Extensive Genetic Analysis of 10 Candidate Genes for Hypertension in Japanese

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Abstract—The identification of genes that contribute to essential hypertension has been hampered because of a lack of statistical power and problems with multiple testing. In the present study, we performed association analyses between the 161 single nucleotide polymorphisms of 10 candidate genes and hypertension in a Japanese population recruited from the Suita Study (n=3654). We found that 5 polymorphisms in the 3 genes (*SLC9A2*, *UMOD*, and *ELN*) were associated with hypertension status, and 4 of these 5 polymorphisms were also associated with blood pressure values with a classical criterion of $P<0.05$. However, when a Bonferroni correction for multiple testing was applied, none of the polymorphisms were associated with blood pressure levels. We also performed association analyses between these 5 polymorphisms and intermediate phenotypes corresponding with the functions of candidate genes, including the renin/aldosterone profile, plasma uric acid levels, and pulse wave velocity. The *ELN* 3′-untranslated region (−/A) polymorphism was found to significantly affect pulse wave velocity, an indicator of arterial stiffness. Associations of the *ELN* 3′-untranslated region (−/A) polymorphism with hypertension and pulse wave velocity were reconfirmed in another set of the study population. Thus, *ELN* seems to contribute to blood pressure regulation by affecting arterial stiffness in Japanese. **(*Hypertension*. 2006;48:901-907.)**

Key Words: hypertension ■ genetics ■ polymorphism ■ elastin

Recent genome-wide linkage scans for hypertension have had limited success in identifying genes that determine blood pressure levels.[1,2] This genome-wide scan strategy is based on the assumption that common diseases can be explained by a combination of common disease alleles. The limited success of the genome-wide scan strategy in hypertension research may indicate several possibilities: (1) hypertension may be explained by multiple rare alleles, (2) the contribution of any single gene to blood pressure variation may be much less than expected and may be very small, (3) noncoding RNA genes may be more important than protein-coding genes, (4) epigenetic alterations may be more important than common polymorphisms of genome DNA, and (5) the effects of and interactions with environmental factors may be quite strong, and these are difficult to include in the model. The lack of maturity in the methods used in genome-wide scans may also contribute to the limited success of previous studies based on this approach.

Although the candidate gene approach may not replace the genome-wide scan strategy, it is an important alternative strategy. Indeed, some of the above-mentioned obstacles may be overcome by a candidate gene approach. The resequencing of candidate genes in a sufficient number of subjects may reveal both common and rare ethnicity-specific polymorphisms. For example, we have found that the W258TER polymorphism in *SLC22A12* is one of the major determinants of uric acid levels in Japanese.[3] The allele frequency of 258TER is 0.0237, and an association study or haplotype analysis with just common alleles would not reveal the importance of this gene in determining uric acid levels in Japanese.

Moreover, by adopting a candidate gene approach, we can incorporate functional aspects of a candidate gene in the analysis. Association with not only a disease phenotype but also with intermediate phenotypes relevant to a candidate gene may strengthen the possible association with the disease phenotype. It may also be possible to incorporate an appropriate environmental factor relevant to a candidate gene in a statistical analysis model to increase the power of significance. For example, the significance of Gitelman's syndrome mutations (inactivating mutations in *SLC12A3*) in blood pressure regulation has been better clarified by including sodium chloride intake in the statistical analysis.[4] We have also reported that the significance of an inactivating mutation of *ALDH2* on blood pressure levels in Japanese can be clarified by including ethanol intake in the analysis.[5]

In the present study, we identified 361 polymorphisms in 10 candidate genes for hypertension by resequencing in 96 to 384 Japanese subjects and performed association studies between 161 polymorphisms and hypertension in a relatively large cohort representing the general population in Japan. We also performed additional association studies between candi-

Received June 16, 2006; first decision July 18, 2006; revision accepted August 11, 2006.

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Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000242485.23148.bb**

date polymorphisms and various phenotypes, including renin activity, aldosterone concentration, uric acid levels, blood pressure values on different occasions, and pulse wave velocity. Although, as mentioned above, the contribution of any single gene to hypertension seems to be small, and, thus, any single study that involves just a few thousand subjects may not necessarily be conclusive,[6] in the present study we have identified an intriguing candidate gene for hypertension, namely *ELN*.

