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Association between regulating synaptic membrane exocytosis 2 gene polymorphisms and degenerative lumbar scoliosis

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Abstract. Degenerative lumbar scoliosis (DLS) is a spinal deformity that develops after skeletal maturity and progresses with age. In contrast to adolescent idiopathic scoliosis, the genetic association of DLS has not yet been elucidated. The purpose of this study was to investigate the association between regulating synaptic membrane exocytosis 2 (RIMS2, OBOE) gene polymorphisms and DLS. Two coding single-nucleotide polymorphisms [rs2028945 (Gln1200Gln) and rs10461 (Ala1327Ala)] of RIMS2 were selected and genotyped by direct sequencing. As a result, the rs10461 was associated with DLS in allele frequencies (P=0.008) and genotype distributions (P=0.006 in the codominant model, 0.018 in the dominant model and 0.029 in the recessive model). In the analysis of haplotypes, two haplotypes exhibited significant differences between the control and DLS groups (CC haplotype, P=0.009 in the codominant model, 0.038 in the dominant model and 0.030 in the recessive model; CT haplotype, P=0.041 in the codominant model and 0.021 in the dominant model). These findings suggest that RIMS2 may be associated with the development of DLS.

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Abbreviations: RIMS2, regulating synaptic membrane exocytosis 2; SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; DLS, degenerative lumbar scoliosis

Key words: degenerative lumbar scoliosis, regulating synaptic membrane exocytosis 2, polymorphism

Introduction

Degenerative lumbar scoliosis (DLS) is defined as a spinal deformity with a Cobb angle of >10˚, which develops after skeletal maturity without a previous history of scoliosis. It generally presents in the lumbar or thoracolumbar part of the spine and may be associated with severe back pain (1,2). The prevalence of DLS increases with age and its incidence in patients >50 years of age was reported to be ~6% (3). The etiology of DLS has not been clearly established. However, the most commonly proposed causes of DLS were ost eoporosis and degenerative diseases of the spinal column (3). Several factors, including life-style, intrinsic mediators and hormonal or genetic factors, are likely to affect the development or the acceleration of DLS (4,5).

Rab3-interacting molecules (RIMs) are presynaptic active zone proteins that perform an essential function in neurotransmitter release (6,7). RIMs were identified as putative effectors for Rab3, which is a synaptic vesicle protein that regulates neurotransmitter release (6,7). RIMs have been shown to interact with multiple synaptic proteins, such as UNC-13 homolog B (*C. elegans*) (UNC13B, Munc13) (8), ELKS/Rab6-interacting/CAST family member 1 (ERC1, ELKS) (9,10), RIM-binding proteins (RIM-BPs) (11), α-liprins and synaptotagmin 1 (12-14). RIMs are encoded by four regulating synaptic membrane exocytosis genes (RIMS1-4), of which RIMS1, RIMS3 and RIMS4 express a single isoform (RIM1α, 3γ and 4γ, respectively), whereas RIMS2 (RAB3IP3, OBOE) expresses three isoforms (RIM2α, RIM2β and RIM2γ) via independent internal promoters (15).

Two single-nucleotide polymorphisms (SNPs) [rs2028945 (Gln1200Gln) and rs10461 (Ala1327Ala)] in the coding region of RIMS2 in DLS were investigated in this study.

Subjects and methods

Subjects and clinical phenotypes. A total of 51 DLS patients and 145 control subjects were enrolled in this study. DLS patients were recruited from the Spine Center of Kyung Hee University East-West Neo Medical Center (Seoul, Korea) and the National Medical Center (Seoul, Korea) and were selected

among ~10,000 patients per year during a 3-year period. Each patient was diagnosed by a specialized spinal surgeon and fulfilled all physical examination and radiographic criteria. The DLS group comprised 44 females and 7 males and the mean age was 68.67±8.00 years [mean ± standard deviation (SD)]. The Cobb angle in the DLS group was 19.36±7.38˚ and the lateral listhesis was 4.84±4.24 mm. A left convex curve was observed more frequently compared to a right convex curve (31 and 20 patients, respectively) (Table I). DLS patients were divided into two subgroups according to clinical characteristics, including Cobb angle (≤30˚ vs. >30˚), lateral listhesis (≤5 vs. >5 mm) and curve direction (left vs. right) (16,17). The control group included 145 patients (124 females and 21 males) who were recruited from a general health check-up program after it was confirmed that they had no clinical evidence of scoliosis or any other severe disorders. The gender and age of the control group were matched with those of the DLS group. This study was conducted according to the Declaration of Helsinki guidelines. Written informed consent was obtained from each individual. The research protocol was approved by the Ethics Review Committee of the Medical Research Institute, Kyung Hee University Medical Center, Seoul, Korea.

