■ Original Article ■

The Genetic Variations of *ESR1* Gene are Associated with Blood Pressure Traits in the Korean Women

Hyun-Seok Jin[1], Jae Woong Sull[2] and Yong-Bin Eom[3,†]

[1]*Department of Medical Genetics, School of Medicine, Ajou University, Suwon 443-721, Korea*
[2]*Department of Biomedical Laboratory Science, College of Health Science, Eulji University, Sungnam 461-713, Korea*
[3]*Department of Biomedical Laboratory Science, Korea Nazarene University, Cheonan 331-718, Korea*

Hypertension is a complex disease that results from the interaction of genetic and environmental influences and heritability is influenced by about one-third to one-half. However, the specific genetic variants determining risk for hypertension are still largely unknown. Here, we performed association analysis to elucidate the possible relations of genetic polymorphisms in *ESR1* gene with blood pressure traits. By examining genotype data of a total of 3,804 women in the Korean Association REsource (KARE) study, we discovered the *ESR1* gene polymorphisms are associated with blood pressure and hypertension. The highest significant polymorphisms were rs2982571 (β=-1.56, P=6.8 $\times$ 10^{-3}) with systolic blood pressure (SBP), rs9322335 (β=-0.61, P=0.013) with diastolic blood pressure (DBP), and rs851985 (OR=0.78, CI: 0.65~0.94, P=8.6 $\times$ 10^{-3}) with hypertension. In the 5 SNPs (rs2982571, rs851985, rs851983, rs851981, and rs851980), their β-values in SBP and/or DBP showed consistent trends with the odds ratios (ORs) of hypertension, and these 5 SNPs were composed with one LD block. Consequently, we found statistically significant SNPs in *ESR1* gene that are associated with both blood pressure and hypertension traits. These results suggested that the individuals with the minor alleles of the 5 SNPs in the *ESR1* gene may be less susceptible to the development of hypertension in the Korean women.

Key Words: Blood pressure, Hypertension, *ESR1*, SNP, Association

INTRODUCTION

Hypertension is caused by various genetic and environmental factors, and genetic polymorphism is understood to be an important factor in the development of hypertension.

Estrogen and estrogen receptors are known to have important physiological roles in endothelial function in men as well as in women. Premature ovarian failure has been shown to be associated with significant endothelial dysfunction (Kalantaridou et al., 2004; Kalantaridou et al.,

2006). In postmenopausal women, estrogen therapy has been shown to enhance endothelium-dependent vasodilation and reduce arterial stiffness in some studies (Gerhard et al., 1998; Sumino et al., 2006). Hypertension incidence and prevalence increase as women enter menopause, suggesting a role of ovarian hormone levels (Harrison et al., 2000; Dubey et al., 2002).

Given the strong genetic contribution to blood pressure (Sneider et al., 2003), and the modulating effects of estrogen on these traits, the *ESR1* is a biologically important candidate gene in hypertension. The human *ESR1* gene (OMIM133430) is located in chromosome 6q25.1 region and encodes an estrogen receptor, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription. The protein localizes to the nucleus where it may form a homodimer or a heterodimer with estrogen receptor

2 (Parker et al., 1993).

There are 2 known estrogen receptors: estrogen receptor α (ESR1) and estrogen receptor β (ESR2). Estrogen is known to have long-term effects on the vasculature (Mendelsohn, 2002). This effects are mediated through estrogen-activated transcription factors, ESR1 (encoded by the *ESR1* gene), which is found in vascular endothelial and smooth muscle cells in both men and women (Karas et al., 1994; Venkov et al., 1996). Functional ESR1 is activated by binding to estrogen response elements located near the promoter sequence of many genes. ESR1 knockout mice express a broad range of changes in vascular physiology that include a lack of response to estrogen on vascular injury (Pare et al., 2002), blood vessel and vascular smooth muscle cell abnormalities, and hypertension (Zhu et al., 2002).

Following the report that an *AGT* gene polymorphism might contribute to the prevalence of hypertension in Caucasians (Jeunemaitre et al., 1992), SNPs in *ESR1* gene have been extensively tested as the genetic factors of blood pressure and hypertension in the Korean population

In this study, we examined the association with genetic variations in *ESR1* gene and hypertension in the Korean population. Notably, this study provides insight into the relation of *ESR1* gene with hypertension.

MATERIALS AND METHODS

Subjects and clinical characteristics

Subjects in the Korean population in the Korean Association REsource (KARE) study were described in more detail by other study (Cho et al., 2009). Briefly 10,038 persons in the Ansung-Ansan prospective community cohorts were recruited. A two-community cohort study in South Korea was initiated beginning in 2001 as part of a major project for the Korean Health and Genome Study (KHGS) in Korea National Health and Genome Study (KNIH). Cohort examinations were conducted biennially. The initial numbers of subjects who were aged 40 to 69 years from Ansung and Ansan were 5018 and 5020, respectively. Of the 10,038 subjects, 1196 were excluded due to poor genotyping data. In addition, to analyze accurate blood pressure traits, 330 subjects who were on drug

treatments such as folk medicine that were likely to influence the blood pressure were also excluded. The remaining 8512 subjects were finally investigated in this study.

