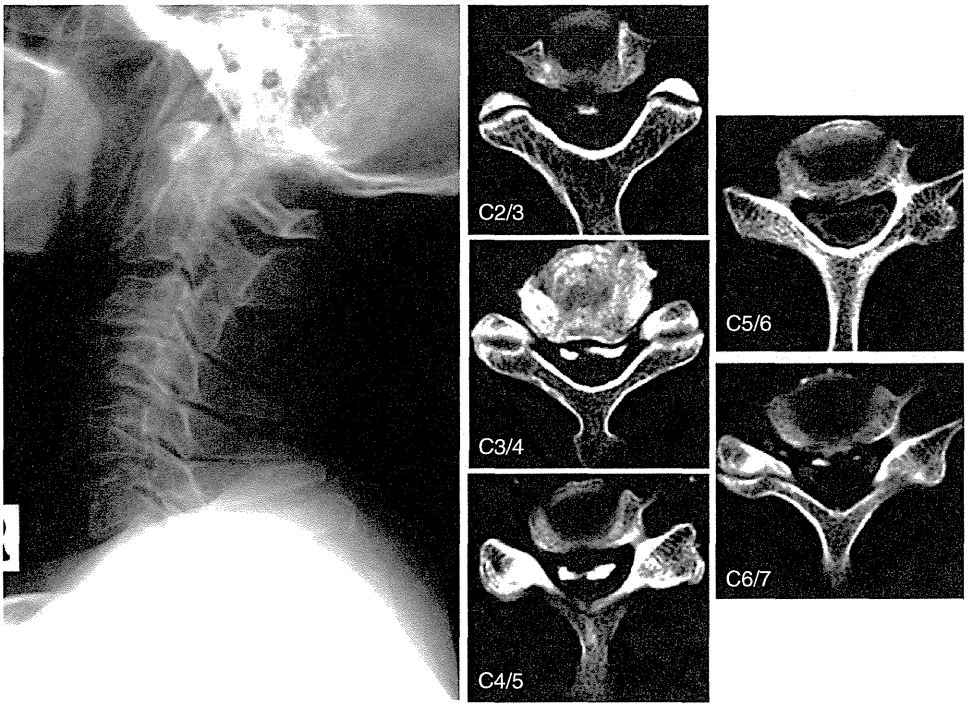

Figure 1. Case 1: A 62-year-old male with C2–C7 ossification of posterior longitudinal ligament. Preoperative radiograph and axial view of computed tomographic myelogram. Severe stenosis was particularly apparent in C3–C4 and C4–C5.

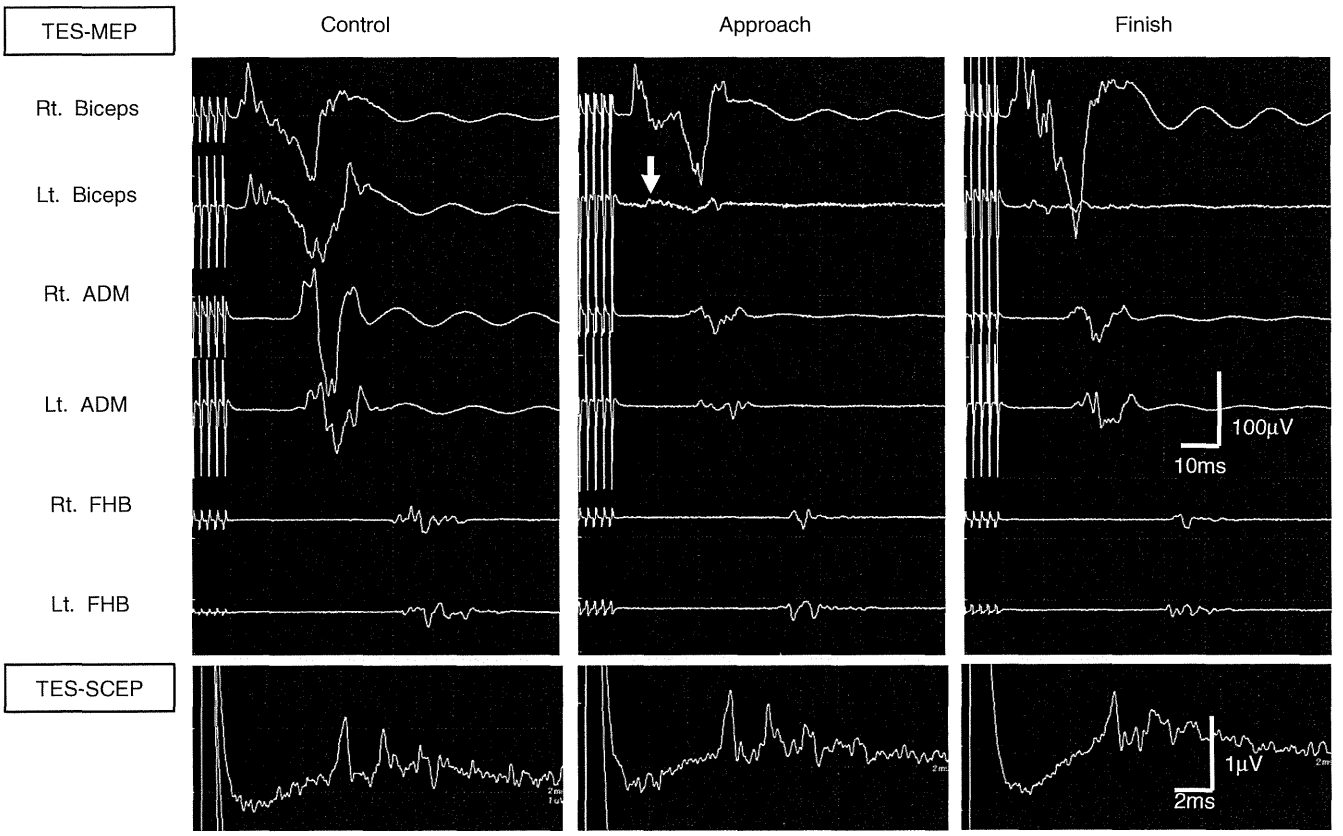

Figure 2. Case 1: TCE-MEP amplitude of the left biceps decreased to 4.5% during exposure as compared with before skin incision (arrow). We did not alert the surgeon for the reasons that wave amplitude, albeit small, did not disappear, the change occurred before decompression, and there was no change in TCE-SCEP. TCE-MEP indicates transcranial electrical motor-evoked potential; ADM, abductor digiti minimi; FHB, flexor hallucis brevis; TCE-SCEP, transcranial electrical simulated spinal cord-evoked potential.

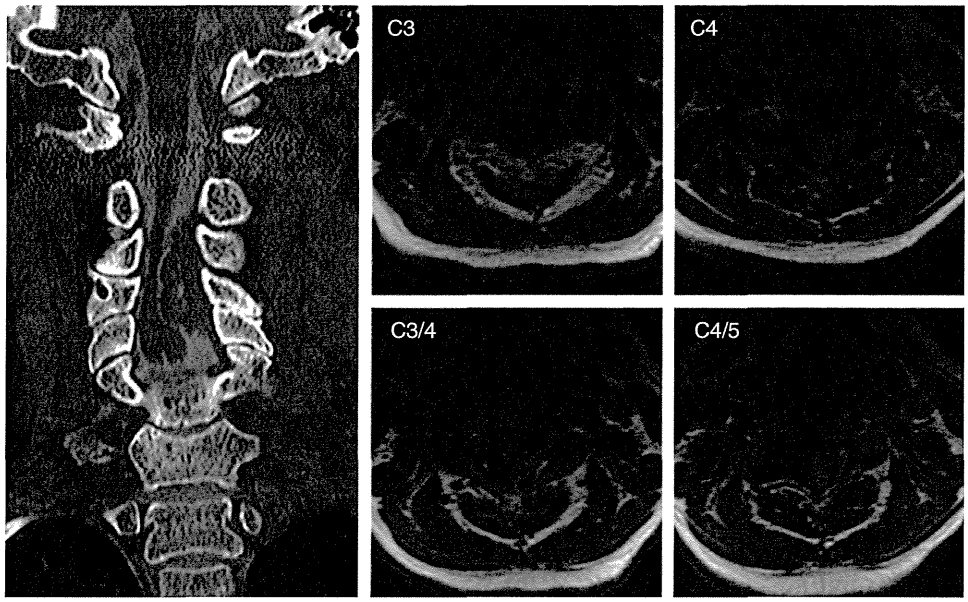

Figure 3. Case 2: A 66-year-old woman with C2–C5 meningioma. Preoperative coronal view of computed tomographic myelogram and axial view of magnetic resonance image.

and spinal segments are shown in Table 2. Disappearance of the TCE-MEP waveform of the spinal tract was seen in 43 cases (12.2%) for which we alerted the surgeon in 14 cases; there were no postoperative motor deficits considered to be caused by spinal tract injury in any case. Disappearance of the TCE-MEP waveform of the spinal segments was seen in 11 cases (3.1%), and we issued a warning in 3 of these cases; there were no postoperative motor deficits in any of the

