The L-type Amino Acid Transporter (LAT1) Expression in Patients with Scoliosis

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Abstract:

Introduction: Amino acid transporters are transmembrane proteins that are known to mediate the transfer of amino acids. As one of the amino acid transporters, LAT1, which is encoded by Slc7a5, mediates the cellular uptake of the essential amino acids. Recently, most studies have focused on examining the relationship between LAT1 and skeletal formation in terms of development. However, little is known regarding the clinical features of LAT1 in the cartilage, which might result in the development of skeletal deformities such as scoliosis. Thus, the aim of this study was to investigate the expression of L-type amino acid transporter 1 (LAT1) and its solute carrier transporter 7a5 (Slc7a5) in patients with pediatric scoliosis and to compare with the relationship between LAT1 and Slc7a5 expression and their clinical features.

Methods: We have prospectively recruited 56 patients who underwent corrective spinal fusion for scoliosis. The patients comprised 40 girls and 16 boys, with a mean age of 13.1 years at the time of surgery. There were 34 idiopathic scoliosis (IS) patients, whereas 22 were congenital scoliosis (CS) patients. During the surgery, an epiphyseal part of the spinous process at apical vertebra was harvested; then, LAT1 and Slc7a5 expressions in the cartilage were evaluated.

Results: As per our findings, LAT1 expression was observed in the cartilage in 60.7% (34 out of 56) of the patients. LAT1 expression in IS patients was 76%, which were statistically higher compared to 36% in CS patients. When compared with LAT1 expression, no statistical difference was noted in terms of age, gender, body mass index (BMI), Cobb angle, and Risser grade. Meanwhile, the mean Slc7a5 expression in IS patients was determined to be significantly higher than that in CS patients. No significant correlation was observed between Slc7a5 expression and age, BMI, and Cobb angle.

Conclusions: LAT1 and Slc7a5 expression in IS and CS patients showed significant differences. These expressions were found to be not correlated with age, stature, and severity of the deformity.

Keywords:

scoliosis, L-type amino acid transporter 1, solute carrier transporter 7a5, idiopathic scoliosis, congenital scoliosis

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Introduction

Scoliosis is a complex deformity defined as three-dimensional curvature of the spine that occurs during periods of growth with a prevalence rate of 2 to 3% among children[1)]. There exist many studies on the pathogenesis of scoliosis; in fact, its etiology has been considered multifactorial and is yet to be fully understood. As per previous epidemiological research, it has been recognized that genetic factors contribute to the development of scoliosis. Recent genome-wide association studies (GWAS) were utilized to investigate the genetic background of adolescent idiopathic scoliosis (AIS). Several candidate genes, such as LBX1, GRP126, PAX1, and TBX1, have been identified to be in-

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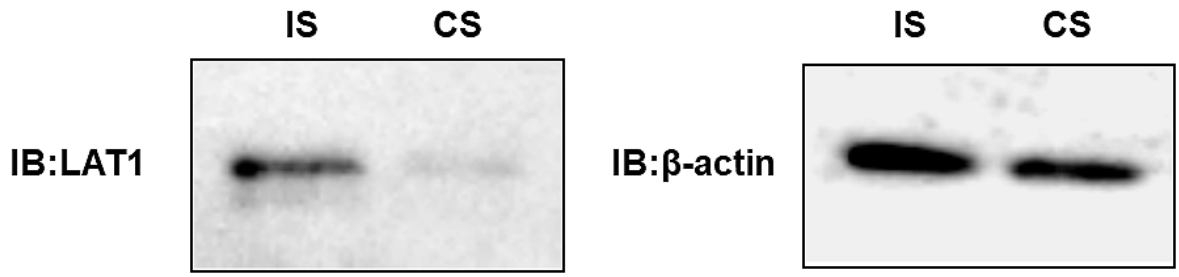

Figure 1. LAT1 expression in the cartilage.
Western blotting analysis of LAT1 protein expression in the cartilage from spinous process. Comparison of LAT1 expression between IS and CS.

volved in the development of AIS[2-5]. Furthermore, a meta-analysis among Japanese population identified 14 susceptible loci associated with AIS[6].

