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Novel Genome-Wide Interactions Mediated via *BOLL* and *EDNRA* Polymorphisms in Intracranial Aneurysm

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Objective : The association between boule (*BOLL*) and endothelin receptor type A (*EDNRA*) loci and intracranial aneurysm (IA) formation has been reported via genome-wide association studies. We sought to identify genome-wide interactions involving *BOLL* and *EDNRA* loci for IA in a Korean adult cohort.

Methods : Genome-wide pairwise interaction analyses of *BOLL* and *EDNRA* involving 250 patients with IA and 296 controls were performed using the additive effect model after adjusting for confounding factors.

Results : Among 512575 single-nucleotide polymorphisms (SNPs), 23 and 11 common SNPs suggested a genome-wide interaction threshold ($p<1.25\times10^{-8}$) involving rs700651 (*BOLL*) and rs6841581 (*EDNRA*). Rather than singe SNP effect of *BOLL* or *EDNRA* on IA development, they showed a synergistic effect on IA formation via multifactorial pair-wise interactions. The rs1105980 of *PTCH1* gene showed the most significant interaction with rs700651 (natural log-transformed odds ratio [lnOR], 1.53; $p=6.41\times10^{-11}$). The rs74585958 of *RYK* gene interacted strongly with rs6841581 (lnOR, -19.91; $p=1.64\times10^{-9}$). Although, there was no direct interaction between *BOLL* and *EDNRA* variants, two *EDNRA*-interacting gene variants of *TNIK* (rs11925024 and rs1231) and *FTO* (rs9302654), and one *BOLL*-interacting *METTL4* gene variant (rs549315) exhibited marginal interaction with *BOLL* gene.

Conclusion : *BOLL* or *EDNRA* may have a synergistic effect on IA formation via multifactorial pair-wise interactions.

Key Words : Boule · Endothelin receptor type A · Genome-wide association study · Intracranial aneurysm.

INTRODUCTION

Intracranial aneurysm (IA) refers to a bulge in the wall of intracranial arteries due to endothelial dysfunction and extracellular matrix remodeling of the hemodynamic response. Although the prevalence of IA is approximately 3% in the general

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population, the mortality rate due to subarachnoid hemorrhage following aneurysm rupture is close to 50%[25,31]. Clinical and radiological studies showed that IA formation and rupture were related to female gender, hypertension, smoking, larger size and posterior circulation aneurysm[3].

The plethora of genome-wide association studies (GWASs) during late 2000's has increased the number of investigations into IA. These genetic studies have identified several candidate genes and loci associated with IA such as *EDNRA*, *GBA*, *CDKN2A/B*, *RBBP8*, *STARD13/KL*, and *SOX17*[1,2,11,29]. GWAS reported differences in the frequency of single-nucleotide polymorphisms (SNPs) based on case-control studies. Accordingly, the missing heritability is explained by independent SNPs involving complex diseases in human including cerebrovascular diseases (CVDs). To address this issue, robust analytical approaches such as meta-analyses, multifactorial interactions, and polygenic risk scoring systems have been performed in many populations[9]. A GWA meta-analysis reported IA-associated risk loci, including new loci (*SLC22A5*, *6q16.1*, *12q21.33*, *PSMA4*, and *NT5C2*) based on 10754 cases and 306882 controls[1]. Two large-scale meta-analyses also reported the successful validation of two IA-associated loci boule (*BOLL*) and endothelin receptor type A (*EDNRA*) in an East Asian population of nearly 20000 individuals[12,13]. However, few studies have reported gene-gene interactions or polygenic risk scores for IA patients. Considering to two previous findings of *BOLL* and *EDNRA* loci, here we estimated the effects of genome-wide gene-gene interactions on IA in a prospective hospital-based cohort study.

MATERIALS AND METHODS

All the study protocols have been approved by the Institutional Review Board and Ethics Committee of Hallym University Chuncheon Sacred Heart Hospital (No. 2016-3, 2019-06-006). The study protocol and design are described in detail elsewhere[11].

