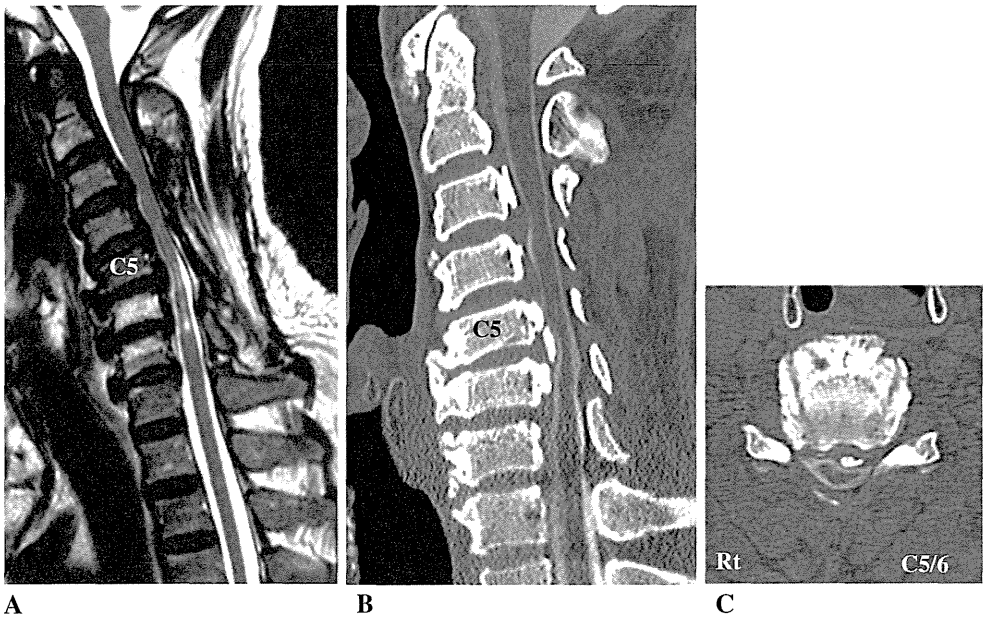

Fig. 1 Case 7. T2-weighted midsagittal MR image (**a**) and a CT midsagittal reconstruction plane (**b**) and CT axial plane at C5–C6 (**c**) showing anterior compression of the spinal cord by ossification of posterior longitudinal ligament (OPLL) at C5–C6

Table 6 Clinical trials using G-CSF injection

Author [ref.]	Sample size	Clinical scenario	G-CSF dose (μg/kg/day)	Route of administration	Duration of G-CSF therapy (days)	Peak WBC count ($\times 10^3$/μl)
Engelmann et al. [2]	23	AMI	10	s.c.	5	42.9 ± 25.7
Ince et al. [5]	15	AMI	10	s.c.	6	55 ± 8
Nefussy et al. [12]	19	ALS	5	s.c.	4	30.0 ± 7.2
Ripa et al. [15]	39	AMI	10	s.c.	6	51 ± 8
Shyu et al. [21]	7	CI	15	s.c.	5	42.9 ± 9.6
Takano et al. [22]	18	AMI	2.5	s.c.	5	29.4 ± 9
Valgimigli et al. [23]	10	AMI	5	s.c.	4	35 ± 11
Zohlnhofer et al. [27]	56	AMI	10	s.c.	5	48 ± 15
Our cases	5	Myelopathy	5	i.v.	5	26.7 ± 10.7
	10	Myelopathy	10	i.v.	5	35.2 ± 7.2

CI cerebral infarction, *AMI* acute myocardial infarction, *ALS* amyotrophic lateral sclerosis, *s.c.* subcutaneous injection, *i.v.* intravenous injection

In the present study, the increase of WBC counts after G-CSF administration was lower than that in other clinical studies using G-CSF [2, 5, 12, 15, 21–23, 26, 27]. One of the reasons for this seems to be that we performed G-CSF therapy for a chronic disease, whereas other studies performed G-CSF therapy for the acute phase of disease. In addition, we suggest that the route of G-CSF administration could contribute to the lower WBC increases in the present study. G-CSF was intravenously administered in our study, while it was subcutaneously administered in other studies [2, 5, 12, 15, 21–23, 26, 27].

In the ten patients enrolled in the second stage of this trial, G-CSF suppressed the progression of myelopathy. In addition, neurological improvements in both motor and sensory functions were obtained in all patients. The study design was open-label, and no control group was instituted. In spite of such limitations, the present results indicate that G-CSF had a neuroprotective effect on worsening symptoms of compression myelopathy.

