ORIGINAL ARTICLE doi: 10.2176/nmc.oa.2017-0138

Neurol Med Chir (Tokyo) 58, 17–22, 2018 Online November 10, 2017

Genetic Risk Assessment of Elastin Gene Polymorphisms with Intracranial Aneurysm in Koreans

Jin Pyeong JEON,[1,2] Eun Pyo HONG,[3] Jeong Eun KIM,[4] Eun Jin HA,[4] Won-Sang CHO,[4] Young-Je SON,[4] Jae Seung BANG,[4] and Chang Wan OH[4]

[1]*Department of Neurosurgery, Hallym University College of Medicine, Chuncheon, Korea;*
[2]*Institute of New Frontier Research, Hallym University College of Medicine, Chuncheon, Korea;*
[3]*Department of Medical Genetics, Hallym University College of Medicine, Chuncheon, Korea;*
[4]*Department of Neurosurgery, Seoul National University College of Medicine, Seoul, Korea*

Abstract

Elastin encoded by elastin gene (*ELN*) is a crucial extracellular matrix protein responsible for arterial resilience. The objective of this study was to identify single nucleotide polymorphisms (SNPs) of *ELN* gene susceptible to intracranial aneurysm (IA) in Korean population. Two SNPs of *ELN* gene, rs2071307 (Gly422Ser) and rs2856728 (intron), were genotyped in 90 patients with IA and 90 age and frequency matched controls. Fisher's exact test was conducted to evaluate allelic association with IA. Of the two SNPs in *ELN* gene, T allele of rs2856728 (intron) showed statistically significant association with increased development of IA (odds ratio [OR]: 2.34, 95% confidence interval [CI]: 1.44–3.81, $P = 7.6 \times 10^{-4}$). However, G allele of rs2071307 (Gly422Ser) had no significant association with the development of IA (OR: 1.27, 95% CI: 1.44–3.81, $P = 0.607$). Interestingly, the odds of having rs2856728 variant was approximately 2-fold higher in males than that in females (OR: 3.46 vs. 1.88, $P < 0.05$). However, none of SNPs showed difference between single and multiple IA in this study. This preliminary study implies that the rs2856728 variant in *ELN* gene polymorphisms might play crucial roles in the development and pathogenesis of IA in Korean population.

Key words: intracranial aneurysm, subarachnoid hemorrhage, elastin, genetic variants

Introduction

Subarachnoid hemorrhage (SAH) has 30-day mortality rate ranging from 36% to 42%.[1,2] Among SAH survivors, up to 50% of them have permanent disability.[3,4] Intracranial aneurysm (IA) accounts for 85% of nontraumatic SAH.[4,5] Hemodynamic studies have shown that elevated wall shear stress (WSS) may lead to IA formation by degenerative endothelial remodeling.[6,7] Structural integrity of the arterial wall can be sustained by extracellular matrix (ECM).[8] Bruno et al.[8] have reported that focal degradation of ECM is related to the formation and growth of aneurysm.

Elastin encoded by elastin gene (*ELN*) is a crucial ECM protein responsible for resilience of arteries.[9] Since arterial wall of IA has decreased level of ECM,[10] *ELN* gene has been studied as a candidate for IA.[11] Although two studies have shown a possible association between *ELN* gene in IA formation,[12,13] positive correlation between *ELN* gene and IA is not revealed in most studies. According to a meta-analysis by Paterakis et al.,[14] *ELN* INT20 1315T > C variants is associated with IA formation (odds ratio [OR]: 0.66). However, its association is not shown in Caucasian population.[13,15,16] Regarding *ELN* EX20 1264G > A, its association with IA formation is not significant in the three studies of Caucasian, although it is significant in one study on East Asians.[11] In addition, higher incidence of IA in Asian compared to that in Caucasian has been reported.[17] These results suggest that *ELN* variants could affect IA formation with ethnical difference. To the best of our knowledge,

Received June 14, 2017; Accepted August 25, 2017

genetic association study of *ELN* polymorphisms in Koreans has not been reported to date. Therefore, the objective of this study was to investigate the association between *ELN* gene polymorphisms and IA in a homogeneous Korean population to provide clue for the pathogenesis of IA in East Asians.