Study populations

The study subjects were enrolled from the multi-institutional biobanks comprising five university hospitals constituting "The First Korean Stroke Genetics Association Research", and including patients diagnosed with CVDs such as IA between March 2015 and December 2020 (https://www.1ksgh.org/)[11,18]. Data derived from 250 patients with IA and 296 controls, which were also used in the first Korean IA GWAS[11], were used in the analysis. The inclusion of patients with IA was based on the following criteria : 1) adult patients more than 18 years of age; 2) patients without other types of CVD such as ischemic stroke, hemorrhagic stroke, and vascular malformation; and 3) patients without any other genetic disorders such as polycystic kidney and moyamoya disease. Control subjects were defined as adults without CVD. Medical and radiological data were collected and updated.

Genotyping and quality controls

Genomic DNA derived from the peripheral blood of the study population was genotyped using the AxiomTH Asian Precision Medicine Research Array (APMRA) (Thermo Fisher Scientific, Waltham, MA, USA). High quality plates were defined by a plate pass rate higher than 95% for samples and the average call rate of passing samples was greater than 99%. Out of 798148 SNPs, 512575 SNPs passed the quality control including genotyping call rate of 95% or higher, minor allele frequency (MAF) of at least 1%, and Hardy-Weinberg equilibrium (HWE) with p-value $\geq 1\times10^{-6}$ [11].

Genome-wide SNP-SNP interactions via *BOLL* and *EDNRA* genes

We investigated genome-wide SNP-SNP interactions using either *BOLL* or *EDNRA* loci associated with IA in previous GWASs and meta-analyses[11-13]. The multivariate analyses of the SNP interactions between rs700651 intron SNP (*BOLL*, 2q33.1) and rs6841581 upstream SNP (*EDNRA*, 4q31.22) on 512574 SNPs were performed using the Contrived Acronym of software for SNP Interactions (CASSI ver. 2.5; https://www.staff.ncl.ac.uk/richard.howey/cassi/index.html)[27]. SNP-SNP interactions were analyzed by choosing target SNP from two given SNP windows (possibly from different pedigree files). Each pair of SNPs that interaction test passes a given significance level (i.e., minimized p-value=1) is returned in the output file with possible extra information such as beta coefficient, standard error, chi-square, and p-value. The CASSI accepted only PLINK binary files in order to perform the calculations as efficiently as possible. The logistic regression epistasis test was available for SNP-SNP interactions in this study even though this program can provide the maximum number

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of 1M terms. Subsequent regression analyses were carried out under the additive effect model and adjusted for 10 covariates including age, sex, hypertension, diabetes mellitus, hyperlipidemia, smoking status, and four genetic ancestry factors. The effect coefficient was estimated using the natural log-transformed scale of odds ratio (i.e., natural log-transformed odds ratio [lnOR]). The multiple comparisons were adjusted for a genome-wide interaction threshold with a *p*-value less than 1.25×10^{-8} (genome-wide *p*-value=5×10^{-8} divided by four tests for interaction). A subsequent power and sample size calculations for each interaction term were estimated under the assumption with GW interaction significance threshold, 1 : 1.2 case-control ratio and information of each SNP (i.e., allele frequency and effect size) through performing the Quanto ver. 1.2.4 program (https://bio.tools/QUANTO). The performance of the large-scale interaction analyses was completed by the multi-tasking supper computer that has a capacities of Intel(R) Xeon(R) CPU E5-2667 v4 (3.20 GHz), 256 GB RAM, and 15 Cores. We performed Manhattan plots of *BOLL* and *EDNRA* interaction using the package of "*qqman*" in R v3.6.1 (https://cran.r-project.org/web/packages/qqman) and regional visualizations of the target SNP's base-pair position ±400 kb regions using LocusZoom ver. 1.3 (https://genome.sph.umich.edu/wiki/LocusZoom_Standalone) written in Python and R[23].

