using an 11-gauge biopsy needle (TrapSystem; Medical Device Technologies, Gainesville, Fla).

The reparative cartilage was stained with hematoxylin-eosin and toluidine blue for general histologic analysis and assessment of metachromasia. The reparative cartilage was judged to be hyaline cartilage if it had an abundant extracellular matrix that showed metachromatric staining with toluidine blue and a glassy appearance. The reparative cartilage was judged to be fibrocartilage if it had randomly distributed bundles of collagen fibers and showed no metachromatric staining with toluidine blue. Each reparative cartilage specimen was classified as hyaline cartilage (predominance of hyaline cartilage), fibrocartilage (predominance of fibrocartilage), or mixed cartilage (approximately equal quantities of hyaline cartilage and fibrocartilage). All cartilage classifications were performed by Chiba University staff pathologists. For the purposes of statistical analysis, fibrocartilage and mixed cartilage were grouped together and classified as other cartilage. The concentrations of GAG in the reparative cartilage and normal cartilage biopsy specimens were measured by using high-performance liquid chromatography (27).

Figure 1

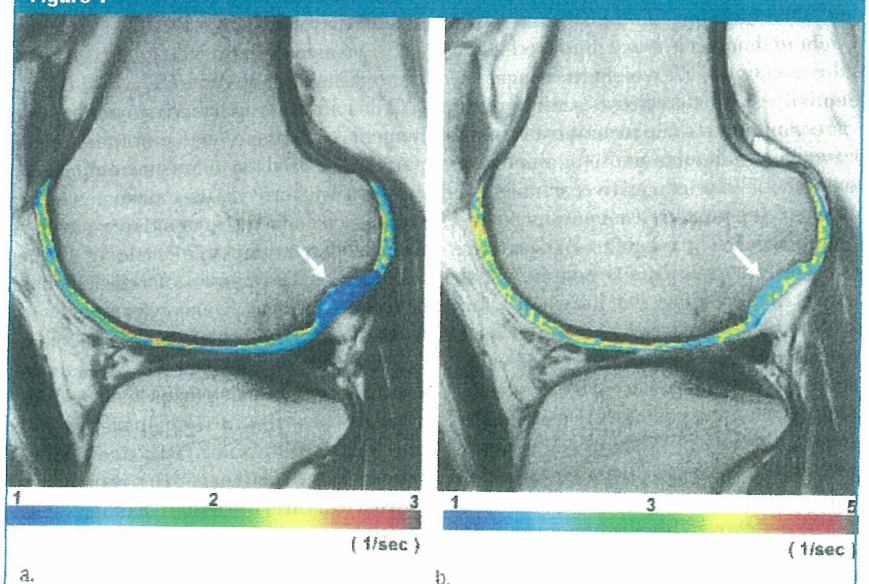

Figure 1: (a) Color-coded MR map of cartilage constructed by using $R1_{pre}$ values. The R1 in the reparative cartilage (arrow) appears to be lower than that in the normal cartilage. (b) Color-coded MR map of cartilage constructed by using $R1_{post}$ values. Arrow = reparative cartilage. Throughout the cartilage, the $R1_{post}$ values have a smaller range than the $R1_{pre}$ values. On the maps and color scales, blue represents areas of low R1 and red represents areas of high R1.

Table 1

$R1_{pre}$, $R1_{post}$, and $\Delta R1$ in Normal and Reparative Cartilage

Cartilage	$R1_{pre}$	$R1_{post}$	$\Delta R1$
Normal	0.99 ± 0.10*	2.16 ± 0.15	1.18 ± 0.16*
Reparative	0.76 ± 0.08*	2.32 ± 0.24	1.56 ± 0.19*

Note.—Data are mean values (in 1/sec) ± standard deviations.

* Differences in values between normal and reparative cartilage were significant ($P < .05$).

Clinical Evaluation

The scoring system of Lysholm and Gillquist (28), a scoring system used for the clinical assessment of knee function, was performed to quantify the clinical status of the patients before the ACI, 1 year after the ACI (mean, 12.4 months ± 0.5 after surgery; range, 12–13 months), and at the time of the last medical examination (mean, 31.4 months ± 9.0 after surgery; range, 23–45 months). This scoring was determined by two authors (Y.W. and A.W., with 12 and 8 years of experience with this scoring system, respectively).

Data and Statistical Analyses

To evaluate the usefulness of delayed gadolinium-enhanced MR imaging for measuring the GAG concentration in reparative cartilage, we calculated the relative $\Delta R1$ (ie, $\Delta R1$ in reparative cartilage divided by $\Delta R1$ in normal cartilage in the same patient) and the relative GAG concentration (ie, GAG concentration in reparative cartilage divided by GAG concentration in normal cartilage in the same patient). We then performed a correlation analysis to determine the relationship between relative $\Delta R1$ and relative GAG concentration. The relationships between relative $R1_{pre}$ ($R1_{pre}$ in reparative cartilage divided by $R1_{pre}$ in normal cartilage in the same patient) and relative GAG concentration and between relative $R1_{post}$ ($R1_{post}$ in reparative cartilage divided by $R1_{post}$ in normal cartilage in the same patient) and relative GAG concentration also were studied.

To assess the usefulness of the rela-

Table 2

GAG Concentrations in Normal and Reparative Cartilage Measured with High-Performance Liquid Chromatography

Cartilage	GAG Concentration
Normal ($n = 7$)	107.9 ± 17.0
Reparative ($n = 9$)	65.9 ± 9.4

Note.—Data are mean values (in micrograms per milligram) ± standard deviations. Differences in values between normal and reparative cartilage were significant ($P < .05$).

Figures 2–4

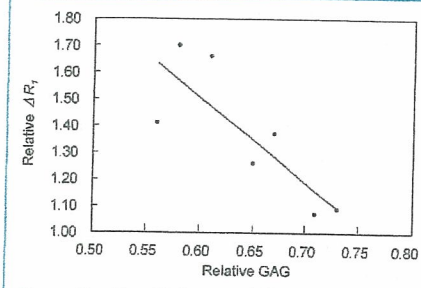

Figure 2: Quantitative correlation of relative ΔR1 and relative GAG concentration. A significant correlation between relative ΔR1 and relative GAG concentration ($r = 0.818$, $P = .024$) was observed.

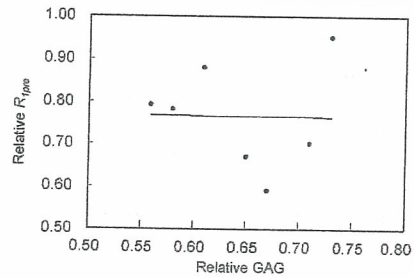

Figure 3: Quantitative correlation of relative $R1_{pre}$ and relative GAG concentration. No significant correlation between relative $R1_{pre}$ and relative GAG concentration ($r = 0.010$, $P = .983$) was observed.

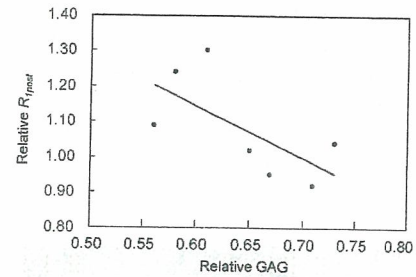

Figure 4: Quantitative correlation of relative $R1_{post}$ and relative GAG concentration. No significant correlation between relative $R1_{post}$ and relative GAG concentration ($r = 0.661$, $P = .106$) was observed.

tive ΔR1 in predicting the histologic type of reparative cartilage, differences in relative ΔR1 between hyaline reparative cartilage and other reparative cartilage were analyzed.

The time from biopsy for histologic and biochemical analyses to MR imaging evaluation varied among the patients. To investigate the possible effect of the length of time between biopsy and MR imaging on the nature of the reparative cartilage, the patients were divided into two groups—those imaged early (≤12 months) and those imaged late (>12 months) after biopsy—and the differences in relative ΔR1 values between the early and late groups were analyzed.

Appropriate statistical tests were used to perform the data analyses and included the Student *t* test for paired or unpaired samples and the Bartlett test for correlation analysis. $P < .05$ indicated statistical significance. Statistical computer software (Statview, version 5; SAS Institute, Cary, NC) was used to perform all statistical analyses.

Results

Image Analysis Findings

In all patients, the maps of the cartilage constructed by using $R1_{pre}$ and $R1_{post}$ values had similar appearances (Fig 1). The $R1_{pre}$ values for reparative cartilage appeared to be lower than those for normal cartilage, and $R1_{pre}$ values differed significantly between reparative and normal cartilage (*t* statistic, 4.94; $P < .05$) (Table 1). In contrast, the $R1_{post}$ values on the calculated maps had a smaller range than the $R1_{pre}$ values throughout the cartilage, and $R1_{post}$ values did not differ significantly between reparative and normal cartilage (*t* statistic, 1.62; $P = .13$) (Table 1). All ΔR1 values for reparative cartilage were higher than the corresponding ΔR1 values for normal cartilage, and ΔR1 values differed significantly between reparative and normal cartilage (*t* statistic, 4.46; $P < .05$) (Table 1).

