

## Assessment of glycosaminoglycan content in intervertebral discs of patients with leg length discrepancy: A pilot study

David Latz<sup>a</sup>, Miriam Frenken<sup>b,\*</sup>, Erik Schiffner<sup>a</sup>, Maxime Knautz<sup>b</sup>, Wolfgang Alois Quante<sup>a</sup>, Joachim Windolf<sup>a</sup>, Jan Peter Grassmann<sup>a</sup>, Pascal Jungbluth<sup>a,1</sup>, Christoph Schleich<sup>b,1</sup>

<sup>a</sup> Department of Trauma and Hand Surgery, University Hospital, Moorenstrasse 5, 40225, Düsseldorf, Germany

<sup>b</sup> Department of Diagnostic and Interventional Radiology, University Dusseldorf, Medical Faculty, D-40225, Dusseldorf, Germany

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### ABSTRACT

**Objective:** Leg length discrepancy (LLD) could be a predisposing factor for early degeneration of lumbar intervertebral discs (IVD). The purpose of this study was to elucidate the molecular effect of LLD on IVDs.  
**Methods:** IVDs of Eleven patients ( $25.6 \pm 4.3$  years) with LLD ( $> 10$  mm) and 14 control subjects ( $23.9 \pm 3.5$  years) were compared using a 3T-MR scanner. Morphological T2-weighted and glycosaminoglycan-chemical-exchange-saturation-transfer (gagCEST) sequences were performed.  
**Results:** No differences in morphological Pfirrmann grading were found ( $p > 0.05$ ). In contrast, nucleus-pulposus-gagCEST-values of L5/S1 were significantly lower ( $p = 0.0008$ ).  
**Conclusion:** Our results suggest that LLD is a predisposing factor for molecular IVD alterations, which are detectable even before morphological pathologies could be found.

## 1. Introduction

Leg length discrepancy (LLD) is a frequent finding during orthopedic physical examination. LLD can be caused by a disproportion of bony structures or altered mechanics of the lower extremities due to muscle tightness or contractures. Mostly, LLD is a cause of structural and functional spine changes.<sup>1</sup> In daily clinical practice, only physical examination is frequently used to determine the clinical LLD, and only in severe cases, radiological examination is mandatory.<sup>2,3</sup> Recent studies suggest that chronic low back pain (LBP) correlates with LLD.<sup>4,5</sup> However, LLD can be found in about two-thirds of the population without physical complaints, and the amount of LLD that has to be treated is still discussed controversially.<sup>6,7</sup> LLD leads to pelvic obliquity and a lateral tilt of the lumbar spine; therefore, it could be responsible for asymmetric loading of the intervertebral discs (IVDs) of the lower back and functional changes in the facet joints.<sup>8</sup> Asymmetric loading of the IVD causes higher tension on the convex side and higher compression on the concave side.<sup>9</sup> This functional change could become structural over time.<sup>10</sup> Some studies propose that LLD could be a predisposing factor for lumbar disc herniation.<sup>11</sup> A well-established and

sensitive method for detecting morphological IVD changes is magnetic resonance imaging (MRI).<sup>12</sup> Glycosaminoglycan chemical exchange saturation transfer (gagCEST) imaging is a noninvasive tool to assess the matrix components of IVDs, glycosaminoglycans (GAGs).<sup>13</sup> Previous findings suggest, that a decrease of GAG content correlates with degeneration of the lumbar discs.<sup>14</sup> Thus, with gagCEST imaging, it could be possible to detect early molecular changes in the lumbar discs in patients with LLD, before they could be visible in standard MRI. Therefore, the purpose of this prospective study was to elucidate the effect of LLD greater than 10 mm on the GAG content of lumbar discs.

## 2. Materials and methods

### 2.1. Subjects

Patients were screened for LLD prospectively for six months in the Department of Trauma and Hand Surgery. Eleven patients aged from 20 to 33 years ( $25.6 \pm 4.3$  years, five female, six male) with LLD greater than 10 mm were compared with 14 control subjects aged from 21 to 30 years ( $23.9 \pm 3.5$  years, seven female, seven male) without LLD. Only

\* Corresponding author.