For quantitative blood pressure traits analysis, 961 subjects who were undergoing antihypertensive treatment were excluded and the remaining 7,551 subjects [3,747 men (49.6%); 3,804 women (50.4%)] were investigated. A case-control study was performed between hypertensive women cases (n=1,058) and normotensive women controls (n=2,390). Hypertensive women cases were recruited -- base on systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg in addition to the subjects who were receiving hypertension medication, were generating a total of 1,058 cases. Normotensive women controls were defined as SBP < 120 mmHg and DBP < 80 mmHg. Clinical characteristics of the subjects are summarized in Table 1. This study was approved by the institutional review board of the Korean National Institute of Health (KNIH). Written informed consent was obtained from all subjects.

Measurement of blood pressure

Blood pressure measurements were taken three times in the supine position using a mercury sphygmomanometer (Baumanometer; W. A. Baum, Copiague, NY, USA) with an appropriate cuff size by trained nurses at clinics, and the average value data was used for this study. Before the first measurement, subjects rested for 5 min, and three measurements were taken at least 2 min apart.

Genotyping and selection of *ESR1* gene SNPs

The detailed genotyping, quality control processes and quantitative traits including SBP and DBP were described in the previous report (Cho et al., 2009). Briefly, most DNA samples were isolated from the peripheral blood of participants and genotyped using the Affymetrix Genome-Wide Human SNP array 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). The accuracy of the genotyping was calculated by Bayesian Robust Linear Modeling using the Mahalanobis Distance (BRLMM) algorithm (Rabbee et al., 2006). Samples that had genotyping accuracies were lower than

Table 1. Basic characteristics of the women subjects in the KARE study

Characteristics	Quantitative trait analysis[*]	Case-control analysis[**]		
		Normotensive	Hypertensive	P value[***]
Number of subjects	3,804	2,390	1,058	
Age (M years $\pm$ SD)	51.59 $\pm$ 8.89	49.15 $\pm$ 8.05	58.20 $\pm$ 7.92	< 0.0001
Body mass index (BMI) (M kg/m^2 $\pm$ SD)	24.68 $\pm$ 3.22	24.27 $\pm$3.08	26.18 $\pm$ 3.38	< 0.0001
Systolic blood pressure (SBP) (M mmHg $\pm$ SD)	114.82 $\pm$ 18.21	103.93 $\pm$ 9.51	141.02 $\pm$ 17.94	< 0.0001
Diastolic blood pressure (DBP) (M mmHg $\pm$ SD)	72.68 $\pm$ 11.33	66.59 $\pm$ 7.65	85.98 $\pm$ 11.24	< 0.0001
Total cholesterol (M mg/dl $\pm$ SD)	190.05 $\pm$ 35.18	185.99 $\pm$ 33.62	200.36 $\pm$ 36.98	< 0.0001
High density lipoprotein cholesterol (M mg/dl $\pm$ SD)	45.91 $\pm$ 9.97	46.38 $\pm$ 9.94	44.05 $\pm$ 10.24	< 0.0001
Triglyceride (M mg/dl $\pm$ SD)	143.53 $\pm$ 86.65	132.38 $\pm$ 78.42	179.74 $\pm$ 103.27	< 0.0001

Abbreviations: M, mean value; SD, standard deviation. [*]Individuals who are not using hypertensive medications. [**]Controls (normotensive), SBP <120 mmHg and DBP <80 mmHg; Cases (hypertensive), SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or antihypertensive medication. [***]Significant differences in characteristics between the normotensive and hypertensive subjects were determined by the two-tailed Student's t-test.

98%, high missing genotype call rates (≥4%), high heterozygosity (>30%), or gender biases were excluded.

The SNPs that we analyzed were selected from the KARE data, based on their positions within the gene boundary (5 kb upstream and downstream of the first and last exons, respectively) (Table 2). The positions of the SNPs were validated in the NCBI database (http://www.ncbi.nlm.nih.gov). The clinical information and geno-type data that we used were graciously provided by the Center for Genome Science, KNIH, Korea Center for Disease Control (KCDC).

Statistical analysis

Most statistical analyses were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and PASW Statistics version 17.0 (SPSS Inc., Chicago, IL, USA). Linear regression was used to analyze SBP and DBP as quantitative traits in the final 3,804 women, controlling for cohort, age and body mass index (BMI) as covariates. The 60 selected SNPs were also analyzed in hypertension case-control studies using logistic regression analysis.

All association tests were based on an additive genetic model, and P-values were not adjusted for multiple tests. Statistical significance was determined at a two-tailed value of $P < 0.05$.

Haploview version 4.2 (Whitehead Institute for Bio-medical Research, Cambridge, MA, USA) was used to examine the structure of the linkage disequilibrium (LD) block (Barrett et al., 2005) using the KARE genotype data for *ESR1* gene. The LD coefficient r^2 was examined between all pairs of biallelic loci (Hedrick et al., 1987).