In this trial, one patient (case 7) did not choose surgery because neurological recovery after the G-CSF administration was evident. In other cases, neurological improvement was also obtained though the degree of improvement differed among individual cases. This result indicated that other cases in addition to case 7 might have been able to avoid surgery. We suggest that by introducing G-CSF neuroprotective therapy, extremely conservative treatment may be possible for patients with worsening symptoms of

compression myelopathy, and surgical treatment can be avoided in some patients.

We had planned a third stage for this clinical trial with G-CSF administration of 15 μg/kg/day for 5 days. Based on the present results, however, we cancelled the third stage because G-CSF therapy at a dose of 10 μg/kg/day caused sufficient neurological improvement. In addition, G-CSF therapy at a dose of 10 μg/kg/day increased WBC counts to 50,000 cells/mm^3 in one patient. Thus, it is possible that G-CSF therapy at a dose of 15 μg/kg/day could cause side effects.

We intend to advance to a phase IIb clinical trial for accurate assessment of the efficacy of G-CSF therapy. Based on the present results, we will use G-CSF at a dose of 10 μg/kg/day for 5 days. The study design will be a multi-center prospective controlled clinical trial, and a control group without G-CSF administration will be incorporated. By undertaking this phase IIb clinical trial, we wish to establish the efficacy of the G-CSF neuroprotective therapy for patients with worsening symptoms of compression myelopathy. To date, there have been no reports of a drug that improves neurological status in patients with worsening symptoms of compression myelopathy. If the efficacy and safety of G-CSF treatment for worsening symptoms of compression myelopathy are established and clinical use of G-CSF neuroprotective therapy is approved, a novel and effective approach for the treatment of this disorder will be available.

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Conflict of interest None.

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Novel surgical technique for ossification of posterior longitudinal ligament in the thoracic spine

Technical note

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Several surgical procedures have been developed to treat thoracic ossification of the posterior longitudinal ligament (OPLL). However, favorable surgical results are not always achieved, and consistent protocols and procedures for surgical treatment of thoracic OPLL have not been established. This technical note describes a novel technique to achieve anterior decompression via a single posterior approach. Three patients with a beak-type thoracic OPLL underwent surgery in which the authors' technique was used. Complete removal of the ossified PLL was achieved in all cases. With the patient in the prone position, the authors performed total resection of the posterior elements at the anterior decompression levels. This maneuver included not only laminectomies but also removal of the transverse processes and pedicles, which allowed space to be created bilaterally at the sides of the dural sac for the subsequent anterior decompression. The thoracic nerves at the levels of anterior decompression were ligated bilaterally and lifted up to manipulate the ossified ligament and the dural sac. An anterior decompression was then performed posteriorly. The PLL was floated without any difficulty. After exfoliation of the adhesions between the ossified ligament and the ventral aspect of the dural sac, the ossified PLL was removed. In every step of the anterior decompression, the space created in the bilateral sides of the dural sac allowed the surgeons to see the OPLL and anterolateral aspect of the dural sac directly and easily. After removal of the ossified PLL, posterior instrumented fusion was performed. This surgical procedure allows the surgeon to perform, safely and effectively, anterior decompression via a posterior approach for thoracic OPLL.
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Key Words • **thoracic spine** • **novel surgical technique** • **ossification of the posterior longitudinal ligament** • **anterior decompression** • **posterior approach**

Ossification of the PLL, which is common in the Asian population, is a well-known cause of cervical myelopathy. It can also appear, although less commonly, in the thoracic spine and cause severe myelopathy.[18] Thoracic myelopathy caused by OPLL in the thoracic spine is usually progressive and responds poorly to conservative therapy, which makes surgery the only effective treatment option. Despite advancement in techniques for surgery of thoracic OPLL, favorable results are not always achieved. In patients with thoracic myelopathy resulting from thoracic OPLL, removal of the ossified PLL is the most effective method of relieving pressure on the spinal cord.[2,4,7,17,19,21] However, the anterior approach for removal of ossified PLL is technically demanding, and postoperative neurological deterioration has been reported.[2,12]

In this technical note, we present a novel technique used to float or remove thoracic ossified PLLs using a posterior approach; we use a single case to illustrate the procedure.

Abbreviations used in this paper: JOA = Japanese Orthopaedic Association; OPLL = ossification of the posterior longitudinal ligament.

