BMI classified by age-strata and sex were identical between completers and non-completers, while mean age of female completers in their 70s was significantly younger than that of female non-completers (completers, 71.7 years (standard deviation (SD), 1.8 years) vs. non-completers 75.1 years (SD; 2.8 years); $p<0.001$).

Table 1 shows the characteristics including anthropometric factors and BMDs at the time of baseline measurement for participants who completed the 10-year follow-up (Table 1). Mean height and weight of the remaining participants were smaller according to age, while BMI did not differ significantly for both men and women in all age groups except men in their 70s.

Height loss and bone loss

Table 2 shows mean change of height, weight, BMI and change rate of BMDs over 10 years by age and gender (Table 2). Height and weight of men and women decreased in all age strata, and these decreases were greatest in subjects in their seventies. BMI in the 50s, 60s and 70s were decreased over 10 years in both genders, but no significant differences were seen among age-strata. BMDs at the lumbar spine and femoral neck decreased except for BMD at the lumbar spine in men.

To clarify associations between height, height change and changes in BMD, multiple regression analysis was performed. Rate of change of BMD (%/year) was used as an objective factor and height at baseline (cm) or change of height (cm/10 years) were used as explanatory factors. Analysis was performed after adjustment for age and female menstrual status at baseline (0, regular; 1, irregular; 2, menopause). In both men and women, no significant relationship was identified between bone loss and height at baseline (lumbar spine: men, $\beta=-0.046$, standard error of the mean (SE)=0.011, P=0.653, $R^2=0.036$; women, $\beta=-0.042$, SE=0.014, P=0.652, $R^2=0.032$; femoral neck: men, $\beta=0.143$, SE=0.014, P=0.149, $R^2=0.125$; women: $\beta=0.078$, SE=0.014, P=0.397, $R^2=0.043$).

Regarding the association between height loss and bone loss over 10 years, no significant relationship was identified between height change and rate of change of BMD at the lumbar spine and femoral neck after adjusting for age in men (lumbar spine: $\beta=0.058$, SE=0.031, P=0.501, $R^2=0.038$; femoral neck: $\beta=0.100$, SE=0.038, P=0.228, $R^2=0.121$). In contrast, among women, significant positive associations were noted between height change and change rate of BMD at the lumbar spine after adjusting for age ($\beta=0.221$, SE=0.039, P=0.012, $R^2=0.069$), while no significant relationship was noted between height change and change rate at the femoral neck ($\beta=0.107$, SE=0.039, P=0.229, $R^2=0.048$).

Height loss and vertebral fractures

As reported elsewhere [21], 32 men and 35 women had suffered from previous VFx at the initial survey. Cumulative incidences of first VFx at follow-up for subjects in their 40s, 50s, 60s and 70s were thus 2.9%, 2.8%, 8.6% and 21.1% in male completers, respectively, and 2.1%, 7.0%, 18.9% and 31.3% in female completers, respectively. Cumulative incidence of first VFx among participants during follow-up increased with age in both men and women, and was higher in women than in men in all age-strata except the 40s.

Table 3 shows differences in height at baseline and height loss between the incident group and non-fracture group. Both height and height loss over the 10 years were

Table 1 Characteristics at the baseline measurement of participants completed 10-year follow-up

				Anthropometric factors			Bone mineral density (g/cm^2)	
Birth cohort	Age strata	N	Age (years)	Height(cm)	Weight(kg)	BMI (kg/m^2)	L2-4	Femoral neck
Men								
1940–1949	40–49	36	44.1 (3.1)	166.5 (5.9)	64.4 (8.9)	23.1 (2.3)	1.19 (0.17)	0.98 (0.16)
1930–1939	50–59	41	53.9 (2.6)	162.0 (5.7)[a]	60.2 (8.0)	22.9 (2.4)	1.15 (0.20)	0.90 (0.18)
1920–1929	60–69	38	63.2 (2.8)	159.4 (5.4)[a]	56.1 (7.5)[a]	22.0 (2.4)	1.03 (0.19)[a]	0.82 (0.12)[ab]
1910–1919	70–79	22	73.2 (2.7)	155.3 (6.5)[ab]	50.0 (8.4)[ab]	20.6 (2.6)[ab]	1.03 (0.20)[a]	0.79 (0.11)[ab]
Women								
1940–1949	40–49	49	44.7 (3.1)	152.5 (4.7)	53.3 (8.4)	22.9 (2.8)	1.18 (0.16)	0.88 (0.12)
1930–1939	50–59	46	54.8 (2.6)	149.6 (5.3)	50.3 (7.4)	22.4 (2.8)	0.99 (0.18)[a]	0.75 (0.12)[a]
1920–1929	60–69	40	64.4 (2.8)	147.4 (5.1)[a]	47.4 (6.8)[a]	21.8 (3.0)	0.86 (0.20)[ab]	0.69 (0.11)[ab]
1910–1919	70–79	27	71.7 (1.8)	143.1 (5.5)[ab]	45.4 (7.7)[a]	22.1 (3.0)	0.79 (0.16)[ab]	0.65 (0.09)[ab]

Mean (SD)

a: Significantly different from values of the birth cohort group born in 1940–1949

b: Significantly different from values of the birth cohort group born in 1930–1939

Table 2 Changes in height, weight, BMI and change rate in bone mineral densities over 10 years by age and gender

Age at initial survey	Change rate of anthropometric factors			Change rate of bone mineral density	
Age strata (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	L2–4 (%/year)	Femoral neck (%/year)
Men					
40–49	−0.73 (2.21)	−0.21 (5.09)	0.17 (2.20)	0.17 (0.69)	−0.26 (0.86)
50–59	−0.54 (2.09)	−0.83 (3.69)	−0.18 (1.38)	0.55 (0.58)	−0.13 (0.84)
60–69	−1.19 (2.41)	−3.01 (4.80)	−0.86 (1.84)	0.01 (0.89)[b]	−0.75 (0.97)[b]
70–79	−1.54 (1.72)	−3.05 (3.88)	−0.84 (1.65)	−0.16 (0.68)[b]	−1.17 (1.09)[ab]
Women					
40–49	−0.69 (1.21)	−0.33 (3.22)	0.06 (1.39)	−0.87 (0.71)	−0.53 (0.70)
50–59	−1.37 (1.18)	−1.74 (3.64)	−0.35 (1.69)	−0.83 (0.75)	−0.53 (0.71)
60–69	−2.06 (2.08)[a]	−2.44 (3.55)[a]	−0.58 (1.69)	−0.48 (0.71)	−0.50 (0.87)
70–79	−3.65 (2.83)[abc]	−3.09 (3.48)[a]	−0.42 (1.76)	−0.48 (1.48)	−1.16 (1.32)[abc]

Mean (SD)
a: Significantly different from values of the age-group in their 40s
b: Significantly different from values of the age-group in their 50s
c: Significantly different from values of the age-group in their 60s

also greater in the group with VFx than without VFx. To clarify associations between height or height change and incidence of VFx after excluding the effects of age, logistic regression analysis was performed. We utilized new VFx over 10 years (1: yes; 0: no) as an objective factor and height at baseline (cm) or change of height (cm/10 years) as explanatory factors. Analysis was performed after adjusting for age and female menstrual status at baseline (0: regular; 1: irregular; 2: menopause). After logistic regression analysis, no significant relationship was identified between VFx and height at baseline in men and women (men: odds ratio (OR) 0.93, 95% confidence interval (CI) 0.81–1.05, P=0.24; women: OR 0.97, 95% CI 0.87–1.08, P=0.58). Furthermore, a non-significant relationship was seen between cumulative incidence of VFx and height loss in men and women (men: OR 1.31, 95% CI 1.00–1.71, P=0.051; women: OR 1.20, 95% CI 0.94–1.53, P=0.14).

Table 3 Comparison of height (cm) at baseline and height loss between the group with new vertebral fractures and the no fracture group

		VFx* over 10 years		
		No (n=116)	Yes (n=9)	P (Yes vs. No)
Men	Height (cm)	161.8 (6.49)	156.4 (7.76)	0.014
	Height loss (cm/10 years)	0.87 (2.08)	2.59 (2.23)	0.019
		No (n=128)	Yes (n=16)	
Women	Height (cm)	149.7 (5.75)	145.9 (6.43)	0.015
	Height loss (cm/10 years)	1.33 (1.78)	2.88 (2.26)	0.002

*VFx: vertebral fractures

Height loss and QOL

Among the 299 subjects who participated in the latest follow-up survey in 2000, 212 answered the QOL questionnaire distributed in 2002 (94 men, 118 women; 70.9%).

Figures 2 and 3 show mean values for utility in EQ5D health states and VAS scores classified by age and gender. Mean utility for EQ5D in men in their 40s (n=30), 50s (n=33), 60s (n=25) and 70s (n=6) were 0.95, 0.87, 0.88 and 0.83, respectively, compared to 0.90, 0.85, 0.81 and 0.77 in women in their 40s (n=42), 50s (n=32), 60s (n=31) and 70s (n=13). VAS values in men were 76.6, 75.1, 72.4 and 63.8, respectively, compared to 77.6, 73.9, 67.6 and 71.7, respectively, in women. Utility of EQ5D decreased according to age in both men and women, while mean VAS scores were lowest for women in their 60s.

