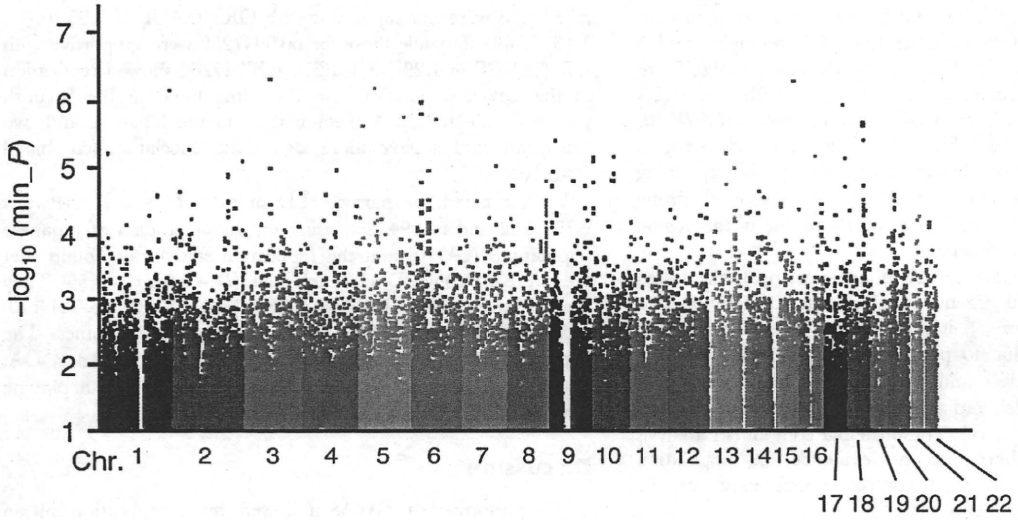

Figure 1. Results of a genome-wide association study ($-\log_{10}$ *P* value plot). Each *P* value is the minimum of Fisher's exact tests for three models: dominant, recessive and allele frequency model.
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SNPs selected for the replication study that had the smallest *P* values (minimum $P<1\times10^{-5}$) were next genotyped in an independent set of 167 Japanese knee OA individuals and 347 Japanese controls from a resident cohort study. Through these studies, only two SNPs, rs7775228 (combined $P=2.43\times10^{-8}$; OR = 1.34; 95% CI = 1.21–1.49) and rs10947262 (combined

Table 2. Association of rs7775228 and rs10947262 with knee osteoarthritis.

SNP (Nearest gene)	Allele	Population	Minor allele Frequency				
			Case	Control	OR (95% CI)[a]	P^b	$P_{het}{}^c$
rs7775228	C/T	Japanese					
(*HLA-DQB1*)		GWAS	0.318	0.379	1.31 (1.18–1.47)	1.38 E-06	
		Replication	0.290	0.385	1.53 (1.15–2.02)	3.07 E-03	
		Combined[d]			1.34 (1.21–1.49)	2.43 E-08	0.33
		European Caucasian					
		Spanish	0.194	0.209	1.10 (0.83–1.45)	0.521	
		Greek	0.094	0.075	0.78 (0.58–1.03)	0.084	
		European combined[e]			0.93 (0.76–1.13)	0.178	0.09
		All combined[f]			–	–	0.003
rs10947262	C/T	Japanese					
(*BTNL2*)		GWAS	0.358	0.419	1.30 (1.16–1.44)	2.45 E-06	
		Replication	0.332	0.422	1.47 (1.12–1.93)	5.74 E-03	
		Combined[d]			1.32 (1.19–1.46)	6.73 E-08	0.40
		European Caucasian					
		Spanish	0.122	0.136	1.13 (0.81–1.59)	0.465	
		Greek	0.068	0.094	1.43 (1.06–1.92)	0.019	
		European combined[e]			1.29 (1.03–1.61)	0.025	0.32
		All combined[f]			1.31 (1.20–1.44)	5.10 E-09	0.63

Odds ratios (ORs) and *P* values for the independence test were calculated by the Mantel-Haenszel method.
[a]OR of the risk allele from the two-by-two allele frequency table.
[b]P values of the Pearson's χ^2 test for the allele model.
[c]Results of the Breslow-Day test.
[d]Meta-analysis of Japanese studies.
[e]Meta-analysis of European studies.
[f]Meta-analysis of all studies.
doi:10.1371/journal.pone.0009723.t002

$P = 6.73 \times 10^{-8}$; OR = 1.32; 95% CI = 1.19–1.46) were significant even after the Bonferroni correction for multiple testing ($P = 1.09 \times 10^{-7}$) (Table 2). The two SNPs showing significant associations are located within a 340-kb region within the HLA locus, including *BTNL2*, *HLA-DRA*, *HLA-DRB5*, *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* (Figure 2). Although the HLA region is known to show extensive linkage disequilibrium (LD) spanning over 7 Mb, only SNPs in the 340-kb region showed strong associations with OA (Figure 2), and SNPs outside of this region did not have significant association.

Application of the Cochrane-Armitage test to all the tested SNPs indicated that the genetic inflation factor lambda was 1.08 for GWAS (Figure 3), implying a low possibility of false positive associations due to population stratification. We also carried out age, gender- and BMI-adjusted analysis using a logistic regression model, and confirmed similar association after adjustment (data not shown). The principal component analysis [13] revealed that there was no evidence for population stratification between the two control groups used for the GWAS (Figure S1).

To check the association of rs7775228 and rs10947262 in different ethnic populations, we examined the association of the SNPs with knee OA in two European Caucasian populations from Greece and Spain. We genotyped a total of 813 OA and 1,071 control subjects (Table 1). We conducted the meta-analysis using the Mantel-Haenszel method. The combined European results for rs7775228 were not significant with OR (95%CI) of 0.93 (0.76–1.13) (Table 2), while those for rs10947262 were supportive with OR (95%CI) of 1.29 (1.03–1.61). rs10947262 showed replication in the Greek population and the same trend in the Spanish population (Table 2). A meta-analysis of the Japanese and two European studies gave more significant association (combined $P = 5.10 \times 10^{-9}$).

We estimated the pairwise LD indexes (D' and r^2) between rs7775228 and rs10947262 using the genotype data of Japanese populations (GWAS and the replication study), and found that they were in strong LD with each other ($D' = 0.82$, $r^2 = 0.56$). They formed two frequent haplotypes (Haplotype I and II; Table 3) accounting for about 90% of all observed chromosomes. The haplotypes were also significantly associated with knee OA; Haplotype I, the most frequent haplotype was a risk haplotype ($P = 1.48 \times 10^{-8}$; OR = 1.33; 95% CI = 1.20–1.46).

Discussion

We performed a GWAS followed by a replication in an independent population using a total of ~4,800 Japanese subjects, and identified two SNPs (rs7775228 and rs10947262) in the HLA class II/III locus associated with susceptibility to knee OA. To our knowledge, this study represents the first GWAS of OA with extensive coverage (~550,000 markers) and definite genome-wide significance even after Bonferroni's correction, which is very

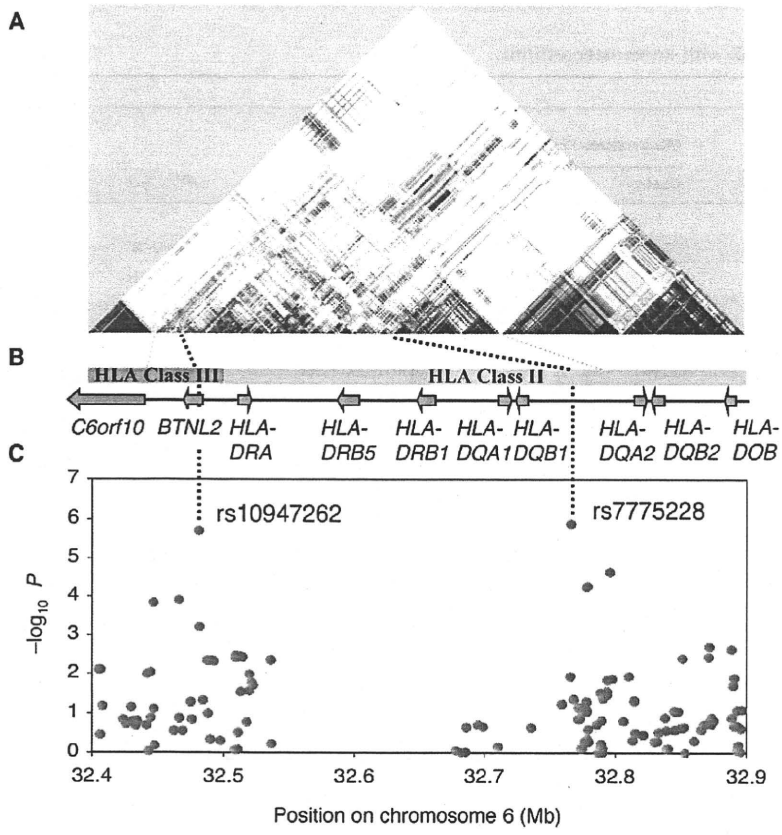

Figure 2. Case-control association analysis and linkage disequilibrium (LD) map of the HLA class II/III region of chromosome 6. (A) The LD map based on D' was drawn using HapMap data release 24 for the JPT population. (B) Genomic structure within the extended HLA-II/III region. (C) Results of GWAS for osteoarthritis in Japanese population. The $\log_{10}$-transformed P values are plotted on the y axis.
doi:10.1371/journal.pone.0009723.g002