Histologic and Biochemical Analysis Findings

The reparative cartilage was classified as hyaline cartilage in five patients (two male, three female; age range, 13–35 years; mean age, 23.6 years), as mixed cartilage in three male patients (age range, 15–27 years; mean age, 19.0 years), and as fibrocartilage in one male patient aged 27 years.

The mean GAG concentration in the reparative cartilage biopsy specimens was significantly lower than that in the normal cartilage biopsy specimens ($P < .05$) (Table 2). In all patients, the GAG concentration in the reparative cartilage was lower than that in the normal cartilage.

Clinical Findings

The patients' knee function scores (Lysholm and Gillquist scoring) improved significantly after ACI, from a mean score of 63.0 ± 12.2 before to a mean score of 94.9 ± 5.1 a year after the surgery (*t* statistic, 7.2; $P < .01$). The mean score had further significantly improved, to 97.7 ± 4.9, at the last medical examination (*t* statistic, 7.9; $P < .01$). No patient had clinical complaints related to the treated knee at the last examination.

Usefulness of Delayed Gadolinium-enhanced MR Imaging of Cartilage for Measuring GAG Concentration

A significant correlation between relative ΔR1 and relative GAG concentration (seven knees, $r = 0.818$, $P = .024$) (Fig 2) was observed. However, no significant correlation between relative $R1_{pre}$ and relative GAG concentration (seven knees, $r = 0.010$, $P = .983$) (Fig 3) or between relative $R1_{post}$ and relative GAG concentration (seven knees, $r = 0.661$, $P = .106$) (Fig 4) was observed.

Differences in Relative ΔR1 between Hyaline Reparative Cartilage and Other Reparative Cartilage

The relative ΔR1 was slightly lower in hyaline reparative cartilage (mean, 1.23 per second ± 0.14 for five knees) than in other reparative cartilage (mean, 1.50 per second ± 0.18 for four knees). This difference was not significant (*t* statistic, 2.20; $P = .06$).

ΔR1 Changes in Reparative Cartilage as a Function of Time between Biopsy and MR Imaging

The mean relative ΔR1 was 1.42 per second ± 0.24 in the early group (two

male patients, two female patients; age range, 15–35 years; mean age, 26.0 years) versus 1.26 per second ± 0.12 in the late group (four male patients, one female patient; age range, 13–27 years; mean age, 18.0 years). This difference was not significant (*t* statistic, 1.10; $P = .31$).

Discussion

The $R1_{pre}$, which can directly affect the evaluation of contrast agent concentration (Eq [1]), is known to vary according to tissue composition. In previous clinical studies in which early degenerative changes in cartilage were evaluated, the differences in $R1_{pre}$ between degenerative cartilage and normal cartilage were so small that the authors concluded that the $R1_{pre}$ had a negligible influence in shortening the acquisition time (23,24,29). In contrast, a comparison between normal cartilage and experimentally prepared cartilage that simulated late degenerative changes revealed noticeable differences in $R1_{pre}$ between degenerated cartilage and normal cartilage (30).

To our knowledge, before the present study, a detailed investigation of the differences in $R1_{pre}$ between reparative cartilage and normal cartilage in patients after ACI had not been performed. Among the currently available cartilage repair methods, ACI has yielded some of the best reported results (15,16). However, the concentration of macromolecular tissue components and the collagen arrangement in reparative cartilage are not equivalent to those in normal cartilage (17–19). In our study, the reparative cartilage after ACI had a lower GAG concentration and a different histologic appearance compared with the normal cartilage, and the $R1_{pre}$ in the reparative cartilage was significantly lower than that in the normal cartilage. Although $R1_{post}$ measurements alone did not enable the detection of differences in GAG concentration between reparative cartilage and normal cartilage, ΔR1 measurements did enable the detection of such differences.

Correlation analysis revealed a significant correlation between relative ΔR1 and relative GAG concentration only. This finding suggests that delayed gadolinium-enhanced MR imaging evaluation of the GAG concentration in reparative cartilage after ACI requires measurement of the ΔR1. Thus, methods that involve the use of $R1_{post}$ measurements only might not be suitable for evaluating reparative cartilage after ACI.

A decreased $R1_{pre}$ in reparative cartilage may result from increased tissue water content, a decreased concentration of macromolecular matrix components, or differences in the collagen network structure (31). Measurement of the $R1_{pre}$ may be necessary for tissue with $R1_{pre}$ values that could differ markedly from the $R1_{pre}$ in the surrounding normal cartilage, tissue such as spontaneously reparative cartilage after a traumatic defect, the reparative cartilage generated with therapeutic intervention, and cartilage with locally advanced degeneration. The necessity of performing both precontrast MR imaging and 2-hour-delay postcontrast MR imaging makes it difficult to routinely use delayed gadolinium-enhanced MR imaging of cartilage in the clinical setting. However, evaluation with postcontrast MR imaging alone might lead to an overestimation of the GAG concentration in reparative cartilage after ACI.

Gillis et al (32) reported that delayed gadolinium-enhanced MR imaging of cartilage has potential as a noninvasive MR imaging technique for monitoring the GAG concentration in autologous cartilage transplants. Their study findings suggest that the GAG concentration in reparative cartilage measured 12 months or longer after ACI is comparable to the GAG concentration in the surrounding normal cartilage. However, these authors performed postcontrast MR imaging only and thus may have overestimated the GAG content in the grafts that they evaluated. In all the patients in our study, GAG concentrations were lower in the reparative cartilage than in the normal cartilage.

Relaxivity, a second parameter that can affect the evaluation of contrast material concentration (Eq [1]), is known to vary as a function of the tissue composition and the magnetic field strength of the MR imaging system (30,33). Measuring relaxivity is difficult in clinically limited situations, and the difference in relaxivity between reparative cartilage after ACI and normal cartilage is unknown. Because differences in relaxivity may result in an underestimation of the anionic gadopentetate dimeglumine concentration as well as the $R1_{pre}$ in reparative cartilage, further investigation is necessary.

Direct measurement of the GAG concentration in reparative cartilage with delayed gadolinium-enhanced MR imaging has been considered as a possible method of tracking the time course of the GAG concentration in reparative cartilage after ACI. However, direct measurement of the GAG concentration with delayed gadolinium-enhanced MR imaging of cartilage is not feasible currently because it requires knowledge of the actual concentration of anionic gadopentetate dimeglumine in the synovial fluid of the knee joint, which is very difficult to measure. Thus, in our study, we used instead the relative ΔR1 as a parameter for comparing GAG concentrations in reparative and normal cartilage, and we investigated whether the ΔR1 correlated with the relative GAG concentration, which is the ratio of the actual tissue GAG concentrations in reparative and normal cartilage. Our analysis revealed a significant correlation between relative ΔR1 and relative GAG concentration, which suggests that the relative ΔR1 may be useful for the quantitative evaluation of reparative cartilage.

Our study had several limitations. First, the sample size was relatively small. In our study, no significant difference in relative ΔR1 between hyaline reparative cartilage and other reparative cartilage or between the early group and the late group was observed. These findings might have been caused by the relatively small number of patients. A larger-scale study is needed.

Second, the time from biopsy for histologic and biochemical analyses to MR imaging evaluation varied among the patients. For some patients, this in-

terval was considerably long because the patients had already undergone second-look arthroscopy with biopsy when the delayed gadolinium-enhanced MR imaging of cartilage technique became available at our institute. If the maturation and degeneration of the reparative cartilage were ongoing processes, then changes in the cartilage composition could have developed between the time of MR imaging and the time of biopsy. The effects of this long interval on the results of our study are unknown; however, we believe that no substantial changes in the cartilage composition occurred during the period between tissue biopsy and MR imaging. One reason for this belief is that the tissue biopsy procedures were performed 12–13 months after surgery, by which time—according to some reports—the maturation of implanted cartilage would have already been completed (34–36). A second reason for this belief is that because our study patients had femoral condyle lesions, which are known to have better clinical results than patellar or trochlear lesions (16), their reparative cartilage may have benefited from an environment that was unfavorable for degeneration. However, performing concurrent biopsy and MR imaging might improve the correlation between GAG concentration and findings of delayed gadolinium-enhanced MR imaging of cartilage.

