



# Histopathological and electron microscopic study in dogs with patellar luxation and skin hyperextensibility

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**ABSTRACT.** Patellar luxation is abnormal displacement of the patella from the femoral trochlear groove. It is seen primarily in small breed dogs and causes pain and limited mobility of the stifle joint. This study aimed to investigate the relationship among patellar luxation, skin extension, and skin collagen fibril diameter. Nine dogs with patellar luxation and five clinically normal dogs were enrolled in the study. We measured the skin extension and investigated the ultrastructure of the skin and patellofemoral ligament by histopathology and transmission electron microscopy. The mean skin extension in dogs with patellar luxation was  $18.5 \pm 5.5\%$  which is greater than the reference value ( $14.5\%$ ). Mean skin extension in controls was  $8.8 \pm 1.7\%$  and was within the normal range. In dogs with patellar luxation, histopathology of the skin and patellofemoral ligament showed sparse and unevenly distributed collagen fibers. Transmission electron microscopy identified poorly organized, irregularly shaped, thin collagen fibrils. Collagen fibril thickness in dogs with patellar luxation was significantly less than fibril thickness in controls ( $P < 0.001$ ). There was a significant negative correlation ( $\rho = -0.863$ ;  $P < 0.001$ ) between skin collagen fibril diameter and skin extension. Skin extension was correlated with patellar luxation and disease severity. Dogs with patellar luxation, joint dysplasia, and hyperextensible skin appear to be pathologically related. This might represent a phenotype of the Ehlers–Danlos syndrome, a hereditary connective tissue disorder in humans.

**KEY WORDS:** Ehlers–Danlos syndrome, patellar luxation, skin hyperextensibility

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Patellar luxation, defined as abnormal displacement of the patella from the femoral trochlear groove, causes pain and muscle contracture, and ultimately limits flexion and extension of the stifle joint. In dogs, patellar luxation results from contracture of the quadriceps femoris muscles, morphological abnormalities in the femur, or abnormalities of the supporting soft tissues of the stifle, such as the patellofemoral ligament [5, 13, 27, 29, 30]. However, the ultrastructural abnormalities of the patellofemoral ligament and other supporting soft tissues of the canine knee joint have not been investigated.

In humans, Ehlers–Danlos syndrome (EDS) is a hereditary connective tissue disease, with abnormalities of the skin, musculoskeletal and cardiovascular systems, and other connective tissues. It is characterized by joint laxity, patellar subluxation, skin hyperextensibility, and fragility [3, 4, 6, 8, 9]. A characteristic histological and ultrastructural feature of EDS is abnormally thin collagen fibrils in connective tissue. Based primarily on clinical findings, EDS has been reported in dogs [2, 7, 16, 17, 21]. There are limited reports providing histological evidence of collagen abnormalities by skin biopsy. Both joint and skin manifestations in EDS in dogs have been rarely reported [10]. We previously reported a case of a toy poodle with bilateral patellar luxation and skeletal hypoplasia accompanied by skin fragility [31]. The dog was diagnosed with EDS based on the clinical signs of fragile skin, and skin hyperextensibility and electron microscopic evidence of abnormal thin collagen fibrils.

In the present study, we report a series of nine dogs that presented with patellar luxation and skin hyperextensibility. We measured the skin extension and investigated the ultrastructure of the skin and patellofemoral ligament by histological evaluation and transmission electron microscopy (TEM). Further, on the basis of results obtained from five control dogs without EDS, we

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**Table 1.** Characteristics of the study population

