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

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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 non-traumatic SAH.5,6 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.
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, 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 were selected for this study. They accounted for linkage disequilibrium (LD, r² < 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).

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).

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer</th>
<th>Primer sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2071307 &amp;</td>
<td>Forward</td>
<td>5′-AATCCATCAG-CATCCCTCAG-3’</td>
<td>395 bp</td>
</tr>
<tr>
<td>rs2856728</td>
<td>Reverse</td>
<td>5′-CAACTCTCCT-CCTGAGCACAT-3’</td>
<td></td>
</tr>
</tbody>
</table>

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

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

*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 benigneffect 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. IEL has a longitudinal arrangement of elastin fiber. Defect of IEL or elastin degradation has been proposed to be related to IA formation. Associations between several ELN gene polymorphisms and sporadic IA in East-Asian and Caucasian populations have been studied. Compared to relative similar proportions of minor allele of rs2071307 in Japanese and Dutch

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Position</th>
<th>Function</th>
<th>Genotype</th>
<th>90 patients with IA and 90 controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>chr.</td>
<td>chr.</td>
<td></td>
<td>Patient, $N$ (%)</td>
</tr>
<tr>
<td>ELN</td>
<td>rs2071307</td>
<td>73,470,714</td>
<td>Gly422Ser</td>
<td>GG</td>
<td>73 (81.1)</td>
</tr>
<tr>
<td></td>
<td>7q11.23</td>
<td>73,470,714</td>
<td></td>
<td>AG</td>
<td>17 (18.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>rs2856728</td>
<td>73,470,782</td>
<td></td>
<td>intron</td>
<td>TT</td>
<td>60 (66.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TC</td>
<td>27 (30.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>3 (3.3)</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>SNP</th>
<th>Group</th>
<th>N/R$^a$</th>
<th>NN/NR/RR$^b$</th>
<th>RAF$^c$</th>
<th>OR$^d$</th>
<th>95% CI$^d$</th>
<th>$P^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patient, $N$</td>
<td>Control, $N$</td>
<td>Patient/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2071307</td>
<td>Male</td>
<td>G/A</td>
<td>23/6/0</td>
<td>31/4/1</td>
<td>0.10/0.08</td>
<td>1.27</td>
<td>0.39–4.17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>A/G</td>
<td>1/11/50</td>
<td>0/15/39</td>
<td>0.91/0.84</td>
<td>1.63</td>
<td>0.71–3.27</td>
</tr>
<tr>
<td>rs2856728</td>
<td>Male</td>
<td>C/T</td>
<td>1/7/21</td>
<td>6/16/14</td>
<td>0.84/0.61</td>
<td>3.46</td>
<td>1.47–8.14</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>C/T</td>
<td>2/20/39</td>
<td>7/20/27</td>
<td>0.80/0.60</td>
<td>1.88</td>
<td>1.03–3.43</td>
</tr>
</tbody>
</table>

$^a$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.
Chinese Han population harboring sporadic IA has higher proportion of minor allele of rs2071307 (allele A). A recent meta-analysis on four studies has shown that INT20 1315T > C variant has protective effect on IA formation (OR = 0.66). 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 > A, INT23 1051 + 24 T > C, and INT4 196 + 71G > A are not associated with IA. 

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. In particular, chromosome 7q11 near D7S2472 in the vicinity of ELN gene is founded to be closely related to IA. 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 × 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. 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. On the contrary, Hofer et al. 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. Mineharu et al. 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. 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. On the contrary, protective aspirin (≥3 times/week) effect on SAH development was more noted in male than female IA patients.

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. Most comparisons have been done between ruptured cases and controls without including unruptured cases. Accordingly, such results could not accurately reflect SAH-specific genetic loci due to the absence of unruptured cases. Yang et al. 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. 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. 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. 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. 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 SOX17 transcription regulator gene have been found to be significantly related to sporadic IA. Compared to relative similar proportion of minor allele of rs2071307 in Japanese and Dutch population, Chinese Han population harboring sporadic IA has higher proportion of minor allele of rs2071307 (allele A). 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. 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.

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Conflicts of Interest Disclosure

The authors report no conflicts of interest.

References


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