We utilized multiple regression analysis using utility of EQ5D health states or VAS scores as an objective factor and height at baseline (cm) or change of height (cm/10 years) as explanatory factors to clarify associations between height and QOL. Analysis was performed after adjusting for age and female menstrual status at baseline

Fig. 2 QOL score classified by age and gender

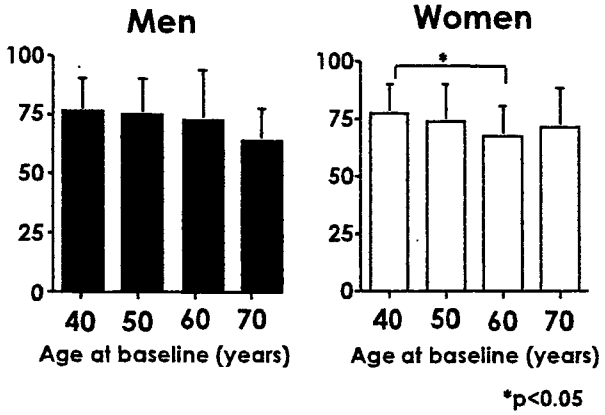

Fig. 3 VAS scores classified by age and gender

(0: regular; 1: irregular; 2: menopause). In both men and women, no significant relationship was identified between utility of EQ5D and height at baseline (men: β=−0.148, SE=0.003, P=0.202, R^2=0.076; women: β=0.127, SE=0.004, P=0.235 R^2=0.048), and height change (men: β=−0.078, SE=0.008, P=0.452, R^2=0.065; women: β=0.053, SE=0.010, P=0.608, R^2=0.038). Regarding VAS scores, height at baseline among men and women was not significantly associated VAS scores (men: β=−0.148; SE=0.003, P=0.202, R^2=0.076; women: β=0.066, SE=0.255, P=0.532, R^2=0.092). In addition, no significant associations were identified between utility of VAS scores and height change (men: βa=−0.148, SE=0.003, P=0.202, R^2=0.076; women: β=0.142, SE=0.698, P=0.160, R^2=0.105).

Discussion

The present study clarified associations between height, height change and bone loss and cumulative incidence of VFx. Furthermore, we assessed the usefulness of height and height change as predictors of future QOL. As a result, we identified significant positive associations between height change and change rate of BMD at the lumbar spine in women after adjusting for age and menstrual status, while no significant relationships were found between height or height change at the femoral neck in either men or women. Regarding associations between height, height change and cumulative incidence of first VFx, both height and height loss over the 10 years were also greater in the group with VFx than in the group without VFx, but the association was less significant in logistic regression analysis after adjusting for age. No significant relationships existed between height, height change and future QOL in men or women.

Particularly among anthropometric measurements, light weight [5–8], weight loss [9, 10] and low BMI [11–13] could suggest a risk of osteoporosis and osteoporotic fractures. Conversely, few investigations have reported that height and height loss are associated with low BMD or bone loss. We have already reported that tall height is associated with greater bone loss over 3 years [22]. Twiss et al. [23] reported that actual height loss is associated with risk factors of osteoporosis, while Thornton et al. [24] evaluated relationship between height change and bone mineral density among 168 healthy women at 50- to 65-years-old, and reported no significant relationships between height change and BMD. Kantor [25] reviewed cross-sectional data from 2,108 women referred for a bone density scan and reported that a height loss of ≥2 inches offers a highly significant predictor of osteoporosis at the hip [25]. As mentioned, investigations into associations between height and bone loss have yielded controversial results, and no data from follow-up studies over periods as long as 10 years have been available. The present study clarified that greater height loss was associated with greater bone loss at the lumbar spine in women. This means that height loss might offer a predictor for greater bone loss, thus indicating a potential high-risk group for future osteoporosis in women. Conversely, the present study failed to identify any significant association between height loss and bone loss at the lumbar spine in men, which is artificial due to the difficulties in measuring BMD at the lumbar spine in men. As observed in the BMD cohort, 35.1% of men and 13.3% of women were diagnosed with osteophytosis more than grade 3 according to Nathan's classification [26, 27]. Such osteophytes might lead to overestimation of BMD in men.

Regarding the relationship between height loss and osteoporotic fractures, Meyer et al. [11] compared mean height among participants of population-based cohort studies established in different countries in Europe, and found that participants in Oslo were taller than those in other European countries. They noted that the taller height of community-dwelling inhabitants might contribute to the higher incidence of hip fracture in Finland, although this suggestion was based on ecological data. Fujiwara et al. [28] suggested that the presence of more than one column of VFx will lead to a decrease of about 2 cm in height. The present study found both height and height loss over the 10 years were also greater in the group with VFx than in the group without VFx, but failed to identify any statistically significant association between height loss and VFx. This might be because the sample size of the BMD cohort was insufficient to detect a significant association. However, height loss (cm/10 years) tended to increase the OR of VFx in both men and women. Loss of height may represent an important clinical sign of vertebral deformation and/or fracture in postmenopausal women and elderly men. Relationships between BMD at the femoral neck and hip fracture were not able to be analyzed because of the low numbers of new hip fractures in subjects. A larger

epidemiological study would be needed to clarify associations between height loss and future osteoporotic fractures.

Regarding relationships between QOL, height and height loss, Martin et al. [29] found that height loss and kyphosis in women are significantly associated with increased physical difficulty in activities of daily life. In addition, some reports have described the influence of osteoporotic VFx on QOL [30–32]. These investigations have shown that patients with higher grades of vertebral deformities displayed low QOL, suggesting that the results of VFx such as height loss are related to QOL, but the direct influence of height loss on QOL remains unclear. The present study could not find any significant association between height loss and QOL, so we concluded that QOL in patients with osteoporosis is impaired by postural deformities, particularly by whole kyphosis, and that spinal mobility exerts a strong effect on QOL in these patients.

Conclusions

The present study identified significant positive associations between height change and change rate of BMD at the lumbar spine in women, while no significant relationships were found between height, height change, cumulative incidence of VFx and future QOL.

In conclusion, changes in measured height might offer a cost-saving indicator of bone loss. Measurement of height should be considered as one potential component in determining risk of comprehensive osteoporosis, but further consideration is required before utilizing this approach as a predictor of future osteoporotic fracture and QOL.

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Original article

High tibial osteotomy using two threaded pins and figure-of-eight wiring fixation for medial knee osteoarthritis: 14 to 24 years follow-up results

Go Omori[1], Yoshio Koga[2], Masunao Miyao[3], Takashi Takemae[4], Takashi Sato[2], and Hiroshi Yamagiwa[5]

[1]Center for Transdisciplinary Research, Niigata University, 2-8050 Igarashi, Niigata 950-2181, Niigata, Japan
[2]Department of Orthopedic Surgery, Niigata Kobari Hospital, Niigata, Japan
[3]Department of Orthopedic Surgery, Nekoyama Miyao Hospital, Niigata, Japan
[4]Rehabilitaton Center Midori Hospital, Niigata, Japan
[5]Division of Orthopedic Surgery, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan

Abstract

Background. High tibial osteotomy (HTO) is an established surgical treatment for medial knee osteoarthritis (OA). Several studies have reported the deterioration of clinical results with time, especially after more than 10 years. The purpose of this study was to evaluate the long-term results after HTO using our originally developed fixation method and to clarify the factors affecting the long-term clinical outcome.

Methods. Sixty-eight HTO treatments in 55 patients were evaluated. Eighteen patients were unable to be analyzed, thus reducing the study to 48 knees in 37 patients. The follow-up rate of the knee joint was 70.6% and the mean follow-up period was 17.1 years. The first evaluation was performed at a mean of 6.5 years postoperatively, and the most recent evaluation was done at more than 10 years postoperative follow-up. A closing-wedge osteotomy was performed, and the osteotomy site was fixed with two threaded pins and a figure-of-eight wiring technique. The Japanese Orthopaedic Association knee rating score (JOA score) was used for the clinical assessment. The change of the femorotibial angle (FTA) and progression of knee OA were radiographically analyzed. The whole knees were subsequently divided into two groups, satisfactory group and unsatisfactory group, according to the JOA score at the most recent follow-up.

Results. The mean JOA score was 59.1 before HTO and 83.1 at the most recent evaluation. In comparing the satisfactory and unsatisfactory groups, the JOA score before HTO was the same, but the JOA score of the unsatisfactory group was significantly lower at the first evaluation. The FTA in the unsatisfactory group was the same as in the satisfactory group preoperatively, but it was significantly larger after HTO. The radiographic OA was significantly progressed at the most recent evaluation, but no difference was observed in the distribution of the preoperative OA grade between the two groups.

Conclusions. HTO with two threaded pins and figure-of-eight wiring fixation showed an acceptable clinical outcome, but careful attention was needed for correction loss in early postoperative periods. In addition, the proper correction angle is necessary in order to achieve satisfactory long-term results.

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Introduction

Osteoarthritis (OA) is the most common form of degeneration of the joints. The knee joint is the key structure in the lower extremity and has much influence on the activity of daily life (ADL) and the quality of life (QOL) in elderly persons. These include standing, walking, running, jumping, stair climbing, deep knee bending such as squatting or Japanese-style sitting, and other lower extremity tasks. Approximately 10% to 15% of people aged 60 years and older have symptomatic knee OA.[1] Therefore, knee OA is a major source of chronic disability and is becoming a serious public health problem.

High tibial osteotomy (HTO) is one of the successful surgical treatments for medial compartment knee OA. HTO was first described by Jackson and Waugh,[2] and it is now widely accepted as an attractive procedure with good pain relief and preservation of knee function. Previous studies of early to midterm results of HTO have shown excellent outcomes in more than 80% of cases.[3–5] However, several studies with long-term follow-up reported that the results of HTO deteriorated with time, especially after more than 10 years. Several factors have been identified as affecting the results of HTO, but they remain controversial. These include sex, age at surgery, body weight, preoperative severity of knee OA, method of osteotomy and fixation, correction angle, amount of preoperative adduction moment, and postoperative period.[6–14]

Among these factors, the type of fixation following osteotomy remains important, and, in the past, the following methods have been reported: bone staples, blade plate with screws, one third tubular plate with a cortical screw (tension bend principle), L-buttress plate, and external fixator.[3,15–20] We developed a fixation method using two threaded pins and figure-of-eight wire and used this method for our consecutive HTO cases.

The purpose of this retrospective study was to assess the long-term results after HTO using our fixation method and to clarify the factors affecting the long-term clinical outcome.