E-mail addresses: [David.Latz@med.uni-duesseldorf.de](mailto:David.Latz@med.uni-duesseldorf.de) (D. Latz), [Miriam.Frenken@med.uni-duesseldorf.de](mailto:Miriam.Frenken@med.uni-duesseldorf.de) (M. Frenken), [Erik.Schiffner@med.uni-duesseldorf.de](mailto:Erik.Schiffner@med.uni-duesseldorf.de) (E. Schiffner), [Maxime.knautz@uni-duesseldorf.de](mailto:Maxime.knautz@uni-duesseldorf.de) (M. Knautz), [Waq@live.de](mailto:Waq@live.de) (W.A. Quante), [Windolf@uni-duesseldorf.de](mailto:Windolf@uni-duesseldorf.de) (J. Windolf), [Jan.Grassmann@med.uni-duesseldorf.de](mailto:Jan.Grassmann@med.uni-duesseldorf.de) (J.P. Grassmann), [Pascal.Jungbluth@med.uni-duesseldorf.de](mailto:Pascal.Jungbluth@med.uni-duesseldorf.de) (P. Jungbluth), [Christoph.Schleich@med.uni-duesseldorf.de](mailto:Christoph.Schleich@med.uni-duesseldorf.de) (C. Schleich).

<sup>1</sup> Pascal Jungbluth and Christoph Schleich are contributed equally.

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subjects without a history of trauma or musculoskeletal diseases of the lower back, hips, or lower limbs were included. In order to identify possible exclusion criteria and to match control subjects and patients according to risk factors for lumbar disc herniation<sup>15–17</sup> (age, gender, height, weight and body mass index (BMI)), a customized questionnaire and written informed consent from each subject was obtained prior to the procedure. After checking for any excluding criteria (musculoskeletal or systemic diseases, chronic drug or alcohol abuse, or treatment of LLD from other health professionals), physical examination was followed by MRI. This study was approved by the institutional review board (3980).

## 2.2. Physical examination

All patients had the initial diagnosis of LLD based on physical examinations and a referral from an orthopedic specialist with 30 years of experience. One single experienced orthopedic surgeon (six years of experience), who was blinded to the initial diagnosis, examined each patient and each control subject. Two clinical methods were used for LLD measurement: 1) an indirect method visualizing the pelvic level using a spirit level (Beckenwasserwaage, Schein Orthopädie Service KG, Remscheid, Germany), which was clipped on the anterior superior iliac spine (ASIS). The degree of LLD was quantified using small heel lifts under the shorter leg and 2) a direct method measuring from bony landmarks with measuring tape. In this study, the distance from the ASIS to the medial malleolus (MM) and the ASIS to the lateral malleolus (LM) were evaluated. Furthermore, to exclude an apparent LLD caused by an asymmetric hypoplastic iliac bone, the distance from the greater trochanter (GT) to MM and GT to LM were determined. To exclude measurement errors caused by contractures, the range of motion (ROM) of the cervical, thoracic, and lumbar spine and of the lower limbs (hip, knee, and upper/lower ankle joints), using a double-armed goniometer (Winkelmesser Goniometer, Kirchner & Wilhelm GmbH + Co. KG, Asperg, Germany), was obtained.<sup>3,18</sup>

## 2.3. Magnetic resonance imaging, T2-weighted and gagCEST

All participants were examined with a whole body 3T MR system (Magnetom Trio, A Tim System, Siemens Healthineers, Forchheim, Germany) in supine position. For signal reception, four channel body matrix coils and a 24 channel spine matrix coil were used. The protocol included a localizer, T2-weighted imaging in sagittal and transversal orientation.

Biochemical imaging was performed with a novel gagCEST sequence, chemical exchange saturation transfer imaging using the spin-lock technique (CESL), and the WASABI (water saturation and B1) method for B0 and B1 field inhomogeneity correction.<sup>19</sup> For gagCEST imaging, several images were acquired with presaturation pulses at different offset frequencies around the bulk water resonance and one reference image without saturation. The spin-lock saturation block was comprised of three radiofrequency (RF) pulses. The first RF pulse flips the magnetization away from the direction of the main magnetic field to the effective field at a specific frequency offset. The second RF pulse is a rectangular pulse, during which the chemical exchange saturation transfer process takes place. The third RF pulse flips the magnetization back to the direction of the main magnetic field. In this study, the presaturation module of CESL was comprised of 10 Gaussian or spin-lock pulses with a pulse duration of 100 ms, B1 amplitude of 1.0, 1.5, and 2.0  $\mu$ T and a duty cycle of 50%. The entire Z-spectrum was acquired with 33 frequency offsets in intervals of 0.3 ppm from  $-4.8$ – $4.8$  ppm. In addition, one reference scan with a frequency offset of  $-300$  ppm was acquired for CESL-spectrum normalization. The WASABI-Z-spectrum was obtained using 49 frequency offsets in a frequency range from  $-2.4$ – $2.4$  ppm with one rectangular-shaped RF pulse (B1 = 4  $\mu$ T and PD = 5 ms) for the presaturation module.<sup>20,21</sup>

Table 1 and Table 2 gives detailed information about the sequence

**Table 1**

Detailed sequence parameters of T2-weighted images.