RESULTS

Detailed information including SNP genotype distribution and HWE *p*-value, is presented in Supplementary Table 1. Out of 512574 SNP interaction terms, 23 and 11 SNPs reached a genome-wide interaction threshold ($p<1.25\times10^{-8}$) with rs700651 intron SNP (*BOLL*, 2q33.1) and rs6841581 upstream SNP (*EDNRA*, 4q31.22), respectively (Fig. 1, Tables 1 and 2). All 34 SNPs showed an MAF above 1% and an HWE *p*-value greater than 0.01 (Supplementary Table 2). Most of the *BOLL*- or *EDNRA*-interacting SNPs showed shared alleles in both patient and control groups (i.e., average of MAF >0.23) without significant association in a single SNP analysis ($0.0<p<1$) (Table 1). These findings suggest that *BOLL* or *EDNRA* may have a synergistic effect on IA formation, via multifactorial pair-wise interactions, rather than involved alone in the IA formation. Among the interactions, the rs1105980 upstream SNP of *PTCH1* gene (9q22.32) showed the most significant interaction with rs700651 (effect, 1.53; $p=6.41\times10^{-11}$) (Fig. 2A). The rs74585958 of *RYK* gene (3q22.2) interacted strongly with rs6841581 (effect, -19.91; $p=1.64\times10^{-9}$) (Fig. 2B). Two strong pair-wise linkage disequilibrium ($r^2>0.95$) were observed in the interaction of rs328025 with rs700855 (*RGPD4*, 2q12.3) and between rs11925024 and rs1231 (*TNIK*, 3q26.31) ($r^2>0.95$, data not shown). Interestingly, two *EDNRA*-interacting gene variants of *TNIK* (rs11925024 and rs1231, $p=1.04\times10^{-8}$ and 1.22×10^{-9}, respectively) and *FTO* (rs9302654, $p=3.78\times10^{-9}$), and one *BOLL*-interacting *METTL4* gene variant (rs549315, $p=4.80\times10^{-10}$) showed marginal interaction with *BOLL* gene ($0.001<p<0.05$). However, there was no direct interaction between *BOLL* and *EDNRA* variants (effect, -0.27; $p=0.301$). When power calculation was estimated by the basis of detail SNP information (Supplementary Tables 2 and 3), two SNPs such as rs74585958 (*RYK*) and rs150664966 (*EIF4H*) showed

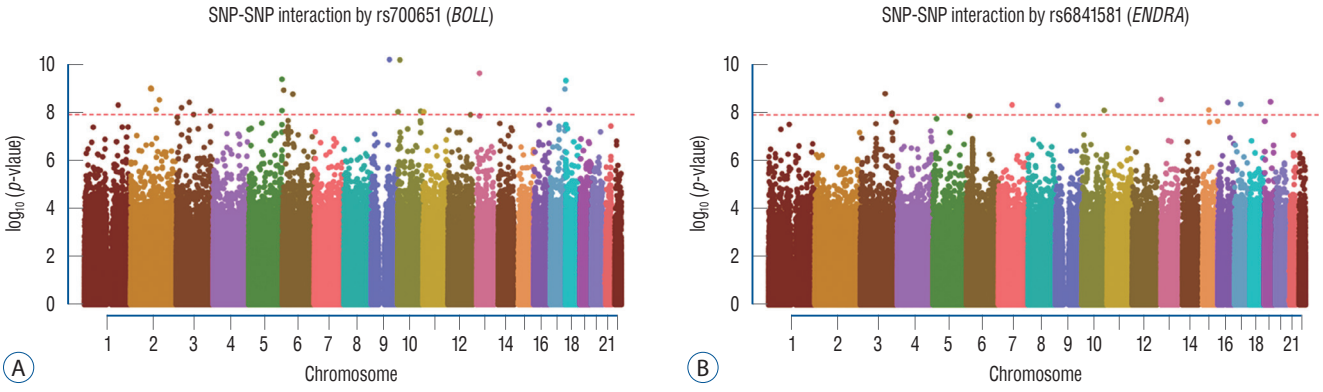

Fig. 1. Manhattan plots of genome-wide interactions with (A) rs700651 (*BOLL*, 2q33.1) and (B) rs6841581 (*EDNRA*, 4q31.22) and their effect on intracranial aneurysm based on the additive effect model. Red line indicates genome-wide significance threshold (interaction $p=1.25\times10^{-8}$). SNP : single nucleotide polymorphism, ENDRA : endothelin receptor type A.

