Table 2 Interobserver reliability of the classification for cervical OPLL

	Specialists	Residents	Total
First round			
Radiographs	0.565	0.476	0.528
Radiographs and CT images	0.667	0.623	0.633
Second round			
Radiographs	0.630	0.526	0.574
Radiographs and CT images	0.733	0.622	0.658

Results

Interobserver and intraobserver reliability of the classification for cervical OPLL (Tables 2, 3)

In the first round, interobserver reliability was 0.528 with radiographs only (specialists 0.565, residents 0.476) and 0.633 using both radiographs and CT images (specialists 0.667, residents 0.623). In the second round, interobserver reliability was 0.574 with radiographs only (specialists 0.630, residents 0.526) and 0.658 using both radiographs and CT images (specialists 0.733, residents 0.622).

In the first round, interobserver reliability with radiographs only showed moderate agreement, but interobserver reliability using both radiographs and CT images showed substantial agreement. In the second round, interobserver reliability was improved in comparison with the first round.

Intraobserver reliability was 0.477 with radiographs only (specialists 0.561, residents 0.392) and 0.605 using both radiographs and CT images (specialists 0.665, residents 0.544). Intraobserver reliability of residents with radiographs only was fair. Intraobserver reliability was improved by additional CT images.

Interobserver and intraobserver reliability of the diagnosis of either cervical OPLL or CSM (Tables 4, 5)

We also evaluated interobserver and intraobserver reliability of the diagnosis of either cervical OPLL or CSM. In the first round, interobserver reliability was 0.743 with radiographs only (specialists 0.758, residents 0.812) and 0.833 using both radiographs and CT images (specialists 0.710, residents 0.753). In the second round, interobserver reliability was 0.787 with radiographs only (specialists 0.817, residents 0.878) and 0.853 using both radiographs and CT images (specialists 0.832, residents 0.823). If the observers are separated into specialists and residents, interobserver reliability obtained using both radiographs and CT images was lower than that with radiographs only in each observer group. But interobserver reliability in all observers using both radiographs and CT images improved in comparison with radiographs only. By additional CT images, interobserver reliability of the diagnosis showed almost perfect agreement.

Table 3 Intraobserver reliability of the classification for cervical OPLL

	Specialists	Residents	Total
Radiographs	0.561	0.392	0.477
Radiographs and CT images	0.665	0.544	0.605

Table 4 Interobserver reliability of the diagnosis of either OPLL or CSM

	Specialists	Residents	Total
First round			
Radiographs	0.758	0.812	0.743
Radiographs and CT images	0.710	0.753	0.833
Second round			
Radiographs	0.817	0.878	0.787
Radiographs and CT images	0.832	0.823	0.853

Table 5 Intraobserver reliability of the diagnosis of either OPLL or CSM

	Specialists	Residents	Total
Radiographs	0.690	0.537	0.613
Radiographs and CT images	0.795	0.808	0.802

Intraobserver reliability of the diagnosis was 0.613 (specialists 0.690, residents 0.537) with radiographs only and 0.802 (specialists 0.795, residents 0.808) using radiographs and CT images. Intraobserver reliability was improved by additional CT images.

Case presentation (Fig. 2)

Case 1

Using only radiograph, ten observers classified this case as CSM. Although the ossification area was unclear by radiograph, 15 observers classified it as cervical OPLL by additional CT image.

Case 2

All 16 observers classified this case as circumscribed cervical OPLL by additional CT image.

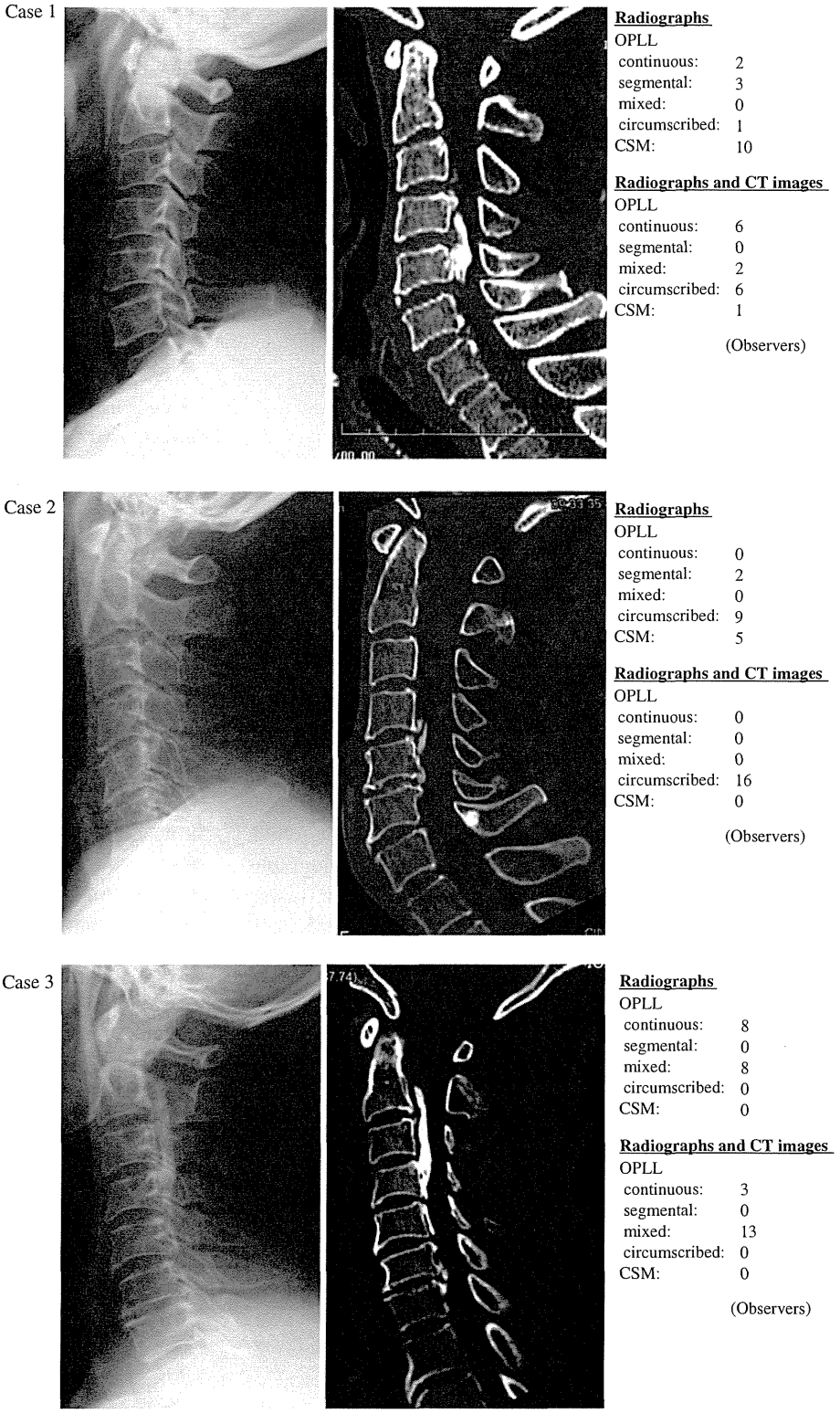

Fig. 2 Case presentation

Case 3

By radiograph, eight observers classified this case as mixed cervical OPLL and the other eight observers classified it as continuous cervical OPLL. By additional CT image, 13 observers classified it as mixed cervical OPLL and the other three observers classified it as continuous cervical OPLL.

Discussion

In Clinical Guidelines for OPLL (Table 1) [10], diagnostic criteria for OPLL include requirements of radiographic images and clinical findings. According to the requirements of radiographic images, the ossification area must be visible in lateral plain radiograph, and a small ossification area that is visible in only CT images is not included in OPLL. If the lower cervical spine is not visible adequately by lateral plain radiograph, CT images are advised.

Chang et al. [12] reported inter- and intra-observer variability of cervical OPLL classification using reconstructed CT images. In their study, the inter- and intra-observer kappa values were 0.51 and 0.67 by the lateral radiograph, 0.70 and 0.85 by 2-D CT images. The reliability of their study was higher than that of our study.

In our study, interobserver and intraobserver reliability of classification for the cervical OPLL was improved by additional CT images. In lateral plain radiographs, facet joints of the cervical spine may overlap with the ossification area. Intervertebral bone spurs may also overlap with circumscribed OPLL. In contrast, we could not observe such overlaps in CT images. Interobserver reliability of specialists was higher than that of residents, and the reliability of residents was remarkably improved in comparison with that of spine surgery specialists by additional CT images. It seems that experience in clinical practice affects the interobserver and intraobserver reliability. The influence of experience in clinical practice may be reduced by CT images.

Interobserver reliability of the diagnosis for cervical OPLL was improved by additional CT images, and intraobserver reliability was improved remarkably. The criteria for OPLL seemed to affect the results. According to the current criteria for OPLL, it is difficult to evaluate an ossification area that is visible on CT image clearly but difficult to identify on plain radiograph.

It is well known that the ossification area is often progressive during the natural course of the disease. Shindo et al. [13] reported the long-term natural course of OPLL. Progression of the ossification was detected in 38 % of segmental OPLL, 75 % of continuous OPLL, and 55 % of mixed OPLL, but it was not related to aggravation of the myelopathy. OPLL often progresses after surgery, which may cause late-onset neurological deterioration. Kawaguchi et al. [14] reported the relationship between the progression of ossification of the OPLL and the clinical results following en bloc cervical laminoplasty. Young patients with mixed and continuous types OPLL had the greatest risk for progression. Some patients had neurological deterioration following an increase in the thickness of the ossification. Hori et al. [15] reported on 55 patients after cervical laminoplasty who were available for serial radiographs for more than 5 years. The patients were divided into three groups according to the pattern of OPLL progression. OPLL progression related to patient age or OPLL type. In genetic study, it was reported that mixed or continuous type OPLL had higher osteogenic differentiation potency than segmental or circumscribed type OPLL [16]. As above, patients with mixed or continuous OPLL had the greatest risk for progression of the ossification area. It is important for prognosis and diagnosis that reliability of the classification for cervical OPLL has high agreement. We will work out the new classification for cervical OPLL by advanced CT images.

CT images were very valuable on the classification and diagnosis for cervical OPLL; however, there were some problems. One problem was the cost. CT images of cervical spine cost about three times more than radiographs of cervical spine in Japan. Another problem was the radiation dosage to the patients. The radiation dose of CT images of cervical spine was about 17 times higher than that of radiographs of cervical spine in our institute (The radiation dosage of CT images of cervical spine was 61.8 mGy and that of radiographs of cervical spine was 3.6 mGy). In terms of the cost and the radiation dosage, CT images were not repeatable test.

This study suggested that interoberver and intraobserver reliability of the classification for cervical OPLL was improved by additional CT images, which also improved intraobserver reliability of the diagnosis for cervical OPLL. We propose that diagnostic criteria for OPLL include both radiographs and CT images.

Acknowledgments We thank the Spine Surgery group in the Department of Orthopaedic Surgery of Hirosaki University Graduate School of Medicine and Drs. Taisuke Nitobe and Naoki Echigoya for providing film samples, and Drs. Takashi Tomita, Syuichi Aburakawa, Takeuchi Kazunari, Akira Saito, Masaki Kishiya, Ryoko Uesato, Takuya Naraoka, Yoshimitsu Hayashi, Kozo Kato, and Yuka Kimura for classifying and making a diagnosis for OPLL.

Conflict of interest None of the authors has any potential conflict of interest.