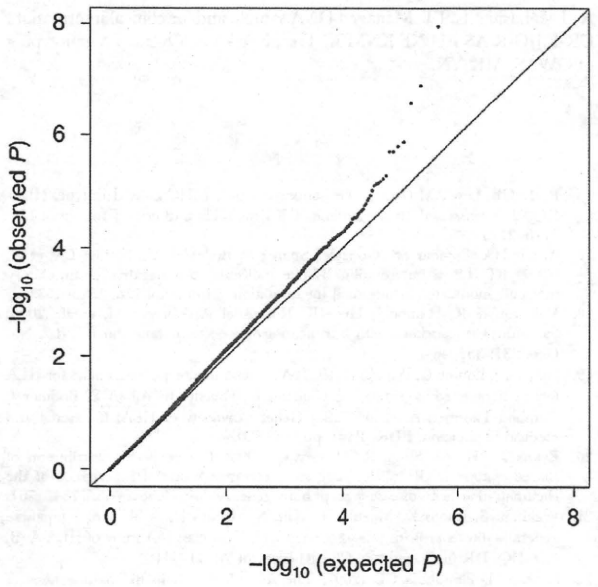

Figure 3. GWAS of knee osteoarthritis. Q-Q plot with Cochrane-Armitage trend *P* in the GWAS. Horizontal and vertical lines represent expected *P* values under a null distribution and observed *P* values, respectively. The genetic inflation factor lambda is 1.08.
doi:10.1371/journal.pone.0009723.g003

conservative. There were no effects of population stratification and confounding factors. Since two groups of controls were used in the GWAS, we evaluated the possibility of genetic heterogeneity between the two groups by a principal component analysis and found it unlikely (Figure S1). Although there was large age difference between the case and control groups of GWAS (Table 1), significant association was observed after the age adjustment.

In the NCBI genome database, rs7775228 and rs10947262 located between upstream region of *HLA-DQA2* and *HLA-DQB1*, and within the intron 1 of *BTNL2*, respectively (Figure 2). *HLA-DQA2* and *HLA-DQB1* encode HLA-DQ α and β chains, which belong to the HLA class II molecules. HLA class II molecules are expressed in antigen presenting cells (B lymphocytes, dendritic cells and macrophages) and play a central role in the immune system by presenting peptides derived from extracellular proteins [14]. The HLA-DQA2 protein is expressed, but at a very low level in comparison with the HLA-DQA1 protein [15,16]. Moreover, the HLA-DQA2 α chain does not dimerize with class II β chains [16]. *BTNL2* encodes butyrophilin-like 2, a member of butyrophilin family that shares sequence homology with the B7 co-stimulatory molecules. BTNL2 regulates T-cell activation through unknown receptor, distinct from CD28 and CTLA-4 [17]. In Japanese population, the haplotype association was more significant than those of respective SNPs (Tables 2 and 3). Therefore, there may be hidden SNP(s) with a lower *P* value than rs7775228 and rs10947262, or the haplotype may be implicated in the OA susceptibility. An association of sarcoidosis with rs2076530, a coding SNP on exon 5 of the *BTNL2* gene has been reported [18], but the SNP was not in LD with rs10947262 ($D' = 0.11$, $r^2 = 0$).

Table 3. Haplotype association analysis for knee osteoarthritis susceptibility SNPs, rs7775228 and rs10947262.

		Frequency		Haplotype effect[b]	
Haplotype[a]		Case	Control	OR (95% CI)	P value
I	TC	0.610	0.541	1.33 (1.20–1.46)	1.48 E-8
II	CT	0.277	0.340	0.74 (0.67–0.83)	5.07 E-8
III	CC	0.077	0.080	0.96 (0.80–1.15)	0.653
IV	TT	0.037	0.040	0.91 (0.71–1.18)	0.475

OR: odds ratio, CI: confidence interval.
[a]Alleles of rs7775228 (C/T) and rs10947262 (C/T) with this order.
[b]A haplotype vs. all others.
doi:10.1371/journal.pone.0009723.t003

The 340-kb region of HLA locus, where the two SNPs are located also includes *HLA-DRA*, *HLA-DRB1*, *HLA-DRB3*, *HLA-DRB4*, *HLA-DRB5* and *HLA-DQA1*. *HLA-DRA*, *HLA-DRB1/3/4/5* and *HLA-DQA1* encode HLA-DR α, β and HLA-DQ α chains, which could also belong to the HLA class II molecules. *HLA-DRB1* is present in all individuals. Allelic variants of *HLA-DRB1* are linked with either none or one of the genes *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* [19]. Among these genes, *HLA-DRB1* is strongly associated with RA. Some subtypes of HLA-DRB1 alleles, such as *0101, *0401, and *0405, is associated with RA [20], but not with generalized OA [21].

Although OA has generally been considered a non-inflammatory disease, accumulating evidences suggest that this is not the case. Inflammation involving activated T cells in the synovial membrane of OA patients is well documented [22]. Recently, we identified a genetic variant of *EDG2* gene encoding lysophosphatidic acid receptor associated with knee OA [23]. A GWAS has identified a genetic variant of the *PTGS2* gene encoding cyclooxygenase-2 involved in risk for knee OA [6]. These genetic associations of genes such as *EDG2* and *PTGS2* underscore the potential role of inflammatory pathways in the pathogenesis of knee OA.

Several studies have suggested associations of OA with HLA class I and class II alleles. Study on generalized OA revealed association with HLA A1-B8 in Caucasian [24] and with HLA-Cw4 in Japanese [21]. An association of the HLA-DRB1*02 alleles with knee and hip OA was identified in a cohort of 106 patients [25]. Interestingly, chondrocyte, which are normally HLA-DP, DQ and DR-negative, become positive for them in OA [26,27], suggesting their function as antigen-presenting cells. Cartilage fragments are mechanically shaved from the joint surface and frequently found in the synovial membrane of OA patients [28]. So, physical interaction between chondrocytes and T cells is conceivable. Peripheral blood T cells from OA patients show significantly higher proliferative responses to autologous chondrocytes [27]. Our results further support the concept that OA is an immunologic disorder.

Supporting Information

Figure S1 Principal component analysis of GWAS samples. Samples in the GWAS and in HapMap database are analyzed by a program of Smartpca [12], and plotted for the first (X axis) and the second (Y axis) principal component (PC), respectively.
Found at: doi:10.1371/journal.pone.0009723.s001 (0.16 MB TIF)

Acknowledgments

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Author Contributions

Wrote the paper: MN SI. Planned and supervised the whole project: SI. Performed the Japanese association study: MN IK TF JD. Managed the European association study: A. Tsezou AG. Helped with statistic analysis: A. Takahashi NK TT. Managed DNA sample and/or clinical information: CR-F JJG-R AS AU NF KNM A. Tsezou AG YN. Oversaw a genotyping of GWAS: MK YN.

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Tophaceous pseudogout in the knee joint mimicking a soft-tissue tumour: a case report

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ABSTRACT

Tophaceous pseudogout in the knee joint is rare. We report an 82-year-old man who presented with a one-year history of pain and swelling of the right knee joint. Treatment with non-steroidal anti-inflammatory drugs and aspiration of the joint effusion had not been effective. The mass continued to enlarge, and the patient had difficulty walking. Radiographs and computed tomography showed meniscal calcification with an abnormal soft-tissue mass surrounded by calcification. After excision, massive calcified deposits were seen both inside and on the surface of the tophaceous pseudogout. The deposits showed birefringence under polarised light, suggestive of calcium pyrophosphate dihydrate crystals. At the 2-year follow-up, the patient could walk independently without knee pain or swelling, although his range of knee motion was slightly limited due to joint contracture that developed before surgery.

Key words: *calcium pyrophosphate; chondrocalcinosis; knee joint; soft tissue neoplasms; synovial membrane*

INTRODUCTION

Pseudogout is caused by deposition of calcium pyrophosphate dihydrate (CPPD) crystals in the articular cartilage or synovium. It predominantly affects the knee, wrist, and hip joints of elderly people. The mechanisms governing deposition and formation of CPPD, together with its biological significance in the human body, remain unclear.[1] Pseudogout of the knee joint sometimes induces destruction of the articular cartilage, resulting in secondary osteoarthritis.

We present a rare case of tophaceous pseudogout of the knee joint. Tophaceous pseudogout (also known as tumoural CPPD crystal deposition disease) is pseudogout that manifests in the form of a mass. It usually occurs in the temporomandibular joint, occasionally in the perispinal tissues, but rarely in the joints of the extremities.[2-5]

CASE REPORT

In October 2007, an 82-year-old man presented to our hospital with a one-year history of pain and swelling in his right knee joint. Treatment with non-steroidal anti-inflammatory drugs and aspiration of the joint

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effusion had not been effective. The mass continued to enlarge, and the patient had difficulty walking.