On the other hand, congenital scoliosis (CS) is defined as a spine deformity at birth, which is an underlying congenital vertebral malformation. The frequency of CS is much lower than AIS in the range of 0.01%-0.05%. CS is also investigated to be a consequence of multifactorial genetic interaction. Previous studies have reported several associated genes, including PAX1 and TBX6; moreover, many other genes are known to cause vertebral malformations[7,8]. Although both types of scoliosis are related to genetic background, these candidate genes explain approximately 5% of the phenotypic variance; thereby, further approach is necessary to elucidate the pathogenesis of scoliosis.

Recently, the relation between amino acid transporters and various pathologies, such as cancer, diabetes, and neurological and kidney disorders, have been investigated[9]. Amino acid transporters are described as transmembrane proteins that mediate the transfer of amino acids and are also involved in gene expression, protein synthesis, and energy metabolism. Being one of the amino acid transporters, L-type amino acid transporter 1 (LAT1), which is encoded by solute carrier transporter 7a5 (Slc7a5), mediates the cellular uptake of essential amino acids. Dysregulation of LAT1 has been mainly reported to be one of the pathologies of cancer expression. Nutrient signal such as amino acid and glucose is considered to be necessary to maintain skeletal formation. As one of them, LAT1 mediates cellular uptake of the essential amino acids[10,11]. Ozaki et al. investigated the relation between LAT1 encoded by Slc7a5 and bone homeostasis[12]. They showed that the osteoclast-specific deletion of Slc7a5 in mice led to osteoclast activation and bone loss in vivo; moreover, Slc7a5 deficiency increased osteoclastogenesis in vitro. On the other hand, this mouse model did not show skeletal deformity (unpublished data). In the skeletal formation, we hypothesized that the dysfunction of cartilage homeostasis could be related with the skeletal deformity including scoliosis. However, contrary to the pivotal function of LAT1 on bone homeostasis, little is known regarding the physiological and pathological importance of LAT1 in cartilage homeostasis, which might affect the development of skeletal deformity including scoliosis. In this study, we addressed the clinical question on whether the patients with various types of scoliosis, which could be attributed to the dysfunction of cartilage homeostasis, could exhibit the different behaviors of LAT1 to investigate the association of LAT1 with various types of scoliosis and to reveal the relationship between LAT1 expression and its clinical features.

Materials and Methods

This research has been approved by the IRB of the authors' affiliated institution. Patients with scoliosis, who underwent corrective spinal fusion from 2017 to 2020 at two centers, were included. Patients were selected based on the following inclusion criteria: age under 20 years, idiopathic and congenital scoliosis, and candidates for posterior scoliosis correction. The patients with syndromic scoliosis were excluded in this study. Informed consent was obtained from all patients and families. Materials were obtained by posterior approach. A part of the spinous process at apical vertebra was exposed and harvested. The bony part of the spinous process was used for bone grafting, whereas the tips of the spinous processes which contain an epiphyseal cartilage was removed and analyzed for this study.

All the specimens were fixed in 70% ethanol and kept refrigerated at −70°. For the evaluation of LAT1, tissues were homogenized in a lysis buffer containing protease inhibitor cocktail. Samples were then subjected to SDS-PAGE, followed by transfer to polyvinylidene difluoride (PVDF) membranes and subsequent immunoblotting. The primary antibodies used was anti-LAT1 antibody (Cell Signaling Technology (#5347)). The primary antibodies were diluted with blocking solution. Fig. 1 shows Western blotting analysis of LAT1 expression. The data was expressed by above or below the detection limit. For the evaluation of Slc7a5, the total RNA was extracted using TRIzol reagent, followed by the synthesis of complementary DNA (cDNA) with reverse transcriptase and oligo-dT primer. The expression was measured using real-time polymerase chain reaction via the following primers: 5'-AAACTGGAACGGTGAAGGTGA-3' and 5'-TGTGTGGACTTGGGAGAGGA-3'. The levels of the genes examined were normalized by *ACTB* as an internal control for each sample using the following primers: 5'-TTC TCCACACTCCACTGACAAA-3' and 5'-AAAGCACAACG

Table 1. Descriptive Data.