References

1. Tsukimoto H (1960) A case report-autopsy of syndrome of compression of spinal cord owing to ossification within spinal canal of cervical spines. Arch Jpn Chir 29:1003–1007
2. Sakou T, Matsunaga S (1996) Ossification of the posterior longitudinal ligament. J Jpn Spine Res Soc 7:437–448 (in Japanese)
3. Terayama K, Kurokawa T, Seki H (1975) National survey of ossification of the posterior longitudinal ligament. In: Investigation Committee 1975 report on the ossification of the spinal ligaments of the Japanese Ministry of Public Health and Welfare, pp 8–33 (in Japanese)
4. Furukawa K-I (2008) Pharmacological aspect of ectopic ossification in spinal ligament tissues. Pharmacol Ther 118:352–358

5. Koga H, Sakou T, Taketomi E et al (1998) Genetic mapping of ossification of the posterior longitudinal ligament of the spine. Am J Hum Genet 62:1460–1467
6. Nakamura I, Ikegawa S, Okawa A et al (1999) Association of the human NPPS gene with ossification of the posterior longitudinal ligament of the spine (OPLL). Hum Genet 104:492–497
7. Tanaka T, Ikari K, Inoue I et al (2003) Genomewide linkage and linkage disequilibrium analyses identify COL6A1, on chromosome 21, as the locus for ossification of the posterior longitudinal ligament of the spine. Am J Hum Genet 73:812–822
8. Investigation Committee on OPLL of the Japanese Ministry of Public Health and Welfare (1981) The ossification of the longitudinal ligament of the spine (OPLL). J Jpn Orthop Assoc 55: 425–440
9. Yonenobu K, Nakamura K, Toyama Y (eds) (2006) OPLL ossification of the posterior longitudinal ligament. Springer, Japan
10. Nakamura K, Shinomiya K, Yonenobu K et al (2005) Clinical guidelines for ossification of the posterior longitudinal ligament (in Japanese). Nankodo, Tokyo
11. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33:159–174
12. Chang H, Kong C-G, Park J-B et al (2010) Inter- and intra-observer variability of a cervical OPLL classification using reconstructed CT images. Clin Orthop Surg 2:8–12
13. Shindo S, Yamaura I et al (2004) Long-term clinical course of ossification the posterior longitudinal ligament in the cervical region. Rinsyo Seikeigeka 39:445–452 (in Japanese)
14. Kawaguchi Y, Kanamori M, Ishihara H et al (2001) Progression of ossification of the posterior longitudinal ligament following en bloc cervical laminoplasty. J Bone Joint Surg Am 83:1798–1802
15. Hori T, Kawaguchi Y, Kimura T et al (2006) How does the ossification area of the posterior longitudinal ligament progress after cervical laminoplasty. Spine 31:2807–2812
16. Kudo H, Furukawa K-I, Toh S et al (2011) Genetic differences in the ostenogenic differentiation potency according to the classification of ossification of the posterior longitudinal ligament of the cervical spine. Spine 36:951–957

Biochemical and Biophysical Research Communications 436 (2013) 698–704

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Immunohistochemical localization of mesenchymal stem cells in ossified human spinal ligaments

Shunfu Chin [a,b], Ken-Ichi Furukawa [a,*], Atsushi Ono [b], Toru Asari [b], Yoshifumi Harada [b], Kanichiro Wada [b], Toshihiro Tanaka [b], Wataru Inaba [c], Hiroki Mizukami [c], Shigeru Motomura [a], Soroku Yagihashi [c], Yasuyuki Ishibashi [b]

[a] Department of Pharmacology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
[b] Department of Orthopaedic Surgery, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
[c] Department of Pathology and Molecular Medicine, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan

ARTICLE INFO

Article history:
Received 4 June 2013
Available online 14 June 2013

Keywords:
Spinal ligament ossification
Mesenchymal stem cells
Immunohistochemistry
Localization
Neovascularization
Chondrocytes

ABSTRACT

Mesenchymal stem cells (MSCs) have been isolated from various tissues and used for elucidating the pathogenesis of numerous diseases. In our previous *in vitro* study, we showed the existence of MSCs in human spinal ligaments and hypothesized that these MSCs contributed to the pathogenesis of ossification of spinal ligaments. The purpose of this study was to use immunohistochemical techniques to analyze the localization of MSCs in ossified human spinal ligaments *in situ*. Ossified (OLF) or non-ossified ligamentum flavum (non-OLF) samples from the thoracic vertebra were obtained from patients who had undergone posterior spinal surgery. Serial sections were prepared from paraffin-embedded samples, and double immunofluorescence staining was performed using antibodies against markers for MSCs (CD73, CD90 and CD105), endothelial cells (CD31), pericytes (α-smooth muscle actin), and chondrocytes (S100). Immunolocalization of MSCs was observed in the perivascular area and collagenous matrix in spinal ligaments. Markers for MSCs and pericytes were co-expressed in the perivascular area. Compared with non-OLF, OLF had a large amount of neovascularization in the fragmented ligament matrix, and a high accumulation of MSCs around blood vessels. The prevalence of MSCs in OLF within collagenous matrix was significantly higher than that in non-OLF. Chondrocytes near the ossification front in OLF also presented expression of MSC markers. MSCs may contribute to the ectopic ossification process of OLF through endochondral ossification.

1. Introduction

Human spinal ligaments adjacent to the spine contribute to its flexibility and stabilization by guiding segmental motion and limiting excessive motion [1,2]. Ossification of spinal ligaments, such as ossification of the posterior longitudinal ligament (OPLL) and the ligamentum flavum (OLF), can lead to narrowing of the spinal canal and eventually cause serious damage to the spinal cord, with patients suffering from various symptoms. These patients require pharmacotherapy and in severe cases surgery may be required to remove the ossified components and release the compression on the spinal cord [3]. The etiology of ectopic ossification of spinal ligaments has been analyzed extensively and linked to various epidemiological, genetic, metabolic, and mechanical factors [4–7]; however, the pathogenesis of the disease is still unknown.

Mesenchymal stem cells (MSCs) have been isolated from various human tissues including muscle, synovium, meniscus, intra-articular ligament, bone marrow, and adipose tissue, among others [8–12]. MSCs with multilineage potential have been used in regenerative therapy [13] and to elucidate the pathogenesis of numerous diseases in animal experimental models [14–16]. Furthermore, a number of studies have separated and identified MSCs in spinal ligaments and focused on the role of the MSCs in the pathogenesis of hypertrophy of spinal ligaments [17].

Ectopic bone formation in spinal ligamentous tissues has been shown to occur through endochondral ossification [18,19]. However, until recently, the source of these cells remained to be clarified. We recently identified the presence of MSCs in human spinal ligaments *in vitro* and showed their capacity to differentiate into the chondrocytic and osteocytic lineages. We hypothesized that these cells may contribute to the pathogenesis of ectopic ossification [20]. Using this previous work as a basis, it is now important to determine the localization of MSCs in ossified spinal ligaments as compared with non-ossified spinal ligaments, with the goal to determine how these cells commit to the ossification site. One possibility is that MSCs undergo chondrocytic differentiation, resulting in spinal ligament ossification. These findings would provide

* Corresponding author. Fax: +81 172 39 5023.
E-mail address: furukawa@cc.hirosaki-u.ac.jp (K.-I. Furukawa).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.019

valuable insight into the treatment of ectopic ossification in spinal ligaments.

Therefore, the purpose of this study was to use immunohistochemical techniques to analyze the localization of MSCs in ossified and non-ossified human spinal ligaments *in situ* and investigate a possible role of MSCs and/or chondrocytes in spinal ligament ossification.

2. Materials and methods

2.1. Clinical diagnosis and spinal ligament samples

Clinical diagnosis was confirmed by X-ray, computed tomography and magnetic resonance imaging of the spine. Samples of the thoracic vertebra ligamentum flavum plaque were obtained *en bloc* from 12 patients. The plaques of ossified ligament tissues and ligamentum flavum (LF) were taken from six patients (four males, two females; mean age at surgery, 69.2 years; range 56–77 years) who underwent posterior decompression surgery for thoracic OLF. As a control, non-ossified LF plaques were obtained from six patients (five males, one female; mean age, 49.8 years, range, 22–81 years) who underwent posterior surgery for spinal tumor, syringomyelia or burst fracture at the thoracic vertebral level. None of the patients had evidence of congenital bone or joint disorders or was positive for rheumatoid factor. The Human Ethics Review Committee of the Hirosaki University Hospital approved the study protocol, and a signed informed consent form was obtained from each patient for all procedures.

2.2. Tissue preparation

Samples were immediately fixed with 10% formaldehyde at 4 °C for 7 days. Samples with ossified tissue or bony tissue were further decalcified with KC-X solution (Falma, Tokyo, Japan) for 4–7 days at room temperature. Then, samples were bisected sagittally in the median plane, and embedded in paraffin. Serial, 4-μm-thick sections were prepared and subjected to hematoxylin and eosin (H&E) staining and immunohistochemical staining, using antibodies against markers for MSCs (CD73, CD90 and CD105), endothelial cells (CD31), pericytes (α-smooth muscle actin (SMA)), and chondrocytes (S100).

2.3. Immunohistochemical staining

Immunohistochemical analysis was performed with fluorescence antibody double staining. Sections were deparaffinized with xylene and treated with ethanol. After washing in phosphate buffered saline (PBS) at room temperature for 5 min, antigen retrieval was performed by heating samples in a PASCAL pressure chamber (Dako Cytomation, Produktionsvej, Glostrup, Denmark) to 125 °C for 3 min in Tris/EDTA buffer (Tris 10 mM, EDTA 1 mM, pH 9.0). After washing with PBS containing 0.01% Tween 20 (PBS-T), the sections were treated with 1% bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO, USA) diluted in PBS-T at room temperature for 30 min to block non-specific protein binding. Next, the samples were incubated overnight at 4 °C with a mixture of two primary antibodies diluted with 1% BSA from the following list of antibodies. Monoclonal anti-CD73 antibody (Abcam, Cambridge, MA, USA; mouse); polyclonal anti-CD73 antibody (Abcam; rabbit); monoclonal anti-CD90 antibody (Abcam; rabbit); and monoclonal anti-CD105 antibody (Dako; mouse) were used to detect the expression of MSC markers. Polyclonal anti-CD31 antibody (Abcam; rabbit) and monoclonal anti-CD31 antibody (Dako; mouse) were used to identify vascular endothelial cells. Polyclonal anti-alpha smooth muscle actin (α-SMA) antibody (Abcam; rabbit) and monoclonal anti-α-SMA antibody (Dako; mouse) were used to identify pericytes. Monoclonal anti-S100 antibody (ab14849; Abcam; mouse) and polyclonal anti-S100 antibody (ab76729; Abcam; rabbit) were used to identify chondrocytes. Sections were then washed with PBS and incubated at room temperature for 2 h with a mixture of two secondary antibodies: Alexa Fluor® 594 donkey anti-mouse IgG (H + L) conjugate and Alexa Fluor® 488 goat anti-rabbit IgG (H + L) conjugate (Life Technologies, Carlsbad, CA). Finally, the sections were lightly counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

2.4. Observation and quantification

Following double immunofluorescence staining, the sections were examined by confocal laser scanning microscope (Digital Eclipse C1si/C1 plus; Nikon Instruments, Japan) equipped with a charge-coupled device (CCD) camera and EZ-C1 3.90 Free Viewer software (Nikon Instruments, Japan). Images were collected sequentially as raw TIFF files and analyzed without further thresholding or filtering (e.g., no background subtraction). MSCs are phenotypically characterized by the expression of CD73, CD90 and CD105 [21]. Thus, in this study, double staining of the pairs of anti-CD73/CD90 antibodies, anti-CD73/CD105 antibodies, and anti-CD90/CD105 antibodies were performed to identify MSCs. For MSC marker expression, we focused on regions surrounding blood vessels, within collagenous matrix, and near the ossification front in ossified ligamentous plaques. Furthermore, double staining of the pairs of anti-CD31 antibody with anti-CD73, anti-CD90, or anti-CD105 antibodies, and the pairs of anti-α-SMA antibody with anti-CD73, anti-CD90, or anti-CD105 antibodies were performed to confirm the association between MSCs with endothelial cells and pericytes in the blood vessel regions.

Next, we calculated the prevalence of MSC marker-positive cells in the collagenous matrix area of all samples and compared the prevalence of the OLF group with the non-OLF control group in the MSC marker pairs of anti-CD73 and anti-CD90 antibodies, anti-CD73 and anti-CD105 antibodies, and anti-CD90 and anti-CD105 antibodies. Prevalence was defined as the ratio of MSC marker-double positive cells to nucleated cells. For each MSC marker pair, three serial sections per sample were prepared and subjected to double immunofluorescence staining. On each section, multiple sites within the collagenous matrix were examined and MSC marker-double positive cells and nucleated cells were counted. Values were expressed as the percentage of MSC marker-double positive cells compared with the total number of DAPI-counterstained cells within each section, counting at least 1000 cells per section.