Subjects and methods

Our indications for HTO were basically as follows: (1) degenerative change was mainly located in medial compartment (medial knee osteoarthritis), (2) normal or mild degeneration in lateral and patello-femoral compartment, (3) patient was younger than 70 years old and had relatively high activity in ADL, and (4) good range of motion and no remarkable knee joint instability. Between 1980 and 1990, HTO was performed in 68 consecutive knees in 55 cases by our senior surgeon (Y. K.). Seven patients died, 6 patients were unable to be evaluated due to the presence of other severe medical illnesses, and 2 patients were lost to follow-up. Three knees in 3 patients were converted to total knee arthroplasty (TKA) at 10 years, 12 years, and 15 years after HTO, respectively. Therefore, the remaining 48 knees in 37 cases were available for the present study, and the follow-up rate of the knee joint was 70.6%. There were 43 knees in 33 women and 5 knees in 4 men. The mean age at HTO was 59 years with a range from 40 to 69 years. The mean follow-up period was 17.1 years, but individual follow-up ranged from 14 to 24 years. The preoperative diagnosis was medial compartment knee OA in all the cases, and the preoperative Kellgren-Lawrence classification[21] showed grade II in 8 knees, grade III in 35 knees, and grade IV in 5 knees. All of the patients were evaluated initially in 1993, with a mean follow-up of 6.5 years, and evaluated at more than 10 years follow-up postoperatively. All of the patients were fully informed about the procedures and gave their informed consent.

Operative procedures and postoperative regimen

In all knees, the closing-wedge interlocking osteotomy through a lateral approach was performed according to the technique described by Ogata.[22] The correction angle was preoperatively determined to allow the mechanical axis, which is the line connecting the center of the femoral head and the ankle joint, to pass through the midpoint of the lateral compartment. The preoperative planning was performed using non-weight-bearing supine radiograph of the whole lower extremity according to Ogata et al.[23] Ogata mentioned that the relative angle of the articular surface (condylar-plateau angle) in the weight-bearing knee changed after osteotomy, and this might give unpredictable results postoperatively. He also found that the condylar-plateau angle in the postoperative standing radiograph was very similar to that seen in the non-weight-bearing supine condition, and recommended that a non-weight-bearing supine radiograph was better for preoperative planning. The femorotibial angle (FTA) that met this condition was around 165° to 168° in the majority of cases. The fibula was resected at the mid portion of the shaft. The osteotomy site was fixed with two threaded pins and a figure-of-eight wiring technique. First, two threaded pins, 2.4 or 3.0 mm in diameter, were inserted from distal and lateral of the osteotomy site to the medial corner of the proximal tibia passing through the medial half of the osteotomy line. Next, figure-of-eight wiring, 0.8 to 1.0 mm in diameter, was placed between the distal end of the pins and lateral wall of the proximal tibia. After the osteotomy site was fixed, leg alignment was checked by X-ray and cancellous bone fragments harvested from the resected bone wedge were grafted to the osteotomy site (Fig. 1). Postoperatively, the knee joint was immobilized with a cast for 6 weeks. Range-of-motion exercise was started after the cast was removed. Partial weight bearing was started 4 weeks after HTO and full weight bearing was allowed at 8 to 10 weeks postoperatively.

Clinical evaluation

All of the patients were directly interviewed and examined. The clinical result was evaluated using the Japanese Orthopedic Association knee rating score (JOA score).[24] The JOA score consisted of four categories and 100 points as full marks: pain and walking (30 points), pain and ascending or descending stairs (25 points), range of motion (35 points), and joint effusion (10 points). In this study, the preoperative JOA score was compared with the JOA score at the first evaluation in 1993 and at the most recent follow-up. Subsequently, the results of the JOA score were classified as excellent if the most recent score was 91 to 100, good if 81 to 90, fair if 71 to 80, and poor if the most recent score was less than 70 points. Furthermore, all knee joints were divided into two subgroups according to the result of the most recent follow-up. The patients who were classified as excellent and good were referred to as the satisfactory group, and the patients who were classified as fair and poor were referred to as the unsatisfactory

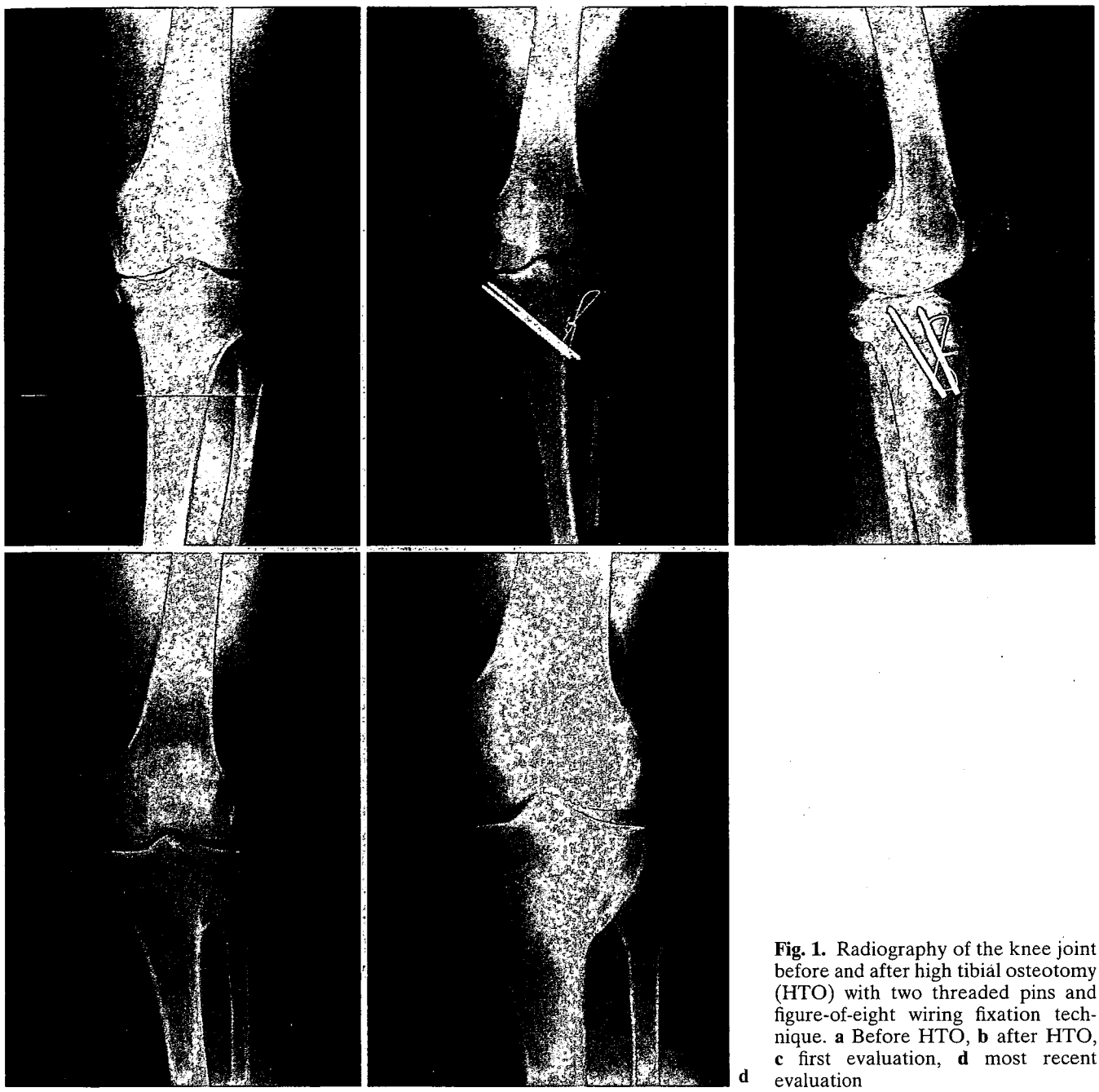

Fig. 1. Radiography of the knee joint before and after high tibial osteotomy (HTO) with two threaded pins and figure-of-eight wiring fixation technique. **a** Before HTO, **b** after HTO, **c** first evaluation, **d** most recent evaluation

group. Thirty-seven knees in 22 patients (1 male, 21 female) were included in the satisfactory group, with an average age at surgery of 57.9 ± 5.0 years and average follow-up period of 14.0 ± 2.9 years. On the other hand, 11 knees in 10 patients (3 male, 7 female) were included in the unsatisfactory group, with an average age at surgery of 60.1 ± 8.7 years and average follow-up period of 14.3 ± 3.1 years. No statistical difference was observed in the demographic data between the two groups.

Radiographic evaluation

The change of FTA and the grades of knee OA according to the Kellgren-Lawrence classification were analyzed with a standing whole-leg X-ray taken before surgery, at 1 to 3 weeks after HTO, and at each follow-up point.

Statistical analysis

The obtained data were expressed as the mean values ± standard deviation (SD). The relationships of analyzed parameters were determined using the paired *t*-test and the Wilcoxon signed rank test. In all analyses, a *P* value of less than 0.05 was considered to be significant.

Results

Clinical results

The mean JOA score of all patients improved significantly from 59.1 ± 7.6 before HTO to 86.3 ± 6.5 at the first evaluation (Table 1). At the most recent follow-up, the JOA score had slightly declined to 83.1 ± 9.3 but this change was not significant. In each category of JOA scores in all patients, the pain and walking score improved from 14.5 ± 5.2 before HTO to 26.6 ± 5.6 at the most recent evaluation, the pain and stairs score from 12.7 ± 6.6 to 20.2 ± 4.9, the score for range of motion from 25.6 ± 4.8 to 27.8 ± 4.6, and the score for joint effusion from 6.3 ± 5.7 to 8.5 ± 4.3. The mean range of motion was 9.3° ± 8.0° fixed flexion to 133.0° ± 18.1° of flexion before HTO, and 2.6° ± 4.3° to 132.5° ± 16.2° of flexion at the most recent evaluation. In comparing the satisfactory group and the unsatisfactory group, the mean JOA score was similar before HTO, but at the first and the most recent evaluation, the JOA score of the unsatisfactory group was significantly lower than that of the satisfactory group. Furthermore, in the unsatisfactory group, the JOA score had significantly declined from first evaluation to the most recent follow-up (Table 1). In the current study, there were two postoperative complications. One patient had peroneal nerve palsy and spontaneously recovered in 3 months after surgery. Another patient had delayed union and autologous iliac bone graft was performed. Final bone union was obtained at 7 months after HTO. These complications did not affect the clinical results.

