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Comparative Gene-Expression Analysis of the Ligamentum Flavum of Patients with Lumbar Spinal Canal Stenosis: Comparison between the Dural and Dorsal Sides of the Thickened Ligamentum Flavum

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Thickening of the ligamentum flavum is the main factor in the development of lumbar spinal canal stenosis (LSCS). Although previous studies have reported factors related to ligamentum flavum thickening, its etiology has not been clarified. Furthermore, it is often difficult to set proper controls to investigate the pathologies of thickening due to differences in patient characteristics, such as age, sex, obesity, and comorbidities. This study aimed to elucidate the pathologies of ligamentum flavum thickening by comparing the dural and dorsal sides of the thickened ligamentum flavum in patients with LSCS. Ligamentum flavum samples were collected from 19 patients with LSCS. The samples were divided into the dural and dorsal sides. The dural side was used as a control to assess the pathologies occurring on the dorsal side. Elastic Masson staining was used to assess the elastic fibres. Gene expression levels were comprehensively assessed using quantitative reverse transcription polymerase chain reaction and DNA microarray analyses. Gene ontology analysis was used to identify biological processes associated with differentially expressed genes. The elastic fibres were significantly decreased on the dorsal side of the thickened ligamentum flavum. Genes related to fibrosis, inflammation, tissue repair, remodeling, and chondrometaplasia, such as *COL1A2, COL3A1, COL5A1, TGFB1, VEGFA, TNFA, MMP2, COL10A1, and ADAMTS4,* were highly expressed on the dorsal side of the thickened ligamentum flavum. The biological processes occurring on the dorsal side of the thickened ligamentum flavum were extracellular matrix organization, cell adhesion, extracellular matrix disassembly, and proteolysis. These are considered important pathologies of ligamentum flavum thickening.

Keywords: genes; ligamentum flavum; lumbar spinal canal stenosis; microarray analysis; thickening
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Introduction

Lumbar spinal canal stenosis (LSCS) is a common disease among older individuals. Compression of nerves due to canal stenosis causes the associated symptoms (Szpalski and Gunzburg 2003). The ligamentum flavum is an elastic ligament connecting the upper and lower vertebrae and forms the posterior and lateral walls of the spinal canal (Schönström and Hansson 1991). Thickening of the ligamentum flavum is considered to be a key factor in the narrowing of the spinal canal, which leads to LSCS (Yoshida et al. 1992). Previous studies have reported that thickening of the ligamentum flavum is due to tissue hypertrophy or buckling (Altinkaya et al. 2011; Yabe et al. 2015, 2022), and that the thickened ligamentum flavum shows a loss of elastic fibres and an increase in collagen fibers (Kosaka et al. 2007; Yabe et al. 2016). These changes are mainly observed to occur on the dorsal side of the ligamentum fla-

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vum, which is thought to be due to the increased load applied on the dorsal layer during lumbar motion (Sairyo et al. 2005; Zhang et al. 2010). Previous reports have identified pathologies occurring in the thickened ligamentum flavum, such as fibrosis, chondrometaplasia, and inflammation, and have reported factors related to these pathologies (Yoshida et al. 1992; Park et al. 2005; Kosaka et al. 2007; Sairyo et al. 2007; Zhong et al. 2011). Furthermore, a few studies have used comprehensive approaches for genetic analysis of the thickened ligamentum flavum (Sairyo et al. 2007; Yabe et al. 2015; Duan et al. 2022; Hayashi et al. 2022).