2.5. Statistical analysis

The prevalence of each MSC marker pair was compared between OLF and non-OLF groups using the Mann–Whitney *U*-test. Statistical analysis was performed with SPSS ver. 12.0J (SPSS Inc., Chicago, IL, USA), and the level of significance was set at a p value of less than 0.05.

3. Results

3.1. Localization of MSCs in blood vessel region

Double immunofluorescence staining for MSC markers (CD73, CD90 or CD105) showed the existence of MSC marker-double positive cells around blood vessels within and surrounding the spinal ligaments for both OLF and non-OLF samples (Fig. 1). In the control non-OLF tissues, few MSC marker-double positive cells were detected sparsely distributed around the blood vessels in intact

ligament tissues (Fig. 1A and C–E). On the other hand, in OLF tissues, there was a large amount of neovascularization in the fragmented ligament matrix (Fig. 1B), and a higher number of MSC marker-double positive cells around blood vessels (Fig. 1F–H). These differences between the OLF group and non-OLF group suggested that there is a close relationship between neovascularization and the accumulation of MSCs during damage ligament repair.

Since these MSC marker-positive cells were located close to blood vessels, we investigated the relationship between MSC marker-positive cells and the presence of vascular endothelial cells and pericytes in these regions with non-OLF samples. Double immunofluorescence staining showed no co-localization between MSC marker-positive cells and CD31-positive endothelial cells (Fig. 2A–C and G). In contrast, double immunofluorescence staining for MSC markers and the pericyte marker, α-SMA, showed co-expression of these two cell types in the perivascular area (Fig. 2D–G). Thus, this staining revealed that MSCs are distinct from endothelial cells, but exist at the perivascular area, possibly in close relationship with pericytes.

3.2. Localization and prevalence of MSCs in collagenous matrix

Next, we investigated the expression of MSC markers within the collagenous matrix, and observed fibroblast-like cells with double positive expression of MSC markers (Fig. 3). In control non-OLF tissues, the collagenous fibers showed regular arrangement (Fig. 3A), but only few fibroblast-like cells were observed that were double positive for the expression of MSC markers (Fig. 3D–F). On the other hand, in OLF tissues, numerous fibroblast-like cells were double positive for MSC markers, situated amongst the irregular arrangement and fragmented collagenous fibers (Fig. 3B and G–I). The statistical analysis showed a significant increase in the prevalence of MSC marker expression coincident with ossified ligament plaques than in non-ossified ligament plaques ($p < 0.05$, each) (Fig. 3C). These results suggest that MSCs migrated from certain locations (such as the perivascular area) to accumulate at micro-injured ligament tissue sites to restore damaged ligamentous tissues.

3.3. Localization of MSCs near the ossification front

In OLF tissue samples, we identified chondrocytes using a characteristic marker of morphology (S100), and identified a large number of chondrocytes around the ossification front (Fig. 4A–E; B shows a higher magnification of A). In addition, we also observed that chondrocytes around the ossification front showed double positive expression of MSC markers (Fig. 4F–H). Together, these observations may suggest a role for MSCs in chondrocyte differentiation or endochondral ossification during the pathogenesis of OLF.

4. Discussion

Human MSCs have been identified in multiple organs *in vivo*. Using various methods of immunodetection, these MSCs have been shown to reside both in alignment with the collagenous matrix and

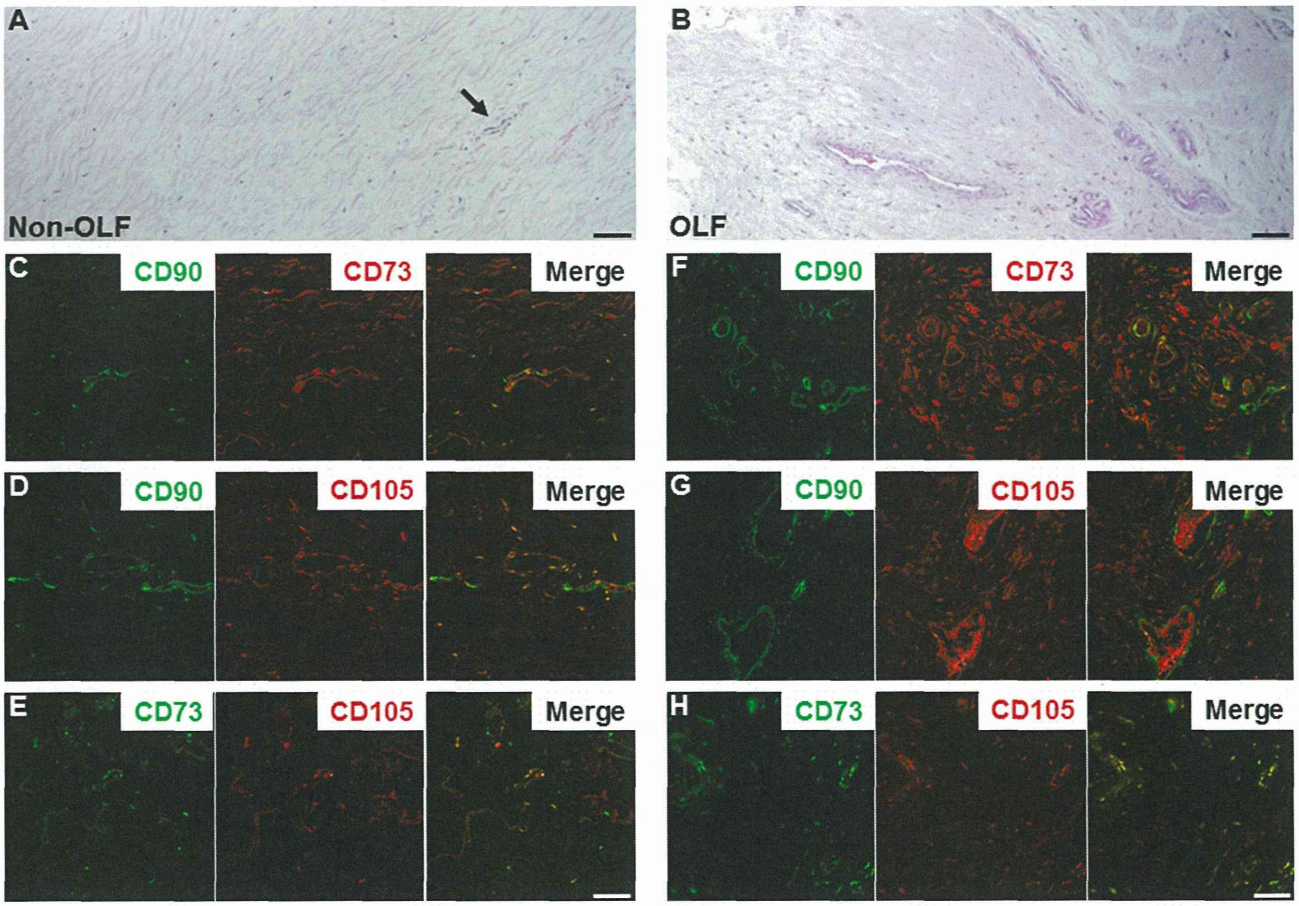

Fig. 1. Mesenchymal stem cell (MSC) marker-double positive cells in blood vessel regions. (A, B) H&E staining of (A) control non-OLF shows the sparse distribution of microvasculature (arrow: blood vessel), whereas a rich neovascularization is observed in the (B) OLF ligament matrix. Representative images of double immunofluorescence staining for MSC markers (CD73, CD90, and CD105) were shown in non-OLF (C–E) and OLF (F–H). The immunoexpression was detected around the blood vessel region. Merged images for CD90 (green) and CD73 (red) (C, F), for CD90 (green) and CD105 (red) (D, G), and for CD73 (green) and CD105 (red) (E, H) are shown. MSC marker-double positive cells are shown in yellow or orange. OLF: ossification of the ligamentum flavum; scale bar = 50 μm.

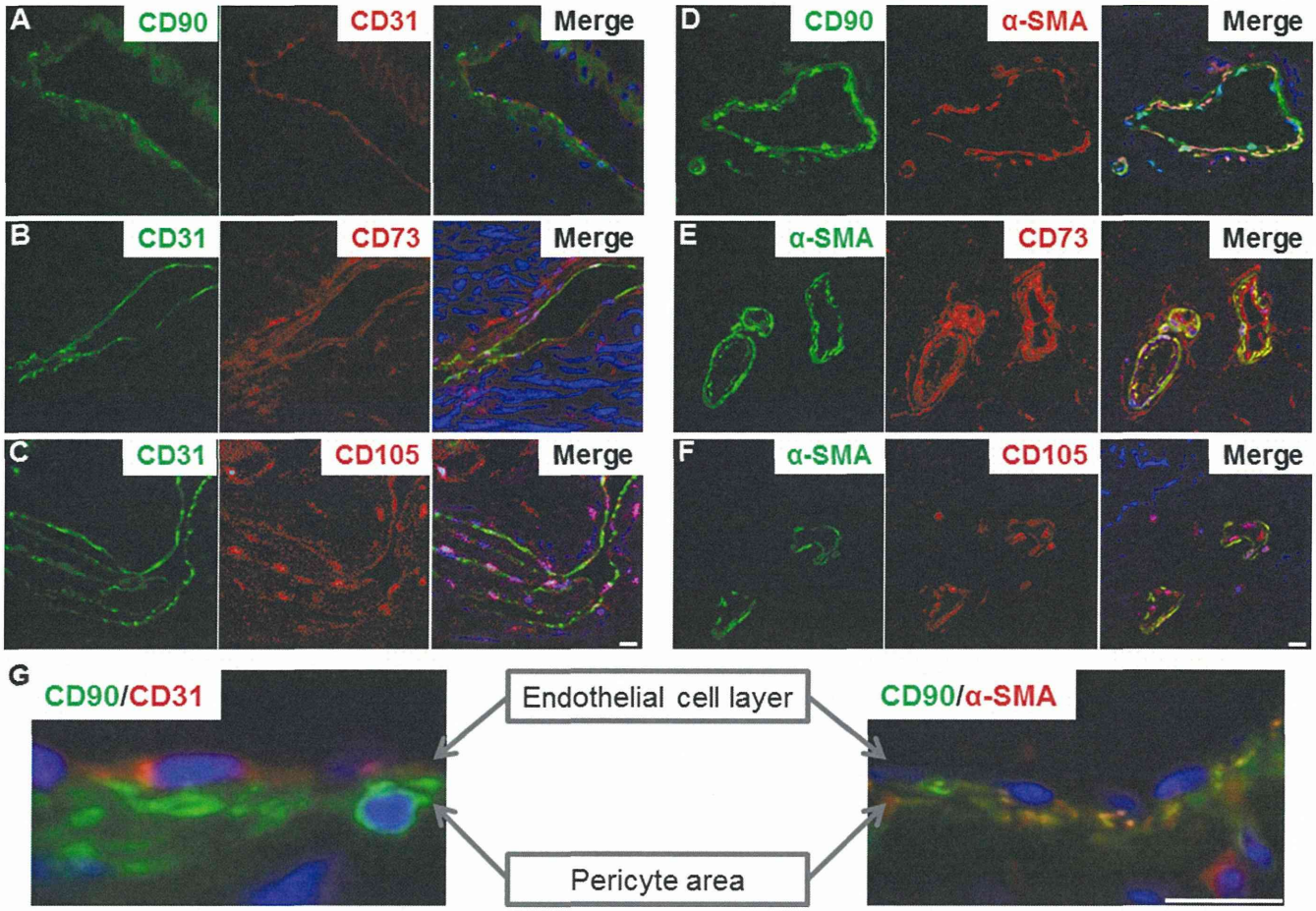

Fig. 2. Relationship between mesenchymal stem cell (MSC) marker (CD73, CD90, and CD105)-positive cells with vascular endothelial cells and pericytes in non-OLF samples. Merged images of double immunofluorescence staining for MSC markers and endothelial cell marker, CD31: (A) CD90 (green) and CD31 (red); (B) CD31 (green) and CD73 (red); (C) CD31 (green) and CD105 (red). Merged images of double immunofluorescence staining for MSC markers and pericyte marker, α-smooth muscle actin (SMA): (D) CD90 (green) and α-SMA (red); (E) α-SMA (green) and CD73 (red); (F) α-SMA (green) and CD105 (red). Enlarged images (G) show an absence of co-localization with CD90-positive cells (green) and CD31-positive cells (red), but co-expression of CD90-positive (green) and α-SMA positive (red) staining (merged, yellow and orange) for cells in the perivascular area. α-SMA: α-smooth muscle actin; scale bar = 10 μm.

adjacent to small blood vessels [11,22,23]. However, until our recent study [20], there was no report to describe the detailed localization of MSCs from human spinal ligaments *in situ*. Here, we build on our previous findings, and show, for the first time, the existence of MSCs in both non-ossified and ossified human spinal ligaments *in vivo*. In spinal ligaments, a distinct pattern of MSC localization was observed, with positive MSC marker expression found in regions of vascularization and within the collagenous matrix. Furthermore, chondrocytes around the ossification front in ossified spinal ligaments showed positive expression of MSC markers.