Methods

Study Population

The selection criteria and design of the Suita Study have been described previously.[7–10] The sample consisted of 14 200 men and women (30 to 79 years of age at enrollment), stratified by gender and 10-year age groups (10 groups and 1420 subjects in each group), who had been randomly selected from the municipal population registry. They were all invited, by letter, to attend regular cycles of follow-up examination (every 2 years). We routinely check 10 to 15 participants per day. DNA from leukocytes was collected from participants who visited the National Cardiovascular Center between April 2002 and March 2004 (N=3654). An additional 289 DNA samples (study population II) were collected between April 2004 and February 2005 from participants who had not visited us between April 2002 and March 2004. All of the participants were Japanese, and only those who gave their written informed consent for genetic analyses were included.

Blood pressure was measured after 10 minutes of rest in a sitting position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were the means of 2 physician-obtained measurements. Physicians obtained detailed personal medical information directly from participants in the Suita Study.

Right and left bilateral brachial-ankle pulse wave velocities (PWVs) were determined using a FORM PWV/ABI device (Colin Co).[11–13] This device has 4 cuffs that use the oscillometric method to simultaneously measure blood pressure levels in both arms and both legs and can automatically calculate the ankle brachial pressure index. The mean blood pressure values of the right and left arms were used as a subject's blood pressure levels in the recumbent position. This device can also record pulse waves by sensors in the cuffs and automatically compute the brachial-ankle PWV values. PWV was measured by a trained medical technician. In the present study, PWV was defined as (right-PWV+left-PWV)/2. This PWV measurement preceded the physician's checkups.

The characteristics of the subjects analyzed in the present study are summarized in Table 1. The diagnosis of hypertension was based on blood pressure measurement (SBP ≥140 mm Hg or DBP ≥90 mm Hg) or the current use of antihypertensive medication.

Selection of Candidate Genes and Polymorphisms

Candidate genes were selected based on their physiological functions. *SCNN1B, KCNJ1, CLCNKB,* and *SLC12A3* have been reported to be responsible for human genetic disorders of renal tubular functions that lead to disturbances in electrolytes and blood pressure homeostasis.[14–17] *UMOD* has been reported to be responsible for juvenile hyperuricemic nephropathy, which is characterized by hyperuricemia, hypertension, and renal failure.[18] *SLC9A3*-knockout mice have been reported to show renal absorptive defect and low blood pressure.[19] *SLC9A2* was selected based on analogy to *SLC9A3*.[20] *ELN*-knockout mice have been reported to show hypertension.[21–23] *PTEN* has been reported to be associated with leptin and insulin signaling[24,25] and may be a key molecule for metabolic syndromes. *GPX3* encodes plasma glutathione peroxidase, a selenocysteine-containing protein with antioxidant properties.[26] A deficiency of plasma glutathione peroxidase has been reported to be responsible for familial juvenile stroke.[27]

The sequences of these candidate genes were determined in 96 to 384 subjects (≥48 hypertensive and 48 normotensive subjects). The promoter region (up to −2Kb) and all of the exons were sequenced. Generally, intronic sequences of ≈300 bp from the splicing donor or acceptor sites were included in the sequencing analyses. The polymorphisms found in the present study are all shown in Table I (available online at http:/hyper.ahajournals.org.).

In a single gene, the degree of linkage disequilibrium (LD) among polymorphisms with frequency >0.10 was calculated using the SNPAlyze statistical package (Dynacom). For *KCNJ1, CLCNKB, SLC12A3, GPX3,* and *PTEN*, polymorphisms with an R^2 value for LD of >0.5 were categorized in 1 group. For *SLC9A2, SLC9A3, SCNN1B, UMOD,* and *ELN*, polymorphisms with an R^2 value for LD of >0.65 were categorized in 1 group. At least 1 representative polymorphism from each group was included in genotyping. Generally, rare polymorphisms were not included in the analysis, except for missense, promoter, or possibly functional polymorphisms. When a polymorphism in one LD group tended to be associated with hypertension with $P<0.1$, other polymorphisms in this LD group were included in the association study to identify the polymorphism that was most closely associated with hypertension. Thus, we determined 161 polymorphisms of the 10 candidate genes in 1880 to 3654 subjects. Polymorphisms were determined by the TaqMan method or PCR, and the details of primers and probes are described in Table II.