Third, because MR imaging was performed after second-look arthroscopy in all patients, the biopsy procedure itself may have produced cartilaginous changes that affected the appearance of and R1 in the reparative cartilage at subsequent MR imaging. We believe the effect of the biopsy on the cartilage repair process was small because we used a small biopsy needle during arthroscopy, which is a minimally invasive procedure. Nonetheless, the optimal time for MR imaging would be just before the biopsy.

In conclusion, ACI is a promising method of treating cartilage injury, and various studies to assess methods of producing reparative cartilage tissue similar to normal hyaline cartilage are underway (16). To improve the ability to obtain stable clinical results and good long-term outcomes with ACI, evaluation of the time course of the cartilage repair with use of an effective noninvasive qualitative method is important. The results of our study indicate that pre- and postcontrast imaging is necessary for delayed gadolinium-enhanced MR imaging evaluation of reparative cartilage after ACI. Additional, larger-scale studies are needed to validate the usefulness of delayed gadolinium-enhanced MR imaging for evaluating reparative cartilage after ACI.

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Molecular backgrounds of age-related osteoporosis from mouse genetics approaches

Hiroshi Kawaguchi

Abstract Backgrounds underlying age-related bone loss can be classified into two categories: systemic abnormality and osteoblast dysfunction. The former includes insufficiency of vitamin D or estrogen, causing a negative balance of calcium metabolism. We propose the contribution of an aging-suppressing gene, *klotho*, as a novel systemic factor, as a mouse deficient in the *klotho* gene exhibits multiple aging phenotypes including osteopenia with a low bone turnover. As a factor intrinsic to osteoblasts, we investigated the role of PPARγ, a key regulator of adipocyte differentiation, based on the facts that osteoblasts and adipocytes share a common progenitor. Heterozygous PPARγ-deficient mice exhibited high bone mass by stimulating osteoblastogenesis from bone marrow progenitors, and this effect became prominent with aging, indicating involvement of PPARγ-dependent bone formation in the pathophysiology of age-related bone loss. The local environment of osteoblasts is mainly controlled by cytokines/growth factors, among which insulin-like growth factor-I (IGF-I) is the most possible candidate whose production and activity are decreased with aging. Bone phenotypes of deficient mice of insulin receptor substrates (IRS-1 and IRS-2), essential molecules for intracellular signaling of IGF-I, revealed that IRS-1 is essential to maintain bone turnover by up-regulating anabolic and catabolic functions of osteoblasts, while IRS-2 is needed to keep the predominance of the anabolic function over the catabolic function. A next task ahead of us will be to elucidate the network system of these factors underlying age-related osteoporosis.

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Keywords Osteoporosis · Klotho · PPARγ · Insulin receptor substrate (IRS)

1 Three major backgrounds of osteoporosis

There are three major backgrounds of osteoporosis in aged women: (1) the peak bone mass during their adolescence was low, (2) the bone loss by menopause due to estrogen deficiency was severe, and (3) the bone loss by ageing thereafter was severe (Fig. 1); each of these has an independent mechanism. Regarding the mechanisms underlying the bone loss by ageing, accumulated evidence has suggested many age-related abnormalities which can be classified into two categories: systemic abnormality and osteoblast dysfunction (Fig. 2). The former includes insufficiency of active vitamin D or estrogen, which decreases calcium absorption from G.I. and kidney, causing a negative balance of calcium metabolism and a secondary hyperparathyroidism [1–8]. The latter can be further divided into abnormalities that occur inside and outside of osteoblasts. As an inside factor, Runx2, a key transcription factor for osteoblast differentiation, is the most probable candidate, since the expression is reported to be suppressed during cellular ageing of osteoblasts [9]; however, there is no *in vivo* evidence of its contribution to age-related bone loss. The local environment outside osteoblasts is mainly controlled by cytokines/growth factors such as insulin-like growth factor-I (IGF-I) [10–12], interleukin-11 [13], transforming growth factor-β [14] and bone morphogenetic proteins [15].

None of these hormones, cytokines, or transcription factor, however, can fully explain the etiology of age-related bone loss. To further investigate its molecular backgrounds, we have been involved in the reverse and

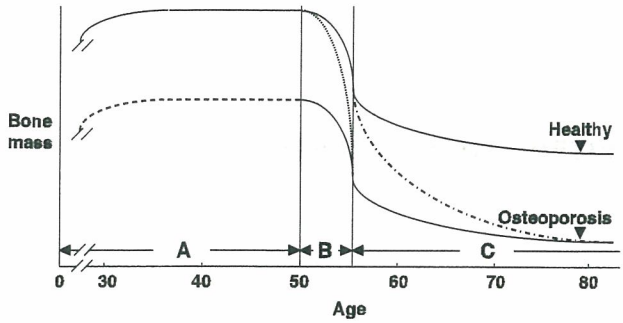

Fig. 1 Three major backgrounds for the etiology of osteoporosis. (A) The peak bone mass in the adolescence. (B) A rapid bone loss after menopause. (C) A gradual age-related bone loss thereafter

forward genetic approaches: the reverse genetics is the approach from a genotype to a phenotype using gene-manipulated mice such as knocked-out or transgenic, while the forward genetic approach is from a disease to the responsible gene using the human genomic analysis, based on the reverse genetic findings. This review summarizes the possible roles of three different types of molecules, a hormone klotho, an osteoblast intrinsic factor peroxisome proliferator-activated receptor-γ (PPARγ), and a local factor IGF-I by way of its adaptor molecule insulin receptor substrates (IRS), in age-related bone loss primarily from our recent mouse genetics approaches.

2 Klotho as a hormone in age-related osteoporosis

In addition to hormones vitamin D and estrogen, we hereby propose the contribution of an ageing-suppressing gene, *klotho*, as a novel systemic factor regulating age-related bone loss. Klotho was originally identified as a mutated gene in a mouse strain that accelerates age-dependent loss

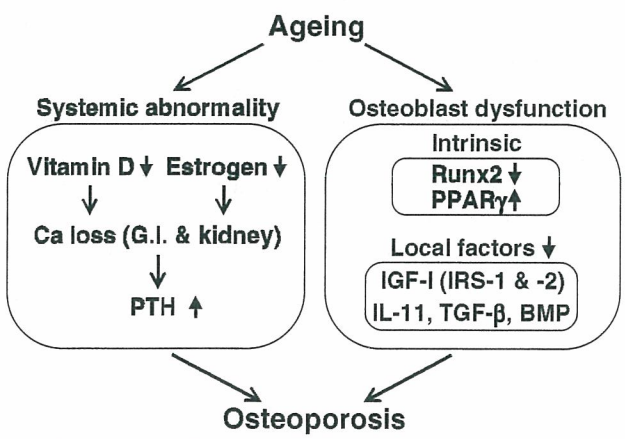

Fig. 2 Possible mechanisms underlying bone loss by ageing. *IL-11* interleukin-11; *TGF-β* transforming growth factor-β; *BMP* bone morphogenetic protein; *G.I.* gastrointestinal; *PTH* parathyroid hormone