Materials and Methods

Subjects

This prospective study included radiologically confirmed 90 IA patients with saccular shape and 90 age- and gender- matched controls from April 2015 to December 2016. Aneurysms which showed non-saccular types such as fusiform, dissection, traumatic, or infectious aneurysms were excluded. The control group consisted of sex- and age- matched patients who underwent computed tomography or magnetic resonance angiography for headache evaluation or medical check-up. Those who had other neurological diseases including arteriovenous malformation, intracranial hemorrhage, and infarction were excluded. Medical records included sex, age, presentation (unruptured IA vs. SAH), multiplicity, hypertension (HTN), diabetes mellitus (DM), hyperlipidemia, smoking,[18] and familial history of aneurysm (vs. sporadic). This study was approved by the Institutional Review Boards (No. 1504-087-665 and 2016-31).

SNP selection and genotyping

Two SNPs, rs2071307 and rs2856728, of *ELN* gene reported in previous studies[11–13] were selected for this study. They accounted for linkage disequilibrium (LD, $r^2 < 0.8$) in Japanese and Chinese of Phase II HapMap data supported by LD TAG SNP Selection (TagSNP) of SNPinfo web server (http://snpinfo.niehs.nih.gov/guide.htm). For genotyping of the two SNPs, genomic DNA was extracted from peripheral blood of 90 patients and 90 controls using HiGeneTM Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea). Primers of the two SNPs (Table 1) were designed using Primer-3 v.0.4.0 program (http://bioinfo.ut.ee/primer3-0.4.0/). Polymerase chain reaction (PCR) was performed in 25 μl volume containing 100 ng genomic DNA, 1.5 μl of each primer (10 pmole/μl), and SolgTM 2X Taq PCR Pre-Mix (Solgent, Daejeon, Korea). Pre-denaturation was done at 95°C for 5 min, 34 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. Amplified fragments were confirmed by 1.5% agarose gel electrophoresis, purified with the SolgTM PCR purification kit (SolGent, Daejeon, Korea), and sequenced using an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Table 1 Primers designed for the two SNPs of elastin (*ELN*) gene

SNP	Primer	Primer sequence	Length
rs2071307 & rs2856728	Forward	5'-AATCCATCAG-CATCCCTCAG-3'	395 bp
	Reverse	5'-CAACTCCTC-CCTGAGCACAT-3'	

Statistical analysis

Baseline characteristics were described as mean ± standard deviation (SD) for age and the number of subjects. Percentage was used to describe other discrete variables. Chi-square and unpaired *t*-tests were used to evaluate difference of clinical variables between patients with IA and controls. Regarding allelic associations between IA and the two SNPs of *ELN* gene, Fisher's exact test was performed to estimate OR with 95% confidence intervals (CIs). Descriptive and association analyses were conducted using STATA software v.11.2 (Stata Corp., College Station, TX, USA). Minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE) were evaluated using Haploview v.4.2 (https://www.broadinstitute.org/haploview/haploview).[19]

Results

Demographic characteristics of the enrolled patients

Baseline characteristics of the 90 patients with IA and 90 controls are summarized in Table 2. There were 61 (67.8%) female patients with IA and 54 (60.0%) female controls ($P = 0.368$). Mean ages of the 90 patients with IA and 90 controls were 57.8

Table 2 Baseline characteristics of patients with intracranial aneurysm (IA) and controls

Variables	IA ($n = 90$)	Controls ($n = 90$)	*P*-value
Female	61 (67.8%)	54 (60.0%)	0.368
Age, years	57.8 ± 10.2	56.6 ± 14.2	0.551
Hypertension	30 (33.3%)	24 (26.7%)	0.331
Diabetes mellitus	11 (12.2%)	9 (10.0%)	0.636
Hyperlipidemia	18 (20.0%)	13 (14.4%)	0.325
Smoking	15 (16.7%)	10 (11.1%)	0.283
Aneurysm rupture	7 (7.8%)		
Multiple aneurysm	30 (33.3%)		

*$P < 0.05$ is significant.

and 56.6 years (P = 0.551), respectively. Seven (7.8%) patients with IA had aneurysm rupture. History of diseases and cigarette smoking showed no statistical difference between the two groups. Thirty (33.3%) patients with IA exhibited multiple aneurysms.