Statistical Analysis and Strategy

Values are expressed as the mean±SD. All of the statistical analyses were performed with the JMP statistical analysis package (SAS Institute, Inc). Associations between genotypes and hypertension category were calculated by logistic analyses that included genotypes (major/heterozygous/minor), age, and body mass index (BMI) as covariates. When the number of homozygous subjects with a minor allele was <5%, *P* values were also recalculated by recategorizing genotypes (major/heterozygous+minor). Because we assessed asso-

TABLE 1. Characteristics of the Study Population

	1880 Subjects			3654 Subjects		
Phenotype	Male	Female	*P* Value	Male	Female	*P* Value
No.	864	1016		1708	1946	
Age, y	66.36±11.04	63.31±11.04	<0.0001	66.13±11.32	63.47±11.12	<0.0001
BMI, kg/m^2	23.22±2.97	22.30±3.15	<0.0001	23.32±2.98	22.38±3.21	<0.0001
SBP, mm Hg	131.76±19.43	128.05±19.82	<0.0001	130.81±19.09	128.26±19.90	<0.0001
DBP, mm Hg	79.68±10.69	76.55±9.91	<0.0001	79.19±10.25	76.51±9.76	<0.0001
HTN-drug, %	27.43	22.66	0.0171	28.22	22.61	<0.0001
HTN, %	47.22	38.23	<0.0001	45.49	38.34	<0.0001

Data are expressed as mean±SD. HTN-drug indicates use of antihypertensive drug; HTN, presence of hypertension.

TABLE 2. Association Between Polymorphisms and Hypertension

SNP No.	Gene	Region	NT/HTN	Major	Hetero	Minor	N	*P*	*P′*
4	*SLC9A2*	Intron6	NT	[−−] 1350 (63.77)	[−+] 688 (32.50)	[++] 79 (3.73)			
			HTN	[−−] 1021 (67.71)	[−+] 430 (28.51)	[++] 57 (3.78)	3625	0.0457	0.0154†
107	*UMOD*	5′region	NT	[AA] 1870 (88.25)	[AG] 240 (11.33)	[GG] 9 (0.42)			
			HTN	[AA] 1363 (90.32)	[AG] 143 (9.48)	[GG] 3 (0.20)	3628	0.106	0.0385
145	*ELN*	Exon 5 (A73V)	NT	[CC] 2017 (95.14)	[CT] 102 (4.81)	[TT] 1 (0.05)			
			HTN	[CC] 1415 (93.52)	[CT] 96 (6.35)	[TT] 2 (0.13)	3633	0.0665	0.0199
158	*ELN*	Intron 31	NT	[CC] 1974 (92.85)	[CT] 147 (6.91)	[TT] 5 (0.24)			
			HTN	[CC] 1365 (90.22)	[CT] 146 (9.65)	[TT] 2 (0.13)	3639	0.005	0.0020*
160	*ELN*	3′UTR	NT	[−−] 1713 (80.65)	[−A] 380 (17.89)	[AA] 31 (1.46)			
			HTN	[−−] 1176 (77.83)	[−A] 308 (20.38)	[AA] 27 (1.79)	3635	0.066	0.0251*

P values were calculated by logistic analyses that included genotype (major/heterozygous/minor), age, and BMI as covariates. When the number of homozygous subjects with a minor allele was <5%, P values (*P′*) were also recalculated by recategorizing genotypes (major/heterozygous+minor). NT and HTN indicate normotensive and hypertensive subjects.

The statistical power was calculated by the latter model: *statistical power >0.70; †statistical power >0.80.

ciations between 161 polymorphisms and hypertension, we adopted the Bonferroni correction for multiple testing.

Residuals were the observed values minus predicted values based on confounding factors. Residuals of blood pressure values were calculated by adjusting for age and BMI. Because the relationship between DBP and age was not linear, DBP levels were adjusted by a polynomial equation for age (n=2). Renin activity and aldosterone concentration were logarithmically transformed to attain a normal distribution, and residuals of log(renin) and log(aldosterone) were calculated by adjusting for sex and age. Residuals of uric acid levels were calculated by adjusting for sex, age, BMI, ethanol consumption per day, triglycerides, and fasting blood glucose. Residuals of PWV were calculated by adjusting for age, waist:hip ratio, pulse rate, ethanol consumption per day, triglycerides, and fasting blood glucose. Power calculations were performed using SamplePower (SPSS Inc). Haplotype analysis was performed with the SNPAlyze Pro statistical package (Dynacom).