	IS	CS	Total	Significance
No. of cases	34	22	56	
Age (years)	14.7±2.8	10.5±4.0	13.1±3.8	p<0.05
Gender (girl/boy)	30/4	10/12	40/16	p<0.05
BMI	18.5±2.9	17.6±2.8	18.2±2.9	N.S.
Main Cobb angle	58.2±12.7	48.5±22.8	54.4±17.9	N.S.
Risser grade	3.5±1.4	1.3±1.8	2.7±1.9	p<0.05
Grade 0	3	13	16	
Grade 1	0	1	1	
Grade 2	3	2	5	
Grade 3	5	2	7	
Grade 4	14	2	16	
Grade 5	9	2	11	

IS, idiopathic scoliosis; CS, congenital scoliosis

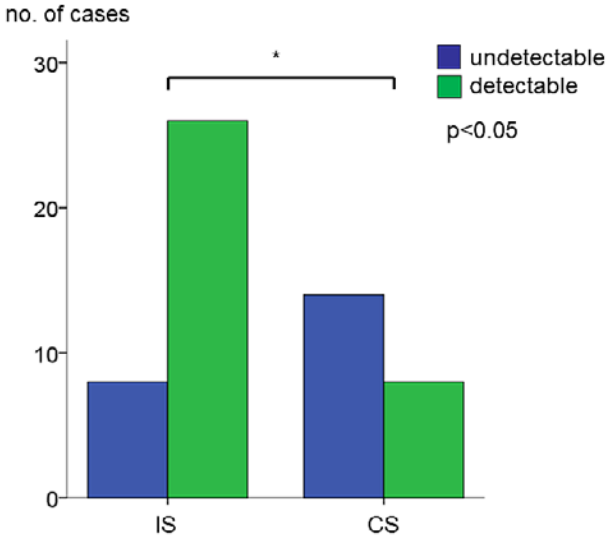

Figure 2. LAT1 expression.
Detected or undetected cases of LAT1 expression on each scoliosis type. LAT1 expression was detected in 34 out of 56 patients. The rate of LAT1 expression in IS patients was 76%, which was statistically higher compared to 36% in CS patients (chi-square test).

ACTGAAAATGC-3'. Slc7a5 expression was calculated by % control of IS patient (case no1).

The characteristics of the patients were collected including initial age, body mass index (BMI), menarche age, past history, and family history. For radiographic evaluation, Cobb angle of the major curve was obtained. Skeletal maturity was measured using the Risser classification.

The SPSS software (version 23.0; SPSS Inc., Chicago, IL) was used for statistical analysis. For comparison of unpaired values among groups, the Mann-Whitney U test was used. To compare the categorical value, chi-square test was used when the cell sizes are expected to be large. The Spearman rank correlation test was used for correlations between variables. Statistical significance was set at P<0.05.

Table 2. LAT1 and Other Factors.

	LAT1 (+)	LAT (−)	Significance
No. of cases	34	22	
Age (years)	13.7±3.5	12.1±4.3	N.S.
Gender (girl/boy)	25/9	15/7	N.S.
BMI	18.4±2.8	17.7±2.9	N.S.
Main Cobb angle	57.8±13.3	49.1±22.6	N.S.
Risser grade	3.0±1.7	2.1±2.0	N.S.

LAT1, L-type amino acid transporter 1

Results

In total, 56 patients (40 females, 16 males) with a mean age at the time of surgery of 13.1±3.8 years were enrolled in this study. There were 34 idiopathic scoliosis (IS), whereas there were 22 CS patients. The mean BMI was 18.2 ±2.9 kg/m^2, and the mean menarche age was 12.3±1.4 years. The average of Risser grade was 2.7±1.9. The distribution of Risser grades was as follows: 16 patients were classified as having grade 0; 1 as grade 1; 5 as grade 2; 7 as grade 3; 16 as grade 4; and 11 as grade 5. The apex of the curve was located at the thoracic spine in 40 patients and at thoracolumbar/lumbar spine in 16 patients. The mean Cobb angles were 54.4±17.9°. There was a statistical difference regarding age, gender, Cobb angle, and Risser grade among two groups (Table 1).