On examination he was found to have a hard, moveable mass, 5x5 cm in size, with a smooth surface and a well-defined margin in the medial para-patellar pouch. His right knee range of motion was limited (-20° extension to 90° flexion).

Radiographs showed a mass with abnormal calcification of the meniscus soft tissue (Fig. 1). Computed tomography revealed irregular and marginal calcification around the mass (4x4x1.5 cm) in the medial para-patellar joint space (Fig. 2).

The initial diagnosis was a synovial osteochondromatosis, which may occur secondarily or haphazardly in combination with pseudogout. Other benign tumours (such as myositis ossificans and a calcified lipoma) or malignant tumours (such as a synovial sarcoma, a leiomyosarcoma or an extra skeletal osteosarcoma) could not be ruled out.[6,7]

An excisional biopsy was performed. The mass was partially connected to the synovium and measured 5x4.5x2 cm in size. Its surface was whitish and glossy, suggestive of fibrous or articular cartilage (Fig. 3). Geometric calcified deposits were observed on the surfaces of the tumour and the joint synovium, but no palpable mass was noted in the synovium. Calcification was also observed on the articular cartilage and meniscus, but no significant degeneration or destruction was seen. The mass was excised as a reactive benign lesion, thus neither a synovectomy nor a meniscectomy was performed.

A histological examination of the excised tumour tissue revealed massive calcified deposits (which were stained dark blue by haematoxylin-eosin) on both the surface (more prominent) and interior of the tumour (Fig. 4). The interstitial matrix resembled fibrous cartilage, or fibrous granulation tissue. The calcified deposits showed birefringence under polarised light, suggestive of CPPD crystals (Fig. 5). The diagnosis was changed to tophaceous pseudogout.

At the 2-year follow-up, the patient could walk independently without knee pain or swelling, although his knee range of motion was limited by a joint contracture that developed prior to surgery.

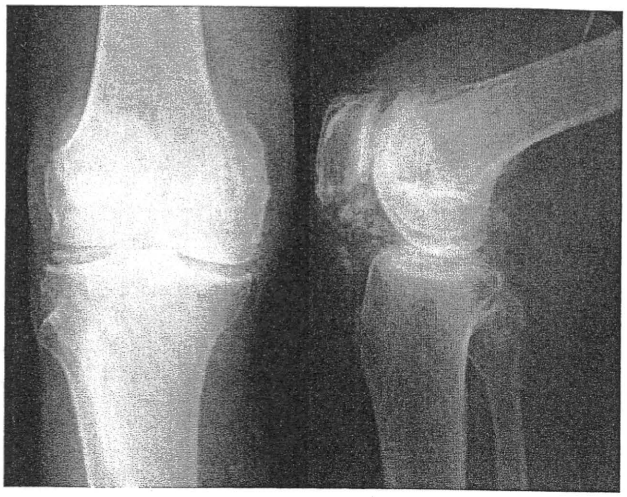

Figure 1 Radiographs of the right knee joint showing a mass with abnormal calcification of the meniscus.

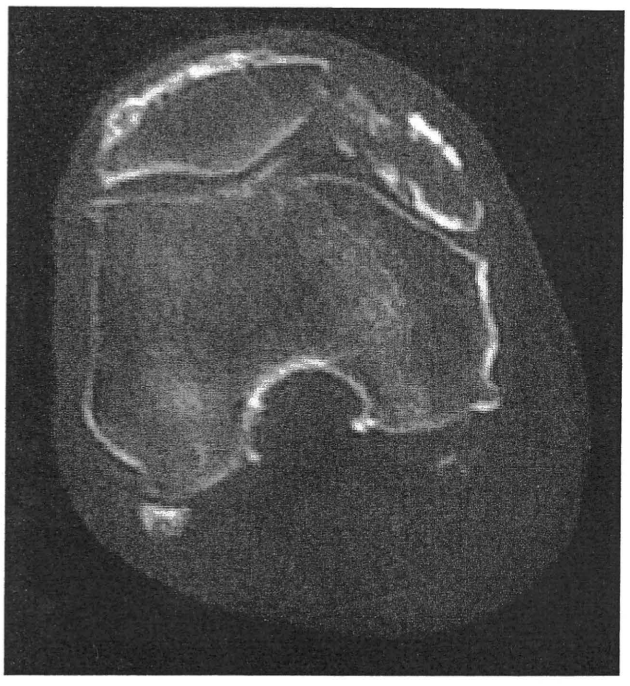

Figure 2 Computed tomography showing irregular and marginal calcification around the mass in the medial para-patellar joint space.

DISCUSSION

Tophaceous pseudogout is a rare disease of unknown aetiology, characterised by CPPD crystal deposition that forms a solitary bulky mass in the joint.[1] The usual site of involvement is the temporomandibular joint; it is rarely reported in the joints of the extremities. Most such cases are misdiagnosed radiographically as bone or soft-tissue tumours.[1,3,8–10]

We reviewed computed tomographic findings of 9 reported cases of tophaceous pseudogout (Table).[2–5,10–14] Seven of the cases exhibited marginal

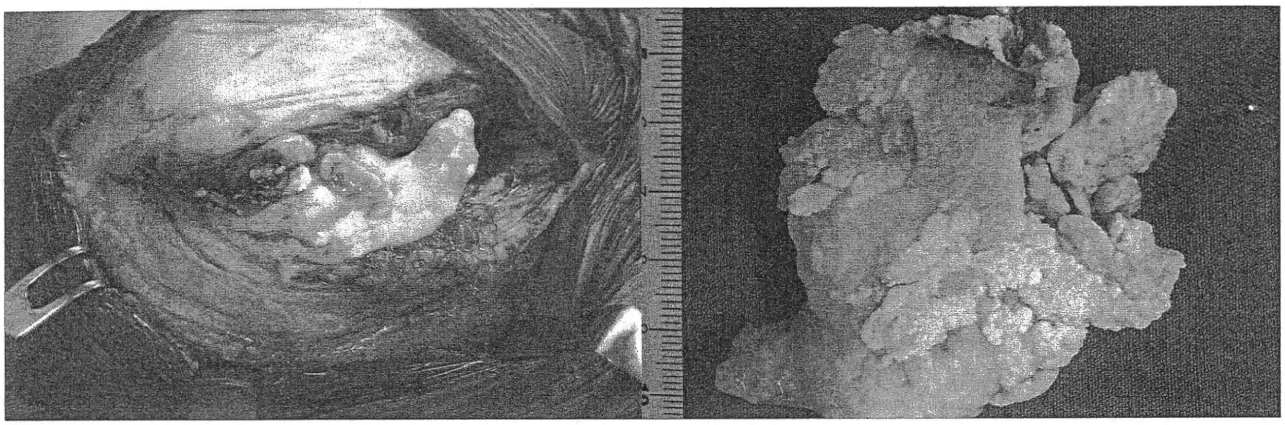

Figure 3 The mass is partially connected to the synovium. Its surface is whitish and glossy. Geometric calcified deposits are observed on the surfaces of the tumour and joint synovium as well as on the articular cartilage and meniscus.

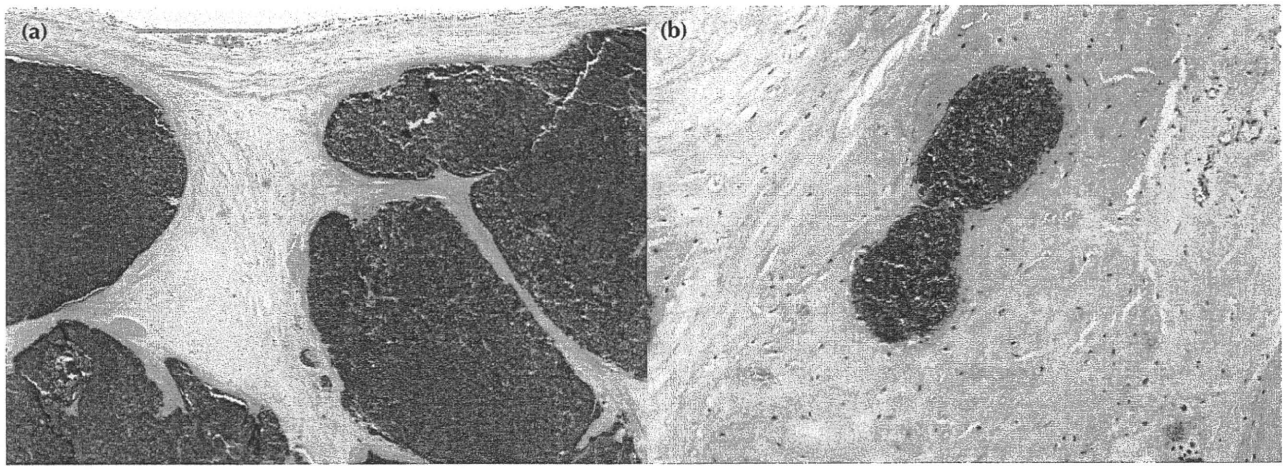

Figure 4 Massive calcified deposits on both **(a)** surface and **(b)** inside of the tophaceous pseudogout (H&E, x4).