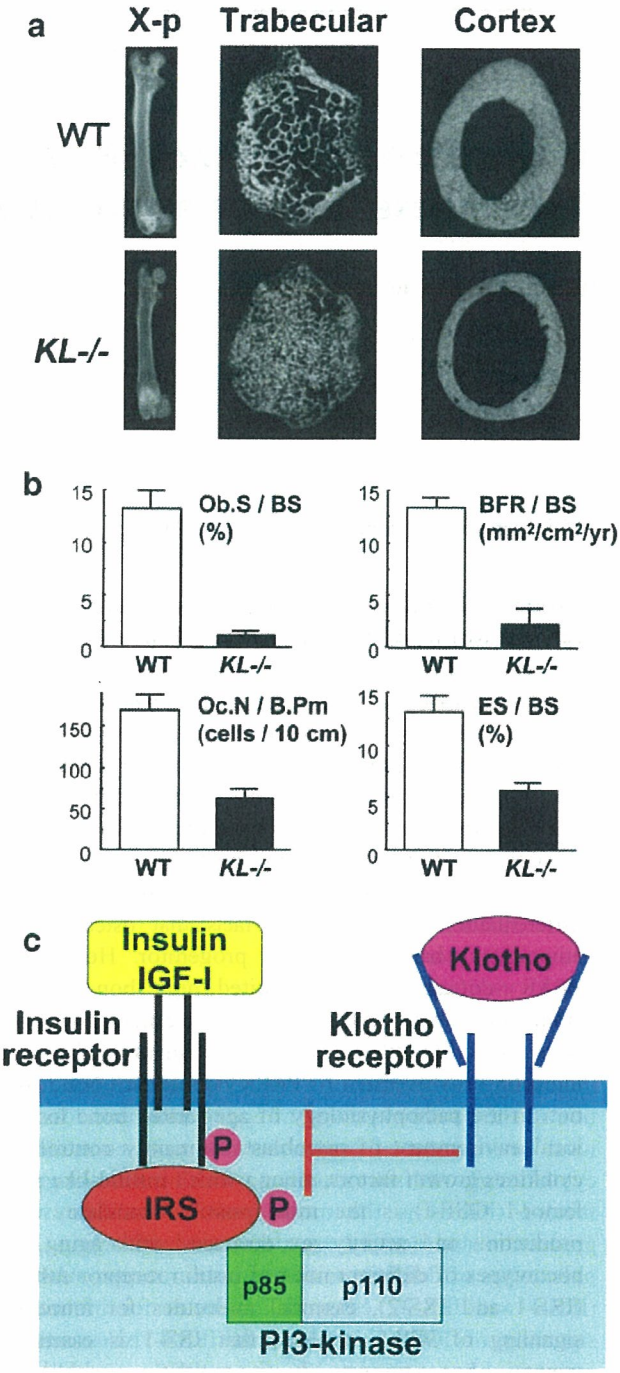

Fig. 3 Bone phenotype of KL−/− mice (a and b) and interaction between klotho and insulin/IGF-I signalings (c) (a) Plain X-ray and CT images of femora of *KL−/−* and WT littermates at 8 weeks of age. (b) Bone histomorphometric analysis of the proximal tibiae of *KL−/−* and WT littermates. *Ob.S/BS* Osteoblast surface per bone surface; *BFR/BS* bone formation rate per bone surface; *Oc.N/B.Pm* osteoclast number per bone perimeter; *ES/BS* eroded surface per bone surface. (c) A scheme of the interaction between klotho and insulin/IGF-I signalings

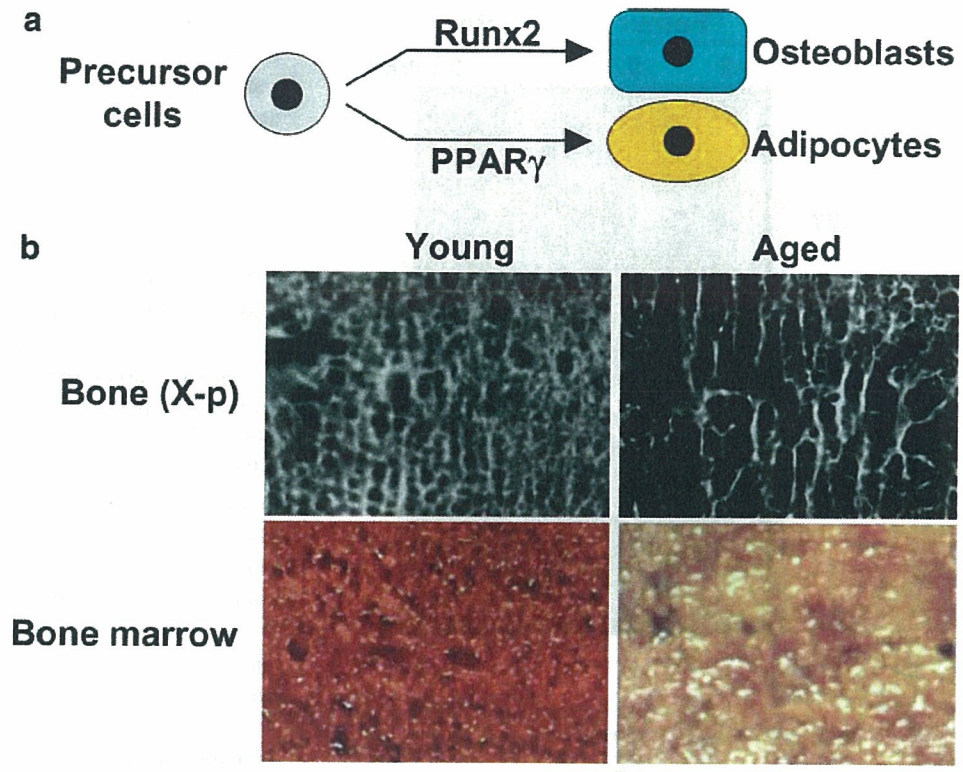

Fig. 4 Backgrounds for possible involvement of PPARγ in age-related bone loss. (a) Mesenchymal precursor cells in bone marrow can differentiate into both osteoblasts and adipocytes through respective key molecules Runx2 and PPARγ. (b) X-ray (*top*) and normal pictures (*bottom*) of bone marrow in proximal femurs of young (34 years) and aged (86 years) females, both of whom underwent surgical operations due to fracture. In the aged marrow, a reciprocal decrease of bone mass and an increase of adipose tissue

of function in multiple age sensitive traits [16]. An insertional mutation that disrupts the 5′promoter region of the *klotho* gene resulted in a strong hypomorphic allele. Mice homozygous for the mutated allele (*KL−/−* mice) exhibited multiple age-related disorders including osteopenia, especially in the cortex bones, just like human senile osteoporosis (Fig. 3(a)), and suffered premature death around 2 months of age [17, 18]. Bone histomorphometric analysis revealed that parameters of both bone formation (Ob.S/BS and BFR/BS) and bone resorption (Oc.N/B.Pm and ES/BS) were lower in *KL−/−* mice in the wild-type (WT) littermates, with predominant decreases of the formation parameters over the resorption parameters (Fig. 3(b)), indicating a state of low bone turnover osteopenia.

The *klotho* gene encodes a single-pass transmembrane protein that is detectable in limited tissues, particularly the distal convoluted tubules in the kidney and the choroid plexus in the brain. Because a defect in the *klotho* gene leads to systemic age-dependent degeneration, the klotho protein may function through a circulating humoral factor that regulates the development of age-related disorders or natural ageing processes [19]. We recently showed that overexpression of klotho can extend life span, and found that the extracellular domain of the klotho protein circulates in the blood and binds to a putative cell-surface receptor [20]. Klotho functions as a hormone that suppresses tyrosine phosphorylation of insulin and IGF-I (Fig. 3(c)). Since extended life span upon negative regulation of insulin and IGF-I signaling is an evolutionarily conserved mechanism to suppress ageing [21], klotho appears to be a peptide hormone to modulate such signaling and thereby mediate insulin metabolism and ageing.

As the forward genetic approach, we examined the association between human *klotho* gene polymorphisms and bone density in postmenopausal women of two genetically distinct racial populations: the Caucasian and the Japanese. Screening of single-nucleotide polymorphisms (SNPs) in the human *klotho* gene identified a total of 11 SNPs, and three of them were common in both populations. Among them, two SNPs: one in the promoter region and one in exon 4 were significantly associated with bone density of the aged postmenopausal women in both populations [22]. Other SNPs in the human *klotho* gene are reported to be associated not only with bone loss [23, 24], but also with altered life span [25] and risk for coronary artery disease [26] and stroke [27]. These results indicate that the *klotho* gene may be involved in the longevity and pathophysiology of age-related disorders including osteoporosis in humans.

3 PPARγ as an osteoblast intrinsic molecule in age-related osteoporosis

Osteoblasts and adipocytes are known to share a common progenitor: multipotential mesenchymal stem cells in bone marrow, being driven by respective key molecules Runx2