		CEST	WASABI
$T_R/T_E$	[ms]/[ms]	14/3.64	14/3.64
Field of view	[mm <sup>2</sup> ]	300 × 300	300 × 300
In-plane resolution	[mm <sup>2</sup> ]	2.3 × 2.3	2.3 × 2.3
Slice thickness	[mm]	5	5
Flip angle	[°]	10	10
Averages		1	1
Basic resolution		128 × 128	128 × 128
Number of slices		1	1
Acquisition duration	[min:sec]	9:51	3:10

**Table 2**

Detailed sequence parameters for spin-lock CEST (three pulses with B1 amplitude of 1.0, 1.5, and 2.0  $\mu$ T) and B0-/B1-field inhomogeneity correction (WASABI).

	T2-weighted imaging (sagittal)	T2-weighted imaging (transversal)
Sequence type	Turbo spin echo	Turbo spin echo
Turbo factor	31	18
TR/TE [ms]	3100/105	4510/113
Field of View (FOV) [mm <sup>2</sup> ]	300 × 300	240 × 240
In-plane resolution [mm <sup>2</sup> ]	1.2 × 1.2	0.8 × 0.6
Slice thickness [mm]	3.0	3.0
Flip angle [°]	160	140
Averages	2	1
Basic resolution	256 × 256	384 × 307
Number of slices	15	54
Acquisition duration [min:sec]	3:39	5:13

parameters. To suppress artifacts caused by the abdominal wall or movement of the bowels, a saturation band was applied anterior to the spine.

## 2.4. Data analysis

One board certified radiologist with six years of experience in musculoskeletal radiology, who was blinded to the gagCEST values, scored all lumbar IVDs according to the Pfirrmann scoring system.<sup>12</sup> A region-of-interest (ROI) analysis was performed for SLRasym evaluation of the nucleus pulposus (NP) and annulus fibrosus (AF). All ROIs were selected by an in-house developed automatic image processing algorithm based on MATLAB software (The Mathworks, Inc., Natick, MA, R2012b).<sup>22</sup> The disc segmentation to divide bone and ligament from disc tissue of the lumbar spine was based on Bayes classification. Every automatically positioned ROI was visually checked by one radiologist with six years of experience in IVD segmentation, blinded to Pfirrmann classification analysis and clinical information. None of the ROIs was repositioned. For data analysis, an in-house developed MATLAB software<sup>23</sup> was generated. A reduction of image noise was performed using an in-plane 3 × 3 Gaussian filter. Z-spectra of the WASABI B0 and B1 maps were shifted pixel-wise according to the obtained frequency offset maps.<sup>23</sup> SLRasym maps were calculated by averaging the asymmetry effect in the offset frequency range of GAG resonances (0.9–1.9 ppm).<sup>24</sup> Pixels with an absolute value of SLRasym > 15% were excluded from further analysis.

## 2.5. Statistical analysis

Statistical analysis was performed using MATLAB. The mean and standard deviations for risk factors for lumbar disc herniation<sup>15–17</sup> (age, gender, height, and weight), physical examination, NP-gagCEST, and AF-gagCEST were calculated. Morphological IVD grading was illustrated according to Pfirrmann score.<sup>12</sup> Kolmogorow-Smirnow-Lilliefors

tests were used to assess normal distribution. Univariate analysis of variance (ANOVA) and Kruskal-Wallis tests were performed to assess statistical differences of the means of the gagCEST values of the different groups and subgroups. P values < 0.05 were assumed to be statistically significant.