Radiographic results

The mean FTA of all patients was corrected from 185.4° ± 4.4° before HTO to 168.2° ± 2.9° postoperatively, and this alignment was maintained at the most recent evaluation. In the satisfactory group, the change of FTA was almost same as the results of all patients. In contrast, the FTA of the unsatisfactory group changed from 185.3° ± 2.1° preoperatively to 170.2° ± 2.3° after HTO, and gradually increased at first evaluation and increased even more at the most recent follow-up. The FTA of the unsatisfactory group was the same as the satisfactory group preoperatively, but was significantly larger at each time of postoperative evaluation (Table 2). Seven of the unsatisfactory group (63.6%) had an FTA larger than 168° (170°: 3 cases, 172°: 3 cases, 173°: 1 case). The radiographic OA of all patients before HTO were classified as follows: 8 knees as Grade II, 35 knees as Grade III, and 5 knees as Grade IV. At the most recent evaluation, the distributions were 1 knee as Grade II, 18 knees as Grade III, and 29 knees as Grade IV. The number of Grade IV OA at the latest evaluation was significantly greater than that of before HTO (Table 3). In comparing the satisfactory group and the unsatisfactory group, no statistical difference was observed in the distribution of preoperative radiographic OA grade (Table 4). At the latest evaluation, the distributions of OA in the satisfactory group were 1 knee in Grade II, 18 knees in Grade III, and 18 knees in Grade IV. On the other hand, in unsatisfactory group, all knees were classified as Grade IV OA.

Table 1. Japanese Orthopaedic Association (JOA) score before high tibial osteotomy (HTO), at the first evaluation, and at the latest evaluation

Classification	Number of knees	JOA score		
		Before HTO	First evaluation[a]	Latest evaluation[b]
All Patients	48	59.1 ± 7.6	86.3 ± 6.5	83.1 ± 9.3
			**	
Satisfactory group	37	59.1 ± 9.1	90.0 ± 5.4	87.3 ± 4.3
			**	* **
Unsatisfactory group	11	59.1 ± 5.8	82.2 ± 7.2	69.1 ± 5.8
			**	*

Data given as mean ± standard deviation
* $P < 0.05$; ** $P < 0.01$
[a] Mean follow-up 6.5 years
[b] Mean follow-up 17.1 years

Table 2. Femorotibial angle (FTA) before HTO, at the first evaluation, and at the latest evaluation

Classification	Number of knees	FTA (degrees)			
		Before HTO	After HTO	First evaluation[a]	Latest evaluation[b]
All Patients	48	185.4 ± 4.4	168.2 ± 2.9	169.1 ± 4.5	169.8 ± 5.2
Satisfactory group	37	185.5 ± 4.8	167.6 ± 2.8 *	168.0 ± 4.1 *	168.4 ± 4.4 **
Unsatisfactory group	11	185.3 ± 2.1	170.2 ± 2.3 *	172.7 ± 3.8 *	174.4 ± 5.2 **

Data given as mean ± standard deviation
* $P < 0.05$; ** $P < 0.01$
[a] Mean follow-up 6.5 years
[b] Mean follow-up 17.1 years

Table 3. Distribution of the radiographic osteoarthritis (OA) grade before HTO and at the latest evaluation

Classification	Number	OA grade[a]		
		Grade II	Grade III	Grade IV
Before HTO	48	8	35	5
Latest evaluation[b]	48	1	18	29**

** $P < 0.01$
[a] Radiographic OA grade according to the Kellgren-Lawrence classification
[b] Mean follow-up 17.1 years

Table 4. Distribution of the preoperative radiographic OA grade between the satisfactory group and the unsatisfactory group

Classification	Number	OA grade[a]		
		Grade II	Grade III	Grade IV
All Patients	48	8	35	5
Satisfactory group	37	8	26	3
Unsatisfactory group	11	0	9	2

[a] Radiographic OA grade according to the Kellgren-Lawrence classification

Discussion

The first purpose of this study was to evaluate our fixation methods. We used two threaded pins and figure-of-eight wire, and the basic concept of this procedure was similar to a tension band or modified tension band fixation as previously described.[17,18] Generally speaking, rigid fixation and early rehabilitation is important for good clinical outcome after HTO,[15,25] and there have been several studies concerning the primary stability of the implants for HTO.[26–28] Flamme et al.[27] tested the initial stability of the following devices: one third tubular plate with a cortical screw, blade plate with screws (Giebel's plate), bone staples, and external fixator. In their study, the highest stability was achieved by the bone staple and external fixator, while Giebel's plate and one third tubular plate were less stable. Recently, we biomechanically evaluated the initial stability of our fixation method and compared it with the bone staple, Giebel's plate, and L-buttress plate. The results of this study indicated that our method showed similar stability to Giebel's plate and the bone staple against compression and bending stress except rotational force.[29] In the present study, we additionally used cast immobilization after HTO in consideration of initial stability of our fixation method, and we clinically experienced 11 of 48 unsatisfactory cases. Furthermore, 7 of the unsatisfactory cases showed correction loss in early postoperative periods. The main reason for this early correction loss is thought to be combination of the lack of initial stability especially against rotational stress and the bone quality of the osteotomy site. Thus, we think the two threaded pins and figure-of-eight wiring fixation is an acceptable fixation procedure for HTO; however, careful attention should be paid to correction loss in the early postoperative periods.

The second purpose of the present study was to evaluate the long-term clinical results after HTO and to determine the factors related to the outcome. There are many studies about the clinical results after HTO. The

majority of authors have reported satisfactory results in the short to midterm, but these results gradually deteriorated over time, especially at more than 10 years after surgery. The reported probability of a good or excellent result after HTO was 75% to 96% after 6 years, 45% to 94% after 10 years, and 46% to 90% after more than 15 years.[3–14] In the current study, the percentage of satisfactory results (excellent or good) after HTO was 93.7% after 6 years and 77.1% after 17 years. Our results had the same tendency of deterioration over a long period as the other studies, but still maintained a favorable result up to 17 years after HTO. We think the main reason for the good clinical outcome in spite of the progression of radiographic OA is that good alignment was maintained in the majority of cases during the follow-up period and the ADL of the patients slowly deteriorated with time. Recently, Koshino et al.[14] evaluated 75 knees with a mean follow-up of 19 years and reported good or excellent results in 90% of their series. Good alignment was described as the most important factor for good long-term clinical results.[14]

There is still considerable discussion about which factors affect the long-term outcome of HTO, and the present study focused on the correction angle at the surgery and the preoperative severity of knee OA. As for the correction angle, previous studies have reported that the optimum clinical outcomes were associated with a correction of 6° to 16° valgus, and an undercorrection less than 5° was strongly related to a high failure rate.[5,8–14] In this study, the mean FTA after HTO was 167.6° in the satisfactory group and 170.2° in the unsatisfactory group. In addition, in the unsatisfactory group, progressive varus recurrence was found at the follow-up. We believe that the most important concept for HTO is to shift the loading axis from the medial compartment to the lateral compartment, and this will lead good long-term clinical outcomes in HTO. In order to achieve this safely, we recommend that we should target a valgus correction of at least 10° for medial compartment knee OA.

In western countries, the patients with advanced knee OA were primarily indicated for total knee arthroplasty. Therefore, there have been few studies that evaluate the relationship between the preoperative severity of the knee OA and the clinical result of HTO. Holden et al.[30] followed 51 knees for 10 years and found no correlation between the clinical results and the radiographic severity of the knee OA preoperatively. Rinonapoli et al.[10] evaluated 60 knees with an average follow-up of 15 years and their multivariate analysis indicated that the length of follow-up and the amount of preoperative osteoarthritis affected the clinical results. On the other hand, there have been many studies about this issue in Japan, because the preservation of range of motion is important for ADL in Japanese people. Yasuda et al.[8] found no statistical difference between the preoperative OA stage and the clinical results, but also described that no stage IV patients obtained good results. Sasazaki et al.[31] compared HTO in mild to moderate OA with advanced OA, and found no clinical difference between the two groups. They also indicated that overcorrection was effective for HTO in advanced OA cases.[31] In this study, the radiographic OA grade of the knee joint was significantly deteriorated at the mean follow-up of 17 years, but no statistical difference was observed regarding the preoperative severity of the radiographic knee OA between the satisfactory and the unsatisfactory group. Furthermore, three of five patients with preoperative Grade IV OA were included in the satisfactory group at the recent follow-up. Therefore, we agree that the mild to moderate stage is expected to have better results after HTO, but we could also expect good clinical outcomes for the advanced stage if the cartilaginous condition of the lateral compartment is acceptably preserved and the proper postoperative alignment is achieved.

We believe that there are two limitations in this study. The first limitation is that this is a retrospective study and the 70.6% follow-up rate is perhaps low even for the long-term periods of more than 10 years. The second limitation is that we used the JOA score for clinical evaluation. The JOA score is a good scoring system and is popular in Japan. In addition, several recent studies about HTO using this scoring system have been published in international journals.[24,32,33] However, even though the JOA score is not a worldwide universal measuring system, we believe that we can compare the result of this study with other clinical reports.

In conclusion, HTO with two threaded pins and figure-of-eight wiring fixation showed an acceptable and good clinical outcome for an average of 17 years of follow-up. The present study also suggests that the proper correction angle is necessary to achieve satisfactory long-term clinical results and HTO is considered to be indicated for the patients with a moderate to advanced stage of medial knee OA.