Table 1. Genome-wide interaction terms by *BOLL* or *EDNRA* polymorphisms on intracranial aneurysm according to *BOLL* interaction *p*-value ranking

Gene	Chr	SNP	BP	M/m	MAF, case/control	lnOR*	*p*-value for GWAS*	lnOR†	*p*-value for interaction†
BOLL	2q33.1	rs700651	198631714	A/G	0.476/0.449	1.42	0.0079	NA	NA
PTCH1	9q22.32	rs1105980	98113635	G/C	0.27/0.294	-0.08	0.5821	1.53	6.41E-11
CCDC3	10p13	rs12412014	12911725	G/C	0.281/0.291	-0.09	0.5145	1.47	6.63E-11
LINC00457	13q13.2	rs1536847	35106975	G/T	0.295/0.329	-0.18	0.2033	1.39	2.37E-10
C5orf60	5q35.3	rs62405726	179069468	G/A	0.318/0.287	0.13	0.3646	1.46	4.21E-10
METTL4	18p11.32	rs549315	2183055	G/A	0.378/0.429	-0.29	0.0389	-1.32	4.80E-10
RGPD4	2q12.3	rs700855	108368694	T/C	0.372/0.328	0.27	0.0454	1.31	9.91E-10
RGPD4	2q12.3	rs328025	108355045	G/A	0.377/0.324	0.31	0.0219	1.25	1.06E-09
MALL	2q13	rs117802391	110862084	C/T	0.036/0.061	-0.77	0.0198	19.57	1.06E-09
LINC01978	17q25.3	rs57851800	77896371	A/C	0.345/0.326	0.16	0.2657	-1.27	1.08E-09
RREB1	6p24.3	rs9505086	7232186	T/C	0.286/0.307	-0.11	0.4279	1.24	1.20E-09
DST	6p12.1	rs117021265	56628021	T/C	0.034/0.024	0.25	0.5494	-18.96	1.75E-09
RPRM	2q23.3	rs5005908	154003680	G/T	0.344/0.27	0.32	0.022	1.26	3.03E-09
FOXP1	3p13	rs878118	71246228	T/G	0.238/0.255	-0.15	0.323	-1.39	3.85E-09
LINC01344	1q25.3	rs12033118	182229747	C/T	0.022/0.027	-0.15	0.7192	-19.75	5.00E-09
RBMS3	3p24.1	rs1979271	29607405	T/A	0.406/0.39	0.07	0.5841	-1.17	6.47E-09
CXCR4	2q22.1	rs189432614	136809235	A/G	0.016/0.025	-1.63	0.0131	-34.81	7.59E-09
CDH13	16q23.3	rs3848296	82550548	G/A	0.192/0.231	-0.16	0.3193	1.46	7.69E-09
RUFY1	5q35.3	rs4075890	178997373	T/C	0.216/0.2	0.1	0.5444	1.46	8.47E-09
EIF2B5	3q27.1	rs4350902	184352200	T/C	0.472/0.492	-0.09	0.5253	-1.18	8.83E-09
PLEKHA1	10q26.13	rs10510110	124192430	C/T	0.399/0.372	0.17	0.2098	1.26	8.96E-09
PFKP	10p15.2	rs58183624	3107217	C/T	0.066/0.044	0.15	0.592	-2.68	9.52E-09
TRIM22	11p15.4	rs7480654	5722839	T/C	0.317/0.284	0.18	0.22	-1.21	9.59E-09
LINC00879	3q11.2	rs4411883	94549686	T/G	0.09/0.111	-0.34	0.1404	-1.91	1.24E-08
TNIK	3q26.31	rs11925024	171014067	A/C	0.145/0.151	-0.15	0.4431	-0.9	0.0008
TNIK	3q26.31	rs1231	171031233	A/T	0.144/0.154	-0.18	0.3537	-0.8	0.0021
FTO	16q12.2	rs9302654	54009545	C/T	0.114/0.144	-0.34	0.088	0.58	0.0334
SLFN11	17q12	rs77814639	33678827	A/G	0.184/0.153	0.28	0.1329	-0.43	0.108
SAP18	13q12.11	rs9509543	21692404	C/T	0.346/0.356	-0.05	0.705	-0.2	0.2731
EDNRA	4q31.22	rs6841581	148401190	A/G	0.13/0.217	0.53	0.0006	-0.27	0.301
SLC7A10	19q13.11	rs11672303	33726375	T/C	0.171/0.154	0.08	0.6556	0.23	0.3238
CACUL1	10q26.11	rs11198727	120767097	A/G	0.382/0.429	-0.13	0.3308	0.17	0.3558
MPDZ	9p23	rs1332064	12942764	T/C	0.354/0.309	0.19	0.1724	0.1	0.5916
UNC13C	15q21.3	rs4774715	55140204	C/T	0.432/0.441	-0.01	0.9362	-0.09	0.6185
RYK	3q22.2	rs74585958	133773362	G/A	0.054/0.041	0.57	0.0817	-0.21	0.6852
EIF4H	7q11.23	rs150664966	73594157	T/C	0.016/0.022	-0.17	0.7152	-0.05	0.9384