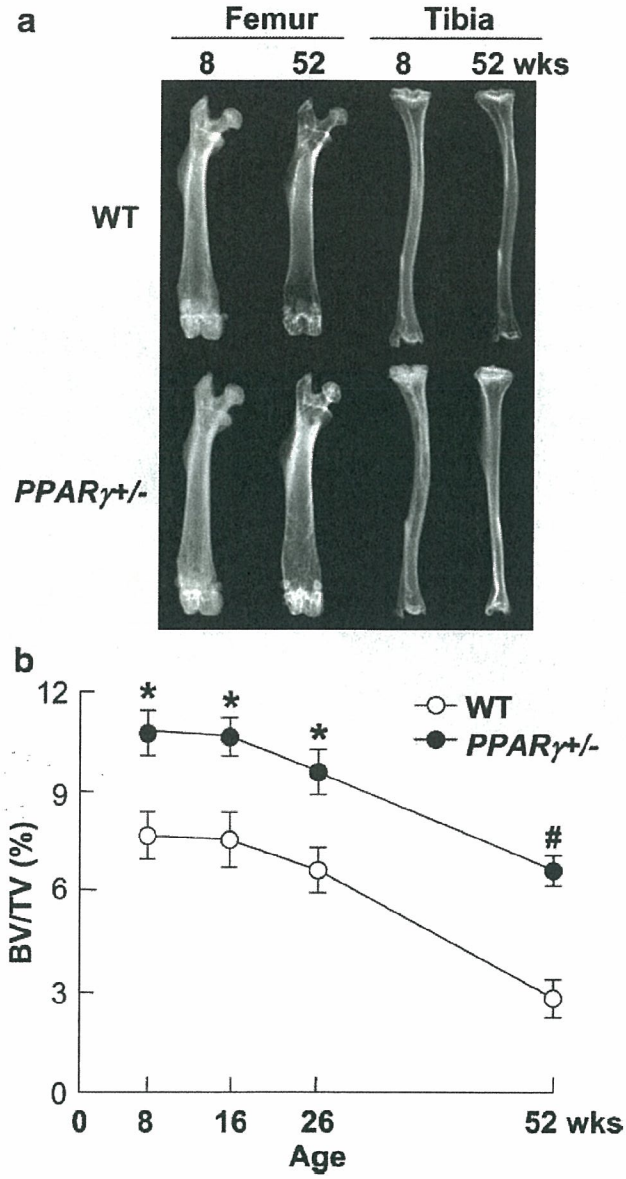

Fig. 5 Radiological analyses of PPARγ +/− and WT littermates at indicated ages. (a) Plain X-ray images of femora and tibiae of representative *PPARγ* +/− and WT littermates at 8 and 52 weeks of age. (b) Time course of trabecular bone volume expressed as percentage of total tissue volume (BV/TV, %) at the distal femora was measured on the CT image. Data are expressed as means (*symbols*)±SEMs (*error bars*) for eight mice/group for *PPARγ* +/− and WT. Significant difference from WT; $*P<0.05$, $\#P<0.01$, determined by post-hoc testing using Bonferroni's method

and PPARγ [28–31] (Fig. 4(a)). In addition, ageing is associated with a reciprocal decrease of osteogenesis and an increase of adipogenesis in bone marrow [32–34] (Fig. 4(b)). Based on these facts, we investigated the physiological role of PPARγ in bone metabolism [35], using heterozygous *PPARγ*-deficient (*PPARγ* +/−) mice [36]. Although they showed no abnormalities in major organs such as brain, heart, liver, spleen or kidney on a standard diet [36, 37], they exhibited high bone mass both at young and old ages (Fig. 5(a)). The time course of the bone volume revealed that bone mass was decreased with ageing in both *PPARγ* +/− and WT littermates; however, the difference of bone volume between the two genotype mice became more prominent at 52 weeks (Fig. 5(b)), indicating the involvement of the PPARγ signaling in the pathophysiology of human age-related osteoporosis [35]. In fact, a previous association study between bone density and a genetic polymorphism of *PPARγ* in postmenopausal women implies the involvement of PPARγ in bone loss [38]. *Ex vivo* culture of bone marrow cells derived from *PPARγ* +/− and WT showed that PPARγ haploinsufficiency caused not only a decrease in the number of adipocytes, but also an increase of osteoblasts, indicating that PPARγ signaling in marrow progenitors functions as a potent suppressor of commitment to osteoblastic lineage [35].

In addition to the role of PPARγ as an intracellular molecule, a recent report suggested that PPARγ could have an antiosteogenic endocrine role, since severely lipodystrophic $PPAR\gamma^{hyp/hyp}$ mice which have a hypomorphic mutation at the PPARγ locus in white adipose tissue [39] showed enhanced bone formation [40]. Adipokines, such as leptin and adiponectin, which are secreted by adipocytes and potently regulate bone metabolism [41–45] might be involved in the PPARγ-related systemic signaling in bone formation.

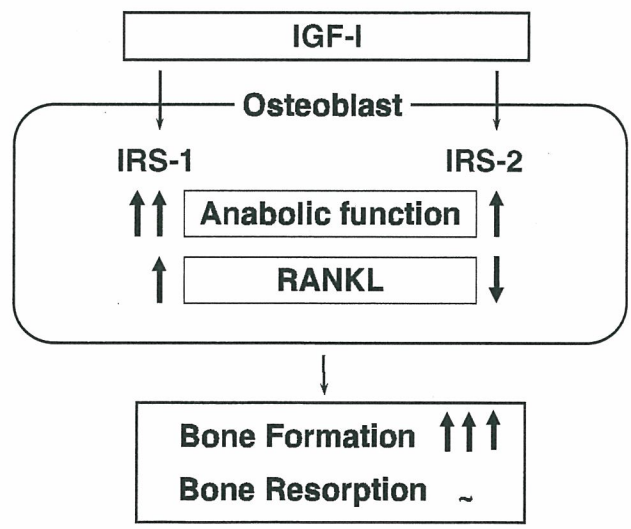

Fig. 6 Mechanism of bone formation by IGF-I through IRS-1 and IRS-2 signalings. Analyses of bones of *IRS-1*−/− and *IRS-2*−/− mice revealed that IRS-1 is important for maintaining both bone anabolic function and catabolic function through RANKL expression in osteoblasts, while IRS-2 increases the anabolic function but decreases the catabolic function. As a balance of the two signalings, IGF-I up-regulates bone formation without affecting bone resorption

4 IRS signaling in age-related osteoporosis

Among cytokines/growth factors of which decreases with ageing have been indicated to be responsible for osteoblast dysfunction, IGF-I is the most probable candidate whose serum level is decreased with ageing and positively related to bone density of aged populations [10–12]. IGF-I initiates cellular responses by binding to its cell-surface receptor tyrosine kinase IGF-I receptor, which then activates essential adaptor molecule IRS followed by downstream signaling pathways like phosphatidylinositol-3 kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs) [46]. The mammalian IRS family contains at least four members: ubiquitous IRS-1 and IRS-2, adipose tissue-predominant IRS-3, and IRS-4 which is expressed in the thymus, brain and kidney. We previously reported that IRS-1 and IRS-2 are expressed in bone [47, 48]. Our further studies on mice lacking the IRS-1 gene (*IRS-1−/−* mice) or the IRS-2 gene (*IRS-2−/−* mice) revealed that these knockout mice exhibited severe osteopenia with distinct mechanisms: *IRS-1−/−* mice showed a low bone turnover in which both bone formation and resorption were decreased [47], whereas *IRS-2−/−* mice showed an uncoupling status with decreased bone formation and increased bone resorption [48]. It therefore seems that under physiological conditions IRS-1 is important for maintaining bone turnover, while IRS-2 for retaining the predominance of anabolic function over catabolic function of osteoblasts. IGF-I may up-regulate bone formation without affecting bone resorption through the balance of the two signalings (Fig. 6).

5 Conclusion

We hereby propose new players, klotho, PPARγ, and IGF-I through IRS-1 and IRS-2 signalings, in the mechanism of age-related osteoporosis, using mouse genetics approaches. These signalings may constitute a network with other molecules like vitamin D, estrogen, Runx2, other cytokines, etc. to maintain bone mass. In addition, there may be a complex interaction among the signalings. For example, considering that the insulin/IGF-I/IRS signaling exhibits a bone anabolic action as described above, the inhibitory action of klotho on the insulin/IGF-I/IRS signaling is inconsistent with the osteopenic phenotype of *KL−/−* mice. We believe that there are signal pathways other than the insulin pathway for the klotho action on bone metabolism. One of the next tasks ahead of us will be to elucidate the network system of these many factors involved in age-related bone loss.

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日本骨粗鬆症学会 平成17年度 研究奨励賞

骨粗鬆症と変形性関節症の因果関係に関する研究（報告）

吉村典子[1)]　雑賀明宏[2)]　岡　敬之[1)]

はじめに

急速な高齢化の進行と余命の延長により，疾病予防の考え方も「いかに長生きするか」から「いかに健康的に長生きするか」へと変化してきた。すなわち生命予後の悪い疾患のみの予防対策から，高齢者の生活の質（quality of life：QOL）を障害する慢性疾患の予防対策にも社会的ニーズが広がってきている。あまたある骨関節疾患の分野で，患者数が極めて多くその予防が急務となる慢性疾患は骨粗鬆症と変形性関節症であると考えられる。

まず骨粗鬆症の患者は現在全国に約1,100万人とも推定されており[1)]，骨代謝疾患のなかではもっとも有病率の高い疾患である。またその一方，高齢化の進行とともに変形性関節症の患者数も急速な増大をみており，厚生労働省の平成16年国民生活基礎調査の結果によると，要支援の原因の2位が関節症であると報告されている[2)]。

骨粗鬆症も変形性関節症もいずれも高齢者のQOLの低下をもたらす。骨粗鬆症のもっとも重篤な合併症である大腿骨頸部骨折は寝たきりの原因ともなり，さらに骨粗鬆症による骨折は生命予後を短縮させる[3)]との報告もある。変形性関節症による関節の疼痛は患者の歩行困難の原因となりQOLの著しい低下をきたす。