In the current study, the existence of MSCs and blood vessels in collagenous matrix was minimal in non-OLF tissues. On the other hand, a high number of MSCs and a large amount of vascularization were observed in OLF tissues. Recently, some researchers have demonstrated that blood vessels are the source or niche of MSCs, providing convincing evidence that angiogenesis is associated with ectopic calcification in human tissues, such as in blood vessel walls, heart valves, and skeletal muscle. It has been hypothesized that angiogenesis may regulate ectopic calcification via various angiogenic factors, cytokines, oxygen and nutrients [24] and that new blood vessels can serve as a conduit for osteoprogenitor cells, which may be derived from the circulation or from pericytes present in the neovessels themselves and have the functions of vessel stabilization, synthesis of matrix proteins, and providing immunological properties. [25,26]. From the results of our current study and in line with previous reports, we consider that vascularization occurs as part of the repair process brought about by mechanical stress that leads to collagen tears and other microdamage in the ligament. Active vascularization derives a large number of MSCs from the circulation or pericytes from capillary walls, and consequently changes the microenvironment of the extracellular matrix by secreting various factors or cytokines. These various growth factors and cytokines create an environment that leads to ectopic ossification within the ligament. However, the precise suite of factors responsible for this process is still unknown. In the future, a better understanding of the underlying mechanisms that link angiogenesis, pericytes, and MSCs should provide a basis for understanding the pathogenesis of ectopic ossification in spinal ligaments.

In spinal ligaments, we showed MSCs localized around blood vessels, coincident with the expression of the pericyte marker (α-SMA) in the perivascular area. However, the MSCs were distinct from the endothelial cells, as indicated by CD31 staining in the endothelial cells layer. In recent years, pericytes that surround blood vessels have been identified in multiple human organs including skeletal muscle, pancreas, adipose tissue, and placenta. Moreover, irrespective of their tissue of origin, long-term cultured pericytes are able to give rise to adherent, multilineage progenitor cells that exhibit the features of MSCs. Some studies have

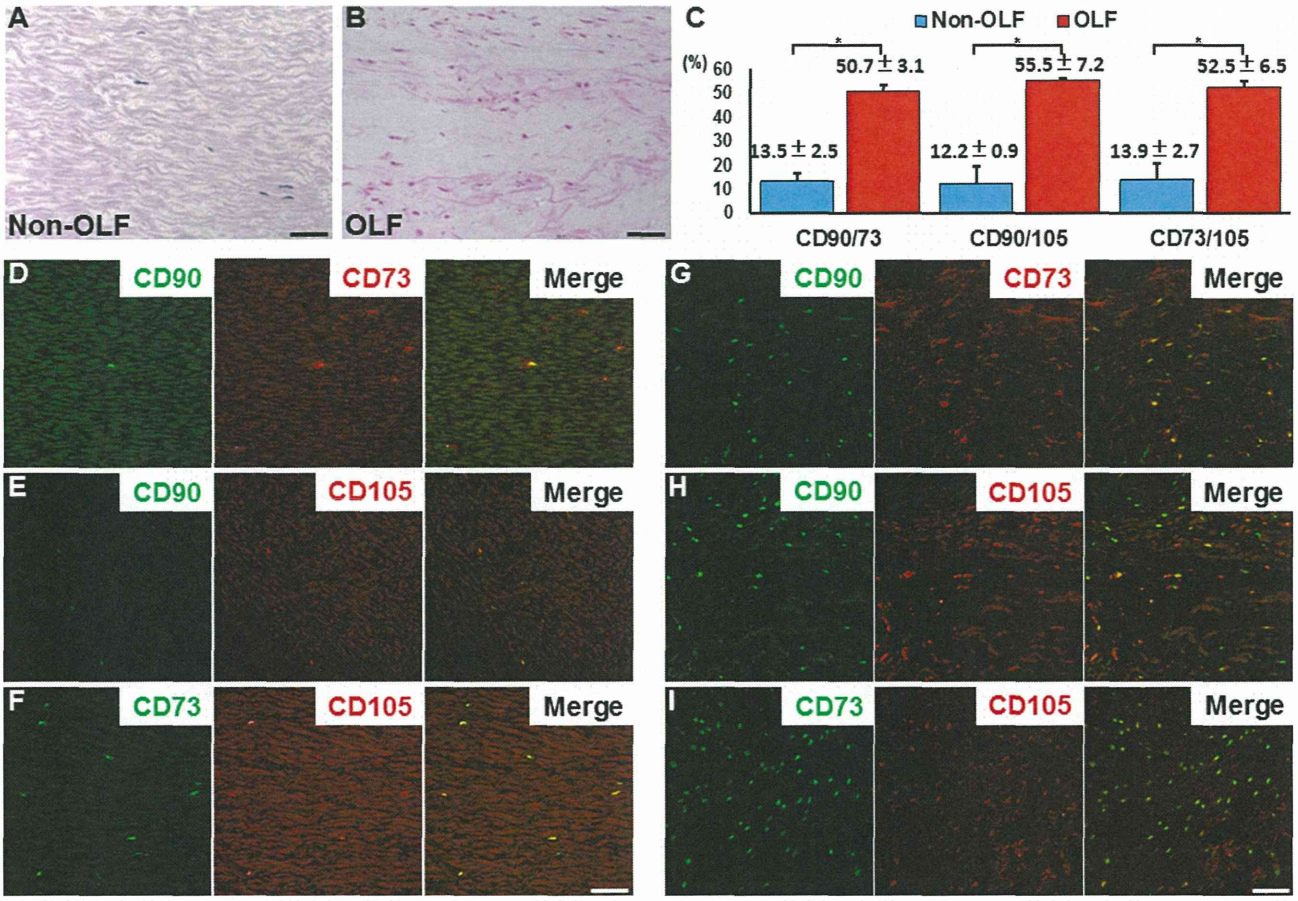

Fig. 3. *Mesenchymal stem cell (MSC) marker-double positive cells in collagenous matrix. In the H&E staining,* (A) *a section of the control non-OLF shows regular arrangement* of fiber bundles, whereas (B) OLF shows marked irregular and fragmented fibers. Representative images of double immunofluorescence staining for MSC markers (CD73, CD90, and CD105) are shown in (D–F) non-OLF and (G–I) OLF. The fibroblast-like cells in the collagenous matrix show immunoexpression of MSC markers. Merged images for (D, G) CD90 (green) and CD73 (red); (E, H) CD90 (green) and CD105 (red); and (F, I) CD73 (green) and CD105 (red). (C) The prevalence of MSC marker-double positive cells in OLF (red) was compared with the prevalence in non-OLF (blue) in all of three MSC marker pairs. Values are the mean ± SEM (standard error of the mean) from six samples per group.*$p < 0.05$, compared with the control. OLF: ossification of the ligamentum flavum; scale bar = 50 μm.

hypothesized that MSCs are pericytes, or could be derived from pericytes [11,24,27]. Overall, the results of the co-expression of the pericyte marker and MSC markers in our study are consistent with the previous studies and support the current hypothesis. Furthermore, we believe that an ancestor of the MSC is firmly associated with human perivascular cells, pericytes in particular.

Near the ossification front, the immunohistochemical analysis revealed the presence of numerous chondrocytes that were also positive for MSC markers. Several studies concluded that the process of ectopic ossification of the spinal ligament occurs through endochondral ossification and clustering of abnormal fibrocartilage or cartilaginous cells [19]. Our previous studies have shown that various cytokines are involved in the presence/development of ectopic ossification in human spinal ligaments, and chondrocytes around the ossification front were stained with the antibody against CTGF/Hcs24, which plays an important role in endochondral ossification and osteogenesis in spinal ligament cells [18,28–32]. Uchida et al. demonstrated that chondrocytes around the ossification front had strong immunoreactivity using antibodies against several transcription factors, including Sox9, Runx2, and Osterix, among others, and demonstrated that chondrocyte differentiation around the ossification front is influenced by these transcription factors [33]. With this in mind, and given the positive expression of MSC markers in chondrocytes, our study supports the involvement of MSCs in the process of ectopic ossification in human spinal ligaments. Future experiments will hope to elucidate the role of MSCs in chondrocyte differentiation and the relationship between the cytokines that induce chondrometaplasia.

There were several limitations in this study. First, we employed double staining instead of triple staining (CD73/CD105, CD90/CD105 and CD90/CD73). Since MSCs have no unique specific marker, identification of the expression of CD73, CD90 and CD105 surface markers is required to verify the cell type. In future studies, triple immunohistochemical staining analysis will provide a more accurate representation of MSC populations for the identification of the MSCs. Second, we only used S100 as a marker for the presence of chondrocytes. As S100 also stains other cells of neural crest origin, additional staining using chondrocyte-specific markers, such as Type II collagen, osteonectin, aggrecan, chondroitin-S or other markers should be considered in future experiments. Third, the current study included a relatively limited number of subjects ($n = 6$) and was not adequately powered to perform all statistical analyses. It would be necessary to conduct a further study with a larger sample size in the future.

In conclusion, our study showed the localization of MSCs in human spinal ligaments in the perivascular area and within the collagenous matrix. In addition, the co-expression of MSC and pericyte markers was observed in the perivascular area. Chondrocytes near the ossification front in OLF were also positive for MSC marker expression. The prevalence of MSCs in OLF was