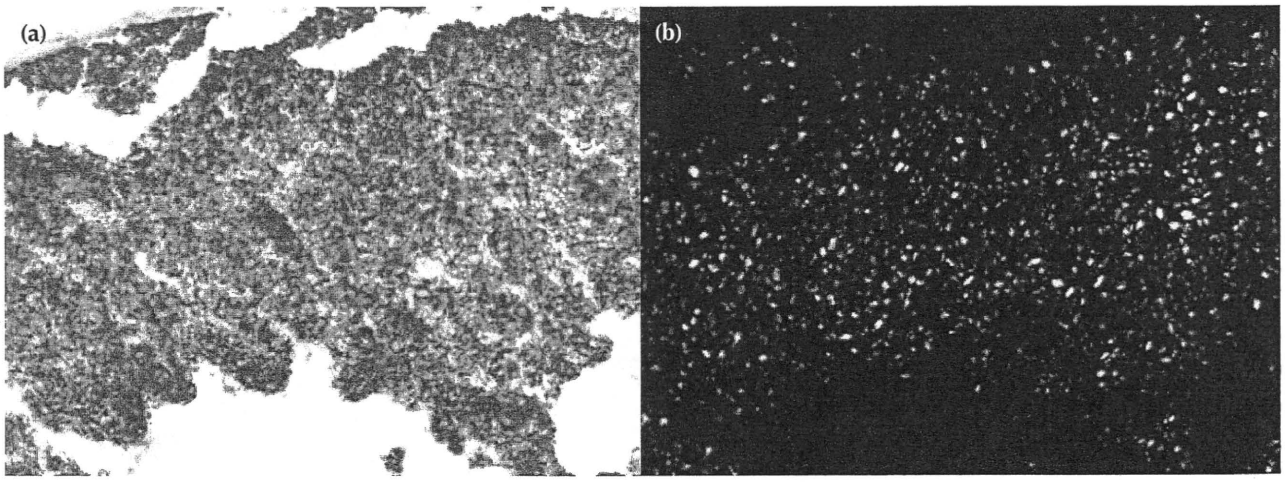

Figure 5 Under **(a)** normal and **(b)** polarised light, the calcified deposits show birefringence suggestive of calcium pyrophosphate dihydrate crystals (H&E, x20).

Table
Reported cases of tophaceous pseudogout

Study	Year	Involved site	Calcification pattern
Pynn et al.[11]	1995	Temporomandibular joint	Marginal
Shaffrey et al.[10]	1995	Spine	Diffuse
Ishida et al.[3]	1995	Hip	Marginal
Kokubun et al.[12]	1996	Spine	Marginal
Muthukumar and Karuppaswamy[13]	2003	Ligamentum flavum	Diffuse
Havitcioglu et al.[2]	2003	Knee	Marginal
Hiroaki et al.[4]	2005	Hip	Marginal
Carlson et al.[14]	2007	Spine	Marginal
Hideki and Mitsuru[5]	2008	Hip	Marginal

calcification, whereas the remaining 2 showed a random calcification pattern. Marginal calcification of the mass may therefore be characteristic of tophaceous pseudogout. Synovial osteochondromatosis usually shows a central ossification pattern. Nonetheless, marginal calcification may also be found in lesions in myositis ossificans and synovial sarcoma.[6]

CPPD deposition is characterised by the presence of numerous refractive, birefringent, rhomboid crystals under polarised light. In patients with gouty arthritis, the hydroxyapatite crystals are needle-like and do not exhibit birefringence.[1] Chondrification and myxomatous change to the stroma around the crystals are often seen in tophaceous pseudogout. We presume that tophaceous pseudogout results from CPPD deposition into the stromal matrix showing chondrification or myxomatous change. These stromal tissues may arise from the synovium as a result of inflammation induced by pseudogout.

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JOURNAL OF
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Case report

Ganglion cyst arising from the posterolateral capsule of the knee

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Introduction

Although ganglion cysts have been observed in various joints, intra-articular ganglion cysts are uncommon. Most intra-articular ganglion cysts of the knee have been reported to originate from the cruciate ligaments and menisci. We report herein a rare case of a ganglion cyst arising from the posterolateral capsule of the knee.

Case report

A 47-year-old woman had experienced right knee pain in the medial and posterior joint for several years. She reported no history of trauma or participation in active sports. Knee pain became worse with full flexion of the knee. Her pain did not improve with conservative treatment, which included nonsteroidal anti-inflammatory drugs, steroid injections, and physical therapy. Physical examination of the right knee showed full range of motion with no effusion. Slight tenderness was present in the medial joint space, but McMurray's and Apley's compression tests yielded negative results. The knee was stable during the Lachman, anterior and posterior drawer, and medial and lateral stress tests.

Plain radiography of the right knee joint showed slight narrowing of the joint space and subchondral sclerosis with no osteophyte formation, which was classified as Kellgren and Lawrence grade I (Fig. 1). Magnetic resonance imaging (MRI) of the right knee revealed a well-defined 20 × 12 mm multilobulated cystic mass located posterior to the posterior cruciate ligament (PCL), with signal hypointensity on T1-weighted imaging and signal hyperintensity on T2-weighted imaging (Fig. 2).

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Arthroscopy of the right knee detected a superficial lesion of the medial femoral condyle, including fissuring and a crack in the medial compartment; no meniscal tear was found. Arthroscopic examination revealed a mass located between the anterior cruciate ligament (ACL) and the PCL. The cyst was punctured and leaked a jelly-like fluid. The mass was excised using an intercondylar notch approach.[1] After dissection was continued posteriorly, the cyst was found to be attached to the posterior capsule of the posterolateral compartment (Fig. 3). Subsequent histological examination was consistent with a diagnosis of a ganglion cyst (Fig. 4).

At follow-up after 12 months, the patient was symptom-free with full range of motion. Follow-up MRI showed no evidence of recurrence.

In regard to this case, each author certifies that his or her institution has approved the human protocol for this investigation; that all investigations were conducted in conformity with ethical principles of research; and that informed consent was obtained.

Discussion

Ganglion cysts are cystic lesions arising from a tendon sheath or joint capsule. Intra-articular ganglion cysts of the knee are uncommon. The reported prevalence of intra-articular ganglion cyst of the knee is 0.2%–1.3% on knee MRI[2–5] and 0.4%–2.0% on knee arthroscopy.[6–8]

Intra-articular ganglion cysts of the knee are usually associated with the cruciate ligaments. Other locations include the meniscus, popliteus tendon, infrapatellar fat pad, and posterior capsule. To the best of our knowledge, there have been only four reported cases of a ganglion cyst arising from the posterior capsule in the posteromedial compartment of the knee.[1,7,9] Unlike other reported cases, ganglion cyst in the present case

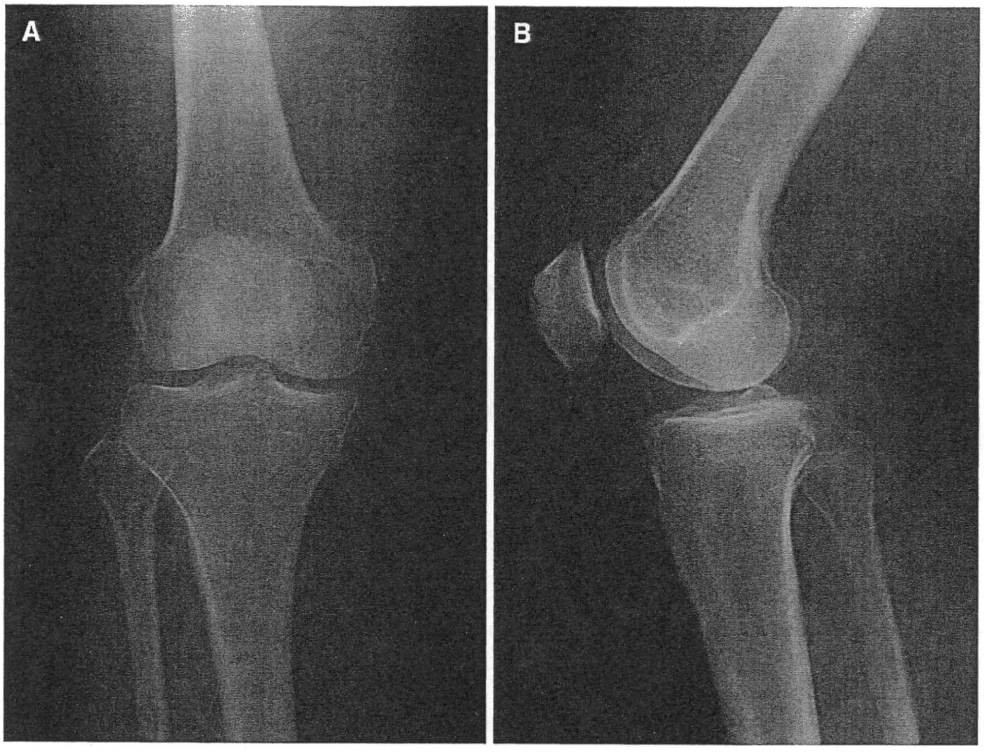

Fig. 1. Preoperative anteroposterior (**A**) and lateral (**B**) plain radiography of the right knee joint shows slight narrowing of joint space and subchondral sclerosis with no osteophyte formation

