 (0.78-1.57)	1.08 (0.76-1.53)
	MCP	1.06 (0.78-1.57)	0.89 (0.49-1.62)
Wrist	Knee	3.64 (2.41-5.49)	2.42 (1.58-3.73)
	Hip	1.51 (0.80-2.85)	1.38 (0.72-2.62)
	Symphysis pubis	1.05 (0.48-2.30)	1.03 (0.47-2.26)
	MCP	6.38 (3.18-12.81)	3.86 (1.88-7.93)
MCP	Knee	1.79 (1.32-2.41)	1.14 (0.83-1.57)
	Hip	1.59 (1.09-2.32)	1.38(0.93-2.03)
	Wrist	2.66 (1.97-3.58)	1.48 (1.06-2.04)
	Symphysis pubis	1.19 (0.78-1.85)	1.10 (0.71-1.72)

1 Adjusted for age (tertiles), gender (female 1, male 0), and BMI (tertiles), and adjusted for OA at distant joint area.

The presence of nodes did not increase the risk of CC on their own (Table 6). Both OA (without nodes) and nodal GOA associated with CC. However, the association between nodal GOA and CC was not significantly stronger than the association between OA and CC alone (Table 6). The OR (95%CI) for association between CC and nodal GOA compared to OA alone was 1.26 (0.99-1.62) (unadjusted); and 1.10 (0.85-1.42) after adjusting for age, gender, and current BMI.

Table 6 Association between nodal status and chondrocalcinosis

		CC +	CC -	OR(95%CI)	aOR (95%CI)[1]
Nodes	OA				
-	-	55	805	1.00	1.00
+	-	7	97	0.94 (0.42-2.15)	1.06 (0.47-2.38)
-	+	257	1347	2.65 (1.93-3.62)	2.79 (2.06-3.79)
+	+	110	457	2.94 (2.05-4.22)	3.52 (2.50-4.97)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles).

Increasing number of knee compartments with structural changes of OA associated with CC on that side (p_{trend}= 0.001) (Table 7). This persisted after adjusting for age, gender and BMI. A similar pattern was observed in the TFJs (p_{trend}= 0.008 in the right and 0.024 in the left knee) (Table 8).

Table 7 Association between number of knee compartments with osteoarthritis and knee chondrocalcinosis

	CC +	CC -	OR (95%CI)	aOR (95%CI) [1]
Right knee				
0	64	1412	1	1
1	117	1221	2.11(1.54-2.90)	1.90 (1.37-2.63)
2	27	303	1.97 (1.23-3.14)	1.83 (1.13-2.97)
3	3	16	4.14 (1.18-14.56)	3.90 (1.09-14.01)
			P_{trend} <0.001	P_{trend}=0.001
Left knee				
0	53	1466	1	1
1	115	1231	2.58 (1.85-3.61)	2.38 (1.68-3.36)
2	22	264	2.31 (1.38-3.85)	2.07 (1.21-3.53)
3	0	16	-/-	-/-
			p_{trend} <0.001	p_{trend}=0.001

[1]Adjusted for age (tertile), gender, and BMI (tertile).

Table 8 Association between number of tibio-femoral joint compartments with osteoarthritis and knee chondrocalcinosis

	CC+	CC-	OR (95%CI)	aOR (95%CI) [1]
Right knee				
0	98	1789	1	1
1	109	1121	1.78 (1.34-2.36)	1.59 (1.18-2.13)
2	4	42	1.74 (0.61-4.95)	1.75 (0.61-5.03)
			P_{trend} <0.001	P_{trend}=0.008
Left knee				
0	92	1803	1	1
1	98	1126	1.71 (1.27-2.29)	1.53 (1.13-2.08)
2	0	45	-/-	-/-
			p_{trend} =0.002	p_{trend}=0.024

[1]Adjusted for age (tertile), gender, and BMI (tertile).

Key results:

- OA at knees, wrists, and MCPJs associates with CC at the same and distant joints.
- OA at hips does not associate with CC at the same or distant joints.
- In those with OA, the additional presence of nodes does not associate with CC.
- Knee CC associates with increasing numbers of knee compartments with OA.

Discussion: In this study, we validated the well established association between CC and OA (McCarty *et al* 1963; Zitnan *et al* 1963). Although the initial reports of OA in joints with CC were hospital based and only had older participants, subsequent community based studies showed that at least at the knee, this association is not due to biased recruitment or confounding by age (Felson *et al* 1989; Sanmarti *et al* 1996; Neame *et al* 2003). However, the association between MCPJ calcification and MCPJ OA, and between OA and CC at joints other than knees, and the inter relationship between OA and CC at distant joints are less well documented. Therefore, this is the first systematic study to examine the association between OA and CC at the same joint, OA at one joint and CC at the opposite joint, and OA at one joint area and CC at a distant joint area regardless of, and independent of OA at the distant joints.

This study shows that OA at the knee, wrist, and any MCPJ in one hand, associates with CC at the same joint. However, there was no such association at the hip. The findings of this study are in keeping with those of Ramonda *et al.* who did not find any association between CC in lower limbs (knee or hip) and radiographic or clinical hip OA (Ramonda *et al* 2009). This suggests that the chondrocyte response or cartilage matrix changes secondary to joint insult differs between different joints. It also suggests that OA is a more diverse and heterogeneous disease than is thought otherwise, and perhaps hip OA is distinct from OA at other joints. This is in keeping with observations that OA at different joints associate with different risk factors and has different radiographic phenotype e.g. osteophytes are uncommon at

the wrist, but common at the knee (Lane *et al* 2011; Bierma-Zeinstra *et al* 2011; Kellgren *et al* 1957). Some other studies also show that there is no association between hip OA and hip CC. A small hospital based case control study showed that CC was no more common in hips with OA than in age matched healthy controls (Menkes *et al* 1985). An autopsy study also found CC to be no more common in those with hip OA than in age and gender matched control hips (Sokoloff *et al* 1988).

Both TFJ and PFJ OA associated with knee CC with overlapping OR (95%CI). This suggests that there is no difference in the compartmental location of OA in knees with and without CC. The findings of this study are supported by a previous hospital based study of 300 knees with symptomatic radiographic OA where both isolated PFJ OA and TFJ OA associated with CC or presence of CPP crystals (Ledingham *et al* 1993a). This observation, however, is not in keeping with previous reports which suggest that in knees with CC, OA occurs more commonly at the PFJs than at the TFJs (Resnick *et al* 1977a; Watt 1983). In another hospital based case-control study, knees with CPPD and OA had more frequent involvement of the PFJ than knees with OA alone (Resnick *et al* 1977a), and isolated PFJ OA was particularly common in knees with CPPD and OA when compared to knees with OA alone (Resnick *et al* 1977a).

We found an association between wrist CC, MCPJ calcification and OA changes at the wrists and MCPJs respectively. The association between wrist CC, MCPJ calcification and OA at these joints has only been reported in case series (McCarty *et al* 1962; McCarty *et al* 1963; Zitnan *et al* 1963)

and has never been systematically examined in a large study. Taken together our findings suggest that OA at one joint associates with CC at the same joint, except at the hip.

Left knee OA associated with right knee CC. A similar association was not observed between right knee OA and left knee CC after adjusting for age, gender, BMI and left knee OA. Wrist or MCPJ OA in one hand associated with wrist CC or MCPJ calcification in the opposite hand after adjusting for age, gender, BMI and OA at the opposite joint. There was no association between OA at one hip and CC at the opposite hip. This suggests that OA increases the risk of CC by a generalized effect at wrists & MCPJs and probably also at the knee but not at the hip.

In keeping with these observations, knee OA associated with CC at all sites examined i.e. hips, wrists, symphysis pubis, and with MCPJ calcification, while hip OA did not associate with CC at any site examined after adjusting for age, gender, BMI and OA at the distant site. Wrist OA associated with knee CC and with MCPJ calcification, while MCPJ OA associated with wrist CC after adjusting for age, gender, BMI and OA at distant sites. This further supports the notion that knee, wrist, and MCPJ OA increase the risk of CC by a generalized effect and that hip OA does not increase the risk of CC at any site.

Similar findings have been reported in earlier smaller studies. A primary care based study of 26 CC cases with CC at either knee, wrists, or MCPJ calcification and 104 controls showed that CC at any site associates with OA at the 1st to 3rd MCPJs (aOR(95%CI) 3.1(1.1-8.8) (Sanmarti *et al*

1996). Several hospital based studies suggest that wrist CC associates with trapezio-scaphoid arthropathy (Stucki *et al* 1999; Donich *et al* 2000; Peter *et al* 2001).

Our findings show that nodal GOA (finger nodes + OA at either knees or hips) associates with CC. However, this association is no stronger than that between OA and CC. This suggests that a generalised predisposition to OA conferred by nodes does not increase the risk of CC. This finding is not in keeping with previous small studies which showed an association between CC and GOA (Gerster *et al* 1975a). The findings in this small study were not adjusted for age, gender and BMI. In our study 23.7% of participants with CC had either Heberden's or Bouchard's nodes. This is lower than previous reports from hospital based studies which suggested that Heberden's nodes occur in 46% - 50% of participants with CPPD (Skinner *et al* 1969; Fam *et al* 1981). This may be because previous studies had older patients.

We found that knee CC associates with increasing number of knee compartments with OA changes. This is in keeping with observations from earlier studies where CPPD associated with multi-compartment OA (aOR(95%CI) 3.31(1.61-6.77))(Ledingham *et al* 1993a) and with bi- or tri-compartment OA (Pattrick *et al* 1993). However, in another study bi-compartmental knee OA was similar in frequency in knees with and without CPPD (45.7% *vs.* 50%) and tricompartmental involvement was less common in knee joints with CPPD and OA than in knee joints with OA alone (6.0% *vs.* 34.6%, $p<0.05$) (Resnick *et al* 1977a). In another study of 58 patients with

CPPD uni-, bi-, and tri-compartmental involvement occurred in 70.7%, 25.9%, and in 3.4 % cases respectively (Bjelle *et al* 1974).

The association between OA at one joint and CC at opposite joints and at distant joints has not been examined before. Similarly, this is the first study to systematically examine the association between nodal GOA and CC. However, the results from this study may be confounded by the participants' hip or knee OA status since the participants have been selected for severe symptomatic large joint OA. In order to minimise any confounding we have adjusted for OA at distant sites.

In summary, we found that OA at one joint associates with CC at the same and distant joints except for hip OA. This suggests the presence of a systemic or generalised predisposition in those with OA. However, this predisposition does not exist for hip OA. There is a hierarchy of systemic effects with knee OA, wrist OA, and MCPJ OA having descending influence on the occurrence of CC at distant sites.

4.3 Body mass index and body shape in 20s to 60s

Aim: The aim of this study was to examine the association between BMI and body shape in each decade from the 3rd to 7th decade, and the risk of CC at any site, at either knee, or at either knee or hip. For this analysis, current height and self-reported weight in each decade were used to calculate the BMI.

Results: There was no association between BMI in the 3rd, 4th and 5th decades of life and CC at any site (Table 9). However, there was a negative association between obesity (BMI ≥30 kg/m^2) in the 6th decade and risk of CC at any site (Table 9). Similarly, there was a negative association between being overweight (BMI ≥25 kg/m^2) or obese (BMI ≥30 kg/m^2) in the 7th decade and risk of CC at any site (Table 9).

Table 9 Association between body mass index and chondrocalcinosis at any joint

BMI	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s				
<25 kg/m^2	329	2084	1.00	1.00
≥25-<30 kg/m^2	78	466	1.06 (0.81-1.38)	0.97 (0.74-1.28)
≥30 kg/m^2	18	110	1.04 (0.62-1.73)	0.96 (0.57-1.61)
30s				
<25 kg/m^2	259	1641	1.00	1.00
≥25-<30 kg/m^2	133	786	1.07 (0.86-1.34)	0.98 (0.78-1.24)
≥30 kg/m^2	34	245	0.88 (0.60-1.29)	0.76 (0.51-1.12)
40s				
<25 kg/m^2	198	1277	1.00	1.00
≥25-<30 kg/m^2	173	964	1.16 (0.93-1.44)	1.03 (0.82-1.30)
≥30 kg/m^2	54	431	0.81 (0.59-1.11)	0.74 (0.53-1.03)
50s				
<25 kg/m^2	151	870	1.00	1.00
≥25-<30 kg/m^2	190	1090	1.00 (0.80-1.27)	0.91 (0.72-1.16)
≥30 kg/m^2	78	625	0.72 (0.54-0.96)	0.68 (0.50-0.92)
60s				
<25 kg/m^2	108	496	1.00	1.00
≥25-<30 kg/m^2	178	991	0.83 (0.64-1.07)	0.72 (0.55-0.95)
≥30 kg/m^2	105	647	0.75 (0.56-1.00)	0.61 (0.45-0.83)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip

There was no association between BMI in the 3rd, 4th and 5th decade and knee CC (Table 10). There was a negative association between obesity (BMI ≥30 kg/m^2) in the 5th and 6th decade and risk of CC at either knee (Table 10). Similarly, there was a negative association between being obese (BMI ≥30 kg/m^2) in the 7th decade and risk of CC at either knee (Table 10).

Table 10 Association between body mass index and knee chondrocalcinosis

BMI	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s				
<25 kg/m^2	186	2225	1.00	1.00
≥25-<30 kg/m^2	50	493	1.21 (0.88-1.68)	1.06 (0.76-1.49)
≥30 kg/m^2	10	118	1.01 (0.52-1.97)	0.89 (0.45-1.75)
30s				
<25 kg/m^2	148	1750	1.00	1.00
≥25-<30 kg/m^2	78	840	1.10 (0.83-1.46)	0.97 (0.72-1.30)
≥30 kg/m^2	20	259	0.91 (0.56-1.48)	0.72 (0.44-1.18)
40s				
<25 kg/m^2	111	1362	1.00	1.00
≥25-<30 kg/m^2	106	1031	1.26 (0.96-1.67)	1.03 (0.77-1.38)
≥30 kg/m^2	29	445	0.78 (0.51-1.19)	0.61 (0.40-0.95)
50s				
<25 kg/m^2	84	936	1.00	1.00
≥25-<30 kg/m^2	123	1156	1.19 (0.89-1.59)	1.00 (0.74-1.36)
≥30 kg/m^2	38	664	0.64 (0.43-0.95)	0.56 (0.37-0.85)
60s				
<25 kg/m^2	62	541	1.00	1.00
≥25-<30 kg/m^2	115	1054	0.95 (0.69-1.32)	0.75 (0.53-1.05)
≥30 kg/m^2	58	693	0.73 (0.50-1.07)	0.56 (0.37-0.84)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee.

There was no association between BMI in the 3rd, 4th and 5th decade of life and CC at either the knee or hip (Table 11). There was a negative association between obesity (BMI ≥30 kg/m^2) in the 6th decade and risk of CC at either the knee or hip (Table 11). Similarly, there was a negative association between being obese (BMI ≥30 kg/m^2) in the 7th decade and risk of CC at either the knee or hip (Table 11).

Table 11 Association between body mass index and knee or hip chondrocalcinosis

BMI	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s				
<25 kg/m^2	244	2166	1.00	1.00
≥25-<30 kg/m^2	63	481	1.16 (0.87-1.56)	1.03 (0.76-1.39)
≥30 kg/m^2	13	115	1.00 (0.51-1.68)	0.92 (0.50-1.65)
30s				
<25 kg/m^2	194	1704	1.00	1.00
≥25-<30 kg/m^2	102	816	1.10 (0.85-1.42)	0.98 (0.76-1.27)
≥30 kg/m^2	24	225	0.83 (0.53-1.29)	0.71(0.46-1.12)
40s				
<25 kg/m^2	147	1326	1.00	1.00
≥25-<30 kg/m^2	134	1002	1.21 (0.94-1.55)	1.04 (0.80-1.34)
≥30 kg/m^2	38	447	0.77 (0.53-1.11)	0.69 (0.47-1.00)
50s				
<25 kg/m^2	112	908	1.00	1.00
≥25-<30 kg/m^2	145	1133	1.04 (0.80-1.34)	0.92 (0.70-1.20)
≥30 kg/m^2	57	646	0.72 (0.51-1.00)	0.66 (0.47-0.93)
60s				
<25 kg/m^2	81	522	1.00	1.00
≥25-<30 kg/m^2	136	1033	0.85 (0.63-1.14)	0.73 (0.54-0.99)
≥30 kg/m^2	74	676	0.71 (0.51-0.99)	0.57 (0.40-0.81)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI(tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip

There was a negative association between body shape >5 in the 3rd and 4th decade of life and CC at any site which persisted after adjusting for age, gender and knee or hip OA. There was no association between body shape in the 5th and 6th decade of life and risk of CC at any site (Table 12). However, there was a negative association between body shape >5 in the 7th decade and risk of CC at any site (Table 12).

Table 12 Association between body shape and chondrocalcinosis at any joint

Body shape	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s				
≤5	383	2301	1.00	1.00
>5	47	403	0.70 (0.51-0.97)	0.70 (0.51-0.97)
30s				
≤5	312	1843	1.00	1.00
>5	117	861	0.80 (0.64-1.01)	0.78 (0.62-0.99)
40s				
≤5	209	1309	1.00	1.00
>5	220	1393	0.99 (0.81-1.21)	0.98 (0.79-1.21)
50s				
≤5	145	793	1.00	1.00
>5	275	1817	0.83 (0.67-1.03)	0.81 (0.64-1.01)
60s				
≤5	95	424	1.00	1.00
>5	297	1716	0.77 (0.60-1.00)	0.68 (0.52-0.89)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI(tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip

There was a negative association between body shape >5 in the 3rd decade of life and knee CC which persisted after adjusting for age, gender and knee OA. There was no association between body shape in the 4th, 5th and 6th decade of life and risk of knee CC (Table 13). However, there was a negative association between body shape >5 in the 7th decade and risk of knee CC (Table 13).

Table 13 Association between body shape and knee chondrocalcinosis

Body shape		CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s					
	≤5	224	2457	1.00	1.00
	>5	25	425	0.65 (0.42-0.99)	0.63 (0.41-0.98)
30s					
	≤5	182	1971	1.00	1.00
	>5	67	910	0.80 (0.60-1.07)	0.74 (0.55-1.00)
40s					
	≤5	123	1394	1.00	1.00
	>5	126	1485	0.96 (0.74-1.25)	0.87 (0.67-1.14)
50s					
	≤5	84	853	1.00	1.00
	>5	162	1928	0.85 (0.65-1.12)	0.78 (0.58-1.04)
60s					
	≤5	56	462	1.00	1.00
	>5	180	1832	0.82 (0.69-1.12)	0.69 (0.50-0.96)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee

There was a negative association between body shape >5 in the 3rd and 4th decade of life and knee or hip CC which persisted after adjusting for age, gender and knee or hip OA. There was no association between body shape in the 5th and 6th decade of life and risk of knee or hip CC (Table 14). However, there was a negative association between body shape >5 in the 7th decade of life and risk of knee or hip CC (Table 14).