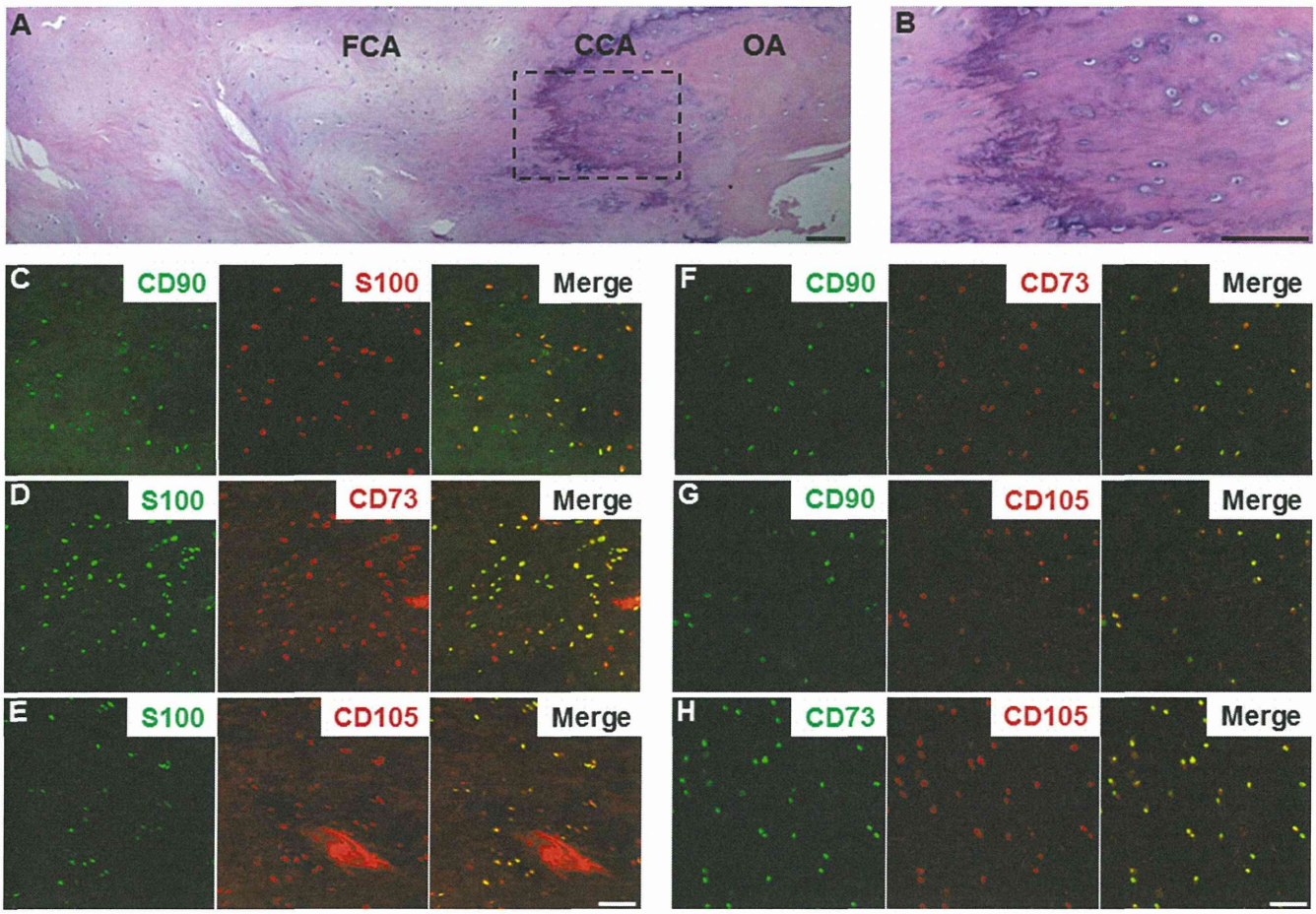

Fig. 4. Mesenchymal stem cell (MSC) marker-double positive cells near the ossification front in OLF. (A) Histological findings using H&E staining of the ossification front show irregular calcification and many chondrocytes. (B) Higher magnification of the boxed area in (A). The chondrocyte-like cells near the ossification front showed immunoexpression of the chondrocyte marker (S100) and MSCs markers (CD73, CD90, and CD105). Merged images of double immunofluorescence staining for S100 and MSCs markers in the calcified cartilage area (CCA) area: (C) CD90 (green) and S100 (red); (D) S100 (green) and CD73 (red); (E) S100 (green) and CD105 (red); (F) CD90 (green) and CD73 (red); (G) CD90 (green) and CD105 (red); and (H) CD73 (green) and CD105 (red). FCA: fibrocartilage area; OA: ossified area; OLF: ossification of the ligamentum flavum; scale bar = 50 μm.

significantly higher than that of non-OLF in collagenous matrix. We suspect that MSCs play a key role in the ectopic ossification process of OLF.

Acknowledgments

This work was supported by a Grant-in-Aid from the Investigation Committee on Ossification of the Spinal Ligaments, the Public Health Bureau of the Japanese Ministry of Labour, Health and Welfare and by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid 24590310). We thank Drs. Kazumasa Ueyama, Naoki Echigoya, Akio Sannohe, Gentaro Kumagai, Toru Yokoyama, Kazunari Takeuchi, Takashi Tomita, Takuya Numasawa, Shuichi Aburakawa, Yoshihito Yamasaki, Taisuke Nitobe, and Kenji Kowatari for their contributions to sample collection, and thank Medical Corporation Seijin Society Ono Hospital for financial support. We also thank Drs. Kenzo Uchida and Takafumi Yayama and Professor Hisatoshi Baba of the Fukui University Department of Orthopaedics and Rehabilitation Medicine for their valuable suggestions.

References

[1] A.D. Olszewski, M.J. Yaszemski, A.A. White III, The anatomy of the human lumbar ligamentum flavum. New observations and their surgical importance, Spine (Phila Pa 1976) 21 (1996) 2307–2312.

[2] S. Ferguson, Biomechanics of the spine, in: N. Boos, M. Aebi (Eds.), Spinal Disorders, Springer Verlag, Berlin, Heidelberg, 2008, pp. 41–66.

[3] K.-I. Furukawa, Pharmacological aspect of ectopic ossification in spinal ligament tissues, Pharmacol. Ther. 118 (2008) 352–358.

[4] K. Ohtsuka, K. Terayama, M. Yanagihara, et al., A radiological population study on the ossification of the posterior longitudinal ligament in the spine, Arch. Orthop. Trauma Surg. 106 (1987) 89–93.

[5] T. Tanaka, K. Ikari, K. Furushima, et al., Genomewide linkage and linkage disequilibrium analyses pinpoint the ossification of the posterior longitudinal ligament of the spine to COL6A1 locus on chromosome 21, Am. J. Hum. Genet. 73 (2003) 812–822.

[6] H. Li, L.S. Jiang, L.Y. Dai, Hormones and growth factors in the pathogenesis of spinal ligament ossification, Eur. Spine J. 16 (2007) 1075–1084.

[7] K.-I. Furukawa, Current topics in pharmacological research on bone metabolism: molecular basis of ectopic bone formation induced by mechanical stress, J. Pharmacol. Sci. 100 (2006) 201–204.

[8] D.J. Prockop, Marrow stromal cells as stem cells for nonhematopoietic tissues, Science 276 (1997) 71–74.

[9] M.F. Pittenger, A.M. Mackay, S.C. Beck, et al., Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.

[10] C. De Bari, F. Dell'Accio, P. Tylzanowski, et al., Multipotent mesenchymal stem cells from adult human synovial membrane, Arthritis Rheum. 44 (2001) 1928–1942.

[11] M. Crisan, S. Yap, L. Casteilla, et al., A perivascular origin for mesenchymal stem cells in multiple human organs, Cell Stem Cell 11 (2008) 301–313.

[12] Y. Segawa, T. Muneta, H. Makino, et al., Mesenchymal stem cells derived from synovium, meniscus, anterior cruciate ligament, and articular chondrocytes share similar gene expression profiles, J. Orthop. Res. 27 (2009) 435–441.

[13] S. Wakitani, K. Imoto, T. Yamamoto, et al., Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees, Osteoarthritis Cartilage 10 (2002) 199–206.

[14] P.P. Lui, Y.M. Wong, Y.F. Rui, et al., Expression of chondro-osteogenic BMPs in ossified failed tendon healing model of tendinopathy, J. Orthop. Res. 29 (2011) 816–821.
[15] Y.F. Rui, P.P. Lui, L.S. Chan, et al., Does erroneous differentiation of tendonderived stem cells contribute to the pathogenesis of calcifying tendinopathy?, Chin Med. J. (Engl.) 124 (2011) 606–610.
[16] Y.F. Rui, P.P. Lui, M. Ni, et al., Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells, J. Orthop. Res. 29 (2011) 390–396.
[17] Y.T. Chen, J.D. Wei, J.P. Wang, et al., Isolation of mesenchymal stem cells from human ligamentum flavum: implicating etiology of ligamentum flavum hypertrophy, Spine (Phila Pa 1976) 36 (2011) E1193–E1200.
[18] Y. Yamamoto, K.-I. Furukawa, K. Ueyama, et al., Possible roles of CTGF/Hcs24 in the initiation and development of ossification of the posterior longitudinal ligament, Spine (Phila Pa 1976) 27 (2002) 1852–1857.
[19] J. Song, J. Mizuno, Y. Hashizume, et al., Immunohistochemistry of symptomatic hypertrophy of the posterior longitudinal ligament with special reference to ligamentous ossification, Spinal Cord 44 (2006) 576–581.
[20] T. Asari, K.-I. Furukawa, S. Tanaka, et al., Mesenchymal stem cell isolation and characterization from human spinal ligaments, Biochem. Biophys. Res. Commun. (2012) 1193–1199.
[21] M. Dominici, K. Le Blanc, I. Mueller, et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.
[22] L. da Silva Meirelles, A.I. Caplan, N.B. Nardi, In search of the in vivo identity of mesenchymal stem cells, Stem Cells 26 (2008) 2287–2299.
[23] A.F. Steinert, M. Kunz, P. Prager, et al., Mesenchymal stem cell characteristics of human anterior cruciate ligament outgrowth cells, Tissue Eng. Part A 17 (2011) 1375–1388.
[24] V.L. Bautch, Stem cells and the vasculature, Nat. Med. 17 (2011) 1437–1443.
[25] G.D.M. Collett, A.E. Canfield, Angiogenesis and pericytes in the initiation of ectopic calcification, Circ. Res. 96 (2005) 930–938.
[26] V.Y. Lounev, R. Ramachandran, M.N. Wosczyna, et al., Identification of progenitor cells that contribute to heterotopic skeletogenesis, J. Bone Joint Surg. Am. 91 (2009) 652–663.
[27] A.I. Caplan, All MSCs are pericytes?, Cell Stem Cell 3 (2008) 229–230
[28] M. Tanno, K.-I. Furukawa, K. Ueyama, S. Harata, et al., Uniaxial cyclic stretch induces osteogenic differentiation and synthesis of bone morphogenetic proteins of spinal ligament cells derived from patients with ossification of the posterior longitudinal ligaments, Bone 33 (2003) 475–484.
[29] H. Ohishi, K.-I. Furukawa, K. Iwasaki, et al., Role of prostaglandin I2 in the gene expression induced by mechanical stress in spinal ligament cells derived from patients with ossification of the posterior longitudinal ligament, J. Pharmacol. Exp. Ther. 305 (2003) 818–824.
[30] K. Iwasaki, K.-I. Furukawa, M. Tanno, et al., Uni-axial cyclic stretch induces Cbfa1 expression in spinal ligament cells derived from patients with ossification of the posterior longitudinal ligament, Calcif. Tissue Int. 74 (2004) 448–457.
[31] T. Iwasawa, K. Iwasaki, T. Sawada, et al., Pathophysiological role of endothelin in ectopic ossification of human spinal ligaments induced by mechanical stress, Calcif. Tissue Int. 79 (2006) 422–430.
[32] M. Kishiya, T. Sawada, K. Kanemaru, et al., A functional RNAi screen for Runx2-regulated genes associated with ectopic bone formation in human spinal ligaments, J. Pharmacol. Sci. 106 (2008) 404–414.
[33] K. Uchida, T. Yayama, H.K. Cai, et al., Ossification process involving the human thoracic ligamentum flavum: role of transcription factors, Arthritis Res. Ther. 13 (2011) R144.

J Orthop Sci (2013) 18:121–129
DOI 10.1007/s00776-012-0329-2

ORIGINAL ARTICLE

Evaluation of locomotive disability using loco-check: a cross-sectional study in the Japanese general population

Eiji Sasaki · Yasuyuki Ishibashi · Eiichi Tsuda · Atsushi Ono · Yuji Yamamoto · Ryo Inoue · Ippei Takahashi · Takashi Umeda · Shigeyuki Nakaji

Received: 20 April 2012 / Accepted: 12 October 2012 / Published online: 1 November 2012

Abstract
Background The purposes of this study were to reveal the prevalence of locomotive syndrome (LS) evaluated by loco-check in the Japanese general population and to analyze the relationship between radiographic knee osteoarthritis (OA) and lumbar spondylosis, metabolic syndrome and LS. Furthermore, we evaluated LS according to functional examinations.
Methods Seven hundred twenty-two volunteers aged 56.6 ± 13.6 years participated in the Iwaki Health Promotion Project in 2010 and were classified into two groups: LS (one or more disabilities) or non-LS (no disability) according to the criteria of LS proposed by the Japanese Orthopaedic Association. Radiographic knee OA and lumbar spondylosis were defined according to the Kellgren-Lawrence grade. Metabolic syndrome was defined as the presence of two or more risk factors in addition to visceral obesity. The prevalence of LS associated with knee OA, lumbar spondylosis and metabolic syndrome was compared statistically. Also, data of six functional examinations were compared between the non-LS and LS groups.
Results The prevalence of LS was 21.2 % in males and 35.6 % in females and increased with aging regardless of gender. The prevalence of LS with knee OA was 48.7 %, with lumbar spondylosis was 33.8 %, and with metabolic syndrome was 43.4 %. The non-LS group had significantly better performance in the functional reach and sit and reach tests than the LS group in males and females by age-adjusted comparison.
Conclusion The prevalence of LS in the general population was higher in females than in males. A strong risk factor for LS was radiographic knee OA. Also, those with LS had loss of skeletal muscle mass, balancing and flexibility. This study showed that evaluation by loco-check was an acceptable tool to detect the early stage of locomotive disability for LS, and interventional prevention for strength, balancing and flexibility would be helpful for those with LS.