Illustrative Case

History. This 63-year-old man was referred to our hospital with a 4-year history of gait disturbance. His symptoms had become gradually progressed in the previous 2 months. Thoracic OPLL had been identified at another hospital, and conservative treatment had been tried without success. His medical history included chronic renal failure requiring dialysis, hypertension, and hepatitis C.

Examination. On admission to our hospital, the patient was suffering from severe thoracic myelopathy. He found walking to be difficult, as muscle strength in the bilateral lower extremities was weakened, so he used a wheelchair. Physical examination showed 20%–50% hyp-

This article contains some figures that are displayed in color online but in black-and-white in the print edition.

algesia below the belly and hyperreflexia at bilateral patellar and Achilles tendons. As a measure of thoracic myelopathy, his preoperative JOA score[9] was 0 (total score of 8 by eliminating the score for bladder function due to his anuric renal failure). Postmyelography CT scanning and MRI revealed an isolated beak-type ossified PLL at the T6–7 level with the spinal cord severely compressed at the same level (Fig. 1). The patient had continuous-type OPLL in the cervical spine; this did not seem to cause his myelopathy. A circumferential decompression and instrumented fusion via a posterior approach was planned for the thoracic OPLL.

Operation. In the prone position, the posterior elements from T-4 to T-9 were exposed via a midline incision. In the first step, total resection of the posterior elements of T-6 and T-7 was performed. This maneuver included not only laminectomies but also removal of the transverse processes and pedicles, which allowed space to be created at the bilateral sides of the dural sac for the subsequent anterior decompression. Thus, removal of the ribs or destruction of costovertebral joints of the rib heads was not necessary. The thoracic nerves at the T-6 and T-7 levels were ligated bilaterally and lifted up to manipulate the ossified ligament and the dural sac. Anterior decompression was performed via the posterior approach. The posterior portions of the vertebral bodies were removed using a diamond bur from the bilateral sides of the dural sac. The surgeons could see the ossified PLL and the anterolateral aspect of the dural sac directly and could use a bur safely in the space created at the bilateral sides of the dural sac (Fig. 2). Using this maneuver, the ossified PLL was floated without any difficulty. After exfoliation of adhesions between ossified ligament and the ventral aspect of the dural sac, the ossified PLL was removed completely (Figs. 2 and 3). In every step of the anterior decompression, the space created at the bilateral sides of the dural sac allowed us to see the ossified PLL and the anterolateral aspect of the dural sac directly and easily. After removal of the ossified PLL, we placed posterior instrumentation at T4–9 and performed posterolateral fusion using local bone chips of the resected laminae and transverse processes (Fig. 2). There was no dural tear or CSF leakage in the procedure.

Postoperative Course. After the surgery, the patient's neurological symptoms were improved. An orthosis was applied for 3 months to achieve posterolateral fusion at T4–9. One month after surgery, the patient could walk using crutches. Postoperative CT myelography showed the complete removal of the ossified PLL and the subarachnoid space in the ventral side of the spinal cord; this indicated that spinal cord decompression had been sufficiently achieved (Fig. 4). At the 1-year follow-up examination, the patient exhibited mild symptoms due to myelopathy such as spastic gait and mild sensory disturbance of the lower extremities. However, he was able to walk without assistance 5 months after the surgery. The JOA score for thoracic myelopathy was 4 and the recovery rate was 50% 1 year after surgery.