Appropriate controls are needed to investigate the pathology of ligamentum flavum thickening. As it is difficult to obtain a normal ligamentum flavum, a non-thickened ligamentum flavum in patients with lumbar disc herniation (LDH) has generally been used as a control. However, patients with LDH were typically younger than those with LSCS in most previous reports analysing the pathologies of ligamentum flavum thickening (Lakemeier et al. 2013; Kamita et al. 2015; Duan et al. 2022). To overcome this limitation, some recent studies have used the dural side of the thickened ligamentum flavum as a control when assessing changes occurring on the dorsal side (Zhang et al. 2010; Saito et al. 2017; Hayashi et al. 2022). Saito et al. (2017) reported that no significant difference in the expression levels of genes related to fibrosis between the ligamentum flavum of LDH patients and the dural side of the ligamentum flavum of LSCS patients. This approach can be useful for clarifying the pathologies of thickening of the ligamentum flavum; however, the number of reports remains small. Thus, this study aimed to elucidate the pathology of ligamentum flavum thickening by comparing the dural and dorsal sides of the thickened ligamentum flavum using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and DNA microarray analysis.

Materials and Methods

This study included ligamentum flavum samples from 19 patients with LSCS who underwent decompressive surgery. The thickness of the ligamentum flavum was assessed using T1-weighted images of magnetic resonance imaging (MRI) as previously described. At the level of the facet joint, the ligamentum flavum was observed as a low-intensity mass on the ventral side of the facet joint, and the thickest portion of this slice was measured (Zhang et al. 2010). The study protocol was reviewed and approved by the institutional review board of our university (approval number: 2022-1-136).

Tissue preparation

The ligamentum flavum was removed en bloc during surgery. The samples were sagittally cut and fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.4). The paraffin-embedded tissue was cut into 5-μm-thick sections and stained using Elastica Masson staining to assess the elastic fibres. Ten areas, 0.25-mm^2 in size (five on the dural side and five on the dorsal side) were chosen from each ligamentum flavum for analysis. The area that stained dark purple within the region-of-interest, which corresponded to elastic fibres, was calculated using ImageJ 1.53 software (National Institutes of Health, Bethesda, MD, USA), as previously described (Fig. 1) (Yabe et al. 2015).

RNA extraction and purification

The 19 ligamentum flavum samples were divided into dural and dorsal sides (Hayashi et al. 2022), cut into small pieces, immersed in 3-ml TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), and immediately frozen in liquid nitrogen. Total RNA was extracted and purified as described previously (Yabe et al. 2015).

Quantitative reverse transcription polymerase chain reaction

Complementary DNA (cDNA) was synthesised using a cloned avian myeloblastis virus first-strand cDNA synthesis kit (Thermo Fisher Scientific). Gene expression was quantitatively assessed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using a LightCycler (Roche Diagnostics, Basel, Switzerland). PCR efficiencies and relative expression levels of genes encoding factors related to fibrosis, chondrometaplasia, and inflammation, relative to that of *EF1A1*, were calculated, as previously described (Pfaffl 2001). The primer sequences are shown in Supplementary Table S1.

DNA microarray analysis

Samples from six patients were used for DNA microarray analysis. Among those, samples from three patients were mixed and used as a "pooled average" sample for efficiency. DNA microarray analysis was performed and evaluated as previously described (Yamayoshi et al. 2009; Yabe et al. 2015). To characterize microarray expression profiles,

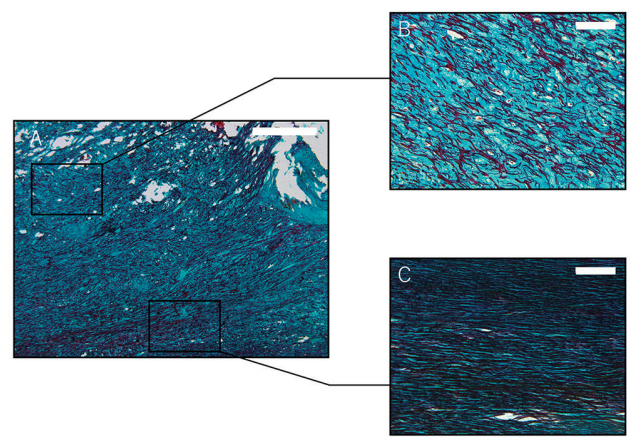

Fig. 1. Elastica Masson staining of the thickened ligamentum flavum (A-C).
Elastic fibres are decreased in the dorsal (B) as compared to the dural side (C) of the ligamentum flavum. Dark purple areas indicate elastic fibres. Scale bars: 500 μm (A), 100 μm (B and C).

