

Assessment of NMDA receptor genes (*GRIN2A*, *GRIN2B* and *GRIN2C*) as candidate genes in the development of degenerative lumbar scoliosis

KI-TACK KIM¹, JINSUNG KIM², YOO JIN HAN², JUN HO KIM³, JONG SEOK LEE⁴ and JOO-HO CHUNG³

¹Department of Orthopedic Surgery, Spine Center, School of Medicine, Kyung Hee University Hospital at Gangdong, Seoul 134-727; ²Department of Physical Medicine and Rehabilitation; ³Department of Pharmacology and Kohwang Medical Research Institute; ⁴Department of Emergency Medicine, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

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Abstract. Degenerative lumbar scoliosis (DLS) progresses with aging after 50-60 years. The genetic association of DLS remains largely unclear. In this study, the genetic association between glutamate receptor, ionotropic, *N*-methyl D-aspartate (*NMDA*, *GRIN*) receptor genes and DLS was investigated. A total of 9 coding single nucleotide polymorphisms (cSNPs) in NMDA receptor genes [*GRIN2A* (rs8049651, Leu425Leu; rs9806806, Tyr730Tyr); *GRIN2B* (rs7301328, Pro122Pro; rs35025065, Asp447Asp; rs1805522, Ile602Ile; rs1806201, Thr888Thr; rs1805247, His1399His); and *GRIN2C* (rs689730, Ala33Ala; rs3744215, Arg1209Ser)] were selected and genotyped using direct sequencing in 70 patients with DLS and 141 healthy controls. Multiple logistic models (codominant, dominant and recessive) were calculated for the odds ratio (OR), 95% confidence interval (CI) and corresponding P-values. The SNPStats, SNPAnalyzer and HelixTree programs were used for the evaluation of the genetic data. Among the SNPs examined, no significant associations were observed between the *NMDA* receptor genes and DLS. When the patients were divided into two groups according to clinical characteristics based on Cobb's angle ($<20^\circ$ or $\geq 20^\circ$) and lateral listhesis (<6 mm or ≥ 6 mm), associations were observed between rs689730 of *GRIN2C* and Cobb's angle (codominant, $P=0.038$; dominant, $P=0.022$) and between rs7301328 of *GRIN2B* and lateral listhesis (codominant, $P=0.003$; dominant, $P=0.015$; recessive, $P=0.015$). These results indicate that the *GRIN2A*, *GRIN2B* and *GRIN2C* genes do not affect the development of DLS. However, the *GRIN2C* gene may be associated with Cobb's angle, while the *GRIN2B* gene may be associated with lateral listhesis.

Introduction

Degenerative scoliosis develops after skeletal maturity without a previous history of scoliosis and typically presents in the lumbar or thoracolumbar spine (1,2). The etiology of degenerative lumbar scoliosis (DLS) remains unclear, although several factors, including hormones, genetics and degenerative diseases of the spine, may be involved (3). It has been suggested that the cause of DLS is associated with disc degeneration and osteoporosis (4).

During an individual's lifetime, modeling, remodeling and repair of the bone occur as a result of the activity of osteoclasts and osteoblasts (5). The control of bone remodeling is performed by a few neurotransmitters, such as glutamate, via corresponding receptors in the bone cells (6). Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS) (7) and acts through a variety of receptors including *N*-methyl-D-aspartate (*NMDA*) receptors. The *NMDA* receptors (*NMDARs*) are composed of *NMDAR1*, *NMDAR2* and *NMDAR3* and are expressed in the bone cells (8,9). In addition, glutamate is a known regulator of the maturation and differentiation of osteoclasts and osteoblasts (10,11).

We hypothesized that genetic polymorphisms in the *NMDAR* genes may be associated with DLS. However, there have been no published studies concerning the association between DLS and single nucleotide polymorphisms (SNPs) of *NMDAR* genes. Therefore, the 9 coding SNPs (cSNPs) of *NMDAR2A* (*GRIN2A*), *NMDAR2B* (*GRIN2B*) and *NMDAR2C* (*GRIN2C*) were analyzed in 70 patients with DLS and 141 control subjects.