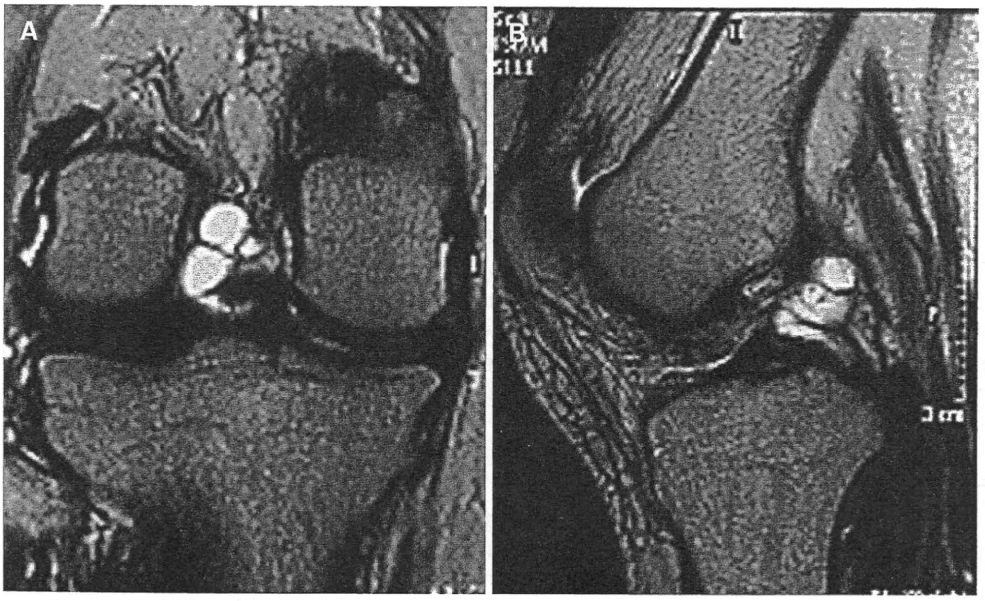

Fig. 2. Preoperative T2-weighted magnetic resonance imaging in the coronal plane (**A**) and sagittal plane (**B**) shows a multilobulated cystic mass posterior to the posterior cruciate ligament

arose from the posterior capsule in the posterolateral compartment of the knee.

Clinical symptoms of an intra-articular ganglion cyst of the knee resemble those of meniscus tears, including pain, swelling, click, limitation of motion, and mechanical blocking. Intra-articular ganglion cysts can be symptomatic or asymptomatic. It was reported that of the 85 cases of intra-articular ganglion cyst of the knee, only 9 were symptomatic; and all the 9 symptomatic patients had no history of trauma.[10] The cause of ganglion cysts may be due to synovial tissue herniation or connective tissue degeneration. Although trauma has been advocated as playing a role in the pathogenesis of ganglion formation, the cause of ganglion cysts remains controversial. In the present case, there was no past history of trauma.

Magnetic resonance imaging has been shown to be an effective method for evaluating cystic lesions of the

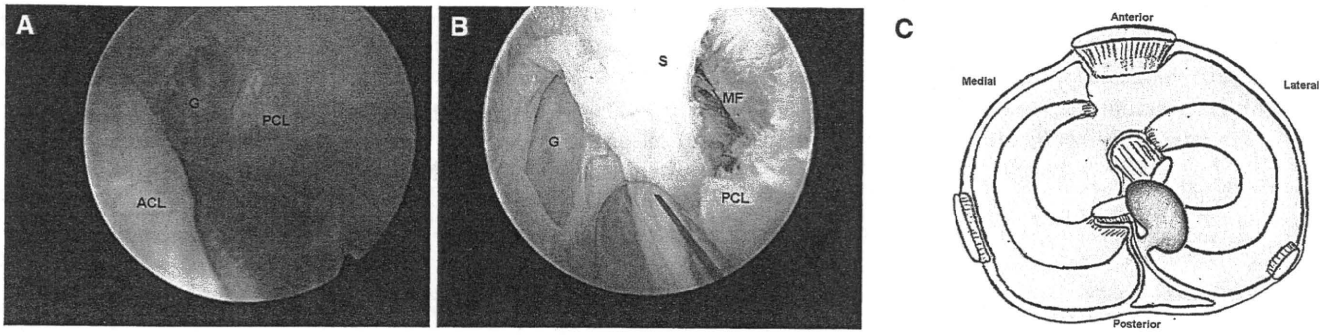

Fig. 3. Arthroscopic view from the anterolateral portal shows the mass located between the anterior cruciate ligament (*ACL*) and posterior cruciate ligament (*PCL*) (**A**) and arising from the posterior capsule of the posterolateral compartment (**B**). **C** Diagram of the ganglion cyst arising from the posterolateral capsule of the knee (**C**). *G*, ganglion; *S*, septum; *MF*, medial femoral condyle

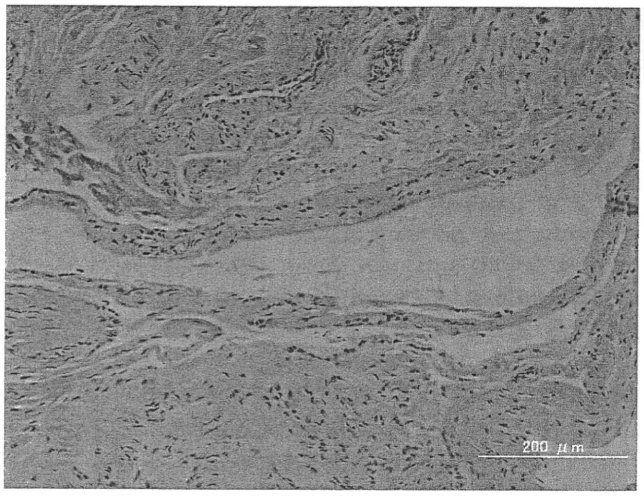

Fig. 4. Histological examination shows that the cystic lesion consists of dense fibrous connective tissue without a synovial cell lining

knee. The appearance on MRI of ganglion cysts arising from the cruciate ligament depends on the origin of the ganglion cyst. Ganglion cysts associated with the PCL reportedly appear as well-defined multilocular cysts along the ligament surface, whereas those associated with the ACL display a fusiform appearance and extend along the course of and interspersed within the fibers of the ligament.[11] In the present case, MRI of the right knee revealed a multilocular cystic mass located posterior to the PCL, simulating a PCL ganglion cyst.

Ganglion cysts of the hand and wrist are well known to be likely to recur with failure to remove the cyst stalk. However, successful treatment of intra-articular ganglion cysts of the knee has been reported after arthroscopic excision or débridement.[7,8,12] Cystic lesions posterior to the PCL are barely visible arthroscopically. In such cases, the establishment of a posteromedial or posterolateral approach is required. In the present case, the ganglion cyst was located in the proximal part of the posterior compartment and was visible between the ACL and PCL from the anterior approach. We thus successfully removed the lesion using an intercondylar approach and confirmed that the cyst was attached to the posterolateral capsule. Careful analysis of the origin of the ganglion cyst was needed to prevent recurrence.

Conclusion

We reported herein a case of intra-articular ganglion cyst arising from the posterolateral capsule of the knee. Precise location of the ganglion cyst is important when planning the operative procedure. An intercondylar notch approach was useful for excising this ganglion cyst in the posterior compartment. In cases of ganglion cyst located in the posterior compartment, the possibility of a ganglion cyst arising from the posterior capsule should be considered along with a PCL ganglion cyst.

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Original article

Clinical and radiological results of calcium phosphate cement-assisted balloon osteoplasty for Colles' fractures in osteoporotic senile female patients

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Abstract

Background. Distal radius fractures in osteoporotic senile female patients often used to be complicated with residual deformity, stiffness, and pain. Recently, however, adequate usage of a palmar locking plate or external fixation has led to fewer subsequent complications. The method proposed here deserves consideration because it is less invasive and more cost-effective.

Methods. A total of 11 Colles' type fractures (AO type A2) in 11 patients (all female; mean age 78 years) were treated. After a closed reduction, the fractures were fixed by percutaneous pinning, as Kapandji previously described. Through a 5-mm longitudinal skin incision on the dorsoulnar aspect of the fracture site, the barrel of a disposable 1-ml syringe was inserted into the fracture site as a port. Next, a pediatric uromatic balloon was introduced into the fracture site and inflated by contrast medium. The balloon inflation enlarged the void of the fracture site. A compression bandage around the fracture site was applied before calcium phosphate cement injection with a cement gun through the port under an image intensifier. The functional and radiological results were evaluated. The mean follow-up period was 16 months (range 12–25 months).

Results. All results were graded as good or excellent within 3 months, and all were graded as excellent at the final follow-up. The average duration of immobilization was 4 weeks with a short forearm cast. The overall postoperative correction loss in ulnar variance was 1.7 mm. Radial inclination and volar tilt showed no postoperative correction loss. The final volar tilt, radial inclination, and ulnar variance were comparable to those of the nonaffected side.

Conclusions. Calcium phosphate cement-assisted balloon osteoplasty is a less invasive procedure and can be clinically justified as a therapeutic option for a Colles' fracture in osteoporotic senile female patients.