E. Sasaki (✉) · Y. Ishibashi · E. Tsuda · A. Ono · Y. Yamamoto · R. Inoue
Department of Orthopaedic Surgery, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
e-mail: e_sasaki@cc.hirosaki-u.ac.jp

E. Sasaki · I. Takahashi · T. Umeda · S. Nakaji
Department of Social Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Introduction

Aging increases locomotive disability associated with loss of muscle strength and balance [1]. These disabilities cause falling easily and fractures, and they can make the elderly become bedridden. The Japanese National Livelihood Survey showed that people aged 65 years or older accounted for 22 % of the population [2], and the country is now facing the advent of a super-aged society earlier and more rapidly than other countries in the world. Furthermore, the causes for using nursing care insurance in 21.5 % of the entire population were articular disease, fractures and fallings. Lower functional activities and performance levels were associated with higher medical care costs and hospitalization days among Japanese community-dwelling elderly individuals [3].

Recently, Nakamura suggested criteria for locomotive syndrome (LS) including seven items using loco-check [4] to assess the locomotive disability based on the activities of

daily living (ADL). However, its validity and relationship with diseases and individual physical function are not clear; meanwhile, Seichi et al. [5] reported the availability of the 25-question Geriatric Locomotive Function Scale to evaluate locomotive disabilities. Although those with severe limitations in ADL visit hospitals, those with mild locomotive disability and postural instability are not followed in the hospital because of the lack of awareness and understanding concerning locomotive disability. Now, it is necessary to decrease the number of bedridden patients or people requiring nursing care; therefore, a new useful tool for early and easy detection of locomotive disability is needed.

Osteoarthritis (OA) and spondylosis are major public health problems among the elderly that affect ADL and quality of life, leading to increased morbidity and mortality [6]. In fact, some researchers reported knee OA and lumbar spondylosis cause locomotive disability, particularly affecting walking ability [7, 8]. However, the prevalence of LS in the Japanese general population is not clear, nor is how knee OA and lumbar spondylosis affect it. Furthermore, while LS is affected by balance, osteoporosis, degenerative diseases such as OA, spondylosis and other chronic diseases, few long-term or multilateral studies have investigated the locomotive disability.

The purposes of this study were to reveal the prevalence of LS evaluated by loco-check in the Japanese general population and to analyze the relationship between radiographic knee OA and lumbar spondylosis, metabolic syndrome and LS. Furthermore, we evaluated LS according to functional examinations.

Materials and methods

A total of 929 volunteers from among 12,000 residents participated in the Iwaki Health Promotion Project in 2010 [9, 10]. This annual project, running since since 2005, is a community-based program to improve average life expectancy by performing general health checkups of the general population in the Iwaki area of Hirosaki city located in western Aomori prefecture, Japan. Physicians, surgeons, orthopedists, gynecologists, urologists, and psychiatrists from Hirosaki University Hospital are involved in this project to investigate diseases and disorders in various fields.

Subjects

All participants answered questionnaires to find those with LS. Patients with a past history causing locomotive disabilities such as stroke, cerebral bleeding, malignant disease, chronic lung disease and ischemic cardiac disease, were excluded so as to investigate locomotive disability focusing on the disability in those without internal diseases. Further exclusion criteria for this study were postoperative patients who had undergone surgery for knee and spine diseases and those not completing the questionnaires. Finally, 727 participants, 264 males and 463 females, were included in this study. The mean ages in male and female participants were 56.3 ± 14.1 (21–86) years and 56.7 ± 13.2 (21–87) years, respectively, and there was no statistically significant difference between them ($p = 0.839$). These subjects included 223 participants aged over 65 years old. Further questionnaires on lifestyle habits such as drinking, smoking and exercise, presence of a marriage partner and level of education were administered.

Anthropometric measurement in LS

The concept and purpose of LS as proposed by Nakamura are to identify a pre-dysfunction group before these individuals require assistance through health screening for locomotive disability [4]. Health screening is done by using self-completed questionnaires called the "loco-check." The loco-check consists of the following seven statements: (1) You cannot put on a pair of socks while standing on one leg. (2) You stumble or slip in your house. (3) You need to use a handrail when going up stairs. (4) You cannot get across the road at a crossing before the traffic light changes. (5) You have difficulty walking continuously for 15 min. (6) You find it difficult to walk home carrying a shopping bag weighing about 2 kg. (7) You find it difficult to do housework requiring physical strength. In this study, participants who check one or more statements are defined as having have LS.

The physical characteristics of participants were investigated regarding height, weight, individual body resistance at 50 kHz (R) using the Tanita MC-190 body composition analyzer (Tanita Corp., Tokyo, Japan) and waist circumference at the navel level. Body mass index (BMI) and skeletal muscle (SM) mass were also calculated. Janssen's regression equation to estimate SM mass was based on the evaluation of the relationship between bioelectrical impedance analysis (BIA) and SM measured by MRI [11]. SM mass (kg) = $[(Ht^2/R \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102$, where Ht is in centimeters; R is in ohms; for gender, men = 1 and women = 0; age is in years. The R^2 and SEE values of the regression equation were 0.86 and 2.7 kg or 9 %, respectively. Furthermore, the skeletal muscle index (SMI) was calculated by SM/$Ht^2 \times 10^2$ (kg/m^2) for standardization of differences influenced by height.

Radiographic knee and lumbar OA

Weight-bearing and anterior-posterior radiographs of the bilateral knees and lateral view of the lumbar spine

including intervertebral levels from L1–L2 to L5–S1 of participants were taken. All knee radiographs were graded by two trained orthopedic surgeons without knowledge about the participants. If their findings were dissociated, they came to a conclusion after consultation with each other. The severity of each knee and intervertebral level was scored based on the Kellgren-Lawrence grade (K-L grade) [12]. The presence of radiographic OA was defined as a K-L grade of 2, 3 or 4. Participants were classified into two groups, OA or non-OA, depending on their worst knee. Also, the presence of radiographic spondylosis was defined as K–L grade 2 or more in at least one intervertebral level.

Metabolic syndrome

Metabolic syndrome was defined according to the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome, with a slight modification, as the presence of two or more risk factors in addition to visceral obesity (waist circumstance: $\geq$85 cm for men and $\geq$90 cm for women). Risk factors are high blood pressure, dyslipidemia (high triglyceride and/or low HDL cholesterol) and hyperglycemia. Instead of fasting blood glucose, HbA1c, which is more sensitive for the diagnosis of hyperglycemia than fasting glucose, was used in this study. The criterion for each risk factor was as follows: high blood pressure, systolic blood pressure $\geq$130 mmHg and/or diastolic blood pressure $\geq$85 mmHg; low HDL cholesterol, HDL cholesterol <40 mg/dl for men and <50 mg/dl for women; high triglyceride, triglyceride $\geq$150 mg/dl; high HbA1c, HbA1c >5.8 %. Subjects under treatment for hypertension, dyslipidemia and diabetes mellitus were also included in the above definitions of risk factors [13]. Blood pressure was measured once by trained nurses after resting in the sitting position. Blood samples were taken in all participants before breakfast in the early morning. Serum triglyceride, serum HDL cholesterol and hemoglobin A1c (HbA1c) were measured by enzymatic methods.

Functional examinations

Six functional examinations were made. The functional reach test (average of three times) can detect the dynamic balance in elderly individuals related to maintaining postural stability during movement [14]. The one-leg standing with eyes opened test (best data from three times), which evaluates the ability to maintain equilibrium during a transition to a small base of support over 10 s, provides the highest combination of sensitivity and specificity for a history of one or more falls [15]. The timed up and go test (best data of two times) evaluates the ability to rise from a seated position in a chair, walk 3 m, turn and walk back, returning to a seated position [16]. Grasping power (two times), the sit and reach test (two times) and response to a falling stick (five times) were evaluated as average values.

Statistical analysis

Data input and calculation were performed with SPSS, version 12.0J (SPSS Inc., Chicago, IL, USA). Age, height, body weight, BMI, waist circumference, SM, SMI, knee and lumbar pain, and lifestyle habits were compared between the LS and non-LS groups by using Mann-Whitney U test and χ^2 test. In the 223 people aged over 65 years old [5], comparisons of positive loco-check rates with or without knee OA, spondylosis and metabolic syndrome were performed using the χ^2 test. Mean values and corrected values by age of the six functional examinations were compared between the LS and non-LS groups using the Bonferroni test. Furthermore, to evaluate the relative risk for LS, logistic regression analysis was performed with the presence of LS as an independent variable and age, BMI, pain, lifestyle habits, presence of a marriage partner and education level as dependent variables. A p value below 0.05 was considered significant in all analyses.

Results

Anthropometric measurements of LS

Fifty-six out of 264 (21.2 %) males and 165 out of 463 (35.6 %) females were classified into the LS group. The prevalence of LS was significantly higher in females than in males ($p < 0.001$). The prevalence of LS tended to increase with age in both males and females (Fig. 1). The anthropometric features of LS were lower body weight ($p = 0.026$), lower SM ($p < 0.001$) and lower SMI ($p = 0.035$) in males and higher BMI, higher waist circumference and lower SM in females ($p < 0.001$, respectively) (Table 1).

Radiographic knee OA and lumbar spondylosis

Presence of radiographic knee OA occurred in 33 (12.5 %) males and 160 (34.6 %) females, and they were classified into the OA group. Also, in the radiographs of the lumbar spine, the presence of radiographic spondylosis was detected in 226 (85.6 %) males and 368 (79.5 %) females. The prevalence of radiographic knee OA was significantly higher in females than in males ($p < 0.001$), and the prevalence of radiographic spondylosis was significantly higher in males than in females ($p = 0.040$). The prevalence of knee OA and spondylosis tended to increase with age, and especially in knee OA, the prevalence prominently increased in both males and females over age 60 (Figs. 2,