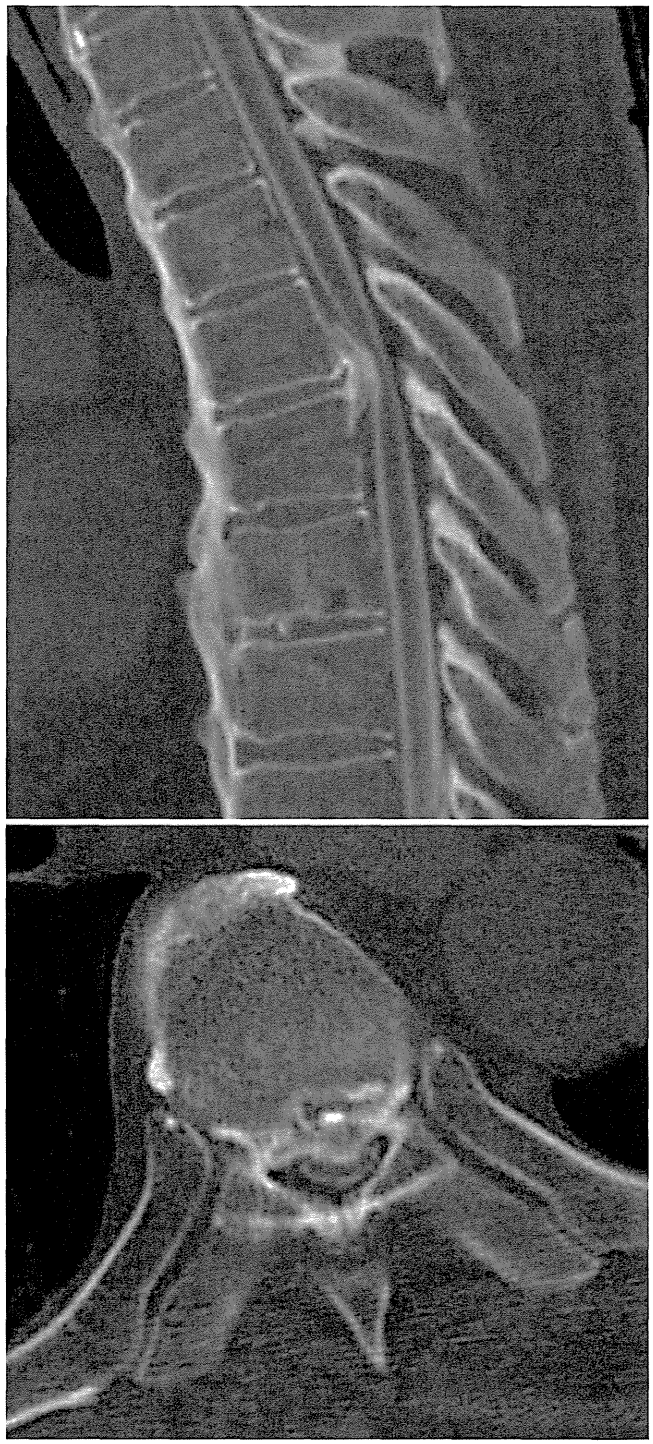

FIG. 1. Preoperative sagittal **(lower)** and axial **(upper)** CT myelograms showing beak-type OPLL at the T6–7 levels.

Results

We used this surgical technique in 3 patients overall who had a beak-type OPLL in the middle thoracic spine. Complete removal of ossified PLL through the anterior decompression levels was achieved in all cases. No intraoperative or postoperative complications were encountered except a minor dural tear in one patient that did not

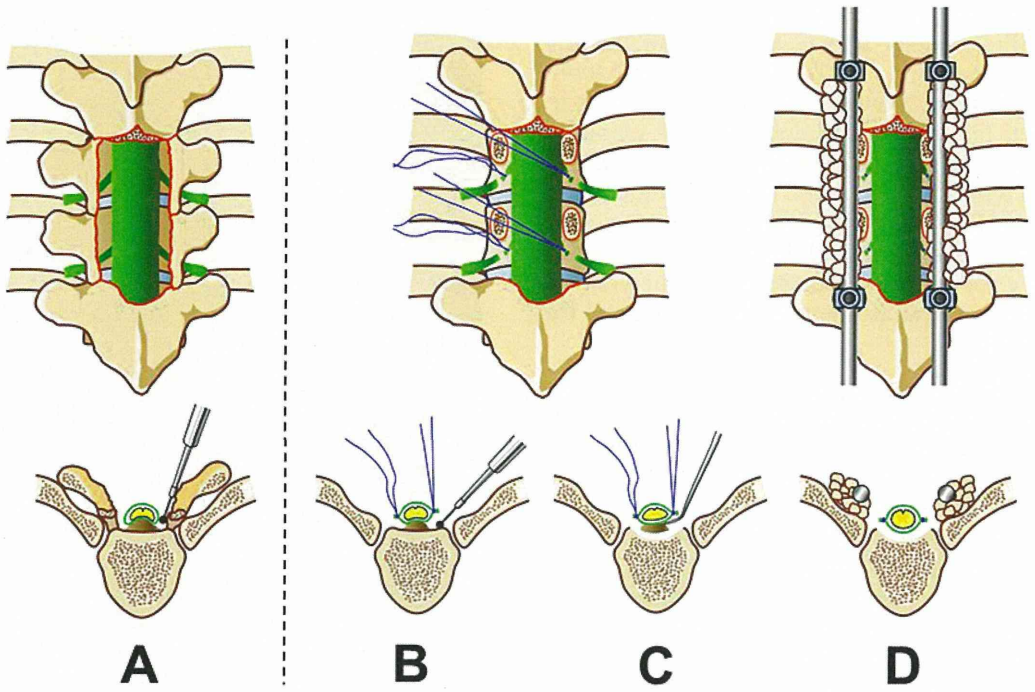

FIG. 2. Schematic diagrams of anterior decompression via a single posterior approach. **A:** The conventional procedure (Ohtsuka method). **B:** This novel technique provides larger space for anterior decompression than the conventional procedure. **C:** Lifting up the ligated nerve roots helps the surgeon manipulate the floating ossified PLL and the dural sac safely. **D:** Posterior instrumented fusion is performed using local bone chips.

result in postoperative CSF leakage. At the most recent follow-up (3–12 months postoperatively), each of the 3 patients exhibited improvement of preoperative neurological symptoms.