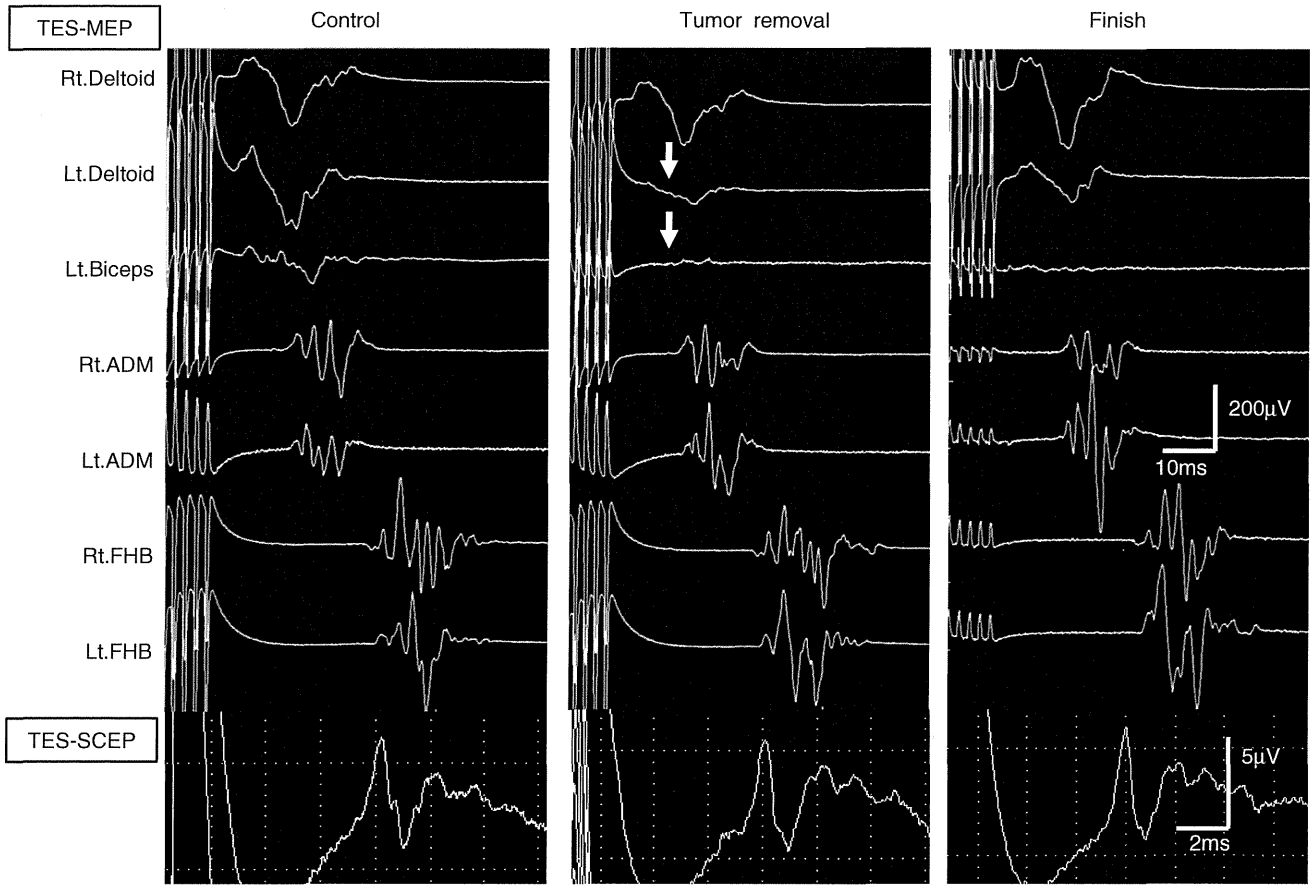

Figure 4. Case 2: When the tumor was resected around the C3–C4 spinal cord from the left side, TCE-MEP amplitude of the ipsilateral deltoid and biceps decreased to 27% and 21%, respectively, as compared with before resection (arrows). We monitored the situation without warning the surgeon because the TCE-MEP waveform did not disappear and there was no change in TCE-SCEP. TCE-MEP indicates transcranial electrical motor-evoked potential; ADM, abductor digiti minimi; FHB, flexor hallucis brevis; TCE-SCEP, transcranial electrical simulated spinal cord-evoked potential.

11 cases. However, as mentioned earlier, 2 cases with no TCE-MEP waveform disappearance showed postoperative motor deficits considered to be the result of spinal segment injury.

DISCUSSION

Intraoperative monitoring of spinal function is becoming an essential technique in spinal surgery as any incorrect operative procedure, no matter how small, may cause serious neurological deficits. Although the technique is susceptible to anesthetics and muscle relaxants, in recent years train stimulation with total intravenous anesthesia has enabled stable measurement of TCE-MEP.[1,2,9,13,19–26] In addition, some studies have proven TCE-MEP monitoring to be more sensitive than several other monitoring techniques for spinal injury.[7,9,27,28] However, the warning threshold for TCE-MEP monitoring is not yet established, and clinically used thresholds differ among institutions.

Research studies have attempted to establish a warning threshold using parameters such as the stimulation threshold value or changes in waveform morphology.[29–37] Calancie *et al*[29–31] suggested the former parameter, but the method is technically complicated because evaluation of the TCE-MEP derivation threshold is necessary at every measurement point. In addition, high-stimulation intensity might be needed to produce an electromyogram from a group of muscles affected by pre- or intraoperative neurological disorder, and the resulting body movement may disturb the surgery. The method using the latter parameter was suggested in 2005 by Quiñones-Hinojosa *et al*.[32] The use of waveform morphology has been investigated only for intramedullary tumors thus far, and further study is required to determine its applicability to compression myelopathy. Some authors have reported using TCE-MEP amplitude as the parameter,[33–37] but the threshold amplitude value has yet to be determined and the waveform changes have not been examined in relation to their origin in the spinal tract and spinal segments.

In this study, in the 2 cases that showed postoperative motor deficits, amplitude decreased intraoperatively to 4.5% or 27% of the control amplitude. Therefore, if we had established the warning threshold uniformly as 30% of the control amplitude, we would likely have prevented postoperative motor deficits in both cases. However, if we had established the warning threshold uniformly at 30% and not divided amplitude changes according to their origin (*i.e.*, spinal tract or spinal segment), 106 (30.3%) of cases would have become positive cases. This is an impractical increase in false-positive cases. As both the cases of postoperative muscle weakness in this study are thought to have resulted from intraoperative segmental injury, we hypothesized that we could reduce the number of false-positive cases by separating the TCE-MEP warning threshold for spinal segment injury from that for spinal tract injury.

One of the important issues in investigating the warning threshold for intraoperative monitoring is that just watching and then checking for postoperative muscle weakness cannot be done from an ethical standpoint in cases when the intraoperative wave change meets the warning criterion. In most past reports, the cases in which an intraoperative warning was given were usually classified as true positive irrespective of whether they involved postoperative motor deficits.[5,6,10,34,35,37] However, in those cases, the waveforms might have recovered spontaneously and postoperative motor deficits might not have occurred even without the warning. We excluded such cases in this study because we do not know of any way to classify true-positive or false-positive cases. In this study, 15 cases were given warnings and no postoperative motor deficits were apparent in any of these cases, and so we excluded them and then established experimentally the warning thresholds for the spinal tract and the spinal segments in 10% brackets from 0% to 50%, the results of which are shown in Tables 3 and 4. We think that establishing the warning threshold for the spinal tract as waveform disappearance enables spinal tract injury to be avoided because no cases of postoperative motor deficits caused by spinal tract injury occurred in our study population. On the basis of this supposition, the 29 cases with intraoperative wave disappearance considered to be caused by spinal tract injury would be classified as false-positive cases for a specificity of 91.3% (Table 3). Moreover, to have prevented spinal segment injury in this study, we would have needed to establish a warning threshold for the spinal segments higher than the 27% seen in case 2. If we had established the warning threshold at 30%, the sensitivity and specificity would have been 100% and 90.4%, respectively

TABLE 3. Warning Threshold for Amplitude Changes Originating in the Spinal Tract

Warning Threshold (%)	True-Positive Cases (Motor Deficit of Tract +, Alarm +)	False-Positive Cases (Motor Deficit of Tract −, Alarm +)	True-Negative Cases (Motor Deficit of Tract −, Alarm −)	False-Negative Cases (Motor Deficit of Tract +, Alarm −)	Sensitivity	Specificity (%)
50	0	113	222	0	...	66.3
40	0	92	243	0	...	72.5
30	0	72	263	0	...	78.5
20	0	57	278	0	...	83.0
10	0	39	296	0	...	88.4
Disappearance	0	29	306	0	...	91.3

TABLE 4. Warning Threshold for Amplitude Changes Originating in the Spinal Segments

Warning Threshold (%)	True-Positive Cases (Motor Deficit of Tract +, Alarm +)	False-Positive Cases (Motor Deficit of Tract −, Alarm +)	True-Negative Cases (Motor Deficit of Tract −, Alarm −)	False-Negative Cases (Motor Deficit of Tract +, Alarm −)	Sensitivity (%)	Specificity (%)
50	2	56	277	0	100	83.2
40	2	49	284	0	100	85.3
30	2	32	301	0	100	90.4
20	1	17	316	1	50	94.9
10	1	10	323	1	50	97.0
Disappearance	0	6	327	2	0	98.2

(Table 4), which we consider suitable for intraoperative spinal monitoring. The results when we apply the "sole warning threshold" or the "warning threshold on the basis of origin" to this series are shown in Table 5: if we had established the warning thresholds as wave disappearance for spinal tract injury and 30% of the control amplitude for spinal segment injury, the sensitivity, specificity, and likelihood rate would have been 100%, 83.7%, and 6.17, respectively.

TCE-MEP is thought to be the compound muscle action potential generated by the firing of anterior horn cells excited by descending conductive spinal cord potentials.[1,9,11–13] Therefore, if the descending potential does not reach the excitation threshold of the anterior horn cells, the waves may disappear, whether the function of the spinal tract is preserved or not. However, changes in TCE-MEP waves of the spinal segments might reflect the extent of surgical invasion more substantially than those of the spinal tract because the changes involve invasion of the distal part rather than the synaptic junction at the anterior horn cells. In addition, because of the multiple innervation of most muscles, TCE-MEP changes caused by surgical invasion on a single spinal segment might be concealed by other unaffected innervation and appear milder than the actual extent of invasion.[22,38] Thus, we hold that a stricter warning threshold is necessary for TCE-MEP changes originating in spinal segments than for those originating in the spinal tract.

Although we used electromyograms of the upper limbs to monitor the spinal segments when the spinal level innervating them was included within the decompressed levels, it is unclear whether the observed changes actually reflected invasion of the spinal segments. When multiple spinal levels were decompressed, the amplitude change might, in reality, have reflected invasion of the spinal tract at a higher spinal level. Because it is difficult in a practical sense to determine whether the changes originate in the tract or segments, we emphasize that the stricter warning threshold for segments should be adopted.

A decrease in TCE-MEP amplitude before exposure was seen in 1 of the false-positive cases in this study. Because no changes were seen on intraoperative monitoring during decompression, the cervical spinal cord might have been compressed by hyperextension of the cervical spine for surgical positioning. In the case of a severely compressed spinal cord particularly, any change in intraoperative monitoring should be viewed more seriously, even if occurring before exposure of the spinal cord. Furthermore, significant change in TCE-MEP amplitude originating from the decompressed spinal segment should be regarded as a warning, even without any changes

TABLE 5. Results of Adopting the "Sole Warning Threshold" and the "Warning Threshold on the Basis of Origin" to This Series

Warning Thresholds	True Positive	False Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)	False-Positive Rate (%)	False-Negative Rate (%)	Likelihood Rate (%)
Sole warning threshold									
Disappearance of waveform	0	33	300	2	0	90.1	9.9	100	0
30%	2	89	244	0	100	73.3	26.7	0	3.75
Warning threshold depending on the cause									
Spinal tract origin: disappearance of waveform									
Spinal segments origin: 30%	2	54	279	0	100	83.7	16.2	0	6.17

in TCE-MEP amplitude originating from other segments and also even when monitoring recordings from a single muscle.