In all patients, LAT1 and Slc7a5 expressions in the cartilage were analyzed from the tips of the spinous processes. LAT1 expression was detected in the cartilage in 34 out of 56 patients. The rate of LAT1 expression in IS patients was 76%, which is statistically higher compared to 36% in CS patients (Fig. 2). When compared with detected and undetected patients, there was no statistical difference among age, genders, BMI, Cobb angle, and Risser grade (Table 2). Regardless of the difference between IS and CS, the expression of Slc7a5 was all can be detected. Fig. 3 shows the Slc7a5 expression depending on the scoliosis type. Slc7a5 expression was noted to be significantly higher in IS pa-

tients than in CS patients. There was no significant correlation between age, BMI, and Slc7a5 expression (Fig. 4). No correlation was also noted between Cobb angle and Slc7a5 expression (Fig. 5).

Discussion

This is the first clinical study to investigate the amino acid transporter expression in patients with scoliosis. This current study showed that LAT1 and Slc7a5 expression was different depending on the type of scoliosis. Also, a statistical difference was noted in terms of the severity of the deformity, gender, and maturity between the two groups which could bias the results (Table 1). On the other hand, additional analysis of LAT1 and Slc7a5 expression showed no statistical difference among these factors (Table 2) and no significant correlation (Fig. 4, 5). Therefore, the results may indicate that LAT1 and Slc7a5 expressions were more influenced by scoliosis type than the severity of the deformity, gender, or maturity.

In the chondrogenic differentiation, paired box gene 1 (Pax1) has also been reported to be one of the regulators of chondrocyte maturation[13]. Recent GWAS study showed that PAX1 was associated with IS in females[4]. Although the etiology between idiopathic scoliosis and CS is different, mutations in PAX1 have been reported with the development of vertebral malformations[7]. Purkiss et al. reported the high incidence of IS in families of children with CS[14]. Although no previous report was found examining the relationship between Slc7a5 and the other genes in the field of spinal deformity, the current results may indicate that any chondrogenic factors would predispose to the development of spinal deformity.

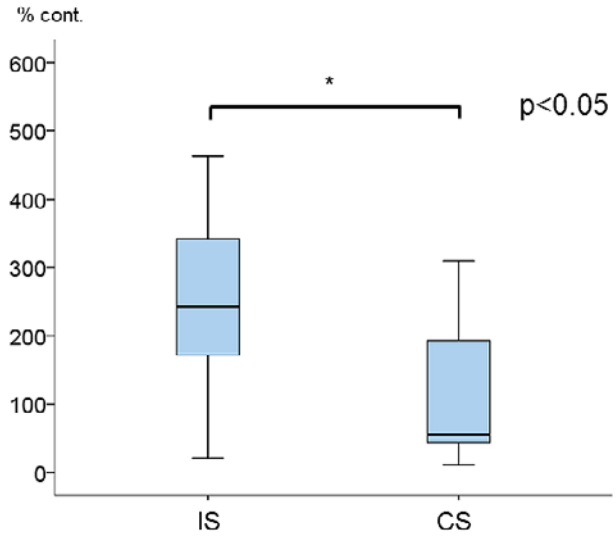

Figure 3. Slc7a5 expression.
Slc7a5 expression on each scoliosis type. Slc7a5 expression in IS patients was significantly higher than that in CS patients. (Mann–Whitney U test).

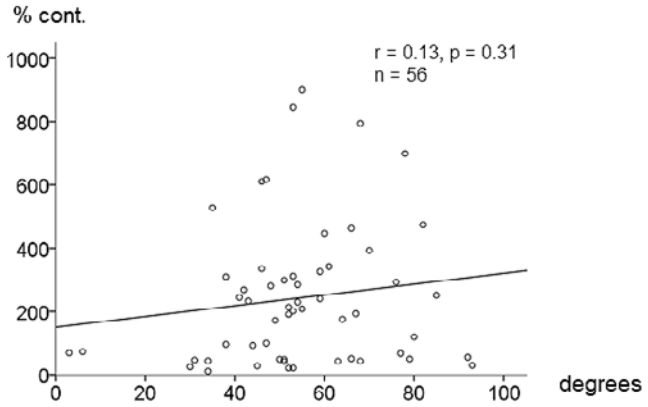

Figure 5. Slc7a5 expression and Cobb angle.
Correlation between Slc7a5 expression and Cobb angle. There was no significant correlation. (Spearman rank correlation test).