Table 1. Demographic data of the samples.

	n	Mean ± SD	Range	P
Total	19			
Age		74.7 ± 6.9	62-86	
Sex				
Male	11			
Female	8			
Disc level				
L2/3	1			
L3/4	6			
L4/5	12			
Ligamentum flavum thickness		6.78 ± 1.17	5.11-8.74	
Ratio of elastic fibers (%)				
Dural side		58.29 ± 3.07	50.80-62.78	< 0.001
Dorsal side		42.46 ± 5.95	30.35-52.09	
DNA Microarray analysis	6			
Age		71.5 ± 5.02	62-77	
Sex				
Male	2			
Female	4			
Disc level				
L2/3	1			
L3/4	2			
L4/5	3			
Ligamentum flavum thickness		5.94 ± 0.57	5.11-6.87	
Ratio of elastic fibers (%)				
Dural side		58.23 ± 2.28	55.65-62.78	0.003
Dorsal side		44.64 ± 5.77	38.26-52.09	

probes whose expression was higher on the dorsal side than on the dural side in all samples and whose mean was more than 1.5-fold upregulated on the dorsal side were selected. The results of microarray analysis were used for Gene Ontology (GO) analysis to assess the biological processes occurring on the dorsal side of the ligamentum flavum. The false discovery rate was set to < 5% in the GO analysis.

Statistical analyses

Differences between the dural and dorsal sides of the ligamentum flavum were compared using a paired t-test (ratio of elastic fibres) and Wilcoxon signed-rank test (qRT-PCR). Data were expressed as mean ± standard deviation (SD). Tests were two-sided, and a P-value of < 0.05 was considered statistically significant.

Results

The demographic data of the participants are shown in Table 1. The mean age of the patients was 74.7 (range 62-86) years and the mean thickness of the ligamentum flavum was 6.78 (range 5.11-8.74) mm. The ratio of elastic fibres on the dorsal side (42.46 ± 5.95%; range 30.35-52.09%) was significantly smaller than that of those on the dural side (58.29 ± 3.07%; range 50.80-62.78%) of the ligamentum flavum (P < 0.001).

Gene expression related to fibrotic, inflammatory, and chondrogenic process

The gene expression levels of *COL1A2*, *COL3A1*, *TGFB1*, *TNFA*, *MMP2*, *COL10A1*, and *ADAMTS4* were significantly higher on the dorsal side than on the dural side of the ligamentum flavum. Expression levels of *COL1A1*, *CTGF*, *PDGFB*, *CYR61*, *IL1B*, *IL6*, *MMP1*, *MMP3*, *TIMP1*, *TIMP2*, *TIMP3*, *COL2A1*, *ACAN*, *ADAMTS5*, and *SOX9* did not differ significantly (Table 2).

Gene expression profiles by microarray analysis

A total of 110 genes were upregulated on the dorsal side of the ligamentum flavum (Supplementary Table S2), and representative genes among these are shown in Table 3. Notably, expression levels of fibrotic factors, such as *COL3A1*, *COL5A1*, and *VEGFA*, were higher on the dorsal side than on the dural side. Furthermore, we identified 10 GO biological process terms on the dorsal side of the ligamentum flavum including extracellular matrix organization, cell adhesion, extracellular matrix disassembly, and prote-

olysis (Table 4).

Discussion

The present study investigated gene expression levels on the dorsal side of the thickened ligamentum flavum as compared to those on the dural side, using qRT-PCR and DNA microarray analysis to clarify the pathology of ligamentum flavum thickening. We found that the elastic fibres were significantly decreased on the dorsal side of the thickened ligamentum flavum as compared to those on the dural side. Expression levels of genes related to fibrosis, inflammation, tissue repair, remodeling, and chondrometaplasia were increased significantly on the dorsal side compared to the dural side. Genes with increased expression were associated with biological processes of extracellular matrix organization, cell adhesion, extracellular matrix disassembly, and proteolysis.