RESULTS

Association analysis with SNPs in *ESR1* gene and blood pressure traits

We informed the *ESR1* gene and its 60 SNPs (Table 2). And, the basic characteristics of study subjects were shown to Table 1. The mean age of the 3,804 study women was 51.59 years. The mean SBP of the 3,804 women was 114.82 $\pm$ 18.21, and the mean DBP was 72.68 $\pm$ 11.33 (Table 1). And, the mean and variance of BMI, SBP, DBP, total cholesterol, high density lipoprotein cholesterol, and triglyceride were all statistically different between case-control groups by Student's t-test in Table 1.

Linear regression analysis was used to associate geno-types with blood pressure traits, controlling for age, BMI, and cohort as covariates. The results of associations on the 60 SNPs of *ESR1* gene in women were listed in Table 2.

The results on the SBP, five SNPs (rs2982571, rs851985, rs851983, rs851981 and rs851980) were significantly associated with SBP in women (Table 2). The SNP rs-2982571 had the highest significance P value (β=-1.56, additive P=6.8 $\times$ 10^{-3}). The results on the DBP, three SNPs (rs2982571, rs851985 and rs9322335) were significantly

Table 2. The association analysis results of SNPs in the *ESR1* gene with blood pressure and hypertension in the KARE women

No.	SNP	Minor allele	MAF	Genotype missing rate	HWE-*p*	Function	SBP (n=3,804)		DBP (n=3,804)		HTN (controls 2,390: cases 1,058)	
							beta ± se	*P* value	beta ± se	*P* value	OR (95% CI)	*P* value
1	rs2982571	A	0.119	0.0002	0.4402	intron	-1.56 ± 0.57	**6.8E-03**	-0.80 ± 0.37	**0.030**	0.80 (0.67~0.96)	**0.017**
2	rs851985	G	0.113	0.0006	0.0398	intron	-1.52 ± 0.58	**9.1E-03**	-0.82 ± 0.38	**0.029**	0.78 (0.65~0.94)	**8.6E-03**
3	rs851983	G	0.108	0.0069	0.1475	intron	-1.39 ± 0.60	**0.020**	-0.71 ± 0.39	0.065	0.80 (0.66~0.97)	**0.023**
4	rs17081604	T	0.024	0.0000	0.5256	intron	1.45 ± 1.24	0.241	1.33 ± 0.80	0.098	1.16 (0.80~1.70)	0.437
5	rs9383591	G	0.379	0.0002	0.6186	intron	0.19 ± 0.39	0.632	0.02 ± 0.25	0.952	1.02 (0.91~1.15)	0.740
6	rs851981	T	0.112	0.0000	0.4185	intron	-1.38 ± 0.59	**0.019**	-0.73 ± 0.38	0.056	0.81 (0.67~0.97)	**0.025**
7	rs851980	G	0.111	0.0011	0.3350	intron	-1.41 ± 0.59	**0.017**	-0.74 ± 0.38	0.051	0.79 (0.65~0.95)	**0.015**
8	rs7772579	C	0.173	0.0000	0.3313	intron	-0.68 ± 0.50	0.168	-0.40 ± 0.32	0.213	0.90 (0.77~1.05)	0.165
9	rs2982565	A	0.068	0.0004	0.8171	intron	-0.95 ± 0.75	0.203	-0.30 ± 0.48	0.538	0.92(0.72~1.16)	0.467
10	rs6899458	A	0.195	0.0002	0.7085	intron	-0.39 ± 0.47	0.416	-0.21 ± 0.31	0.497	0.95 (0.82~1.10)	0.483
11	rs2982561	T	0.166	0.0013	0.4268	intron	-0.61 ± 0.51	0.230	-0.16 ± 0.33	0.628	0.89 (0.76~1.05)	0.164
12	rs3020348	T	0.243	0.0002	0.0167	intron	-0.15 ± 0.45	0.734	-0.03 ± 0.29	0.906	0.95 (0.83~1.10)	0.500
13	rs2982554	G	0.243	0.0000	0.0151	intron	-0.14 ± 0.45	0.760	-0.02 ± 0.29	0.933	0.95 (0.83~1.10)	0.506