Many previous studies have been conducted on the warning threshold for TCE-MEP monitoring, but few of these studies involved such a large, nonselective, consecutive patient series as this study. Furthermore, the amplitude changes have never been examined on the basis of anatomical origin. Given the findings of this study, we recommend that the warning threshold for amplitude changes be split into those originating from the spinal tract and spinal segments. Specifically, the warning threshold for spinal segment injury should be 30% of the control TCE-MEP amplitude when the spinal level innervating it is within the spinal level exposed to surgical invasion; for the spinal tract, the warning threshold should be the disappearance of the TCE-MEP waveform when the innervating spinal level is lower than the level exposed to surgical invasion. We think that such measures can reduce false-positive cases and enable cervical surgeries to be performed safely and more smoothly.

This research has some limitations. Further study is needed to determine the warning threshold for sensory disturbance because somatosensory-evoked potential was not used during our surgeries. Our series seems to include more cases showing intraoperative TCE-MEP changes compared with past reports. This may be due to intrapatient variability in the quantity of anesthetics used in this study, and a muscle relaxant was used in some of the earlier cases. Furthermore, all of our patients had compressive cervical myelopathy, which is known to show frequent intraoperative TCE-MEP changes.[34,37] We used only a constant stimulation of 200 mA, which was the maximum power output of our stimulator. Use of higher intensity stimulation might have produced different results. It is difficult to decide a specific value for the warning threshold on the basis of only 2 false-negative cases. We think it more important to distinguish between a warning threshold related to spinal tract injury and that related to spinal segment injury at this point in time, and determining a clear numerical value for the warning threshold warrants further study. Our patient series did not include those with intramedullary tumor, so further research is required on the warning threshold in surgeries for intramedullary tumors that cannot avoid direct invasion of the spinal cord.

➢ Key Points

- ❑ Two of 357 cases presented with postoperative motor deficit when we established disappearance of the TCE-MEP waveform as the sole warning threshold.
- ❑ If we had established waveform disappearance as the warning threshold for the spinal tract and 30% of the control amplitude for spinal segments, we should have experienced no postoperative motor deficits and could have reduced false-positive cases (sensitivity: 100%, specificity: 83.7%).
- ❑ The warning threshold on the basis of origin should make cervical surgery smoother and safer.

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CERVICAL SPINE

Five-year Follow-up Evaluation of Surgical Treatment for Cervical Myelopathy Caused by Ossification of the Posterior Longitudinal Ligament

A Prospective Comparative Study of Anterior Decompression and Fusion With Floating Method Versus *Laminoplasty*

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Study Design. Prospective, comparative clinical study.

Objective. To compare the clinical outcome of anterior decompression and fusion with floating method and laminoplasty in the treatment of cervical myelopathy caused by ossification of the posterior longitudinal ligament (OPLL).

Summary of Background Data. There have been no reports that have accurately and prospectively compared surgical outcomes after anterior decompression and posterior decompression.

Methods. For cervical myelopathy caused by OPLL, we performed anterior decompression and fusion with floating method (ADF) in 1997, 1999, 2001, 2003, and 2004 and French-door laminoplasty (LAMP) in 1996, 1998, 2000, and 2002 at one institution. Twenty patients in the ADF group and 22 patients in the LAMP group were evaluated for 5 years' follow-up. The following criteria were evaluated: operation time, blood loss, complications, and Japanese Orthopedic Association score. For radiographic evaluation, canal narrowing ratio of OPLL, lordotic angle at C2–C7, and postoperative progression of the ossified lesion were measured.

Results. The operation time in the ADF group was longer than that in the LAMP group. The average blood loss showed no statistical difference between the 2 groups. Complications occurred in 5 cases in the ADF group, but none occurred in the LAMP group. The mean Japanese Orthopedic Association score system for cervical myelopathy and the recovery rate in the ADF group were superior to those in the LAMP group, especially for cases with greater than 50% of the spinal canal compromised by OPLL or kyphotic alignment of the cervical spine, preoperatively. Postoperative progression of OPLL was observed in 5% of the ADF group and 50% of the LAMP group.

Conclusion. ADF is considered especially suitable for cases with massive OPLL and preoperative kyphotic alignment of the cervical spine, although it leads to a higher incidence of surgery-related complications compared with LAMP.

Key words: anterior decompression and fusion, anterior floating method, laminoplasty, prospective study, ossification of the posterior longitudinal ligament **Spine 2012;37:367–376**

Since it was first reported in 1960,[1] ossification of the posterior longitudinal ligament (OPLL) has been recognized as a common cause of cervical myelopathy, especially in Japan. As OPLL of the cervical spine develops, the cervical cord is compressed from the anterior aspect, resulting in myelopathy. Although the origins and pathophysiological mechanisms of OPLL are not entirely understood,[2–4] several surgical options have been established to address the neurological sequelae.[5]

An anterior decompression and fusion method with floating method (ADF), especially the technique of anterior corpectomy and floating of the ossification of the OPLL introduced by Yamaura,[6] can provide an immediate decompression effect on the spinal cord. Posterior decompression is achieved by shifting the spinal cord posteriorly.[7–11] Extensive cervical laminectomy and laminoplasty are used to decompress the neural elements posteriorly when there is extensive involvement of the cervical spine.

There have been many retrospective studies comparing the surgical outcomes of anterior and posterior decompression.[12–20] However, there is no report that has prospectively compared the surgical outcomes of anterior decompression and posterior decompression. Therefore, we performed a prospective study comparing ADF and laminoplasty (LAMP) for cervical myelopathy caused by OPLL with a 5-year follow-up by 1 surgical group at 1 institution.

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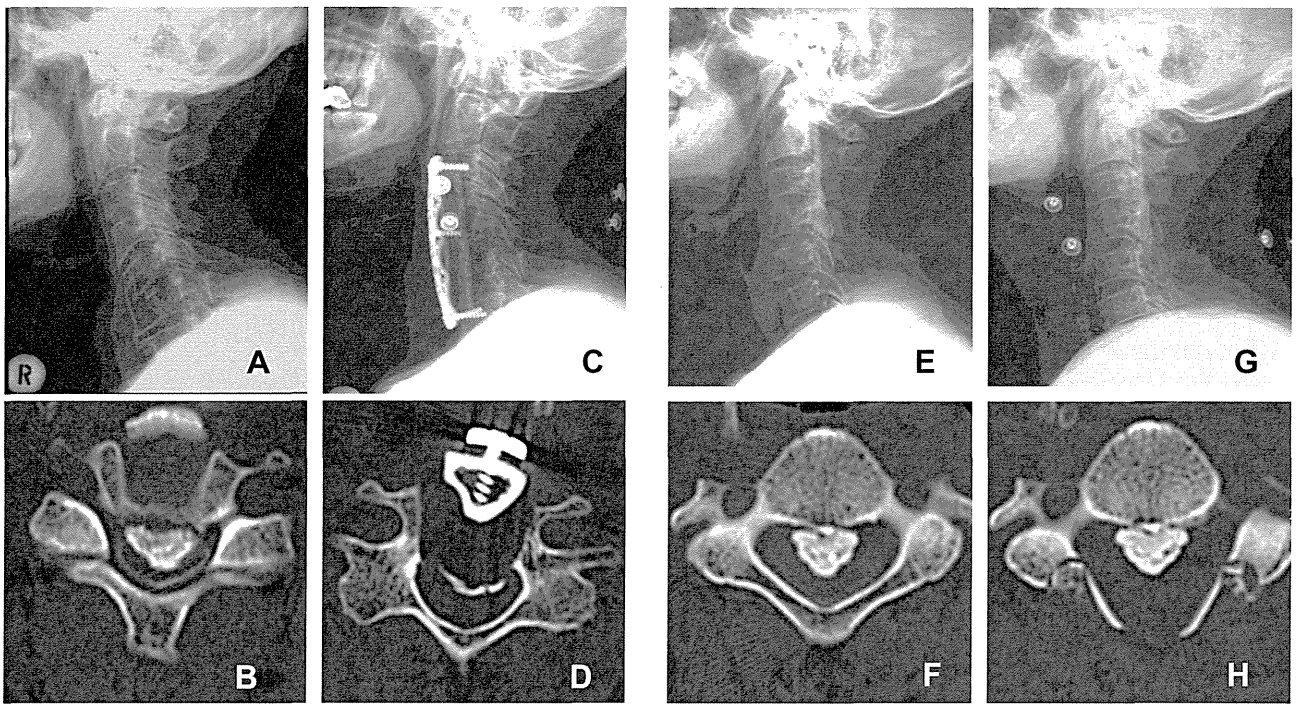

Figure 1. Preoperative and postoperative plain radiographs and computed tomographic images after myelography. Preoperative plain radiograph (**A**), computed tomographic image after myelography at the C4/C5 level (**B**), and plain radiograph (**C**) and computed tomographic image at the C4/C5 level (**D**) 2 weeks after a C3–C7 anterior decompression and fusion with floating method. Preoperative plain radiograph (**E**), plain computed tomographic image at the C4 level (**F**), and plain radiograph (**G**) and plain computed tomographic image at the C4 level (**H**) 2 weeks after a C3–C7 French-door laminoplasty.

MATERIALS AND METHODS

Materials

This study was a prospective, comparative, single-institution trial of 2 surgical procedures for the treatment of cervical myelopathy caused by OPLL. Consecutive patients treated for cervical myelopathy caused by OPLL at our hospital between 1996 and 2004 were included. Patients with myelopathy caused by cervical disc herniation or spondylosis, patients with a history of previous cervical spine surgery or injury, and patients who had OPLL that extended to the C1/C2 level and compressed the cervical cord were excluded.

Choice of Surgical Procedure

After informed consent was obtained from 51 patients, the patients were divided into 2 groups on the basis of the year of treatment. A total of 22 patients were enrolled in the ADF group in 1997, 1999, 2001, 2003, and 2004, and 29 patients were enrolled in the LAMP group in 1996, 1998, 2000, and 2002.