Table 14 Association between body shape and knee or hip chondrocalcinosis

Body shape		CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
20s					
	≤5	291	2390	1.00	1.00
	>5	32	418	0.63 (0.43-0.92)	0.62 (0.42-0.91)
30s					
	≤5	239	1914	1.00	1.00
	>5	83	894	0.74 (0.57-0.97)	0.70 (0.53-0.91)
40s					
	≤5	155	1362	1.00	1.00
	>5	167	1444	1.02 (0.81-1.28)	0.96 (0.76-1.22)
50s					
	≤5	106	821	1.00	1.00
	>5	209	1881	0.87 (0.68-1.12)	0.82 (0.64-1.06)
60s					
	≤5	69	449	1.00	1.00
	>5	223	1788	0.81 (0.61-1.08)	0.70 (0.52-0.94)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip.

Key results:

- There was a negative association between high BMI in late adult life, and CC at any site, at either knee or hip, and at any knee.
- There was a negative association between body shape >5 in both early, and late adult life and CC at any site, at either knee or hip, and at any knee.

Discussion: There was a negative association between being overweight or obese in the 60s and having CC at any site, at any knee or at either knee or hip. This validates our observation that there is a negative association between current BMI and CC. Moreover, there was a negative association between obesity in the 50s and CC at any site, at any knee or at either knee or hip. There was no association between being overweight in the 50s and CC at any site, at any knee or at either knee or hip. Similarly, there was no association between being overweight or obese in the 20s, 30s or 40s with CC at any site, at any knee or at either knee or hip. This suggests a lack of influence of early life obesity on the subsequent development of CC. This is quite contrary to the fact that early life obesity associates with the development of OA in later life.

We also found a negative association between body shape >5 in the the 60s and CC at any site, at any knee or at either knee or hip. This further supports our finding that there is a negative association between increasing BMI in the 60s and CC at any site, at any knee or at either knee or hip. However, contrary to the findings from the self-reported early life BMI and

risk of CC analysis, we found a negative association between body shape >5 in the 20s and 30s and CC at any site, at either knee or at either knee or hip. This may be as most people who have a body shape >5 in their early adult life continue to have a similar body shape later on.

This is the first report of a negative association between high BMI in later life, increasing body shape in later life and CC. These findings further support the negative association between current BMI and CC. The mechanism underlying this association is unclear but possible contributing factors have been discussed in Chapter 4.1.

4.4 Bone mineral density and soft tissue calcification

Aim: The aim of this analysis was to examine the association between BMD, peri-articular calcification (on knee radiographs), vascular calcifications (on pelvic radiographs) and CC.

Results: There was an association between lower tertiles of metacarpal index (a measure of cortical BMD) and CC at any site (Table 15). However, there was no association between BMD according to calcaneal DEXA (a measure of cancellous BMD) and CC at any site (Table 15).

Table 15 Association between bone mineral density and chondrocalcinosis

	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Metacarpal index				
Tertile 1 (>0.51-0.83)	109	927	1.00	1.00
Tertile 2 (0.43-0.51)	127	913	1.18 (0.90-1.55)	1.04 (0.79-1.38)
Tertile 3 (0.43-0.01)	190	846	1.91 (1.48-2.46)	1.36 (1.02-1.79)
Calcaneal DEXA				
Z-score >1	202	1320	1.00	1.00
Z-score -1 to +1	189	1192	1.04 (0.84-1.28)	1.04 (0.83-1.99)
Z-score <-1	40	192	1.36 (0.94-1.97)	1.35 (0.91-1.99)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip.

There was an association between peri-articular calcification around the knee, and vascular calcification visualised on the pelvic radiograph with CC at any site (Table 16).

Table 16 Association between soft tissue calcification, vascular calcification and chondrocalcinosis

	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Soft –tissue calcification (knee radiograph)				
Absent	355	2458	1.00	1.00
Present	84	253	2.30 (1.75-3.01)	1.67 (1.25-2.23)
Vascular calcification (pelvis radiograph)				
Absent	412	2621	1.00	1.00
Present	33	97	2.16 (1.44-3.26)	1.84 (1.18-2.84)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0). OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip.

The association between CC and pelvic vascular calcification, soft-tissue calcification at the knee, and low cortical BMD are independent of one another (Table 17).

Table 17 Assocation between a calcifying phenotype and chondrocalcinosis

Risk factor	aOR (95%CI)[1]
Peri-articular calcification (knee radiograph)	1.85 (1.38-2.46)
Vascular calcification (pelvis radiograph)	1.75 (1.12-2.73)
Cortical BMD	
1st tertile	1.00
2nd tertile	0.74 (0.57-0.95)
3rd tertile	0.70 (0.53-0.93)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and OA (present 1, absent 0), and for each other. OA was defined as present if K&L score was ≥3 at either knee, or ≥2 at either hip.

Key results:

- There is a negative association between cortical BMD and CC.
- There is an association between vascular calcification, soft-tissue calcification, and CC.

Discussion: We found a negative association between cortical BMD and CC. This is a novel finding that has not been reported before. The negative association persisted after adjusting for confounding factors such as age, gender, BMI, and knee or hip OA. This negative association between CC and MCI – a measure of cortical BMD - is particularly interesting. This is as OA which associates with CC has variously been associated with either a high (Nevitt *et al* 1995; Jones *et al* 1995; Arden *et al* 1999; Sower *et al* 1999; Bergink *et al* 2003) or a normal BMD in cross-sectional studies (Hannan *et al* 1993; Hochberg *et al* 1994; Schneider *et al* 2002). However, progression of OA is associated with loss of BMD in longitudinal studies. For example, in a prospective study, women who subsequently developed hand OA had higher MCI at baseline but had a greater loss of MCI than women who did not develop hand OA (Sowers *et al* 1991). Similar findings were reported from the Rotterdam study (Burger *et al* 1996), the Chingford study (Bettica *et al* 2002), and more recently by Guler-Yuksel *et al* 2011. In keeping with these studies knee OA patients with high BMD or a gain in BMD had a lower risk of progression of knee OA in the Framingham study (Zhang *et al* 2000). Thus, the association between CC and low MCI suggests that CC identifies a subset of patients who have had a rapid progression of their OA.

To understand the reasons underlying this association between CC and low BMD, it is important to revisit the biochemical basis of CPPD; the effect of PPi on crystal nucleation and growth; and the in-utero bone deposition. CPPD results from high synovial (or interstitial) fluid PPi levels. This ePPi is predominantly produced by articular chondrocytes, and articular

chondrocytes from joints with CPPD secrete more PPi than those from joints without CPPD (Ryan *et al* 1981; Rosenthal *et al* 1993). High ePPi levels inhibit hydroxyapatite and other BCP crystal nucleation and growth (Cheng *et al* 1983; Thouverey *et al* 2009). Provided the high PPi secreting phenotype of the articular chondrocytes is also present in the foetal pool of chondrocytes, bones in people who later develop CPPD will be exposed to high interstitial fluid PPi when they are being first deposited by the process of enchondral ossification in-utero - except for some parts of the skull and some flat bones like the clavicle which are deposited by intra-membranous ossification. This phenomenon is likely to be present in-utero as the articular and growth plate chondrocytes arise from a common pool of embryonic chondrocytes. This may therefore predispose the individual to a low BMD (Cheng *et al* 1983; Thouverey *et al* 2009).

There was a trend towards a negative association between low trabecular BMD and CC. However, this was not statistically significant. The fact that CC associates with low cortical BMD but not with low trabecular BMD may be related to the fact that patients with CPPD have a high middle fragment PTH concentration and high PTH preferentially associates with loss of mineral content from the cortical bone (Pawlotsky *et al* 2008; Mosekilde 2008). The elevated concentration of middle fragment PTH in patients with CPPD who are not known to have hyperparathyroidism, suggests that patients with CPPD may have high SF PPi levels due to a local functional ‘hyperparathyroidism’ at the tissue level (Pawlotsky *et al* 2008). As hyperparathyroidism associates predominantly with loss of cortical bone

(Mosekilde 2008), this explains our finding of a statistically significant negative association between cortical BMD and CC, but not between cancellous BMD and CC. For the same reasons this difference in association may be due to a type II error, and a large sample size may be needed to detect statistically significant differences in trabecular BMD between those with and without CC.

We found an association between CC at any site and peri-articular calcification around the knees. This persisted after adjusting for age, gender, BMI and hip or knee OA. Soft-tissue calcification has been reported in between 13.5% – 40.0% patients with CPPD (**Error! Reference source not found.**) which is not dissimilar to our finding that 23.7% patients with CC have peri-articular soft-tissue calcification at the knee. In an age-matched hospital based case-control study carried out those with GOA, tendon calcification associated with CC (Gerster *et al* 1977). In another age and gender matched study, tendon calcification associated with CC plus OA (Gerster *et al* 1984). However, the findings from this study may be confounded by OA. Our study is larger than the earlier studies and we have adjusted for possible confounding factors such as age, gender, BMI and OA. These findings suggest that patients with CC have a tendency towards calcification which is not restricted to the cartilage itself and which may involve both BCP and CPP crystal deposition.

We found an association between CC at any site and vascular calcification on pelvic radiographs. This persisted after adjusting for age, gender, BMI, and hip or knee OA. In a previous study using knee

radiographs, extra-articular vascular calcification associated with knee CC (OR (95%CI) 2.27 (1.45-4.13)), however, this association was lost after adjusting for age (aOR (95%CI) 1.43 (0.77-2.64)) (Neame *et al* 2003). Other hospital-based case series have also reported vascular calcification in patients with CPPD. However, these studies lack a control group and are confounded by age. This is the largest study to examine the association between CC and vascular calcification and to adjust for confounding factors such as age, gender, BMI and knee or hip OA. Our findings suggest that patients with CC have a tendency for calcification which is not restricted to the cartilage or to the soft-tissues but extends to the vasculature as well. This suggests the presence of a calcifying phenotype. This is in keeping with the bone forming phenotype described by Rogers *et al* (Rogers *et al* 1997; Rogers *et al* 2004). In two large skeletal studies using more than 300 cadevera each they reported an association between osteophytosis and enthesophyte formation which was independent of age, gender, and BMI (Rogers *et al* 1997; Rogers *et al* 2004). However, we did not examine the association between enthesophytosis and CC in this study. Indeed, the tendency to calcification may extend to predispose the participant to the formation of salivary and renal stones. Therefore, a study including information about enthesophytosis, stone formation, and calcification at other sites e.g. the shoulder, is required to confirm the association between a calcifying phenotype and CC.

We report an association between CC and vascular calcification. Vascular calcification results from hydroxyapatite deposition in intima (in

atherosclerosis) and media (in age related arteriosclerosis, diabetes, and end-stage renal failure) of blood vessels. Vascular calcification may occur due to a combination of the following - deficiency of calcification inhibitors (e.g. matrix Gla protein, osteopontin, PPi); osteochondrogenic differentiation of vascular smooth muscle cells driven by lipids, phosphate, and inflammations; apoptosis which results in formation of apoptotic bodies that serve as foci for crystal deposition; matrix degradation by matrix metalloproteinases, and elastolysis; circulating pro-nucleating factors; and altered calcium phosphate homeostasis (Giachelli 2009). It is possible that high extracellular PPi in patients with CC is hydrolyzed to phosphate in vascular wall which is transported intracellularly (by Type 3 Na dependent PiT-1 protein) promoting an osteochondral transformation of the vascular smooth muscle cells thus resulting in vascular calcification. Elevated extracellular phosphate is a key driver of osteochondral differentiation of the vascular smooth muscle cells (Giachelli 2009). Alternatively, a circulating pro-nucleating factor, and lack of a crystallization inhibitor may explain the association between vascular calcification, and CC. The association between vascular calcification and CC cannot be explained on the basis of elevated extracellular PPi alone as elevated PPi itself would be expected to inhibit vascular calcification by preventing calcium crystal nucleation and growth; and by inhibiting the osteochondral differentiation of vascular smooth muscle cells (Johnson *et al* 2006). The validity of these findings is also supported by reports of negative association between BMD and arterial calcification (Schulz *et al* 2004; Naves *et al* 2008). As shown in table 38, the association between BMD, vascular calcification and CC is not due to confounding alone.

4.5 Diuretics

Aim: The overall aim of this study was to establish the association between exposure to thiazide or loop diuretics and CC at any site. The specific objectives were to:

1) investigate the association between exposure to thiazide or loop diuretics and CC, and
2) examine the relation between number of years on thiazide or loop diuretics and CC.

Methods: Individuals who were on a diuretic for less than 3 month, those who received potassium sparing diuretics alone, and those who were unsure of the name of their diuretic were excluded from this analysis. Information about the diuretic's name, month and year of starting and stopping treatment was obtained from the GOAL database. This was used to calculate the number of years on each diuretic. The duration of treatment with loop or thiazide diuretics were summated for each individual. In order to examine the dose-response relation between number of years on thiazide or loop diuretics and risk of CC, the duration of exposure to thiazide or loop diuretics was summated and converted to tertiles.

Results: 925 individuals reported diuretic use for more than 3 months. Of these, 45 who were unsure of the diuretic's name, and 7 who were on potassium sparing diuretic alone were excluded from the analysis. Fifty-one individuals received two, and two individuals received three different diuretic treatment cycles. On unadjusted analysis, exposure to loop or thiazide diuretics associated with CC (Table 18). However, there was no association

between exposure to loop or thiazide diuretics and CC after adjusting for age, gender, BMI and knee or hip OA (Table 18). Similarly, there was no association between duration of exposure to diuretics and CC at any site (Table 18).

Table 18 Association between diuretic use and chondrocalcinosis

Risk factor		CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Diuretic					
None		282	1948	1.00	1.00
Loop or thiazide		141	724	1.35 (1.08-1.68)	1.12 (0.89-1.42)
Number of years on loop or thiazide diuretic					
Tertile 1	≤2 years	33	199	1.00	1.00
Tertile 1	3-6 years	58	259	1.35 (0.85-2.15)	1.29 (0.80-2.08)
Tertile 1	≥7 years	46	254	1.09 (0.67-1.77)	0.98 (0.60-1.62)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and knee or hip OA (K&L score ≥3 at either knee, or ≥2 at either hip).

Key result:

- No association between diuretic exposure and CC after adjusting for age, gender, BMI, and OA

Discussion: There was no association between exposure to loop or thiazide diuretics and CC. There was an association between exposure to loop or thiazide diuretic and CC at any site on univariate analysis but this became non-significant after adjusting for age, gender, BMI and knee or hip OA. The findings of this study are not in agreement with those of a previous community based case-control study where diuretic use associated with knee CC (OR (95%CI) 2.69 (1.38-5.27)), and the association still remained significant after adjusting for age and gender (aOR (95%CI 2.07 (1.02-4.19))(Neame *et al* 2003). However, adjustment for BMI, which associates with hypertension and congestive cardiac failure, two of the commonest indications for diuretics, and adjustments for OA which associates with CC were not carried out in this study. Therefore, the findings from the earlier study may be confounded by BMI and OA.

The association between diuretic use and CC is hypothesisied to be mediated by diuretic-induced hypomagnesemia. As potassium sparing diuretics do not lead to hypomagnesemia they were excluded from this analysis, *a priori*. However, the findings from this analysis were unchanged when patients on potassium sparing diuretics alone were included in this analysis. Our findings therefore suggest that there is no true association between CC and diuretic use.

This lack of association between diuretic use and CC observed in our study is supported by a lack of any dose-response relation between number of years on loop or thiazide diuretics and risk of CC. However, the results of our study need to be interpreted with caution since this is a hospital based

study where controls were selected from other departments including renal and cardiovascular departments which have higher risk of using diuretics and further community based studies are necessary to draw a firm conclusion about the association between diuretic use and CC.

4.5 Local risk factors for knee chondrocalcinosis

4.5.1 Injury and surgery

Aim: The overall aim of this study was to examine the association between any category of knee trauma and knee CC. The specific objectives of this analysis were to examine the association between 1) previous arthroscopy (with or without meniscectomy), 2) previous meniscectomy, 3) self-reported significant knee injury and knee CC.

Results: There was an association between previous arthroscopy (with or without meniscectomy), previous meniscectomy, and CC at the same knee on joint-based analysis (Table 19). This persisted after adjusting for age, gender, BMI, and knee OA. A similar association was observed on person-based analysis (Table 19).

Table 19 Association between knee surgery and knee chondrocalcinosis

	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Right knee				
Arthroscopy +/- meniscectomy				
-	170	2643	1.00	1.00
+	39	288	2.11 (1.46-3.04)	2.00 (1.45-2.75)
Left knee				
Arthroscopy +/- meniscectomy				
-	172	2869	1.00	1.00
+	16	83	1.79 (1.19-2.66)	1.59 (1.04-2.05)
Either knee				
Arthroscopy +/- meniscectomy				
-	193	2548	1.00	1.00
+	57	338	2.18 (1.63-2.92)	1.75 (1.28-2.40)
Right knee				
Meniscectomy				
-	191	2845	1.00	1.00
+	18	86	3.12 (1.84-5.29)	2.31 (1.33-4.01)
Left knee				
Meniscectomy				
-	172	2869	1.00	1.00
+	16	83	3.22 (1.84-5.61)	2.62 (1.45-4.72)
Either knee				
Meniscectomy				
-	219	2737	1.00	1.00
+	31	149	2.60 (1.72-3.90)	1.93 (1.25-2.98)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and knee OA (K&L score ≥3 at index or either knee as appropriate)

There was an association between self-reported significant joint injury in one knee and CC at the same joint on joint-based analysis (Table 20). This persisted after adjusting for age, gender, BMI and knee OA. A similar association was observed on person-based analysis (Table 20).

Table 20 Association between knee injury and knee chondrocalcinosis

		CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Right knee Injury					
	-	163	2561	1.00	1.00
	+	46	370	1.95 (1.38-2.76)	1.69 (1.18-2.42)
Left knee Injury					
	-	153	2640	1.00	1.00
	+	35	312	1.94 (1.32-2.85)	1.67 (1.12-2.02)
Either knee Injury					
	-	170	2278	1.00	1.00
	+	80	608	1.76 (1.33-2.33)	1.51 (1.12-2.02)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and knee OA (K&L score ≥3 at index or either knee as appropriate).