### 3. Results

#### 3.1. Subjects

Of 25 participants, no one had previously received treatment for LLD from other health professionals for at least one year. There were no significant differences with regard to typical risk factors for developing a lumbar disc herniation<sup>15–17</sup> between patients with LLD and controls: age (patients with LLD: 25.6 ± 4.3 years vs. controls: 23.9 ± 3.5 years, p > 0.05), gender (patients with LLD: 5 female, 6 male vs. controls: 7 female, 7 male), height (patients with LLD: 177.0 ± 8.6 cm vs. controls: 174.5 ± 9.0 cm, p > 0.05), weight (patients with LLD: 73.6 ± 15.5 kg vs. controls: 67.9 ± 11.3 kg, p > 0.05) and BMI (patients with LLD: 23.3 ± 3.1 kg/m<sup>2</sup> vs. controls: 22.2 ± 2.2 kg/m<sup>2</sup>, p > 0.05).

#### 3.2. Physical examination

The average LLD was 12 mm (range 10–20 mm). No participant had contractures or a loss of ROM of the cervical, thoracic, or lumbar spine or of the lower limbs (hip, knee, or upper/lower ankle joints).

#### 3.3. Magnetic resonance imaging, T2-weighted and gagCEST

Of 11 patients with LLD and 14 control subjects without LLD, 125 lumbar IVDs (L1–S1) were successfully imaged with biochemical imaging. Subjects with bulging or herniated discs were excluded. No IVD had to be excluded due to motion artifact.

Morphological IVD grading was performed according to the Pfirrmann<sup>12</sup> classification. Of 125 IVDs, 37 IVDs were scored Pfirrmann grade 1, 86 IVDs Pfirrmann grade 2, and 2 IVDs Pfirrmann grade 3. No degenerated discs, Pfirrmann grade 4 and 5, were scored. In the level L1/2, three discs were scored Pfirrmann grade 1 and 22 discs grade 2. In level L2/3 and in level L3/4, eight discs were graded Pfirrmann score 1 and 17 IVDs Pfirrmann score 2. In level L4/5, eight IVDs were scored Pfirrmann grade 1, 15 discs Pfirrmann grade 2, and 2 IVDs Pfirrmann score 3. In level L5/S1, 10 IVDs were graded Pfirrmann score 1 and 15 discs Pfirrmann grade 2.

No significant differences in morphological Pfirrmann grading were found between LLD patients and healthy controls (p > 0.05).

The mean NP-gagCEST value of the L5/S1 IVD in patients with LLD greater than 10 mm was 1.57 ± 1.19, while for controls, it was 4.46 ± 2.24. NP-gagCEST values of L5/S1 IVDs in patients with LLD greater than 10 mm were significantly lower when compared to subjects without LLD (p = 0.0008; Figs. 1–3).

All other disc levels showed no significant difference between participants with LLD and controls without LLD (p > 0.05). Additionally,

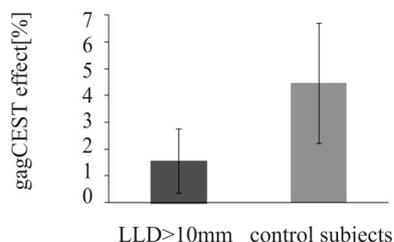


Fig. 1. Comparison of NP-gagCEST values of L5/S1 IVD between patients with LLD > 10 mm and control subjects.

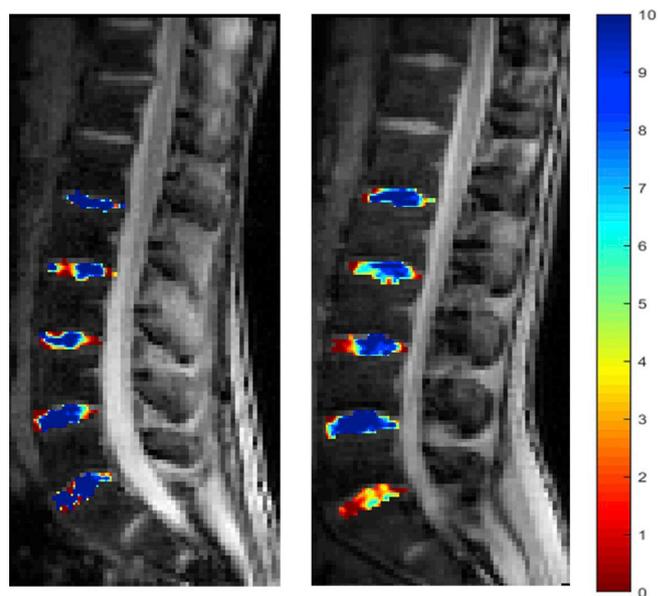


Fig. 2. Color-coded gagCEST map with high GAG content in blue and low GAG content in red of the lumbar spine (L1–S1). On the left side, a participant without leg length discrepancy (LLD) with high GAG content (blue IVDs), and on the right side, a patient with LLD demonstrating molecular alteration of the lumbar disc on level L5/S1 with low GAG content in red and orange. Pfirrmann grading illustrates no morphological disc degeneration with scores of 1 or 2.