Operative Procedures

The level in the spinal cord where the injury occurred was diagnosed by spinal cord–evoked potentials recorded from an epidural electrode and radiographic findings before surgery. All surgeries were performed while monitoring spinal cord–evoked potentials. In the ADF group, ADF, as described by Yamaura,[6] was performed (Figure 1A–D). After corpectomy was performed by removing discs and vertebral bodies, the OPLL was cut very thin and allowed to float away from the dural sac without removal. The cervical spine was reconstructed using an autologous bone graft from the ilium or fibula, fixed internally by a plate and screw system, ambulated after 2 postoperative days, and fixed externally using a collar for 2 to 3 months. The decompression and fusion levels were based on the findings of preoperative spinal cord–evoked potentials and radiographic studies. The average decompression and fusion level was 3.1 intervertebral discs (range: 1–5). In the LAMP group, expansive French-door laminoplasty, as described by Miyazaki and Kirita,[8] was performed (Figure 1E–H). The paravertebral muscles were detached from the spinous processes on both sides and the processes at C3–C6 were removed. The laminae at C3–C6 were split at the midline, and bilateral gutters were made by using a high-speed air-burr drill. The bilateral laminae were kept open by anchor sutures to the capsule of the facet joint. Superior lamina at C7 was fenestrated. Small bone chips made from the spinous processes were inserted into the bilateral gutters. For patients who had OPLL extending to the C2/C3 level, inferior lamina at C2 was fenestrated. The patients were ambulated after 2 postoperative days and fixed externally using a collar for 2 to 4 weeks. The average decompression level was 4.5 intervertebral discs (range: 4–5).

Outcome Measures

The operative procedures were evaluated for the time of operation, blood loss, and perioperative complications. Neurological recovery was evaluated using the Japanese

TABLE 1. Japanese Orthopedic Association Score System for Cervical Myelopathy
I Upper extremity motor function
0: Unable to feed oneself with any tableware including chopsticks, spoon, or fork, and/or unable to fasten buttons of any size
1: Can manage to feed oneself with spoon and/or fork but not chopsticks
2: Either chopsticks feeding or writing is possible but not practical, and/or large buttons can be fastened
3: Either chopsticks feeding or writing is clumsy but practical, and/or cuff buttons can be fastened
4: Normal
II Lower extremity motor function
0: Unable to stand up and walk by any means
0.5: Able to stand up but unable to walk
1: Unable to walk without a cane or other support on level ground
1.5: Able to walk without support but with a clumsy gait
2: Walks independently on level ground but needs support on stairs
2.5: Walks independently when going upstairs but needs support when going downstairs
3: Capable of walking fast but clumsily
4: Normal
III Sensory function
A. Upper extremity
0: Complete loss of touch and pain sensation
0.5: 50% or below of normal sensation and/or severe pain or numbness
1: Over 60% of normal sensation and/or moderate pain or numbness
1.5: Subjective numbness of a slight degree without any objective sensory deficit
2: Normal
B. Lower extremity
Same as A
C. Trunk
Same as A
IV Bladder function
0: Urinary retention and/or incontinence
1: Sensory of retention, dribbling, thin stream, and/or incomplete continence
2: Urinary retardation and/or pollakiuria
3: Normal
Total score for normal = (I + II + III + IV) = 17
Recovery rate = (postoperative score – preoperative score) × 100 / (17 – preoperative score)

Orthopedic Association score system for cervical myelopathy (C-JOA score; Table 1) and the recovery rate of the Japanese Orthopaedic Association Cervical Myelopathy (C-JOA) score, which is calculated using Hirabayashi's method.[7] Radiological evaluations included measuring the canal narrowing ratio (CNR) of the OPLL (Figure 2), the lordotic angle at C2–C7, and the postoperative progression of the OPLL lesion in the lateral view of a plain radiograph.

Statistics

Statistical analyses were performed using the Student *t* test for continuous variables, and the Mann-Whitney *U* test or the χ^2

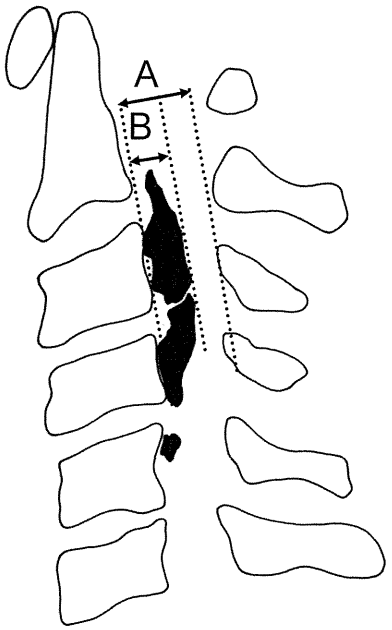

Figure 2. Canal narrowing ratio. (**A**) Proper anteroposterior diameter of the spinal canal. (**B**) Thickness of the ossification at the level of the greatest canal narrowing. The canal narrowing ratio is defined as **B** divided by **A**.

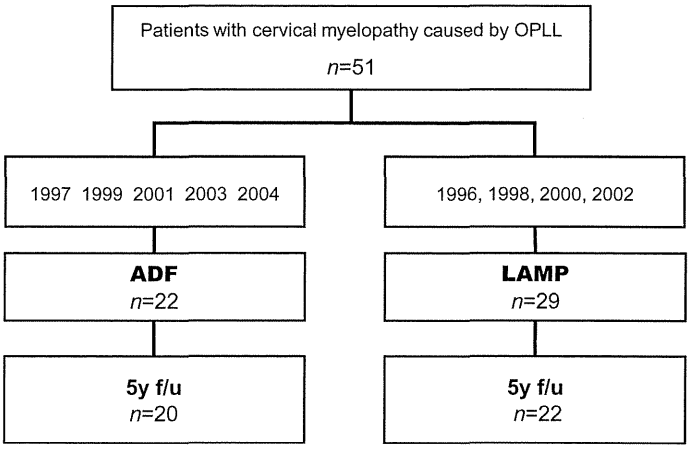

Figure 3. Allocation of the study for cervical myelopathy caused by ossification of the posterior longitudinal ligament (OPLL). Of the 51 patients, 42 completed the 5-year follow-up in this series. The remaining 9 patients could not be followed, and data are missing for these patients. There were 20 patients in the anterior decompression and fusion with floating method group and 22 patients in the laminoplasty group.

test for discrete variables. Significance was set at $P < 0.01$ or $P < 0.05$.

RESULTS

Of the 51 patients, 42 completed the 5-year follow-up in this series (follow-up rate, 82.4%). Data for every annual follow-up were available for these 42 patients. The remaining 9 patients could not be followed, and data are missing for these patients. The patients included in the study presented with continuous (n = 9), segmental (n = 12), or mixed (n = 21) OPLL types. There were 33 males and 9 females. The average age at the time of surgery was 59.5 years (range: 39–80 years). There were 20 patients in the ADF group and 22 patients in the LAMP group (Figure 3).

The preoperative surveys are shown in Table 2. The age, CNR, and C-JOA score showed no statistically significant differences between the 2 groups.

The operative surveys between the 2 groups are shown in Table 2. The average time of operation was 300.3 minutes in the ADF group and 183.2 minutes in the LAMP group. The time of operation in the ADF group was significantly longer than that in the LAMP group ($P < 0.01$). The average blood loss was 292.8 g in the ADF group and 289.6 g in the LAMP group. The blood loss showed no statistical difference between the 2 groups. Complications that occurred in the ADF included 2 cases of dislocation of the bone graft, 2 cases of delayed union, and 1 case of dyspnea by hematoma; however, there were no complications in the LAMP group. We performed salvage operations for 3 cases (2 cases of dislocation of bone graft and 1 case of dyspnea by hematoma) out of the 5 cases with complications in the ADF group. Neurological deterioration early after the operation, such as spinal cord injury or C5 nerve root palsy, did not occur in either group.

The postoperative changes in the mean C-JOA score and the recovery rate of the C-JOA score between the 2 groups are shown in Table 2 and Figure 4. The mean recovery rate of C-JOA score at the 5-year follow-up time point was 71.4% in the ADF group and 55.3% in the LAMP group. The C-JOA score and the recovery rate of the C-JOA score in the ADF group were superior to those in the LAMP group after the 4-year time point ($P < 0.05$).

To investigate the relationship between the CNR and neurological recovery, we divided the patients into 2 subgroups on the basis of the CNR of the OPLL and then compared the C-JOA score in the ADF group with that in the LAMP group for each subgroup. The preoperative C-JOA score showed no statistical difference between the ADF and the LAMP groups in both subgroups. The postoperative change in the recovery rate of C-JOA score between the 2 subgroups is shown in Table 3 and Figure 5. In those patients with a CNR less than 50%, the mean recovery rate of C-JOA score at the 5-year time point was 70.2% in the ADF group and 64.0% in the LAMP group. In those patients with a CNR less than 50%, the C-JOA score showed no statistical difference between the ADF and LAMP groups. In contrast, in those patients with a CNR equal to 50% or more, the mean recovery rate of C-JOA score at the 5-year time point was 72.9% in the ADF group and 41.2% in the LAMP group. In those patients with a CNR equal to 50% or more, the recovery rate of C-JOA score in the ADF group was superior to that in the LAMP group after the 4-year time point ($P < 0.05$).

Next, to investigate the relationship between the preoperative cervical alignment and neurological recovery, we divided these patients into 2 subgroups on the basis of the C2–C7 lordotic angle of the preoperative cervical spine: greater than 5° (prelordosis subgroup), or 5° or less (prekyphosis subgroup). We then compared the C-JOA score in the ADF group with

TABLE 2. Preoperative Surveys and Clinical Outcomes in ADF and LAMP Group

	ADF Group	LAMP Group	*P*
Number of cases	20	22	
Age (year), mean ± SD	59.5 ± 9.3 (42–80)	58.4 ± 9.6 (39–79)	ND
CNR (%), mean ± SD	43.4 ± 16.6 (17–71)	46.9 ±16.1 (23–79)	ND
Preoperative C-JOA score (points), mean ± SD	11.4 ± 2.8 (6.5–16)	10.9 ± 2.3 (6–14)	ND
Time of operation (min), mean ± SD	300.3 ± 78.6*	183.2 ± 41.1	<0.01
Blood loss (g), mean ± SD	292.8 ± 192.8	289.6 ± 215.8	ND
Complications (cases)	Dislocation of bone graft (2)		
	Delayed union (2)		
	Dyspnea by hematoma (1)		
C-JOA score (points)/ recovery rate (%)			
1 year, mean ± SD	14.1 ± 2.9 / 54.0 ± 30.4	14.8 ± 1.4 / 62.6 ± 24.6	ND
2 years, mean ± SD	14.7 ± 2.6 / 64.8 ± 26.0	14.8 ± 1.2 / 62.2 ± 21.1	ND
3 years, mean ± SD	14.8 ± 2.1 / 62.7 ± 27.0	14.3 ± 1.5 / 54.1 ± 25.7	ND
4 years, mean ± SD	15.0 ± 2.3 / 68.0 ± 30.3**	13.8 ± 2.1 / 48.7 ± 21.7	<0.05
5 years, mean ± SD	15.1 ± 2.2 / 71.4 ± 26.0**	14.0 ± 2.6 / 55.3 ± 29.6	<0.05

*$P < 0.01$ versus *LAMP group by the Student t test.*

**$P < 0.05$ versus *LAMP group by the Mann-Whitney U test.*

ADF indicates anterior decompression and fusion with floating method; C-JOA, Japanese Orthopedic Association for cervical myelopathy; CNR, canal narrowing ratio of OPLL; LAMP, laminoplasty; ND, not significant difference.

that in the LAMP group for each subgroup. The preoperative C-JOA score showed no statistical difference between the ADF and LAMP groups in both subgroups. The postoperative change in the recovery rate of C-JOA score between the 2 subgroups is shown in Table 3 and Figure 6. In the prelordosis subgroup, the mean recovery rate of C-JOA score at the 5-year follow-up point was 67.2% in the ADF group and 61.8% in the LAMP group. In the prelordosis subgroup, the C-JOA score showed no statistical difference between the ADF and LAMP groups. In contrast, in the prekyphosis subgroup, the mean recovery rate of C-JOA score at the 5-year follow-up point was 76.6% in the ADF group and 42.4% in the LAMP group. In the prekyphosis subgroup, the recovery rate of C-JOA score in the ADF group was superior to that in the LAMP group after the 4-year follow-up point ($P < 0.05$).