No.	Breed	Age (years)	Sex	Weight (kg)	Patellar grade	Surgery	Skin (harvest)	Joint (harvest)
1	Miniature pinscher	2	Castrated	3.8	MPL (R2 L2)	Patellar luxation	○	N/A
2	Toy poodle	2	Castrated	2.1	MPL (L2)	Patellar luxation	○	N/A
3	Toy poodle	5	Male	5.5	MPL (R2)	Patellar luxation	○	N/A
4	Italian greyhound	2	Castrated	5.1	MPL (R2 L2)	Patellar luxation	○	N/A
5	Toy poodle	1	Spayed	1.4	MPL (R2 L2)	Patellar luxation	○	○
6	Chihuahua	6	Female	2.1	MPL (R2 L2)	Patellar luxation	○	○
7	Chihuahua	3	Spayed	3.5	MPL (R4 L4)	Patellar luxation	○	○
8	Toy poodle	3	Female	2	MLPL (bilateral)	Patellar luxation	○	○
9	Toy poodle	6	Female	2.8	MLPL (bilateral)	Patellar luxation	○	○
1	Toy poodle	1	Male	4.5	Control	Castration	○	N/A
2	Jack Russell terrier	5	Male	7.5	Control	Castration	○	N/A
3	Standard poodle	4	Male	25.5	Control	Castration	○	N/A
4	Toy poodle	2	Female	5.5	Control	Spaying	○	N/A
5	Mixed breed	3	Castrated	7.8	Control	Femoral fracture	○	○

Note: This case series included nine dogs with patellar luxation, seven with medial patellar luxation (MPL), and two with medial-lateral patellar luxation (MLPL). ○ indicates that the sample was collected; L=left; R=right.

investigated the relationship among patellar luxation, skin extension index, and skin collagen fibril diameter.

## MATERIALS AND METHODS

### Study population

The protocols used in this study conformed to the guidelines of an institutional animal care and use committee (IACUC). Informed consent was obtained from all owners. Nine adult dogs (Table 1) with patellar luxation were enrolled in the study. Inclusion criteria were: 1) difficulty walking and signs suggestive of pain; 2) patellar dislocation on palpation; 3) patellar luxation identified on radiographic images; 4) indication for surgical repositioning of the knee joint (non-responsiveness to nonsteroidal anti-inflammatory analgesics); and 5) exclusion of Cushing's syndrome and hypothyroidism by cytometry, biochemical tests, and measurement of cortisol and thyroxine concentrations. All nine dogs exhibited patellar luxation [5, 13, 26, 27, 29, 30] of grades II–IV. While all dogs (dogs 1–9) exhibited medial patellar luxation (MPL), dogs 8 and 9 presented with medial-lateral patellar luxation (MLPL), a condition in which MPL progresses and becomes severe [1, 11]. The control group included five dogs of similar size and breed with clinically normal skin and joints that did not meet the inclusion criteria. Four of the control dogs were referred for neutering (orchectomy or ovariectomy), and only skin samples could be obtained from these dogs. The fifth control dog presented with a traumatic femoral fracture, enabling us to biopsy of the patellofemoral ligament and the skin.

### Surgical management

After premedication with atropine (0.5 mg, atropine sulfate; Mitsubishi Tanabe Parma, Osaka, Japan), anesthesia was induced with intravenous propofol (1%; 20 ml; Mylan Pharmaceutical, Osaka, Japan). After endotracheal intubation, anesthesia was maintained by isoflurane (2%; Isoflu, DS Pharma Animal Health, Osaka, Japan). For surgical treatment of patellar luxation, a skin incision was made on the medial side of the stifle. After confirming that the articular capsule was relaxed, an incision in the lateral articular capsule was made, followed by trochlear sulcoplasty, tibial tuberosity transposition, and cerclage of the femoropatellar fascia. Perioperative pain was managed with subcutaneous morphine (10 mg; morphine hydrochloride injectable solution, Takeda Chemical, Osaka, Japan) and robenacoxib (2%; Novartis Animal Health, Tokyo, Japan). Antibiotic treatment involved intravenous administration of cefazolin sodium (20 mg/kg/0.1 ml; Fujita Pharmaceutical, Tokyo, Japan) before induction of anesthesia, with two additional doses on the day of surgery. In addition, orbifloxacin (5%; DS Pharma Animal Health) was administered subcutaneously.

### Measurement of skin extension

Skin extension was measured as previously described [7, 16, 17]. The “maximum distance achieved by stretching the skin in the lower back vertically without causing pain” was measured, and the percentage was calculated by dividing the measured value of extension with the “length of the body ranging from the occipital crest to the root of the tail” and multiplying by 100. Skin extension values of  $\leq 14.5\%$  were considered normal, while those  $> 14.5\%$  were considered abnormal and indicative of hyperextensibility.