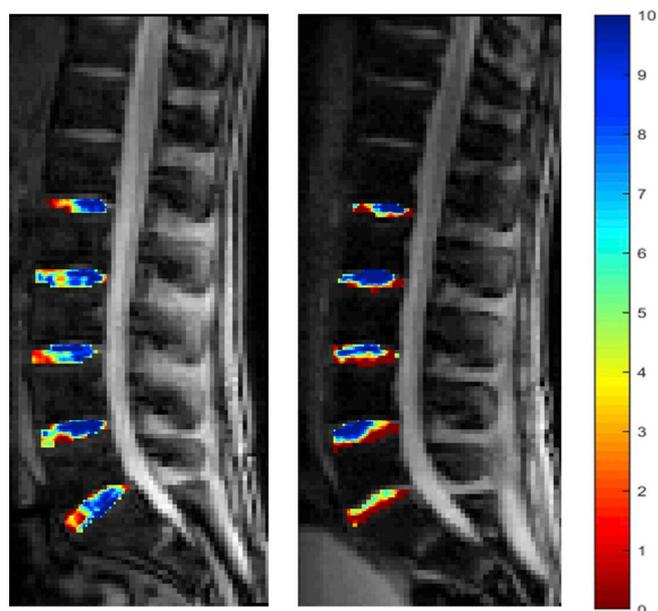


Fig. 3. Color-coded gagCEST map with high GAG content in blue and low GAG content in red of the lumbar spine (L1–S1). Another example of a participant without LLD on the left side compared to a patient with LLD on the right side. On level L5/S1, the patient with LLD (right side) showed significantly lower GAG content compared to control participant. All discs revealed no morphological disc degeneration with Pfirrmann score 1 or 2 (not shown in this picture).

no significant difference between the two groups was found for AF.

### 4. Discussion

NP-gagCEST values of L5/S1 IVDs in patients with LLD were significantly lower compared to healthy subjects. In contrast, no morphologically significant differences could be found. Our results indicate

that LLD correlates with molecular alterations of the L5/S1 lumbar disc in asymptomatic healthy young adults with MRI morphologically inconspicuous IVDs and facet joints. It is known that the rhythmic load on the spine supports the metabolism of the IVD,<sup>25</sup> whereas a permanent static one-sided compressive load worsens the disc's nutrition.<sup>26</sup> LLD leads to pelvic obliquity and a lateral tilt of the lumbar spine; therefore, it could be responsible for a static one-sided compressive load of the IVD.<sup>8,10</sup> A poor nutrient supply to the IVD correlates with a loss of matrix production and matrix degradation and could lead to early disc degeneration.<sup>27,28</sup> Young adults with uncorrected scoliosis are at higher risk of recurrent lumbar disc herniation.<sup>29</sup> Additionally, there is evidence about a significant correlation of the side of radiculopathy to the shorter leg in patients with lumbar disc herniation and LLD.<sup>30</sup> Our findings are in line with previous studies that suggest a loss of GAG in the NP before morphological IVD degeneration occurs<sup>24</sup> and with increasing grade of morphological degeneration.<sup>13,31</sup> Additionally, it is known that degeneration of the IVD occurs before degeneration of the facet joints.<sup>32</sup> The assessment of IVD via gagCEST is a highly sensitive method to detect the early stages of disc degeneration.<sup>33</sup> GAG loss is a central part of disc degeneration.<sup>34</sup> Thus, with gagCEST, detection of early and potentially reversible degeneration of the IVD may be possible.<sup>35–37</sup> The lower NP-gagCEST values of L5/S1 IVDs in patients with LLD could be interpreted as a predisposing factor for lumbar disc protrusion or herniation because of the direct correlation between the compression resistance of the IVD and the number of GAG molecules bound to the core protein<sup>38</sup>. So far, it is unknown if treatment of LLD, for example with shoe inserts, could prevent lumbar disc herniation or could change potentially reversible lower NP-gagCEST values. Noteworthy, IVD herniation of L5/S1 occurs at younger ages, whereas IVD herniation of L4/L5 and especially L3/L4 occur at older ages.<sup>39</sup> Some studies suggest that degeneration of the lumbar spinal column progresses from caudal to rostral.<sup>32</sup> Tayler et al. made the conclusion that the proteoglycans of L5/S1 turned over faster than the proteoglycans of the adjacent lumbar discs because of its proximity to the rigid segment of the sacrum.<sup>40</sup> Arguably, this could be a reason why a compensation for LLD and pelvic obliquity is affecting L5/S1 first. However, there is no consensus regarding the amount of LLD that should be treated by physical therapy and shoe inserts.<sup>7</sup> Some studies have shown that patients with LLD > 25 mm functioned well athletically and without any complaints.<sup>41</sup> In contrast, newer studies suggest that mild LLD (< 20 mm) causes compensatory changes during gait. Furthermore, these authors pointed out that these compensatory changes during gait were unable to prevent the effects of mild LLD on pelvic obliquity.<sup>42</sup> Harvey et al. demonstrated that LLD of 10 mm or more is associated with prevalent and symptomatic knee osteoarthritis in the shorter leg.<sup>43</sup> In line with that, Defrin et al. showed that shoe inserts can significantly reduce pain intensity and functional disability in patient with LLD < 10 mm.<sup>44</sup>