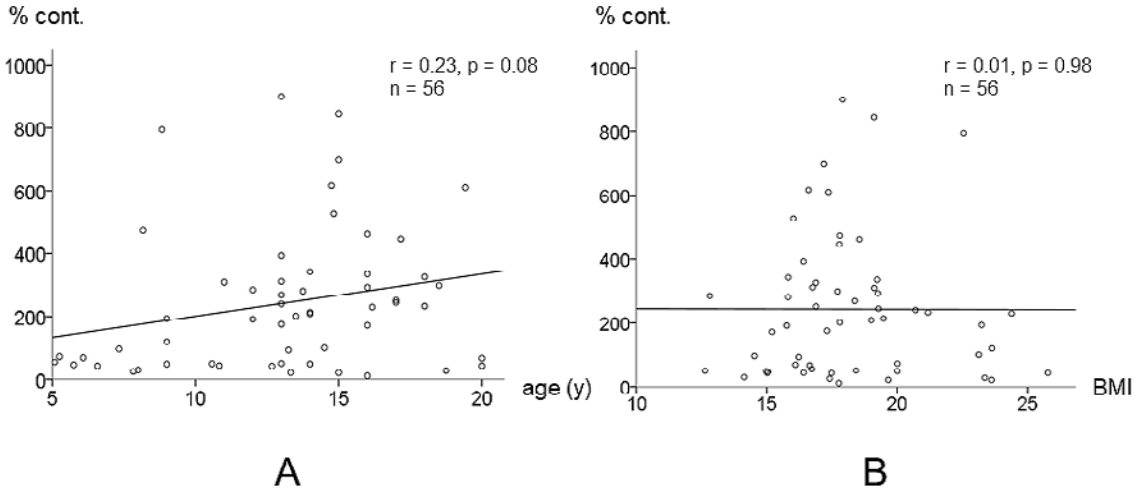

Figure 4. Slc7a5 expression and age (A) and BMI (B).
Correlation between Slc7a5 expression and age (A) and BMI (B). Both parameters did not show significant correlation. (Spearman rank correlation test).

In the development of spinal column, the relationships between spinal growth plates and scoliosis were investigated. Bylski-Austrow et al. investigated spinal growth plate histomorphometry between scoliosis patients and autopsy controls[15], wherein it was showed that the hypertrophic chondrocyte heights in the vertebral growth plates in patients with scoliosis were lower than that in autopsy controls without scoliosis. Zhu et al. analyzed the histomorphology of the spinal growth plates in IS and CS[16], wherein they showed that the proliferative chondrocytes in the anterior spinal column in IS patients were more active than that of the posterior column, whereas the difference found in IS patients was not observed in CS patients. They concluded that two types of scoliosis may have different growth kinetics affecting the curve development. LAT1 and Slc7a5 expressions in the cartilage between AIS and CS patients were found to be statistically different (Fig. 2, 3), which might support one of the hypotheses. In the clinical and basic features of IS and CS, Giampietro et al. hypothesized that IS and CS share an underlying genetic mechanism, whereas a single genetic defect may result in a predisposition to different types of spinal deformities[17]. We reasoned from previous reports and this current study that the difference between IS and CS may reflect one of the functional effects of the participation of Slc7a5 in a developmental role. Currently, the results of this study do not directly link to clinical application. However, we hope that the results will help us clarify the pathogenesis of scoliosis, which could only be explained in 5% of scoliosis patients at present.

This study has several limitations. First, we could not obtain the epiphysis of the spinous processes as an age-matched control without scoliosis. Additionally, the patients with mild or moderate scoliosis were not also investigated same as the controls without scoliosis. Second, the sample size of each type was small. Finally, we did not investigate other candidate genes previously reported to be involved in the development of scoliosis. Thereby, further study with larger sample size and with the relationship between the previously reported genes and Slc7a5 would be warranted.

In conclusion, L-type amino acid transporter 1 (LAT1) and solute carrier transporter 7a5 (Slc7a5) expression in IS and CS patients showed significant differences. These expressions did not relate to age, stature, and severity of the deformity. The different behaviors between IS and CS might reflect the functional effects through cartilage formation in the developmental phase.

Conflicts of Interest: The authors declare that there are no relevant conflicts of interest.

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Ethical Approval: This study was approved by the Ethics Committee Institution of Kanazawa University Hospital (No. 2017-156).

Informed Consent: Informed consent for publication was obtained from all participants in this study.

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