



# Change in MRI findings of medial collateral ligament injury in adolescent baseball players with no clinical symptoms over time

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## Abstract

**Objective** Elbow screening of adolescent baseball players began in 2014 using ultrasound, palpation, and dedicated magnetic resonance imaging (MRI). We frequently encountered subjects showing MCL injury on MRI but no clinical symptoms. We assessed variations in asymptomatic MCL injury findings at follow-up MRI, and clarified the pathogenesis of these findings.

**Materials and methods** Using a 0.2-T-dedicated MRI, 30 subjects with asymptomatic MCL injury at initial MRI who agreed to follow-up MRI were included. We classified the findings at repeat MRI as follows: (a) disappeared, (b) better, and (c) worse.

**Results** There were 6, 16, and 8 subjects in groups a, b, and c, respectively. The average age at follow-up was 14.0, 12.1, and 12.4 years in groups a, b, and c, respectively. There were significant differences between groups a and b and between groups a and c. Average height at follow-up of groups a, b, and c was 1.64, 1.52, and 1.57 cm, respectively, with a statistically significant difference between groups a and b. The average size of the short axis of the MCL of subjects in group a was 2.0 cm on the dominant side and 1.5 cm on the contradominant side ( $P < 0.04$ ).

**Conclusion** Players with asymptomatic MCL injury can continue to play baseball with no limitations, as these findings usually disappear around the age of 14.0 years, when the growth spurt occurs. This finding may be a precursor of ‘adaptation’, which is generally observed in high school baseball players, suggesting that the MCL adapts as growth occurs.

**Keywords** Dedicated MRI · Asymptomatic MCL injury · Elbow screening · Adaptation · Medial epicondyle

## Introduction

Adolescent Japanese baseball players experience a high prevalence of elbow injuries [1–4]. In Japan, screening for baseball-related elbow injuries in adolescence started in the early 2000s. Screening is carried out mainly by ultrasonography (US) and palpation [5–7]. It is not feasible to use magnetic resonance imaging (MRI) as a screening tool for a number of reasons, although it has the highest contrast resolution compared to other imaging modalities: first, the MRI machine is fixed and cannot be moved from its sealed space; second, MRI scanners are expensive, and scheduling of patients for

examination is difficult, and third, subjects are required to remain still within an enclosed space, which may be very stressful for adolescents [8].

Dedicated MRI has been available since the early 2000s. Unlike the whole-body scanner, this is specialized for scanning small joints, and subjects insert only the body part required for examination into this machine. This open-type machine is favorable for scanning adolescents as there is less associated stress.

Ultrasonography is adequate for screening of elbow injuries because it can evaluate the elbow dynamically and functionally; however, assessment of the MCL parenchyma, including identification of mild inflammation or subtle injury, is unsatisfactory. In contrast, MRI can provide a qualitative evaluation of the MCL with high-contrast, high-resolution images despite the lack of dynamic assessment. We hypothesized that dedicated MRI can complement US, increasing the accuracy of elbow screening; therefore, we started elbow screening using US, palpation, and dedicated MRI in 2014.

Our screening yielded unexpected findings. The medial collateral ligament (MCL) is generally not injured in

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adolescent baseball players because it is relatively more strain-resistant than the medial epicondyle; however, we frequently encountered findings of MCL injury on MRI with no clinical symptoms. Signal intensity change, change in size (short axis), and morphological change (bowing, elongation, fluffing, or spiculation) of the MCL were frequently observed. Previous reports in the literature also demonstrated the same tendency [9, 10], and Tanaka et al. reported this condition as ‘asymptomatic MCL injury’ [10]. This finding provided a clinical dilemma, as its significance was difficult to determine, as was any suggestion to their parents as to further management.

Although we frequently identified subjects with ‘asymptomatic MCL injury’, no subject showed a clinical MCL injury until the end of 2017; therefore, the significance of this finding on MRI remains obscure. It is also unpredictable how this finding may vary, because the literature on MRI imaging of MCL injuries in adolescent baseball players is extremely limited.

We thus began performing follow-up MRI imaging of subjects who showed asymptomatic MCL injuries on the initial examination. As MRI can be performed under the same condition every time (i.e., positioning or parameters), images can be evaluated very objectively. MRI is considered to be the most favorable modality for observation of change in the MCL over time, as compared to US and palpation.