SNP selection and genotyping. We investigated SNPs in the coding region of RIMS2. The related information was obtained from the SNP database (www.ncbi.nlm.nih.gov/SNP, dbSNP BUILD131). The SNPs with unknown heterozygosity, minor allele frequencies <10% and no data regarding Asian populations (rs17854256, rs76323676 and rs61753732) were excluded. Consequently, rs2028945 (Gln1200Gln) and rs10461 (Ala1327Ala) were selected for this investigation. Genomic DNA was extracted from the blood sample of each subject using Qiagen DNA Extraction kit (Qiagen, Tokyo, Japan) following the manufacturer's instructions and was amplified using the primers for each SNP of RIMS2 (Table II). PCR products were sequenced by an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA). Sequence data were analyzed using SeqMan II software (DNASTAR, Inc., Madison, WI, USA).

Statistical analysis. The Hardy-Weinberg equilibrium (HWE) was assessed using SNPStats (http://bioinfo.iconcologia.net/index.php) (18). The allele frequencies of each SNP were compared by the Pearson's Chi-square test. The effect of SNP genotypes was analyzed using the codominant, dominant and recessive models. Logistic regression analysis was used to calculate the odds ratio (ORs), 95% confidence intervals (CIs) and P-values with controlling age and gender as covariables. The linkage disequilibrium (LD) was assessed using Haploview software version 4.1 (Broad Institute, Cambridge, MA, USA). LD block was constructed using the Gabriel method (19,20). The association of SNPs and haplotypes was analyzed using SNPStats, HapAnalyzer version 1.0 (http://hap.ngri.go.kr/), and HelixTree (Golden Helix Inc., Bozeman, MT, USA) software.

Statistical analysis was performed using the package of SPSS Statistics software version 17.0 (SPSS Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Table I. Demographic characteristics of the study subjects.

Characteristic	Control group	DLS group
Number of subjects (n)	145	51
Male/female (n)	124/21	44/7
Age (mean ± SD, years)	65.57±8.65	68.67±8.00
Cobb angle (degrees)		19.36±7.38
Lateral listhesis (mm)		4.84±4.24
Curve direction (left/right)		31/20

DLS, degenerative lumbar scoliosis; SD, standard deviation.

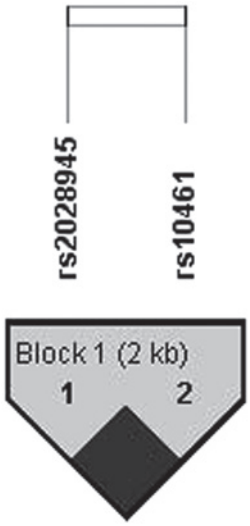

Figure 1. Linkage disequilibrium block between rs2028945 and rs10461 in regulating synaptic membrane exocytosis 2 (RIMS2).

Results

The clinical characteristics of control subjects and DLS patients are summarized in Table I. The mean age of the control and the DLS group was 65.57±8.65 (mean ± SD) and 68.67±8.00 years, respectively. In the DLS group, the average Cobb angle was 19.36±7.38˚ and the length of the lateral listhesis was 4.84±4.24 mm. Patients with a left convex curve outnumbered those with a right convex curve (31 and 20, respectively) (Table I).

The genotype distributions of rs2028945 and rs10461 were in HWE equilibrium (P>0.05). In an analysis of the allele frequencies on two SNPs, the C allele of rs10461 was more frequently encountered in the DLS group compared to the control group (OR=1.857; 95% CI, 1.174-2.937 and P=0.008) (Table III). In an analysis of the genotype distributions, the rs10461 was associated with DLS (OR=1.93; 95% CI, 1.20-3.10 and P=0.006 in the codominant model; OR=2.62; 95% CI, 1.12-6.13 and P=0.018 in the dominant model, and OR=2.25; 95% CI, 1.10-4.61 and P=0.029 in the recessive model). The rs2028945 was not significantly different between the control and the DLS groups (Table IV). One LD block was constructed between rs2028945 and rs10461 with the Gabriel method (Fig. 1). Of the haplotypes in the block, the

Table II. Primer sequences used for each SNP in RIMS2.