Materials and methods

Subjects. The DLS group comprised 70 unrelated individuals (10 males and 60 females), aged 52-82 years (mean \pm SD, 67.63 ± 7.05 years). The DLS patients were selected from the Spine Center of Kyung Hee University East-West Neo Medical Center (Seoul, Korea) and the National Medical Center (Seoul, Korea), out of $\sim 9,000$ patients per year over three years. Each patient was diagnosed by two specialized spine surgeons

Correspondence to: Dr Joo-Ho Chung, Department of Pharmacology and Kohwang Medical Research Institute, School of Medicine, Kyung Hee University, Dongdaemun-Gu, Kyunghee-Daero 26, Seoul 130-701, Republic of Korea
E-mail: jhchung@khu.ac.kr

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Table I. Characteristics of DLS and control subjects.

Factor	DLS (n=70)	Controls (n=141)
Age (years, mean \pm SD)	67.63 \pm 7.05	66.4 \pm 8.23
Gender (male/female)	10/60	25/116
Cobb's angle ($^{\circ}$, mean \pm SD)	17.07 \pm 2.95	NA
Lateral listhesis (mm, mean \pm SD)	4.78 \pm 2.30	NA

DLS, degenerative lumbar scoliosis; NA, not applicable.

Table II. Sequences of primers for cSNPs of *GRIN2A*, *GRIN2B* and *GRIN2C* genes.

Gene	SNP	Direction	Sequence (5'→3')	Size (bp)
<i>GRIN2A</i>	rs8049651,	Forward	GATTTGCCTCTCCAGAAATCAG	321
	Leu425Leu	Reverse	CTATTTCAAAGGGTTGGGCACG	
	rs9806806	Forward	TGCATGCATTTACCTCCTAACA	380
	Tyr730Tyr	Reverse	AATGGGAGCAGATAGGAAGCTGA	
<i>GRIN2B</i>	rs7301328,	Forward	CGAGAAAGATGATTTCCACCAT	391
	Pro122Pro	Reverse	GATTAATTGCTGGCCTATCCAC	
	rs35025065,	Forward	GGTCTGTTGCCCTGCTTTATTTTC	322
	Asp447Asp	Reverse	CAGGTTCCATTGATTTTCTTCC	
	rs1805522,	Forward	TGAGAGTCCCTGGAAAAGAGAG	392
	Ile602Ile	Reverse	CCAGAAACCTGGTCCACATATT	
	rs1806201,	Forward	TTGTGGTCATTTCTAGCCTCTC	371
	Thr888Thr	Reverse	CCGAACGTTCTCTCTACCTCAC	
rs1805247,	Forward	CCTACGCCACATGTTTGAGAT	381	
His1399His	Reverse	GGTTTTTGTGTTAGGCACACA		
<i>GRIN2C</i>	rs689730,	Forward	AACCTCTGTCTCTCTCCCTGTG	394
	Ala33Ala	Reverse	GGAGATGAAGTCAAGGATCTGG	
	rs3744215,	Forward	CTGTCTGTCTCACCTTCCACC	392
	Arg1209Ser	Reverse	CACCATGATTTGAGGCTACTGA	

SNP, single nucleotide polymorphisms.

and all patients fulfilled the physical examination and radiographic criteria (Cobb's angle, $>10^{\circ}$). Informed consent was obtained from all individuals according to the Declaration of Helsinki guidelines. The study was approved by the Ethics Review Committee of the Medical Research Institute, Kyung Hee University Medical Center (Seoul, Korea). The control group comprised 141 individuals (25 males and 116 females), aged 53-85 years (mean \pm SD, 66.74 \pm 8.23 years). Control group individuals were recruited following a general health check-up program to confirm that they had no clinical evidence of DLS according to radiographic criteria. The ages and genders of the control group individuals were matched to those of the DLS group (Table I).