The purpose of our study was to use dedicated MRI to follow ‘asymptomatic MCL injury’ findings, which has not been previously reported. Moreover, we also aimed to analyze the correlation between the change in MRI findings and various parameters including age, body weight, height, and player position.

## Methods

### Subjects

Since 2014, 30 subjects who showed abnormal findings in the MCL of the dominant elbow on initial examination, and for whom permission for follow-up MRI could be obtained from their parents, were included in our study. The second author contacted all candidates who showed abnormal findings on initial MRI by e-mail or cellphone. After detailed explanation of the purpose of additional follow-up scanning, candidates freely decided whether to participate in follow-up scanning or not. In total, 30 participants and their parents agreed to participate in our study.

All 30 subjects had continued playing baseball during the follow-up period. To recruit the study subjects, we placed an advertisement in a local magazine and newspaper in the initial study. Dedicated MRI images were obtained in both the initial

and the follow-up studies. The follow-up durations were from 6 months to 2 years.

The subjects were active baseball players, defined as those who play on a regular basis, and who were asymptomatic at the time of examination. Players who were not active because of elbow pain or tenderness on examination were excluded in the two studies. Subjects completed a questionnaire regarding age, height, weight, and player position at each examination. In the initial MRI, we recruited 9–13 years old, which is the most prevalent age for elbow injury. Our study was approved by the Ethics Committee of our institution. We obtained written informed consent from the parents of the study participants prior to all examinations.

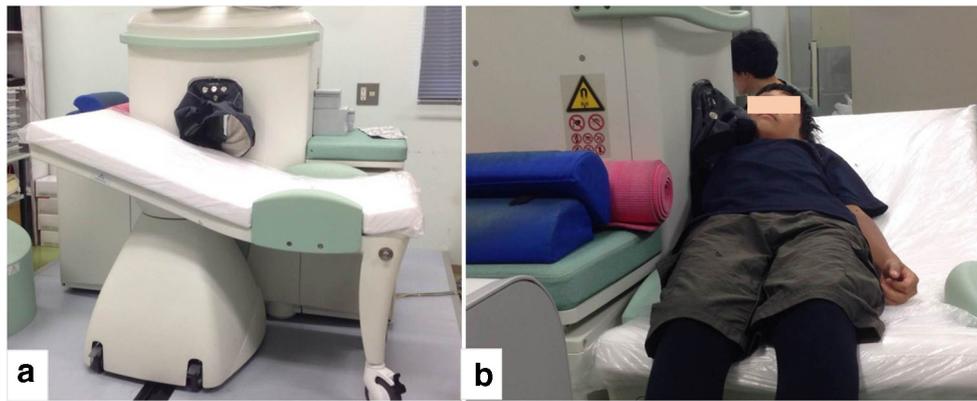
### MRI

An open-type 0.2 T MRI C-SCAN (Esaote Inc., Genova, Italy) was used in our study (Fig. 1). Scanning parameters were selected to accurately diagnose MCL injury. In sequence 1 (dominant side), gradient echo coronal images were obtained with the following parameters: TR, 500 ms; TE, 18 ms; slice thickness, 3 mm; gapless; matrix size, 256 × 192 mm; field of view (FOV), 180 × 180 mm; flip angle (FA), 75°; and scan time, 1 min 39 s. In sequence 2 (dominant side), coronal images were obtained with the short TI inversion recovery technique using the following parameters: TR, 1,000 ms; TE, 25 ms; slice thickness, 3 mm; matrix size, 256 × 192 mm; FOV, 180 × 180 mm; FA, 90°; inversion time, 80 ms; and scan time, 3 min 35 s. In sequence 3 (dominant side), gradient echo sagittal images were obtained using the following parameters: slice thickness, 4.5 mm; gapless; the other parameters were the same as those in sequence 1. After we scanned the aforementioned sequences 1–3, we also scanned the elbow of the nondominant side to acquire a normal reference image as sequence 4. The scanning parameters for sequence 4 were the same as those in sequence 1.

In these sequences, the sequence 1 image was primarily used for assessing the MCL, whereas the sequence 4 image was used for identifying abnormal findings based on the interpretation of the sequence 1 image. The sequence 2 image was used for assessing early osteochondritis dissecans, bone edema, and fluid collection, and sequence 3 was also used for assessing osteochondritis dissecans, as a screening of the elbow joint of adolescent baseball players.