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Introduction

Fracture of the distal radius is one of the most common skeletal injuries in senile female patients. Given the general conditions of these patients, quite often an invasive surgical method should be avoided. The key problem is continuous correction loss inside the cast after reduction during conservative treatment. Residual deformity leads to disabling stiffness and pain of the wrist.

Calcium phosphate cement (CPC) is an injectable biocompatible bone substitute that has been used for various applications in orthopedic surgery and has proven effective as a bone filler in the orthopedic field.[1,2] However, its mechanical properties do not provide solid mechanical strength for rigid fixation, so proper internal fixation, cast, or external fixations[3,4] are usually required. Hidaka et al. reported their clinical and radiological results after usage of CPC in distal radius fractures.[2] Their data showed mild correction loss in middle-aged women after a 1-year follow-up. Because we frequently deal with elderly patients, we tried to inject CPC into the enlarged fracture cavity after using a pediatric uromatic balloon. The purpose of this study was reassessment of the usefulness of CPC for distal radius fractures in elderly female patients.

Patients and methods

Between September 2004 and August 2007, CPC-assisted balloon osteoplasty was performed for Colles' fractures in 11 osteoporotic senile female patients. All patients met the following criteria: (1) more than 12 months of follow-up; (2) age >60 years; (3) availability of complete data of the physical examination and radiography; and (4) operation and follow-up performed by a single surgeon (S.I.). Informed consent was obtained from all patients. The patients were all female, with an

average age of 78 years (range 61–93 years). All fractures occurred as a result of a fall from a standing height. All were dorsally displaced fractures of the distal aspect of the radius and classified as Colles type fractures (AO type A2).

Operative technique

The patient was placed in the supine position under axillary anesthesia without a pneumatic tourniquet. With the affected side of the shoulder abducted 90° and the affected elbow fully extended, a countertraction pad was placed under the axilla area. A Chinese finger trap was then applied to the thumb, index, and middle fingers; and traction was manually applied. Reduction was confirmed by portable fluoroscopy.

A small longitudinal stab skin incision was made on the dorsoradial aspect of the fracture site for the first pin, which was a 1.6-mm Kirschner wire. A small artery forceps was then used to dissect the tissue down to the fracture site, as Kapandji previously described.[5,6] Next, using the same technique, a second 1.6-mm Kirschner wire was inserted into the volar-radial aspect of the fracture site. Although good reduction was obtained, a void or cavity usually appeared at the fracture site (Fig. 1a).

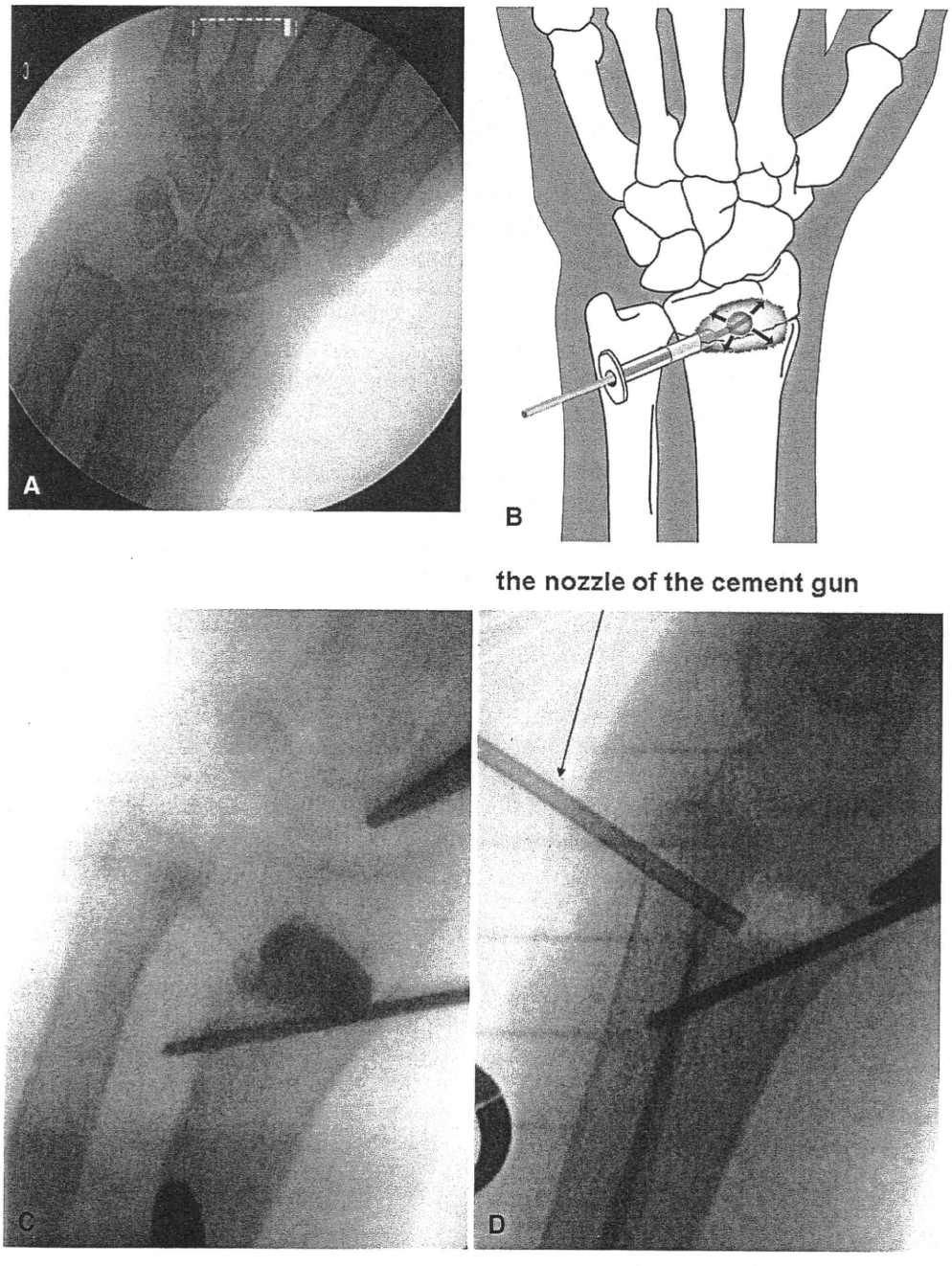

Fig. 1. **a** Postreduction image showing the fracture void. **b** The barrel of a 1-ml syringe was shortened to about 4 cm, inserted via a small stab skin incision into the fracture cavity, and then used as a port for the procedures. **c** Radiological image shows a pediatric uromatic balloon that was introduced into the radiological void and inflated with contrast medium. About 2 cc of contrast medium was required to inflate the balloon fully to create the cavity. **d** Image shows the void of the fracture site enlarged by balloon inflation just before injection of calcium phosphate cement (CPC). The radiopaque hollow tubular structure is the nozzle of the cement gun. It was passed through the syringe into the fracture cavity

A small longitudinal stab skin incision was made on the dorsoulnar aspect of the fracture site for insertion of a 3-mm prod. Care was taken to avoid tendon or soft tissue injury. Halfway through the fracture, the prod was directed to the cavity caused by bone crush. A disposable 1-ml syringe (JMS, Tokyo, Japan) was cut in half, and the barrel on the plunger side was inserted into the opening of the fracture site along the 3-mm prod. After removing the prod, the syringe was used as a port for the following procedures (Fig. 1b). After irrigating the inside of the fracture cavity, a pediatric uromatic balloon was introduced into the radiological void and inflated by contrast medium (Fig. 1c). This procedure ensures that the balloon is inflated within the fracture void and allows one to determine how much CPC is necessary so the appropriate CPC kit can be selected. Usually, about 2 cc of contrast medium was required to inflate the balloon fully to enlarge the cavity. As this balloon could provide pressure sufficient to enlarge the fracture void by crushing osteoporotic cancellous bone and forcing it outward, the balloon inflation not only ejected hematoma but also enlarged the void of the fracture site by compressing the cancellous bone from within (Fig. 1d). CPC (Biopex; Mitsubishi Materials, Tokyo, Japan) with a powder/liquid ratio of 3.3 was prepared and then injected into the fracture site by a cement gun after 1 min of kneading.[2,4,7,8] A compression bandage around the fracture site was used to prevent CPC leakage into the soft tissue. Theoretically, the fracture site was open to the air through the port, enabling the surplus CPC stained with blood to be spontaneously removed through the port syringe, which has a wider diameter than the cement gun; the diameters of the cement gun and of the port of a 1-cc syringe are 3.5 mm and 5.5 mm, respectively. We then injected 3 cc of CPC. As most of the surplus CPC flowed out of the syringe, little leakage occurred.

After the operation, a short forearm cast was applied. One day postoperatively, active finger motion was recommended. The volar-radial Kirschner wire was removed 3 weeks postoperatively, the forearm cast was removed 4 weeks postoperatively, and the dorsoradial wire was removed 6 weeks postoperatively.

Patient evaluation

The function of the affected wrist was evaluated at 6 weeks, 3 months, and at the final follow-up visit (>12 months after the operation). The mean follow-up period was 16 months (range 12–25 months). Clinical results were evaluated objectively based on the radiological findings, range of motion (ROM) of the wrist and forearm, grip strength, and complications. Radial inclinations, volar tilt, and ulnar variance were measured in sequential radiographs.