Genetic associations of 2 *ELN* polymorphisms with IA

Results of genotype and allele frequencies with HWE P-value and associations of the two *ELN* polymorphisms, rs2071307 (Gly422Ser) and rs2856728 (intron), in 90 patients with IA and 90 healthy controls are shown in Table 3. *ELN* polymorphism rs2071307 with benign effect in the genome was not significantly associated with IA formation (OR: 1.27; 95% CI: 0.64–2.49, P = 0.607). However, the major "T" allele of rs2856728 was strongly associated with the risk of developing IA (OR: 2.34; 95% CI: 1.44–3.81). It was more frequent among IA patients compared to that in the control group in the current study ($P = 7.6 \times 10^{-4}$).

Genetic difference of the two *ELN* polymorphisms was compared between two gender groups. Results are shown in Table 4. The coding variant rs2071307 was not significantly different between the two gender groups. It showed no significant association with developing IA (in male group, OR: 1.27, 95% CI: 0.39–4.17; in female group, OR: 1.63, 95% CI: 0.71–3.27). On the other hand, the rs2856728 variant was significantly associated with developing IA in both gender groups (P = 0.004 in male and P = 0.048 in female). The T allele in this variant showed an approximately 2-fold high risk in males compared to that in females (male, OR: 3.46; female, OR: 1.88). However, no SNP showed an association with single or multiple aneurysms in the 90 IA patients ($P > 0.1$, data not shown).

Discussion

Due to absence of the external elastic lamina, internal elastic lamina (IEL) is a major contributor to the strength of the cerebral arterial wall.[20] IEL has a longitudinal arrangement of elastin fiber. Defect of IEL or elastin degradation has been proposed to be related to IA formation.[21] Associations between several *ELN* gene polymorphisms and sporadic IA IN East-Asian and Caucasian populations have been studied.[11–13] Compared to relative similar proportions of minor allele of rs2071307 in Japanese and Dutch

Table 3 Associations between elastin (*ELN*) polymorphisms and intracranial aneurysm (IA)

Gene chr.	SNP function	Position	Genotype	90 patients with IA and 90 controls								
				Patient, N (%)	Control, N (%)	HWE p[a]	Allele	Patient, N (%)	Control, N (%)	OR[b]	95% CI[b]	P[b]
ELN	rs2071307	73,470,714	GG	73 (81.1)	70 (77.8)	1.00	G[c]	163 (90.6)	159 (88.3)	1.27	0.64–2.49	0.607
7q11.23	Gly422Ser		AG	17 (18.9)	19 (21.1)		A	17 (9.4)	21 (11.7)			
			AA	0 (0)	1 (1.1)							
	rs2856728	73,470,782	TT	60 (66.7)	41 (45.6)	0.35	T[c]	147 (81.7)	118 (65.6)	2.34	1.44–3.81	7.6×10^{-4}
	intron		TC	27 (30.0)	36 (40.0)		C	33 (18.3)	62 (34.4)			
			CC	3 (3.3)	13 (14.4)							

Chr.: chromosome, [a]HWE p: Hardy-Weinberg equilibrium P-value for control group, [b]odds ratio (OR), 95% confidence interval (CI). The P-value was estimated from allelic association analysis using Fisher's exact test. [c]Tested allele in association analysis.

Table 4 Gender difference between elastin (*ELN*) polymorphisms and intracranial aneurysm

SNP	Group	N/R[a]	NN/NR/RR[b]		RAF[c]	OR[d]	95% CI[d]	P[d]
			Patient, N	Control, N	Patient/ control			
rs2071307	Male	G/A	23/6/0	31/4/1	0.10/0.08	1.27	0.39–4.17	0.766
	Female	A/G	1/11/50	0/15/39	0.91/0.84	1.63	0.71–3.27	0.298
rs2856728	Male	C/T	1/7/21	6/16/14	0.84/0.61	3.46	1.47–8.14	0.004
	Female		2/20/39	7/20/27	0.80/0.69	1.88	1.03–3.43	0.048

[a]N/R: non-risk/risk allele type, [b]NN/NR/RR: non-risk homozygote/heterozygote/risk homozygote genotypes, [c]RAF: risk allele frequency in patients and controls, [d]odds ratio (OR), 95% confidence interval (CI). The P-value was estimated from allelic association analysis using Fisher's exact test.