Discussion

The surgical outcome after thoracic OPLL compares unfavorably with that after cervical OPLL.[9] This is because of anatomical factors and the pathophysiology of the thoracic ossified PLL: 1) the thoracic spine is naturally kyphotic, and posterior decompression is less effective because backward movement of the spinal cord is restricted; 2) the spinal cord is more vulnerable at the site of compression because of its relative avascularity;[8] and 3) the ossified ligament adheres strongly to the ventral aspect of the dural sac, increasing the difficulty of directly removing the OPLL and increasing the risk of spinal cord injury during decompression maneuvers.

A variety of surgical procedures have been developed to treat thoracic OPLL: 1) posterior decompressive laminectomy or laminoplasty; 2) posterior decompression and instrumentation; 3) anterior decompression via an anterior approach; 4) 2-stage circumferential decompression via a combined posterior-anterior approach; and 5) anterior (circumferential) decompression via a posterior approach. Each procedure has advantages and disadvantages.

Laminectomy or laminoplasty can be relatively successful for thoracic OPLL at the upper thoracic spine because the spinal curvature is usually lordotic or only slightly kyphotic at the cervicothoracic junction. Thus, dorsal shift and decompression of the spinal cord can be expected by posterior decompression alone.[9] The use of instrumentation allows correction of kyphosis or prevention of progression of kyphosis, and stabilization of the spine, thus enhancing and maintaining the decompressive effect.[20] However, posterior decompression with or without instrumented fusion has not always produced satisfactory results because the ossified PLL remains and may still compress the spinal cord due to the limitation of posterior shift of the spinal cord.[11,16]

Anterior decompression via an anterior route is the best approach in the treatment of thoracic myelopathy caused by thoracic OPLL on the concave side of the spinal cord. Fujimura et al.[3] analyzed the surgical outcomes after anterior decompression and fusion in 48 patients, reporting favorable overall results. However, postoperative neurological deterioration has been reported in several studies.[2,12] The anterior approach for thoracic OPLL is technically demanding and opening the thoracic cage can place considerable surgical stress on the patient. If CSF leaks into the thoracic cavity, it can be difficult to regulate.[3,12]

Tomita and colleagues[6,17] reported good surgical outcomes for circumferential decompression via a combined posterior and anterior approach. In a multiinstitutional retrospective study in Japan, although statistically not significant, the surgical outcomes using the Tomita method tended to be more favorable than those achieved using other surgical procedures.[9] This surgical procedure makes it possible to remove or float the ossified PLL safely and completely compared with either an anterior or posterior single approach. However, it has not been the standard surgical method for thoracic OPLL because of

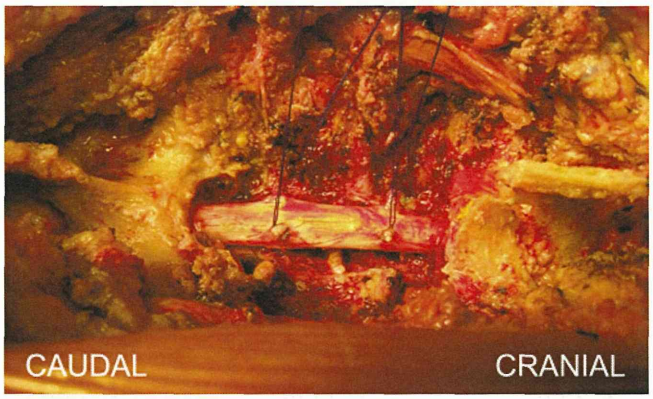

FIG. 3. Intraoperative view after the removal of the ossified PLL. The surgeon can see the anterolateral side of the dural sac and the ossified PLL directly, lifting the ligated nerve roots.

the technical demands and the surgical stress caused by this 2-stage surgery.