### Histopathological assessment

Tissue samples were obtained during surgery. In dogs with patellar luxation, skin from the caudal back was sampled using a 6-mm punch biopsy. Sample from the patellofemoral ligament were obtained in five of nine dogs. In the control dogs, a skin

**Table 2.** Skin extension and skin collagen fibril diameter

No.	Skin extension (%)	n	Diameter of collagen fibrils (nm)		
			Average	SD	
1	MPL <sup>a)</sup>	15.2	100	75.57	8.30
2	MPL <sup>a)</sup>	15.0	100	87.45	14.08
3	MPL <sup>a)</sup>	14.7	100	61.92	7.78
4	MPL <sup>a)</sup>	15.2	100	85.45	13.40
5	MPL <sup>a)</sup>	19.0	100	80.46	9.44
6	MPL <sup>a)</sup>	18.5	100	55.45	8.92
7	MPL <sup>a)</sup>	21.2	100	52.28	11.57
8	MLPL <sup>b)</sup>	19.2	100	73.51	10.13
9	MLPL <sup>b)</sup>	30.8	100	*	*
1	Control	12.2	100	96.82	7.81
2	Control	7.8	100	99.13	12.58
3	Control	9.0	100	105.78	16.24
4	Control	8.8	100	114.32	16.23
5	Control	8.3	100	102.29	16.97
Patellar luxation total		18.5	900	71.51**	18.81
Control total		8.8	500	103.67**	15.58

Note: Skin collagen fibril diameter is significantly smaller in dogs with patellar luxation than controls ( $P < 0.001$ ). a) MPL=medial patellar luxation; b) MLPL=medial-lateral patellar luxation; \*Collagen fibrils with large diameters maintaining a circular shape could not be confirmed. \*\*Significant difference ( $P < 0.001$ ).

sample from the caudal back was obtained during orchiectomy or ovariohysterectomy. In addition, a patellofemoral ligament sample was obtained from the control dog with the femoral fracture. Samples were fixed in 10% formalin, embedded in paraffin, sliced in 5- $\mu$ m-thick sections, and stained with hematoxylin-eosin (HE) and Masson's trichrome (MT). Histological evaluation was performed with a biological microscope (BA 210 EINT, SHIMADZU, Tokyo, Japan). We focused on epidermal and dermal thickness and collagen fiber structure and organization. Images were acquired using the built-in digital camera.

### TEM

Skin and patellofemoral ligament tissue specimens were prefixed in 4% glutaraldehyde in a 0.1-M phosphate buffer solution, followed by postfixation in osmium tetroxide. The fixed specimens were dehydrated in alcohol, embedded in the epoxy resin Epon, and sliced into ultrathin sections. Longitudinal and transverse sections were then examined by TEM (JEOL-1200 EX or JEM 1400plus, JEOL Ltd., Tokyo, Japan), with focus on collagen fibrils in the patellofemoral ligament and the mid-dermis of skin samples. From the TEM photographs of each sample, the diameter of 100 randomly selected collagen fibrils was measured using image analysis software [15] (Image J; National Institutes of Health, Bethesda, MD, U.S.A.), and the mean values were calculated [9, 14]. Collagen fibril diameter of the patellar luxation and control groups was then compared, and the relationship between collagen fibril diameter and skin extension was evaluated.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD), with  $P$  values  $< 0.05$  considered significant. The relationship between patellar luxation and skin collagen fibril diameter was evaluated using a mixed-effects linear regression analysis. The model included group (patellar luxation or control) as a fixed effect and the subject as a random effect. The association between the mean skin collagen fibril diameter and skin extension was evaluated using Spearman's rank correlation analysis. Statistical analyses were performed using a statistical software package (SPSS 19.0 for Windows; SPSS Inc., Chicago, IL, U.S.A.).

### Statement of the data availability

The data analyzed in this study are included in the main article.