population,[12,13] Chinese Han population harboring sporadic IA has higher proportion of minor allele of rs2071307 (allele A).[11] A recent meta-analysis on four studies,[11,13,15,16] has shown that INT20 1315T > C variant has protective effect on IA formation (OR = 0.66).[14] However, the protective effect of INT20 1315T > C variants is mainly found in East-Asian population. The other three studies did not yield an association between INT20 1315T > C and IA. Other variants such as EX20 1264G > 4, INT23 1051 + 24 T > C, and INT4 196 + 71G > A are not associated with IA.[14]

Conflicting results on the association between *ELN* and IA have been published. Genome-wide linkage analysis of IA in 104 Japanese with affected sib pairs has revealed possible associations of chromosome 5q, 7q, and 14q with IA formation.[12] In particular, chromosome 7q11 near *D7S2472* in the vicinity of *ELN* gene is founded to be closely related to IA.[12] Although no association between IA and 14 SNPs in *ELN* has been noted, haplotype at INT20/INT23 has been significantly observed in IA than that in controls ($P = 3.81 \times 10^{-6}$). Exon 22 of the *ELN* (minor allele frequency 0% in SAH and 2.8% in control) has been found to be associated with SAH presentation in Dutch sporadic aneurysms.[13] Haplotypes of INT5/EX22 and INT4/EX22 are found to be significantly associated with SAH presentation. In both cases, major allele (G-G haplotypes) is more evident in SAH patients than that in controls.[13] On the contrary, Hofer et al.[15] have reported that there is no significant relationship between the two SNPs (INT 20 and INT23) of *ELN* and IA in Central Europe. Haplotype frequencies of INT20/INT23 was not associated with IA either ($P = 0.45$). For Caucasian population, 10 SNPs of *ELN* are not associated with SAH at allele or haplotype level.[22] Mineharu et al.[23] have also reported that there is no association between alleles or haplotypes of *ELN* and IA in a Japanese population.

In this study, we identified two polymorphisms, rs2071307 (Gly422Ser) and rs2856728 (intron), in *ELN* gene susceptibility to develop IA in a Korean population. Among these variants, Yang et al.[11] have reported that minor A allele of rs2071307 is significantly associated with increased risk of IA in a Chinese population. However, this variant showed an insignificant association with IA formation ($P = 0.607$) in the present study. However, the T allele of rs2856728 intron variant showed a constant association with IA in both Chinese and Korean populations (OR: 2.12 and 2.34, respectively; $P < 0.001$). Interestingly, the risk of developing IA was found to be influenced by rs2856728 variant in the current study. This risk was significantly higher ($P < 0.05$) in males than that in females in the current study of a Korean population. Previous studies have revealed that female gender was a risk factor for IA formation, in particular postmenopausal age. The incidence of SAH was also higher in female patients. Such data imply that estrogen may contribute to the biological process of IA.[24] On the contrary, protective aspirin (≥3 times/week) effect on SAH development was more noted in male than female IA patients.[25,26] Therefore, genetic factors and gender difference should be further evaluated in IA formation in the future work.

Results such as familial IA (vs. sporadic IA), SAH presentation (vs. unruptured IA), and allele frequencies in enrolled patients might have conflicting results.[11] Most comparisons have been done between ruptured cases and controls without including unruptured cases.[13] Accordingly, such results could not accurately reflect SAH-specific genetic loci due to the absence of unruptured cases. Yang et al.[11] have analyzed *ELN* gene allele between ruptured and unruptured aneurysms. In their study, minor allele of rs2071307 was found to be significantly associated with ruptured aneurysms (31.3% vs. 23.2% in ruptured cases; OR: 1.51, $P = 0.013$), while rs2856728 was not significantly associated with aneurysm rupture (OR: 0.88, $P = 0.48$). Khurana et al.[27] also reported that three tandem endothelial nitric oxide (*eNOS*) gene variations such as promoter SNP (T-786C), intron-4 27-base pair variable number of tandem repeats, and exon-7 SNP (G894T) were more prone to aneurysm rupture in Caucasian. However, these data was not replicated in Japanese patient sample.[28] Although, ruptured aneurysms showed significantly increased lymphocytes and natural killer cells, similar histological findings of atherosclerotic lesions with cellular infiltration of macrophage and proliferating smooth muscle cells were noted in both ruptured and unruptured aneurysms.[29,30] Accordingly, we have included both ruptured and unruptured aneurysms. In our study, both SNPs showed no difference between rupture and non-rupture groups among the 90 IA patients. Four loci (1p34.3-p36.13, 7q11, 19q13.3, and Xp22) have been replicated in other studies of familial IA.[31] SNPs of chromosome 4 near endothelin receptor A gene at chromosome 9 within cyclin-dependent kinase inhibitor 2B antisense inhibitor gene and at chromosome 8 near *SOX*17 transcription regulator gene have been found to be significantly related to sporadic IA.[32] Compared to relative similar proportion of minor allele of rs2071307 in Japanese[12] and Dutch population,[13] Chinese Han population harboring sporadic IA has higher proportion of minor allele of rs2071307 (allele A).[11] In our study,