Key results:

- Self-reported meniscectomy, arthroscopy (with or without meniscectomy), and self-reported knee injury associates with knee CC independent of age, gender, BMI, and knee OA.

Discussion: There was an association between self-reported arthroscopy (with or without meniscectomy), meniscectomy, and knee injury with CC at the same joint. This persisted after adjusting for confounding factors such as age, gender, BMI, and OA at that joint.

The previously reported association between prior meniscectomy in one knee and CC at the same joint was validated (Doherty *et al* 1982). In that study there was a five-fold increase in the risk of CC in knee joints with previous meniscectomy compared to the opposite knee after a mean follow-up of 25 years, (Doherty *et al* 1982). Similar results were found on comparing the index knee with a past history of meniscectomy with data from an age and gender matched control group (Doherty *et al* 1982).

Two case series also support an association between previous meniscectomy and knee CC. In a hospital based case series, 18 of 76 patients with knee CC were young (mean age 43.1 years) and all 18 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 patients only and an underlying predisposition to CC was absent (de Lange *et al* 1985). Similarly 65.5% of 85 patients with CPPD identified in a review of

3,228 arthroscopies had a past history of knee injury, meniscectomy or other knee surgery (Fisseler-Eckhoff *et al* 1992).

There was an association between self-reported significant knee injury and knee CC which persisted after adjusting for age, gender, BMI and knee OA. This is supported by previous reports that patients who develop knee CC at a young age almost always have a prior history of knee injury (Fisseler-Eckhoff *et al* 1992). Several small case series also suggest that repeated joint injury in the setting of generalised (Bird *et al* 1978), and localised (Settas *et al* 1982) hypermobility lead to CPPD. However, all joints with CPPD in these two studies also had changes of knee OA suggesting that CPPD may be secondary to OA rather than a direct result from joint injury. Thus, the present study is the first large study to systematically examine the association between self-reported significant knee injury and risk of CC. Moreover, in this study we adjusted for knee OA so the findings are unlikely to be confounded by OA.

There are several mechanisms by which knee injury, arthroscopy or meniscectomy may lead to CC. These may all lead to chondrocyte death or cartilage degeneration which in turn may serve as a nidus for crystal deposition. Moreover, meniscectomy alters the load distribution across the knee (Song *et al* 2008). This leads to 50% increase in mechanical load on the central parts of the tibial articular hyaline cartilage (Song *et al* 2008). As mechanically loaded chondrocytes secrete more PPi, this may lead to CPPD (Graff *et al* 2007). Moreover, the central weight-bearing tibial hyaline articular cartilage becomes dessicated and chronically deformed (Song *et al* 2008).

Finally, meniscectomy itself leads to knee OA (Englund *et al* 2009) and OA associates with CC. The association between meniscectomy and CC could therefore be mediated by OA. However, this is unlikely to be the sole reason for the association between meniscectomy and knee CC since we adjusted for knee OA.

There are several caveats to this study. Firstly, information about knee injury, arthroscopy, and meniscectomy was self-reported and a validation by a search of medical records was not attempted. Secondly, significant inter-personal variation is likely to exist in the interpretation of significant knee injury. It is also possible that some people who self-report significant knee injury actually had injury to peri-articular structures. Moreover, information about self-reported knee injury, arthroscopy and/or meniscectomy may be affected by recall bias.

4.5.2 Mechanical knee loading

Aim: The overall aim of this analysis was to examine the association between knee malalignment, occupational knee loading and knee CC. The specific objectives were to examine the association between:

1) self-reported early adult life frontal plane knee malalignment,
2) self-reported current knee malalignment,
3) current radiographic knee malalignment,
4) occupational risk factors for knee loading,

and knee CC.

Methods: Current knee malalignment and knee malalignment in their 20s was self-reported separately using a validated line diagram instrument (Ingham *et al* 2010). Knee radiographs were scored for frontal plane knee malalignment using the methods described by Krause *et al* (Kraus *et al* 2005). Intra-class coefficient (ICC) was calculated to estimate the intra-rater reliability for measurement of frontal plane knee mal-alignment. Once knee radiographs from the first 100 GOAL participants (serial numbers 0 - 100) had been scored for frontal plane knee malalignment, 20 knee radiographs belonging to 10 consecutive GOAL participants were randomly selected for repeat measurements. This process was repeated for 10 consecutive participants between GOAL serial numbers 1500-1600, and 3000-3100. Repeat measurements were performed on the same work-station five days after initial measurements with the reader being unaware of the readings from the first session.

Results: The *k* statistic for intra-rater reliability for the measurement of frontal plane knee malalignment were: 1st set (GOAL serial number 0-100) 0.98 (0.95-0.99); 2nd set (GOAL serial number 1500-1600) 0.96(0.89-0.98); and 3rd set (GOAL serial number 3000-3100) 0.99 (0.97-1.00).

Self-reported frontal plane knee malalignment in the 20s associated with knee CC on both unadjusted and adjusted analysis (Table 21). Further analysis was carried out to examine the association between self-reported varus or valgus knee mal-alignment in the 20s and knee CC. While there was an association between self-reported varus knee malalignment in the 20s and knee CC there was no association between self-reported valgus knee malalignment in the 20s and knee CC (Table 21). There was no association between self-reported current frontal plane knee malalignment and knee CC (Table 21). Similarly, there was no association between self-reported current varus or valgus knee malalignment and knee CC (Table 21).

Table 21 Association between self-reported knee malalignment and knee chondrocalcinosis

	CC+	CC-	OR (95%CI)[1]	aOR (95%CI)[2]
Knee alignment in 20s				
Straight	121	1669	1	1
Any malalignment	26	166	2.16 (1.37-3.40)	2.10 (1.32-3.35)
Varus knee	22	124	2.45 (1.50-3.99)	2.33 (1.40-3.88)
Valgus knee	4	42	1.31 (0.46-3.72)	1.29 (0.44-3.74)
Knee alignment current				
Straight	130	1692	1	1
Any malalignment	26	289	1.17 (0.76-1.82)	1.17 (0.75-1.82)
Varus	18	153	1.53 (0.91-2.58)	1.37 (0.81-2.34)
Valgus	8	136	0.77 (0.37-1.60)	0.85 (0.40-1.80)

[1] Adjusted for age, gender, knee OA (K&L score ≥3 at either knee), and BMI in 20s or current BMI as appropriate.

For the analysis reported in table 42 above, participants self-reported a single measure of frontal plane knee malalignment which was derived from, and applied to, both their knees (i.e. varus, neutral, or valgus). While knee alignment in early adult life is symmetrical in the great majority of cases the same may not be true of knee alignment in late life, especially in patients with knee OA. Therefore we measured frontal plane knee malalignment for all GOAL participants in order to ensure that the lack of association between current knee mal-alignment and CC is not confounded by a difference in knee alignment between the two knees. This allowed a joint-based analysis of association between current frontal plane knee malalignment and knee CC.

Current radiographic varus but not valgus knee malalignment was associated knee CC. However, the association was dependen on knee OA (Table 22).

Table 22 Association between radiographic knee malalignment and knee chondrocalcinosis

Knee malalignment	CC +	CC -	OR (95%CI)	aOR (95%CI)[1]	aOR (95%CI)[2]
Right knee					
Straight (-2^o to $+2^o$)	101	1759	1	1	1
Any varus or valgus	98	1114	1.53 (1.15-2.04)	1.38 (1.03-1.86)	1.00 (0.72-1.39)
Varus ($<-2^o$)	81	886	1.59 (1.18-2.16)	1.42 (1.04-1.95)	0.99 (0.70-1.41)
Valgus ($>2^o$)	17	228	1.30 (0.76-2.21)	1.26 (0.72-2.10)	0.96 (0.55-1.67)
Left knee					
Straight (-2^o to $+2^o$)	78	1746	1	1	1
Any varus or valgus	107	1155	2.07 (1.54-2.80)	1.96 (1.44-2.69)	1.41 (0.99-1.99)
Varus ($<-2^o$)	92	934	2.21 (1.61-3.01)	2.09 (1.50-2.89)	1.39 (0.97-2.01)
Valgus ($>2^o$)	15	221	1.52 (0.86-2.69)	1.48 (0.83-2.66)	1.16 (0.64-2.11)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and [2] also for knee OA (K&L score ≥3 at either knee).

Next, the absolute deviation from neutral alignment was compared between knees with and without CC (Table 23). On unadjusted analysis knees with CC showed a greater deviation from neutral than knees without CC. The difference was statistically significant after adjusting for age, gender and BMI using covariate analysis. However, after further adjustment for knee OA the magnitude of absolute deviation was not significantly different in knees with and without CC.

Table 23 Magnitude of knee malalignment and chondrocalcinosis

	Magnitude of deviation in degrees from the neutral (+/-180 degrees)		
	Mean (95% CI)	Mean (95% CI)[1]	Mean (95% CI)[2]
Right knee			
CC +	3.24 (2.88-3.61)	3.10 (2.74-3.46)	2.67 (2.36-2.99)
CC -	2.43 (2.33-2.53)	2.44 (2.35-2.53)	2.46 (2.38-2.54)
Left knee			
CC +	3.27 (2.89-3.65)	3.12 (2.75-3.50)	2.57 (2.25-2.90)
CC -	2.45(2.35-2.55)	2.46 (2.36-2.55)	2.49 (2.40-2.57)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and [2] also for knee OA (K&L score ≥3 at either knee).

The presence of an occupational risk factor associated with knee CC (Table 24). Of the five occupational risk factors considered, kneeling and heavy work whilst standing associated with knee CC whereas squatting, lifting 25 kg loads and lifting very heavy loads did not associate with CC (Table 24).

Table 24 Association between occupational risk and knee chondrocalcinosis

		CC +	CC -	OR (95%CI)	aOR (95%CI)[1]
Kneeling					
	-	186	2363	1.00	1.00
	+	64	528	1.56 (1.15-2.10)	1.37 (1.00-1.89)
Squatting					
	-	162	1982	1.00	1.00
	+	88	904	1.19 (0.91-1.56)	1.10 (0.42-1.46)
Heavy work while standing					
	-	186	2358	1.00	1.00
	+	64	528	2.57 (1.15-2.15)	1.45 (1.06-2.00)
Lift 25 kg lots					
	-	191	2316	1.00	1.00
	+	59	570	1.26 (0.92-1.70)	1.15 (0.83-1.61)
Lift very heavy					
	-	200	2454	1.00	1.00
	+	50	432	1.42 (1.03-1.97)	1.35 (0.95-1.92)
Any risk factor					
	-	120	1646	1.00	1.00
	+	130	1240	1.44 (1.11-1.86)	1.37 (1.04-1.81)

[1]Adjusted for age (tertiles), gender (female 1, male 0), BMI (tertiles), and knee OA (K&L score ≥3 at either knee).

Key results:

- Early-life but not current knee malalignment associates with knee CC.
- Occupational knee loading associated with knee CC.

Discussion: This study shows that self-reported varus knee malalignment in the 20s is associated with increased risk of subsequent development of knee CC. The association between self-reported varus knee malalignment in the 20s and knee CC was independent of age, gender, BMI in the 20s and knee OA. However, there was no association between current knee malalignment and knee CC, suggesting that knee malalignment is less likely to be a concurrent condition or a consequence of the knee CC. Biomechanically demanding occupational risks also associated with knee CC.

The association between early adult life knee malalignment and knee CC has not been studied before. Two small case-control studies and a hospital-based case series give conflicting results for the association between current knee mal-alignment and knee CC. In a survey of 58 ambulatory active residents of a home for the elderly (>70 years) varus knee mal-alignment (>10^{o}) associated with CC (predominantly at the knee) (Ellman *et al* 1975). There was no difference in prevalence of clinical knee arthropathy between the groups (Ellman *et al* 1975). In a hospital based case series of acute geriatric inpatients 31% of participants with CPPD had varus, valgus or fixed flexion deformities at the knee (Wilkins *et al* 1983). However, a subsequent case-control study of 45 CC cases and 23 OA controls did not find an association between current knee mal-alignment and knee CC (Hansen *et al* 1984).

We did not find an association between self-reported valgus knee alignment in the 20s and knee CC. This may be due to the small number of participants (n=46) who reported valgus knee mal-alignment in their 20s. We

also showed an association between knee loading due to occupational use and knee CC on multivariate analysis.

There are several caveats to this study. Firstly, this is a case-control study executed by reconstituting the study groups of GOAL, which was primarily designed to identify risk factors for severe large joint OA. Therefore, this sample does not represent the general population. Secondly, as there is a high prevalence of knee OA and related risk factors the result may be confounded. However, we adjusted for knee OA and related risk factors to minimise any bias. Thirdly, there were only 162 knee CC cases which reduced the study power. Finally, information about knee malalignment in the 20s, weight in the 20s and occupational exposure were self-reported and potentially subject to inaccuracy and recall bias. Because of such caveats it is desirable for this observation to be confirmed in other studies.

In conclusion, early life varus knee mal-alignment associates with knee CC. This is not restricted to the mechanically loaded tibiofemoral compartment and is independent of age, knee OA and other risk factors.

4.6. Genetic risk factors

Aim: The overall aim of this analysis was to examine the association between SNPs in genes involved in:

1. PPi metabolism – *ANKH, TNAP, PC1*

2. Iron overload - *HFE* and *transferrin* gene, and CC.

Results: Seventeen SNPs were selected for genotyping (**Error! Reference source not found.**). The rationale for the selection of these SNPs has been discussed previously. Genotyping was carried out using the Taqman^{R} assay and was outsourced to AstraZeneca and Kbiosciences. All SNPs were in Hardy-Weinberg equilibrium (Table 25). Two SNPs - *TNAP* 571 in *TNAP* and rs28933977 in *ENPP1* - had a minor allele frequency of 0.3% and 1.2% respectively. By definition, SNPs are present in ≥5% of the population tested. As these two were present in <5% of the GOAL population, they were regarded as mutations for the purpose of this study and not as SNPs.

Table 25 Hardy-Weinberg equilibrium of selected single nucleotide polymorphisms

Gene	SNP	Minor allele frequency	Chi-square[1]	*P*
ANKH				
	-4bp G to A	0.09	2.50	>0.05
	rs3045	0.1	1.79	>0.05
	rs39968	0.30	1.50	>0.05
	rs875525	0.26	0.06	>0.05
TNAP				
	rs3200254	0.11	1.9	>0.05
	rs4654760	0.07	0.02	>0.05
	TNAP571	0.01	0.01	>0.05
ENPP1				
	rs858342	0.25	3.71	>0.05
	rs943003	0.42	1.78	>0.05
	rs1044498	0.13	1.24	>0.05
	rs1800949	0.26	0.04	>0.05
	rs28933977	0.04	0.29	>0.05
HFE				
	rs1800562	0.08	0.03	>0.05
	rs1799945	0.15	0.06	>0.05
TF				
	rs1799852	0.09	0.65	>0.05
	rs2280673	0.36	0.92	>0.05
	rs3811647	0.35	0.48	>0.05

[1] degrees of freedom (d.f.) = 2

On univariate analysis two of the four SNPs in *ANKH* (-4bpG>A 5'UTR, and rs3045) associated with CC (Table 26). This persisted after adjusting for age, gender, BMI and knee or hip OA. The adjusted genotype OR (95%CI) for association between one additional minor allele of -4bp G>A 5'UTR in *ANKH* and CC at any site was 1.39 (1.08-1.77), p=0.009. However, the association became statistically insignificant after Bonferroni correction for multiple testing was applied (p=0.135).

Similarly, the adjusted genotype OR (95%CI) for association between an additional minor allele of rs3045 in *ANKH* gene and CC at any site was 1.32 (1.05-1.66), p=0.018. The association became statistically insignificant after Bonferroni correction for multiple tests was applied (p=0.270).

Table 26 Univariate analysis of association between single nucleotide polymorphisms in *ANKH* and chondrocalcinosis

SNP	CC +			CC -			Genotype OR (95%CI)	P
	1:1	1:2	2:2	1:1	1:2	2:2		
-4bpG>A 5'UTR	347	89	4	2255	414	14	1.36 (1.07-1.73)	0.013
rs3045	333	102	5	2163	496	23	1.28 (1.02-1.60)	0.030
rs39968	224	189	30	1269	1161	246	0.89 (0.76-1.05)	0.160
rs875525	232	173	38	1479	1035	170	0.89 (0.76-1.04)	0.152

On univariate analysis none of the two SNPs in *TNAP* selected for genotyping associated with CC (Table 27).

Table 27 Univariate analysis of association between single nucleotide polymorphisms in *TNAP* and chondrocalcinosis

SNP	CC +			CC -			Genotype OR (95%CI)	P
	1:1	1:2	2:2	1:1	1:2	2:2		
rs3200254	342	82	7	2109	510	38	0.99 (0.79-1.25)	0.957
rs4654760	386	58	1	2358	330	12	1.01 (0.76-1.35)	0.955

On univariate analysis none of the five SNPs in *ENPP 1* selected for genotyping associated with CC (Table 28).

Table 28 Univariate analysis of association between single nucleotide polymorphisms in *ENPP1* and chondrocalcinosis

SNP	CC +			CC -			Genotype OR (95%CI)	P
	1:1	1:2	2:2	1:1	1:2	2:2		
rs1044498	324	108	6	2037	593	37	1.07 (0.86-1.32)	0.564
rs28933977	406	30	1	2465	216	3	0.81 (0.55-1.20)	0.826
rs1800949	245	167	30	1457	1034	182	0.98 (0.83-1.16)	0.298
rs858342	251	163	24	1482	1043	151	0.92 (0.78-1.10)	0.365
rs943003	162	202	79	924	1287	486	1.06 (0.86-1.32)	0.401

On univariate analysis none of the two SNPs in *HFE* commonly associated with haemochromatosis associated with CC (Table 29). Similarly none of the three SNPs in transferrin gene which result in increased total body iron stores associated with CC.

Table 29 Univariate analysis of association between single nucleotide polymorphisms in *HFE, transferrin* and chondrocalcinosis

Gene	SNP	CC +			CC -			Genotype OR (95%CI)	p
		1:1	*1:2*	*2:2*	*1:1*	*1:2*	*2:2*		
HFE									
	rs1800562	376	61	4	2271	391	12	1.01 (0.77-1.33)	0.937
	rs1799945	301	124	13	1939	673	55	1.20 (0.99-1.46)	0.070
Transferrin									
	rs1799852	367	63	4	2162	442	26	0.89 (0.69-1.15)	0.372
	rs2280673	189	197	54	1115	1190	344	0.97 (0.83-1.12)	0.664
	rs3811647	170	205	54	1122	1211	312	1.08 (0.92-1.26)	0.348

Key results:

- There was no association between SNPs in genes involved in PPi metabolism and CC.