14	rs3020306	A	0.242	0.0000	0.6897	intron	-0.49 ± 0.44	0.258	-0.16 ± 0.28	0.581	0.91 (0.79~1.04)	0.157
15	rs1856057	C	0.287	0.0000	0.5664	intron	-0.24 ± 0.42	0.557	-0.14 ± 0.27	0.592	0.94 (0.83~1.07)	0.352
16	rs9322330	A	0.276	0.0000	0.8835	intron	0.22 ± 0.42	0.607	0.07 ± 0.27	0.787	1.02 (0.90~1.16)	0.789
17	rs9371551	T	0.297	0.0086	0.6219	intron	0.32 ± 0.41	0.435	0.15 ± 0.27	0.585	1.00 (0.89~1.14)	0.946
18	rs6916835	G	0.294	0.0015	0.8598	intron	-0.56 ± 0.41	0.174	-0.15 ± 0.27	0.573	0.90 (0.80~1.03)	0.116
19	rs6939257	T	0.294	0.0000	0.9156	intron	-0.57 ± 0.41	0.171	-0.17 ± 0.27	0.526	0.90 (0.80~1.03)	0.117
20	rs9371552	T	0.362	0.0000	0.1628	intron	-0.09 ± 0.40	0.825	-0.04 ± 0.26	0.890	1.03 (0.91~1.16)	0.663
21	rs2248586	C	0.137	0.0002	0.8526	intron	-0.85 ± 0.54	0.117	-0.43 ± 0.35	0.219	0.80 (0.67~0.95)	**0.012**
22	rs2347759	G	0.297	0.0047	0.5500	intron	-0.54 ± 0.41	0.192	-0.13 ± 0.27	0.630	0.92 (0.81~1.04)	0.187
23	rs3844508	C	0.288	0.0097	0.8296	intron	0.03 ± 0.42	0.937	-0.02 ± 0.27	0.936	1.13 (0.99~1.28)	0.067
24	rs9371557	C	0.299	0.0000	0.8612	intron	-0.24 ± 0.41	0.558	-0.25 ± 0.26	0.340	0.94 (0.83~1.07)	0.339
25	rs9340817	T	0.256	0.0032	0.2987	Intron	0.03 ± 0.44	0.953	0.08 ± 0.28	0.780	1.01 (0.89~1.16)	0.838
26	rs712221	T	0.437	0.0009	0.3406	intron	0.47 ± 0.38	0.217	0.29 ± 0.25	0.241	1.12 (0.99~1.26)	0.076
27	rs1709183	T	0.395	0.0000	0.7591	intron	0.32 ± 0.38	0.399	-0.07 ± 0.25	0.781	1.05 (0.93~1.18)	0.413
28	rs11155819	G	0.170	0.0000	0.5332	intron	-0.18 ± 0.50	0.719	-0.17 ± 0.32	0.598	1.00 (0.86~1.17)	0.956
29	rs9322335	G	0.400	0.0009	0.6036	intron	-0.68 ± 0.38	0.077	-0.61 ± 0.25	**0.013**	0.92 (0.82~1.04)	0.168
30	rs11155820	G	0.054	0.0408	0.3756	intron	-1.12 ± 0.85	0.188	-0.04 ± 0.55	0.942	0.89 (0.69~1.16)	0.387
31	rs7772475	A	0.102	0.0011	0.0307	intron	-0.01 ± 0.63	0.992	0.02 ± 0.41	0.966	0.99 (0.82~1.21)	0.960
32	rs9397453	T	0.101	0.0479	0.8041	intron	-0.66 ± 0.64	0.298	-0.15 ± 0.41	0.720	0.98 (0.80~1.19)	0.813
33	rs4870061	T	0.473	0.0006	0.8832	intron	0.49 ± 0.38	0.189	0.02 ± 0.24	0.937	1.05 (0.93~1.18)	0.411
34	rs1801132	G	0.481	0.0000	0.8373	synonymous	0.32 ± 0.38	0.401	0.05 ± 0.24	0.842	1.03 (0.92~1.16)	0.629
35	rs9397459	A	0.094	0.0058	0.0469	intron	-0.42 ± 0.64	0.505	-0.08 ± 0.41	0.837	0.99 (0.81~1.21)	0.906
36	rs3020314	T	0.200	0.0002	0.1299	intron	0.22 ± 0.47	0.633	0.41 ± 0.30	0.172	1.06 (0.91~1.22)	0.465
37	rs3020394	A	0.373	0.0116	1.0000	intron	0.40 ± 0.39	0.313	0.36 ± 0.25	0.156	1.12 (0.99~1.27)	0.061
38	rs1884051	T	0.476	0.0000	0.3040	intron	0.11 ± 0.38	0.775	0.31 ± 0.25	0.205	1.11 (0.98~1.24)	0.093
39	rs9383951	G	0.091	0.0002	0.2489	intron	-0.48 ± 0.65	0.460	0.02 ± 0.42	0.971	0.99 (0.81~1.21)	0.885
40	rs2144025	G	0.432	0.0002	0.4737	intron	-0.42 ± 0.38	0.277	-0.14 ± 0.25	0.581	0.93 (0.83~1.05)	0.244
41	rs12664544	G	0.319	0.0024	0.9462	intron	0.42 ± 0.40	0.298	0.13 ± 0.26	0.615	1.01 (0.89~1.14)	0.928