7 (7.8%) patients presented with SAH and one (1.1%) patient had family history of SAH. Accordingly, differences in ethnicity and aneurysm status should be carefully considered when interpreting study results.

Conclusions

Despite a small sample size, rs2856728 variant in *ELN* gene polymorphisms was found to be significantly associated with IA in this study. This might imply a sufficient statistical power due to its large effect size.[33] Furthermore, we found that *ELN* polymorphism might play crucial roles in the development and pathogenesis of IA in Koreans. The major "T" allele of rs2856728 highly increased the risk of IA formation. When determining the effect of this variant on the development of IA, genetic difference between gender groups needs to be considered. Further studies using large-scale independent population are needed to validate our novel findings.

Acknowledgment

We would like to thank Sung-Eun Kim for help with the data collection and valuable contribution to the statistical analysis. This research was supported by a grant (0320140150, 2014-1266) from Seoul National University Hospital, and partly by National Research Foundation of Korea grant funded by the Ministry of Science, Information and Communication Technologies and Future Planning of the Korea Government (No. 2017M3A9E8033223).

Conflicts of Interest Disclosure

The authors report no conflicts of interest.

References

1) Ingall T, Asplund K, Mähönen M, Bonita R: A multinational comparison of subarachnoid hemorrhage epidemiology in the WHO MONICA stroke study. *Stroke* 31: 1054–1061, 2000
2) Sandvei MS, Mathiesen EB, Vatten LJ, et al.: Incidence and mortality of aneurysmal subarachnoid hemorrhage in two Norwegian cohorts, 1984–2007. *Neurology* 77: 1833–1839, 2011
3) Rose MJ: Aneurysmal subarachnoid hemorrhage: an update on the medical complications and treatments strategies seen in these patients. *Curr Opin Anaesthesiol* 24: 500–507, 2011
4) D'Souza S: Aneurysmal Subarachnoid Hemorrhage. *J Neurosurg Anesthesiol* 27: 222–240, 2015
5) Priebe HJ: Aneurysmal subarachnoid haemorrhage and the anaesthetist. *Br J Anaesth* 99: 102–118, 2007
6) Jeong W, Rhee K: Hemodynamics of cerebral aneurysms: computational analyses of aneurysm progress and treatment. *Comput Math Methods Med* 2012: 782801, 2012
7) Hoi Y, Meng H, Woodward SH, et al.: Effects of arterial geometry on aneurysm growth: three-dimensional computational fluid dynamics study. *J Neurosurg* 101: 676–681, 2004
8) Bruno G, Todor R, Lewis I, Chyatte D: Vascular extracellular matrix remodeling in cerebral aneurysms. *J Neurosurg* 89: 431–440, 1998
9) Debelle L, Alix AJ: The structures of elastins and their function. *Biochimie* 81: 981–994, 1999
10) Chyatte D, Reilly J, Tilson MD: Morphometric analysis of reticular and elastin fibers in the cerebral arteries of patients with intracranial aneurysms. *Neurosurgery* 26: 939–943, 1990
11) Yang S, Wang T, You C, et al.: Association of polymorphisms in the elastin gene with sporadic ruptured intracranial aneurysms and unruptured intracranial aneurysms in Chinese patients. *Int J Neurosci* 123: 454–458, 2013
12) Onda H, Kasuya H, Yoneyama T, et al.: Genomewide-linkage and haplotype-association studies map intracranial aneurysm to chromosome 7q11. *Am J Hum Genet* 69: 804–819, 2001
13) Ruigrok YM, Seitz U, Wolterink S, Rinkel GJ, Wijmenga C, Urbán Z: Association of polymorphisms and haplotypes in the elastin gene in Dutch patients with sporadic aneurysmal subarachnoid hemorrhage. *Stroke* 35: 2064–2068, 2004
14) Paterakis K, Koutsias S, Doxani C, et al.: Variants of the elastin (ELN) gene and susceptibility to intracranial aneurysm: a synthesis of genetic association studies using a genetic model-free approach. *Int J Neurosci* 127: 567–572, 2017
15) Hofer A, Hermans M, Kubassek N, et al.: Elastin polymorphism haplotype and intracranial aneurysms are not associated in Central Europe. *Stroke* 34: 1207–1211, 2003
16) Krex D, König IR, Ziegler A, Schackert HK, Schackert G: Extended single nucleotide polymorphism and haplotype analysis of the elastin gene in Caucasians with intracranial aneurysms provides evidence for racially/ethnically based differences. *Cerebrovasc Dis* 18: 104–110, 2004
17) Ohaegbulam SC, Dujovny M, Ausman JI, Diaz FG, Malik GM: Ethnic distribution of intracranial aneurysms. *Acta Neurochir* (*Wien*) 106: 132–135, 1990
18) Jeon JP, Cho YD, Rhim JK, et al.: Extended monitoring of coiled aneurysms completely occluded at 6-month follow-up: late recanalization rate and related risk factors. *Eur Radiol* 26: 3319–3326, 2016
19) Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263–265, 2005
20) Mimata C, Kitaoka M, Nagahiro S, et al.: Differential distribution and expressions of collagens in the cerebral aneurysmal wall. *Acta Neuropathol* 94: 197–206, 1997