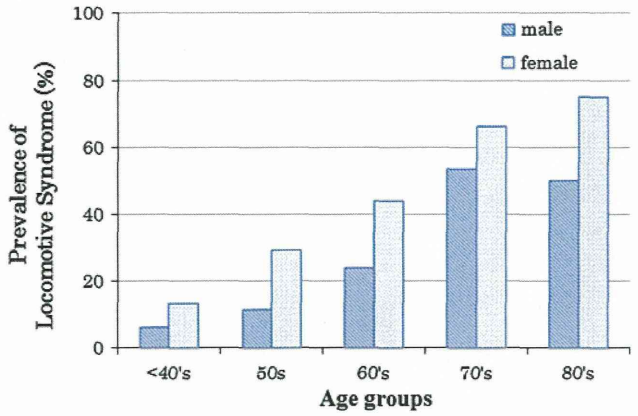

Fig. 1 The prevalence of locomotive syndrome among age groups

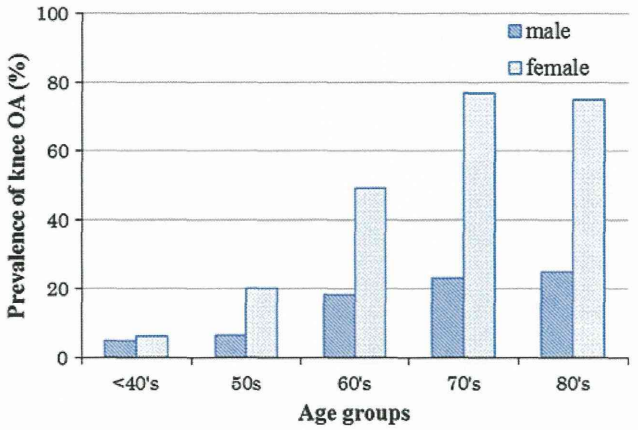

Fig. 2 The prevalence of knee OA among age groups

Table 1 Comparison of characteristics between non-LS and LS patients

	Male			Female		
	Non-LS (n = 208)	LS (n = 56)	p value	Non-LS (n = 298)	LS (n = 165)	p value
Age (years old)	53.7 ± 13.6	66.1 ± 12.0	0.000	52.8 ± 12.9	63.6 ± 10.8	0.000
Height (cm)	167.5 ± 6.8	164.0 ± 6.5	0.000	155.5 ± 5.7	151.8 ± 6.4	0.000
Body weight (kg)	66.2 ± 10.0	62.5 ± 8.0	0.026	53.6 ± 8.1	54.2 ± 8.5	0.497
BMI (kg/m^2)	23.6 ± 3.0	23.6 ± 3.0	0.517	22.2 ± 3.0	23.5 ± 3.4	0.000
Waist circumference (cm)	83.3 ± 8.1	83.3 ± 6.6	0.705	79.3 ± 8.9	83.3 ± 9.5	0.000
SM (kg)	25.6 ± 3.1	23.8 ± 2.8	0.000	16.3 ± 2.1	15.5 ± 2.1	0.000
SMI (kg/m^2)	9.1 ± 0.8	8.8 ± 0.7	0.035	6.7 ± 0.7	6.7 ± 0.7	0.638
Knee pain (%)	20.2	32.1	0.058	30.2	63.0	0.000
Low back pain (%)	42.3	57.1	0.048	37.9	60.6	0.000
Drinking habit (%)	73.1	76.8	0.575	30.9	18.2	0.003
Smoking habit (%)	32.2	30.4	0.791	11.1	7.9	0.271
Fitness habit (%)	34.6	35.7	0.878	36.6	29.1	0.103

The age, height, body weight, BMI, waist circumference, SM and SMI values are the mean ± SD. Knee pain, low back pain, drinking, smoking and fitness habit data are the percentage. p values below 0.05 indicate a significant difference of the mean value between male and female non-LS and LS groups using the Mann-Whitney U test and the pain and lifestyle habit data using the χ^2 test, respectively

3). The positive rates of all LS items except for "You cannot put on a pair of socks while standing on one leg," "You cannot get across the road at a crossing before the traffic light changes" and "You have difficulty walking continuously for 15 min" were significantly higher in the OA group than in the non-OA group. Regarding the relationship between spondylosis and LS, none of the LS items showed significant differences (Table 2).

Metabolic syndrome

The prevalence of metabolic syndrome was 17.0 % in males, 8.2 % in females and higher in males than in females (Fig. 4). Loco-check applied to metabolic syndrome was significant for the statements "You cannot put on a pair of socks while standing on one leg" and "You need to use a handrail when going upstairs" (Table 2). Except for diastolic pressure in females, there were no significant differences in the risk factors for metabolic syndrome between the LS and non-LS groups in age-adjusted comparisons (Table 3).

Functional examinations

Although the male non-LS group had significantly better results in all functional examinations than the LS group (timed up and go test: $p = 0.014$; the other tests: $p < 0.001$, respectively), there were significant differences only in the functional reach ($p = 0.030$) and sit and reach test ($p = 0.002$) by age-adjusted analysis (Table 4). In

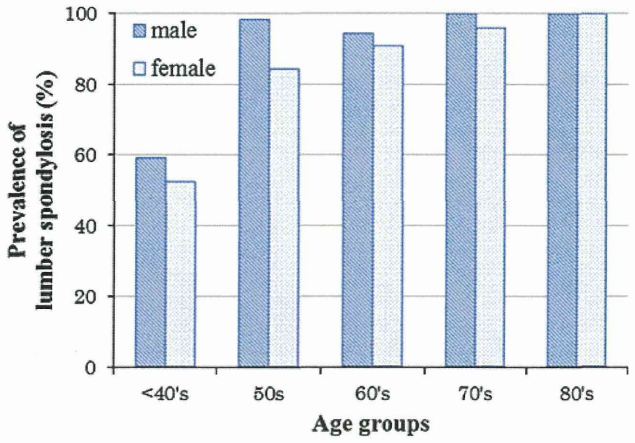

Fig. 3 The prevalence of lumbar spondylosis among age groups

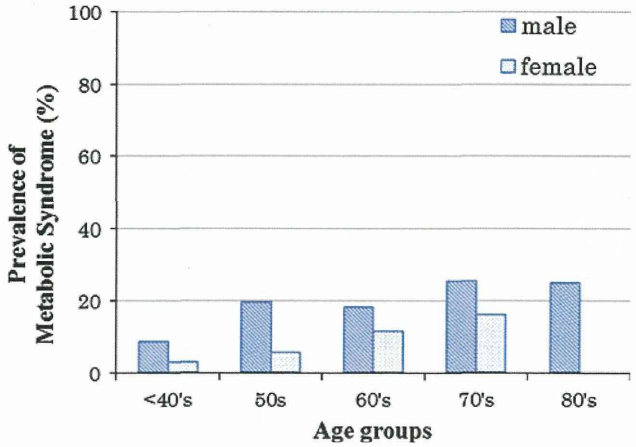

Fig. 4 The prevalence of metabolic syndrome among age groups

Table 2 Comparison of the positive rate of LS items in the knee and lumbar OA group and metabolic syndrome group

LS items	Knee OA (%)		Spondylosis (%)		Metabolic syndrome (%)	
	−	+	−	+	−	+
You cannot put on a pair of socks while standing on one leg	35.2	44.9	33.3	40.7	37.4	53.7†
You stumble or slip in your house	18.1	30.5†	11.1	25.2	23.1	31.7
You need to use a handrail when going upstairs	12.4	35.6†	11.1	25.2	22.0	36.6†
You cannot get across the road at a crossing before the traffic light changes	3.8	4.2	0.0	4.2	4.4	2.4
You have difficulty walking continuously for 15 min	5.7	11.0	0.0	8.9	9.3	4.9
You find it difficult to walk home carrying a shopping bag weighing about 2 kg	6.7	15.3†	11.1	11.2	10.4	14.6
You find it difficult to do housework requiring physical strength	7.6	22.0†	22.2	15.0	14.3	19.5
Prevalence of LS	45.7	63.6†	33.3	56.1	53.3	63.4

The values are the positive rate (%) of LS items

† p values below 0.05 indicate the significant difference of the mean value in the knee OA group, spondylosis group and metabolic syndrome group using the χ^2 test, respectively

females, although the non-LS group had significantly better results in five functional examinations than the LS group (response to a falling stick: $p = 0.002$; the other tests: $p < 0.001$, respectively) except for the sit and reach test ($p = 0.070$), there were significant differences except for in the response to a falling stick and timed up and go test by age-adjusted analysis.

Risk factors of LS

Odds ratios of gender, age, BMI, low back pain, knee pain, lifestyle habits, presence of a partner in marriage and education for the presence of LS were estimated using logistic regression analysis. Female gender, aging, obesity, low back pain and knee pain were the risk factors for LS. Among these risk factors, knee pain increased the risk of LS the most (Table 5).

Discussion

This study showed that the prevalence of LS evaluated by loco-check was higher in females than males. Also, the prevalence of LS increases with age regardless of gender, and this is absolutely so for locomotive disabilities because we strictly excluded chronic diseases such as stroke, cerebral bleeding, malignant disease, chronic lung disease and ischemic cardiac disease in order to evaluate the relationship between locomotive disability caused by musculoskeletal dysfunction and pain.

Determining which instruments best evaluate locomotive disability in the Japanese general population is controversial. Various tools detect locomotive disability [17]; generally, these are classified into basic ADL, instrumental activities of daily living (IADL) and mobility. The JOA proposed the LS criteria as a tool for health screening of

Table 3 Comparison of data of laboratory examinations and physiological examinations between non-LS and LS groups

	Male				Female			
	Non-adjusted		Adjusted		Non-adjusted		Adjusted	
	Non-LS	LS	Non-LS	LS	Non-LS	LS	Non-LS	LS
Hemoglobin A1c (%)	5.2 ± 0.6	5.4 ± 0.7	5.2 ± 0.0	5.2 ± 0.1	5.1 ± 0.4	5.3 ± 0.5*	5.2 ± 0.0	5.2 ± 0.0
Triglyceride G (mg/dl)	118.6 ± 105.6	97.3 ± 80.3*	117.3 ± 7.1	102.0 ± 14.2	78.2 ± 45.5	84.7 ± 41.4*	79.4 ± 2.6	82.6 ± 3.6
Total-cholesterol (mg/dl)	199.4 ± 33.5	193.4 ± 30.3	199.4 ± 2.3	193.6 ± 4.6	203.0 ± 33.0	205.9 ± 29.6	205.2 ± 1.9	202.0 ± 2.6
HDL-cholesterol (mg/dl)	59.5 ± 15.3	59.4 ± 13.8	59.9 ± 1.0	59.5 ± 2.1	68.8 ± 16.4	65.1 ± 15.2*	68.5 ± 1.0	65.8 ± 1.3
LDL-cholesterol (mg/dl)	114.9 ± 30.0	112.3 ± 24.9	115.0 ± 2.0	111.8 ± 4.1	114.6 ± 28.8	120.3 ± 25.9*	116.9 ± 1.6	116.1 ± 2.2
Systolic blood pressure (mmHg)	127.6 ± 18.9	136.6 ± 18.4*	129.1 ± 1.2	131.0 ± 2.4	122.2 ± 20.2	129.4 ± 20.4*	125.3 ± 1.1	123.9 ± 1.5
Diastolic blood pressure (mmHg)	74.9 ± 11.9	74.4 ± 10.3	75.0 ± 0.8	73.8 ± 1.6	70.3 ± 12.9	70.3 ± 12.3	71.3 ± 0.7	68.6 ± 1.0*

All values are the mean ± standard deviation and corrected mean ± standard error of the mean adjusted by age. Differences between LS and non-LS groups were evaluated by the Bonferroni method regarding non-adjusted data and adjusted data, respectively. p values below 0.05 indicate the significant difference of the mean value (*) between the non-LS and LS groups

Table 4 Comparison of physical performance data between non-LS and LS groups

	Male				Female			
	Non-adjusted		Adjusted		Non-adjusted		Adjusted	
	Non-LS	LS	Non-LS	LS	Non-LS	LS	Non-LS	LS
Grasping power (kg)	44.7 ± 7.9	38.7 ± 7.9*	43.8 ± 0.5	41.9 ± 0.9	27.9 ± 4.4	25.4 ± 4.5*	27.4 ± 0.3	26.2 ± 0.3*
Floor-seated anterior bend test (cm)	41.8 ± 10.5	34.8 ± 12.0*	42.7 ± 0.8	37.1 ± 1.5*	45.4 ± 8.0	43.4 ± 10.0	46.6 ± 0.5	44.2 ± 0.7*
Response to a falling stick (cm)	28.0 ± 5.5	31.8 ± 6.2*	28.5 ± 0.4	30.1 ± 0.7	29.0 ± 5.5	30.8 ± 5.1*	29.7 ± 0.3	29.7 ± 0.4
Functional reach (cm)	34.3 ± 5.5	30.1 ± 5.5*	33.8 ± 0.3	32.1 ± 0.7*	32.9 ± 4.5	30.2 ± 5.1*	32.4 ± 0.3	31.1 ± 0.4*
One-leg standing with eye opening (s)	62.6 ± 18.6	50.3 ± 25.7*	61.1 ± 1.3	55.7 ± 2.7	64.5 ± 15.8	50.0 ± 27.9*	61.7 ± 1.1	54.7 ± 1.6*
Timed up and go test (s)	9.1 ± 4.5	9.5 ± 1.9*	9.3 ± 0.3	9.1 ± 0.6	8.7 ± 3.1	9.4 ± 1.6*	8.8 ± 0.2	9.2 ± 0.2

All values are the mean ± standard deviation and corrected mean ± standard error of the mean adjusted by age. Differences between LS and non-LS groups were evaluated by the Bonferroni method regarding non-adjusted data and adjusted data, respectively. p values below 0.05 indicate a significant difference of the mean value (*) between the non-LS and LS groups

locomotive disability in order to detect early disability, prevent patients from becoming bedridden and reduce the cost of medical or nursing care. Loco-check mainly measures IADL and mobility, and it makes possible identifying a pre-dysfunction state in daily living before an individual requires assistance. However, no previous reports have described the reliability and validity of loco-check. However, Seichi et al. [5] reported that a 25-question geriatric locomotive function scale is a reliable and valid system for evaluating locomotive disabilities. Unpublished data indicate a positive rate for loco-check, showing very high sensitivity based on the 25-question geriatric locomotive function scale as a screening tool according to our statistical analysis.