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Regional Differences in Chondrocyte Metabolism in Osteoarthritis

A Detailed Analysis by Laser Capture Microdissection

Naoshi Fukui,[1] Yasuko Ikeda,[1] Toshiyuki Ohnuki,[1] Nobuho Tanaka,[1] Atsuhiko Hikita,[1] Hiroyuki Mitomi,[1] Toshihito Mori,[1] Takuo Juji,[1] Yozo Katsuragawa,[2] Seizo Yamamoto,[3] Motoji Sawabe,[3] Shoji Yamane,[1] Ryuji Suzuki,[1] Linda J. Sandell,[4] and Takahiro Ochi[1]

Objective. **To determine the change in metabolic activity of chondrocytes in osteoarthritic (OA) cartilage, considering regional difference and degree of cartilage degeneration.**

Methods. **OA cartilage was obtained from knee joints with end-stage OA, at both macroscopically intact areas and areas with various degrees of cartilage degeneration. Control cartilage was obtained from age-matched donors. Using laser capture microdissection, cartilage samples were separated into superficial, middle, and deep zones, and gene expression was compared quantitatively in the respective zones between OA and control cartilage.**

Results. **In OA cartilage, gene expression changed markedly with the site. The expression of cartilage matrix genes was highly enhanced in macroscopically intact areas, but the enhancement was less obvious in the degenerated areas, especially in the upper regions. In contrast, in those regions, the expression of type III collagen and fibronectin was most enhanced, suggesting that chondrocytes underwent a phenotypic change there. Within OA cartilage, the expression of cartilage matrix genes was significantly correlated with *SOX9* expression, but not with *SOX5* or *SOX6* expression. In OA cartilage, the strongest correlation was observed between the expression of type III collagen and fibronectin, suggesting the presence of a certain link(s) between their expression.**

Conclusion. **The results of this study revealed a comprehensive view of the metabolic change of the chondrocytes in OA cartilage. The change of gene expression profile was most obvious in the upper region of the degenerated cartilage. The altered gene expression at that region may be responsible for the loss of cartilage matrix associated with OA.**

Osteoarthritis (OA) is a disease characterized by a progressive loss of cartilage matrix that often extends over a decade. During the long course of the disease, chondrocytes undergo obvious metabolic changes. A variety of changes are known to occur that have 2 distinctive aspects. First, the anabolic activity of chondrocytes is strongly enhanced in OA. Following the initial reports more than 4 decades ago (1), an increasing number of studies have shown that the expression of virtually all cartilage components is up-regulated in OA cartilage (2–13). The increased anabolism may be a repair response of the chondrocytes that counteracts the loss of cartilage matrix (2–4). Second, in OA, chondrocytes undergo phenotypic changes. Because of this,

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[1]Naoshi Fukui, MD, PhD, Yasuko Ikeda, DVM, Toshiyuki Ohnuki, Nobuho Tanaka, BS, Atsuhiko Hikita, MD, PhD, Hiroyuki Mitomi, MD, PhD, Toshihito Mori, MD, Takuo Juji, MD, Shoji Yamane, PhD, Ryuji Suzuki, DVM, PhD, Takahiro Ochi, MD, PhD: National Hospital Organization Sagamihara Hospital, Sagamihara, Japan; [2]Yozo Katsuragawa, MD: International Medical Center of Japan, Tokyo, Japan; [3]Seizo Yamamoto, MD, PhD, Motoji Sawabe, MD, PhD: Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan; [4]Linda J. Sandell, PhD: Washington University School of Medicine, St. Louis, Missouri.

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Address correspondence and reprint requests to Naoshi Fukui, MD, PhD, Clinical Research Center, National Hospital Organization Sagamihara Hospital, Sakuradai 18-1, Sagamihara, Kanagawa 228-8522, Japan. E-mail: n-fukui@sagamihara-hosp.go.jp.

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chondrocytes in OA cartilage express matrix genes that are not expressed in normal cartilage, such as type I and type III collagens (5–10). Since the induction of these genes also occurs during the dedifferentiation of chondrocytes in vitro, the phenotypic changes in OA have an aspect resembling that of the dedifferentiation process (9). The phenotypic changes also show a characteristic of developmental reversal, since the expression of type IIA procollagen, a prechondrogenic splicing variant of the type II collagen gene, is observed in OA (11,12). In contrast, the presence of type X collagen in OA cartilage has persuaded investigators that chondrocytes are undergoing hypertrophic changes there (13,14).

Because of the diversity in gene expression, it is currently difficult to obtain a comprehensive idea of the metabolic changes in OA. This diversity may stem from a topographic variation of the pathology. Since cartilage pathology differs obviously from site to site within OA cartilage, it is likely that the metabolic changes in the chondrocytes also differ by areas related to that pathology (4,9,10,15). The regional differences of chondrocyte metabolism may be important to our understanding of the mechanism of disease progression. For example, a focal decline of the matrix synthesis in OA cartilage may play a critical role in the loss of cartilage matrix (3,4,9).

Conventionally, the regional differences of cellular metabolism in OA have been evaluated primarily by histologic methods, so the comparison among the areas has not been quantitative. Laser capture microdissection (LCM) is an innovative technology that enables the isolation of a specific area of tissue by its histologic features (16). Coupled with real-time polymerase chain reaction (PCR), the use of LCM allowed us to perform a quantitative evaluation of the multiple genes expressed in specific regions of OA cartilage. Thus, this study has revealed, for the first time, a comprehensive view of the changes in metabolic activity of chondrocytes in OA cartilage.

MATERIALS AND METHODS

Tissue procurement. This study was performed with the approval of the Human Ethics Review Committees of the participating institutions. For material collection, informed consent was obtained in writing from each subject or family of the donor. OA cartilage samples were obtained from 32 end-stage OA knee joints of 30 patients (mean age 70.3 years [range 56–88 years]) within 4 hours after surgery. The diagnosis of OA was based on the criteria for knee OA of the American College of Rheumatology (17). Control cartilage samples were obtained from 18 nonarthritic knee joints from 16 donors (mean age 82.3 years [range 67–89 years]) within 24 hours after death. The donors had no known history of joint disease or serious trauma, and the normality of the joint was confirmed macroscopically at the time samples were obtained. Knee cartilage in aged donors usually undergoes some degeneration, even though the donors did not have any problems with the joints. Therefore, we obtained control cartilage samples from the knees even when the cartilage showed some signs of degeneration, as long as the degeneration was superficial and limited to small areas (<20% of total cartilage area). Control ligaments, bone tissues, and menisci were also harvested from these joints.

Laser capture microdissection. In each OA joint, cartilage tissues were harvested from 2–5 sites in femoral condyles showing various degrees of cartilage degeneration. In each control joint, cartilage samples were harvested from 2–4 sites in the weight-bearing areas of the femoral condyles. The cartilage samples were cut above the calcified zone, which was confirmed under a microscope at the time of laser microdissection. Immediately after harvest, the cartilage samples were embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan), snap-frozen in liquid nitrogen, and then stored at −80°C until used.

In preparation for LCM, 20–40-μm–thick frozen sections were cut from the cartilage tissues along a plane vertical to the joint surface. The sections were first treated with 0.5*M* EDTA (pH 8.0) for 3 minutes, dehydrated with graded concentrations of ethanol, and clarified with xylene. All reagents were prepared RNase-free, and the entire process was completed within 30 minutes to minimize RNA degradation.

Under an LCM device (PixCell IIe; Arcturus, Mountain View, CA), each frozen section was divided into cartilage zones based on its histologic features (18,19). Cartilage samples from preserved areas contained 3 zones (superficial, middle, and deep) and were separated into these respective zones. For the cartilage from degenerated areas, the number of zones in the section differed from 3 to 1, depending on the severity of the cartilage pathology. A section containing all 3 zones was separated into the 3 respective zones. When a superficial zone was lost to the disease, the section was divided into 2 zones, the middle and deep zones (Figure 1). If a section contained only a deep zone, it was used directly for RNA extraction without microdissection. At each tissue procurement, the appropriateness of zone isolation was confirmed under a microscope.

Analysis of gene expression. Immediately after LCM, RNA was extracted from the tissues using an RNeasy Micro kit (Qiagen, Hilden, Germany) with routine use of DNase I (Qiagen). Complementary DNA (cDNA) was synthesized using Sensiscript reverse transcriptase (Qiagen). Gene expression was evaluated quantitatively by real-time PCR on a LightCycler (Roche Diagnostics, Basel, Switzerland). Gene-specific primers and probes were prepared (a list of primer and probe sequences is available at http://www.hosp.go.jp/~sagami/rinken/crc/index.html), and the process of PCR was monitored by either SYBR Green or hybridization probes. LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics) or LightCycler FastStart DNA Master Hybridization Probe (Roche Diagnostics) was used for PCR. The PCR protocol was as follows: 95°C for 10 minutes to activate *Taq* polymerase, then 40 cycles of 95°C for 10 seconds, melting temperature for the individual gene for 15 seconds (a list of melting temperatures for the individual genes is available at

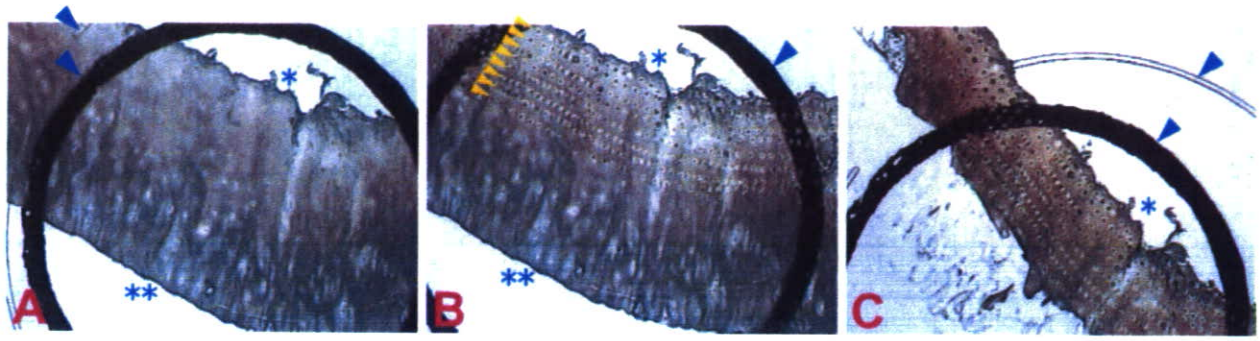

Figure 1. Separation and acquisition of cartilage zone by laser capture microdissection (LCM). **A,** A tissue section was set on an LCM device, and a transparent plastic film was placed on the section. Cartilage zones were identified through the film. **B,** The zone of interest was fixed to the film by shooting with a laser. The area was shot multiple times until the entire zone was anchored to the film. Arrays of spots indicated by **yellow arrowheads** are the laser shot marks. **C,** After laser shooting, any unnecessary area of the section was removed, and only the zone of interest that had adhered to the film was obtained. Acquisition of a middle cartilage zone from a section containing middle and deep zones is shown. The superficial zone of this section was already lost to disease. **Single** and **double asterisks** indicate the top and bottom of the section, respectively. Transparent and bold black arcs indicated by **blue arrowheads** are the marks on the plastic film. (Original magnification × 2.)

http://www.hosp.go.jp/~sagami/rinken/crc/index.html), and 72°C for 6 seconds.