### MRI diagnosis

Image interpretation was performed independently by two experienced clinicians by visual inspection in random order. One was a radiologist specializing in musculoskeletal imaging, with 18 years of experience, and the other was an orthopedic surgeon specializing in sports medicine, with 12 years of experience. The assessment of the MCL was performed in the



**Fig. 1** Overview photographs of pediatric imaging. **a** Overview photograph of compact MRI dedicated to limb imaging. **b** Pediatric imaging with right arm inserted into and immobilized in the machine; MRI imaging is being performed. Since there is no barrier between the

face and surroundings, there is no sense of claustrophobia, and the subjects are able to converse with their parents. MRI magnetic resonance imaging

coronal images of GRE (sequence 1). The findings from the non-dominant side (sequence 4) were used as a normal reference (Fig. 2a).

Three evaluation criteria were used:

1. Swelling of the MCL: lengthening of the short axis of the MCL with irregular shape was defined as swelling (Fig. 2b).
2. Morphological changes in the MCL: morphological change of the entire MCL (Fig. 2c).
3. Signal changes within the MCL: elevation of signal intensity in the ligament at any site (Fig. 2d).

We diagnosed subjects as having asymptomatic MCL injury if the subject showed at least one of the above described diagnostic criteria. Next, 30 subjects who showed asymptomatic MCL injury in the initial study and for whom permission could be obtained for follow-up MRI were evaluated. We compared the MRI findings of these patients in the initial and follow-up studies.

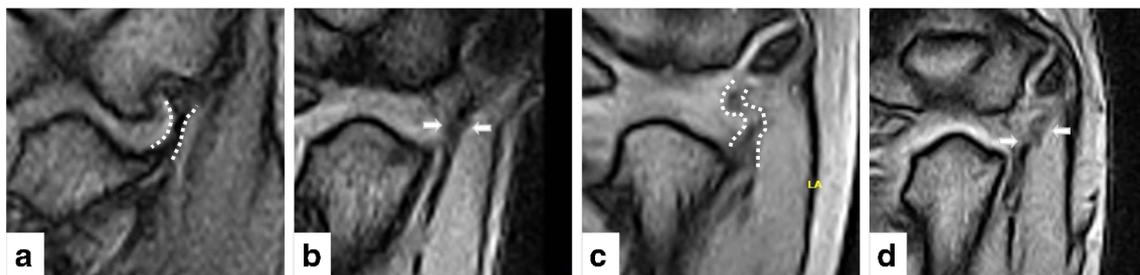
Assessments were categorized as (a) disappeared, (b) better, or (c) worse compared to the initial study. When all

findings tended to disappear, subjects were included in group a. When findings tended to be obscure, subjects were included in group b. When findings tended to be emphasized, subjects were included in group c. Initially, we included group d (no significant change); however, as there were no cases of category d, we omitted it from our study. All MRI interpretations were assessed visually. When the diagnosis was different between the two interpreters, the final diagnosis was made by consensus.

### Statistical analysis

After all evaluations, the agreement rate and kappa coefficient of diagnosing of features 1–3 and groups a–c between the two interpreters was calculated for each of the three assessments of each subject.

To clarify the association between the change of MRI and aging, imaging intervals, height, weight, and main position during follow-up period, we calculated the averages of these elements for groups a–c. Analysis of variance and the Steel–Dwass test were used to compare the three groups, and the significance threshold was  $P < 0.05$ .



**Fig. 2** Examples of findings on gradient echo coronal images. **a** MCL of contradominant side. The ligament has a smooth appearance, and the signal within the ligament is uniform. **b** MCL of dominant side. The arrows indicate the area of MCL swelling. **c** MCL of dominant side.

The ligament exhibits signal changes as well as marked laxity and morphological changes. **d** MCL of dominant side. Ligament laxity is mild, but there are high signal intensity changes within the ligament. MCL medial collateral ligament

### Additional measurement of MCL thickness

After we finished all examinations and statistical analyses, we hypothesized that the MCL in group a might be a precursor of ‘adaptation’ of the MCL found in high school and professional baseball players, which has previously been reported [11, 12]. Therefore, we performed additional measurements of the MCL for subjects in group a.