SNP	Sequence (5'-3')	Product size (bp)
rs2028945		
Sense	TCCTCACTGAACACTCATTCAGG	543
Antisense	CTCAGCCCAGTGGAATCTTTAAC	
rs10461		
Sense	AGATCATCGTCTGGGGAGATTA	343
Antisense	CCCTAGAAACAGGCTCAGAAGA	

SNP, single-nucleotide polymorphism; RIMS2, regulating synaptic membrane exocytosis 2.

Table III. Genotype and allele frequencies of RIMS2 polymorphisms in the control and DLS groups.

		Control		DLS				
SNP	Type	No.	(%)	No.	(%)	Model	OR (95% CI)	P-value
rs2028945	Genotype							
Gln1200Gln	C/C	49	33.8	20	39.2	Codominant	0.72 (0.45-1.16)	0.18
	C/T	69	47.6	26	51.0	Dominant	0.79 (0.40-1.53)	0.48
	T/T	27	18.6	5	9.8	Recessive	0.44 (0.16-1.23)	0.09
	Allele							
	C	167	57.6	66	64.7		1	
	T	123	42.4	36	35.3		0.74 (0.46-1.18)	0.21
rs10461	Genotype							
Ala1327Ala	T/T	45	31.0	8	15.7	Codominant	1.93 (1.20-3.10)	**0.006**
	T/C	71	49.0	25	49.0	Dominant	2.62 (1.12-6.13)	**0.018**
	C/C	29	20.0	18	35.3	Recessive	2.25 (1.10-4.61)	**0.029**
	Allele							
	T	161	55.5	41	40.2		1	
	C	129	44.5	61	59.8		1.857 (1.174-2.937)	**0.008**

P-values were calculated by logistic regression analysis following adjustment for age and gender. Bold numbers indicate significant association. RIMS2, regulating synaptic membrane exocytosis 2; SNP, single-nucleotide polymorphism; DLS, degenerative lumbar scoliosis; OR, odds ratio; CI, confidence interval.

CC and CT haplotypes were associated with the risk of DLS (CC haplotype: P=0.009 in the codominant model, P=0.038 in the dominant model and P=0.030 in the recessive model; CT haplotype: P=0.041 in the codominant model and P=0.021 in the dominant model) (Table IV).

The results obtained in this study were compared with different ethnic populations by searching the human SNP database (www.ncbi.nlm.nih.gov/SNP; dbSNP BUILD 131), which includes the genotype frequencies of each SNP. Genotype distributions in our control subjects were similar to those in Asian populations, particularly the Chinese population (Table V).

Discussion

DLS develops *de novo* in adulthood and presents more frequently in the elderly (3). With increasing life expectancy, DLS may complicate degenerative spondylolisthesis, lateral listhesis or spinal stenosis, in which the neural elements are compressed by bone and soft tissue, leading to nerve root ischemia (2). Therefore, patients with accelerated DLS may develop severe back pain and neurological deficits (3). Despite a variety of studies on DLS, the etiology remains unknown. Therefore, the potential of RIMS2 as a candidate gene was evaluated. Our results demonstrated that rs10461 (Ala1327Ala) was significantly associated with DLS. It was hypothesized that the C allele of rs10461 may be a risk factor in the development of DLS (Table III). In addition, two haplotypes (CC and CT) exhibited significant differences between the control and DLS groups. The results indicated that RIMS2 may be associated with DLS. However, RIMS2 polymorphisms were not associated with the clinical features of DLS (Cobb angle, lateral listhesis and curve direction) (data not shown).

Table IV. Haplotype distributions of RIMS2 polymorphisms (rs2028945 and rs10461) in the control and DLS groups.