The amount of specific cDNA was quantified with a standard curve based on the known amounts of PCR product. When SYBR Green I was used for monitoring, melting curves were routinely recorded to verify singularity of the product. A previous study showed that *GAPDH* is expressed at similar levels in chondrocytes in normal and OA cartilage (8). Consistently, the result of our preliminary experiment indicated that the expression of *GAPDH* and *ACTB* (a gene coding β-actin) was highly correlated in cartilage samples from OA and control knees. Thus, in this study, *GAPDH* was used as the internal standard for gene expression, and cDNA levels were expressed as the ratio of gene expression:*GAPDH* expression.

Statistical analysis. Pearson's correlation and paired *t*-tests were calculated with the SAS software package (SAS Institute, Cary, NC). For some data, statistical differences were determined by an analysis of variance followed by a Scheffe's post hoc test. *P* values less than 0.05 were considered significant.

RESULTS

Up-regulated expression of cartilage matrix molecules at different regional intensities in OA cartilage. In each OA joint, cartilage was harvested from femoral condyles, both from macroscopically intact areas and

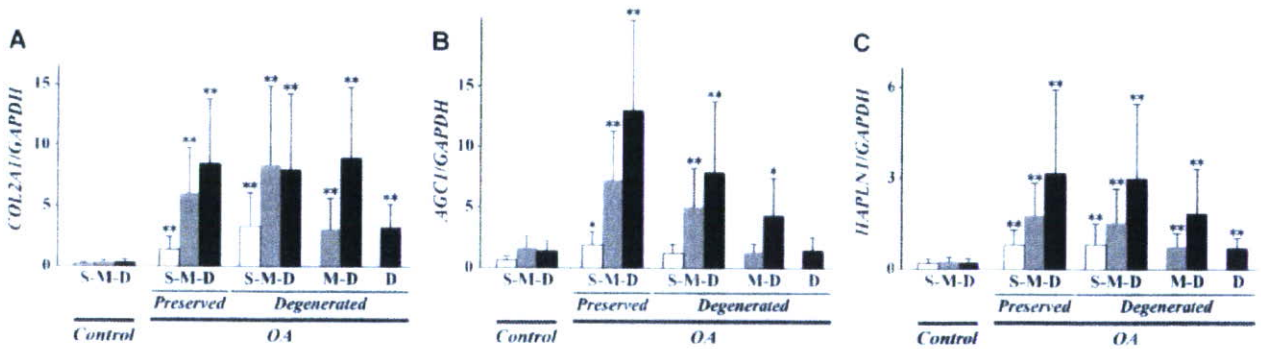

Figure 2. Expression of cartilage matrix genes in osteoarthritic (OA) and nonarthritic (control) cartilage. Cartilage samples obtained from nonarthritic knee joints and knee joints with end-stage OA were divided into superficial (S), middle (M), and deep (D) zones by laser capture microdissection, and expression of cartilage matrix genes was evaluated in the respective zones. In OA joints, cartilage samples were harvested from macroscopically intact areas (preserved) and areas with various degrees of cartilage degeneration (degenerated). The latter samples were divided into 3 groups (S-M-D, M-D, and D) according to the zones retained at the site. Expression of the genes coding type II collagen (*COL2A1*) (**A**), aggrecan (*AGC1*) (**B**), and link protein (*HAPLN1*) (**C**) is shown as ratios of the expression of *GAPDH*. Each bar represents the results from at least 16 samples. Values are the mean and SD. * = $P < 0.05$; ** = $P < 0.01$, versus the corresponding zone in control cartilage.

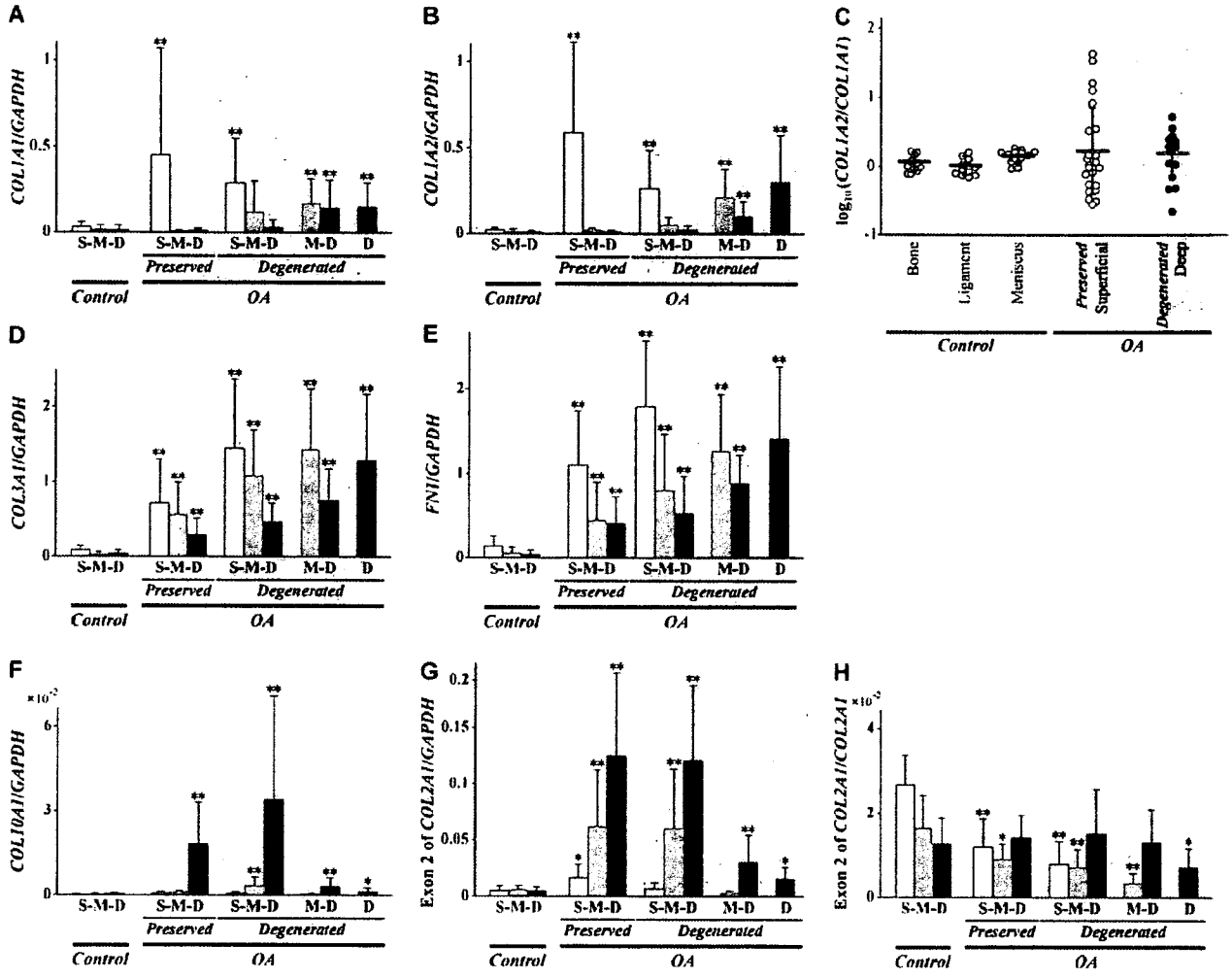

Figure 3. Expression of minor cartilaginous genes in OA and control cartilage. A and B, Expression of *COL1A1* (A) and *COL1A2* (B) in control and OA cartilage is shown as ratios of the expression of *GAPDH*, as described in Figure 2. C, The expression ratios of *COL1A2* to *COL1A1* were obtained in the superficial zone in preserved areas and in the deep zone in degenerated areas where the zone was directly exposed to the joint cavity, and were compared with those obtained in bone, ligaments, and menisci harvested from control joints. Ratios are shown in logarithmic values. D–F, Expression of genes coding type III collagen (*COL3A1*) (D), fibronectin (*FN1*) (E), and type X collagen (*COL10A1*) (F) is shown as ratios of the expression of *GAPDH*. G and H, Expression of exon 2 of *COL2A1* gene is shown as ratios of the expression of *GAPDH* (G) and by ratio to the total expression of *COL2A1* (H). S-M-D, M-D, and D under the respective groups of bars indicate the zone(s) retained in the samples. Each bar represents the results from at least 11 samples. Values are the mean ± SD. * = $P < 0.05$; ** = $P < 0.01$, versus the corresponding zone in control cartilage. See Figure 2 for definitions.

from areas showing macroscopic signs of degeneration. In this study, such areas were designated "preserved" and "degenerated" areas, respectively. OA and control cartilage samples were separated into 3 cartilage zones by LCM, and gene expression was evaluated in the respective cartilage zones by real-time PCR, considering the zonal difference and the severity of cartilage degeneration.