SNP genotyping. The cSNPs of *GRIN2A*, *GRIN2B* and *GRIN2C* were searched. Information relating to the cSNPs was obtained from the SNP database (www.ncbi.nlm.nih.gov/SNP; dbSNP Build 130) of the National Center for Biotechnology Information (NCBI). cSNPs with unknown heterozygosity

and minor allele frequencies ($<5\%$) were excluded. Finally, 9 cSNPs were selected [*GRIN2A* (rs8049651, Leu425Leu; rs9806806, Tyr730Tyr); *GRIN2B* (rs7301328, Pro122Pro; rs35025065, Asp447Asp; rs1805522, Ile602Ile; rs1806201, Thr888Thr; rs1805247, His1399His); and *GRIN2C* (rs689730, Ala33Ala; rs3744215, Arg1209Ser)] for analysis. Genomic DNA was extracted from blood samples collected in EDTA using a commercially available Qiagen DNA extraction kit (Qiagen, Tokyo, Japan). Genomic DNA was amplified using primers for each cSNP (Table II). The PCR products were sequenced using an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis. The Hardy-Weinberg equilibrium (HWE) was assessed using SNPStats (<http://bioinfo.iconcologia.net/snpstats>) (12). Multiple logistic regression models (codominant, dominant and recessive) were used to calculate the odds

Table III. Genotype frequencies of cSNPs in *GRIN2A*, *GRIN2B* and *GRIN2C* genes.

Gene	SNP	Genotype	DLS		Control		Models	OR (95% CI)	P-value
			n	Freq.	n	Freq.			
<i>GRIN2A</i>	rs8049651, Leu425Leu	C/C	61	0.87	123	0.87	Codominant	0.88 (0.40-1.94)	0.76
		C/T	9	0.13	16	0.11	Dominant	0.99 (0.42-2.35)	0.99
		T/T	0	0.00	2	0.01	Recessive	0.00 (0.00-NA)	0.17
	rs9806806, Tyr730Tyr	C/C	63	0.90	120	0.85	Codominant	0.60 (0.26-1.37)	0.20
		C/G	7	0.10	18	0.13	Dominant	0.64 (0.25-1.59)	0.32
		G/G	0	0.00	3	0.02	Recessive	0.00 (0.00-NA)	0.11
<i>GRIN2B</i>	rs7301328, Pro122Pro	C/C	12	0.18	31	0.22	Codominant	0.86 (0.57-1.29)	0.46
		G/C	32	0.47	61	0.43	Dominant	0.91 (0.49-1.69)	0.77
		G/G	24	0.35	49	0.35	Recessive	0.69 (0.32-1.46)	0.32
	rs1805522, Ile602Ile	A/A	5	0.07	7	0.05	Codominant	0.93 (0.57-1.51)	0.76
		G/A	16	0.23	41	0.29	Dominant	0.84 (0.45-1.56)	0.58
		G/G	49	0.70	93	0.66	Recessive	1.22 (0.36-4.16)	0.75
	rs1806201, Thr888Thr	A/A	14	0.21	34	0.24	Codominant	0.89 (0.58-1.36)	0.60
		G/A	37	0.54	72	0.51	Dominant	0.95 (0.48-1.87)	0.88
		G/G	17	0.25	34	0.24	Recessive	0.77 (0.38-1.57)	0.47
	rs1805247, His1399His	A/A	46	0.66	91	0.65	Codominant	0.84 (0.50-1.40)	0.50
		A/G	23	0.33	42	0.30	Dominant	0.96 (0.53-1.77)	0.91
		G/G	1	0.01	8	0.06	Recessive	0.20 (0.02-1.68)	0.08
<i>GRIN2C</i>	rs689730, Ala33Ala	C/C	36	0.54	81	0.58	Codominant	1.28 (0.77-2.13)	0.34
		C/T	26	0.39	56	0.40	Dominant	1.14 (0.63-2.06)	0.66
		T/T	5	0.07	3	0.02	Recessive	3.31 (0.76-14.48)	0.10
	rs3744215, Arg1209Ser	G/G	26	0.39	44	0.31	Codominant	0.97 (0.64-1.47)	0.88
		G/T	27	0.40	74	0.52	Dominant	0.75 (0.41-1.39)	0.36
		T/T	14	0.21	23	0.16	Recessive	1.38 (0.66-2.91)	0.40