In these subjects, the thickness at the widest portion of the ligament was measured in the slice in which the ligament was most clearly depicted on the operator console in the follow-up MRI. The measurements were performed by the two individuals described in the preceding, the average values were calculated, and the dominant and contradominant sides were compared using the *t*-test.

### Results

In total, 315 adolescents participated in our elbow screening. One hundred out of 315 subjects (32%) showed ‘asymptomatic MCL injury’ at the initial study. Permission for follow-up MRI was obtained from the parents of 30 out of 100 subjects.

Of the 30 individuals, six subjects showed (a) disappeared, 16 showed (b) better, and 8 showed (c) worse findings. Typical cases of each group are presented in Figs. 3, 4, and 5. Details and changes in the initial and follow-up MRI findings, ages, imaging intervals, height, weight, and main position during the follow-up period are summarized in Table 1.

The average age at initial scan was 12.5, 11.3, and 11.3 years in groups a, b, and c, respectively, showing no significant difference, whereas the average age at follow-up was 14.0, 12.1, and 12.4 years in groups a, b, and c, respectively. There were significant differences in the age at follow-up between groups a and b ( $P < 0.01$ ) and between groups a and c ( $P = 0.02$ ).

The average imaging interval of groups a, b, and c was 18.5, 9.1, and 12.4 months, respectively. The average height

at follow-up of groups a, b, and c was 1.64, 1.52, and 1.57 cm, respectively; there was a statistically significant between groups a and b ( $P = 0.04$ ). The average weight at follow-up of groups a, b, and c was 55.1, 43.7, and 49.2 kg, respectively, showing no statistically significant differences.

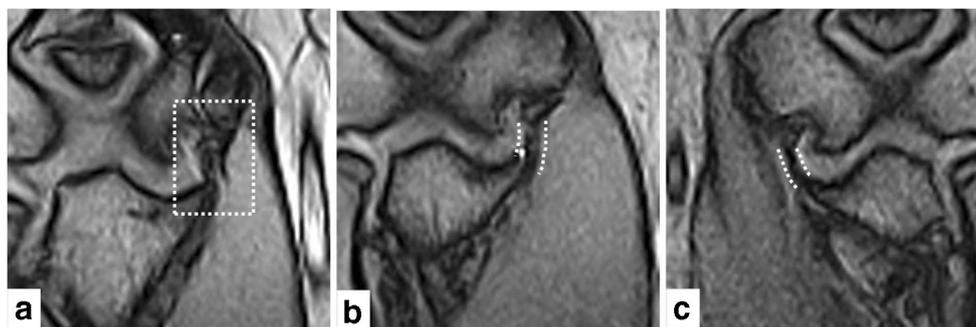
The change in the height/imaging interval (centimeter/month) of groups a, b, and c was 0.56, 0.61, and 0.73, respectively, showing no statistically significant differences. The change in the weight/imaging interval (kilogram/month) of groups a, b, and c was 0.45, 0.49, and 0.38, respectively, also showing no statistically significant difference.

The number of pitchers and players whose first position was pitcher was 16. Among 16 pitchers, 3 of 6 (50%) showed a, 10 of 16 (63%) showed b, and 3 of 8 (38.0%) showed c. There were no statistical predilections among a-c in pitchers. The number of fielders also showed the same tendency among a-c.

The agreement rates and Kappa coefficients between the two interpreters are as follows: (1) 95.8 and 0.77, (2) 92.4 and 0.77, (3) 86.5 and 0.81, (a) 94.4 and 0.79, (b) 72.2 and 0.71, and (c) 90.5 and 0.65, respectively.

Additionally, for group a, we measured the ligament thickness at follow-up MRI: the average thickness was 2.0 mm on the dominant side and 1.5 mm on the contradominant side, representing a significant difference ( $P < 0.04$ ). This suggested slight thickening in the dominant side compared to the contradominant side in the actual measurement, despite the disappearance of swelling by visual assessment.