Table 2. Continued

No.	SNP	Minor allele	MAF	Genotype missing rate	HWE-*p*	Function	SBP (n=3,804)		DBP (n=3,804)		HTN (controls 2,390: cases 1,058)	
							beta ± se	*P* value	beta ± se	*P* value	OR (95% CI)	*P* value
42	rs1569788	T	0.442	0.0099	0.9524	intron	-0.37 ± 0.38	0.325	-0.01 ± 0.25	0.980	1.04 (0.92~1.17)	0.534
43	rs9340955	A	0.011	0.0090	1.0000	intron	2.57 ± 1.82	0.158	1.12 ± 1.18	0.343	1.31 (0.75~2.28)	0.338
44	rs9340958	T	0.075	0.0354	0.5892	intron	0.08 ± 0.73	0.915	0.42 ± 0.47	0.374	0.92 (0.72~1.16)	0.465
45	rs9341004	C	0.309	0.0277	0.0003	intron	0.48 ± 0.40	0.239	0.07 ± 0.26	0.783	0.99 (0.88~1.13)	0.916
46	rs3020368	A	0.079	0.0017	0.4807	intron	-0.26 ± 0.70	0.715	0.25 ± 0.45	0.585	1.01 (0.81~1.25)	0.966
47	rs6930355	G	0.336	0.0017	0.1223	intron	0.44 ± 0.39	0.265	0.11 ± 0.25	0.680	0.98 (0.87~1.11)	0.728
48	rs6557192	A	0.335	0.0011	0.0523	intron	0.50 ± 0.39	0.206	0.12 ± 0.25	0.651	0.98 (0.87~1.11)	0.770
49	rs1884152	T	0.335	0.0002	0.0565	intron	0.49 ± 0.39	0.214	0.10 ± 0.25	0.682	0.98 (0.87~1.11)	0.802
50	rs2273206	A	0.335	0.0206	0.0031	intron	0.36 ± 0.39	0.363	0.05 ± 0.25	0.831	0.97 (0.86~1.10)	0.622
51	rs2273207	C	0.314	0.0002	0.0448	intron	0.42 ± 0.40	0.290	0.01 ± 0.26	0.960	0.98 (0.87~1.11)	0.739
52	rs2207396	T	0.205	0.0101	0.7517	intron	-0.21 ± 0.47	0.648	-0.31 ± 0.30	0.301	1.02 (0.88~1.18)	0.795
53	rs3798571	C	0.343	0.0004	0.0293	intron	0.45 ± 0.39	0.245	0.17 ± 0.25	0.505	0.99 (0.88~1.12)	0.896
54	rs3778080	C	0.313	0.0019	0.0346	intron	0.36 ± 0.40	0.371	-0.01 ± 0.26	0.972	0.97 (0.86~1.10)	0.625
55	rs3798573	C	0.332	0.0000	0.0642	intron	0.53 ± 0.39	0.178	0.11 ± 0.26	0.666	0.99 (0.88~1.12)	0.913
56	rs9479191	C	0.332	0.0019	0.0316	intron	0.54 ± 0.39	0.168	0.12 ± 0.25	0.642	0.99 (0.88~1.12)	0.928
57	rs3778089	A	0.332	0.0004	0.0552	intron	0.54 ± 0.39	0.173	0.11 ± 0.26	0.658	0.99 (0.88~1.12)	0.928
58	rs750686	C	0.450	0.0032	0.0753	intron	-0.32 ± 0.37	0.395	0.02 ± 0.24	0.928	1.00 (0.89~1.12)	0.950
59	rs9322359	A	0.317	0.0163	0.1090	intron	0.29 ± 0.40	0.478	-0.02 ± 0.26	0.938	0.97 (0.86~1.10)	0.609
60	rs3798577	G	0.387	0.0004	0.8288	3' UTR	-0.43 ± 0.39	0.260	-0.41 ± 0.25	0.104	1.04 (0.93~1.17)	0.497

Abbreviations: SNP, sigle nucleotide polymorphism; MAF, minor allele frequency, HWP-*p*, Hardy-Weinberg equilibrium *P* value; beta, regression coefficient; se, standard error; OR, odds ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension. Controls were the subjects with SBP < 120 mmHg and DBP < 80 mmHg, and hypertension cases were the subjects with SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or antihypertensive medication. Statistically significant values (*P* < 0.05) are indicated in bold and underline.

associated with DBP in women (Table 2). The SNP rs9322335 had the highest significance *P* value (β=-0.61, additive *P*=0.013). The two SNPs (rs2982571, and rs-851985) had commonly significance in both SBP and DBP, and their effects were shown the same directions.

With significant 5 SNPs, we further analysis of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride (TG). There was no association with total cholesterol, and triglyceride, but 3 SNPs (rs2982571, rs851981, and rs851980) were associated with high density lipoprotein cholesterol (Table 3). We also analyzed the association between the significant 5 SNPs of *ESR1* and blood pressure traits in the Korean men, but there is no association result (Table 4).

Association analysis with SNPs in *ESR1* gene and hypertension

The 6 SNPs of *ESR1* were associated with hypertension status. The highest significant SNP in hypertension was rs851985 (OR=0.78, CI: 0.65~0.94, additive *P*=8.6 × 10^{-3}) (Table 2). Furthermore, the other 4 SNPs (rs2982571, rs851983, rs851981 and rs851980) in the *ESR1* gene were consistently associated with both blood pressure and hypertension; SBP, and/or DBP, and hypertension (Table 2). In all the 5 SNPs, their β-values in SBP and/or DBP showed consistent trends with the odds ratios (ORs) of hypertension, and these 5 SNPs were composed with one LD block (Fig. 1).