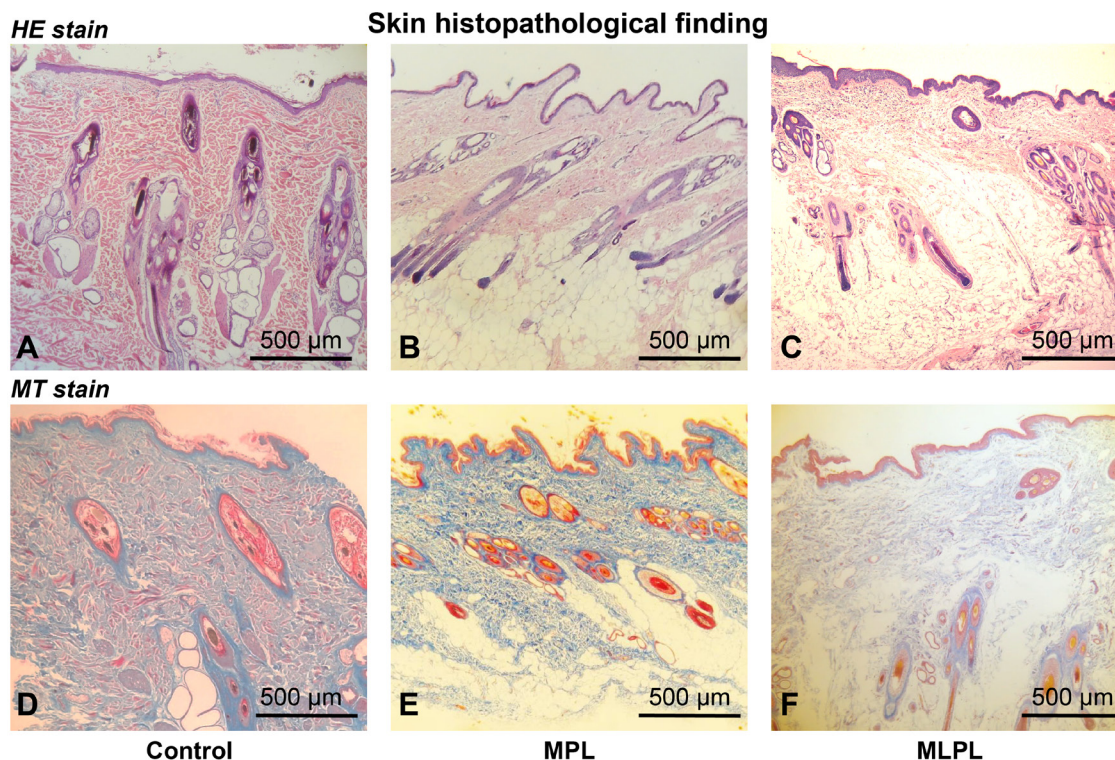
## RESULTS

### Skin extension

The skin extension was  $18.5 \pm 5.5\%$  in dogs with patellar luxation and  $8.8 \pm 1.7\%$  in controls. Skin extension of all dogs in the patellar luxation group was greater than the established normal value (14.5%), and less than 14.5% in all control dogs (Table 2).

### Histopathological findings

Dogs with patellar luxation exhibited thinner epidermis and dermis, with sparse and unevenly distributed collagen fibers in the skin (Fig. 1). Similarly, the patellofemoral ligament sections of dogs with patellar luxation exhibited unevenly distributed collagen



**Fig. 1.** Histopathological findings of the skin in the control and patellar luxation (MPL and MLPL) groups. Relative to the control samples (A, D), the medial patellar luxation (MPL) (B, E) and medial-lateral patellar luxation (MLPL) (C, F) the epidermis and dermis was thinner, with sparse and unevenly distributed collagen fibers. A–C: hematoxylin-eosin staining; D–F: Masson’s trichrome staining.

fibers, with marked gaps between the fibers; in contrast, the control group exhibited dense, uniformly distributed collagen fibers (Fig. 2).

#### TEM findings

In the controls, cross and longitudinal sections of skin revealed bundles of closely packed collagen fibrils (mean diameter,  $103.67 \pm 15.58$  nm; Fig. 3). In contrast, skin from dogs with MPL (Fig. 3) exhibited loosely packed bundles of significantly thinner collagen fibrils (mean diameter,  $71.51 \pm 16.49$  nm;  $P < 0.001$ ; Table 2). Dogs with MLPL (dogs 8 and 9) exhibited heterogeneity in collagen fibril diameter. In dog 9, the collagen fibrils were disorganized, and no bundle-like structures were identified (Fig. 3).