21) Miskolczi L, Guterman LR, Flaherty JD, Hopkins LN: Saccular aneurysm induction by elastase digestion of the arterial wall: a new animal model. *Neurosurgery* 43: 595–600; discussion 600–601, 1998
22) Kaushal R, Woo D, Pal P, et al.: Subarachnoid hemorrhage: tests of association with apolipoprotein E and elastin genes. *BMC Med Genet* 8: 49, 2007
23) Mineharu Y, Inoue K, Inoue S, et al.: Association analysis of common variants of ELN, NOS2A, APOE and ACE2 to intracranial an eurysm. *Stroke* 37: 1189–1194, 2006
24) Amenta PS, Medel R, Pascale CL, Dumont AS: Elucidating sex differences in cerebral aneurysm biology and therapy: the time is now. *Hypertension* 68: 312–314, 2016
25) Wiebers DO, Whisnant JP, Huston J, et al.; International Study of Unruptured Intracranial Aneurysms Investigators: Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. *Lancet* 362: 103–110, 2003
26) Chalouhi N, Starke RM, Correa T, et al.: Differential sex response to aspirin in decreasing aneurysm rupture in humans and mice. *Hypertension* 68: 411–417, 2016
27) Khurana VG, Meissner I, Sohni YR, et al.: The presence of tandem endothelial nitric oxide synthase gene polymorphisms identifying brain aneurysms more prone to rupture. *J Neurosurg* 102: 526–531, 2005
28) Krischek B, Kasuya H, Akagawa H, et al.: Using endothelial nitric oxide synthase gene polymorphisms to identify intracranial aneurysms more prone to rupture in Japanese patients. *J Neurosurg* 105: 717–722, 2006
29) Kosierkiewicz TA, Factor SM, Dickson DW: Immunocytochemical studies of atherosclerotic lesions of cerebral berry aneurysms. *J Neuropathol Exp Neurol* 53: 399–406, 1994
30) Krischek B, Tatagiba M: The influence of genetics on intracranial aneurysm formation and rupture: current knowledge and its possible impact on future treatment. *Adv Tech Stand Neurosurg* 33: 131–147, 2008
31) Ruigrok YM, Rinkel GJ: Genetics of intracranial aneurysms. *Stroke* 39: 1049–1055, 2008
32) Alg VS, Sofat R, Houlden H, Werring DJ: Genetic risk factors for intracranial aneurysms: a meta-analysis in more than 116,000 individuals. *Neurology* 80: 2154–2165, 2013
33) Hong EP, Park JW: Sample size and statistical power calculation in genetic association studies. *Genomics Inform* 10: 117–122, 2012

Address reprint requests to: Jeong Eun Kim, MD, PhD, Department of Neurosurgery, Seoul National University College of Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea.
e-mail: eunkim@snu.ac.kr