*These were estimated by generalized linear model after adjusting for age, sex, hypertension, diabetes, hyperlipidemia, and smoking in the previous GWAS. †These were estimated after *BOLL* by 500 K SNPs interactions by performing CASSI (Contrived Acronym of software for SNP Interactions) program after adjusting for age, sex, hypertension, diabetes, hyperlipidemia, and smoking. Chr : chromosome, SNP : single-nucleotide polymorphism, BP : base-pair position, M/m : major/minor allele type, MAF : minor allele frequency, lnOR : natural log-transformed odds ratio, GWAS : genome-wide association study, NA : not available

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Table 2. Genome-wide interaction terms by *BOLL* or *EDNRA* polymorphisms on intracranial aneurysm according to *EDNRA* interaction *p*-value ranking

Gene	Chr	SNP	BP	M/m	MAF, case/control	lnOR*	*p*-value for GWAS*	lnOR†	*p*-value for interaction†
EDNRA	4q31.22	rs6841581	148401190	A/G	0.13/0.217	0.53	0.0006	NA	NA
RYK	3q22.2	rs74585958	133773362	G/A	0.054/0.041	0.57	0.0817	-19.91	1.64E-09
SAP18	13q12.11	rs9509543	21692404	C/T	0.346/0.356	-0.05	0.705	1.85	2.87E-09
SLC7A10	19q13.11	rs11672303	33726375	T/C	0.171/0.154	0.08	0.6556	2.16	3.55E-09
FTO	16q12.2	rs9302654	54009545	C/T	0.114/0.144	-0.34	0.088	-3.1	3.78E-09
SLFN11	17q12	rs77814639	33678827	A/G	0.184/0.153	0.28	0.1329	-18.5	4.48E-09
EIF4H	7q11.23	rs150664966	73594157	T/C	0.016/0.022	-0.17	0.7152	20.91	4.80E-09
MPDZ	9p23	rs1332064	12942764	T/C	0.354/0.309	0.19	0.1724	1.75	5.10E-09
UNC13C	15q21.3	rs4774715	55140204	C/T	0.432/0.441	-0.01	0.9362	-1.68	7.74E-09
CACUL1	10q26.11	rs11198727	120767097	A/G	0.382/0.429	-0.13	0.3308	1.72	8.06E-09
TNIK	3q26.31	rs11925024	171014067	A/C	0.145/0.151	-0.15	0.4431	-2.71	1.04E-08
TNIK	3q26.31	rs1231	171031233	A/T	0.144/0.154	-0.18	0.3537	-2.86	1.22E-08