The average C2–C7 lordotic angle of the cervical spine was increased from 11.7° preoperatively to 16.4° at the 5-year follow-up point in the ADF group, but it was decreased from 14.3° preoperatively to 8.7° at the 5-year follow-up point in the LAMP group (Table 4). The lordotic angle of the cervical spine in the ADF group was larger than those in the LAMP group after surgery ($P < 0.05$) and was maintained for 5 years.

A postoperative kyphotic change of the cervical spine was defined as a decrease in the C2–C7 lordotic angle greater than 5° at the 5-year follow-up point compared with that at the preoperative period. This was not observed in the ADF group, but it was observed in 11 cases (50.0%) in the LAMP group (Table 4).

A postoperative progression of the ossified OPLL lesion was defined as more than half of 1 vertebral body axially or more than 2 mm in thickness at the 5-year follow-up point compared with measurements taken just after the operation in the lateral view of a plain radiograph. The postoperative progression of the ossified OPLL lesion at the 5-year follow-up point was observed in 1 case (5.0%) in the ADF group, but in 11 cases (50.0%) in the LAMP group (Table 4).

Immediately after surgery, none of our patients deteriorated in either group. During the 5-year follow-up period, however, neurological deterioration, defined as a decrease in the C-JOA score greater than 1 point at follow-up compared with the patient's maximum score, was not observed in the ADF group compared with deterioration in 5 cases in the LAMP group. Of these 5 cases, neurological deteriorated factors were postoperative progression of the OPLL in 2 cases, postoperative instability or kyphotic change in 2 cases, and unknown in 1 case (Table 5).

DISCUSSION

There have been many retrospective studies comparing the surgical outcomes of anterior and posterior decompression surgery. Some reports have concluded that neurological recovery after anterior decompression was superior to that after posterior decompression.[15,16] In contrast, other reports either have not shown any significant difference in the surgical outcomes between anterior and posterior decompression or suggested that posterior decompression was superior.[17,18] However, there has been no study that has prospectively compared the surgical outcomes after anterior or posterior decompression. Therefore, we performed a prospective study comparing ADF and LAMP for the treatment of cervical myelopathy caused by OPLL by 1 surgical group at 1 institution. In our study, the overall recovery rate in the C-JOA score in the ADF group was superior to that in the LAMP group after the 4-year follow-up point.

For patients with CNR 50% to 60% or more, several reports said that neurological recovery after laminoplasty was

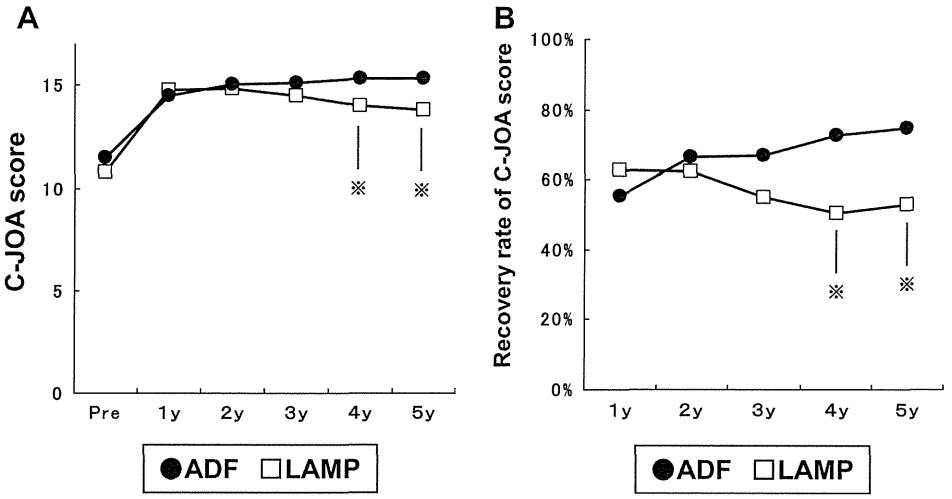

Figure 4. Postoperative changes in the Japanese Orthopaedic Association cervical myelopathy (C-JOA) score (**A**) and the recovery rate of the C-JOA score (**B**) between the 2 groups. ※: $P < 0.05$.

poor and that ADF yielded a better outcome.[13,14,21,22] Similarly, in our study, the C-JOA score in the ADF group was superior to that in the LAMP group in the cases with a CNR equal to 50% or more, but the C-JOA score showed no statistical difference between the ADF and LAMP groups in the cases with a CNR less than 50%. The posterior method can safely

TABLE 3. Comparisons With ADF and LAMP Group in Subgroups

	CNR Less Than 50% Subgroup			CNR 50% or More Subgroup		
	ADF Group (n = 11), Mean ± SD	LAMP Group (n = 13), Mean ± SD	*P*	ADF Group (n = 9), Mean ± SD	LAMP Group (n = 9), Mean ± SD	*P*
Preoperative C-JOA score (points)	11.9 ± 2.2	10.5 ± 2.2	ND	10.9 ± 3.3	11.4 ± 2.3	ND
Recovery rate (%)						
1 year	66.4 ± 31.6	68.1 ± 17.0	ND	42.1 ± 19.8	54.3 ± 32.6	ND
2 years	71.1 ± 28.1	73.7 ± 10.5	ND	53.8 ± 26.5	44.8 ± 21.5	ND
3 years	71.2 ± 27.6	61.3 ± 25.6	ND	56.3 ± 24.7	43.3 ± 23.4	ND
4 years	74.6 ± 30.6	54.9 ± 21.8	ND	63.5 ± 29.4*	38.2 ± 18.6	<0.05
5 years	70.2 ± 25.5	64.0 ± 30.7	ND	72.9 ± 28.1*	41.2 ± 22.8	<0.05
	Prelordosis Subgroup			Prekyphosis Subgroup		
	ADF Group (n = 11), Mean ± SD	LAMP Group (n = 15), Mean ± SD	*P*	ADF Group (n = 9), Mean ± SD	LAMP Group (n = 7), Mean ± SD	*P*
Preoperative C-JOA score (points)	10.7 ± 3.2	11.4 ± 2.3	ND	12.3 ± 2.0	9.9 ± 2.1	ND
Recovery rate (%)						
1 year	45.6 ± 34.6	66.7 ± 23.2	ND	56.8 ± 29.1	54.9 ± 27.1	ND
2 years	51.4 ± 32.9	62.7 ± 23.3	ND	73.5 ± 17.4	61.2 ± 17.9	ND
3 years	56.8 ± 33.5	55.5 ± 28.4	ND	70.8 ± 16.3	51.4 ± 21.6	ND
4 years	59.2 ± 36.2	52.5 ± 20.5	ND	78.9 ± 18.3*	42.4 ± 24.0	<0.05
5 years	67.2 ± 30.8	61.8 ± 25.1	ND	76.6 ± 19.3*	42.4 ± 35.6	<0.05

**P < 0.05 versus LAMP group by the Mann-Whitney U test.*

†Prelordosis was defined as C2–C7 lordotic angle of the preoperative cervical spine more than 5°.

‡Prekyphosis was defined as C2–C7 lordotic angle of the preoperative cervical spine 5° or less.

C-JOA indicates Japanese Orthopedic Association for cervical myelopathy; CNR, canal narrowing ratio of OPLL; ND, not significant difference.

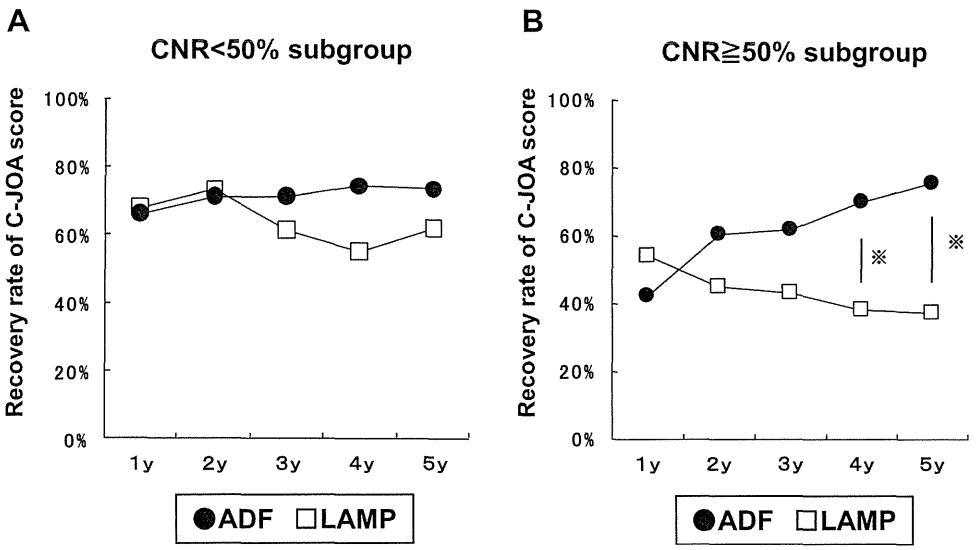

Figure 5. Postoperative changes in the recovery rate of the Japanese Orthopaedic Association cervical myelopathy (C-JOA) score in the canal narrowing ratio (CNR) less than 50% subgroup (**A**) and in the CNR 50% or more subgroup (**B**) in the anterior decompression and fusion with floating method and laminoplasty groups. ※: $P < 0.05$.

achieve the decompression of the spinal cord that results from extensive OPLL, but there is a limitation. In particular, it does not always produce the expected space for a locally protruded OPLL, which causes a highly narrowed spinal canal. For patients with a more severely compromised spinal canal, especially CNR greater than 50%, our data suggest that ADF is the better choice.