This study has several limitations. One limitation is the limited number of participants. Nevertheless, the results of this study seem to be promising for further evaluation in a larger population. The second limitation is that LLD was determined only by clinical and not radiological examination. We minimized measurement errors by using two clinical methods for LLD assessment (indirect and direct methods). Furthermore, when using the direct method, the average value of two separate tape measurements were used.<sup>45</sup> In daily clinical practice, physical examination is frequently used to determine the clinical LLD, and only in severe cases, radiological examination is mandatory. The tape measurement method has equal validity with radiological examination (Pearson product moment correlation 0.98, intertester reliability 0.99).<sup>2</sup> Therefore, previous studies suggest that the physical exam may be more clinically relevant in daily routine than radiological examination.<sup>46</sup> In this study, we tried to elucidate the effect of LLD on GAG content in lumbar IVDs as a predisposing factor for degeneration or even herniation. Therefore, a relatively homogeneous, young patient-collective was examined, since lumbar IVD herniations are

uncommon in the first two decades of life, with a peak of prevalence in the fourth decade.<sup>47</sup>

For gagCEST and Pfirrmann classification no intra- and inter-observer agreement was performed. However, gagCEST analysis was performed automatically with an established segmentation algorithm, and Pfirrmann classification is known to enable excellent intra- and inter-reader agreement.<sup>22,23</sup>

## 5. Conclusion

This study supports the hypothesis that LLD could be a predisposing factor for early molecular alterations of the lumbar disc of L5/S1. Furthermore, lower gagCEST values of the lumbar disc of L5/S1 caused by LLD were observed before any morphological pathologies were detectable. So far, it is unknown if these molecular alterations of the lumbar disc of L5/S1 are reversible. The effect of shoe inserts and physical therapy on the gagCEST values of the lumbar discs of patients with LLD could be the basis for a prospective, long-term follow-up study.

## Declarations

### *Ethics approval and consent to participate*

There was a positive vote from the ethics committee of the Medical Faculty (3980).

### *Consent for publication*

Written informed consent from each subject was obtained prior to the procedure.

### *Conflicts of interest*

The authors declare that they have no conflict of interests.

### *Funding*

Not applicable.

### *Authors' contributions*

All authors were major contributors concerning the management of the patients, review of the articles and manuscript preparation. All authors read and approved the final manuscript.

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The last and 2nd-last author contributed equally.

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## Abbreviations

- LLD: Leg length discrepancy  
 LBP: Low back pain  
 IVD: Intervertebral disc  
 MRI: Magnetic resonance imaging  
 gagCEST: Glycosaminoglycan chemical exchange saturation transfer  
 GAG: Glycosaminoglycan  
 MM: Medial malleolus  
 LM: Lateral malleolus  
 GT: Greater trochanter  
 ROM: Range of motion  
 CEST: Chemical exchange saturation transfer  
 CEST-: Chemical exchange imaging with spin-lock technique  
 WASABI: Water saturation and B1  
 RF: Radiofrequency  
 NP-: Nucleus pulposus  
 AF: Annulus fibrosus  
 BMI: Body mass index