METTL4	18p11.32	rs549315	2183055	G/A	0.378/0.429	-0.29	0.0389	0.59	0.033
PTCH1	9q22.32	rs1105980	98113635	G/C	0.27/0.294	-0.08	0.5821	-0.6	0.058
MALL	2q13	rs117802391	110862084	C/T	0.036/0.061	-0.77	0.0198	-1.58	0.0762
CXCR4	2q22.1	rs189432614	136809235	A/G	0.016/0.025	-1.63	0.0131	1.02	0.2116
LINC01344	1q25.3	rs12033118	182229747	C/T	0.022/0.027	-0.15	0.7192	0.79	0.2679
BOLL	2q33.1	rs700651	198631714	A/G	0.476/0.449	1.42	0.0079	-0.27	0.301
LINC01978	17q25.3	rs57851800	77896371	A/C	0.345/0.326	0.16	0.2657	-0.29	0.3135
PLEKHA1	10q26.13	rs10510110	124192430	C/T	0.399/0.372	0.17	0.2098	-0.26	0.335
PFKP	10p15.2	rs58183624	3107217	C/T	0.066/0.044	0.15	0.592	0.56	0.3378
LINC00457	13q13.2	rs1536847	35106975	G/T	0.295/0.329	-0.18	0.2033	0.25	0.3692
TRIM22	11p15.4	rs7480654	5722839	T/C	0.317/0.284	0.18	0.22	-0.26	0.3718
CDH13	16q23.3	rs3848296	82550548	G/A	0.192/0.231	-0.16	0.3193	-0.31	0.3982
FOXP1	3p13	rs878118	71246228	T/G	0.238/0.255	-0.15	0.323	0.25	0.4035
LINC00879	3q11.2	rs4411883	94549686	T/G	0.09/0.111	-0.34	0.1404	-0.37	0.4182
RGPD4	2q12.3	rs328025	108355045	G/A	0.377/0.324	0.31	0.0219	-0.19	0.4897
RUFY1	5q35.3	rs4075890	178997373	T/C	0.216/0.2	0.1	0.5444	-0.21	0.5058
RGPD4	2q12.3	rs700855	108368694	T/C	0.372/0.328	0.27	0.0454	-0.18	0.5209
CCDC3	10p13	rs12412014	12911725	G/C	0.281/0.291	-0.09	0.5145	-0.14	0.6273
RPRM	2q23.3	rs5005908	154003680	G/T	0.344/0.27	0.32	0.022	-0.13	0.6388
RBMS3	3p24.1	rs1979271	29607405	T/A	0.406/0.39	0.07	0.5841	0.13	0.6541
RREB1	6p24.3	rs9505086	7232186	T/C	0.286/0.307	-0.11	0.4279	0.06	0.825
DST	6p12.1	rs117021265	56628021	T/C	0.034/0.024	0.25	0.5494	0.16	0.8283
C5orf60	5q35.3	rs62405726	179069468	G/A	0.318/0.287	0.13	0.3646	-0.02	0.9398
EIF2B5	3q27.1	rs4350902	184352200	T/C	0.472/0.492	-0.09	0.5253	-0.01	0.9793