Table 5 Evaluation of odds ratios of LS according to physical feature, pain, lifestyle habits, presence of a partner in marriage and education

	p value	Odds ratio	95 % CI
Gender	0.004	2.057	1.266–3.341
Age (years old)	<0.001	1.082	1.062–1.103
BMI (kg/m^2)	0.033	1.068	1.005–1.134
Low back pain	0.001	1.869	1.286–2.717
Knee pain	<0.001	2.708	1.850–3.965
Smoking habit	0.085	1.632	0.935–2.849
Drinking habit	0.730	0.925	0.596–1.437
Exercise habit	0.103	0.713	0.475–1.071
A marriage partner	0.829	1.050	0.673–1.638
Level of education	0.872	0.983	0.803–1.204

Logistic regression analysis was performed with the presence of LS as a dependent variable and gender, age, BMI, low back pain, knee pain, smoking, drinking and fitness habits, presence of a partner in marriage and education as the independent variables. A *p* value below 0.05 was considered significant

Knee pain and low back pain were significantly related to LS. Knee OA decreased the range of motion and quadriceps strength and increased knee pain and self-reported limitation in activities [18]. Furthermore, elderly with radiographic OA accompanied by frequent pain had difficulty in stair climbing, walking a mile and housekeeping. Our results corroborated that being in the knee OA group had a greater impact on walking disabilities such as climbing stairways, balancing on one leg, and having a tendency to fall down or slip in the house; these patients had difficulties with housekeeping and carrying a burden in ADL. Also, the result that low back pain was a risk factor for LS is supported by a previous report that disc degeneration was strongly related to low back pain and individual activities such as having difficulty standing up from a chair or one-leg standing [8].

Being in the LS group was significantly related to the loss of skeletal muscle mass. It is considered that sarcopenia, defined as an age-related loss in skeletal muscle mass by Rosenberg [19], plays a very important role in locomotive disability in the elderly [20]. Reduced muscle strength increases body sway, gait disturbance and risk of falls [21]. Also, metabolic syndrome could be a good predictor of locomotive disability. Obesity is a very important risk factor of both knee OA and metabolic syndrome. Previous study has showed that metabolic syndrome is related to knee OA [6, 10]. In this study, the association between metabolic syndrome and LS items was significant in one-leg standing and stair climbing, and these results are related to the presence of obesity in those with metabolic syndrome.

Balancing and flexibility in the elderly were important factors for LS according to age-adjusted analysis. Non-adjusted data of all six functional examinations in the LS group also showed worse results than in the non-LS group in the study by Yoshimura [22]. These results suggested that the LS group would have problems not only in walking ability and muscle strength, but also in the balancing and flexibility needed for smooth responses to various ADLs, although aging had a major impact on these physical factors. Previous studies focused on evaluation of gait ability and found that reduced walking speed affects mortality; the same can be said for grasping power as a sign of systemic muscle strength [23]. Results of this study showed the importance of balancing and flexibility not only for walking ability but also for muscle strength. This suggested that improving balance and flexibility could contribute to preventing locomotive disability using interventional approaches. This is supported by a report from Lord [24] showing that 12 months of balance exercise can reduce the frequency of falls for women aged 60–85 years. Furthermore, 2 years of home interventional exercise decreased mortality significantly, with risk of dying rates eight times lower than in controls [25]. Early detection and appropriate intervention for locomotive disability are the most important factors for managing it.

There were several limitations. First, other joints involved in OA were not evaluated in this study. Joint pain is considered an important factor causing locomotive disability [7, 26, 27]. Buchman et al. [28] suggested that the risk of disability increases with the number of painful joints (neck, back, hand, hip, knee or foot pain). Second, this study was performed in a limited region, which may not be representative of Japan as a whole. Muraki et al. [29] reported that dwelling in a mountainous community was a risk factor for knee OA in the ROAD study. Third, because this study was cross sectional, the natural course of LS was not discussed. We think the number of people with LS having severe disability needs to be determined as well as how many are in a bedridden condition. The mortality rate could also be assessed by a longitudinal cohort study. Finally, possibly differences in results were less than estimated because of a selection bias as a result of the many voluntary participants who may be more health consciousness.

Despite these limitations, this general population-based study clearly showed that LS according to loco-check was strongly associated with knee OA and knee pain. Those who had radiographic knee OA were at risk of incurring locomotive disability. Furthermore, this is the first study, as far as we know, to provide broad-ranging analysis of locomotive syndrome. It is suggested that the LS criteria proposed by the JOA can be used as an acceptable tool to detect the early stage of locomotive disability.

Conclusions

The prevalence of LS in the general population was higher in females than in males. A strong risk factor for LS was the presence of radiographic knee OA. Those with LS had loss of skeletal muscle mass, balancing and flexibility. This study showed that LS evaluated by loco-check was an acceptable tool to detect the early stage of locomotive disability.

Acknowledgments This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 18200044), Japanese Society for the Promotion of Science (no. 21500676) and JOA-Subsidized Science Project Research from the Japanese Orthopaedic Association.

Conflict of interest The authors declare no conflict of interest.

References

1. Daubney ME, Culham EG. Lower-extremity muscle force and balance performance in adults aged 65 years and older. Phys Ther. 1999;79:1177–85.
2. Ministry of Health, Labour and Welfare. Outline of the result of National Livelihood Survey 2007. http://www.mhlw.go.jp/toukei/saikin/hw/k-tyosa/k-tyosa07/4-2.html.
3. Yang G, Niu K, Fujita K, Hozawa A, Ohmori-Matsuda K, Kuriyama S, Nakaya N, Ebihara S, Okazaki T, Guo H, Miura C, Nagatomi R. Impact of physical activity and performance on medical care costs among the Japanese elderly. Geriatr Gerontol Int. 2011;11:157–65.
4. Nakamura K. The concept and treatment of locomotive syndrome: its acceptance and spread in Japan. J Orthop Sci. 2011; 16:489–91.
5. Seichi A, Hoshino Y, Doi T, Akai M, Tobimatsu Y, Iwaya T. Development of a screening tool for risk of locomotive syndrome in the elderly: the 25-question Geriatric Locomotive Function Scale. J Orthop Sci. 2012;17:163–72.
6. Yoshimura N, Muraki S, Oka H, Kawaguchi H, Nakamura K, Akune T. Association of knee osteoarthritis with the accumulation of metabolic risk factors such as overweight, hypertension, dyslipidemia, and impaired glucose tolerance in Japanese men and women: the ROAD study. J Rheumatol. 2011;38:921–30.
7. Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum. 1998;41:778–99.
8. Ohtori S, Ito T, Yamashita M, Murata Y, Morinaga T, Hirayama J, Kinoshita T, Ataka H, Koshi T, Sekikawa T, Miyagi M, Tanno T, Suzuki M, Aoki Y, Aihara T, Nakamura S, Yamaguchi K, Tauchi T, Hatakeyama K, Takata K, Sameda H, Ozawa T, Hanaoka E, Suzuki H, Akazawa T, Suseki K, Arai H, Kurokawa M, Eguchi Y, Suzuki M, Okamoto Y, Miyagi J, Yamagata M, Toyone T, Takahashi K. Evaluation of low back pain using the Japanese Orthopaedic Assiciation Back Pain Evaluation Questionnaire for lumbar spinal disease in a multicenter study: differences in scores based on age, sex, and type of disease. J Orthop Sci. 2010;15:86–91.
9. Inoue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, Takahashi I, Danjo K, Umeda T, Nakaji S, Toh S. Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. Osteoarthr Cartil. 2011;19:51–7.
10. Inoue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, Takahashi I, Danjo K, Umeda T, Nakaji S, Toh S. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the Japanese general population. J Orthop Sci. 2011;16:704–9.
11. Janssen I, Heymsfield SB, Baumgartner RN, Ross R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol. 2000;89:465–71.
12. Kellgren JH, Lawrence RC. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16:494–502.
13. Wakabayashi I. Cross-sectional relationship between alcohol consumption and prevalence of metabolic syndrome in Japanese men and women. J Atheroscler Thromb. 2010;30:695–704.
14. Duncan PW, Weiner DK, Chandler J, Studenski S. Functional reach: a new clinical measure of balance. J Gerontol. 1990;45: M192–7.
15. Jacobs JV, Horak FB, Tran VK, Nutt JG. Multiple balance tests improve the assessment of postural stability in subjects with Parkinson's disease. J Neurol Neurosurg Psychiatry. 2006;77: 322–6.
16. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc. 1991;39:142–8.
17. Rodrigues MA, Facchini LA, Thumé E, Maia F. Gender and incidence of functional disability in the elderly. Cad Saude Publica. 2009;25(Suppl 3):S464–76.
18. van Dijk GM, Veenhof C, Spreeuwenberg P, Coene N, Burger BJ, van Schaardenburg D, van den Ende CH, Lankhorst GJ, Dekker J. Prognosis of limitations in activities in osteoarthritis of the hip or knee: a 3-year cohort study. Arch Phys Med Rehabil. 2010;91:58–66.
19. Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr. 1997;127:990S–1S.
20. Pahor M, Kritchevsky S. Research hypotheses on muscle wasting, aging, loss of function and disability. J Nutr Health Aging. 1998;2:97–100.
21. Sinaki M, Brey RH, Hughes CA, Larson DR, Kaufman KR. Balance disorder and increased risk of falls in osteoporosis and kyphosis: significance of kyphotic posture and muscle strength. Osteoporos Int. 2005;16:1004–10.
22. Yoshimura N, Oka H, Muraki S, Akune T, Hirabayashi N, Matsuda S, Nojiri T, Hatanaka K, Ishimoto Y, Nagata K, Yoshida M, Tokimura F, Kawaguchi H, Nakamura K. Reference values for hand grip strength, muscle mass, walking time, and one-leg standing time as indices for locomotive syndrome and associated disability: the second survey of the ROAD study. J Orthop Sci. 2011;16:768–77.
23. Cooper R, Kuh D, Hardy R. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. BMJ. 2010;341:c4467.
24. Lord SR, Ward JA, Williams P, Strudwick M. The effect of a 12-month exercise trial on balance, strength, and falls in older women: a randomized controlled trial. J Am Geriatr Soc. 1995;43:1198–206.
25. Gitlin LN, Hauck WW, Dennis MP, Winter L, Hodgson N, Schinfeld S. MPH6 long-term effect on mortality of a home intervention that reduces functional difficulties in older adults: results from a randomized trial. J Am Geriatr Soc. 2009;57: 476–81.
26. McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Knee pain and disability in the community. Br J Rheumatol. 1992;31:189–92.
27. Holla JF, Steultjens MP, Roorda LD, Heymans MW, Ten Wolde S, Dekker J. Prognostic factors for the two-year course of activity

limitations in early osteoarthritis of the hip and/or knee. Arthritis Care Res. 2010;62:1415–25.
28. Buchman AS, Shah RC, Leurgans SE, Boyle PA, Bennett DA. Musculoskeletal pain and incident disability in community-dwelling older adults. Arthritis Care Res. 2010;62:1287–93.
29. Muraki S, Oka H, Akune T, Mabuchi A, En-yo Y, Yoshida M, Saika A, Suzuki T, Yoshida H, Ishibashi H, Yamamoto S, Nakamura K, Kawaguchi H, Yoshimura N. Prevalence of radiographic knee osteoarthritis and its association with knee pain in the elderly of Japanese population-based cohorts: the ROAD study. Osteoarthritis Cartilage. 2009;17:1137–43.