従来，骨粗鬆症は骨量の減少がその疾患の主体であり，変形性関節症は骨棘の形成が認められ骨量自体は増加することが多いなどエックス線所見や病態が真逆の状態を呈し，疫学的研究からも危険因子が相反する報告がみられることから，それぞれ独立した疾患であると考えられてきた。しかし最近，骨粗鬆症と変形性腰椎症の併存例において，変形性腰椎症による腰椎の高骨密度がかならずしも脊椎骨折の危険性を減少させないこと[4)]，また椎間disc space narrowingは椎体骨折のリスクをあげるとする報告[5,6)]がなされ，骨粗鬆症予防のためには変形性関節症を要因の一つとして考慮する必要が示唆されるようになってきている。しかしながら現在のところ，骨粗鬆症と変形性関節症については，骨粗鬆症が変形性関節症の要因となるのか，変形性関節症が骨粗鬆症を引き起こすのか，あるいはそれぞれがお互いの合併症となっているのか，または単に病態を修飾しているだけの併存にすぎないのかなど，それぞれの因果関係についてはほとんどわかっていない。

われわれは，和歌山県山村部のM村において，1990年に骨粗鬆症予防のためのコホート集団を設定し，dual energy X-ray absorptiometry（DXA）を用いて腰椎，大腿骨近位部の骨密度を測定し，問診票を用いたインタビュー形式による詳細な生活習慣調査を実施してきた[7)]。さらに同年整形外科医の協力のもとに脊椎および股関節のエックス線撮影を実施し[8)]，骨粗鬆症のみならず変形性関節症の予防に努めてきた。今回，われわれは，コホートに継続的に行ってきた骨密度測定およびエックス線撮影調査結果と，生活習慣の変容に

Key words：骨粗鬆症，変形性関節症，QOL，DXA

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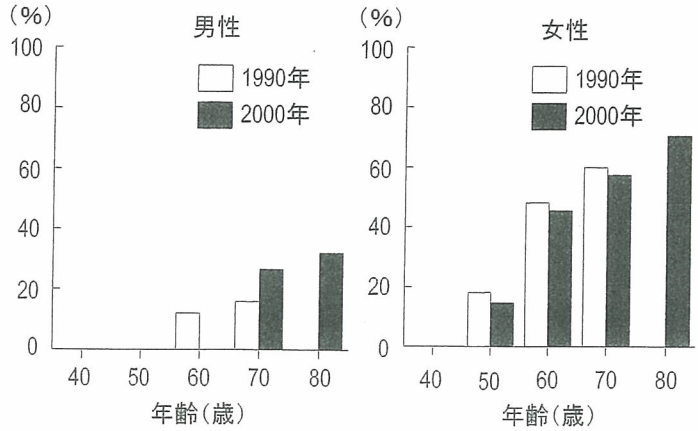

図1　骨粗鬆症有病率の推移

関する問診票調査の結果を，今まで蓄積してきたデータに有機的にリンクすることにより，ベースラインにおける骨粗鬆症および変形性関節症の有病率，および追跡中の骨粗鬆症および変形性関節症の発生率を推定し，骨粗鬆症と変形性関節症の因果関係を明らかにすることを目的として本研究を行ったので報告する。

1　方　　法

和歌山県の山村部に位置するM村では，1989年に40～79歳の全住民1,543人（男性716人，女性827人）を対象にコホートを設定し，既往歴，食生活，運動習慣，飲酒，喫煙，女性の月経に関する事項などからなる125項目の問診票調査を行い，総合的健康管理に役立てている（ベースライン調査）。この集団から1990年に40～79歳の男女各年代50人，計400人をランダムに選び，1990年にDXA（Lunar DPX）を用いて腰椎L_{2-4}，大腿骨近位部（大腿骨頸部，Ward三角，大転子）の骨密度を測定した。さらにその追跡調査として1993年，1997年，2000年にも同じDXAを用い，同対象者に対して，再度腰椎L_{2-4}，大腿骨近位部の骨密度を測定した。さらに1990年と10年目にあたる2000年には，参加者の同意と整形外科医の協力のもとに，脊椎および股関節のエックス線写真を撮影した。

この骨密度結果から日本骨代謝学会の骨粗鬆症診断基準を用いて骨粗鬆症か否かを判定した。発生率については，初回調査時に骨粗鬆症でなかったものが，10年後の各検診時に骨粗鬆症の範疇に入っていた場合を骨粗鬆症新発生とし，10年間の累積発生率を求めた。

また10年間に得られた腰椎側面撮影のエックス線フィルムを一人の整形外科医が再読影し，変形性関節症の重要な診断基準の一つである骨棘症（osteophytosis）であるか否かを判断した。osteophytosisの診断はNathanの分類を用い[9]，腰椎側面写真でNathanの分類が3度以上であると診断したものをosteophytosisありとした。この結果からベースラインのosteophytosisの有病率と10年間における累積発生率を計算した。さらにこれらの結果から骨粗鬆症とosteophysosisとの因果関係を明らかにした。

2　結　　果

1）腰椎骨粗鬆症の有病率と累積発生率

初回調査に参加した40～79歳の対象者400人のうち10年目の追跡調査に参加したのは299人（男性137人，女性162人：74.8%）であった。

ベースラインおよび10年目の追跡調査における骨粗鬆症の有病率を，腰椎骨密度を診断部位として求めたところ，**図1**のごとく男女とも年齢とともに増加傾向にあった。ベースラインと追跡調査の結果を同年代で比較すると，骨粗鬆症有病率は，女性では2000年のほうが低い傾向にあるが有意差はみられなかった（**図1**）。

一方，ベースライン時に骨粗鬆症が認められなかった男性186人，女性137人について，その後

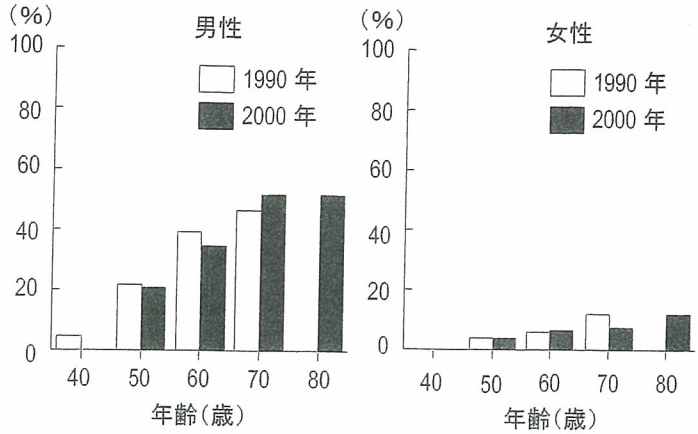

図2　osteophytosis有病率の推移

の10年間における骨粗鬆症の累積発生率を腰椎でみると，1990年で男性40歳代0%，50歳代0%，60歳代11.4%，70歳代11.9%となり，女性では40歳代から順に14.0%，31.7%，19.2%，20.0%となり，骨粗鬆症の累積発生率も女性のほうが高く，女性では50歳代にもっとも高かった。

2）腰椎 osteophytosis の有病率と累積発生率

初回調査に参加した40〜79歳の対象者400人のうちエックス線検査に同意したのは390人（男性194人，女性196人：97.5%）であった。さらに10年目の追跡調査に参加した299人については全員がエックス線調査に同意した。しかしエックス線写真を読影するうえで，椎体の同定が困難など条件不良なものが認められたためそれらを除外し，結果として292人（男性132人，女性160人：73%）分のエックス線写真を読影した。

1990年における住民の腰椎 osteophytosis の有病率を**図2**に示す。男女を比較するといずれの年代でも男性のほうが高かった。

次に1990年の段階で osteophytosis を認めなかった男性141人，女性185人について，その後の10年間の osteophytosis の累積発生率をみると，男性40歳代から11.1%，23.7%，19.3%，14.8%となり，女性で4.2%，4.3%，6.4%，4.7%となり，累積発生率も男性が高かった。

3）腰椎 osteophytosis と骨粗鬆症の関係

osteophytosis と骨粗鬆症の因果関係を明らかにするために，ベースライン時にすでに骨粗鬆症と診断されていたものを解析から除いて，10年後の骨粗鬆症の発生の有無を目的変数に，ベースライン調査時の osteophytosis の有無を説明変数にし，年齢を調整してロジスティック回帰分析を行い，将来の骨粗鬆症発生に対する osteophytosis のリスクをオッズ比でみたところ，男性では0.06（$p=0.017$）となり，osteophytosis は有意にリスクを低減していた。女性では，2.54（$p=0.292$）と osteophytosis を認めるほうが将来の骨粗鬆症のリスクを上昇させていたが有意ではなかった。

逆にベースライン時に osteophytosis を認めたものを解析から除き，10年後の osteophytosis の発生の有無を目的変数に，ベースライン時の骨粗鬆症の有無を説明変数にし，年齢を調整してロジスティック回帰分析を行い，将来の osteophytosis 発生に対する骨粗鬆症のリスクをオッズ比でみたところ，男女とも関連を認めなかった。