Consequently, these results suggested that the individuals with the minor alleles of the 5 SNPs in the *ESR1* gene may be less susceptible to the development of hypertension in

Table 3. The association analysis results of 5 SNPs in the *ESR1* gene with total cholesterol, HDL and TG in the KARE women (n=3,804)

SNP	Minor allele	MAF	Genotype missing rate	HWE-*p*	Function	Total cholesterol		HDL		TG	
						beta ± se	*P* value	beta ± se	*P* value	beta ± se	*P* value
rs2982571	A	0.119	0.0002	0.4402	intron	1.84 ± 1.17	0.118	0.69 ± 0.34	**0.043**	-2.26 ± 2.91	0.436
rs851985	G	0.113	0.0006	0.0398	intron	1.80 ± 1.19	0.130	0.64 ± 0.35	0.063	-2.40 ± 2.94	0.414
rs851983	G	0.108	0.0069	0.1475	intron	1.69 ± 1.22	0.164	0.59 ± 0.35	0.095	-2.12 ± 3.02	0.483
rs851981	T	0.112	0.0000	0.4185	intron	1.86 ± 1.20	0.122	0.69 ± 0.35	**0.049**	-2.03 ± 2.98	0.495
rs851980	G	0.111	0.0011	0.3350	intron	1.78 ± 1.20	0.140	0.71 ± 0.35	**0.043**	-2.37 ± 2.98	0.427

Abbreviations: SNP, sigle nucleotide polymorphism; MAF, minor allele frequency, HWP-*p*, Hardy-Weinberg equilibrium *P* value; beta, regression coefficient; se, standard error; HDL, high density lipoprotein cholesterol; TG, Triglyceride. Statistically significant values ($P < 0.05$) are indicated in bold and underline.

Table 4. The association analysis results of 5 SNPs in the ESR1 gene with blood pressure and hypertension in the KARE men

SNP	Minor allele	MAF	Genotype missing rate	HWE-*p*	Function	SBP (n=3,747)		DBP (n=3,747)		HTN (controls 2,062: cases 910)	
						beta ± se	*P* value	beta ± se	*P* value	beta ± se	*P* value
rs2982571	A	0.119	0.0002	0.4402	intron	0.60 ± 0.54	0.270	0.57 ± 0.39	0.141	1.11 (0.93~1.32)	0.256
rs851985	G	0.113	0.0006	0.0398	intron	0.64 ± 0.55	0.247	0.44 ± 0.39	0.261	1.13 (0.94~1.35)	0.197
rs851983	G	0.108	0.0069	0.1475	intron	0.87 ± 0.56	0.121	0.60 ± 0.40	0.134	1.19 (0.99~1.42)	0.065
rs851981	T	0.112	0.0000	0.4185	intron	0.66 ± 0.56	0.234	0.46 ± 0.40	0.247	1.14 (0.95~1.37)	0.146
rs851980	G	0.111	0.0011	0.3350	intron	0.63 ± 0.56	0.263	0.45 ± 0.40	0.260	1.15 (0.96~1.37)	0.139

Abbreviations: SNP, sigle nucleotide polymorphism; MAF, minor allele frequency, HWP-*p*, Hardy-Weinberg equilibrium *P* value; beta, regression coefficient; se, standard error; OR, odds ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension. Controls were the subjects with SBP <120 mmHg and DBP <80 mmHg, and hypertension cases were the subjects with SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or antihypertensive medication.

the Korean women.

DISCUSSION

In this study, we had investigated the genetic variation of *ESR1* gene are associated with blood pressure and hypertension in the 3,804 Korean women (Table 1). As a result, we investigated 5 SNPs with both blood pressure and hypertension (Table 2, Fig. 1). Moreover, the 5 significant SNPs had negative beta values in blood pressure, and those means that the carrier of minor allele had low blood pressure, and also resistance for hypertension. Therefore these 5 SNPs would be contribute low value of blood pressure, and slow the pathogenesis of hypertension.

As already mentioned, the five *ESR1* polymorphisms investigated in the present study are in strong linkage disequilibrium, and they are located in the forepart of *ESR1* genetic region. Therefore, it could be differentially expressed to mRNA depending on the SNPs. Limitations of this study included the lack of detailed covered SNPs in the *ESR1* gene, but this report has the value of association study for *ESR1* and blood pressure traits.