Discussion: There was no evidence for any association between SNPs in *ANKH*, *TNAP*, *ENPP1*, *HFE* and *transferrin* genes and CC after adjusting for possible confounding factors and correction for multiple testing.

We found an association with CC at any site for two of the five SNPs in the ANKH gene (-4bpG>A 5'UTR, and rs3045) on univariate analysis and after adjusting for age, gender, BMI and knee or hip OA. However, as there were 17 SNPs in our study we applied the Bonferroni correction for multiple testing and when this was done the association became statistically insignificant.

Although not statistically significant after Bonferroni correction our findings are in keeping with the previous observation from a smaller hospital based UK study that there is an association between -4bpG>A 5'UTR SNP in the ANKH gene and sporadic CC (Zhang *et al* 2005). However, corrections for multiple tests were not applied in the earlier study. Association between minor alleles of rs3045 in the *ANKH* and CC has not been studied before. This association is of particular interest to the genetics of CPPD as the minor allele of rs3045 has been shown to reduce intracellular PPi levels in vitro studies of human articular chondrocytes suggesting increased ePPi (Peach *et al* 2007). We failed to show any association between the minor allele of rs3045 and CC after Bonferroni correction. It is possible that a larger study with more cases and therefore more power is needed to detect these associations.

Recently a small study of 25 individuals (10 CC+, 15 CC-) belonging to 8 Slovakian kindreds with familial CC ruled out any monogenic influence of

mutations in the ANKH gene on the occurrence of their familial CC (Couto *et al* 2011). However, the study had insufficient power to rule out any oligo- or polygenic influence of mutations in the ANKH gene in these families and does not relate to more common, apparently sporadic CC.

We did not find an association between SNPs in *TNAP* and *ENPP1* with CC. This is in keeping with previous reports of a lack of association between SNPs in these genes and CC (Zhang *et al* 2007).

The association between CC and haemochromatosis is based on reports of florid CC and an atypical structural arthropathy at a younger age in haemochromatosis (Sahinbegovic *et al* 2010). Just over one third of patients with haemochromatosis and iron overload have CC in at least one joint (Sahinbegovic *et al* 2010). Whilst haemochromatosis results in CC it is a rare cause of sporadic CC itself (Alizadeh *et al* 2007).

We did not find any association between rs1800562 and rs1799945, the two *HFE* SNPs associated with the majority of cases with haemochromatosis and CC. The association between these SNPs in the *HFE* gene and sporadic CC has been previously examined in two studies. A small hospital based study from the UK reported an association between homozygosity for the minor allele of rs1800562 in the *HFE* gene and CC (Timms *et al* 2002). The relative risk was 3.4 (p=0.037). There was no association between the minor allele at rs1799945, rs1800562 and CC. The relative risk was not corrected for possible confounding factors like age and OA and correction for multiple testing was not carried out. However, the relative risk for the association between the minor alleles of rs1800562 and

CC would become non-significant if correction for multiple testing were undertaken.

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 (Alizadeh *et al* 2007). Our findings and those from the Rotterdam study suggest that there is no association between *HFE* genotypes that lead to haemochromatosis and common CC.

The lack of association between minor alleles of rs1800562 and rs1799945 and CC may be due to the fact that both these SNPs have poor penetrance and it is the amount of iron overload, not the presence or absence of these mutations that increases the risk of CC. In community based studies 75% - 91% of men and 40% - 72% of women with homozygous minor alleles at rs1800562 have biochemical evidence of iron overload in the form of a raised ferritin or transferrin saturation (Rossi *et al* 2004). However, only 1% of community dwelling adults homozygous for the minor allele at rs1800562 have evidence of clinical haemochromatosis (Rossi *et al* 2004). Other risk factors such as viral hepatitis and alcohol intake increase the risk of iron overload in those with generic risk of haemochromatosis. We did not find any association between minor alleles at rs1799852, rs2280673, and rs3811647 - the three transferrin gene SNPs reported to associate with iron overload - and CC. This again may be related to poor penetrance of these genes.

rs28933977 (one of the SNPs in *ENPP1*) selected for genotyping had a minor allele frequency <5% in the GOAL population. This is not surprising

as the minor allele frequency of this SNPs ranged between 2.3% and 5.0% in other studies (NCBI reference assembly 2011). rs28933977 has a strong influence on PPi level. Homozygosity for the minor allele of rs28933977 associates with generalised arterial calcification in infancy (GACI) - a rare condition due to a loss of function mutation in *ENPP 1* gene that presents with hydrops fetalis, congestive cardiac failure in the newborn, arterial calcification and soft-tissue calcification due hydroxyapatite deposition (Dlamini *et al* 2009). For these reasons it was hypothesized that the minor allele of rs28933977 would associate negatively with CC. However, the minor allele frequency of this SNP in the GOAL population was very low and therefore it was excluded from further analysis of association between SNPs and CC.

Similarly *TNAP571* is the commonest mutation to cause the mild phenotype of hypophosphatasia in adults. However, the population prevalence of this mutation has not been determined before. As it has a strong influence on PPi levels we included it in our list of SNPs to be genotyped. We hypothesized that it would associate with CPPD via elevation of ePPi levels. However, nucleotide change at this locus was uncommon in the GOAL population and therefore it was excluded from further analysis of association between SNPs and CC.

This is a large study of possible genetic risk factors for CC. There are several strengths of this study. Firstly, all cases and controls were adequately characterised. Controls were adequately screened for absence of radiographic CC. This is unlike previous studies in which healthy blood

donors were selected as controls without specifically screening for the absence of CC. The analysis was adjusted for factors which may associate with CC, specifically: age; gender, which may associate with iron overload in those with genetic risk factors for haemochromatosis; and OA, which may associate with both CC and haemochromatosis. Moreover, correction for multiple testing was applied to reduce the chance of a type I error. However, it is possible that we failed to detect a statistically significant difference due to the strict correction criteria for multiple testing. At the beginning of this study, we intended to carry out gene-environment interactions. However, since we found no convincing evidence of a significant association between candidate SNPs and CC at any site we did not progress to examination of gene-environment interactions. In retrospect, it is apparent that we would have detected a statistically significant association between SNPs in *ANKH* and CC if we had selected fewer candidate SNPs for genotyping. However, it was difficult to restrict the number of SNPs for genotyping as the selection process was hypothesis driven and we had valid reasons to select the SNPs that were finally selected for genotyping.

Chapter 5 Radiographic phenotype of arthropathy associated with chondrocalcinosis

5.1 Radiographic phenotype of arthropathy in joints with osteoarthritis and chondrocalcinosis

Aim: The overall aim of this analysis was to examine the radiographic phenotype of structural arthropathy in joints with OA and CC.

Statistical analysis: The radiographic phenotype of cases with CC plus radiographic OA at a joint was compared with the radiographic phenotype of those with radiographic OA alone at the same joint. Right and left sides were compared separately.

5.1.1 Radiographic phenotype of knee arthropathy associated with knee chondrocalcinocis

Results: In those with radiographic knee OA the presence of CC in one knee did not associate with a greater summated osteophyte score in the same knee (Table 30).

Table 30 Association between summated knee osteophyte score and knee chondrocalcinosis

Summated osteophyte score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right knee				
1 (0-8)	42	428	1	1
2 (9-14)	36	362	1.01 (0.64-1.62)	1.09 (0.68-1.75)
3 (15-36)	53	412	1.31 (0.86-2.01)	1.45 (0.94-2.24)
Left knee				
1 (0-8)	40	417	1	1
2 (9-14)	37	360	1.07 (0.67-1.71)	1.31 (0.69-1.78)
3 (15-38)	45	411	1.14 (0.73-1.79)	1.26 (0.80-1.99)

[1]Adjusted for age, gender, and BMI.

The presence of CC in one knee did not associate with a more severe summated joint space narrowing score in the same knee (Table 31).

Table 31 Association between summated joint space width score at knee and knee chondrocalcinosis

Summated joint space width score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right knee				
1 (0-4)	55	498	1	1
2 (5)	41	370	1.00 (0.66-1.54)	1.02 (0.67-1.57)
3 (6-15)	35	333	0.95 (0.61-1.49)	0.99 (0.63-1.55)
Left knee				
1 (0-4)	34	380	1	1
2 (5)	37	273	1.59 (0.96-2.48)	1.59 (0.97-2.61)
3 (6-15)	28	290	1.08 (0.64-1.82)	1.05 (0.62-1.80)

[1]Adjusted for age, gender, and BMI.

In those with radiographic knee OA the presence of CC in one knee associated with attrition in the same knee on unadjusted analysis (Table 32). This persisted after adjusting for age, gender and BMI (Table 32).

Table 32 Association between knee attrition and knee chondrocalcinosis

Attrition	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right knee				
-	118	1145	1	1
+	13	57	2.21 (1.18-4.16)	2.33 (1.24-4.34)
Left knee				
-	108	1129	1	1
+	14	58	2.52 (1.36-4.67)	2.55 (2.37-4.76)

[1]Adjusted for age, gender, and BMI.

Key results: Please see Figure 43 on page 267

Discussion: In this study, we examined the phenotype of structural arthropathy associated with knee CC + OA by comparing the individual radiographic features of OA in knees with OA plus CC versus those with OA alone. Data for right and left knees were analysed separately. There was an association between knee CC and attrition in knee joints with radiographic OA. This association was present at each knee, and persisted after adjusting for age, gender, and BMI. However, there was no association between the presence of knee CC and either greater summated osteophyte scores or greater summated joint space narrowing scores compared to knees with isolated radiographic OA.

We found that in knee joints with OA the additional presence of CC associated with attrition. This is consistent with previous reports of an association between attrition and presence of CPP crystals in those with symptomatic radiographic knee OA (OR (95%) 1.92 (1.53 - 2.45) (Ledingham *et al* 1993a). The findings of our study are also supported by a study examining the association between synovial fluid inorganic PPi levels and short term radiographic outcome of knee OA (Doherty *et al* 1996). In this study, participants with bony attrition at baseline had high synovial fluid PPi compared to those without bony attrition (median (95%CI) 14.8 (13.0 -18.6), and 7.8 (6.6 – 9.6) µmol). Additionally, there was an association between high synovial fluid PPi levels at baseline and attrition at follow up (p=0.003) (Doherty *et al* 1996). The strong association between CPP crystals and attrition is supported by another prospective study in which the presence of

CPP crystals increased the risk of attrition in symptomatic radiographic knee OA patients after a median 2 years (range 1 - 5 years) of follow up (a(OR) 95% CI 2.41 (1.33-4.39) (Ledingham *et al* 1995). These findings also accord with earlier anecdotal observations of destructive arthropathy in those with CC - so-called *pseudo-neuropathic OA*. Severe joint destruction with fragmentation of subchondral bone (Genant 1976), collapse of articular surfaces (Martel *et al* 1981) and resulting formation of intra-articular loose bodies (Resnick *et al* 1977a) which overall may mimic neuropathic arthropathy are reported to occur with CC (Resnick *et al* 1977a; Dieppe *et al* 1982; Watt 1983).

We found that in knee OA, the additional presence of CC did not associate with a greater summated osteophyte score. This is not in keeping with some previous observations (Resnick *et al* 1977a; Pattrick *et al* 1993; Neame *et al* 2003). For example, in a community based cross-sectional study, Neame *et al* showed an association between knee CC and radiographic knee OA and suggested that this association is due mainly to predisposition to osteophytosis at the knee (Neame *et al* 2003). However, it is important to realise that there are subtle differences in the main objective between the present study and the previous study by Neame *et al* (Neame *et al* 2003). The main objective of the current study is to examine if presence of CC alters the radiographic OA phenotype at a particular knee, while Naeme *et al* examined the association between CC and knee OA and then further explored their data to see which radiographic phenotype of OA could explain

this association (Neame *et al* 2003). Therefore their findings do not directly contradict our findings.

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 features score than those without CC (Musacchio *et al* 2011). The differences were statistically significant when the population was stratified by gender (age-adjusted p=0.003) (Musacchio *et al* 2011). 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 does not suggest that participants with knee OA plus CC have a distinct radiographic phenotype compared to OA alone.

We found no association between knee CC and joint space narrowing in joints with radiograpahic knee OA. This is in keeping with previous observations which showed no association between knee CC and joint space narrowing at the knee (Neame *et al* 2003; Pattrick *et al* 1993 ; Ledingham *et al* 1993a ; Ellman *et al* 1981b).

The findings in the current study are supported by those of a smaller hospital based case-control study of 21 cases with knee OA and CC and 21 age matched controls with OA alone, in which osteophytosis, joint space narrowing, subchondral sclerosis and subchondral cysts were no more common in knee joints with CC plus OA than in knees with OA alone (Hansen *et al* 1984).

The findings of our study are also broadly supported by another study that examined the association between synovial fluid inorganic PPi levels and short term radiographic outcome of knee OA (Doherty *et al* 1996). In this study synovial fluid PPi was measured in 135 knee OA patients referred to hospital for knee pain. There was no association between synovial fluid PPi levels at baseline and joint space narrowing, osteophytes, sclerosis or cysts (Doherty *et al* 1996). However, in some studies, an association between presence of sclerosis (OR (95%CI) 1.73 (1.41 - 2.12), and cystic change (OR (95%CI) 1.34 (1.14-1.57) and CPP crystals in knee joints has also been reported (Ledingham *et al* 1993a).

Several prospective studies have examined the association between CPPD and progression of radiographic features of OA. In a hospital based study of symptomatic knee OA patients the presence of CPP crystals did not associate with progressive joint space loss, osteophytosis, sclerosis or cyst formation at 2 year follow-up (Ledingham *et al* 1995). In community based studies the presence of CC protected against, or had no effects on progressive joint space narrowing in those with knee OA (aRR (95%CI) for progressive joint space narrowing 0.4 (0.2-0.7) in the Boston OA Knee study, and 0.9 (0.6-1.5) for the Health ABC Study)) (Neogi *et al* 2006). Similarly, in a study examining the association between synovial fluid inorganic PPi levels (a surrogate for risk of CPPD) and radiographic outcome of knee OA at a median follow-up of 2.5 years there was a negative association between high synovial fluid PPi levels at baseline and an increase in global radiographic score for OA (p=0.038) and osteophyte score (p=0.002), and no association

with progressive joint space loss (p=0.19) or cysts (p=0.41) (Doherty *et al* 1996). These findings suggest that CC plus OA may have a milder OA phenotype.

Although, this is the largest study to examine if the presence of knee CC alters the OA phenotype, there are several limitations of this study. Firstly, we did not collect information about sclerosis and cysts at the knee and are therefore unable to examine the association of these features with knee CC in knees with OA. Secondly, patients with radiographic knee OA were selected for severe hip or knee OA which limits the generalisability of these findings.

In summary we found that in those with knee OA the additional presence of knee CC associates with attrition at the knee. However, in those with knee OA, there was no association between knee CC and either an increased summated osteophyte score or summated joint space narrowing score at the knee.

5.1.2 Radiographic phenotype of hip arthropathy associated with hip chonsrocalcinosis

On unadjusted analysis there was a negative association between CC in right hip and the summated osteophyte score at the same joint (Table 33). The negative association persisted after adjusting for age, gender and BMI. However, the association was not significant on the left side.

Table 33 Association between summated hip osteophyte score and hip chondrocalcinosis

Summated osteophyte score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
1 (0-3)	19	233	1	1
2 (4-6)	15	417	0.44 (0.22-0.88)	0.45 (0.23-0.92)
3 (7-9)	5	229	0.27 (0.10-0.73)	0.26 (0.09-0.71)
Left hip				
1 (0-3)	9	202	1	1
2 (4-5)	5	254	0.44 (0.15-1.34)	0.44 (0.14-1.37)
3 (6-9)	5	328	0.34 (0.11-1.04)	0.34 (0.11-1.04)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was a negative association between CC and joint space narrowing in the right hip (Table 34). There was a similar association between CC and joint space narrowing on the left side but this was not statistically significant (Table 34).

Table 34 Association between joint space width at hip and hip chondrocalcinosis

Minimum joint space width (mm): tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
1 (2.35-4.27)	16	160	1	1
2 (0.5-2.30)	4	177	0.23 (0.08-0.69)	0.21 (0.07-0.66)
3 (0.00-0.00)	19	544	0.35 (0.17-0.70)	0.33 (0.17-0.68)
Left hip				
1 (2.43-4.55)	7	162	1	1
2 (0.68-2.42)	3	168	0.46 (0.17-1.25)	0.39 (0.10-1.53)
3 (0.00-0.00)	9	454	0.42 (0.11-1.63)	0.44 (0.16-1.22)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one hip and subchondral cysts in the same hip (Table 35).

Table 35 Association between hip subchondral cyst and chondrocalcinosis

Subchondral cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
-	23	424	1	1
+	16	456	0.65 (0.34-1.24)	0.64 (0.33-1.23)
Left hip				
-	9	395	1	1
+	10	390	1.13 (0.45-2.80)	1.11 (0.45-2.77)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was a negative association between CC and subchondral sclerosis in the right hip (Table 36). There was a negative association between CC and subchondral sclerosis in the left hip but this was not statistically significant (Table 36).

Table 36 Association between subchondral sclerosis at hip and hip chondrocalcinosis

Subchondral Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
-	9	105	1	1
+	30	775	0.45 (0.21-0.98)	0.41 (0.19-0.91)
Left hip				
-	4	112	1	1
+	15	674	0.62 (0.20-1.91)	0.63 (0.20-1.94)

[1]Adjusted for age, gender, and BMI.

There was no association between CC and global opinion of atrophic or hypertrophic bone response (Table 37).

Table 37 Association between bony response in hip osteoarthritis and hip chondrocalcinosis

	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
Neither	34	685	1	1
Atrophic	5	134	0.75 (0.29-1.96)	0.78 (0.30-2.04)
Hypertrophic	0	61	-/-	-/-
Left hip				
Neither	15	600	1	1
Atrophic	3	139	0.86 (0.25-3.02)	0.87 (0.25-3.05)
Hypertrophic	1	47	0.85 (0.11-6.58)	0.95 (0.12-7.43)

[1]Adjusted for age, gender, and BMI.

Key results: Please see Figure 43 on page 267

Discussion: In this study we examined the phenotype of structural arthropathy associated with hip CC in hips with OA by comparing the individual radiographic features of OA in hips with OA plus CC and that of hips with OA alone. Data for right and left hips were analysed separately. On the right side there was a negative association between summated osteophyte score, minimum joint space width, subchondral sclerosis and hip CC in hips with radiographic OA. This association persisted after adjusting for age, gender and BMI. There was a similar association on the left side but this was not statistically significant. There was no association between cysts, overall pattern of bony response and CC in hips with OA.