The thickness of the ligamentum flavum increases with aging, along with fibrosis and loss of elastic fibres in the tissue (Sairyo et al. 2005). A previous report showed that fibrosis was more severe in the ligamentum flavum of LSCS patients than in that of LDH patients on both the dural and dorsal sides; however, the difference was much clearer on the dorsal side of the ligamentum flavum (Zhang et al. 2010). Another study also reported that fibrotic changes occurred with loss of elastic fibres in the ligamentum flavum of LDH and LSCS patients (Yabe et al. 2015). Saito et al. (2017) reported that the gene expression levels of factors related to fibrosis, such as *COL1A1*, *COL1A2*, *COL3A1*, *TGFB1*, *CTGF*, and *PDGFA*, were similar in the non-thickened ligamentum flavum and the dural side of the thickened ligamentum flavum, and were significantly higher in the dorsal side of the thickened ligamentum flavum. Although the dural side of the thickened ligamentum flavum shows mild fibrotic changes, we used it as a control, instead of the non-thickened ligamentum flavum, to investigate the pathologies of ligamentum flavum thickening. Thus, this uses paired comparisons and removes the effect of individual patient characteristics, such as age, sex, obesity, and comorbidities, such as diabetes mellitus, which are related to thickening of the ligamentum flavum (Altinkaya et al. 2011; Shemesh et al. 2018; Takashima et al. 2018). In this study, the mean ratio of elastic fibres was 42.96% on the dorsal side, which was significantly lower than that on the dural side (58.29%), indicating that more apparent pathological changes occurred on the dorsal side of the thickened ligamentum flavum.

Table 2. Gene expressions in the ligamentum flavum.

	Dural side	Dorsal side	
	Mean ± SD		P
Fibrosis			
COL1A1	6.07 ± 4.38	13.02 ± 18.50	0.31
COL1A2	3.30 ± 3.66	7.62 ± 6.83	*0.011*
COL3A1	3.46 ± 4.68	11.04 ± 13.51	*0.035*
TGFB1	1.37 ± 1.02	2.71 ± 1.85	*0.044*
CTGF	1.18 ± 0.82	1.41 ± 0.68	0.36
PDGFB	1.91 ± 2.87	4.59 ± 9.87	0.12
CYR61	1.52 ± 1.16	1.52 ± 1.17	0.82
Inflammation			
TNFA	1.63 ± 2.10	3.68 ± 2.60	*0.004*
IL1B	1.53 ± 1.30	1.83 ± 2.44	0.67
IL6	1.39 ± 0.96	1.17 ± 0.98	0.34
MMPs and TIMPs			
MMP1	5.36 ± 8.84	7.89 ± 10.64	0.084
MMP2	2.54 ± 3.62	9.61 ± 12.54	*0.025*
MMP3	1.96 ± 2.66	3.70 ± 6.38	0.088
TIMP1	1.86 ± 2.10	2.46 ± 2.51	0.42
TIMP2	1.26 ± 1.49	2.07 ± 4.59	0.50
TIMP3	4.12 ± 8.34	5.47 ± 11.31	0.16
Chondrogenesis			
COL2A1	2.14 ± 2.70	3.49 ± 3.42	0.25
COL10A1	1.88 ± 2.27	3.69 ± 3.16	*0.008*
ACAN	2.09 ± 1.79	4.22 ± 3.67	0.059
SOX9	2.47 ± 2.26	3.01 ± 4.09	0.97
ADAMTS4	1.59 ± 2.02	2.83 ± 1.91	*0.028*
ADAMTS5	3.53 ± 3.50	5.30 ± 5.32	0.20

Italic values significant at $P < 0.05$.