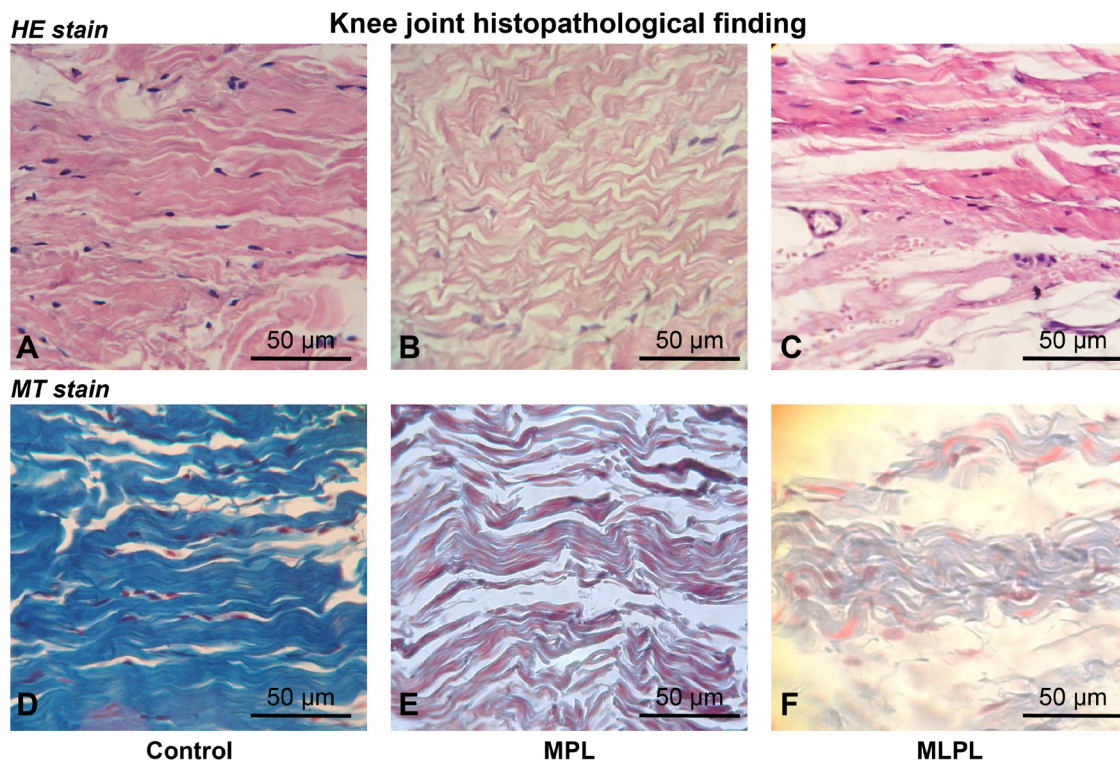
The cross and longitudinal sections of the patellofemoral ligament of the control dog revealed thick collagen fibrils (mean diameter, 150 nm), interspersed with a few fibrils of smaller diameter (Fig. 4). In contrast, the patellofemoral ligaments of dogs with patellar luxation (Fig. 4) exhibited significantly thinner collagen fibrils (mean diameter, 75 nm). Dogs with MLPL (dogs 8 and 9) exhibited heterogeneity in collagen fibril diameter, with increased spacing between the fibrils (Fig. 4). In dog 9, the diameter of petal-like collagen fibrils, consisting of tattered cross-section of collagen fibrils resembling a petal, could not be measured. Intergroup comparison of the patellofemoral ligament collagen fibril diameter was not performed because of the small sample sizes (patellar luxation,  $n=5$ ; control,  $n=1$ ).