Compared to the knee, relatively few studies have reported on the radiographic phenotypes of OA associated with hip CC. Interpretation of reported associations is difficult since only two of these studies have a control group (Resnick *et al* 1977a; Ledingham *et al* 1992).

We found a negative association between summated osteophyte score, minimum joint space width and hip CC in the context of radiographic right hip OA. There was a trend towards a similar association on the left side but this was not statistically significant. This suggests the presence of a less bone forming and milder hip OA phenotype in those with CC plus OA than in those with OA alone. The findings of this study are supported by a previous hospital based case-control study in which hips with CPPD plus OA had a milder OA phenotype than hips with OA alone (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 those with hip OA alone. Joint space narrowing was present in 29% of hips with CPPD plus OA and in all hips with OA alone. In a subsequent hospital based cross-sectional study involving patients with symptomatic radiographic hip OA, hip CC associated with an atrophic bone response at the hip (Ledingham *et al* 1992). The findings from this study support our observation of a negative association between summated osteophytes score and sclerosis with CC in hips with OA. However, there was no association between hip CC and hip osteophytosis in this study (Ledingham *et al* 1992).

We found no association between CC and a global score for atrophic or hypertrophic bony response in hips with OA. This is not in keeping with the above mentioned findings of a negative association between osteophytosis, sclerosis and CC in hips with OA reported in this study. This may be as a single global score for bony response to hip OA is not as sensitive as the summated osteophyte score, or the ordinal score of subchondral sclerosis for the definition of bony response in hips with OA. The lack of association between CC and a global score for atrophic or hypertrophic bony response in hips with OA is also discordant with the previous observation of an association between atrophic bone response and CC in hips with OA (Ledingham *et al* 1992). This lack of association in the current study may be due to only a few hips being categorised as having an overall atrophic or hypertrophic OA phenotype.

We found no association between cysts and CC in hips with OA. This is in keeping with the findings from a hospital based cross-sectional study of

patients with symptomatic hip OA in which there was no association between subchondral cysts and hip CC (Ledingham *et al* 1992). However, the findings of this earlier study and our study are not in agreement with those of a small hospital based post-mortem study. In that study of 8 CPPD plus OA hips and 42 hips with OA alone Resnick *et al.* reported on the universal presence of multiple 'geodes' in joints with CPPD plus OA while joints with OA alone had no cysts or just single cysts (Resnick *et al* 1977b). 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 did not have a greater summated global hip OA radiographic features score than those without CC (Musacchio *et al* 2011). The lack of association between the radiographic features score at the hip and CC could be explained by a lack of association between CC and hip OA itself.

This is the largest study to examine whether the presence of hip CC alters the hip OA phenotype. However, the generalisability of these findings are limited by the fact that patients with radiographic hip OA were selected for hip or knee OA severe enough to warrant referral to the hospital for joint replacement surgery.

In summary we found that in those with hip OA the additional presence of hip CC associates negatively with osteophytosis, minimum joint space width, and sclerosis in the right hip. However, in hips with OA there was no association between hip CC and cysts, an atrophic bone response or a hypertrophic bone response.

5.1.3 Radiographic phenotype of hand arthropathy associated with wrist chondrocalcinosis

5.1.3.1 Wrist arthropathy

On unadjusted and adjusted analysis there was no association between CC and the summated osteophyte score at the same wrist (Table 38).

Table 38 Association between summated osteophyte score at wrist and wrist chondrocalcinosis

Summated osteophyte score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI) [1]
Right wrist				
1 (0-1)	36	138	1	1
2 (2)	5	19	1.10 (0.30-3.15)	0.97(0.34-2.83)
3 (3-4)	4	18	0.85 (0.30-2.91)	0.80 (0.25-2.55)
Left wrist				
1 (0-1)	38	133	1	1
2 (2)	2	13	0.54 (0.12-2.49)	0.47 (0.10-2.26)
3 (3-4)	4	15	0.93 (0.29-2.98)	0.95 (0.29-3.07)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC and summated joint space narrowing score in the same wrist (Table 39).

Table 39 Association between summated joint space width score at wrist and wrist chondrocalcinosis

Summated joint space width score:≤median and >median (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right wrist				
1 (2)	22	109	1	1
2 (3-12)	23	66	1.73 (0.89-3.34)	1.71 (0.87-3.34)
Left wrist				
1 (2)	21	95	1	1
2 (3-12)	23	65	1.60 (0.82-3.13)	1.54 (0.78-3.06)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and cysts in the same wrist (Table 40).

Table 40 Association between wrist cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI) [1]
Right wrist				
-	38	157	1	1
+	7	19	1.52 (0.60-3.88)	1.64 (0.62-4.33)
Left wrist				
-	51	261	1	1
+	8	23	1.66 (0.67-4.10)	1.49 (0.59-3.75)

[1]Adjusted for age, gender, and BMI.

On unadjusted analysis there was an association between CC in the right wrist, and subchondral sclerosis in the same wrist (Table 41). This persisted after adjusting for age, gender and BMI (Table 41). There was a similar association between CC and subchondral sclerosis in the left wrist but this was not statistically significant (Table 41).

Table 41 Association between wrist sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right wrist				
-	16	99	1	1
+	29	78	2.30 (1.17-4.54)	2.09 (1.03-4.22)
Left wrist				
-	16	83	1	1
+	28	78	1.86 (0.94-3.70)	1.75 (0.86-3.55)

[1]Adjusted for age, gender, and BMI.

5.1.3.2 Radio-carpal joint arthropathy

On unadjusted and adjusted analysis there was no association between CC at wrist and osteophyte score at radiocarpal joint on same side (Table 42).

Table 42 Association between radio-carpal joint osteophyte score and wrist chondrocalcinosis

Osteophyte score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right radio-carpal joint				
1 (0-1)	33	99	1	1
2 (2)	1	15	0.20 (0.03-1.57)	0.20 (0.03-1.63)
3 (3-4)	1	2	1.50 (0.13-17.08)	1.35 (0.11-16.08)
Left radio-carpal joint				
1 (0-1)	25	87	1	1
2 (2)	3	8	1.28 (0.30-5.44)	1.31(0.32-5.39)
3 (3-4)	0	2	-/-	-/-

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between wrist CC and joint space narrowing at radiocarpal joint on same side (Table 43).

Table 43 Association between radio-carpal joint space width score and wrist chondrocalcinosis

Joint space width score: ≤median and >median (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right radiocarpal joint				
1 (2)	25	84	1	1
2 (3)	10	32	1.05 (0.45-2.43)	0.99 (0.41-2.39)
Left radiocarpal joint				
1 (2)	22	79	1	1
2 (3)	6	18	1.23 (0.42-3.57)	1.20 (0.42-3.38)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between wrist CC and radio-carpal joint cysts on the same side (Table 44).

Table 44 Association between radio-carpal joint cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right radio-carpal joint				
-	31	106	1	1
+	4	10	1.37 (0.40-4.66)	1.18 (0.33-4.21)
Left radio-carpal joint				
-	22	86	1	1
+	6	11	1.80 (0.57-5.64)	2.13 (0.71-6.40)

[1]Adjusted for age, gender, and BMI.

On unadjusted analysis there was an association between wrist CC and sclerosis in radio-carpal joint on the left side (Table 45). This persisted after adjusting for age, gender and BMI (Table 45).

Table 45 Association between radio-carpal joint sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right radio-carpal joint				
-	10	58	1	1
+	10	58	2.50 (1.10-5.67)	2.29 (0.98-5.33)
Left radio-carpal joint				
-	5	40	1	1
+	23	57	3.29 (1.14-9.52)	3.23 (1.13-9.21)

[1]Adjusted for age, gender, and BMI.

5.1.3.3 Mid-carpal joint arthropathy

On unadjusted and adjusted analysis there was no association between wrist CC and osteophyte score at the mid-carpal joint on the same side (Table 46).

Table 46 Association between mid-carpal joint osteophyte score and wrist chondrocalcinosis

Osteophyte score	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right mid-carpal joint				
1 (0)	30	11	1	1
2 (1)	1	0	-/-	-/-
Left mid-carpal joint				
1 (0)	33	12	1	1
2 (1)	1	1	2.75(0.16-47.52)	1.50 (0.07-34.82)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between wrist CC and joint space narrowing in the mid-carpal joint on the same side (Table 47).

Table 47 Association between mid-carpal joint space width score and wrist chondrocalcinosis

Joint space width score: ≤median and >median (range)	CC + OA	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right mid-carpal joint				
1 (2)	7	17	1	1
2 (3)	4	14	0.69 (0.17-2.86)	0.48 (0.10-2.38)
Left mid-carpal joint				
1 (2)	8	19	1	1
2 (3)	5	15	0.79 (0.21-2.92)	0.82 (0.20-3.41)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and cysts in the mid-carpal joint on the same side (Table 48).

Table 48 Association between mid-carpal joint cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI) [1]
Right mid-carpal joint				
-	9	27	1	1
+	2	4	1.50 (0.23-9.61)	1.25 (0.16-10.10)
Left mid-carpal joint				
-	12	29	1	1
+	1	5	0.48 (0.05-4.59)	0.58 (0.06-5.99)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and mid-carpal sclerosis on the same side (Table 49).

Table 49 Association between mid-carpal joint sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right mid-carpal joint				
-	7	13	1	1
+	4	18	0.41 (0.10-1.71)	0.37 (0.08-1.84)
Left mid-carpal joint				
-	4	16	1	1
+	9	18	2.00 (0.52-7.77)	2.73 (0.62-12.10)

[1]Adjusted for age, gender, and BMI.

5.1.3.4 Common carpometacarpal joint arthropathy

Only 7 common CMCJs on the right side had definite joint space narrowing. None of these had CC in the right wrist, precluding any analysis of association between radiographic phenotype of OA at the right common CMCJ, and right wrist CC. Similarly, only 14 common CMCJs on the left side had definite joint space narrowing. None of those with sclerosis, cysts, high osteophyte score, and a low joint space narrowing score had wrist CC. This precluded the analysis of association between radiographic phenotype of OA at the common CMCJ, and wrist CC on the left side.

5.1.3.5 1st CMCJ arthropathy

On unadjusted and adjusted analysis there was no association between CC in one wrist and the summated osteophytes score in the 1st CMCJ on the same side (Table 50).

Table 50 Association between 1st CMCJ osteophytosis and wrist chondrocalcinosis

Osteophyte score	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right 1st CMCJ				
0-1	2	27	1	1
2	27	227	1.61 (0.36-7.13)	1.38 (0.30-6.22)
3	32	179	2.41 (0.55-10.66)	2.05 (0.46-9.20)
Left 1st CMCJ				
0-1	7	34	1	1
2	31	241	0.63 (0.26-1.53)	0.59 (0.24-1.48)
3	34	169	0.98 (0.40-2.40)	0.94 (0.38-2.33)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and 1st CMCJ joint space narrowing on the same side (Table 51).

Table 51 Association between 1st CMCJ joint space width and wrist chondrocalcinosis

Joint space width score	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right 1st CMCJ				
0-1	5	65	1	1
2	45	307	1.91 (0.73-4.99)	1.95 (0.74-5.15)
3	11	58	2.47 (0.81-7.52)	2.26 (0.73-7.01)
Left 1st CMCJ				
0-1	5	62	1	1
2	47	313	1.82 (0.71-4.87)	1.97 (0.75-5.22)
3	19	66	3.57 (1.26-10.14)	3.45 (1.20-9.93)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was an association between CC in the left wrist and cysts in the 1st CMCJ on the same side (Table 52). Table 52There was an association between CC and 1st CMCJ sclerosis in the right wrist but this was not statistically significant (Table 52).

Table 52 Association between 1stCMCJ cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right 1st CMCJ				
-	40	332	1	1
+	21	101	1.73 (0.97-3.06)	1.69 (0.95-3.03)
Left 1st CMCJ				
-	61	495	1	1
+	26	97	2.18 (1.31-3.62)	2.20 (1.31-3.71)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and sclerosis in the 1st CMCJ on the same side (Table 53).

Table 53 Association between 1st CMCJ sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)
Right 1st CMCJ				
-	11	112	1	1
+	50	321	1.59 (0.80-3.15)	1.41 (0.71-2.85)
Left 1st CMCJ				
-	14	116	1	1
+	58	327	1.47 (0.79-2.73)	1.35 (0.72-2.53)

[1]Adjusted for age, gender, and BMI.

5.1.3.6 ST joint arthropathy

On unadjusted and adjusted analysis there was no association between CC in one wrist and osteophytosis in the STJ on the same side (Table 54).

Table 54 Association between STJ osteophytosis and wrist chondrocalcinosis

Osteophyte score	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right STJ				
0-1	20	183	1	1
2	25	149	1.54 (0.82-2.87)	1.56 (0.83-2.93)
3	10	24	3.81 (1.60-9.10)	3.74 (1.55-9.03)
Left STJ				
0-1	23	197	1	1
2	27	124	1.87 (1.02-3.40)	1.96 (0.98-3.61)
3	5	32	1.34 (0.40-3.77)	1.24 (0.43-3.53)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC at one wrist and joint space narrowing in the STJ on same side (Table 55).

Table 55 Association between STJ joint space width and wrist chondrocalcinosis

Joint space width	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right STJ				
0-1	0	10	1	1
2	29	202	-/-	-/-
3	26	144	-/-	-/-
Left STJ				
0-1	2	4	1	1
2	20	186	0.22 (0.04-1.25)	0.19 (0.03-1.14)
3	33	162	0.41 (0.07-2.32)	0.38 (0.07-2.21)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and cysts in the STJ on the same side (Table 56).

Table 56 Association between STJ cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right STJ				
-	54	340	1	1
+	1	16	0.39 (0.05-3.03)	0.42 (0.05-3.21)
Left STJ				
-	54	341	1	1
+	1	12	0.53 (0.07-4.13)	0.58 (0.07-4.60)

[1]Adjusted for age, gender, and BMI.

On unadjusted analysis there was an association between CC in one wrist and STJ sclerosis on the same side (Table 57). This association persisted after adjusting for age, gender and BMI.

Table 57 Association between STJ sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right STJ				
-	3	64	1	1
+	52	292	3.80 (1.15-12.55)	3.53 (1.06-11.75)
Left STJ				
-	4	78	1	1
+	51	275	3.62 (1.27-10.32)	3.61 (1.26-10.37)

[1]Adjusted for age, gender, and BMI.

5.1.3.7 MCPJ arthropathy

On unadjusted and adjusted analysis there was no association between CC in one wrist and MCPJ osteophytosis on the same side (Table 58).

Table 58 Association between MCPJ osteophytosis and wrist chondrocalcinosis

Summated osteophyte score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right 2nd -5th MCPs				
1 (0-2)	8	47	1	1
2 (3-4)	16	77	1.22 (0.49-3.07)	1.21 (0.48-3.05)
3 (5-10)	6	53	0.67 (0.22-2.06)	0.66 (0.21-2.05)
Left 2nd -5th MCPs				
1 (0-2)	14	101	1	1
2 (3)	4	47	0.61 (0.19-1.97)	0.65 (0.20-2.13)
3 (4-9)	7	81	0.62 (0.24-1.62)	0.60 (0.23-1.57)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and MCP joint space narrowing on the same side (Table 59).

Table 59 Association between MCPJ joint space narrowing and wrist chondrocalcinosis

Summated joint space width score: tertiles (range)	CC + OA	OA	OR (95%CI)	aOR (95%CI)[1]
Right 2nd -5th MCPs				
1 (2-3)	12	78	1	1
2 (4)	7	53	0.86 (0.32-2.32)	0.87 (0.32-2.38)
3 (5-10)	11	46	1.55 (0.64-3.81)	1.47 (0.59-3.63)
Left 2nd -5th MCPs				
1 (2)	8	94	1	1
2 (3)	7	56	1.47 (0.51-4.27)	1.42 (0.49-4.16)
3 (4-9)	10	79	1.49 (0.56-3.95)	1.43 (0.63-3.82)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was an association between CC in the right wrist and MCPJ cysts on the same side (Table 60). There was no association between CC in the left wrist and MCPJ cysts on the left (Table 60).

Table 60 Association between MCPJ cyst and wrist chondrocalcinosis

Cyst	CC + OA	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right 2nd -5th MCPJs				
-	31	275	1	1
+	16	67	2.12 (1.10-4.10)	2.16 (1.10-4.21)
Left 2nd -5th MCPJs				
-	19	189	1	1
+	6	41	1.46 (0.55-3.87)	1.49 (0.56-4.03)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and MCPJ sclerosis on the same side (Table 61).

Table 61 Association between MCPJ sclerosis and wrist chondrocalcinosis

Sclerosis	CC + OA	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right 2nd -5th MCPJs				
-	20	176	1	1
+	27	167	1.42 (0.77-2.63)	1.34 (0.72-2.50)
Left 2nd -5th MCPJs				
-	12	117	1	1
+	13	114	1.11 (0.49-2.54)	1.09 (0.47-2.54)

[1]Adjusted for age, gender, and BMI.

On unadjusted and adjusted analysis there was no association between CC in one wrist and the number of MCPJs with OA changes on the same side (Table 62).

Table 62 Association between number of MCPJs with osteoarthritis and wrist chondrocalcinosis

Number of MCPJs with definite joint space narrowing	CC + OA	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right				
<2	31	295	1	1
≥2	20	118	1.61 (0.88-2.94)	1.49 (0.81-2.75)
Left				
<2	30	355	1	1
≥2	10	67	1.77 (0.82-3.78)	1.80 (0.83-3.92)

[1]Adjusted for age, gender, and BMI

Key results: Please see Figure 43, 44 on page 267, 268

Discussion: In this study we examined the phenotype of structural arthropathy associated with wrist CC in hand joints with OA. We compared the individual structural radiographic features in hand joints with OA plus wrist CC and those with OA alone. Thus we examined whether wrist CC plus OA at the wrist, or 1st CMC joint, or ST joint, or MCPJs associated with a different OA phenotype in terms of osteophytes, joint space narrowing, cysts and sclerosis compared to hands with OA at these joints but with no wrist CC. Data for right and left sides were analysed separately.

The main findings were:

- In wrists, 1st CMCJs, STJs, and MCPJs with OA the presence of wrist CC did not associate with greater osteophytosis or more joint space narrowing.

- In the right wrist and both STJs with OA the presence of wrist CC associated with subchondral sclerosis. There was no association between wrist CC and subchondral sclerosis in the left wrist, both 1st CMCJs and MCPJs with OA.