3　考　　察

本研究では，わが国の骨関節疾患のなかでももっとも頻度が高く，高齢者の QOL に及ぼす影響が大きいと考えられる骨粗鬆症と変形性関節症の関連について解析した。今回の結果からは，骨粗鬆症の有病率は年齢とともに上昇するが，この10年間で同年代を比較しても上昇は認められないことを明らかにした。さらに腰椎の骨密度を指標として，骨粗鬆症の累積発生率を求め，それが50歳代女性においてもっとも高いことを明らか

にした。

われわれはM村における追跡研究から，骨密度の変化率を求め，腰椎骨密度の変化率は50歳代女性においてもっとも大きいことを報告してきた[10]。さらに，腰椎骨密度変化率は女性ホルモンの減少と関連があることも報告していた[11]。今回の腰椎骨粗鬆症の累積発生率が50歳代女性においてもっとも大きいことは，閉経と強い関係があることは容易に類推される。またその後の60，70歳代においても約20%が10年の間に骨粗鬆症を発症することが明らかとなったことは，高齢者における骨粗鬆症対策が急務であることも示唆している。われわれは骨量の低下の度合いが女性において腰椎と大腿骨では異なることを報告し，大腿骨の骨密度低下は高齢者に大きいことを示した[10]が，診断部位が異なれば有病率の推移と発生率は異なる分布を示す可能性がある。そのため，われわれは今後腰椎のみならず大腿骨の骨密度を用いて累積発生率，および発生率を明らかにしていく予定である。

今回の研究では，変形性関節症の構成要素として重要と思われるosteophytosisに的を絞って読影を行い，一般住民におけるosteophytosisの有病率の推移と累積発生率を推定し，osteophytosisは男性に多く，その累積発生率は50歳代に多いことを明らかにした。しかし今回の結果はdisc space narrowingの有無やKellgren-Lawrence分類を解析に入れておらず，変形性関節症については十分吟味したとはいえない。今後さらに詳しく読影を進め，前記の項目についてもその有病率の推移と累積発生率を推定し，変形性関節症の予防につなげたいと考える。

骨粗鬆症と変形性関節症の関連については，最近，膝変形性関節症が椎体骨折および非椎体骨折のリスクを増加させるとするRotterdam studyの報告[4]や脊椎のdisc space narrowingが閉経後女性の椎体骨折の骨折リスクを増加させるとするOFELY studyの報告[5,6]があり，骨粗鬆症の危険因子として新たな要因を考える必要性を提示した。しかしわが国においてはこのような報告はまだ認められていなかった。今回の解析では，年齢を調整したロジスティック解析によって，骨粗鬆症あるいは変形性関節症（osteophytosis）の因果関係とそのリスクの大きさを示した。今回の結果からは，男性においてはベースラインにosteophytosisが存在するほうがむしろ骨粗鬆症の発生は少なく，ベースライン調査時の骨粗鬆症の有無はその後のosteophytosisの発生には関与していなかった。今後この結果が他の集団でも一致性をもつのかについての検証が必要となる。さらにRotterdamの報告でみられるような他部位の変形性関節症が骨粗鬆症の危険因子となるのかどうかについても今後検討を行う予定である。

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Ⅲ.実践される治療法

関節軟骨破壊と修復の関節マーカーによる評価

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Key words：rheumatoid arthritis, osteoarthritis, joint marker, cartilage matrix

Basic step

変形性関節症（osteoarthritis；OA）や関節リウマチ（rheumatoid arthritis；RA）は，関節機能障害によりQOLの低下をきたす代表的疾患である。OAに対しては有病率が高いことから，多数の患者のなかから将来の関節破壊の進行例を予知し，厳重な経過観察を行うとともに，適応があれば骨切り術などの関節温存手術を積極的に勧めるなどの治療を行うことが必要となる。RAについても関節破壊の急速な進行が危惧される症例には，発症後早期に生物学的製剤の使用開始を考慮するべきである。

関節疾患の病態は従来，画像診断によって評価されてきたが，関節液，血液，尿中などに存在する軟骨や滑膜などの関節構成体の代謝に関連する分子である関節マーカーによる診断，評価が行われている[1]。関節の機能上，最も重要な組織である軟骨の代謝状態を反映するマーカーは厳密には軟骨マトリックスの破壊を反映するもの（破壊マーカー），合成を反映するもの（修復マーカー），その両者を反映するもの（代謝回転マーカー）に分類される。

マーカー分子の測定体液中，関節液が該当する関節の代謝を最も直接的に反映する。関節液から血中に移行したマーカー分子が当該関節の状態をどれほど反映するかについては，マーカー分子の生体内での産生量，分布，代謝回転によって異なる。たとえば，cartilage oligomeric matrix protein（COMP）は軟骨内に存在するマイナープロテインであるために，正常代謝回転で常に一定量が血中に遊離されるアグリカン分子などと異なり，血中で測定しても当該関節に由来する割合が高く，その関節の病態を有意に反映する可能性が高い。

軟骨基質に由来するマーカーでは，脊椎の状態にも注意を要する。すなわち脊椎には椎間関節が多数存在し，軟骨も多量に存在するので，

Evaluation of destruction and repair process of articular cartilage using joint markers　0286-5394/06/¥400/論文/JCLS

H. Yamada, A. Kanaji, S. Kato, H. Date, H. Ichinose, H. Sugimoto：藤田保健衛生大学整形外科

変形性脊椎症などを合併している症例ではデータが修飾を受けやすい。

軟骨破壊のマーカー

アグリカン由来フラグメント

硝子軟骨の主要基質であるアグリカンから遊離された断片(フラグメント)がマーカーとして測定されている。軟骨破壊の初期にはⅡ型コラーゲンよりもアグリカンが先に破壊されるので，関節液中のアグリカンフラグメントは軟骨破壊のよい指標である。アグリカン分子を構成する側鎖のなかでKSは軟骨特異性がきわめて高い。これらのアグリカンフラグメントは合成と破壊の両者の影響を受けるので，厳密には代謝回転マーカーである。血中アグリカン濃度については，アグリカン自身が全身の関節軟骨に豊富に存在するマトリックスであるので，単一関節からの遊離，血中への移行で上昇する程度は高くなく，臨床的な実用性は低い。

コラーゲンの分解産物

Ⅱ型コラーゲン分子は，MMP-1，-8，-13などの蛋白分解酵素によって特異的に切断されたのち，さらに低分子化されていくが，この過程で生じるコラーゲン分子のNおよびC末端に存在するⅡ型コラーゲン架橋テロペプチド(NTX-Ⅱ，CTX-Ⅱ)を尿中で測定する手法が開発されている。これらのテロペプチドは軟骨破壊を反映するマーカーとされている。

その他のマーカー

COMPはトロンボスポンジン族に属する糖蛋白質であり，軟骨以外にも靱帯，腱，滑膜などに存在する。COMPの関節軟骨における機能は不明であるが，Ⅱ型コラーゲン線維の安定化に寄与していると考えられている。COMPは関節液中だけでなく血中でも評価可能なマーカーであることが特徴的である。OAなどの関節破壊疾患においては，MMP-13などの作用により，低分子化されたCOMPの比率が多い。COMPは厳密には軟骨の代謝回転を反映するマーカーとされているが，とくに破壊の影響を受けやすい。

軟骨修復のマーカー

軟骨修復すなわちマトリックス合成の指標としては，Ⅱ型コラーゲンの合成マーカーが知られている。Ⅱ型コラーゲンは軟骨細胞内でプロコラーゲンとして合成されたのち，細胞外に遊離されN，C両末端が切断されてから線維化される。この過程で生じるC末端(pCOLⅡ-C，コンドロカルシン)はⅡ型コラーゲン合成と1：1の関係にあり，その合成の指標といえる。関節液中のpCOLⅡ-CはOAでは修復能の亢進を反映して高値を示すが，RAでは炎症性サイトカインなどの影響により，軟骨細胞のマトリックス合成能が低下していることを反映して低値を示す。

Advance step

軟骨破壊マーカーの疾患における動態

股関節OAの関節液中ケラタン硫酸(KS)は病期の進行に伴い濃度が低下し，病期進行に伴う残存軟骨量の減少，および軟骨の代謝活性の低下を反映している(**図1**)[2]。股関節OAでは関節裂隙の狭小化進行が早い症例(1年間に1mm以上の狭小化進行)で尿中のCTX-Ⅱレベルが高いことが報告されている(**図2**)。これは尿中のCTX-