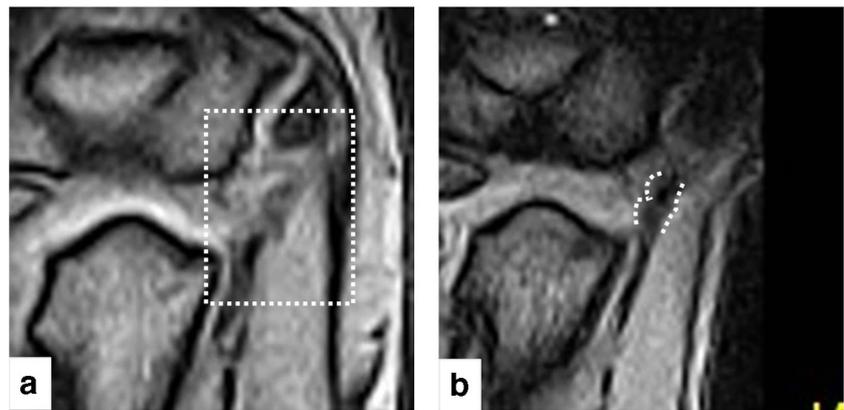
We present typical examples of disappeared (Fig. 3), better (Fig. 4) and worse (Fig. 5) cases, showing actual MRI images. Case No. 5 in Table 1 showed swelling, increased signal intensity, and morphological change in the MCL on initial examination (Fig. 3a); however, after 1 year and 8 months, these findings resolved with the MCL showing a smooth appearance (Fig. 3b), whereby ligament thickness was 2.81 mm on the right (dominant side; Fig. 3b) and 1.57 mm on the left (contradominant side; Fig. 3c). The MCL of case No. 10 was also abnormal, showing swelling and elevation of signal



**Fig. 3** Case where MCL changes disappeared on gradient echo coronal images. **a** Swelling, high signal intensity, and morphological changes are observed in the MCL at the initial examination. **b** Swelling, high signal intensity, and morphological change resolved, and the ligament has a

smooth appearance at second examination with ligament thickness of 2.81 mm. **c** No remarkable findings are observed in the MCL of the contradominant side at second examination, with ligament thickness of 1.57 mm. MCL medial collateral ligament

**Fig. 4** Case where MCL changes were better on gradient echo coronal images. **a** Marked MCL swelling and elevation of signal intensity are observed in the MCL at the initial examination. **b** Only mild swelling and slight elevation of signal intensity are observed in the ligament at second examination. MCL medial collateral ligament



intensity (Fig. 4a); only mild swelling and slight elevation of signal intensity were found in the ligament after 8 months (Fig. 4b). Case No. 26 exhibited marked MCL swelling and elevation of signal intensity (Fig. 5a); however, after 1 year and 5 months, findings had progressed, with enlargement of the MCL and increased signal intensity. Clinically, this patient did not experience any pain.

## Discussion

In cases of ‘asymptomatic MCL injury’, various changes can be identified on follow up MRI, including disappeared, better, and worse radiographic findings. Our results suggest there might be a correlation between cases of ‘disappeared’ findings and the subject’s age.

The MCL is generally not injured by throwing during adolescence because it is relatively more strain-resistant than the medial epicondyle at this age. However, according to some reports [9, 10] in the literature that describe elbow screening of adolescent baseball players using dedicated MRI, many subjects show MCL injury on MRI in spite of no clinical symptoms, whereby the prevalence is quoted to be as high as 50%. Tanaka et al. described this condition as ‘asymptomatic MCL injury.’ They hypothesized that asymptomatic MCL injury might be caused by repetitive

minor trauma resulting from throwing, which is too slight to be noticed by the players [10].

Although different evaluation criteria for MCL injury were used in our study than Tanaka et al. (we used three elements and they used eight), our study also showed a high rate (32%) of asymptomatic MCL injury. We thus concluded that the finding of MCL injury on MRI with no clinical symptoms is not uncommon in daily practice.

Our results clarify that asymptomatic MCL injury can vary in terms of progression, where some may disappear, some may get better, and some may get worse on follow-up MRI. In particular, we wondered why some pitchers showed ‘disappear’ or ‘better’ results on follow-up MRI despite continued throwing. This suggests that the position has no relation to the outcome of asymptomatic MCL injury.

We analyzed and clarified the players’ ages in relation to the outcomes. Six ‘disappear’ subjects (a) had the highest average age of 14.0 years compared to those of the ‘better’ (12.1) and ‘worse’ (12.4) subject groups. We found that asymptomatic MCL injury tends to disappear at approximately 14.0 years of age.