By *in silico* analysis of the 5 significant SNP in the TRANSFAC database (http://www.cbrc.jp/research/db/TFSEARCH.html), we found that the minor allele of rs851983 contained AML-1a transcription factor binding site (85.4 scoring point) and the major allele of rs851981 contained AP-1 transcription factor binding site (85.6 scoring point). Because these SNPs are located in the site of between 5' UTR and exon1 of *ESR1* gene, expression
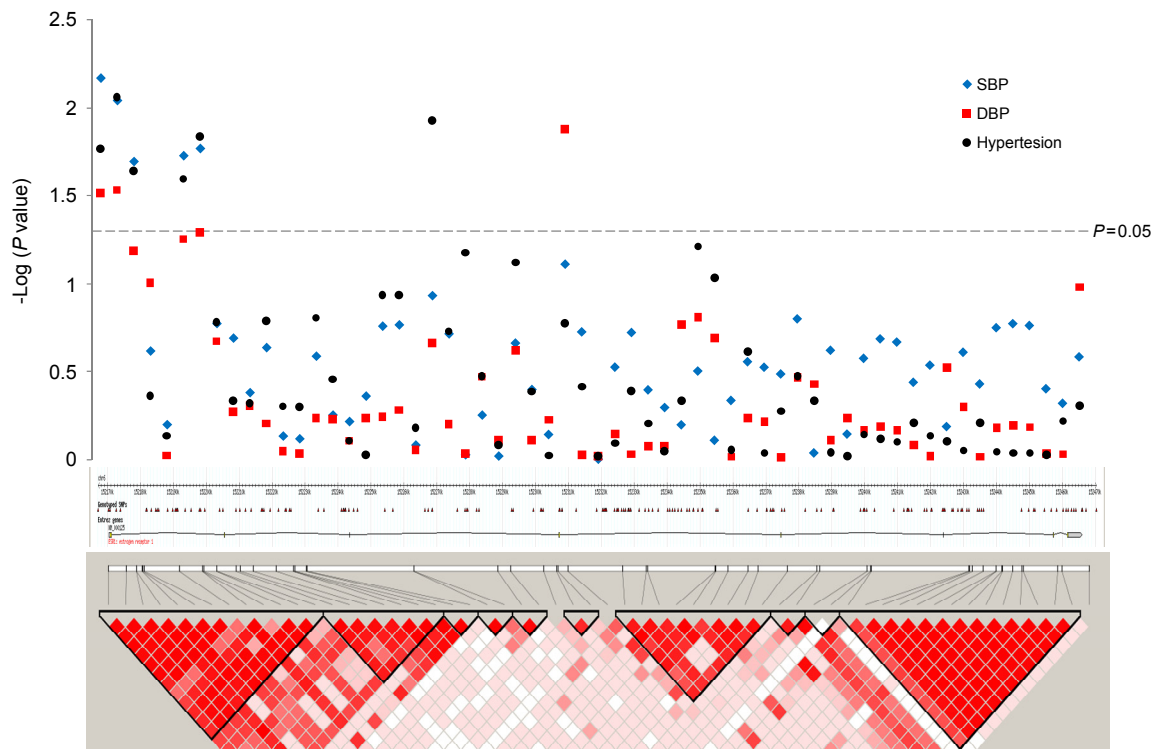
Fig. 1. The plot of *P*-values of the studied 60 SNPs, the gene structure and linkage disequilibrium blocks in the *ESR1* gene. The top panel shows the plots of *P*-values of the studied 60 SNPs in the *ESR1* gene in the quantitative blood pressure traits analysis and the logistic case-control analysis of the KARE women. Standard significant *P*-value threshold (*P*=0.05) are indicated by the dotted lines. The middle panel shows the physical position of *ESR1* on chromosome 6. The bottom panel shows a Haploview of LD (r^2) based on genotyping data from 3,804 KARE women and are generated by using the Haploview program.

level of the *ESR1* gene may be influenced by binding of the transcription factors.

Therefore, polymorphism in the *ESR1* gene may affect the expression or the function of *ESR1* gene. In women, estrogen levels and *ESR1* gene polymorphisms seem to play a determinant role in hypertensive pathology. It is possible that the effects of estrogen to vascular cells, mediated by ESR1, differ due to the ESR1 variant forms that have different transcriptional effects than the 'wild-type' receptor (Matsubara et al., 1997; Maruyama et al., 2000).

Although the exact mechanism whereby the *ESR1* gene polymorphism may affect the vasculature is unclear, it is possible that this variant alters *ESR1* transcript length or RNA splicing causing significant heterogeneity in *ESR1* mRNA transcripts. It has been shown previously that alternative splicing may result in variants with deletions of exons encoding regions of the hormone-binding domain with truncated forms of ESR1 discovered in many tissues

including vascular endothelium and showing altered ligand-activation properties (Moriarty et al., 2006).

Estrogen receptors are expressed in a wide range of tissues, including macrophages, vascular smooth muscle, and vascular endothelial cells (Mendelsohn and Karas, 1999). Estrogen receptors regulate gene expression by both estrogen-dependent and estrogen-independent mechanisms that result in activation of transcription.

The *ESR1* gene has been shown to mediate 3 effects of estrogen on the vessel wall: acceleration of re-endothelialization (Brouchet et al., 2001), alteration of endothelial nitric oxide production (Pendaries et al., 2002), and inhibition of the vascular injury response (Pare et al., 2002). These studies also demonstrated the importance of estrogen receptors in cardiovascular physiology.