We first conducted an association study between 161 polymorphisms and hypertension in 1880 subjects. When a polymorphism in one gene showed an association of $P<0.05$ with hypertension, all of the polymorphisms of this gene were determined in 3654 subjects. Polymorphisms with $P<0.05$ were then subjected to an association study between the polymorphisms and blood pressure values. Blood pressure values were measured twice at the checkups: one was determined automatically in the recumbent position by the PWV measurement device (N=3409), and the other was measured in the sitting position by a physician (N=3654). Polymorphisms with an association of $P<0.05$ in any of the blood pressure values were subjected to an additional association study. We measured renin activity and the aldosterone concentration to assess the sodium balance, which was intended to identify functional polymorphisms of genes relevant to tubular functions (*SLC9A2, SLC9A3, SCNN1B, KCNJ1, CLCNKB,* and *SLC12A3*). Renin/aldosterone profiles were assessed in 843 consecutive subjects who visited us during 2003. We also measured uric acid levels and PWV (aortic stiffness) as part of the routine checkups. These phenotypes were intended to identify functional polymorphisms of *UMOD* and *ELN*, respectively.

Results

Association Analysis With Hypertension

We found 361 polymorphisms in the 10 candidate genes. The allele frequencies and surrounding sequences are shown in Table I. We found 35 missense polymorphisms in the 10 candidate genes. The allele frequencies of most of these missense polymorphisms were very low, and, thus, we could not confirm whether these rare polymorphisms were functional based only on the association study.

We selected 161 polymorphisms for an association study, as described in the Methods section. All of the results of the association studies of the 10 candidate genes are shown in Table III. One polymorphism (No. 4) in *SLC9A2*, 1 polymorphism (No. 107) in *UMOD*, and 3 polymorphisms (Nos. 145, 158, and 160) in *ELN* show an association of $P<0.05$ with hypertension (Table 2). However, none of the polymorphisms were associated with hypertension when a correction for multiple testing (Bonferroni) was applied. Power calculations are also indicated in Table 2. Only polymorphism 4 in *SLC9A2* had statistical power >0.80. We also performed haplotype-based association analyses between these 3 genes and hypertension with polymorphisms with allele frequencies of >0.1. However, haplotype analyses did not increase the power of the association for any of the genes (data not shown). With the sample size in the present study (N=3654), odds of ≥1.15 can be reliably detected (>0.80) in polymorphisms with allele frequency >0.1. Associations between 161 polymorphisms and hypertension were also analyzed separately in male and female subjects for reference. Thirteen polymorphisms gave associations of $P<0.05$ with hypertension in male and/or female subjects (Table IV). In the present study, we focused on the 5 polymorphisms with $P<0.05$ in the total study population.

Association Analysis With Blood Pressure Values

We observed $P<0.05$ associations in 5 polymorphisms in 3 genes (No. 4 in *SLC9A2,* No. 107 in *UMOD,* and Nos. 145, 158, and 160 in *ELN*) in the total study population (Table 2). However, the associations between the polymorphisms in *SLC9A2* and *UMOD* and blood pressure values were not strong (Table V). Moreover, the associations between these 2 polymorphisms and blood pressure levels in the recumbent position were negative (Table VI). These results may indicate that the contributions of these 2 polymorphisms with blood pressure regulation are either very small or spurious. On the other hand, the 3 polymorphisms (Nos. 145, 158, and 160) in *ELN* were associated with blood pressure values in the sitting