#### Correlation between skin extension and skin collagen fibril diameter

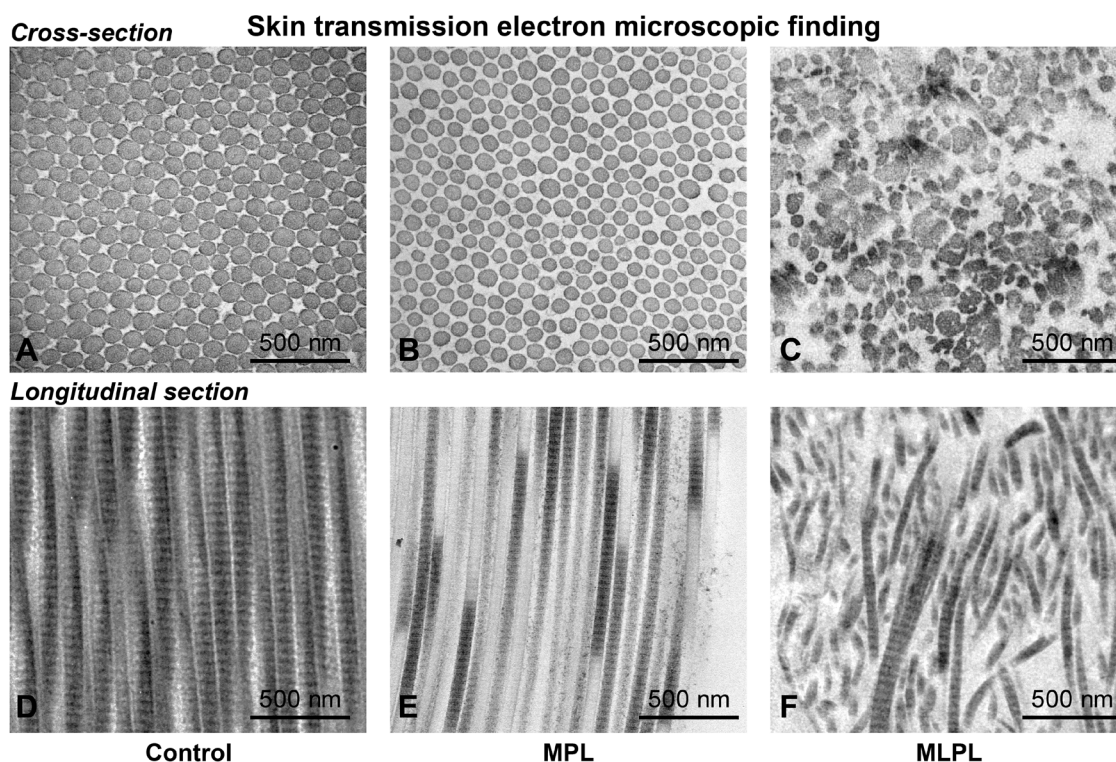
There was a statistically significant and negative correlation ( $\rho = -0.863$ ;  $P < 0.001$ ) between skin extension and skin collagen fibril diameter (Fig. 5).