Some surgeons have obtained relatively favorable surgical outcomes using anterior decompression via a posterior approach.[1,14] However, Takahata et al.[15] reported a relatively high rate of surgical complications, which included a dural tear in 40% and neurological deterioration in 33% of the patients. It was supposed that these complications were related. The space and field of view can be insufficient for the maneuver in anterior decompression via a posterior approach.

Our technique for anterior decompression is a modification of the conventional anterior decompression via a posterior approach. This surgical procedure has several advantages: 1) it has the advantages of the conventional posterior approach (for example, less surgical stress, indication for extensive lesion, control of CSF leakage, and removal of the ossified ligamentum flavum, which frequently accompanies thoracic OPLL); 2) it provides more space at the bilateral sides of the dural sac for maneuvering in the anterior decompression than the conventional posterior approach[1,14] (Fig. 2); 3) it allows the surgeon to see the OPLL and the anterolateral aspect of the dural sac directly, and it allows the lifting up of the ligated nerve roots, which can help the surgeon manipulate the ossified PLL and the dural sac safely; and 4) it allows easier kyphosis correction with posterior instrumentation because the thoracic spine at the decompressed levels becomes more flexible after removal of the transverse processes and pedicles than is achieved via the conventional posterior approach. Kyphosis correction is the maneuver to reduce the compressive pressure created by the ossified PLL on the spinal cord;[6,10] it is especially useful if complete removal of the ossified ligament cannot be achieved and still compresses the spinal cord.

As a disadvantage, our surgical procedure poses a risk of ischemic spinal cord injury because thoracic nerve roots are ligated at the anterior decompression levels. We used intraoperative spinal cord monitoring in all the 3 patients. There were not any significant changes detected on monitoring in the patients after ligation of 2 pairs of thoracic nerve roots. On the basis of the results of total spondylectomy for spine tumors, Murakami et al.[13] reported that surgeons are allowed to sacrifice up to 3 pairs of thoracic nerve roots, even at the level of the artery of Adamkiewicz, without causing ischemic neurological deterioration. In animal studies, we have reported that ligation of bilateral segmental arteries at 4 or more consecutive levels in the thoracic spine carries a risk of ischemic spinal cord dys-