Ⅱが高い症例では，軟骨Ⅱ型コラーゲンの分解が急速であり，その結果として軟骨破壊が高度であることを示している[3]。

OA関節液中においてはCOMPは正常より高値を示すが，早期OAで最も高く病期が進行するに従い低下していく[4]。COMPはアグリカンやⅡ型コラーゲンのような関節軟骨の主要マトリックスではなく，その機能も明らかでないので，COMPの軟骨からの遊離が主要マトリックス破壊とどの程度，相関しているかは重要である。大腿骨頭壊死(ION)は骨頭の圧潰により急激な機械的軟骨破壊をきたし，OAやRAなどとは軟骨の破壊プロセスが異なっている疾患である。関節液中のCOMPとKSの関係を股OAとIONで

図1　OA関節液中におけるケラタン硫酸(KS)濃度
変形性股関節症の関節液中KSはX線上の病期の進行に伴い低下する。

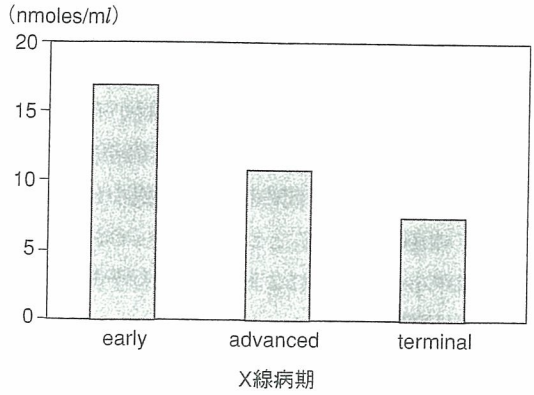
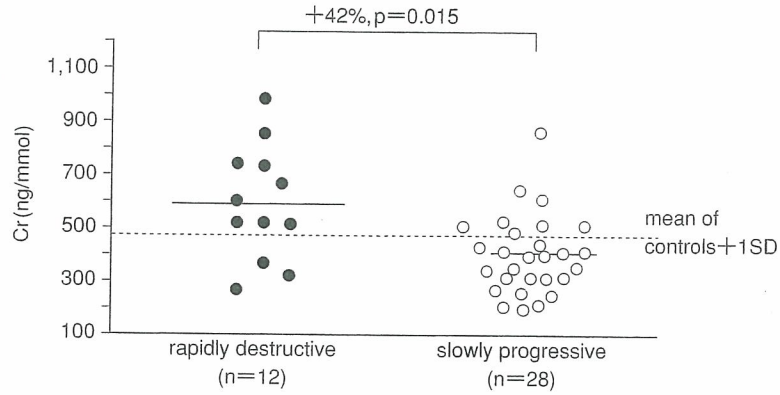
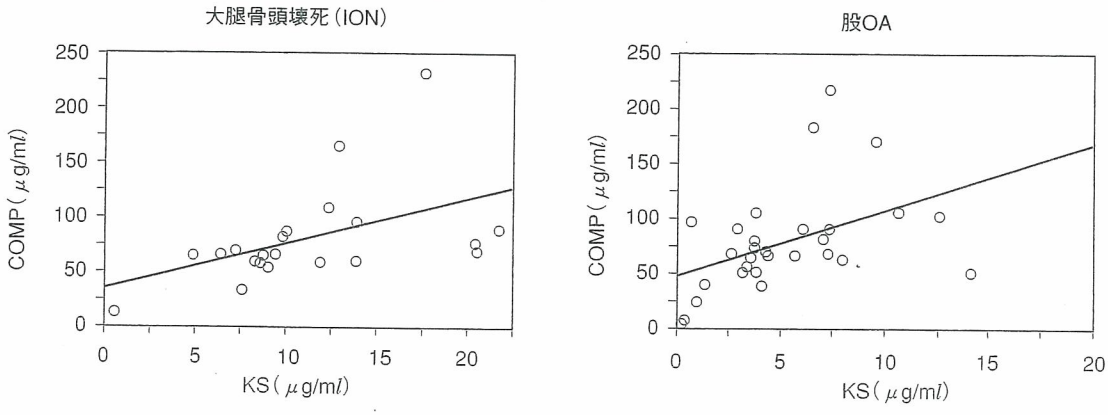


図2　股OAにおける尿中CTX-Ⅱと関節症進行の関係
CTX-Ⅱは進行の早い群(1年間の関節裂隙狭小化が1mm以上)で有意に高値を示していた。



(文献[3]より)

図3　股OAとIONにおける関節液中のCOMPとKSの関係
両マーカーは股OAおよびIONのいずれの疾患でも相関していた。



(文献[5]より)

別々に検討した結果では，両マーカーは股OAおよびIONのいずれの疾患でもよく相関していた(**図3**)[5]。

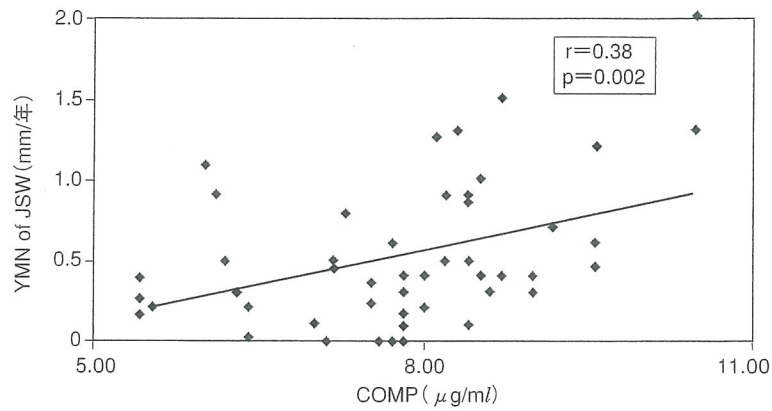
この結果は，軟骨破壊の機序にかかわらず，関節液中COMPはKSと同様の動態をとること，すなわちCOMPは軟骨の機能維持上，重要なアグリカンと類似した動態を示すことを示唆している。また，股OAの血中COMPレベルが初診時に高いものほど，年間の関節裂隙狭小化が大きいことが報告されており，血中COMPはOAの関節破壊進行予知に有用とされている(**図4**)[6]。

軟骨修復マーカーの疾患における動態

初期膝OAの関節液中pCOLⅡ-C濃度は肥満度や大腿脛骨外側角(FTA)と正の相関を有する(**図5**)[7]。これはOAにおいては機械的ストレスの増大に応じてⅡ型コラーゲンの産生が高まっていること，すなわち修復能が亢進していることを示している。膝OA関節液中のpCOLⅡ-Cレベルは4年間の関節裂隙狭小化と有意な正の相関があることが報告されており，関節液中のpCOLⅡ-CによりOAの進行予知が可能である(**図6**)[8]。

図4　股OAにおける初診時の血中COMPと年間の関節裂隙狭小化

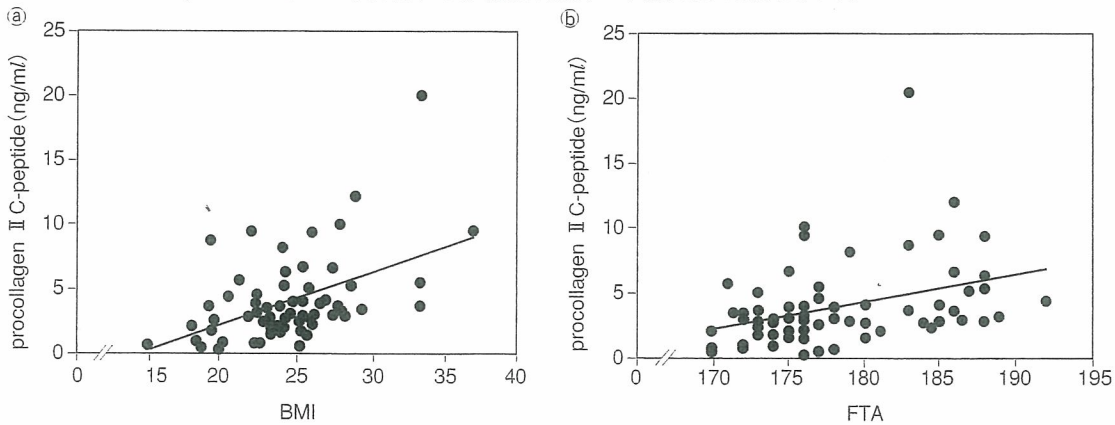
初診時の血中COMPレベルが高いものほど1年間の関節裂隙狭小化が大きい。



(文献[6]より)

図5　膝OAにおける関節液中pCOLⅡ-CとBMIおよびFTAの相関

初期膝OAの関節液中pCOLⅡ-C濃度は肥満度(ⓐ)や大腿脛骨外側角(FTA)(ⓑ)と正の相関を有する。



(文献[7]より)