From our research, genetic association of the *ESR1* gene has been searched in the genetic association databases (HuGe Navigator: http://hugenavigator.net). They had

presented that the *ESR1* gene polymorphisms associated with the neoplasm, osteoporosis, obesity, cardiovascular disease, diabetes etc including hypertension. Figtree *et al.* had reported that the polymorphism located in promoter region of *ESR1* gene was significantly associated with left ventricular hypertrophic response to hypertension (Figtree et al., 2007). The other article had published that the polymorphisms of *ESR1* were associated with DBP in Western women (Peter et al., 2005). These reports are in accord with our results for the promoter region's SNP were associated with blood pressure traits in women.

In summary, we investigated the presence of blood pressure traits-associated SNPs in *ESR1* gene. And, we found statistically significant SNPs that are associated with blood pressure traits. Therefore, this study suggests *ESR1* gene could be related to pathogenesis of hypertension in Korean women.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Barrett JG, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005. 21: 263-265.

Brouchet L, Krust A, Dupont S, Chambon P, Bayard F, Arnal J. Estradiol accelerates reendothelialization in mouse carotid artery through estrogen receptor α but not estrogen receptor β. Circulation 2001. 103: 423-428.

Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet. 2009. 41: 527-534.

Dubey RK, Oparil S, Imthurn B, Jackson EK. Sex hormones and hypertension. Cardiovasc Res. 2002. 53: 688-708.

Figtree GA, Kindmark A, Lind L, Grundberg E, Speller B, Robinson BG, Channon KM, Watkins H. Novel estrogen receptor alpha promoter polymorphism increases ventricular hypertrophic response to hypertension. J Steroid Biochem Mol Biol. 2007. 103: 110-118.

Gerhard M, Walsh BW, Tawakol A, Haley EA, Creager SJ, Seely EW. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation 1998. 98: 1158-1163.

Harrison-Bernard LM, Raij L. Postmenopausal hypertension. Curr Hypertens Rep. 2000. 2: 202-207.

Hedrick PW. Gametic disequilibrium measures: Proceed with caution. Genetics. 1987. 117: 331-341.

Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A. Molecular basis of human hypertension: Role of angiotensinogen. Cell 1992. 71: 169-180.

Kalantaridou SN, Naka KK, Papanikolaou E, Kazakos N, Kravariti M, Calis KA. Impaired endothelial function in young women with premature ovarian failure: normalization with hormone therapy. J Clin Endocrinol Metab. 2004. 89: 3907-3913.

Kalantaridou SN, Naka KK, Bechlioulis A, Makrigiannakis A, Michalis L, Chrousos GP. Premature ovarian failure, endothelial dysfunction and estrogen-progestrogen replacement. Trends Endocrinol Metab. 2006. 17: 101-109.

Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation 1994. 89: 1943-1950.

Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T. Lack of an association of estrogen receptor alpha gene polymorphisms and transcriptional activity with Alzheimer disease. Arch Neurol. 2000. 57: 236-240.

Matsubara Y, Murata M, Kawano K, Zama T, Aoki N, Yoshino H. Genotype distribution of estrogen receptor polymorphisms in men and postmenopausal women from healthy and coronary populations and its relation to serum lipid levels. Arterioscler Thromb Vasc Biol. 1997. 17: 3006-3012.

Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999. 340: 1801-1811.

Mendelsohn ME. Genomic and nongenomic effects of estrogen in the vasculature. A J Cardiol. 2002. 90: 3F-6F.

Moriarty K, Kim KH, Bender JR. Minireview: estrogen receptor-mediated rapid signaling. Endocrinology 2006. 147: 5557 5563.

Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon R, Mendelsohn ME. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. Circ Res. 2002. 90: 1087-1092.

Parker MG, Arbuckle N, Dauvois S, Danielian P, White R. Structure and function of the estrogen receptor. Ann NY Acad Sci. 1993. 684: 119-126.

Pendaries C, Darblade B, Rochaix P. The AF-1 activation-function of ER-α may be despensable to mediate the effect of estradiol on endothelial NO production in mice. Proc Natl Acad Sci USA. 2002. 99: 2205-2210.

Peter I, Shearman AM, Zucker DR, Schmid CH, Demissie S, Cupples LA, Larson MG, Vasan RS, D'Agostino RB, Karas RH, Mendelsohn ME, Housman DE, Levy D. Variation in estrogen-related genes and cross-sectional and longitudinal blood pressure in the Framingham Heart Study. J Hypertens. 2005. 23: 2193-2200.

Rabbee N, Speed TP. A genotype calling algorithm for Affymetrix SNP arrays. Bioinformatics 2006. 22: 7-12.

Snieder H, Harshfield GA, Treiber FA. Heritability of blood pressure and hemodynamics in African- and European-American youth. Hypertension 2003. 41: 1196-1201.

Sumino H, Ichikawa S, Kasama S, Takahashi T, Kumakura H, Takayama Y. Different effects of oral conjugated estrogen and transdermal estradiol on arterial stiffness and vascular inflammatory markers in postmenopausal women. Atherosclerosis 2006. 189: 436-442.

Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. Circulation 1996. 94: 727-733.

Zhu Y, Bian Z, Lu P, Karas RH, Bao L, Cox D. Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. Science 2002. 295: 505-508.