## DISCUSSION

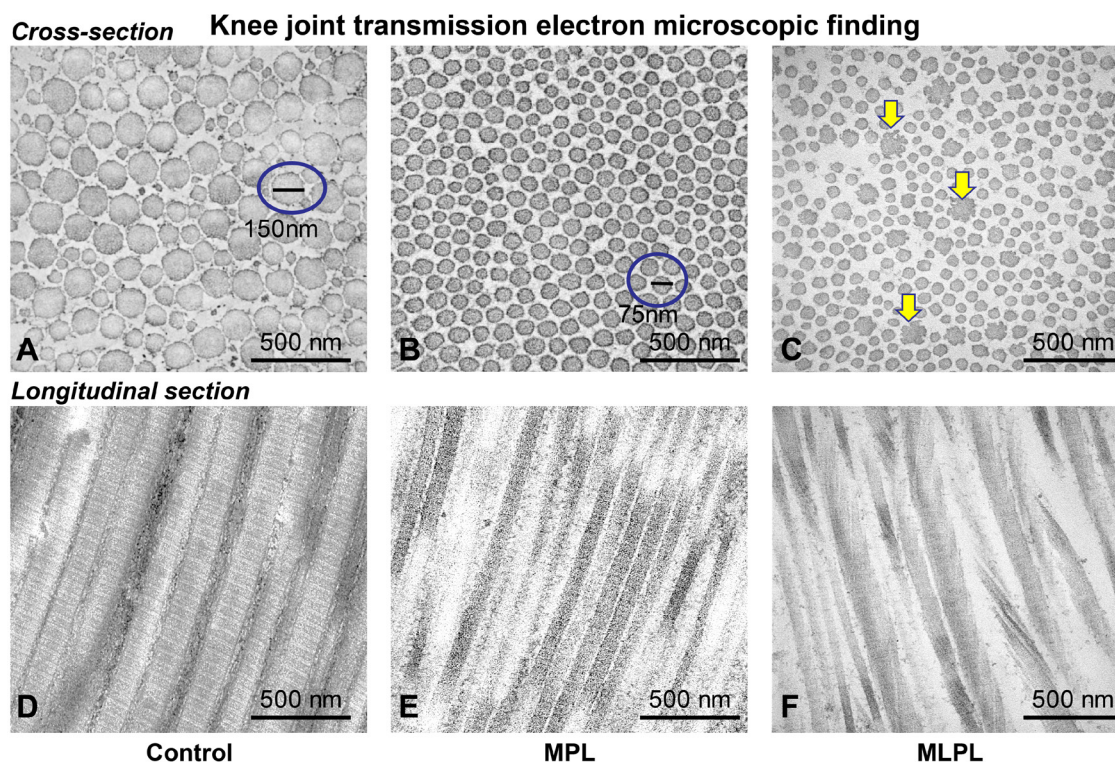
Ligaments and tendons in newborn animals consist only of uniform small-diameter collagen fibrils, while those in mature animals are composed of two types of collagen fibrils. In mature animals, the majority of fibrils are large-diameter collagen fibrils, which are resistant to tension, while a small proportion of fibrils are of small-diameter [22, 25]. The patellofemoral ligament of the control dog exhibited predominantly large-diameter collagen fibrils, while collagen fibrils in dogs with patellar luxation were poorly organized and significantly thinner. Additionally, dogs with patellar luxation exhibited structural abnormalities in collagen fibrils (i.e., noticeably thin collagen fibrils; varying fibril size and shape; and irregular distribution). Consistent with these findings, collagen fibril abnormalities have been reported in dogs with EDS [2, 21, 24]. The present findings are consistent with



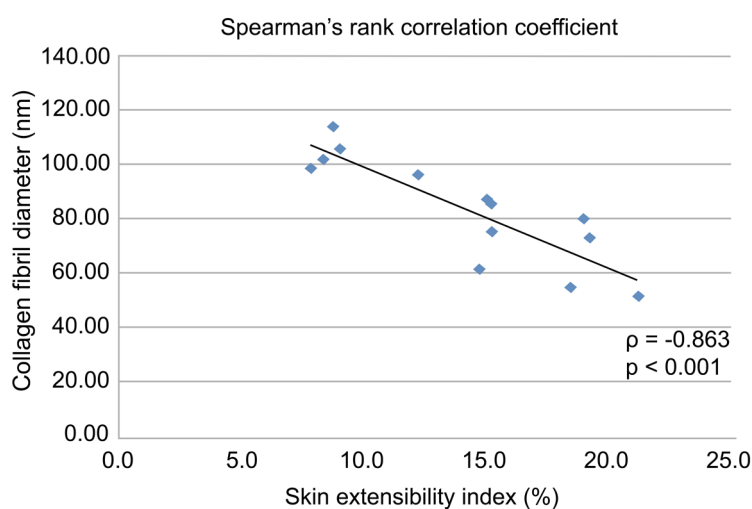
**Fig. 2.** Histopathological findings of the patellofemoral ligament in a control dog and dogs with medial patellar luxation (MPL) and medial-lateral patellar luxation (MLPL). While control samples exhibited dense and uniformly distributed collagen fibers (A, D), the MPL (B, E) and MLPL (C, F) samples exhibited unevenly distributed collagen fibers, with marked gaps between the fibers. A–C: hematoxylin-eosin staining, D–F: Masson's trichrome staining.