*These were estimated by generalized linear model after adjusting for age, sex, hypertension, diabetes, hyperlipidemia, and smoking in the previous GWAS. †These were estimated after *BOLL* by 500 K SNPs interactions by performing CASSI (Contrived Acronym of software for SNP Interactions) program after adjusting for age, sex, hypertension, diabetes, hyperlipidemia, and smoking. Chr : chromosome, SNP : single-nucleotide polymorphism, BP : base-pair position, M/m : major/minor allele type, MAF : minor allele frequency, lnOR : natural log-transformed odds ratio, GWAS : genome-wide association study, NA : not available

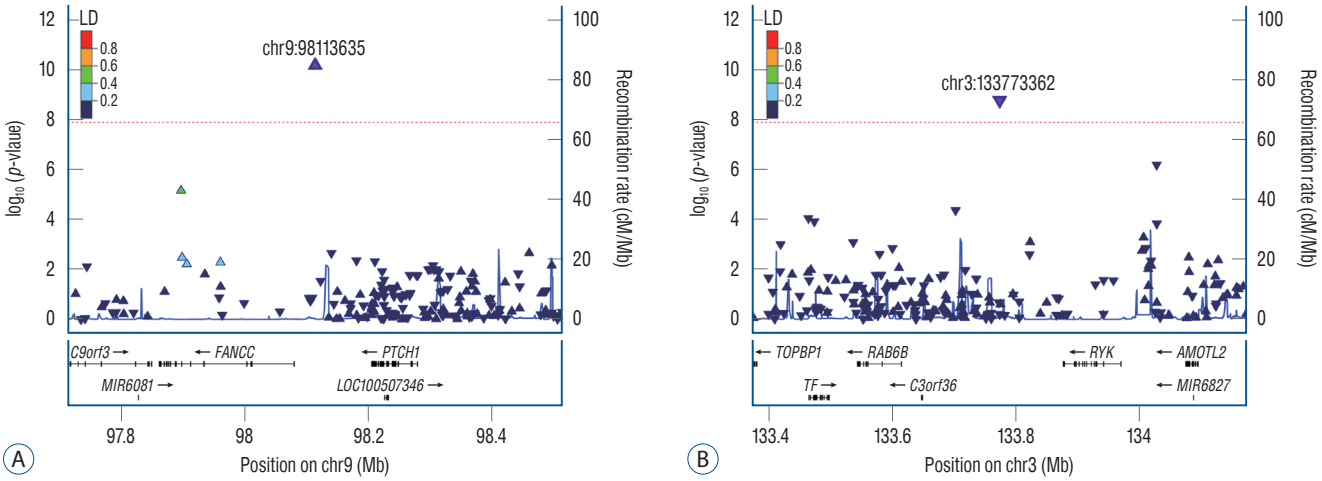

Fig. 2. Regional association plots of (A) rs1105980 (*PTCH1*, 9q22.32) interacting with rs700651 (*BOLL*, 2q33.1) and (B) rs74585958 (*RYK*, 3q22.2) interacting with rs6841581 (*EDNRA*, 4q31.22) at position ±400 kb and the effect on intracranial aneurysm (IA). X-axis indicates the chromosomal position (mega base, Mb) and Y-axis did -$\log_{10}$ transformed *p*-value and recombination rate, respectively. Purple triangles of rs1105980 (p=6.41×10^{-11}) and rs74585958 (p=6.41×10^{-9}) represent the most significant interactions with *BOLL* and *EDNRA*, respectively. Other up or down triangles denote other variants within the target variant ±400 kb regions. Up and down triangles indicate positive and negative effect sizes on IA formation, respectively. Each color shows pair-wise linkage disequilibrium with either rs1105980 or rs74585958. *PTCH1* : patched 1, *RYK* : receptor-like tyrosine kinase.

sufficient statistical power among *EDNRA*-interacting loci (i.e., 0.885 and 0.805, respectively). The rs11672303 (*SLC7A10*) of *EDNRA*-interacting loci showed a marginal statistical power of 78.9%. In contrast, no SNP reached sufficient statistical power threshold of 80% in interaction terms by the *BOLL* locus (i.e., power <50%).

DISCUSSION

Although *BOLL* gene, the G allele of rs700651 associated with risk showed a significant genome-wide overall effect on IA in multi-ethnic integrative meta-analyses (p=1.05×10^{-8})[12], its pathgeneic mechanism remains to be identified. Most studies related to *BOLL* involved spermatogenesis due to its role in germ cell development or cancer[17,22]. *BOLL* is a well-known gene associated with normal germ cell development[26]. The gene is predominantly expressed in secondary spermatocytes[28]. However, few studies investigated the role of *BOLL* in CVD including IA. Harrod et al.[10] reported that estrogen deficiencies may lead to IA by interrupting the inflammatory response. In reality, earlier age at menopause increased the risk of IA, suggesting the association between estrogen deficiency and IA pathogenesis[6]. Thus, in the case of *BOLL* gene, additional studies are needed to determine the protective effect of male hormones against the IA development. The *EIF2B5* gene that interacts with *BOLL* in the current GW interaction and network analyses exhibited a homologous inhibition of cell translation. The differential expression of *EIF2B5* was moderate in human tissues and cells. Brady et al.[4] reported that intron retention in *EIF2B5* inhibited protein translation in hypoxic cancer cells. In the case of abdominal aortic aneurysms, there was no meaningful *EIF2B5* network[16]. Based on a review of the current literature, it is difficult to elucidate the contribution of the two genes to IA pathogenesis. Thus, a further *in vivo* study is needed to investigate the function of the two genes in IA formation.