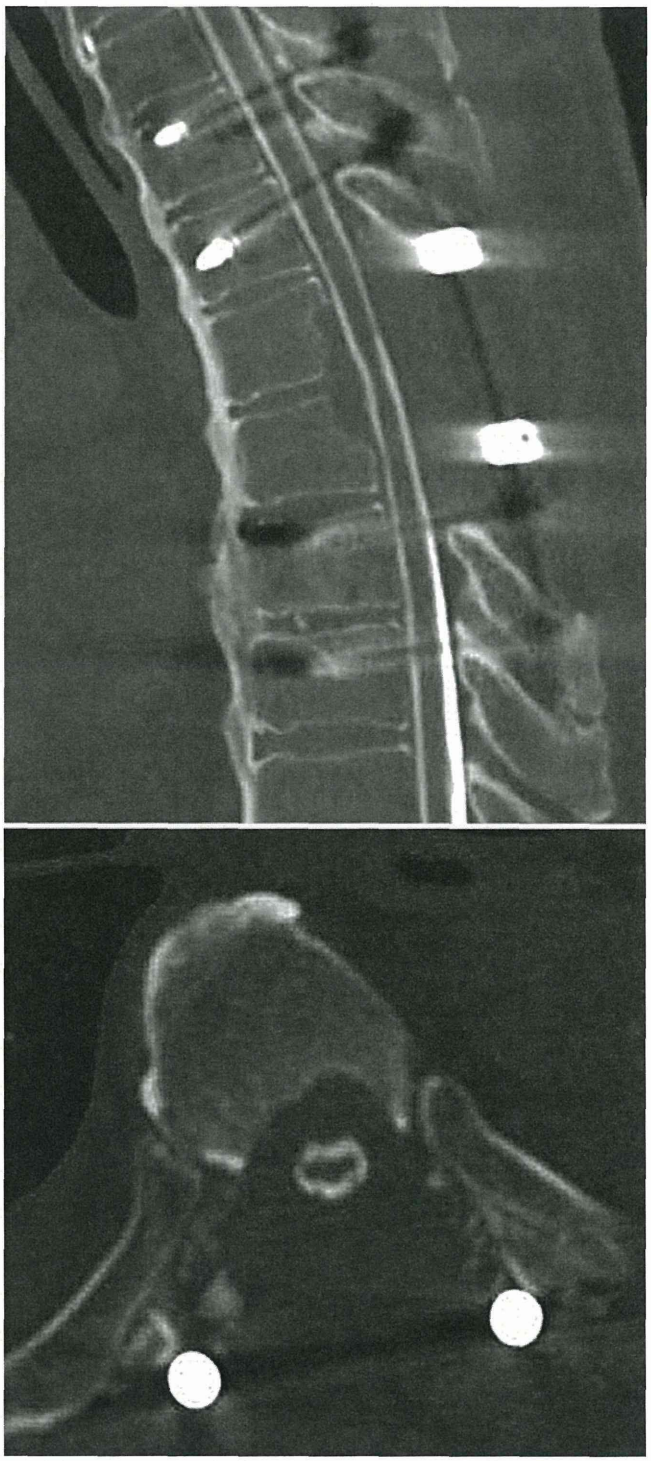

FIG. 4. Postoperative sagittal **(upper)** and axial **(lower)** CT myelograms showing complete removal of the ossified PLL and circumferential decompression of the spinal cord at the T6–7 level.

function.[5] Based on these points, the current technique for anterior decompression can be indicated for thoracic OPLL at 3 consecutive levels or fewer. Despite the limitation in indication, this procedure can be undertaken at every level in the thoracic spine and is especially useful in cases of beak-type OPLL, which is known to be the most complicated surgically.[11,16]

Conclusions

This surgical procedure allows the surgeon to perform, safely and effectively, anterior decompression via a posterior approach for thoracic OPLL. It can be applied at every level in thoracic spine and could become a standard procedure for thoracic OPLL.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Kato, Murakami. Acquisition of data: Kato, Hayashi. Analysis and interpretation of data: Kato, Demura, Yoshioka. Drafting the article: Kato. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Kato. Study supervision: Murakami, Tsuchiya.

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ORIGINAL ARTICLE

Initiation and progression of ossification of the posterior longitudinal ligament of the cervical spine in the hereditary spinal hyperostotic mouse (*twy/twy*)

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Abstract
Introduction Ossification of the posterior longitudinal ligament (OPLL) is a significantly critical pathology that can eventually cause serious myelopathy. Ossification commences in the vertebral posterior longitudinal ligaments, and intensifies and spreads with the progression of the disease, resulting in osseous projections and compression of the spinal cord. However, the paucity of histological studies the underlying mechanisms of calcification and ossification processes remain obscure. The pathological process could be simulated in the ossifying process of the ligament in mutant spinal hyperostotic mouse (*twy/twy*). The aim of this study is to observe that enlargement of the nucleus pulposus followed by herniation, disruption and regenerative proliferation of annulus fibrosus cartilaginous tissues participated in the initiation of ossification of the posterior longitudinal ligament of *twy/twy* mice.

Materials and methods The mutant *twy/twy* mice (6 to 22-week-old) were used in the present study. The vertebral column was analyzed histologically and immunohistochemically.

Results We observed that the enlargement of the nucleus pulposus followed by herniation, disruption and regenerative proliferation of annulus fibrosus cartilaginous tissues participated in the initiation of ossification of posterior longitudinal ligament of *twy/twy* mice. In this regards, the cells of the protruded hyperplastic annulus fibrosus invaded the longitudinal ligaments and induced neovascularization and metaplasia of primitive mesenchymal cells to osteoblasts in the spinal ligaments of *twy/twy* mice.

Conclusion Since genetic mechanisms could play a role in human OPLL, the age-related enlargement of the nucleus pulposus in the *twy/twy* mouse may primarily occur as a result of overproduction of mucopolysaccharide matrix material induced by certain genetic abnormalities.

Keywords Ossification of the posterior longitudinal ligament (OPLL) · *Twy/twy* mouse · Nucleus pulposus herniation · Annulus fibrosus · Enchondral ossification

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Introduction

Ossification of the posterior longitudinal ligament (OPLL) is a pathological condition that can cause serious myeloradiculopathy [2, 29]. Ossification commences in the vertebral posterior longitudinal ligaments, with a particular predilection for the cervical area, but intensifies and spreads with the progression of the disease, resulting in osseous projections and compression of the spinal cord [3, 24]. OPLL was previously considered to be specific to Asian people [15] and did not attract attention in Europe or

the United States. However, because of the reports that about half of the patients with diffuse idiopathic skeletal hyperostosis (DISH) (Forestier disease), which is well known in Europe and the United States, had OPLL, this disease has been recognized as a subtype of DISH [21, 22]. A number of epidemiological [18, 20], metabolic [25, 26], mechanical [4, 16, 28], and biological factors are suspected to contribute to the development as well as progression of OPLL. In addition, gene analysis [6, 14, 23] has been applied to clarify the underlying genetic background because of the high prevalence of OPLL in certain countries and/or races. Thus, recent research on OPLL involves association and/or genome-wide linkage analyses [6, 8, 23] to determine the candidate genes, and proteomics analysis for detecting causative peptides in the ossifying plaque.