Regarding the influence of the preoperative cervical alignment, Suda *et al*[23] reported that local kyphosis reduces the outcome of expansive open-door laminoplasty for cervical spondylotic myelopathy and that anterior decompression or posterior correction of kyphosis as well as laminoplasty should be considered when patients have local kyphosis exceeding 13°. In our study, the C-JOA score in the ADF group was superior to that in the LAMP group with kyphotic alignment of the preoperative cervical spine, and the C2–C7 lordotic angle of the cervical spine was increased in the ADF group after operation and maintained for 5 years. ADF is a surgical procedure that could improve the cervical alignment; therefore, for cervical myelopathy caused by OPLL with kyphotic alignment of the cervical spine, ADF should be considered.

Postoperative kyphotic change after posterior decompression has been detected. Kato *et al*[24] reported that 47% of patients after laminectomy demonstrated a change in cervical alignment during 10 years. Iwasaki *et al*[25] reported that postoperative progression of kyphotic deformity was observed in 8% of patients after laminoplasty during 10 years. In addition, late neurological deterioration occurred because of progressive kyphosis during long-term follow-up of laminoplasty.[22,26] In our study, the average angle of cervical lordosis decreased after LAMP, and postoperative kyphotic changes were observed in 50% of the LAMP and caused late-term neurological deterioration in 1 case.

Postoperative progression of the ossified OPLL lesion after surgery has been reported. Iwasaki *et al*[25] reported that postoperative progression of the ossified lesion after laminoplasty was observed in 70% of the patients across a 10-year follow-up, but only 3% of the patients were found to have related neurological deterioration. Matsuoka *et al*[27] reported that a

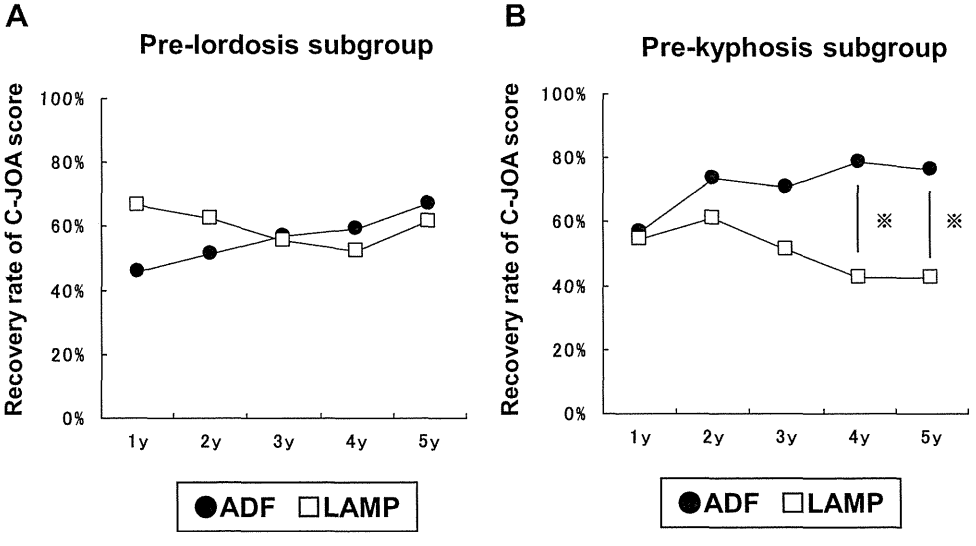

Figure 6. Postoperative changes in the recovery rate of the Japanese Orthopedic Association score in the C2–C7 lordotic angle more than 5° subgroup (prelordosis subgroup) (**A**) and in the 5° or less subgroup (prekyphosis subgroup) (**B**) in the anterior decompression and fusion with floating method and laminoplasty groups. ※: $P < 0.05$.

TABLE 4. Radiological Evaluations in ADF and LAMP Group

		ADF Group	LAMP Group	*P*
C2–C7 angle (°), mean ± SD	Preoperative	11.7 ± 11.1	14.3 ± 10.0	ND
	2 years	16.1 ± 8.4*	9.2 ± 9.2	<0.05
	5 years	16.4 ± 7.5*	8.7 ± 11.3	<0.05
Postoperative kyphotic change (cases [ratio])		0 (0%)**	11 (50.0%)	<0.01
Postoperative progression of OPLL (cases [ratio])		1 (5.0%)**	11 (50.0%)	<0.01

*$P < 0.05$ versus *LAMP group by Mann-Whitney U test.*

**$P < 0.01$ versus *LAMP group by* χ^2 *test.*

†*Postoperative kyphotic change was defined as a decrease in the C2–C7 lordotic angle greater than 5° at the 5-year follow-up point compared with that in the preoperative period.*

‡*Postoperative progression of OPLL was defined as more than half of 1 vertebral body axially or more than 2 mm in thickness at the 5-year follow-up point compared with that at just after operation.*

JOA indicates Japanese Orthopedic Association; ND, not significant difference; OPLL, ossification of the posterior longitudinal ligament.

marked postoperative progression of the ossified lesion after ADF was noted in 16.7% of the patients across a 10-year follow-up period. Tomita *et al*[28] reported that postoperative progression of OPLL ossification after posterior surgery occurred more frequently than after anterior surgery. In our study, postoperative progression of the ossified OPLL at the 5-year follow-up period was observed in 5.0% of the ADF group and in 50.0% of the LAMP group. As in other reports, postoperative progression of OPLL ossification after posterior surgery occurred more frequently than after anterior surgery, and it caused late neurological deterioration in 2 cases. Postoperative progression of OPLL ossification, including new growth, may be an important factor in the deterioration after posterior surgery.

In our study, within a 3-year follow-up period, neurological recovery did not show a statistical difference between the ADF and LAMP groups. After the 4-year time point, however, we found that the neurological recovery of the ADF group was superior to that of the LAMP group, especially for cases with a massive OPLL and a preoperative kyphotic alignment

TABLE 5. Cases With Late-Term Neurological Deterioration*

Age	Sex	Location, Type of OPLL	CNR, %	Operative Procedure	Prekyphosis†	Postoperative Kyphotic Change‡	Postoperative Progression of OPLL§	Follow-up Time Point of Neurological Deterioration, year	Neurological Deteriorated Factors
71	Male	C3, C4, C5–C6	48	LAMP	–	–	+	4	Unknown
61	Female	C5–C6	27	LAMP	+	–	+	3	New growth of OPLL at C7/T1
56	Male	C2–C5	55	LAMP	–	+	–	2	Instability at C5/C6
63	Male	C4, C5–C6	38	LAMP	–	+	+	4	Instability at C4/C5 and postoperative kyphotic change
58	Male	C3–C4, C5–C6	69	LAMP	+	+	+	3	Postoperative progression of OPLL at C4/C5

**Late-term neurological deterioration was defined as a decrease in the C-JOA score at the follow-up point greater than 1 point compared with their maximum point.*

†*Prekyphosis was defined as C2–C7 lordotic angle of the preoperative cervical spine 5° or less.*

‡*Postoperative kyphotic change was defined as a decrease in the C2–C7 lordotic angle greater than 5° at the 5-year follow-up point compared with that in the preoperative period.*

§*Postoperative progression of OPLL was defined as more than half of 1 vertebral body axially or more than 2 mm in thickness at the 5-year follow-up point compared with that at just after operation.*

CNR indicates canal narrowing ratio of OPLL; LAMP, laminoplasty; OPLL, ossification of the posterior longitudinal ligament.

of the cervical spine. One reason for such a late time difference of clinical outcome is thought to be long-term neurological recovery. In the ADF group, the C-JOA score showed a trend to increase year after year slightly, but not in the LAMP group. To obtain this long-term neurological recovery, it may be important to improve the environment around the cord by stabilization with fixation and improvement of cervical alignment and prevention of the progression of the ossified lesion. Another reason for such a late time difference in clinical outcomes is thought to be late-term neurological deterioration. During the 5-year follow-up period, neurological deterioration was not observed in the ADF group but was evident in the LAMP group. Therefore, our data suggest that ADF is a more favorable approach, at least within the first 5 years in view of late neurological deterioration.

ADF, however, was found to have less satisfactory outcomes with the incidence of nonunion, graft trouble, and other complications, and this technique has a long and difficult learning curve.[29–32] Therefore, posterior decompression is recognized as a comparatively safe procedure. Given that the ADF group had a longer operating time and more complications than the LAMP group, the posterior surgery could safely achieve the decompression of the spinal cord for patients who have preservation of cervical lordosis and a small OPLL. In addition, Houten and Cooper[33] reported case series of laminectomy and lateral mass fusion for CSM and OPLL, resulting in neurological recovery equal to ACDF without serious complications. The systematic review also indicated that laminectomy and fusion demonstrated significant improvement of neurological function without postoperative deformity.[34] Posterior decompression with fusion should be enrolled as another surgical option in a future study.

Both anterior and posterior decompressive techniques have merit and may be considered to be appropriate in certain clinical situations. From the results of this prospective comparative study, ADF is indicated in cases with more significant canal compromise and can be used effectively in patients with reduced cervical lordosis. This technique is, however, associated with the risk of nonunion, graft displacement, and in this series, around a 15% chance of requiring revision surgery.

➢ Key Points

- ❑ We performed a prospective study comparing ADF and LAMP for cervical myelopathy caused by OPLL with a 5-year follow-up by 1 surgical group at 1 institution.
- ❑ ADF had a longer operating time and more complications than LAMP.
- ❑ For cervical myelopathy caused by OPLL with more than 50% of the canal compromised, the neurological recovery after ADF was superior to that after LAMP.
- ❑ For cervical myelopathy caused by OPLL with kyphotic alignment of the preoperative cervical spine, the neurological recovery after ADF was superior to that after LAMP.

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Runx2 Haploinsufficiency Ameliorates the Development of Ossification of the Posterior Longitudinal Ligament

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Abstract

Ossification of the Posterior Longitudinal Ligament (OPLL) is a disease that is characterized by the ectopic calcification of the ligament; however, the pathogenesis of OPLL remains to be investigated. We attempted to identify the *in vivo* role of Runx2, a master regulator of osteoblast differentiation and skeletal mineralization, in the pathogenesis of OPLL. The expression of Runx2 in the ligament was examined using *in situ* hybridization and immunohistochemistry and by monitoring the activity of a *LacZ* gene that was inserted into the *Runx2* gene locus. To investigate the functional role of Runx2, we studied *ENPP1*$^{ttw/ttw}$ mice, a mouse model of OPLL, that were crossed with heterozygous *Runx2* mice to decrease the expression of Runx2, and we performed histological and quantitative radiological analyses using 3D-micro CT. Runx2 was expressed in the ligament of wild-type mice. The induction of Runx2 expression preceded the development of ectopic calcification in the OPLL-like region of the *ENPP1*$^{ttw/ttw}$ mice. Runx2 haploinsufficiency ameliorated the development of ectopic calcification in the *ENPP1*$^{ttw/ttw}$ mice. Collectively, this study demonstrated that Runx2 is expressed in an OPLL-like region, and its elevation is a prerequisite for developing the complete OPLL-like phenotype in a mouse model of OPLL.