Previous studies have shown an increase in the expression of several types of collagens in the thickened as compared to the non-thickened ligamentum flavum (Yabe et al. 2015; Hur et al. 2017; Takeda et al. 2021). In particular, the levels of collagen types I and III show a positive correlation with the cross-sectional area of the ligamentum flavum (Takeda et al. 2021), and these are considered to be the main collagens that are increased in the thickened ligamentum flavum. The present study showed that the gene expression levels of *COL1A2* and *COL3A1* were significantly higher on the dorsal side than on the dural side of the thickened ligamentum flavum. The result indicates an activated fibrotic process on the dorsal side of the thickened ligamentum flavum, which has also been reported by other studies comparing the dural and dorsal sides of the thickened ligamentum flavum (Saito et al. 2017; Hayashi et al. 2022). The dorsal side of the ligamentum flavum is exposed to a higher load than the dural side during lumbar movement, which gradually damages the ligament tissue (Sairyo et al. 2005).

In the process of repairing tissue, TGF-β1 plays an important role of inducing extracellular matrix protein synthesis (Cunliffe et al. 1996; Park et al. 2001). Furthermore, MMP-2 degrades the extracellular matrix proteins, such as elastin and denatured collagens, during tissue remodeling (Longo et al. 2002; Visse and Nagase 2003). Additionally, TNF-α is a proinflammatory cytokine that plays a role in the process of wound healing (Wilgus et al. 2003). The gene expression levels of *TGFB1*, *MMP2*, and *TNFA* were significantly increased on the dorsal side of the thickened ligamentum flavum in the present study. These factors have

Table 3. Representative genes increased in the dorsal side compared to the dural side of the ligamentum flavum in microarray analysis.

Systematic name	Gene name	Description
NM_012098	*ANGPTL2*	Homo sapiens angiopoietin like 2 (ANGPTL2), mRNA [NM_012098]
NM_001276	*CHI3L1*	Homo sapiens chitinase 3 like 1 (CHI3L1), mRNA [NM_001276]
NM_001025199	*CHI3L2*	Homo sapiens chitinase 3 like 2 (CHI3L2), transcript variant 3, mRNA [NM_001025199]
NM_000090	*COL3A1*	Homo sapiens collagen type III alpha 1 chain (COL3A1), mRNA [NM_000090]
NM_000093	*COL5A1*	Homo sapiens collagen type V alpha 1 chain (COL5A1), transcript variant 1, mRNA [NM_000093]
NM_001848	*COL6A1*	Homo sapiens collagen type VI alpha 1 chain (COL6A1), mRNA [NM_001848]
NM_058174	*COL6A2*	Homo sapiens collagen type VI alpha 2 chain (COL6A2), transcript variant 2C2a, mRNA [NM_058174]
NM_032888	*COL27A1*	Homo sapiens collagen type XXVII alpha 1 chain (COL27A1), mRNA [NM_032888]
NM_004460	*FAP*	Homo sapiens fibroblast activation protein alpha (FAP), transcript variant 1, mRNA [NM_004460]
NM_054034	*FN1*	Homo sapiens fibronectin 1 (FN1), transcript variant 7, mRNA [NM_054034]
NR_110708	*LINC01534*	Homo sapiens long intergenic non-protein coding RNA 1534 (LINC01534), transcript variant 2, long non-coding RNA [NR_110708]
NM_002318	*LOXL2*	Homo sapiens lysyl oxidase like 2 (LOXL2), mRNA [NM_002318]
NM_002422	*MMP3*	Homo sapiens matrix metallopeptidase 3 (MMP3), mRNA [NM_002422]
NM_004995	*MMP14*	Homo sapiens matrix metallopeptidase 14 (MMP14), mRNA [NM_004995]
NM_001257096	*PAX1*	Homo sapiens paired box 1 (PAX1), transcript variant 2, mRNA [NM_001257096]
NM_198389	*PDPN*	Homo sapiens podoplanin (PDPN), transcript variant 2, mRNA [NM_198389]
NM_001040058	*SPP1*	Homo sapiens secreted phosphoprotein 1 (SPP1), transcript variant 1, mRNA [NM_001040058]
NM_001025370	*VEGFA*	Homo sapiens vascular endothelial growth factor A (VEGFA), transcript variant 6, mRNA [NM_001025370]

Table 4. Characteristic biological events increased in the dorsal side compared to the dural side of the ligamentum flavum.