SNP, single nucleotide polymorphism; cSNPs, coding SNPs; DLS, degenerative lumbar scoliosis; freq, frequency; OR, odds ratio; 95% CI, 95% confidence interval; NA, not applicable.

ratio (OR), 95% confidence interval (CI) and corresponding P-values, with age and gender as covariables. To analyze the associations of cSNPs and haplotypes, SNPStats, HapAnalyzer and HelixTree (Golden Helix, Inc., Bozeman, MT, USA) were used.

Results

Of the nine cSNPs of *GRIN2A*, *GRIN2B* and *GRIN2C* genes examined, eight were polymorphic and one (rs35025065) was monomorphic (Table III). The genotype distributions of the eight cSNPs were in HWE ($P > 0.05$, data not shown).

As shown in Table III, there was no significant difference in the genotypic frequency of polymorphism rs8049651 between the DLS (CC 87%, CG 13% and GG 0%) and control groups (CC 87%, CG 11% and GG 1%; $P = 0.76$). Additionally, the other seven cSNPs were not associated with DLS. Via the Gabriel method (13), two cSNPs (rs1805247 and 1806201 of *GRIN2B*) were used to construct a one linkage disequilibrium (LD) block (data not shown). Three haplotypes in the block had frequencies > 0.1 . However, there was no significant difference in haplotype frequencies between the DLS and control groups (data not shown).

However, when DLS patients were divided into two groups according to clinical characters based on Cobb's angle ($< 20^\circ$ or $\geq 20^\circ$) and lateral listhesis (< 6 mm or ≥ 6 mm), associations were observed between the *GRIN2B* and *GRIN2C* polymorphisms and clinical characteristics. As shown in Table IV, rs689730 of *GRIN2C* was associated with Cobb's angle in the codominant ($P = 0.038$; OR, 2.72; 95% CI, 0.98-7.55) and dominant models ($P = 0.022$; OR, 3.79; 95% CI, 1.15-12.54). The present study showed that the CT or TT genotype frequency with Cobb's angle $\geq 20^\circ$ was ~2-fold higher than with Cobb's angle $< 20^\circ$. In the analysis of lateral listhesis, rs7301328 of *GRIN2B* showed significant differences in the codominant ($P = 0.0027$; OR, 3.21; 95% CI, 1.41-7.29), dominant ($P = 0.015$; OR, 3.91; 95% CI, 1.22-12.51) and recessive models ($P = 0.015$; OR, 5.39; 95% CI, 1.26-23.08). The results indicated that the CC genotype frequency with lateral listhesis ≥ 6 mm was ~4-fold higher than with lateral listhesis < 6 mm (Table IV).

Discussion

A number of individuals present degenerative diseases of the spine, such as spondylolisthesis, lateral listhesis, spinal stenosis and degenerative scoliosis with increasing age (3,14). Scoliosis

Table IV. Genotype frequencies of rs689730 and rs7301328 in DLS patients with Cobb's angle or lateral listhesis.

A, Cobb's angle								
SNP	Genotype	<20°		≥20°		Model	OR (95% CI)	P-value
		n	Freq.	n	Freq.			
GRIN2C, rs689730, Ala33Ala	C/C	15	0.75	21	0.45	Codominant	2.72 (0.98-7.55)	0.038
	C/T	4	0.20	22	0.47	Dominant	3.79 (1.15-12.54)	0.022
	T/T	1	0.05	4	0.09	Recessive	1.82 (0.18-17.97)	0.590

B, Lateral listhesis								
SNP	Genotype	<6 mm		≥6 mm		Model	OR (95% CI)	P-value
		n	Freq.	n	Freq.			
GRIN2B, rs7301328, Pro122Pro	C/C	3	0.08	9	0.32	Codominant	3.21 (1.41-7.29)	0.003
	G/C	18	0.45	14	0.50	Dominant	3.91 (1.22-12.51)	0.015
	G/G	19	0.48	5	0.18	Recessive	5.39 (1.26-23.08)	0.015

DLS, degenerative lumbar scoliosis; SNP, single nucleotide polymorphism; freq, frequency. OR, odds ratio; 95% CI, 95% confidence interval.

is a spinal deformity with lateral curvature or angulation and may be accompanied by rotation of the affected vertebrae in the vertical axis (15).