This study protocol and publication were approved by the committee on ethics and the institutional review board of Oyamada Memorial Spa Hospital.

Results

At the final follow-up, finger motion was not hampered, the radiological loss of reduction was minimal, and the average grip strength was 8 kg compared with 9 kg on the nonaffected side. No complications were observed. Average grip strengths at 6 postoperative weeks and 3 months were 3 and 5, respectively. In terms of ROM, the average values for extension, flexion, radial deviation, and ulnar deviation on the affected side were 56, 39, 18, and 29, respectively, compared with 55, 67, 25, and 31 on the nonaffected side. All patients were graded as good or excellent within 3 months, and all were graded as excellent at the final follow-up. The radiological follow-up data are shown in Table 1.

The average ulnar variance was –0.4 mm right after surgery, and it had increased to 1.3 mm at the final follow-up. This was statistically significant ($P < 0.05$). The final values of radial inclination, volar tilt, and ulnar variance were comparable to those of the nonaffected side ($P = 0.2485$, $P = 0.9407$, and $P = 0.5536$, respectively). Radial inclination and volar tilt showed no postoperative correction loss.

Illustrative case

An 88-year-old woman sustained a distal radius fracture in her left wrist. The distal radius was dorsally displaced and accompanied by comminution of the dorsal cortex and significant shortening of the radius (Fig. 2a). Four days after injury, CPC was injected after closed reduction and percutaneous pinning using our ballooning technique (Fig. 2b). At 15 months after surgery, finger motion was not hampered, and the ROM of her wrist and forearm was restored.

Table 1. Radiological follow-up data

Parameter	Preop.	Postop.	Final	Contralateral
Ulnar variance (mm)	2.6	–0.4	1.3	1.0
Radial inclination (°)	8.2	22.7	20.0	23.3
Volar tilt (°)	–21.4	3.7	6.7	6.0

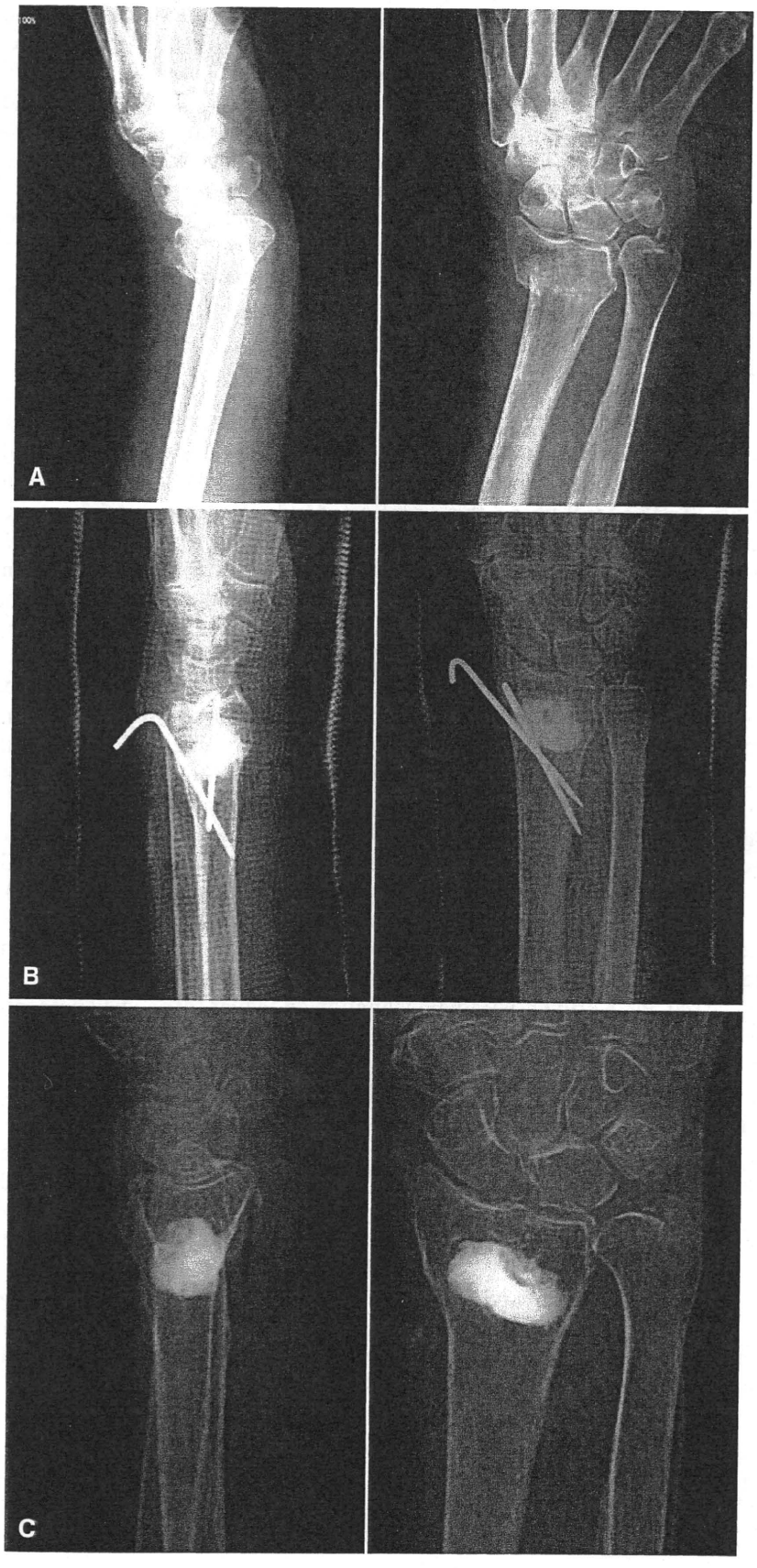

Fig. 2. a Presurgical radiographs of the wrist show a typical Colles' fracture in an elderly women with osteoporosis. **b** Postoperative radiographs show good reduction without CPC leakage. Radial inclination was 27°; ulnar variance was 1 mm; and volar tilt was 2°. **c** Radiographs at 15 months after surgery show minimal radiological change. Radial inclination was 25°; ulnar variance was 1 mm; and volar tilt was 2°

Grip strength on both sides was 8 kg. In terms of the ROM of the wrist and forearm, the extension, flexion, radial deviation, and ulnar deviation values on the affected side were 45, 45, 15, and 25, respectively, compared with 55, 55, 25, and 30 on the right side.

Radiological loss of reduction was minimal considering her age and osteoporotic bone (Fig. 2c). Preoperatively, the radial inclination, volar tilt, and ulnar variance were 2, –15, and 4, respectively. The postoperative ulnar variance was 1 mm immediately after surgery. In this case, reduction loss was not observed, and ulnar variance was unchanged at the final follow-up. The final radial inclination and volar tilt values were 25 and 2, respectively, and were comparable to those on the right side.

Discussion

The choice of external fixation or percutaneous pinning for unstable Colles' fracture is a matter of controversy. Many investigators have compared the clinical and radiological outcome of these therapeutic methods,[9,10] and numerous reports have suggested a loss of reduction with or without statistical significance after removing the pins or external fixation.[9,10] Ludvigsen et al. reported that although external fixation and percutaneous pinning produced the same outcome for unstable Colles' fractures they concluded that the latter were better not only in terms of cost-effectiveness but also in light of complications such as stiffness, pin tract infections, pin loosening, and reflex sympathetic dystrophy.[10] We believe that most senile Japanese women cannot endure external fixators, and sometimes the fracture site is vulnerable to infection as it cannot be kept clean.

Arora et al. compared nonoperative treatment and the volar locking plate and found no significant difference in the functional scores between the two groups.[11] Although obvious deformity was evident in 77% of the nonoperative group, the pain level was significantly less for the nonoperative group. This finding implies the presence of subclinical or hidden complications. Hence, we eventually concluded that the combination of percutaneous pinning and usage of Biopex might be crucial to avoid complications and maintain reduction until bone union in Colles' type fractures (AO type A2). Biopex is not frequently applied because of possible complications, its chemical and mechanical properties, and cost-effectiveness. However, it is useful with our method.

In terms of cost-effectiveness, our method costs about half that of the locking plate system. For example, 3 cc of Biopex is priced at about 60000 yen, whereas one locking plate and six screws cost about 140000 yen.

One of the advantages of our technique was fewer complications, including tendon injuries and carpal tunnel syndrome.[12,13] Although our series was small, we did not experience any complications. A possible complication was related to usage of CPC. However, the use of the compression bandage successfully minimized CPC leakage. In addition, our technique is not technically demanding, whereas open reduction/internal fixation is demanding. When acceptable reduction is obtained, even inexperienced surgeons can achieve the same results. However, when filling the forearm fracture site with CPC, due care should be taken to avoid leakage into the joint space, tendon sheath, or carpal tunnel. A compression bandage around the fracture site is recommended to prevent CPC leakage in cases of ordinary Colles' fracture but not in the case of an arteriovenous fistula in the forearm of a hemodialysis patient.[14] This simple procedure and careful injection with the cement gun under an image intensifier resulted in minimal CPC leakage.