Term	Genes
GO:0015671~oxygen transport	NM_001003938, NM_005331, NM_000519, NM_000517, NM_000559
GO:0042744~hydrogen peroxide catabolic process	NM_001003938, NM_005331, NM_000519, NM_000517, NM_000559
GO:0001525~angiogenesis	NM_001109, NM_001025370, NM_054034, NM_004460, NM_004995, NM_012098, NM_014909
GO:0098869~cellular oxidant detoxification	NM_001003938, NM_005331, NM_000519, NM_000517, NM_000559
GO:0030198~extracellular matrix organization	NM_000090, NM_022137, NM_004995, NM_032888, NM_002422, NM_000093
GO:0007155~cell adhesion	NM_002318, NM_054034, NM_001848, NM_004460, NM_013230, NM_003248, NM_058174, NM_001040058, NM_000093
GO:0030324~lung development	NM_000090, NM_001025370, NM_004995, NM_001276, NM_198389
GO:0022617~extracellular matrix disassembly	NM_001911, NM_001109, NM_004995, NM_002422
GO:0015670~carbon dioxide transport	NM_000519, NM_000517, NM_000559
GO:0006508~proteolysis	NM_001911, NM_001109, NM_004460, NM_004995, NM_014909, NM_002003, NM_002422

also been reported to be increased in the thickened as compared to the non-thickened ligamentum flavum (Hur et al. 2017; Sugimoto et al. 2018; Duan et al. 2022). These results support the theory that mechanical stress on the tissue causes tissue injury and that repair processes lead to thickening of the ligamentum flavum (Sairyo et al. 2005; Yabe et al. 2015). On the other hand, we found no significant difference in the expression of genes related to fibrosis and inflammation, such as *COL1A1*, *CTGF*, *PDGFB*, *CYR61*, *IL1B*, *IL6*, *MMP1*, *MMP3*, *TIMP1*, and *TIMP2*, some of which have been reported to be increased in the thickened as compared to the non-thickened ligamentum flavum (Zhang et al. 2010; Lakemeier et al. 2013; Yabe et al. 2015; Duan et al. 2022). The difference in methodology, such as the age distribution of patients, samples set as controls, and the duration of disease, might affect these results.

Chondrometaplasia is another pathological process seen in the thickened ligamentum flavum (Yoshida et al. 1992; Kosaka et al. 2007; Yabe et al. 2015). Although the number of reports is small, some studies have shown increased collagen type II and aggrecan, which are cartilage matrix proteins, in the thickened as compared to the non-thickened ligamentum flavum, in both gene and protein analysis (Kamita et al. 2015; Yabe et al. 2015). Cartilage matrix is often seen at the insertion of the ligament, which is considered to be induced by mechanical stress (Yoshida

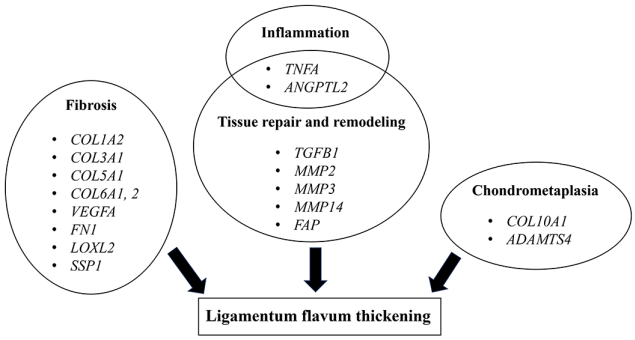

Fig. 2. Pathologies of ligamentum flavum thickening shown in this study.