The G allele of the rs6841581 located near the 5'-untranslated region of *EDNRA* (4q31.22) gene was associated with IA[13]. However, detailed mechanisms of IA mediated by *EDNRA* have yet to be reported to determine the direct effect or an indirect effect. Rats with pulmonary hypertension showed higher expression of *EDNRA* genes[21]. Endothelial injury, followed by disruption of collagen and elastin synthesis contribute to IA[15]. Chronic hypertension per se may induce the structural changes. Thus, the inflammtory response to increased hemodynamic stress following the disruption of cerebral arteries mediated by *EDNRA* may result in IA. In our study, among the *EDNRA*-interacting genes, the *MPDZ*-centered interaction between *EIF4H*, *SAP18*, and *UNC13C* was

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observed. *MPDZ* is a tight junction protein, which modulates notch signaling during angiogenesis by controlling ligand recruitment to adherent junctions[8,24]. Feldner et al.[8] reported that loss of *MPDZ* decreased ependymal cell integrity and caused hydrocephalus. Ependymal cells are mainly responsible for electrolyte transport between brain parenchyma and the CSF. Adult ependymal cells are highly differentiated. Ependymal cells lining the lateral ventricles are quiescent under normal physiological conditions[5]. However, after stroke, adult ependymal cells are transformed into radial glial cells in the subventricular zone[30]. IA formation and growth occur within the CSF space surrounding the cerebral arteries. Accordingly, a further study is required to investigate the relationship between IA and CSF mediated by ependymal cells.

In our study, most variants did not show a significant association with IA via single SNP-based GWAS, although they exhibited significant associations with *BOLL* or *EDNRA* via multiple interactions terms. It is widely believed that a single SNP often has small effect on disease phenotypes including IA, thus it cannot fully account for the genetic susceptibility, in particular stroke. Therefore, identification of SNP interactions that are associated with disease is increasing to interpret the genetic basis of the disease susceptibility[20]. Although several loci related to *BOLL* or *EDNRA* did not pass genome-wide significance in a single SNP analysis, the loci might have a synergy effect on IA development by interacting with the genes. In this study, we aimed to investigate gene-gene interaction using the previous GWAS data for Korean patients with IA for the first time[11]. However, due to the relative small number of the enrolled patients and possible false positives[14], we did not perform all possible pairwise SNP-SNP interaction and inevitably focused on two IA candidates of *BOLL* and *EDNRA* by referring to previous studies[12,13]. Nevertheless, we required further replication GWAS and exhaustive searching for SNP-SNP interaction in a large dataset of GWAS[19]. In addition, further molecular functional study including the estimation of protein levels to validate our interaction results between *BOLL* (or *EDNRA*) and several loci in the future.

The study has some limitations. First, although we identified novel loci for IA, their functional role was not investigated. The role of most of the 34 novel genes interacting with *BOLL* and *EDNRA* in our study has yet to be analyzed in IA or other CVDs. Second, the study had a potential sample size limitation of multiple interaction terms with either *BOLL* or *EDNRA* loci, which have been replicated in IA susceptibility involving Korean adults including 250 patients with IA and 296 controls. In addition, there is a possibility that the results of this IA genetic study are limited to the Korean population. Therefore, we may warrant these findings in the second stage GWA meta-analysis and interaction test. Nevertheless, this study evaluates the first multiple genome-wide SNP-SNP interactions by IA-targeting genes such as *BOLL* and *ENDRA*, which have been validated in previous studies[12,13]. Disease is caused by various biological pathways and it is difficult to explain it based on a GWAS, which focused on differences between individual paired loci[7]. Accordingly, a study of genetic interactions and additional insights into various compensatory functional modules is needed to elucidate complex diseases such as IA. In summary, it is necessary to develop a general framework for mapping complex genetic networks of IA using GWAS data combined with clinically relevant risk factors.

CONCLUSION

Genome-wide interaction between IA and *BOLL* or *EDNRA* revealed 34 novel loci, which were likely to be associated with IA. Common susceptibility variants and their interacting factors can be used to determine the inter-individual status of IA formation. The novel gene-gene interactions reported in this study need to be corroborated via larger prospective cohort studies.

AUTHORS' DECLARATION

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Informed consent

This type of study does not require informed consent.

Author contributions

Conceptualization : JPJ, EPH; Data curation : JJL, SN, HCK, JKR, JJP; Formal analysis : EPH; Funding acquisition : JPJ; Methodology : DHY, BJK, HY; Project administration : JPJ; Visualization : EPH; Writing - original draft : JPJ, EPH; Writ-

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Data sharing

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• Supplementary materials

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