- In the left 1st CMCJ and right hand MCPJs with OA the presence of wrist CC on the same side associated with cysts. There was no association between wrist CC and cysts in the right 1st CMCJ and left hand MCPJs with OA. There was no association between wrist CC and cysts in both STJs and wrists with OA.

Previous studies support our observation that there is no association between osteophytes at wrist and wrist CC in wrists with OA (Bourqui *et al.* 1983, Riestra *et al.* 1985). However, the rest of our findings do not concur with previously published studies. For example, in a hospital based case-control study patients with CPPD plus OA had 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), $p<0.01$, Mann-Whitney U test) (Riestra *et al* 1985). Subchondral cysts were also significantly more common in those with CPPD and GOA (OA at 6 or more joint sites) than in those with GOA alone (Bourqui *et al* 1983). Also these studies do not suggest that there is an association between wrist sclerosis and CC in the context of OA. However, previously published case–series support the observed association between sclerosis and wrist CC in wrists with OA (Resnick *et al* 1974; Donich *et al* 2000).

We report that in STJs with OA, the additional presence of wrist CC associated with STJ sclerosis in each hand. There was no association between wrist CC and cysts, osteophytosis and joint space narrowing in STJs with OA. The association between STJ OA and wrist CC is well established (Stucki et al. 1999, Donich et al. 2000, Peter et al. 2001). Our findings of more sclerosis in ST joints with OA and wrist CC in part agree with those of Peter *et al* who reported that the structural changes in the ST joint in patients with CPPD were ‘atrophic’ with a predominance of joint space narrowing and subchondral sclerosis with little or no osteophytosis (Peter *et al* 20001). In another study the overall radiographic grade of STJ

OA was higher in patients with CPPD than in those with OA alone (Riestra *et al* 1985).

We report that in MCPJs with OA the additional presence of wrist CC associated with cysts in the right hand and that there was a similar but statistically non-significant association in the left hand. There was no association between wrist CC and sclerosis, osteophytosis and joint space narrowing in MCPJs with OA. Our findings are in agreement with those from a hospital based case-control study in which hand x-rays of 46 patients with CPPD at 2 or more joint sites and GOA (defined as radiographic OA at six or more sites) were compared with hand x-rays of 46 patients with generalized OA alone (Bourqui *et al* 1983). The additional presence of CPPD in those with generalized OA 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).

Our findings and those of Bourqui *et al* suggest that MCPJ cysts are potentially the only true structural radiographic phenotype associated with wrist CC in those with MCPJ OA (Resnick *et al* 1974; Adamson *et al* 1983; Resnik *et al* 1983). The earlier reports of association between CPPD and severe subchondral sclerosis, joint space narrowing and florid osteophytosis at the MCPJs may be confounded by OA itself (Resnick *et al* 1974; Adamson *et al* 1983; Resnik *et al* 1983). Our study is also broadly in agreement with findings from an earlier hospital based case-control study in which cases with CPPD had significantly greater overall structural changes at MCPJs than cases with OA without CPPD (mean (S.D.) score 4.62 (3.53) vs. 3.60

(3.29) in primary OA, $p<0.05$, Mann-Whitney U test) (Riestra *et al* 1985). Differences between individual radiographic features of OA were not reported in this study.

We report that in 1st CMCJs with OA the additional presence of wrist CC associated with cysts in the left hand and that there was a similar but statistically non-significant association in the right hand. The association between 1st CMCJ cysts and wrist CC has not been reported before. There was no association between wrist CC and sclerosis, osteophytosis and joint space narrowing in 1st CMCJs with OA. Our findings are discordant with those from an earlier hospital based case-control study where patients with CPPD were compared to those with OA. Patients with CPPD had significantly less overall structural changes at the trapezio-metacarpal joint compared to patients with OA without CPPD (mean (S.D.) score 5.39 (5.36) *vs.* 7.15 (5.55), $p<0.03$, Mann-Whitney U test) (Riestra *et al* 1985). Moreover, participants with CPPD had less severe joint space narrowing than those with OA without CPPD at the trapezio-metacarpal joints (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), compared to 2.63 (1.73) in OA without CPPD ($p<0.035$) (Riestra *et al* 1985).

In summary, we report that in those with OA at wrists, 1st CMCJs, STJs, and MCPJs the additional presence of wrist CC does not associate with osteophytosis and joint space narrowing, but associates with sclerosis at the right wrist, both STJs, and with cysts at the right hand MCPJs and left 1st CMCJ. This is the largest study to examine the radiographic phenotype of

hand OA associated with wrist CC. However, the findings have limited generalisability as most of the participants were selected for large joint OA severe enough to warrant consideration of joint replacement surgery.

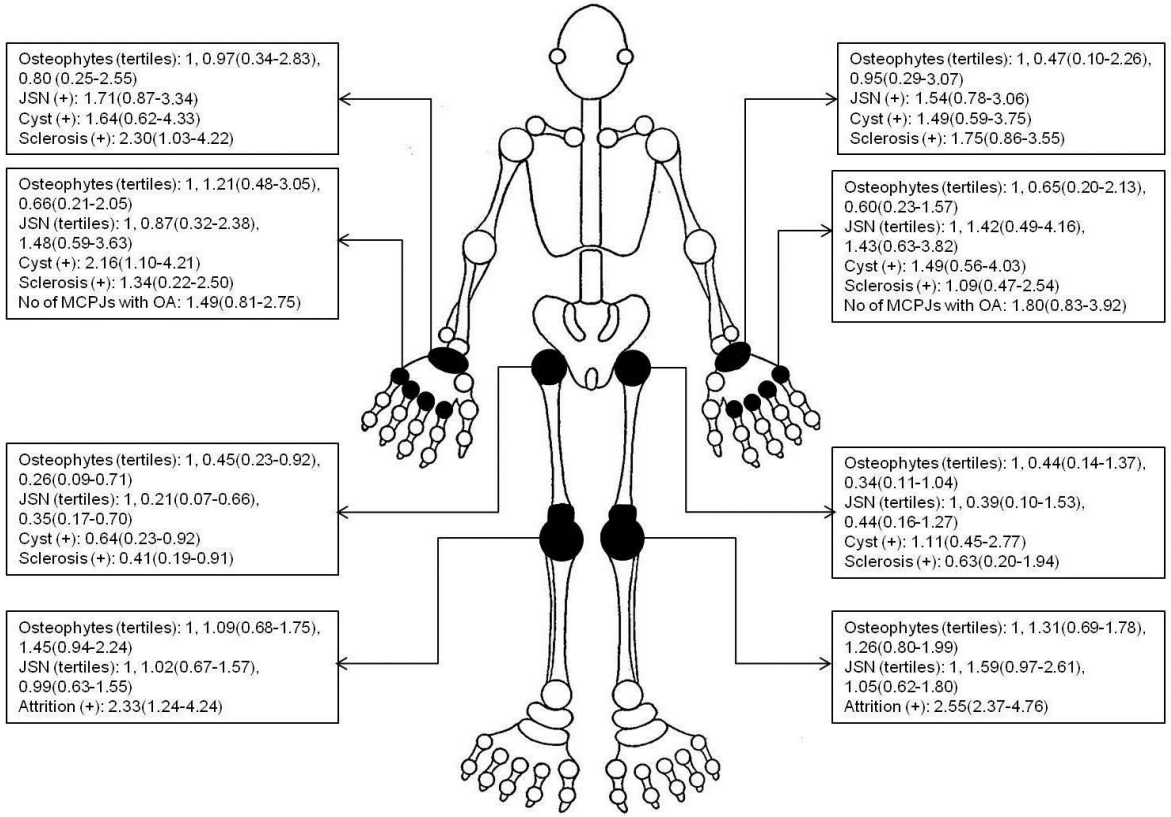

Figure 1 Radiographic phenotype of knee, hip and wrist osteoarthritis associated with chondrocalcinosis – local effects

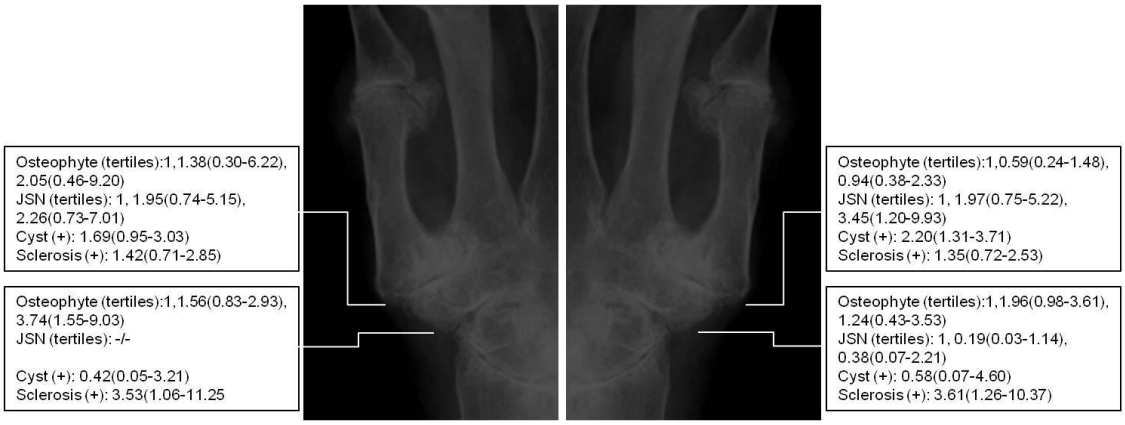

Figure 2 1st CMC and ST joint osteoarthritis associated with wrist chondrocalcinosis

5.2 Radiographic phenotype of arthropathy in osteoarthritic joints with chondrocalcinosis at distant joints

Aim: The aim of this analysis was to examine the association between distant joint CC and radiographic phenotype of structural arthropathy at the index joint, for those radiographic phenotypes at the index joint for which an association exists with index joint CC.

Statistical analysis: If any association was found between CC at a joint and a distinct radiographic phenotype at the same joint in those with OA at that joint (i.e. local effects present) further analysis was carried out to examine the association between CC at distant joints and that particular radiographic structural change at the index joint in those with OA at that particular joint. In order to reduce confounding by local effects participants with CC at the index joint were excluded from this analysis.

Result: On unadjusted analysis there was an association between distant joint CC and attrition at the knee (Table 63). This association remained significant after adjusting for age, gender and BMI (Table 63).

Table 63 Association between knee attrition and distant chondrocalcinosis

Attrition	OA + distant CC	OA	OR (95%CI)	aOR (95%CI) [1]
Right knee				
-	151	1231	1	1
+	13	44	2.41 (1.27-4.57)	2.27 (1.16-4.36)
Left knee				
-	162	1230	1	1
+	14	45	2.36 (1.27-4.40)	2.21 (1.12-4.29)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and the summated osteophyte score at the hip (Table 64).

Table 64 Association between summated osteophyte score at hip and distant chondrocalcinosis

Summated osteophyte score: tertiles(range)	OA + distant CC	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
1 (0-3)	28	204	1	1
2 (4-6)	53	363	1.06 (0.65-1.74)	1.12 (0.68-1.84)
3 (7-9)	20	209	0.70 (0.38-1.28)	0.72 (0.39-1.34)
Left hip				
1 (0-3)	34	166	1	1
2 (4-5)	27	227	0.58 (0.34-1.00)	0.61 (0.35-1.05)
3 (6-9)	40	288	0.12 (0.41-1.11)	0.76 (0.46-1.27)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and minimum joint space width at the hip (Table 65).

Table 65 Association between minimum joint space width at hip and distant chondrocalcinosis

Minimal joint space width (mm): tertiles(range)	OA + distant CC	OA	OR (95%CI)	aOR (95%CI) [1]
Right hip				
1 (2.35-4.27)	19	141	1	1
2 (0.5-2.30)	20	156	0.97 (0.56-1.68)	0.99 (0.57-1.72)
3 (0.00-0.00)	63	480	0.99 (0.49-1.86)	0.91 (0.46-1.80)
Left hip				
1 (2.43-4.55)	21	141	1	1
2 (0.68-2.42)	28	138	1.36 (0.74-2.51)	1.28 (0.69-2.39)
3 (0.00-0.00)	53	401	0.89 (0.52-1.52)	0.88 (0.52-1.52)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and subchondral sclerosis at the hip (Table 66).

Table 66 Association between subchondral sclerosis at hip and distant chondrocalcinosis

Subchondral Sclerosis	OA + distant CC	OA	OR (95%CI)	aOR (95%CI)[1]
Right hip				
-	44	265	1	1
+	85	702	0.73 (0.49-1.08)	0.70 (0.47-1.04)
Left hip				
-	51	369	1	1
+	95	600	1.15 (0.80-1.65)	1.11 (0.77-1.61)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and sclerosis at the wrist (Table 67).

Table 67 Association between wrist sclerosis and distant chondrocalcinosis

Sclerosis	OA + distant CC	OA	OR (95%CI)	aOR (95%CI)[1]
Right wrist				
-	6	92	1	1
+	8	69	1.78 (0.59-5.36)	1.64 (0.52-5.18)
Left wrist				
-	10	72	1	1
+	5	71	0.51 (0.17-1.56)	0.44 (0.14-1.40)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and 1st CMCJ cysts (Table 68).

Table 68 Association between 1stCMCJ cyst and distant chondrocalcinosis

Cyst	OA + distant CC	OA	OR (95%CI)	aOR (95%CI)[1]
Right CMC1				
-	62	430	1	1
+	14	91	1.07 (0.57-1.99)	1.07 (0.57-2.00)
Left CMC1				
-	53	439	1	1
+	15	82	1.52 (0.82-2.82)	1.53 (0.82-2.82)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and ST joint sclerosis (Table 69).

Table 69 Association between STJ sclerosis and distant chondrocalcinosis

Sclerosis	OA + distant CC	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right STJ				
-	25	155	1	1
+	32	279	0.71 (0.41-1.24)	0.66 (0.37-1.17)
Left STJ				
-	21	170	1	1
+	26	265	0.74 (0.40-1.36)	0.79 (0.43-1.46)

[1]Adjusted for age, gender, and BMI.

On both unadjusted and adjusted analysis there was no association between distant joint CC and MCPJ cysts (Table 70).

Table 70 Association between MCPJ cyst and distant chondrocalcinosis

Cyst	OA + distant CC	OA	OR (95%CI)[1]	aOR (95%CI)[2]
Right 2nd -5th MCPs				
-	34	306	1	1
+	12	62	1.74 (0.85-3.55)	1.70 (0.83-3.49)
Left 2nd -5th MCPs				
-	45	322	1	1
+	11	41	1.92 (0.92-4.00)	2.15 (1.01-4.58)

[1]Adjusted for age, gender, and BMI.

Key result: CC at distant joints associates with attrition at the knee.

Discussion: This is the first large study to examine the association between distant joint CC and radiographic phenotype of OA at the index joint. We found that in those with knee OA, CC at distant joints associates with attrition at both the right and the left knee. However, in those with hip OA there was no association between CC at distant joints and osteophytosis, joint space narrowing or subchondral sclerosis. Similarly in those with wrist OA, ST joint OA, 1st CMCJ OA and MCPJ OA there was no association between CC at distant joints and sclerosis at wrists and ST joints, and cysts at 1st CMCJ and MCPJs respectively.

The presence of an association between distant joint CC and attrition in those with knee OA is a novel finding which has not been reported before. This suggests the presence of a distinct radiographic phenotype in those with knee OA and distant CC. The finding is supported by previous observations of destructive arthropathy - so-called *pseudo-neuropathic OA* - in those with CC at local or distant joints. This suggests that there is a subset of patients with CC who develop severe OA, which is in agreement with previous reports of rapidly progressive hip OA in patients with CC at distant sites (Menkes *et al* 1985). Such an association also explains the cases with severe destructive arthropathy reported in earlier hospital based studies. However it is possible that those people with knee attrition who had CC at distant joints also had CC or CPPD at the knee, but this was not visualised on plain radiographs due to advanced joint damage, and the resulting lack of articular cartilage. This finding therefore needs to be validated in a study where

synovial fluid and cartilage from knees with attrition is available to define the presence of CPPD and BCP crystals locally.

Chapter 6 Key findings, and study limitations

6.1 Key findings

The well established GOAL database was used to examine the prevalence and distribution of CC in knee, pelvis and hand radiographs, to determine the constitutional and environmental risk factors for CC, and to examine the radiographic phenotype of OA associated with CC.

6.1.1 Radiographic distribution of chondrocalcinosis

The overall prevalence (95%CI) of CC at any site (knees, pelvis, and hands) was 13.7% (12.5 – 14.9%) in the GOAL population. The knee was the commonest site of CC (8.0%). Other sites in descending order of frequency were the wrists (6.9%), hips (5.0%), symphysis pubis (3.6%) and MCPJs (1.5%). CC was not any more likely to occur on the right side than on the left side at each joint area examined. CC was significantly more likely to be bilateral at all joint sites except for the hips.

Most previous reports suggest that in individuals without CC at the knee CC at other joints does not occur or is very rare. However, two small 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). In our study 42.0% of participants with CC at any site did not have knee CC. Therefore, we have demonstrated convincingly that CC often occurs at other joints in the absence of knee CC.

Even more interesting was the observation that just over 80% of participants with CC could be detected on a knee and pelvis radiograph, or on a knee and wrist radiograph. Thus it can be concluded that in order to

adequately screen for the presence or absence of CC in any individual all three joint areas i.e. the knees, pelvis and wrists should be x-rayed. However, it remains possible that this may still miss some participants with CC at other sites (e.g. shoulders, ankles).

In accord with previous reports knee CC was more likely to be present in the lateral TFJ than in the medial TFJ compartment. The absolute difference in prevalence (95% CI) between lateral TFJ and medial TFJ compartment was 15.2% (8.1 - 22.1%) at the right knee and 15.4% (7.1 - 23.0%) at the left knee. Similarly, CC was more common in fibrocartilage than in hyaline cartilage at the knee. The absolute difference in prevalence (95%CI) between CC of fibrocartilage and CC of hyaline cartilage was 34.6% (26.9 – 42.3%) at the right knee, and 32.6% (23.3 - 40.4%) at the left knee. These findings agree with previous studies and provide face validity to our study.

We showed that joints with CC cluster together more than would be expected by chance alone, and that CC at one joint associates with CC at distant joints. This was true for all joint pairs examined and persisted after adjusting for age, gender, BMI and OA at the distant joint. Of even greater significance was the observation that at knees, hips, and wrists bilateral CC associated with CC at distant joints when compared to unilateral CC. All these observations suggest the presence of a systemic predisposition to CC. Thus we can conclude that participants with CC have a generalised constitutional predisposition to CC and that this is independent of well established risk factors such as age and OA.