Histochemical studies of OPLL have demonstrated certain characteristics including the presence of several different phenotypic osteoblasts in ligament cells obtained from non-ossified sites, high alkaline phosphatase (ALP) activity, parathyroid hormone- and prostaglandin E2-stimulated increases in cAMP, and responses to both calcification and 1,25-dihydroxycholecalcifenol (1,25-(OH)2D3) [10]. In addition to these systemic predispositions, multiple local factors have been proposed for the pathogenesis of OPLL. Using immunohistochemical techniques, Kawaguchi et al. [11] demonstrated the presence of bone morphogenetic protein-2 (BMP-2) inducing cartilage and bone formation, and transforming growth factor-beta (TGF-β) stimulating bone formation in the ossified ligaments of OPLL. While these findings are interesting, the tissues examined in their report were mostly surgically resected materials or autopsy specimens from patients with a late stage disease. Furthermore, abnormal enchondral ossification [1, 5] may play a role in OPLL, but because of the paucity of histological studies the underlying mechanisms of calcification and ossification processes remain obscure.

To clarify the pathogenesis of OPLL and to develop new treatments to combat the ossification of the ligaments, reliable animal models are necessary. The tiptoe walking Yoshimura mouse (twy) was first introduced by Hosoda et al. [9]. The mode of inheritance is autosomal recessive with complete penetrance. Progression of ectopic ossification in the mice is monitored by the contracture of the limb joints, which leads to characteristic "tiptoe" walking. The mouse exhibits ossification of various soft tissues such as tendons, cartilage, and ligaments in the extremities and the spine, in particular the ossification of the spinal ligaments is similar to that seen in human OPLL [7, 19]. The ossification occurs immediately after weaning and progresses within a short period of time.

The present study was designed to investigate serial histological changes in the longitudinal ligaments leading to the ossification in the *twy/twy* mouse spinal ligaments. We also studied immunohistological changes in the area around the intervertebral discs, vertebral endplate and the posterior longitudinal ligaments of *twy/twy* mice.

Materials and methods

Experimental animals

Thirty-one *twy/twy* mice (Central Institute for Experimental Animals, Kawasaki, Japan), 6- to 22-week-old, weighing 25–31 g (mean ± standard deviation, 28 ± 3 g) were used in the present study. Mice were confirmed to have OPLL in the cervical spine by contact microradiography (Softex-CMR; Softex, Osaka, Japan), at the time of commencement of the study when they were 6 weeks old and at 10, 18 and 22 weeks of age when killed. The mutant *twy/twy* mice were maintained by brother–sister mating of heterozygous mice (*+/twy*) and the animal exhibits paravertebral ossification and demonstrates prominent cervical OPLL at 10–14 weeks of age, eventually presenting with extensive spinal ankylosis, involving both the anterior and posterior vertebral columns [7, 9, 19]. Institute of Cancer Research (ICR) mice, age-matched with the *twy/twy* mice, were used as controls ($n = 15$). The Ethics Review Committee for Animal Experimentation of our University approved the experimental protocol.

Casting of microvascular mesh with carbon black gelatin

After anesthesia with an intraperitoneal injection, the animals were exsanguinated through cardiac puncture, perfusion of Ringer lactate (Lactec, Ohtsuka, Tokyo) together with carbon-black gelatin solution consisting of India ink (Kuretake, Nara, Japan) The vertebral column was dissected en bloc and then bisected sagittally in the median plane followed by fixation with 10% buffered formaldehyde at 4°C for 48 h. The specimen was further decalcified for 7–14 days at 4°C in 0.5 M EDTA, and embedded with paraffin. Serial 4 μm thick sections were stained with hematoxylin-eosin (HE).

Immunohistochemical staining

Serial 4 μm-thick sections were prepared from the paraffin-embedded specimens, deparaffinized with xylene and replaced with ethanol. After washing with water, the intrinsic peroxidase was blocked with 0.3% H_2O_2 solution. The sections were irradiated three times, using a microwave oven (500 W, ER-245, Toshiba, Tokyo). Then they were reacted with BLOCKING (LSAB kit, Lot. No. 00075,