TABLE 3. Association Analysis Between *ELN* Polymorphisms and PWV

	145 *ELN* (GCTTG(C/T)GGGTG)				
Phenotype	CC	CT	TT	*P*	*P′*
N(-drug)	3199 (2408)	188 (137)	3 (2)		
PWV	1622.28±404.69	1597.56±365.31	1798.50±519.62	0.5347	0.4721
Residual-PWV 1 (total)	0.93±295.60	−15.72±279.39	−167.61±297.85	0.4642	0.3862
Residual-PWV 1 (-drug)	1.38±278.71	−16.64±263.43	−278.47±114.70	0.2784	0.3688
Residual-PWV 2 (total)	1.59±260.33	−25.47±233.43	−203.79±179.83	0.1506	0.1224
Residual-PWV 2 (-drug)	1.78±242.60	−28.16±212.90	−285.26±7.08	0.0909	0.1099
Residual-PWV 1 (-drug)					
	158 *ELN* (IMS-JST110834)				
Phenotype	CC	CT	TT	*P*	*P′*
N (-drug)	3125 (2352)	264 (195)	7 (5)		
PWV	1618.35±399.92	1654.15±439.71	1606.36±209.74	0.3808	0.1755
Residual-PWV 1 (total)	−1.80±292.99	19.03±314.85	73.96±293.58	0.4367	0.2333
Residual-PWV 1 (-drug)	−2.58±275.56	30.22±301.04	79.20±331.29	0.2324	0.0962
Residual-PWV 2 (total)	−0.74±258.27	8.57±264.11	9.08±240.24	0.8505	0.5693
Residual-PWV 2 (-drug)	−1.06±240.29	12.71±247.60	31.74±269.15	0.7135	0.422
	160 *ELN* (CGCTC(−/A)TAGCA)				
Phenotype	[−][−]	[−] A	AA	*P*	*P′*
N(-drug)	2701 (2032)	639 (482)	51 (35)		
PWV	1610.18±391.78	1652.19±434.97	1751.17±482.63	0.0038	0.0040
Residual-PWV 1 (total)	−8.81±292.50	28.28±329.39	76.99±359.85	0.0036	0.004
Residual-PWV 1 (-drug)	−10.34±273.14	39.46±314.40	36.85±327.86	0.0017	0.0004
Residual-PWV 2 (total)	−5.42±251.38	17.10±285.22	46.28±274.80	0.0608	0.0254
Residual-PWV 2 (-drug)	−5.24±270.35	20.40±270.35	15.11±261.28	0.0991	0.0318

Associations between the ELN polymorphisms and PWV (cm/sec) were investigated. Residuals of PWV1 were calculated by adjusting for age, waist:hip ratio, pulse rate, ethanol consumption per day, triglycerides, and fasting blood glucose. Residuals of PWV2 were calculated by adjusting for the above-mentioned confounding factors plus systolic blood pressure in the recumbent position at the time of PWV measurement. Numbers of subjects without antihypertensive medication are shown in parentheses.

position at $P<0.05$ (Table V). Moreover, 158 and 160 were associated with blood pressure values in the recumbent position at $P<0.05$ (Table VI).

Association Analysis With Intermediate Phenotypes

A functional polymorphism in *SLC9A2* may be expected to alter sodium homeostasis and the renin/aldosterone profile. *UMOD* has been shown to be responsible for familial juvenile hyperuricemic nephropathy,[16] and functional polymorphism of this gene is expected to be associated with plasma uric acid levels. Polymorphism 4 in *SLC9A2* was not associated with the renin/aldosterone profile (Table VII). Polymorphism 107 in *UMOD* was not associated with uric acid levels (Table VIII). Thus, we could not obtain additional support for the hypothesis that these polymorphisms are associated with blood pressure status.

Elastin is a major component of vascular tissues, and functional polymorphisms in *ELN* may influence the characteristics of vascular tissues.[21,22] Thus, we assessed the association of elastin polymorphisms with PWV, which is thought to reflect aortic stiffness. Multiple regression analysis indicated that PWV was influenced by various factors including age, waist:hip ratio, SBP, pulse rate, fasting blood glucose, ethanol consumption, and triglycerides. The *ELN* (−/A) polymorphism (No. 160) significantly influenced the residuals of PWV after adjusting for these confounding factors (Table 3). The adjustment by SBP seems to be an overadjustment, because high PWV (high stiffness) will lead to high SBP ($R^2=0.425$). Nonetheless, we show 2 adjustments of PWV in Table 3 (with and without SBP as a confounding factor). Although polymorphism 158 gave smaller *P* values when blood pressure values in the sitting position were used (Table V), polymorphism 160 gave smaller probability values when blood pressure values in the recumbent position were used (Table VI). Overall, polymorphism 160 seems to be more consistently associated with blood pressure values and PWV than polymorphism 158.

The significance of the *ELN* (−/A) polymorphism (160) was re-examined in study population II. The characteristics of this population are shown in Table 4. The *ELN* (−/A) polymorphism (160) was associated with hypertension, blood pressure values, and PWV with a classical criterion of $P<0.05$.