6.1.2 Risk factors for chondrocalcinosis

The associations between several well established and several putative risk factors for CC were examined in a case-control study in which cases with CC were compared with controls without CC.

We found that increasing age but not gender associated with CC. This concurs with previous studies. We also found a statistically significant association between lower current BMI and CC and this novel finding was examined further. The association between CC and current BMI was examined by the site of CC. In this analysis the negative association remained statistically significant at the knees and symphysis pubis and there was a trend towards a negative association at the hips. Thus we can conclude that there is a negative association between current BMI and CC and that this is primarily driven by a negative association between current BMI and CC at the knee, symphysis pubis, and possibly also at the hips. A similar negative association was observed between BMI in the 60s and CC at any site, knees or hips and knees alone. A weaker negative association was present in the 50s. However, there was no association between BMI in early adult life (20s, 30s) and CC at any site, at knees or hips, or knees alone.

We found that OA associated with CC within the same joint at all joints examined except the hip. At the wrists and MCPJs OA in one hand associated with CC on the contralateral side. A similar association was found between left knee OA and right knee CC. However, such an association was not present between right knee OA and left knee CC. In keeping with the finding that there was no association between CC and OA at the same hip,

there was no association between OA in one hip and CC in the contra-lateral hip. We also found that knee OA associated with CC at all distant joints and that hip OA did not associate with CC at any of the distant joints examined. Wrist OA associated with knee CC and MCPJ calcification, and MCPJ OA associated with wrist CC. These findings suggest that, except for hip OA, OA at other sites associates with CC at contralateral or distant joints. This suggests that the association between OA and CC is mediated by both a local and a systemic predisposition. At the knee the presence of CC associated with a greater number of compartments with OA – supporting the view that CC associates with severe arthropathy at the knee.

We also found an association between CC at any site and low cortical BMD. We confirmed the previously reported association between soft-tissue calcification and CC. Vascular calcification on pelvic radiographs and peri-articular calcification on knee radiographs associated with CC. However, there was no association between diuretic use and CC.

At the knee we found that self-reported knee injury, arthroscopy and/or meniscectomy, occupational joint loading and knee malalignment in the 20s associated with knee CC. However, there was no association between current knee malalignment and knee CC.

This work aimed to establish genetic risk factors for CC. This was done by selecting genes that were good candidates for CC susceptibility and determining whether SNPs in these genes associated with CC. These genes were known to be involved in PPi metabolism or with iron overload. The SNPs in these genes were selected based on an opportunistic selection

strategy as has been described before. The -4bp G>A transformation in *ANKH* and rs3045 associated with CC at any site. However, the association lost significance after correcting for multiple testing. SNPs in other genes, namely *TNAP*, *ENPP1*, and *HFE/TFR* did not associate with CC. For all SNPs assessed there was a good biological rationale for examining their association with CC and one of these SNPs had previously been associated with CC. However, we found no convincing evidence for an association between SNPs in these genes and CC. This analysis adds data to the relatively few candidate gene association analyses undertaken in people with apparently sporadic CC.

6.1.3 Radiographic phenotype of osteoarthritis associated with chondrocalcinosis

In this study, we examined the radiographic phenotype of OA in joints with CC. This was done by comparing the radiographic phenotype of OA in joints with OA plus CC with that of joints with OA alone.

We found that in joints with OA the association between presence of CC and OA phenotype varied according to the joint studied. However, unlike previous reports at no site did the presence of CC associate with greater osteophytosis or greater joint space narrowing.

We found that in knees with OA the additional presence of knee CC associated with attrition but did not associate with greater osteophytosis or with more joint space narrowing. Unfortunately the knee radiographs were not scored for presence of sclerosis or cysts so we are unable to comment on any association between knee CC and these radiographic features.

Hips with OA and CC seemed to have a milder OA phenotype than hips with OA alone. In hips with OA the additional presence of hip CC associated negatively with osteophytes score, minimum joint space width and sclerosis on the right side but not on the left side. In hips with OA there was no association between hip CC and cysts on either sides.

In wrists with OA the additional presence of wrist CC associated with sclerosis in the right but not in the left side. However, in wrists with OA there was no association between presence of CC and osteophytosis, joint space narrowing or cysts. In keeping with the findings at the wrists, in STJs with OA the additional presence of wrist CC associated with sclerosis on both sides. Similarly in STJs with OA there was no association between presence of

wrist CC and osteophytosis, joint space narrowing or cysts. In MCPJs with OA the additional presence of wrist CC associated with MCPJ cysts in the right hand but not in the left hand. In 1st CMCJs with OA the additional presence of wrist CC associated with cysts in the left hand but not in the right hand.

Finally, in those instances where local effects of CC on OA phenotype were present we examined whether CC at distant joints associated with specific OA phenotypes at index joints. We found that in knees with OA the presence of CC at distant joints associated with attrition, suggesting the existence of a destructive knee OA phenotype in patients with CC at any site. None of the other associations tested were statistically significant.

6.2 What do the findings suggests?

This study supports the concept that CC occurs more commonly in the fibrocartilage than in the hyaline articular cartilage. However, it disproves the concept that CC does not occur at other joints in the absence of knee involvement. This study also suggests that CC occurs due to a systemic predisposition which is at least partly mediated by OA. The finding that OA associates with CC locally and at distant joints suggests that the association between OA and CC is underlined by a global cartilage matrix change or chondrocyte response which predisposes to CC at the index, and distant joints. This association was however not present for hip OA – a finding which suggests that OA is a diverse disease and that hip OA is distinct from knee, or wrist OA.

This study validates age as an independent association of CC. It reports a novel association between low cortical BMD, and CC. This suggests that in the GOAL population, CC identifies a subset of OA patients who may have had a rapid progression of OA, or indeed the possibility that CC associates with a low BMD. However, this finding should be confirmed in a community based prospective study before any firm conclusions are drawn. The finding of an association between vascular calcification, soft-tissue calcification, and CC suggests the presence of a calcifying phenotype in patients with CC which should be further explored in community based studies with information about calcification at other joints e.g. the shoulder, and stone formation at other sites like the urinary tract, biliary tree etc. The lack of an association between SNPs in *ANKH, TNAP, ENPP1, HFE, and*

Transferrin genes suggests that there is at the most a very small contribution of heredity to the risk of CC.

Apart from OA other local joint specific factors also associate with CC. For example, mechanical loading of the knee, early-life knee malalignment, knee injury, knee arthroscopy and/or meniscectomy associate with CC and this association was independent of OA at that joint. This suggests that these exposures associate with CC over and above their association with OA, and that the association between these risk factors and CC is not mediated by OA. Current knee malalignment did not associate with CC as it is commonly a consequence of knee OA. On the other hand, current and recent obesity associated negatively with CC, while there was no association between self-reported bodyweight in the early adult life and CC. This suggests that low BMI is a non-causal association of CC.

Specific radiographic phenotypes of structural arthropathy associated with CC in joints with OA, and this varied according to the index joint. For example, in knees with OA, the additional presence of CC associated with attrition; and in hips with OA, the additional presence of CC associated with less osteophytosis, sclerosis, and joint space narrowing; but in radiocarpal and scapho-trapezium joints with OA, the additional presence of CC associated with subchondral sclerosis. This together with the association between distant joint CC and knee attrition suggests that CC associates with a less bone forming phenotype of OA at the knee and hip but not at the radiocarpal or the scapho-trapezium joint. This is not surprising as OA has different phenotype and risk factors at different joints (Lane *et al* 2011;

Bierma-Zeinstra *et al* 2011; Kellgren *et al* 1957). The fact that OA has different phenotypes in different joints is also supported using data from the GOAL study. For example data from tables 51 and 59 show that while summated wrist osteophyte score ≥3 (maximum possible score 9, minimum possible score 0) occurred in 10.1% right wrist, and in 9.3% left wrist, summated knee osteophyte score ≥15 (maximum possible score 40, minimum possible score 0) was much more common occurring in 34.9% right knee, and in 34.8% left knee respectively. And similarly, using data from tables 52 and 60 it is clear that while summated wrist joint space narrowing score ≥3 (maximum possible score 12, minimum possible score 0) occurred in 40.5% right wrist, and in 43.1% left wrist; summated knee joint space narrowing score ≥6 (maximum possible score 20, minimum possible score 0) occurred in 35.3% right knee, and in 30.5% left knee respectively. Thus while both marked osteophytosis and severe joint space narrowing commonly occurs in knee joints with OA, osteophytosis is rare at wrists with OA.

6.3 Strengths and weakness of the study

This is the largest study to date to examine the radiographic distribution of CC, its risk factors, and the radiographic phenotype of OA associated with CC. This is also the first large study of CC to include radiographs of all the common sites of CC, specifically knees, pelvis and hands. Previous studies examining the radiographic distribution of CC were restricted to one or two joint areas or to symptomatic joints, and studies in which all three regions have been x-rayed were small and included hospital in-patients or out-patients. Thus our study has better internal validity than previous studies (i.e. we are more certain that the findings are true, and not due to bias, or confounding) with respect to the radiographic distribution of CC at the knees, pelvis and hands than previous studies.

This study examined the risk factors of CC in a systematic manner. Cases with CC were compared with controls without CC. Unlike previous case-control studies the controls were screened for the absence of CC at all common sites of CC. Accurate characterization of cases and controls provides greater internal validity to this study. This has not been the case in previous small studies. Moreover, in order to avoid any confounding, associations between risk factors and CC were adjusted for age, gender and BMI. As radiographs of knee, pelvis, and hands are adequate to identify patients with CC at all joints, we are confident that none of the controls have CC at any site (Resnick *et al* 1977a).

This is the largest study to examine the radiographic phenotype of OA associated with CC. Several previous small studies have reported varying

associations between structural changes and CC. This may reflect a true association with CC or result from a combination of healthcare seeking, ascertainment, and reporting biases. The current study compares the structural phenotype of OA associated with CC to that of OA alone at a particular joint, thereby reducing any possible impact of healthcare seeking, ascertainment, and reporting biases. Moreover, the associations were adjusted for age, gender and BMI which may alter the OA phenotype. Thus the results of this study provide robust data to inform the debate concerning radiographic phenotypes associated with CC.

However, there are several caveats to this study. Firstly, participants with knee or hip OA in GOAL study (comprising over 2/3rd of the GOAL population) were recruited largely from joint replacement lists of patients with symptomatic large joint OA. Therefore the very old patients with OA and those with co-morbidities which preclude joint replacement surgery may not have been recruited into GOAL. Participants without radiographic and clinical OA at hips and knees (GOAL 'controls') were selected from patients who had undergone an IVU at the CHN and had no radiographic OA in their hips. Therefore, participants in this study do not represent a random sample of community dwelling adults. Thus selection bias is likely to reduce the external validity of the findings i.e. make it less generalisable when applied to other populations e.g. to the general population.

The manner of selection of GOAL 'controls' introduces a non-random selection bias which may affect the association between exposure and event of interest. However, any impact of this bias is likely to be minimal as the

demographic profile of GOAL controls is comparable to that of several community based healthy UK cohorts (Kate Limer, Thesis submitted to the University of Nottingham 2007). Moreover suspected nephrolithiasis which is the commonest indication for IVU is not reported to associate with either CC or with OA. However, other crystal deposition diseases have been associated with CC. Thus, it is possible that a generalized deficiency of crystallization inhibitors may predispose to both chondrocalcinosis and urinary stone formation. Such a phenomenon may bias the GOAL controls by falsely increasing the prevalence of CC compared to the general population. This theoretical concern is unlikely to be a major factor as the prevalence of knee CC in GOAL controls (4.2%) was comparable to that reported in population based studies of prevalence of knee CC in the absence of radiographic knee OA (Felson *et al* 1989). In the Framingham study, knee CC was reported to occur in 5.8% of community dwelling elderly participants without radiographic knee OA (Felson *et al* 1989). The slightly higher prevalence of knee CC in the Framingham study compared to the GOAL controls may be related to the fact that participants in the Framingham study were older (mean age 73 years) than GOAL controls (mean age 65 years). However, bias may still exist since GOAL controls have a lower prevalence of hip or knee OA and higher prevalence of renal, cardiovascular problems and diabetes than the general population.

As two-thirds of GOAL participants have symptomatic hip or knee OA severe enough to warrant referral for joint replacement surgery and the remaining participants have no radiographic or clinical features of hip or knee

OA, this study population with an artificially inflated prevalence of severe large joint OA is not representative of a community based sample of OA patients. This again reduces the external validity of the findings.

Since OA associates with CC the prevalence of CC reported in this study should be interpreted with caution and may have limited generalisability to the general population. However, this study has external validity in that the prevalence of CC reported in this study is in keeping with that observed in large community based radiographic surveys (Felson et al. 1989; Sanmarti *et al* 1993; Ramonda *et al* 2009).

GOAL was primarily designed to examine genetic risk factors for large joint OA and to explore gene-environmental interaction using a case-control design. Participants were selected for the presence or absence of symptomatic large joint OA. The current study was carried out by reconstituting the original case (symptomatic large joint OA) and control (no clinical or radiographic large joint OA) groups and therefore may be confounded by OA. However, we have attempted to minimize any possible effects of confounding by adjusting the analysis for hip or knee OA.

The finding that sporadic CC results, at least in part, from a systemic predisposition to CC that is independent of well recogniosed risk factors for CC such as age and OA, and of other systemic predispositions to OA such as gender and BMI, may just be a manifestation of a ‘generalised predisposition to OA’. Therefore, this finding should be validated in a community based study where the recruitment is independent of the OA status of the participants.

It is not possible to score radiographs for OA changes with the observer blinded for CC and vice versa. Therefore joint radiographs were scored for OA features and for the presence/absence of CC at the same sitting. This introduces the possibility of detection bias in the detection of CC since OA is known to associate with CC. This may be expected to falsely inflate the prevalence of CC in the GOAL population. However, the impact of any such bias in detection of CC would appear to be minimal since the prevalence of CC reported in this study is in keeping with what has been published before, and the OR for association between large joint OA and CC reported in this study are comparable to those reported in other community based studies. Furthermore the radiographs were assessed and scored by a single observer long before the questions that are addressed in this thesis were posed.

For the reasons cited above, measurement bias may operate when scoring structural changes in joints with CC. However, the presence of a measurement bias is unlikely as most analyses of association between specific radiographic phenotype associated with OA and CC at a joint showed that there was no association between the two.

There are several caveats to the analysis of association between risk factors and CC. Since two-thirds of the GOAL population have severe symptomatic large joint OA and because OA associates with CC, some of the risk factors for CC identified in this study could simply be due to confounding by OA. However, we have minimised any confounding by adjusting the analyses for OA at hips or knees using multivariate logistic

regressions. Moreover, the validity of the associations between risk factors and CC is supported by the fact that the ORs for association between well recognised risk factors for CC such as age and OA reported in this study are very similar to those reported in previous large community based studies. For these reasons we believe that the risk factors for CC identified in this study are likely to be valid and generalizable to the general population.

Information about several risk factors for CC such as weight in early adult life (used to calculate early adult life BMI), early adult life body shape, diuretic use, early adult life knee mal-alignment, lifelong occupation, knee injury and arthroscopy and/or meniscectomy were self reported. They may be affected by inaccurate recall and differential misclassification bias. Validation of the self-reported data has not been carried out. Patients with symptomatic OA may be more likely to report a previous exposure to such factors than those without symptomatic OA which may result in a differential misclassification bias. Similarly during the course of administering the questionnaire the research nurse may have become aware of the disease state (OA vs not OA) of the participant which may result in observer or interviewer bias. Since CC associates with OA, differential misclassification bias or observer/interviewer bias may limit the internal validity of our findings. While non-differential misclassification bias carries a risk of obscuring real differences, differential misclassification bias compromises the study's internal validity. However, to minimise the risk of differential misclassification bias the analyses were adjusted for the presence of hip or knee OA, which is the level at which this differential misclassification bias is likely to operate.

Any risk of observer/interviewer bias was reduced by regular training and feedback to the research nurses.

All participants in the GOAL study were included in this study and no formal power calculations were carried out. Therefore we are unable to say if the lack of statistically significant associations reported in this study results from a type 2 error. This may be especially true of the genetic association analyses. Common diseases like CC are multifactorial and any genetic contribution is likely to be oligo- or polygenic so the contribution from individual SNPs is relatively very small. Therefore a large number of cases are required to detect any genetic effect.

Although it is desirable to conduct a prospective cohort study to examine risk factors for CC this would be prohibitively expensive due to the rarity of some risk-factors and the long latency for the development of CC. A case-control study allows the rapid and relatively inexpensive examination of a large number of potential risk factors. However, because this is a case control study it can only examine associations between risk factors and CC. Although retrospective questioning was employed a prospective study is necessary to establish causality.

All radiographs were scored for presence or absence of CC, for the severity of OA, for individual structural features of OA and for radiographic knee mal-alignment by a single trained observer. However, there was excellent intra-rater reliability and inter-rater agreement for the scoring of CC; and good to excellent intra-rater reliability for scoring radiographic changes of OA. Also a validation study to establish that CC in GOAL participants is

predominantly due to CPP crystals was not carried out - the synovial fluid of cases with CC and controls without CC was not examined for the presence of CPP or other crystals. However, a majority of CC patients in previous studies have been shown to have CPP crystals. For the same reason we are unable to say what proportion of controls without CC had CPP crystals in the current study.

6.3 Recommendations for future research

The findings of this study re-confirm several well established risk factors for CC such as age, OA and meniscectomy. However, we also report several novel associations of CC such as low BMI, knee mal-alignment in early adult life, low cortical BMD and soft-tissue and vascular calcifications, all of which need validation in a large community based survey in which the recruitment of participants is independent of their OA status. Such a large community based survey of radiographs, preferably combined with ultrasound of wrists, knees and hips for CC (ultrasound being more sensitive for detection of CC at peripheral joints (Ellabban *et al* 2011) would also validate our observation that CC frequently occurs at other joints in the absence of knee CC, and that individuals with CC have a systemic albeit joint specific predisposition to CC. Such a study can also be used to validate our observations on the OA phenotype associated with CC at an index joint.

CC is a relatively insensitive marker of CPPD. A study examining the relation between CPP crystals (obtained by aspiration, arthroscopy or surgical removal during joint replacement) and the OA phenotype is required to further establish the OA phenotype associated with CPPD at an index joint. The findings from such a study will further validate the novel risk factors for CC identified in this study. The synovial fluid PPi in these joints could also be measured and its relation with body weight, BMI and cortical and cancellous BMD could be examined in order to explain the observed association between low BMI, low cortical BMD and CC.