Compared with that in the control cartilage, the expression of type II collagen was strongly up-regulated in all areas in OA cartilage (Figure 2A). The up-regulation was most apparent in the deep zone, where the expression was ~20-fold that in the corresponding zone of the control cartilage. In contrast, the level of up-regulation was considerably reduced in the upper part of the degenerated cartilage. Where the zones were

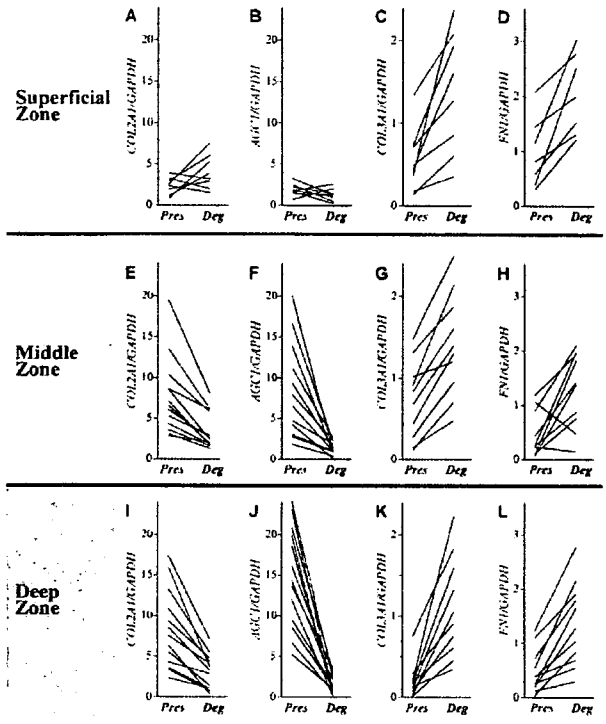

Figure 4. Comparison of gene expression between preserved (Pres) and degenerated (Deg) areas. In each osteoarthritic joint, the expression of 4 genes was compared in the respective cartilage zones between the preserved and degenerated areas. For the middle and deep zones, expression in the degenerated area was determined where the zones were directly exposed to the joint cavity due to the loss of the upper zone(s) to the disease. Expression of *COL2A1* (A, E, and I), *AGC1* (B, F, and J), *COL3A1* (C, G, and K), and *FN1* (D, H, and L) in the superficial, middle, and deep zones is shown. In these graphs, each line represents the expression in a single joint. Results from 7–13 joints are shown as the ratio of gene expression to *GAPDH* expression.

directly exposed to the joint cavity due to the loss of the upper zone(s) to the disease, the expression levels in the middle and deep zones were almost half of those in the preserved areas.

The expression of aggrecan was also enhanced in OA cartilage (Figure 2B). Similar to type II collagen, the increase was most obvious in the deep zone of the preserved area but was less intense in the degenerated area. In this gene, the regional change of expression was more obvious than that in type II collagen. Thus, in the middle and deep zones exposed to the joint cavity in degenerated areas, the expression was virtually unenhanced, and the expression levels were similar to those in the control cartilage. The expression of link protein presented a regional change similar to that of aggrecan, although the decline in the degenerated area was less apparent (Figure 2C).

Spatially distinctive patterns in OA cartilage shown by expression of minor cartilaginous genes induced by OA. In OA, there is enhanced expression of several genes that are not expressed at substantial levels in normal cartilage. Types I, III, and X collagen and fibronectin are among those genes (5,9,13,14,20–22), which are termed minor cartilaginous genes in this report. A change in alternative splicing also occurs in OA, and there is induced expression of exon 2 of type II collagen gene, which is not expressed in healthy adult cartilage (11,12). Therefore, we evaluated the expression of these genes and the exon in OA and control cartilage, paying special attention to regional differences.

In accordance with previous reports (6–8,23), the expression of type I collagen genes, *COL1A1* and *COL1A2*, was induced in OA cartilage (Figures 3A and

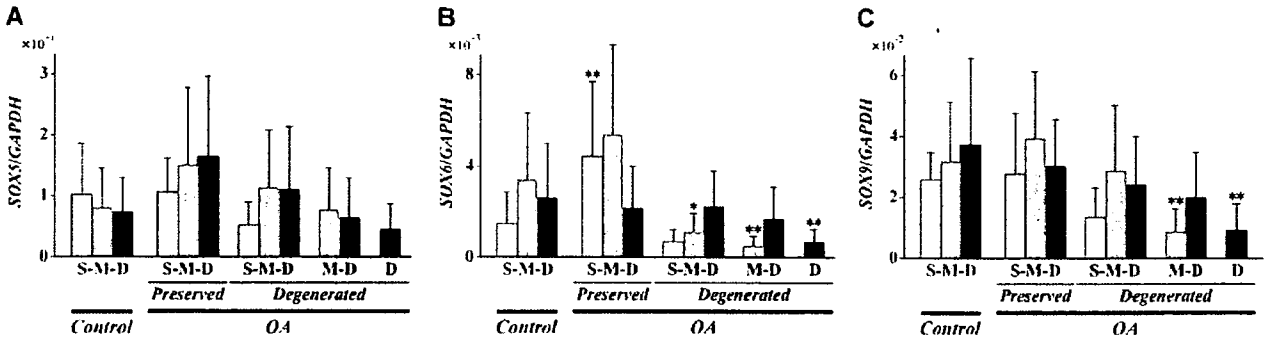

Figure 5. Expression of *SOX* genes in OA and control cartilage. Expression of *SOX5* (A), *SOX6* (B), and *SOX9* (C) in control and OA cartilage is shown as ratios of the expression of *GAPDH*, as described in Figure 2. S-M-D, M-D, and D under the respective groups of bars indicate the zone(s) retained in the samples. Each bar represents the results from at least 13 samples. Values are the mean and SD. * = $P < 0.05$; ** = $P < 0.01$, versus the corresponding zone in control cartilage. See Figure 2 for definitions.

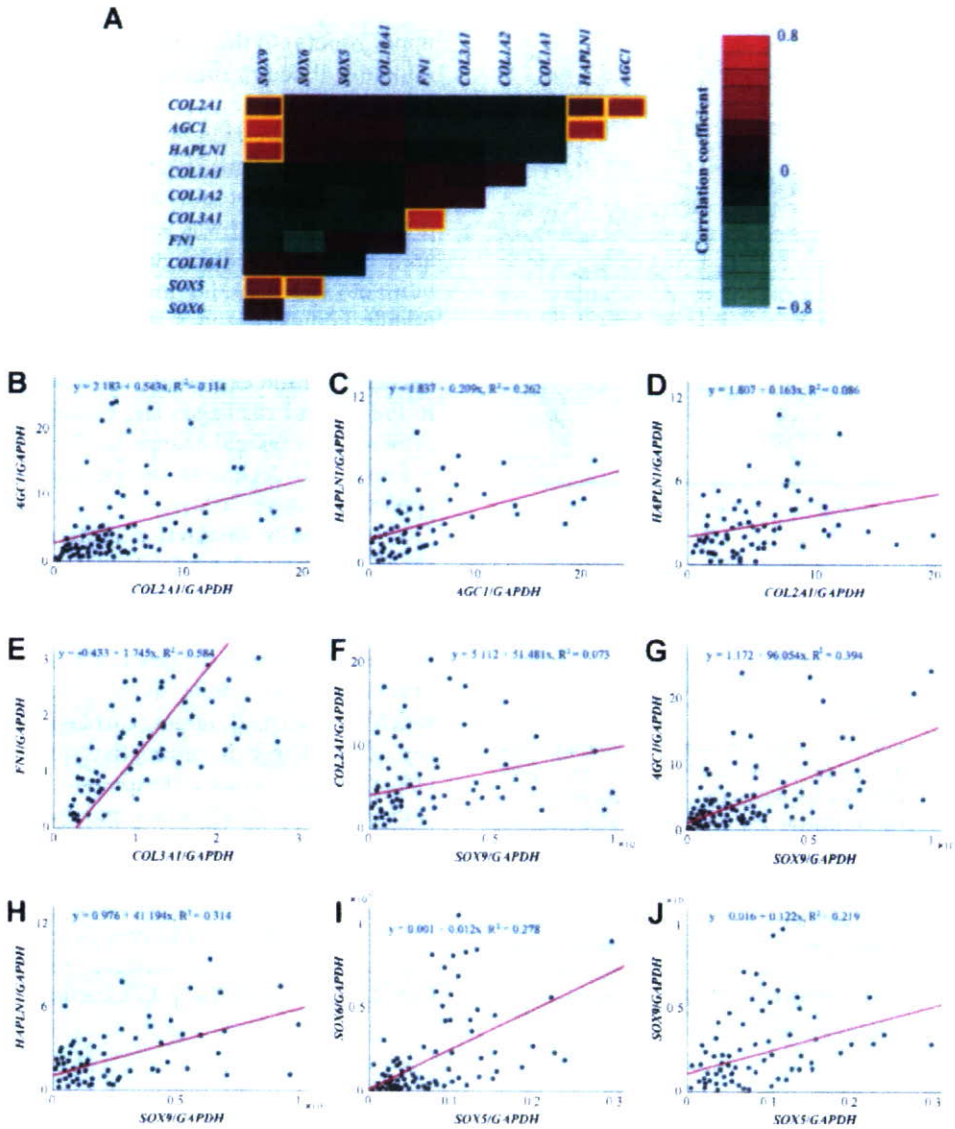

Figure 6. Correlation of gene expression in osteoarthritic (OA) cartilage. Expression of cartilage matrix genes, minor cartilaginous genes induced by the disease, and 3 cartilage-related *SOX* genes was determined at various sites of OA cartilage, and a correlation of expression was investigated among the genes. **A,** Correlation coefficients among the genes are shown by a heat map. Red and green colors indicate positive and negative correlations, respectively. Yellow square frames indicate significant correlations of expression. **B–J,** Correlation of gene expression is shown by scattergrams. Significant correlations were found between *COL2A1* and *AGC1* (**B**), *AGC1* and *HAPLN1* (**C**), *COL2A1* and *HAPLN1* (**D**), *COL3A1* and *FN1* (E), *SOX9* and *COL2A1* (F), *SOX9* and *AGC1* (G), *SOX9* and *HAPLN1* (H), *SOX5* and *SOX6* (I), and *SOX5* and *SOX9* (J), with the strongest correlation between *COL3A1* and *FN1*.