Since 2000, a balloon-like device has been used in the United States to achieve reduction of vertebral body fractures. This procedure has been called "kyphoplasty."[15–17] Our use of a pediatric uromatic balloon is a clinical application of "kyphoplasty" to a Colles' fracture. We speculated that a balloon could create a cavity for CPC injection and be helpful for hemostasis. Such a balloon could provide pressure sufficient to enlarge the fracture void by crushing osteoporotic cancellous bone and forcing it outward. CPC leaks in the direction of pressure lower than at the CPC injection site. Thus, when the 3.5-mm cement gun is inserted through the port of a 1-cc syringe with a diameter of 5.5 mm, the fracture site is, theoretically speaking, open to the air through the port; surplus CPC stained with blood was expected to be spontaneously removed through the port syringe, which has a much greater diameter than that of the cement gun.

Surgeons in various countries might wonder whether the same procedure can be performed using domestically available CPC. To address this issue, we provide data comparing Biopex and Norian (Norian Co., CA, USA), both of which are approved in the United States.[14,18,19]

Required time for primary hardening, the final compressive strength, and the time required for final hardening when using Biopex R and Norian SRS are 6–10 and 8–9 min, respectively; 80 and 50 MPa, respectively; and 72 and within 24 h, respectively. As Norian SRS is quite similar to Biopex, it can be clinically applied with our method.

In conclusion, CPC-assisted balloon osteoplasty proved to be a safe therapeutic procedure clinically applicable for Colles' fractures in aged and/or osteoporotic patients.

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New Sequence Variants in HLA Class II/III Region Associated with Susceptibility to Knee Osteoarthritis Identified by Genome-Wide Association Study

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Abstract

Osteoarthritis (OA) is a common disease that has a definite genetic component. Only a few OA susceptibility genes that have definite functional evidence and replication of association have been reported, however. Through a genome-wide association study and a replication using a total of ~4,800 Japanese subjects, we identified two single nucleotide polymorphisms (SNPs) (rs7775228 and rs10947262) associated with susceptibility to knee OA. The two SNPs were in a region containing HLA class II/III genes and their association reached genome-wide significance (combined $P = 2.43 \times 10^{-8}$ for rs7775228 and 6.73×10^{-8} for rs10947262). Our results suggest that immunologic mechanism is implicated in the etiology of OA.

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Introduction

We are living in the "Bone and Joint Decade" (http://www.boneandjointdecade.org/). As the WHO initiative shows, bone and joint diseases are serious problems all over the world, putting us under severe medical, economical and social burden. Osteoarthritis (OA; MIM 165720) is one of the most common diseases among them. OA affects synovial joints of all over the body, mainly knee, hip, hand and spine. OA is characterized by progressive loss of articular cartilage and, often, proliferation of synovium and bone, which lead to pain, loss of joint function and disability. More than tens of millions patients in the world are suffering from this non-lethal, but intractable disease, and the number is relentlessly increasing; however, its etiological picture remains unclear and we have no fundamental treatment for it.

OA is a polygenic disease. Both environmental and genetic factors contribute to its etiology and pathogenesis [1]. To understand its genetic factor, identification of its susceptibility gene(s) must be the first step. Many OA susceptibility genes identified by candidate-gene association studies have been reported, but only a few have supporting functional evidence and replication of the results in different populations [1,2]. Large-scale association studies including the genome-wide association study (GWAS) using high-density single nucleotide polymorphisms (SNPs) have been reported by a few groups in Asia and Europe [3–6], but only a gene fulfilled genome-wide significance level [2]. The genetic basis of OA susceptibility remains largely uncharacterized. To identify OA susceptibility gene(s), we conducted a GWAS for knee OA and identified two SNPs with genome-wide significance level.

Methods

Samples

Characteristics of each cohort group are shown in Table 1. Case samples of GWAS for the Japanese population were obtained from

Table 1. Basal characteristics of the subjects.

Cohort	Source	Platform	Number of samples	Nationality	Female (%)	Age (mean +/− sd)	BMI (mean +/− sd)	Severity[a] (% severe OA)
GWAS								
knee OA	RIKEN	Illumina HumanHap550	899	Japanese	759 (84.4)	71.6+/−7.6	24.9+/−3.6	76.5
control	ORC+BioBank Japan	Illumina HumanHap550	3,396	Japanese	1,491 (43.9)	52.5+/−15.2	22.5+/−3.7	-
Replication								
Japanese								
knee OA	RIKEN	Invader assay	167	Japanese	124 (74.3)	73.8+/−6.1	24.5+/−3.3	48.5
control	RIKEN	Invader assay	347	Japanese	223 (64.3)	65.9+/−8.7	22.3+/−2.7	-
European Caucasian								
knee OA	Santiago de Compostela	SNaPshot	243	Spanish	197 (81.1)	68.0+/−5.7	32.8+/−4.8	ND[b]
control	Santiago de Compostela	SNaPshot	426	Spanish	165 (38.7)	68.4+/−9.1	28.3+/−3.8	-
knee OA	University of Thessaly	SNaPshot	570	Greek	468 (82.1)	65.8+/−8.7	29.1+/−3.3	77.1
control	University of Thessaly	SNaPshot	645	Greek	417 (64.6)	59.5+/−11.6	25.4+/−3.7	-

OA: osteoarthritis, ORC: Osaka-Midosuji Rotary Club.
[a]Kellegren-Laurence grade ≥3 was considered as severe OA.
[b]All cases underwent TKR (total knee replacement) surgery.
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several medical institutes in Japan, as previously described [5,7]. Knee OA was diagnosed on the basis of clinical and radiographic findings using previously described criteria [5,7]. Rheumatoid arthritis (RA) and polyarthritis associated with autoimmune diseases were excluded, as were secondary OA due to crystal deposition (gout and pseudogout), posttraumatic OA and infection-induced OA. Patients who had clinical and radiographic findings suggestive of skeletal dysplasias, including overt short stature, multiple symmetric involvements of epiphyses and a definitely positive Mendelian family history were also excluded from the study. The control groups consisted of 3,396 individuals that were registered in the Leading Project for Personalized Medicine in the Ministry of Education, Culture, Sports, Science and Technology, Japan as the subjects with diseases unrelated to OA and the volunteers in the Osaka-Midosuji Rotary Club, Osaka, Japan [8]. For replication study, we recruited population-based cohorts from inhabitants of Odai and Minami-ise town (previously Miyagawa village and Nansei town, respectively in the Mie prefecture in Japan) [9]. The Spanish and Greek knee OA and control populations were recruited as described previously from the Hospital Clinico de Santiago, the Departments of Biology and Genetics and of Orthopaedics, University of Thessaly and the Institute of Musculoskeletal Sciences [10]. All the participants provided written informed consent. This research project was approved by the ethical committees at Center for Genomic Medicine (formerly, SNP Research Center), RIKEN and the participating institutions.

SNP genotyping

For the GWAS, we genotyped 906 patients with OA and 3,396 controls using Illumina HumanHap550v3 Genotyping BeadChip. After excluding seven cases with call rate of <0.98, we applied SNP QC (call rate of ≥0.99 in both cases and controls and P value of Hardy-Weinberg equilibrium test of $\geq 1.0\times10^{-6}$ in controls). Finally, 459,393 SNPs on autosomal chromosomes passed the QC filters and were further analyzed. Among the SNPs analyzed in the GWAS, we selected top 15 SNPs showing the smallest P values ($P<1\times10^{-5}$) for the replication study using an independent 514 Japanese subjects from a resident cohort. SNPs with minor allele frequency of ≤0.1 in both case and control samples were excluded from the further analysis. In the replication analysis, we genotyped SNPs using the multiplex PCR-based invader assay (Third Wave Technologies) or by direct sequencing of PCR products using ABI 3700 DNA analyzers (Applied Biosystems), or by SNaPshot Multiplex System (Applied Biosystems) according to manufacturers' protocols.

Statistical analysis

In the GWAS and replication analyses, we applied Fisher's exact test to two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model, and a recessive-effect model. We conducted the meta-analysis using the Mantel-Haenszel method. We examined heterogeneity among studies by using the Breslow-Day test. Significance levels after the Bonferroni correction for multiple testing were $P=1.09\times10^{-7}$ (0.05/459,393). Age, gender- and BMI-adjusted odds ratios were obtained by logistic regression analysis [11]. Odds ratios and confidence intervals were calculated using the risk allele as a reference. We analyzed the haplotype association using Haploview software [12]. We conducted a principal component analysis to detect population stratification [13].

Software

For general statistical analysis, we used R statistical environment version 2.6.1 or Microsoft Excel. Drawing the LD map, estimation of haplotype frequencies and analysis of haplotype association were performed by Haploview software.

Results

To identify genetic variants that determine OA susceptibility, we conducted a GWAS in Japanese knee OA. We examined 906 individuals with knee OA and 3,396 control individuals (Table 1) using Illumina HumanHap550v3 Genotyping BeadChip. After confirming the data quality, we compared the results of 459,393 SNPs between cases and controls by Fisher's exact test for three genetic models: allelic, dominant or recessive (Figure 1). Fifteen