Complicated DLS induces severe back or leg pain with neurological deficits by compressing neural elements and causing nerve root ischemia (16). Despite uncertainty with regard to the etiology of DLS, degeneration of the spinal column occurs in the development of the disease (3). Considering that the etiology of DLS is unclear, the identification of candidate genes associated with the development of DLS may potentially be used to identify at-risk individuals. Therefore, the potential of *NMDAR2* genes as candidate genes in the development of DLS was investigated through a case-control analysis in the current study.

Glutamate is the most prominent excitatory neurotransmitter in the CNS. It has been studied intensively in a number of neuropathological conditions, including stroke, epilepsy and neurodegenerative diseases (17,18). Glutamate exerts its effects through ionotropic ligand-gated ion channels and metabotropic G-protein-coupled glutamate receptors. The ionotropic receptors are further divided into NMDA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors (19-21). Spencer *et al* (6) conducted a review of NMDA-type glutamate signalling.

In the current study, 9 cSNPs in the genes *GRIN2A*, *GRIN2B* and *GRIN2C* were analyzed to assess the association between these polymorphisms and DLS. The *GRIN2A*, *GRIN2B* and *GRIN2C* genes are located on chromosomes 16p13.2, 12p12 and 17q25, respectively. In the coding regions of each gene, 15, 22 and 14 cSNPs have been discovered, respectively. Of these 51 cSNPs, 9 exhibited heterozygosity >0.1 (www.ncbi.nlm.nih.gov/SNP, dbSNP Build 130) and were selected for analysis in this study. Overall, none of the genotype frequencies or haplotype distributions of the

NMDRs (*GRIN2A*, *GRIN2B* and *GRIN2C*) were observed to be associated with DLS.

Several studies have shown associations between SNPs of *GRIN2A* and/or *GRIN2B* and neurological or psychiatric diseases, such as Huntington's disease (22), schizophrenia (23), bipolar disorder (24) and Alzheimer's disease (25). In addition, Kim *et al* (26) reported that rs1806201 of *GRIN2B* was associated with alcoholism in a Korean population, but rs18054247 was not. However, Tadic *et al* (27) reported that rs1806201 was not associated with alcohol dependence, alcohol withdrawal-induced seizures and delirium tremens in a Caucasian population.

The possible associations between nine cSNPs of *GRIN2A*, *GRIN2B* and *GRIN2C* genes and DLS were investigated, but the results were negative. To compare each genotype frequency in various ethnic populations, the human SNP database (www.ncbi.nlm.nih.gov/SNP, dbSNP Build 130) was searched. The genotype distributions of all cSNPs in the control group were similar to those of the Asian populations, particularly the Japanese population (data not shown).

When the patients were divided into two groups according to clinical characteristics (Cobb's angle and lateral listhesis), *GRIN2B* and *GRIN2C* were associated with the severity of DLS. As shown in Table IV, rs689730 of *GRIN2C* was associated with Cobb's angle in the codominant (P=0.038) and dominant models (P=0.022). Furthermore, rs7301328 of *GRIN2B* was associated with lateral listhesis in the codominant (P=0.003), dominant (P=0.015) and recessive models (P=0.015).

The present results indicate that *GRIN2A*, *GRIN2B* and *GRIN2C* do not affect the development of DLS in the Korean population. However, significant associations were observed between *GRIN2C* and Cobb's angle and between *GRIN2B* and lateral listhesis in DLS.

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