Our finding that CC associates with low cortical BMD needs to be validated in a community based study using standard measures of BMD. Further studies examining the levels of bone formation markers, bone resorption markers, vitamin D, parathyroid hormone and calcium levels in patients with CC are necessary to elucidate the reasons underlying an association between CC and low BMD.

Studies to date do not convincingly support any significant genetic contribution to the occurrence of sporadic CPPD. However, our findings and those from a hospital based case control study of participants with CC and healthy blood donors suggest that SNPs in *ANKH* may have a role in sporadic CPPD. Detection of individual genetic contributions to common complex disorders requires a large study with sufficient power to examine the association between SNPs in *ANKH* and CPPD. As CC is an insensitive marker of CPPD the cases and controls in such a study may be better characterised by ultrasound in a community based survey, or by CPP crystal identification in synovial fluid or joint tissues in participants with established or end-stage OA.

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Appendix

i. Published work

The work done in this PhD has resulted in following publications and presentations:

1. Abhishek A, Doherty S, Maciewicz RA, Muir KR, Zhang W, Doherty M. Self-reported knee malalignment in early adult life as an independent risk for knee chondrocalcinosis. Arthritis Care Res (Hoboken). 2011 Nov;63 (11):1550-7.

2. Abhishek A, Doherty M. Pathophysiology of articular chondrocalcinosis--role of ANKH. Nature Review Rheumatology. 2011 Feb;7 (2):96-104.

3. Ingham SL, Moody A, Abhishek A, Doherty SA, Zhang W, Doherty M. Development and validation of self-reported line drawings for assessment of knee malalignment and foot rotation: a cross-sectional comparative study. BMC Medical Research Methodology. 2010 Jun 18;10:57.

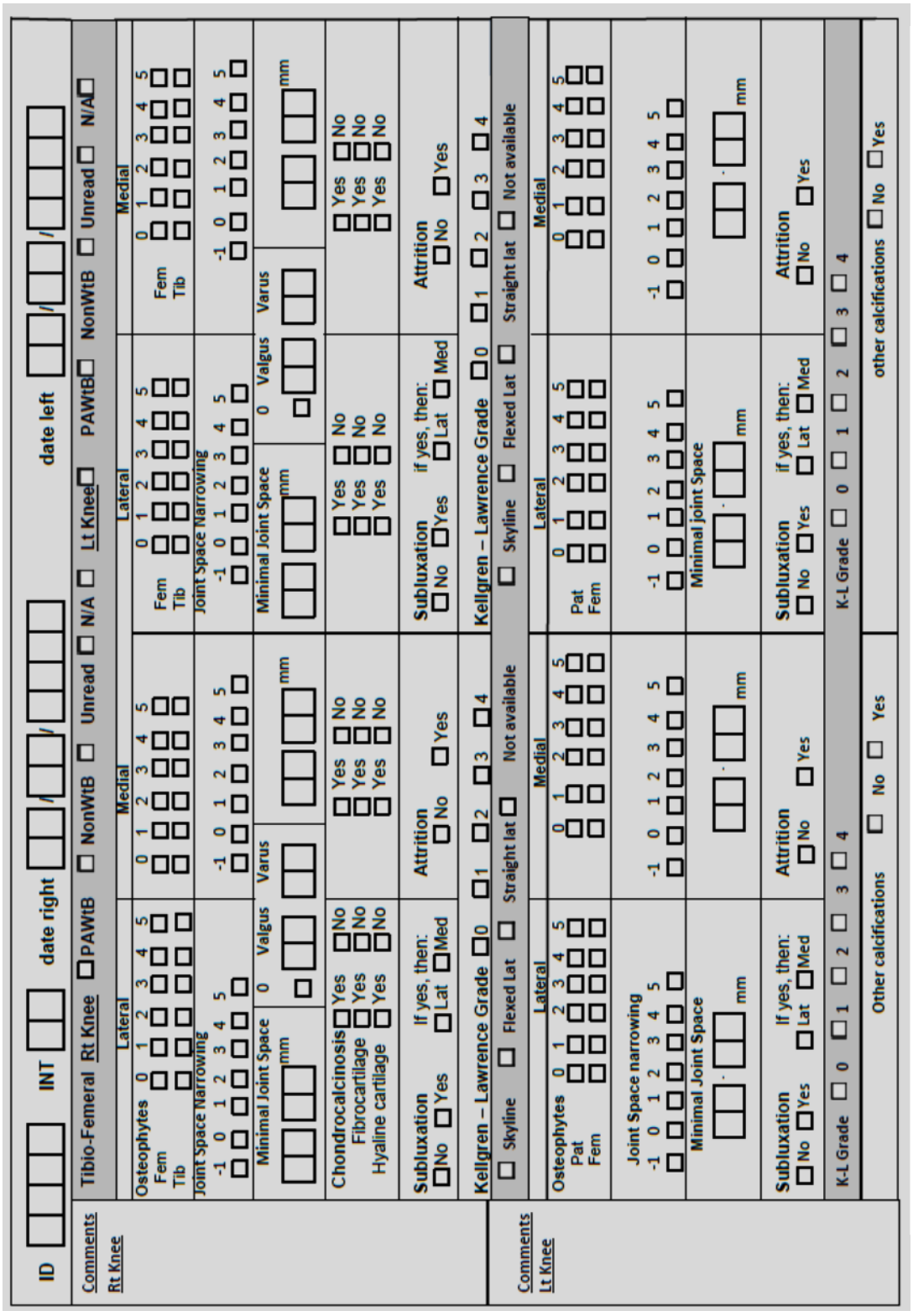
4. Abhishek A, Doherty S, Maciewicz RA, Muir KR, Zhang W, Doherty M. Evidence that a systemic predisposition to chondrocalcinosis exists and is mediated by osteoarthritis at most but not all joints. EULAR2012.

5. Abhishek A, Doherty S, Maciewicz RA, Muir KR, Zhang W, Doherty M. Does chondrocalcinosis associate with a different radiographic phenotype of osteoarthritis? EULAR2012.

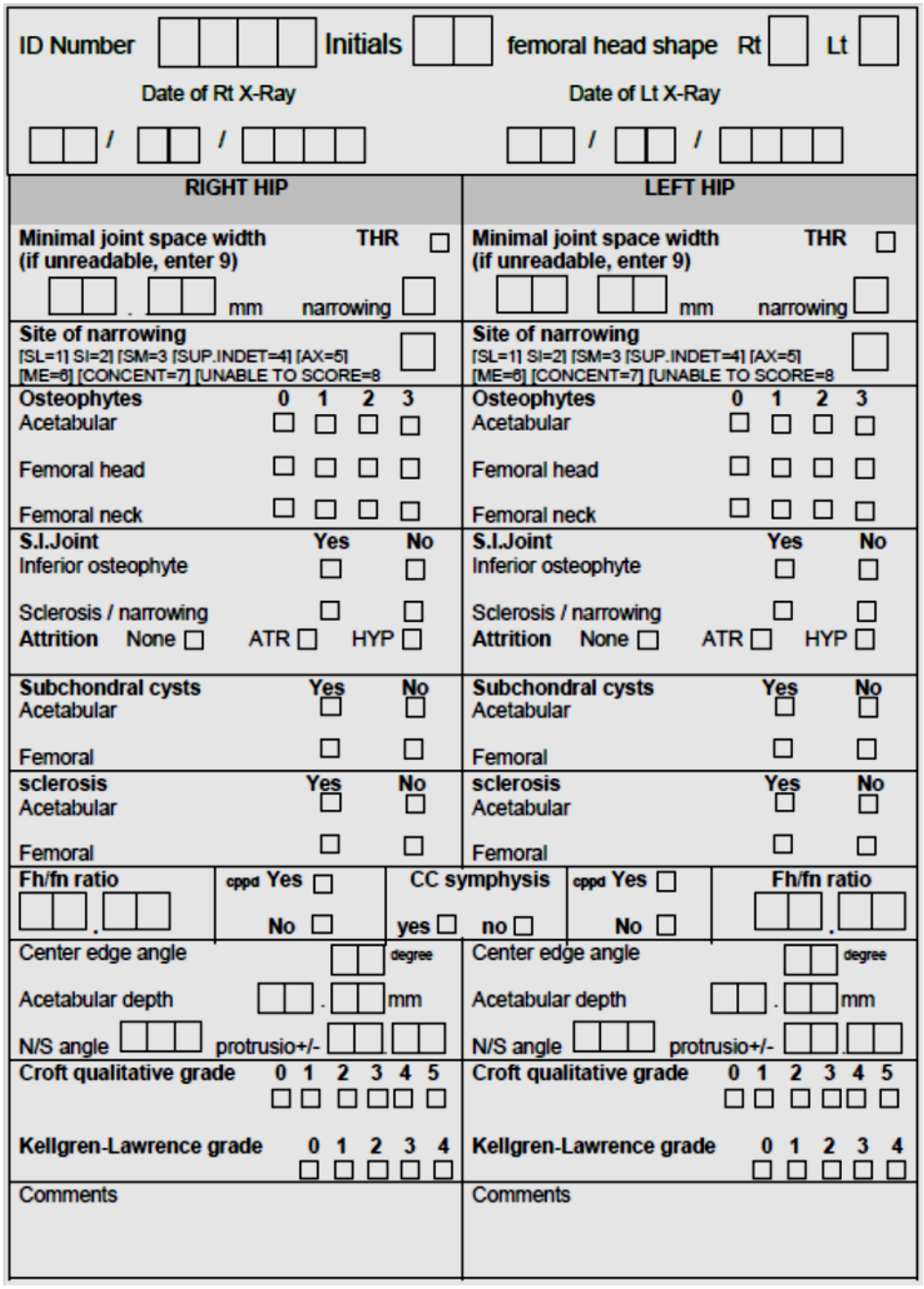
6. Abhishek A, Doherty S, Maciewicz RA, Muir KR, Zhang W, Doherty M. Chondrocalcinosis a fresh look at established and novel risk factors. EULAR2012.

7. Abhishek A, Doherty S, Maciewicz RA, Muir KR, Zhang W, Doherty M. Chondrocalcinosis frequently occurs at the hips and wrists in the absence of knee involvement. EULAR2012.

ii. Template for recording osteoarthritis phenotype scores



Template for scoring knee radiographs



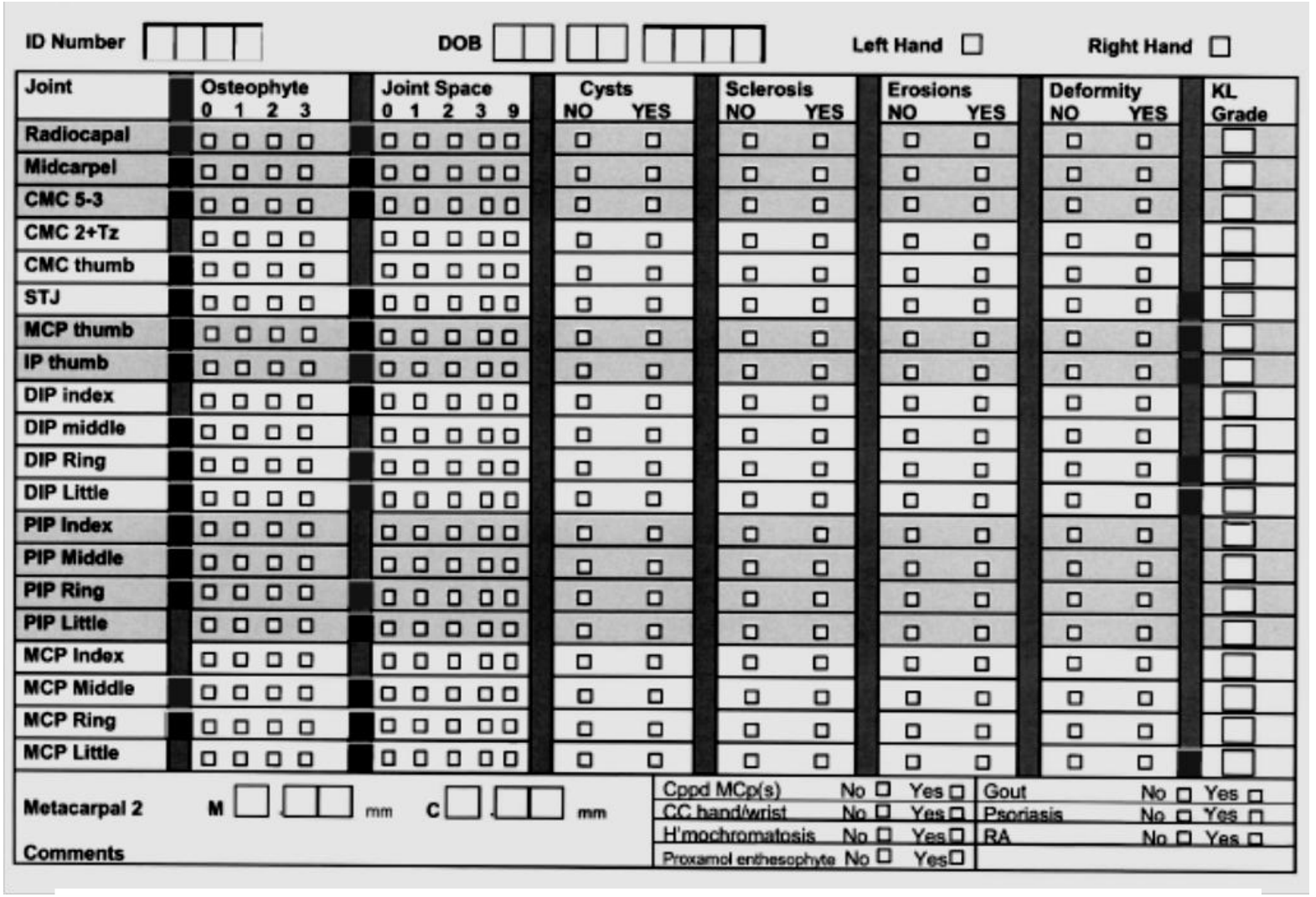
ID Number ☐☐☐☐ Initials ☐☐ femoral head shape Rt ☐ Lt ☐

Date of Rt X-Ray ☐☐ / ☐☐ / ☐☐☐☐

Date of Lt X-Ray ☐☐ / ☐☐ / ☐☐☐☐

RIGHT HIP	LEFT HIP
Minimal joint space width (if unreadable, enter 9) ☐☐.☐☐ mm narrowing ☐ THR ☐	**Minimal joint space width (if unreadable, enter 9)** ☐☐.☐☐ mm narrowing ☐ THR ☐
Site of narrowing [SL=1] SI=2] [SM=3 [SUP.INDET=4] [AX=5] [ME=6] [CONCENT=7] [UNABLE TO SCORE=8 ☐	**Site of narrowing** [SL=1] SI=2] [SM=3 [SUP.INDET=4] [AX=5] [ME=6] [CONCENT=7] [UNABLE TO SCORE=8 ☐
Osteophytes 0 1 2 3 Acetabular ☐ ☐ ☐ ☐ Femoral head ☐ ☐ ☐ ☐ Femoral neck ☐ ☐ ☐ ☐	**Osteophytes** 0 1 2 3 Acetabular ☐ ☐ ☐ ☐ Femoral head ☐ ☐ ☐ ☐ Femoral neck ☐ ☐ ☐ ☐
S.I.Joint Yes No Inferior osteophyte ☐ ☐ Sclerosis / narrowing ☐ ☐ **Attrition** None ☐ ATR ☐ HYP ☐	**S.I.Joint** Yes No Inferior osteophyte ☐ ☐ Sclerosis / narrowing ☐ ☐ **Attrition** None ☐ ATR ☐ HYP ☐
Subchondral cysts Yes No Acetabular ☐ ☐ Femoral ☐ ☐	**Subchondral cysts** Yes No Acetabular ☐ ☐ Femoral ☐ ☐
sclerosis Yes No Acetabular ☐ ☐ Femoral ☐ ☐	**sclerosis** Yes No Acetabular ☐ ☐ Femoral ☐ ☐
Fh/fn ratio ☐☐.☐☐ cppd Yes ☐ No ☐ CC symphysis yes ☐ no ☐	cppd Yes ☐ No ☐ **Fh/fn ratio** ☐☐.☐☐
Center edge angle ☐☐ degree Acetabular depth ☐☐.☐☐ mm N/S angle ☐☐☐ protrusio+/- ☐☐.☐☐	Center edge angle ☐☐ degree Acetabular depth ☐☐.☐☐ mm N/S angle ☐☐☐ protrusio+/- ☐☐.☐☐
Croft qualitative grade 0 1 2 3 4 5 ☐ ☐ ☐ ☐ ☐ ☐ **Kellgren-Lawrence grade** 0 1 2 3 4 ☐ ☐ ☐ ☐ ☐	**Croft qualitative grade** 0 1 2 3 4 5 ☐ ☐ ☐ ☐ ☐ ☐ **Kellgren-Lawrence grade** 0 1 2 3 4 ☐ ☐ ☐ ☐ ☐
Comments	Comments

Template for scoring hip radiographs



Template for scoring hand radiographs

iii. Intra-rater reliability for scores of structural radiographic changes of OA

Scoring of radiographs commenced in January 2006. Reproducibility was assessed in June 2006. A sample of 20 subjects with different scores of knee OA, and different scores of hip OA was selected since this would ensure there was a fair distribution of subjects with different degrees of severity of radiographic features of OA. The scorer was blinded to previous scores. Kappa (for dichotomous data), weighted Kappa (for categorical readings) and ICC (for continous data) were calculated as appropriate.

Joint	Radiographic feature	Intra-rater agreement*
Knee		
	Kellgren - Lawrence grade	0.78
	Osteophytes - femoral lateral	0.64
	Osteophytes - tibial lateral	0.90
	Osteophytes - femoral medial	0.92
	Osteophytes - tibial medial	0.91
	Joint space narrowing – lateral	0.87
	Joint space narrowing – medial	0.84
Hip		
	Kellgren - Lawrence grade	0.89
	Osteophytes - acetabular	0.84
	Osteophytes - femoral head	0.89
	Osteophytes - femoral neck	0.60
	Joint space narrowing	0.93
	Minimal joint space width	0.98
	Subchondral cysts – acetabular	1.0
	Subchondral cysts – femoral	1.0
	Sclerosis – acetabular	1.0
	Sclerosis – femoral	0.88

iv. Illustration of method for measuring radiographic frontal plane knee alignment

The radiographic frontal plane knee alignment was measured using the method described by Krause *et al* (Kraus *et al* 2005). This has been described in methods (see section 2.1.6), and is illustrated in the following knee radiograph -