The odds ratio of [(−/A)+(A/A)] over (−/−) genotypes for hypertension was estimated to be 1.28 (95% CI: 1.08 to 1.52; $P=0.0040$ adjusted by age and BMI) in the total population consisting of study population II and the original

TABLE 4. Association Analysis in the Study Population II

Phenotype	[−][−]	[−] A+AA	*P*
N (N of -D)	237 (182)	50+2 (35+1)	
M, %	44.7	36.5	Ns
Age	62.8±12.1	64.3±12.2	Ns
BMI, kg/m^2	22.8±3.3	22.8±3.4	Ns
HTN, %	33.3	53.9	0.0062*
HTN-drug, %	23.2	30.8	Ns
SBP, mm Hg	121.0±17.9	132.1±20.0	<0.0001
DBP, mm Hg	74.1±10.5	79.2±11.6	0.0023
Res SBP, mm Hg	−1.8±17.0	8.5±17.4	0.0001
Res DBP, mm Hg	−0.9±10.2	4.0±10.7	0.0024
PWV, cm/s	1597.0±394.6 (n=187)	1833.6±565.8 (n=41+1)	0.0015
Res PWV, cm/s	−21.9±283.6	98.2±348.8	0.008
SBP(-D), mm Hg	118.1±17.5	126.8±20.4	0.008
DBP(-D), mm Hg	73.4±10.6	77.8±12.8	0.028
Res SBP(-D), mm Hg	−1.4±16.8	7.0±18.8	0.0078
Res DBP(-D), mm Hg	−0.7±10.4	3.7±11.9	0.0233
PWV(-D), cm/s	1503.3±337.8 (n=144)	1691.4±539.6 (n=27+1)	0.016
Res PWV(-D), cm/s	−15.0±242.5 (n=144)	79.0±332.1 (n=27+1)	0.0804

The characteristics of study population II are shown according to the genotypes of polymorphism 160 in *ELN*. [−][−], [−] A, and [AA] indicate homozygous genotype of deletion allele, heterozygous genotype, and homozygous genotype of A insertion allele, respectively. Ns indicates not significant; NT, normotensive subjects; HTN, hypertensive subjects. Data are expressed as mean±SD. Res indicates residuals, and residuals were calculated as in the "Methods" section. SBP(-D), DBP(-D), and PWV(-D) indicate the values of subjects without antihypertensive medication. Numbers of subjects without antihypertensive medication are shown in parentheses (N of -D).

**P* value was calculated by a logistic analysis that included age and BMI as covariates.

population (N=3920). The statistical power of this association was 0.87. The LD structure and the sites of polymorphisms of *ELN* are shown in Table IX.

Discussion

In the present study, we identified 361 polymorphisms in 10 candidate genes for hypertension by resequencing and performed association studies between 161 polymorphisms and hypertension in a relatively large cohort representing the general population in Japan. None of the polymorphisms significantly affected blood pressure levels when the Bonferroni correction was applied. However, one of the polymorphisms in the elastin gene (*ELN*) was nominally associated not only with blood pressure levels but also with PWV. The strong association with this intermediate phenotype seems to strengthen the hypothesis that *ELN* influences blood pressure levels in Japanese.

We used the presence or absence of hypertension as a primary trait, because the definition of hypertension is relatively straightforward, although there is some ambiguity in defining subjects with antihypertensive treatment as having hypertension. On the other hand, there are many ambiguities in the use of blood pressure values as a trait. Although a correction method has been advocated,[28] there is no consensus on how to handle the blood pressure values of subjects with antihypertensive treatment. In our study population, ≈60% of the hypertensive subjects had been treated. Any statistical results will be biased by including or excluding these subjects in the analysis. However, associations with not only hypertension but also with blood pressure values will strengthen the hypothesis that the polymorphism contributes to blood pressure regulation.

In the present study, we measured blood pressure twice under different conditions. First, we measured blood pressure levels in both arms and both legs automatically with a device to measure brachial-ankle PWV in the recumbent position. Blood pressure was then measured in the sitting position by physicians 1 or 2 hours after PWV measurement. Although polymorphism 158 was more strongly associated with blood pressure values in the sitting position, polymorphism 160 was more strongly associated with blood pressure values in the recumbent position. Both of these polymorphisms are in mild LD (R^2=0.22). A haplotype-based association study with these 2 polymorphisms did not increase the power of association. Thus, it seems difficult to conclude which polymorphism is fundamentally associated with blood pressure levels based only on blood pressure levels.