**Fig. 3.** Transmission electron microscopic findings of cross (A–C) and longitudinal (D–F) sections of the skin. While the control group (A, D) exhibited thick bundles of closely packed collagen fibrils (mean diameter,  $103.67 \pm 15.58$  nm), dogs with patellar luxation (B, E: medial patellar luxation (MPL); C, F: medial-lateral patellar luxation (MLPL)) exhibited loosely packed bundles of significantly thinner collagen fibrils (mean diameter,  $71.51 \pm 16.49$  nm), with the MLPL samples showing disorganized fibrils and heterogeneity in collagen fibril diameter.



**Fig. 4.** Transmission electron microscopic cross sections (A–C) and longitudinal sections (D–F) of the patellofemoral ligament. While the control group (A, D) mainly exhibited thick collagen fibrils (black line indicates mean diameter, 150 nm) interspersed with a few fibrils of smaller diameters, dogs with patellar luxation (B, E: medial patellar luxation (MPL)) had significantly thinner collagen fibrils (black line indicates mean diameter, 75 nm), with the MLPL samples showing heterogeneity in collagen fibril diameter (yellow arrows) and increased spacing between the fibrils (C, F: medial-lateral patellar luxation (MLPL)).



**Fig. 5.** There is a statistically significant negative correlation ( $\rho = -0.863$ ;  $P < 0.001$ ) between skin extension and skin collagen fibril diameter, as assessed by Spearman's rank correlation.

collagen fibril abnormalities of the skin reported in *COL5A1*-deficient mice with experimentally-induced EDS [23, 28, 32] and in humans with classic- and joint-hypermobility-type EDS [12]. The atypical petal-like collagen fibrils observed in the patellofemoral ligaments of dogs with the MLPL phenotype have not been reported in humans with EDS.

Skin extension exhibited excellent correlation with EDS progression. While the control group exhibited skin extension within the normal range, all dogs with patellar luxation exhibited increased skin extension, and dogs with MLPL had a greater increase.

Significant differences in skin collagen fibril diameter were also observed between groups. A statistically significant and negative correlation was observed between skin extension and skin collagen fibril diameter. In humans, the patella has a support mechanism to prevent lateral luxation. The patellofemoral ligament is involved in control of the stifle joint [18–20]. In the present study, congenital collagen abnormalities may be a cause of patellar luxation.

EDS is a group of hereditary diseases in which metabolic and structural abnormalities of the collagen in connective tissues lead to skin hyperextensibility and fragility; capillary and vascular fragility accompanied by an increased tendency to bleed; and abnormal hypermobility of ligaments and joints. Depending on the major symptoms, EDS can be classified into six types: classic, joint-hypermobility, vascular, kyphoscoliotic, arthrochalasia, and dermatosparaxis [7]. Genetic analysis suggests that not all patients with Ehlers Danlos fit this classification scheme [8, 10, 12, 15]. Patellar luxation and skin hyperextensibility in the present case series is consistent with the clinical presentation of all types of EDS with the exception of the vascular type. Similar collagen abnormalities in the skin and patellofemoral ligaments of dogs with patellar luxation indicates a pathological relationship between joint dysplasia and skin hyperextensibility in dogs and suggests that this is a phenotype of EDS. These results also indicate the possibility of using dogs with patellar luxation as animal models for EDS.

This study has several limitations. First, it is a relatively small case series, and we were limited in our ability to find matching controls and obtain ligament biopsies. Further, each group comprised small-sized breeds of dogs. Nevertheless, we obtained consistent results in terms of skin extension and collagen fibril diameter across each group, which indicates that these parameters are useful in differentiating between normal controls and dogs with patellar luxation/EDS.

In conclusion, patellar luxation in dogs is associated with collagen fibril abnormalities similar to those observed in humans with EDS. These findings might be useful in the future, not only in veterinary medicine, but also in developing an animal model of human EDS. Future studies should aim at characterization of dogs with patellar luxation using genetic analyses to identify the molecular mechanisms of collagen abnormalities and further establish the relationship between patellar luxation and EDS.

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