Kooima et al. reported MCL thickening with regular shape in professional baseball players with no clinical symptoms [11]. Hurd et al. also reported the same findings in high school baseball players [12]. They hypothesized that this

**Fig. 5** Case where MCL changes worsened on gradient echo coronal images. **a** MCL swelling and elevation of signal intensity are observed in the MCL at the initial examination. **b** Marked MCL swelling and expansion of the high signal intensity area are observed at second examination. MCL medial collateral ligament



**Table 1** Changes in demographic data and magnetic resonance imaging (MRI) findings between initial and follow-up MRI scans

No.	Initial/follow-up findings			Change in findings	Age at initial scan	Age at follow-up	Imaging interval (months)	Height at follow-up (cm)	Weight at follow-up (kg)	The change of height/imaging interval (cm/months)	The change of weight/imaging interval (kg/months)	Position
	MCL swelling	MCL morphological changes	MCL signal changes									
1	±	±	±	Disappeared	12.3	13.6	16	1.657	52	0.82	0.76	Fielder
2	±	-/-	±	Disappeared	11.6	12.8	14	1.53	43.45	1.02	0.82	Fielder
3	±	-/-	±	Disappeared	13.3	14	9	1.695	60	0.25	0.29	Fielder/pitcher
4	±	±	-/-	Disappeared	12.6	14.3	21	1.6	38	0.84	0.56	Pitcher/fielder
5	±	±	±	Disappeared	12.5	14.2	20	1.71	77	0.21	-0.39	Pitcher/catcher
6	±	-/-	±	Disappeared	12.7	15.3	31	1.65	60	0.2	0.65	Pitcher
7	+/+	-/-	+/+	Better	11.3	11.8	6	1.54	50	0.17	0.51	Fielder
8	+/+	-/-	+/+	Better	11.6	12.8	15	1.6	47.2	0.94	0.72	Pitcher
9	+/+	+/+	+/+	Better	11.1	12.3	15	1.55	48	0.81	0.65	Pitcher/fielder
10	+/+	-/-	+/-	Better	12.5	13.2	8	1.55	37.5	0.45	0.53	Fielder
11	+/+	-/-	+/+	Better	9.7	10.3	7	1.5	53	0.49	0.87	Pitcher
12	+/-	-/-	+/+	Better	11.8	12.5	8	1.54	43	0.53	0.53	Catcher
13	-/+	+/+	+/+	Better	12.7	13.6	11	1.72	70	0.59	0.37	Catcher/fielder
14	+/-	-/-	+/+	Better	11.4	12.5	13	1.6	50	0.96	0.8	Pitcher/fielder
15	+/+	-/-	+/-	Better	11.4	11.8	5	1.44	32	0.34	0.25	Pitcher
16	+/-	-/-	+/+	Better	11.6	12	5	1.48	38	1.08	0.17	Pitcher/fielder
17	-/-	+/-	+/+	Better	11.4	12.1	8	1.5	47	0.66	0.33	Pitcher
18	+/+	-/-	+/-	Better	11.1	11.8	8	1.44	34	0.25	0.25	Pitcher/fielder
19	+/+	+/-	+/+	Better	11.5	12.8	16	1.6	53	1.17	0.78	Catcher/fielder
20	+/-	-/-	+/+	Better	9.5	10.2	8	1.34	32	0.39	0.39	Pitcher/fielder
21	-/-	+/+	-/-	Better	12	12.6	7	1.485	35	0.56	0.56	Fielder
22	-/-	+/+	-/-	Better	10	10.5	6	1.39	30	0.38	0.1	Pitcher/fielder
23	+/+	-/+	+/+	Worse	11.1	12.8	20	1.75	70	1.17	0.63	Pitcher/fielder
24	+/+	-/-	+/+	Worse	11.3	12.5	14	1.53	45	0.96	0.4	Pitcher/fielder
25	+/+	-/-	+/+	Worse	12.3	13.4	13	1.55	43	0.82	0.52	Fielder
26	+/+	-/+	+/+	Worse	10.7	11.9	14	1.486	40.7	0.6	0.9	Pitcher/fielder
27	+/+	-/+	-/-	Worse	11.8	12.8	10	1.63	50	0.1	0.2	Fielder/pitcher
28	+/+	-/-	-/+	Worse	10.8	11.4	6	1.5	34	0.83	-0.5	Fielder/pitcher
29	+/+	-/-	-/+	Worse	12.5	13	6	1.54	41	0	0.33	Fielder
30	+/+	-/-	-/+	Worse	9.9	11.3	16	1.58	70	0.5	0.75	Fielder/pitcher

+ positive - negative

phenomenon may be ‘adaptation,’ namely the ligament thickened as an adaptation to playing baseball; however, the time when this ligament thickening starts remains unclear. In our study, all abnormal findings related to the MCL tended to disappear at approximately age 14. Moreover, typical cases showed ligament thickening (Fig. 3), which resembled the cases shown in Kooima’s and Hurd’s reports.