et al. 1992; Gao et al. 1996). COL10A1 is expressed during the chondrogenic differentiation process (Zimmermann et al. 2008). ADAMTS4 is a proteolytic enzyme that cleaves aggrecan and is induced in the cartilage (Song et al. 2007). The gene expression levels of *COL10A1* and *ADAMTS4* were significantly increased on the dorsal side of the thickened ligamentum flavum in the present study, which were firstly shown as the factors related to ligamentum flavum thickening. Repetitive loading of the ligamentum flavum, particularly on the dorsal side, may explain this phenomenon. However, we found no significant difference in the expression of other genes related to chondrogenic processes, such as *COL2A1*, *ACAN, ADAMTS5*, and *SOX9*, which may also be due to the methodology used in the present study.

Microarray analysis indicated that some biological processes occurred more on the dorsal side of the thickened ligamentum flavum, including extracellular matrix organization, cell adhesion, extracellular matrix disassembly, and proteolysis. Among the representative genes increased on the dorsal side of the thickened ligamentum flavum, some factors have also been reported to be increased in the thickened as compared to the non-thickened ligamentum flavum, such as ANGPTL2 (Nakamura et al. 2014; Hur et al. 2017), COL3A1 (Yabe et al. 2015; Hur et al. 2017), COL6A1 and 2 (Takeda et al. 2021), FN1 (Kamita et al. 2015; Yabe et al. 2015), MMP-3 (Lakemeier et al. 2013), and VEGFA (Hur et al. 2015). These factors are related to fibrosis and repair processes in the tissue. Further, among the other genes increased on the dorsal side, collagen type V is a regulatory fibril-forming collagen that is overexpressed in fibrosis (Mak et al. 2016). COL5A1 was reported to be increased on the dorsal side of the thickened ligamentum flavum as compared to the dural side in a previous report (Hayashi et al. 2022). CHI3L1 and CHI3L2 (Funkhouser and Aronson 2007), FAP (Lay et al. 2019), LOXL2 (Yang et al. 2016), MMP-14 (Snyman and Niesler 2015), PDPN (Honma et al. 2012), and SSP1 (osteopontin) (Weber et al. 2012) have also been reported to be related to fibrosis and tissue repairing or remodeling processes and were implicated as factors related to thickening of the ligamentum flavum in the present study. Furthermore, COL27A1 (Hjorten et al. 2007) and LINC01534 (Wei et al. 2021) are expressed in cartilaginous tissue, while PAX1 is related to the chondrogenic differentiation process (Takimoto et al. 2019). These factors indicated chondrometaplasia occurring on the dorsal side of the thickened ligamentum flavum in this study. The results of the microarray analysis showed that synthesis and degradation of the extracellular matrix including collagens, and chondrometaplasia are induced on the dorsal side of the ligamentum flavum, which supports the theory that repetitive mechanical stress leads to thickening of the ligamentum flavum.

The present study had some limitations. First, the dural side of the thickened ligamentum flavum, which also demonstrated loss of elastic fibres, was used as a control. Although the dorsal side showed more severe pathological changes than the dural side, this approach may have affected our results. Second, LSCS has a long duration, and gene expression levels can differ depending on the duration of the disease, which was not assessed in this study. Finally, protein expression levels were not assessed. No previous studies did protein analysis using the dural side of the thickened ligamentum flavum as a control. Proteome analysis using this method can be useful to clarify the pathologies of ligamentum flavum thickening, which should be done in future studies.

In conclusion, the present study comprehensively compared the dorsal and dural sides of the thickened ligamentum flavum using gene expression analysis and showed increased expression levels of genes related to fibrosis, inflammation, tissue repair and remodeling, and chondrometaplasia on the dorsal side. These are considered important pathologies of ligamentum flavum thickening (Fig. 2).

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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