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Introduction

Ossification of the Posterior Longitudinal Ligament (OPLL; OMIM 602475) is common in East Asia, with a rate of incidence of 2 to 4% [1,2]. At present, the cause of OPLL remains unclear. Previous reports suggested that OPLL is a multifactorial disease that results from several factors, including a history of trauma, infection, diabetes and HLA antigens [1]. Of note, the predominance of OPLL in a specific ethnic group, such as the Japanese population, suggests that OPLL might arise from hereditary factors [2]. In fact, the incidence of OPLL increases significantly to approximately 30% among family members of second-order relatives of the affected patient and up to 85% in siblings of an affected monozygotic twin [2]. Moreover, there are several reports showing the association of SNPs in several genes and the incidence of OPLL by population-based case-control study. Those include Bone Morphogenetic Protein 4(BMP4) SNPs in Chinese population [3], interleukin 15 receptor alpha(IL15RA) SNPs in Korean patients [4], collagen 6A1(COL6A1) SNPs in Chinese Han population [5] and Transforming Growth Factor-β1 (TGFβ1) SNPs in Japanese patients [6]. However, because multiple genetic and environmental factors are related to the development of OPLL, no causal genetic mutation for the OPLL has been identified [7].

Pathological examinations revealed that the affected lesion in OPLL exhibits characteristics of ectopic bone formation (i.e., the existence of osteoblasts), including a lamellar bone structure that contains well-developed Haversian canals and marrow cavities [8], suggesting that bone formation plays a role in the onset and progression of OPLL.

Runx2 is a master regulator of osteoblastogenesis and, thereby, a regulator of the cells that are responsible for bone formation [9]. Runx2 is essential for the differentiation of osteoblasts from mesenchymal cells, and the forced expression of Runx2 transdifferentiates fibroblasts into osteoblasts. Moreover, *Runx2*−/− mice completely lack osteoblasts, lamellar bone and marrow cavities [10], i.e., the characteristic of affected regions in OPLL, throughout their bodies. However, the pathophysiological role of Runx2 in the development of OPLL has remained unknown.

In this study, we used *ENPP1*$^{ttw/ttw}$ mice [11], a mouse model of OPLL, and Runx2 mutant mice to investigate the role of Runx2 in OPLL. We found that Runx2 is induced prior to the formation of ectopic bone in OPLL and that Runx2 haploinsufficiency ameliorates OPLL-associated ectopic calcification.

Materials and Methods

Animals

Enpp1$^{ttw/ttw}$ mice were obtained from the Central Institute for Experimental Animals. *Runx2*$^{+/-}$ mice have been described previously [10]. We housed all mice under a 12-hr light/dark cycle with *ad libitum* access to standard food and water. We determined the genotypes of the mice by polymerase chain

reaction (PCR). (A list of the PCR primer sequences is available upon request.) All animal experiments were performed with the approval of the Animal Study Committee of Tokyo Medical and Dental University (Permit No. 2011-136) and conformed to all relevant guidelines and laws.

Histological Aalysis

For histological examination, after dissection, the tissue samples were fixed immediately in 4% paraformaldehyde/phosphate-buffered saline, then dehydrated with gradually increasing concentrations of ethanol and embedded in paraffin. After fixation, the tissue samples from the adult mice were decalcified in 20% EDTA for two weeks before being embedded in paraffin. For LacZ staining, the spines from heterozygous *Runx2-* mice were fixed in 0.2% paraformaldehyde at room temperature for 30 minutes, and then stained overnight in X-Gal solution, as previously described [12]. Immunohistochemical staining using antibody against Runx2 was performed using the avidin-biotin-peroxidase complex method with the ABC Rabbit IgG Kit (VECTOR Laboratories), as previously described. The anti-Runx2 antibodies have been described previously [13]. *In situ* hybridization was performed using ^{35}S-labeled riboprobes and the standard protocol, as described previously [13].

Micro-computed Tomography Analysis

We obtained three-dimensional images of the cervical spine using micro-computed tomography (micro-CT, ScanXamte-E090, Comscantecno Co. Ltd., Tokyo, Japan). Each spine was placed in a plastic tube, and images were reconstructed from 750 projections. Ectopic ossification was quantitatively analyzed using bone analysis software (TRI/3D-BON, Ratoc System Engineering Co. Ltd., Tokyo, Japan).

Statistical Analysis

All data are presented as the mean ± s.d. (n = 8 or more). We performed the statistical analyses using the Student's *t*-test. Differences were considered statistically significant when $P<.05$. The results are representative of more than four individual experiments.

Results

As an initial measure of the contribution of Runx2 to ligament development, we analyzed the expression of Runx2 in the prospective ligaments of spine including posterior ligament of the vertebrae at the atlanto-occipital area of mouse embryos using three different experimental techniques. First, we performed an in situ hybridization analysis using Runx2 as a probe. At birth, *Runx2* was expressed in vertebrae and at the edge of the vertebrae, which corresponds to a future ligament (Fig. 1A). Next, we took advantage of the *LacZ* allele that was inserted into the *Runx2* locus [10] by performing LacZ staining of spines isolated from heterozygous Runx2 mice to monitor the expression of Runx2 in developing mouse skeletons at birth. Consistent with our in situ hybridization results, we observed robust expression of Runx2 in both the vertebral body and the adjacent ligament (Fig. 1A). To confirm that Runx2 is expressed in the ligament, we performed immunohistochemistry using an antibody against Runx2 and observed Runx2 protein in the ligament (Fig. 1A). These results demonstrate that Runx2 is expressed in ligament cells.

Next, to address the functional relevance of Runx2 in the development of OPLL, we studied the expression of Runx2 in ectopically calcified lesions, which resemble human OPLL lesions, in $ENPP1^{ttw/ttw}$ mice, a mouse model of OPLL [11]. $ENPP1^{ttw/ttw}$ mice are a useful model of ossification in OPLL, which is caused by a point mutation in the *ENPP1* nucleotide pyrophosphatase gene [11]. ENPP1 regulates soft-tissue calcification and bone mineralization by producing inorganic pyrophosphate, a major inhibitor of calcification [11]. $ENPP1^{ttw/ttw}$ mice did not exhibit any overt abnormalities from birth through four weeks of age. At eight weeks of age, abnormal gait, rigidity of the vertebral column and stiffness of the limb joints developed, as previously reported (Fig. 1B) [11]. A histological examination revealed that an ectopically ossified OPLL-like region developed by eight weeks of age; no calcification was evident at four weeks of age, although proliferation of the ligament cells was noted at that age (Fig. 1C). Because Runx2 is essential for bone formation and mineralization, we tested whether Runx2 expression was observed at four weeks of age in the proliferating cells of the ligament that were subsequently mineralized. In fact, Runx2 expression was clearly observed in the ligament at four weeks (Fig. 1D), the age at which the ligament was not calcified (Fig. 1C). Thus, Runx2 expression precedes the development of an OPLL-like region in the ligament, suggesting that Runx2 may play a role in the development of OPLL.

Next, to address the functional role of Runx2 in OPLL, we tested whether decreasing Runx2 expression affects the development of the OPLL-like region in the $ENPP1^{ttw/ttw}$ mice. Accordingly, we generated $ENPP1^{ttw/ttw}$ mice carrying a single allele of Runx2 ($ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice) by mating $ENPP1^{ttw/ttw}$ mice with heterozygous Runx2 mice. A histological examination revealed that the abnormal proliferation of cells in the posterior longitudinal ligament region was substantially lower in the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice than in the $ENPP1^{ttw/ttw}$ mice (Fig. 2A). Moreover, the ectopically calcified OPLL-like region was significantly smaller in the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice (Fig. 2B). An immunohistochemical analysis confirmed that Runx2 expression was lower in the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice than in the $ENPP1^{ttw/ttw}$ mice (Fig. 2C). To test rigorously whether Runx2 haploinsufficiency affects the disease progression of OPLL, we quantified the ectopically ossified region in the OPLL model using reconstructed 3D images obtained using micro-CT (Fig. 3A). We noted that all of the $ENPP1^{ttw/ttw}$ mice exhibited ectopic ossification of the cruciform ligament at the atlanto-occipital area by eight weeks of age (Fig. 2B). Therefore, we quantified the volume of the calcified cruciform ligament. In fact, the volume of calcified ligament in the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice was less than half of that in the $ENPP1^{ttw/ttw}$ mice (Fig. 3B). In accordance with that observation, Bone Mineral Content (BMC) of calcified ligament in the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice was significantly decreased compared to that in the $ENPP1^{ttw/ttw}$ mice (Fig. 3C). Interestingly, volumetric Bone Mineral Density (vBMD) was not significantly different between the $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice and the $ENPP1^{ttw/ttw}$ mice(Fig. 3D), indicating that only the areasize of ectopic bone formation was decreased in $ENPP1^{ttw/ttw}/Runx2^{+/-}$ mice, while mineral apposition to extracellular matrices per unit volume was not overtly changed. Collectively, these results clearly demonstrate that the removal of one allele of Runx2, which led to a decrease in Runx2 expression, ameliorated the progression of the OPLL-like region that was observed in the $ENPP1^{ttw/ttw}$ mice.