Most boys experience a growth spurt at approximately 14 years of age; therefore, we hypothesize that asymptomatic MCL injury tends to disappear around the time of the growth spurt, and adaptation also starts around this time. Indeed, our additional measurement test of MCL thickness at follow-up MRI in group a showed an obviously thicker ligament in the

dominant elbow than in the contradominant elbow. Tanaka et al. hypothesized that “asymptomatic MCL injury” might be a precondition to the “adaptation” [10]. Our results might support their hypothesis.

Adaptation is not only found in the ligament but also in the medial epicondyle. Gunenheim et al. reported that the medial epicondyles of little league pitchers tended to be longitudinally larger on the dominant side compared to the contradominant side [13]. Hang et al. also found that the medial epicondyle tended to be larger on the dominant side in pitchers as well as catchers [14]. Yoshizawa et al. reported that the lower pole of cartilage of the medial epicondyle tended to be larger on the dominant side [15]. We hypothesize that these

are all adaptations of bone and cartilage in the medial epicondyle of baseball players.

At the age of a growth spurt, the chondroid matrix changes bony structures at the attachment of the MCL. At the same time, the MCL grows rapidly with thickening of the ligamentous fiber axis curing the scarring from asymptomatic MCL injury. The rigidity of the lower pole of the medial epicondyle and MCL interactively affect each other at this age. We consider that all these changes in the MCL and medial epicondyle that occur at the time of the growth spurt might explain the mechanism of adaptation.

In our study, there was no statistically significant difference in height and weight change among the three groups. We hypothesize that the reason for this is that group a had the longest scan interval. We assume that the increasing rate of height and weight of group a suggests the possibility that these subjects are in the pre-growth spurt era. In fact, the height and weight at follow-up scan was largest in group a, which confirms that these subjects were indeed undergoing a growth spurt at follow-up scan.

The subjects in groups b and c showed no clinical symptoms despite positive MRI findings, and they were all under the age of 14 years. Therefore, we hypothesize that asymptomatic MCL injury might show variable changes, either better or worse, but could be ultimately be expected to result in adaptation during the growth spurt. We thus conclude that subjects who show asymptomatic MCL injury at the initial elbow screening with no other clinical manifestation do not need to limit baseball-related training, and no therapeutic intervention is necessary.

As throwing form is slightly different, our results might apply to handball players or other thrower players. Although there have been no related reports in the literature, our theory might apply to the aforementioned sports.

As for the associated findings around the MCL, there was no case showing abnormal findings in the flexor muscles, including the common flexor, although its attachment was the same as the MCL and it is also related to valgus lability. Based on osseous stress of the medial epicondyle, there was also no case showing bone marrow edema in our study. Although there was no imaging abnormality, a correlation between valgus lability, muscle strength, and bone stress is possible, and we should pay attention to changes in the flexors and bone marrow edema in future studies.

In conclusion, asymptomatic MCL injury findings can vary over time in active baseball players. It is not related to position as a pitcher or nonpitcher. Around the time of the growth spurt, abnormal findings in this group tend to disappear; this may represent a precondition of ‘adaptation as a baseball player’.

The major limitation of our study is the fact that this study was performed without a clinical MRI machine, but with a low field MRI. MRI acquired by a 1.5 T or 3.0 T machine

would show much better images than those in our study. Whole body MRI can certainly provide more detailed information; however, we believe that diagnostic accuracy was sufficient because we used images of the healthy contra-dominant side as a reference during interpretation, and the actual agreement rate was sufficiently high. Moreover, three previously published studies of elbow screening using dedicated MRI also showed a high agreement rate at interpretation [9–11]. The second limitation is the small number of subjects (30); 70 candidates did not undergo follow-up study; however, candidates decided voluntarily whether to participate in additional follow-up scanning or not, which might have reduced selection bias.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Ethical approval** All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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