Elastin is one of the major components of vascular tissues, and derangement of this molecule is suspected to confer large vessel stiffness that will lead to elderly systolic hypertension.[22,23] Aortic stiffness has been reported to be reliably assessed by PWV.[11,12,29] If *ELN* polymorphisms were really functional, these polymorphisms would be expected to influence large vessel stiffness and, consequently, PWV. Polymorphism 160, but not 158, was associated with PWV. The polymorphisms that affect blood pressure levels should nat-

urally influence PWV under the hypothesis that elastin influences blood pressure by affecting the stiffness of the arterial wall. Thus, the present results suggest that the *ELN* 3′-untranslated region (−/A) polymorphism (160) is more likely to contribute to blood pressure regulation than intronic polymorphism 158. The additional association study in study population II strongly supports the notion that this polymorphism is associated with blood pressure levels through its influence on large vessel stiffness.

Elevated arterial stiffness is an important predictor of cardiovascular outcome in a variety of populations,[29–31] and reducing arterial stiffness seems to confer a prognostic benefit.[32] Intriguingly, angiotensin-converting enzyme inhibitors have been reported to have effects in addition to antihypertensive effects on arterial wall structure and to contribute to the reduction of arterial stiffness by increasing the ratio of elastin:collagen.[33] Moreover, some of the statins have been reported to reduce aortic stiffness without any effects on blood pressure.[34] Thus, such elastogenic therapies or preventions may be optimal for subjects with the *ELN* (A+) 3′-untranslated region allele.

In the present study, we did not obtain additional support for polymorphism 4 in *SLC9A2* and polymorphism 107 in *UMOD*. However, a lack of association with the renin/aldosterone profile or uric acid levels does not exclude the possibility that the polymorphism is associated with blood pressure levels. Validation of these associations should be performed in other study populations with adequate statistical power.

Strengths and Limitations of the Present Study

The strengths of the present study are: (1) a relatively large community-based sample, (2) assessment of various possible confounding factors, (3) resequencing of 10 candidate genes in ≥96 subjects to catalog polymorphisms in Japanese, and (4) characterization of several interesting intermediate phenotypes, including PWV. Although the sample size of ≈4000 subjects is not small, it may not be large enough to detect genes that contribute to blood pressure regulation through small effects. In the present study, we performed an association study in the total study population including both male and female subjects. However, the genetic basis of common diseases is not thought to be identical between male and female subjects. It would be appropriate to analyze male and female subjects separately. However, this would reduce the statistical power. Thus, in the present study, we focused on polymorphisms that were nominally associated with hypertension in the total study population. The significance of the polymorphisms listed in Table IV awaits further investigation.

The mean ages of the male and female subjects were 66±11 and 63±11 years, respectively. Because the phenotypes of elderly subjects are suspected to be influenced by unidentified diseases compared with those of younger subjects, younger subjects might be more appropriate for a genetic association analysis.

Because we only sequenced promoter and exon regions and not the vast majority of intronic and intergenic regions, it is possible that some other polymorphisms may be causal and may be more intimately associated with hypertension. Thus, negative results in the present study should not imply that candidate genes with negative results do not also contribute to hypertension.

Perspectives

As illustrated in the present study, the candidate gene approach is laborious and provides a limited yield. The candidate gene approach may not be efficient and effective for identifying disease-associated polymorphisms. Some methodologic innovations, such as a reliable and cost-effective resequencing microarray, may be required for future studies using the candidate gene approach.

On the other hand, the materials and tools necessary to conduct genome-wide association studies, which include single nucleotide polymorphism description, tag single nucleotide polymorphism selection, and several platforms for genotyping, have recently been established, and several successful examples have already been reported.[35] Thus, because of recent developments in genome-wide association studies, attempts to identify polymorphisms that contribute to common diseases seem to be entering a highly productive phase.

Acknowledgments

We express our gratitude to the participants of the study.

Sources of Funding

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

Disclosures

None.

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Hypertension. 2006;48:901-907; originally published online September 18, 2006;
doi: 10.1161/01.HYP.0000242485.23148.bb
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563