Discussion

In this manuscript, we show that Runx2 is expressed in the prospective ligament in mice. We also demonstrate that Runx2 expression is induced in the ectopically ossified area in $ENPP1^{ttw/ttw}$ mice prior to the appearance of a calcified OPLL-like region. Finally, we demonstrate that decreasing Runx2 expression amelio-

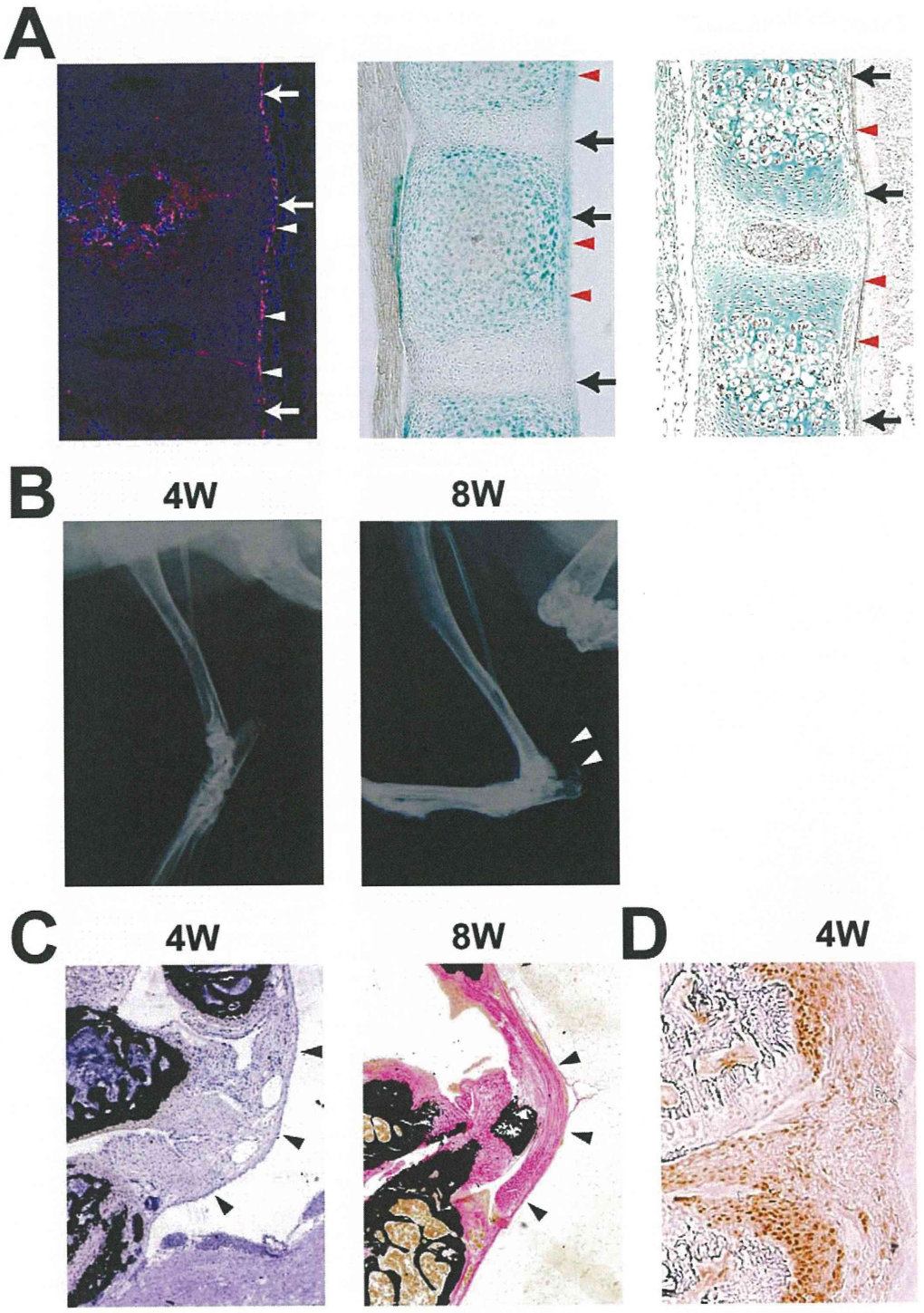

Figure 1. Expression of Runx2 in calcified ligament. A, Runx2 expression (arrowheads) in the posterior longitudinal ligament (arrows). *In situ* hybridization of *Runx2* in wild-type (WT) mouse vertebrae at birth (left). LacZ staining in WT mouse vertebrae at birth (middle). Immunohistochemistry of Runx2 in WT mouse vertebrae at embryonic day 16.5 (right). B, Radiographic assessment of the development of calcification of the ligament in an *Enpp1*$^{ttw/ttw}$ mouse at 4 and 8 weeks of age. Note an appearance of calcification at 8weeks (arrowheads) C, Histological assessment of the cruciform ligament (arrowheads) at the atlanto-occipital area in an *Enpp1*$^{ttw/ttw}$ mouse at 4 and 8 weeks of age. D, Immunohistochemical staining of Runx2 at the posterior longitudinal ligament in an *Enpp1*$^{ttw/ttw}$ mouse at 4 weeks of age. Note that Runx2 was expressed in an area corresponding to the prospective calcification.
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rates the progress of the OPLL-like region. Although OPLL is characterized by ectopic ossification of the posterior longitudinal ligament, the molecular pathogenesis underlying this ossification in vivo was not known [1]. In this study, we demonstrate for the first time that normal Runx2 expression is necessary to achieve the full development of an OPLL-like region in *ENPP1*$^{ttw/ttw}$ mice.

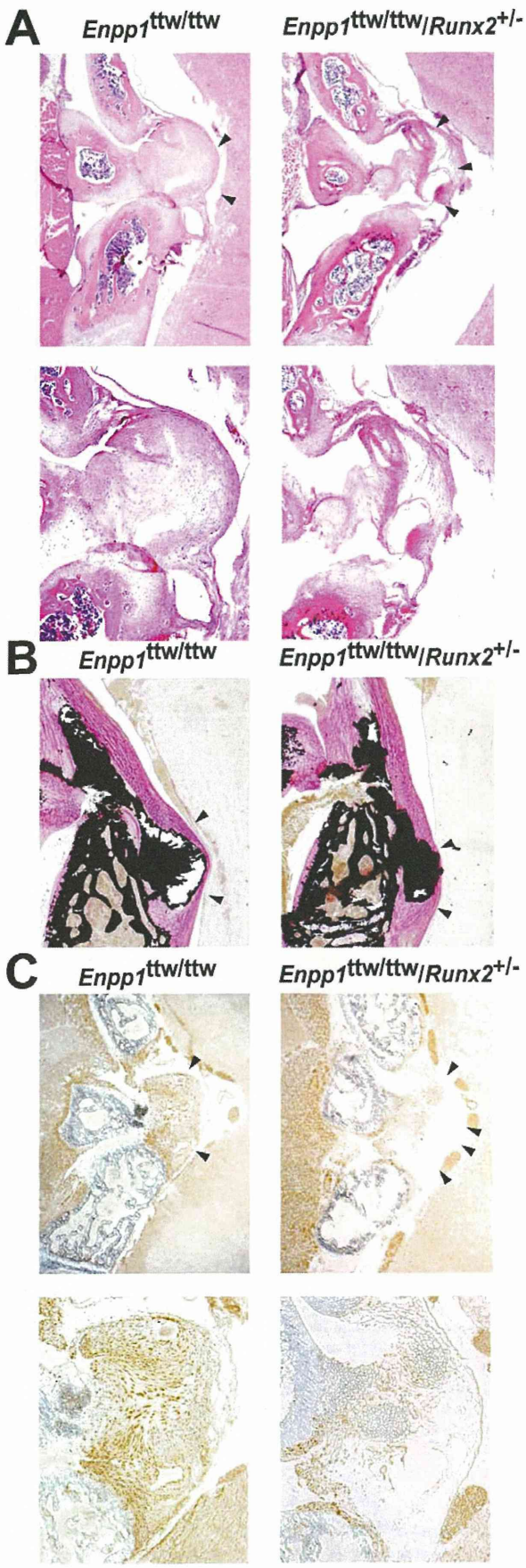

Figure 2. Runx2 haploinsufficiency ameliorates the development of OPLL. A–C, Histological (A and B) and immunohistochemical (C) analyses of the cruciform ligament at the atlanto-occipital area in *Enpp1*$^{ttw/ttw}$ mice at 8 weeks (A and B) or 4 weeks (C) of age with (right) or without (left) Runx2 haploinsufficiency. (A: H&E staining; B: von Kossa staining.) Note a decrease in calcified region (B) and Runx2 immunoreactivity (C) in *ENPP1*$^{ttw/ttw}$/*Runx2*$^{+/-}$ mice. Bottom panels are higher magnification images.(A and C).
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We previously reported that mechanical loading specifically induces Runx2 within the Runx family and that Runx haploinsufficiency ameliorates the intervertebral disc degeneration that is caused by mechanical loading [14]. Interestingly, it is well known that an increase in mechanical loading accelerates the progression of OPLL in human patients [2]. Moreover, previous studies reported that mechanical stress induces Runx2 expression in spinal ligament cells isolated from OPLL patients [15]. Thus, Runx2 induction by mechanical loading may be a common cause of skeletal degeneration, including ectopic ossification and disk degeneration.

We also previously reported that the continuous expression of Runx2 in chondrocytes by the α1(II) collagen promoter-driven *Runx2* transgene led to ectopic bone formation in permanent cartilage (where bone formation is not observed normally) [12]. However, we failed to detect worsening of the OPLL-like region in the *ENPP1*$^{ttw/ttw}$ mice using the α1(II) collagen promoter-driven *Runx2* transgene (Iwasaki and Takeda, unpublished observation). This observation can be explained by the fact that the ectopically ossified area in the *ENPP1*$^{ttw/ttw}$ mice was not caused by endochondral bone formation [12]. However, given that abnormal chondrocyte proliferation occurs in the affected ligaments of human OPLL patients [1], the putative induction of Runx2 in these chondrocytes may accelerate the progression of OPLL in human OPLL patients.

Interestingly, a recent report suggested that various SNPs in the *Runx2* gene may be associated with an elevated incidence of OPLL in the Han population via an unidentified mechanism [16]. A detailed molecular analysis to investigate whether these SNPs affect Runx2 function is needed. It is also demonstrated that Runx2 expression is enhanced in cells isolated from spinal ligaments in OPLL patients compared to non-OPLL patients [17,18], however, it remains unknown if altered Runx2 expression is the cause or the result of ossification of the ligament.

Although Runx2 expression was observed in ligaments of wild-type mice at birth, wild-type mice do not usually develop ectopic calcification, as is observed in *ENPP1*$^{ttw/ttw}$ mice. Thus, it is possible that Runx2 induction is not sufficient for the development of an OPLL-like phenotype. More importantly, molecule(s) that prevent ectopic calcification–notably, ENPP1–should exist in the ligament of wild-type mice, and these factors may be absent in human OPLL patients. Additional studies are needed to identify these molecules.

Currently, the molecular mechanism underlying the induction of Runx2 in the calcification of prospective ligaments is not known. To date, multiple pathways (e.g., Wnt/LRP5/β-catenin and BMP/Smads) and transcription factors (e.g., MSX2, DLX5, and twist) that regulate the expression and function of Runx2 have been identified [9]. Moreover, promyelotic leukemia zinc finger, a transcription factor which is an upstream regulator of Runx2 and promotes osteoblastic differentiation, is highly expressed in cells isolated from OPLL patients [19].

Therefore, it will be interesting to investigate whether the expression of these machineries that regulate Runx2 expression is altered in *ENPP1*$^{ttw/ttw}$ mice and/or human OPLL patients. In