B). However, their induction levels varied markedly among samples, and practically no induction was observed in approximately half of the samples. Within the samples with detectable expression, these genes were expressed in the superficial zones in less degenerated areas and in the middle and deep zones in severely degenerated areas. Interestingly, although these genes showed similar patterns of expression within OA cartilage, their expression levels often differed considerably. The loss of coordinated expression was apparent when

the expression ratio of *COL1A2* to *COL1A1* was compared between OA cartilage and other normal tissues containing type I collagen as a major component (Figure 3C). While the expression ratio of *COL1A2* to *COL1A1* was between 0.7 and 1.7 in the bone, ligament, or meniscus tissues obtained from nonarthritic joints, the ratio in OA cartilage ranged widely from 0.2 to 44. The poor coordination in expression suggests that the expression of type I collagen genes could be induced by an aberrant mechanism(s) in OA cartilage.

In contrast to type I collagen, the induction of type III collagen messenger RNA (mRNA) was consistently observed in OA samples. Within OA cartilage, the expression of type III collagen was most intense in the upper region of degenerated cartilage (Figure 3D). The expression of another gene, fibronectin, was consistently induced in OA cartilage. The regional change of fibronectin expression was very similar to that of type III collagen expression (Figure 3E).

Unlike type I or type III collagen, the induction of type X collagen was observed primarily in the deep zone (Figure 3F). The induction was weaker than that of type I or type III collagen as judged by the ratios of expression to that of *GAPDH*, and the level of induction was considerably different among OA samples; the expression was virtually absent in approximately half of the samples. Interestingly, the expression of type X collagen was more obvious in the less degenerated areas than in the more degenerated areas where the superficial zone was lost to the disease.

Consistent with previous reports, the expression of exon 2 of the *COL2A1* gene was obviously increased in OA cartilage when evaluated by the ratio of its expression to that of *GAPDH* (Figure 3G). However, the expression of exon 2 relative to total *COL2A1* expression was rather reduced in OA cartilage (Figure 3H). Thus, it was assumed that the appearance of type IIA procollagen might not be the result of a phenotypic change in the chondrocytes as previously speculated (11,12), but is more likely to be associated with the up-regulation of type II collagen expression.

Chondrocytes at the upper part of degenerated cartilage undergo a phenotypic change. Next, we compared gene expression between preserved areas and degenerated areas in the respective cartilage zones of the respective OA joints. In the superficial zone, the expression was compared in each sample between the preserved and degenerated areas (i.e., between the 2 regions in the superficial zone without and with macroscopic degeneration). In the middle and deep zones, the comparison was performed in each sample between the preserved areas and the degenerated areas where the zones were directly exposed to the joint cavity.

The result clearly indicated that a shift occurred in the pattern of gene expression at the upper region of degenerated cartilage (Figure 4). In the degenerated areas in the middle and deep zones, the expression of cartilage matrix genes (type II collagen and aggrecan) was suppressed, while the expression of minor cartilaginous genes (type III collagen and fibronectin) was enhanced. In the superficial zone, the expression of minor cartilaginous genes was induced similarly in the degenerated areas, although the suppression of cartilage matrix gene expression was not apparent. In spite of considerable differences in expression levels among the samples, the shift of gene expression was consistently observed in almost all OA samples. Thus, the chondrocytes are considered to undergo a phenotypic change at the upper region of degenerated cartilage, no matter in which cartilage zone the cells reside.

Expression of *SOX* genes in OA and control cartilage. During chondrogenic differentiation, the expression of cartilage matrix genes is regulated by the transcriptional factors *SOX5*, *SOX6*, and *SOX9* (24). In order to estimate the involvement of these molecules in the change of chondrocyte metabolism in OA, their expression was investigated (Figure 5). In OA cartilage, the expression of *SOX* genes tended to be reduced in the degenerated areas, particularly in the upper region of the degenerated cartilage. The reduction was most obvious with *SOX6*, followed by *SOX9*, and was least apparent with *SOX5*. In the preserved areas, the expression of *SOX5* and *SOX6* tended to be increased above control levels, although this trend was not observed with *SOX9*. These regional changes of *SOX* expression within OA cartilage suggested that the altered *SOX* gene expression might be related to the change in matrix gene expression in OA.

Correlation of gene expression in OA cartilage. In an attempt to understand the mechanism(s) underlying the altered gene expression in OA cartilage, a possible correlation of gene expression was investigated (Figure 6A). The expression of 3 cartilage matrix genes correlated significantly. The expression of type II collagen was significantly correlated with that of aggrecan ($r = 0.110$, $P = 0.0081$) (Figure 6B), and a stronger correlation was observed between aggrecan and link protein ($r = 0.512$, $P < 0.0001$) (Figure 6C). A significant correlation was also observed between type II collagen and link protein ($r = 0.294$, $P < 0.0001$) (Figure 6D), implying that the expression of these genes might be modulated by a common factor(s) in OA cartilage.

In contrast, no significant correlation was found between the expression of cartilage matrix genes and minor cartilaginous genes induced by the disease in any combination (from $P = 0.102$ to $P = 0.991$) (Figure 6A).

Among the 5 minor cartilaginous genes evaluated, a significant correlation was observed only between type III collagen and fibronectin ($r = 0.764, P < 0.0001$) (Figure 6E). Therefore, the expression of minor cartilaginous genes was assumed to occur without any association in OA cartilage, except for that of type III collagen and fibronectin. Interestingly, the correlation between type III collagen and fibronectin was stronger than any other relationship observed in this study, suggesting the presence of certain link(s) in their expression. In fact, we have obtained data indicating that the expression of type III collagen in human OA cartilage could be induced, at least partly, through the activation of $\alpha 5\beta 1$ integrin by fibronectin (Fukui N: unpublished observation).

Next, a possible correlation of expression was investigated between the *SOX* genes and the 3 cartilage matrix genes. Although no significant correlation was found between *SOX5* or *SOX6* and the matrix genes (from $P = 0.072$ to $P = 0.857$) (Figure 6A), the expression of all 3 matrix genes was significantly correlated with that of *SOX9* (Figures 6F–H). The correlation was strongest with aggrecan ($r = 0.627$, $P < 0.0001$), followed by link protein ($r = 0.560, P < 0.0001$), and was weakest with type II collagen ($r = 0.270, P = 0.013$). The expression of *SOX* genes was not correlated with that of the minor cartilaginous genes in any combination (from $P = 0.436$ to $P = 0.959$) (Figure 6A). Meanwhile, the expression of *SOX* genes was mutually correlated. Significant correlations were observed between *SOX5* and *SOX6* ($r = 0.527, P < 0.0001$) (Figure 6I) and between *SOX5* and *SOX9* ($r = 0.468$, $P = 0.001$) (Figure 6J), although the correlation between *SOX6* and *SOX9* was not significant ($P = 0.728$).

DISCUSSION

The result of this study has provided a comprehensive view of the change in metabolic activity of the chondrocytes in OA. The profile of gene expression differed considerably with the site, depending on the cartilage zone and the extent of cartilage degeneration. In the macroscopically intact areas of OA cartilage, the expression of cartilage matrix genes was markedly enhanced, particularly in the middle and deep zones. This observation was consistent with the results of previous studies using in situ hybridization (3,4,6,9), in which the enhanced matrix synthesis was considered to be a reparative response that attempts to reconstitute the impaired cartilage matrix (2–4). Meanwhile, the up-regulation of cartilage matrix genes was less obvious in the degenerated areas, particularly in the upper regions. Instead, at those regions, the expression of type III collagen and fibronectin was most enhanced. The shift in gene expression was apparent when the profile of gene expression was compared between preserved and degenerated areas in each OA joint (Figure 4).

This shift in gene expression could be significantly involved in the progression of the disease. First, in OA, cartilage matrix is lost primarily from the surface of degenerated cartilage (25), and that loss of matrix could be accelerated by the reduced cartilage matrix synthesis in the surface region (4,9). Second, matrix loss may be facilitated by the induction of type III collagen synthesis. Although this collagen could be a minor component of normal articular cartilage (26–28), it may diminish the quality of cartilage matrix when expressed in excess through the inhibition of proper matrix organization (28,29). Third, fibronectin is known to cause an intense catabolic response in chondrocytes and synoviocytes when cleaved into fragments (30). Therefore, the induction of this protein at the site of enhanced catabolism may be even more significant in the progression of the disease. Taking these findings together, the shift in matrix gene expression at the upper region of degenerated cartilage could be a critical event in OA pathology. Since the shift of gene expression was observed in virtually all OA samples, the regulation of cellular metabolism at that site may be an effective strategy in the future to delay or inhibit disease progression.

Compared with type III collagen and fibronectin, the expression of the other minor cartilaginous genes was less pronounced in OA cartilage in terms of areas, intensities, and frequencies. The induction of type I collagen mRNA was highly variable among OA samples, and, even when expressed, *COL1A1* and *COL1A2* mRNA were often induced at different intensities. The expression of type I collagen in human OA cartilage has remained controversial in previous studies. Although our result of *COL1A1* expression was consistent with several reports (4,6,9), it was discordant with another report regarding the area of expression (31). Further, while we observed the expression of *COL1A2* in human OA cartilage, it was not detected in an earlier study (9). The revealed discrepancy between *COL1A1* and *COL1A2* expression may account for these contradictions in the literature. Likewise, there has been a controversy regarding the induction of *COL10A1* ex-