	Control			DLS					
Haplotype	No.	(%)	Freq	No.	(%)	Freq	Model	OR (95% CI)	P-value
HAP1 (CC)									
HH	29	20.0		29	20.0		Codominant	1.86 (1.16-2.96)	**0.009**
H -	71	49.0	0.44	71	49.0	0.60	Dominant	2.42 (1.05-5.56)	**0.038**
- -	45	31.0		45	31.0		Recessive	2.18 (1.08-4.41)	**0.030**
HAP2 (TT)									
HH	27	18.6		27	18.6		Codominant	0.74 (0.46-1.18)	0.208
H -	69	47.6	0.42	69	47.6	0.35	Dominant	0.79 (0.41-1.53)	0.486
- -	49	33.8		49	33.8		Recessive	0.48 (0.17-1.31)	0.150
HAP3 (CT)									
HH	4	2.8		4	2.8		Codominant	0.38 (0.15-0.96)	**0.041**
H -	30	20.7	0.13	30	20.7	0.05	Dominant	0.28 (0.09-0.83)	**0.021**
- -	111	76.6		111	76.6		Recessive	0.70 (0.08-6.46)	0.757

P-values were calculated by logistic regression analysis following adjustment for age and gender. Bold numbers indicate significant association. RIMS2, regulating synaptic membrane exocytosis 2; SNP, single-nucleotide polymorphism; DLS, degenerative lumbar scoliosis; Freq, frequency; OR, odds ratio; CI, confidence interval.

Table V. Genotype distributions of RIMS2 polymorphisms among ethnic populations.

	Populations				
SNP	Korean	European	Chinese	Japanese	Sub-Saharan African
rs2028945					
C/C	0.338	0.833	0.267	0.182	0.850
C/T	0.476	0.167	0.489	0.341	0.133
T/T	0.186	-	0.244	0.477	0.017
rs10461					
C/C	0.200	0.414	0.222	0.159	0.153
C/T	0.490	0.483	0.356	0.341	0.610
T/T	0.103	0.103	0.422	0.500	0.237

From dbSNP BUILD131 (www.ncbi.nlm.nih.gov/SNP). RIMS2, regulating synaptic membrane exocytosis 2; SNP, single-nucleotide polymorphism.

RIMS comprise four members, RIMS1-4. RIMS1 and RIMS2 are 512.7 and 747.9 kb in size, respectively, whereas RIMS3 and RIMS4 are 15.4 and 54.5 kb, respectively (8,15). RIMS1 expresses RIM1α; the gene may encode a single isoform. By contrast, RIMS2 encodes three isoforms (RIM2α, RIM2β and RIM2γ); besides the exon for the α isoform, two additional separate exons in RIMS2 may encode the N-termini of RIM2β and RIM2γ through β- and γ-specific promoters, respectively. RIMS3 and RIMS4 express a single isoform, RIM3γ and RIM4γ, respectively (8,15). The α-RIMs (RIM1α and RIM2α) contain the full complement of domains which are the N-terminal Zn^{2+}-finger domain, the central PDZ and C_2A domains and the C-terminal C_2B domain (15). They are able to bind to Rab3 and Munc13 proteins (13,21,22) and closely interact with α-liprins (13), which in turn regulate the active zone structure (23,24). Therefore, α-RIMs are considered to be essential for regulating neurotransmitter releases (8,13,25,26), some of which are involved in the neurobiology of schizophrenia (27,28).

The exocytosis of neurotransmitter-filled synaptic vesicles is under tight regulation in presynaptic nerve terminals. RIMs may mediate the regulation of exocytosis via interacting with multiple synaptic proteins, such as Rab3 (6,7), Munc13 (8), ELKS (9,10), RIM-BPs (11), α-liprins and synaptotagmin 1 (12-14). In addition, RIMs are indirectly connected with the active zone proteins Piccolo and Bassoon via ELKS (29). In a previous study, the experimental loss of α-RIMs resulted in the severe impairment of motor control, profound defects of synaptic transmission at neuromuscular junctions and increment of irregularly distributed motor synapses in skeletal muscle fibers (8). These results suggested

that RIMs may affect the function of the neuromuscular system. However, no published studies assessing the potential role of RIMs in bone degeneration or the genetic association of RIMS polymorphisms in DLS are available. We demonstrated that RIMS2, known as a regulatory gene for synaptic membrane exocytosis in the central nervous system, may be a candidate gene associated with the risk of DLS in the Korean population. However, additional studies including a larger number of subjects and different populations are required to confirm these findings. In conclusion, the rs10461 in RIMS2 was associated with DLS in the Korean population. This finding suggests that RIMS2 may affect the development of DLS.

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