Common Variants in the ATP2B1 Gene Are Associated With Susceptibility to Hypertension

The Japanese Millennium Genome Project

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Abstract—Hypertension is one of the most common complex genetic disorders. We have described previously 38 single nucleotide polymorphisms (SNPs) with suggestive association with hypertension in Japanese individuals. In this study we extend our previous findings by analyzing a large sample of Japanese individuals (n=14 105) for the most associated SNPs. We also conducted replication analyses in Japanese of susceptibility loci for hypertension identified recently from genome-wide association studies of European ancestries. Association analysis revealed significant association of the *ATP2B1* rs2070759 polymorphism with hypertension ($P=5.3\times10^{-5}$; allelic odds ratio: 1.17 [95% CI: 1.09 to 1.26]). Additional SNPs in *ATP2B1* were subsequently genotyped, and the most significant association was with rs11105378 (odds ratio: 1.31 [95% CI: 1.21 to 1.42]; $P=4.1\times10^{-11}$). Association of rs11105378 with hypertension was cross-validated by replication analysis with the Global Blood Pressure Genetics consortium data set (odds ratio: 1.13 [95% CI: 1.05 to 1.21]; $P=5.9\times10^{-4}$). Mean adjusted systolic blood pressure was highly significantly associated with the same SNP in a meta-analysis with individuals of European descent ($P=1.4\times10^{-18}$). *ATP2B1* mRNA expression levels in umbilical artery smooth muscle cells were found to be significantly different among rs11105378 genotypes. Seven SNPs discovered in published genome-wide association studies were also genotyped in the Japanese population. In the combined analysis with replicated 3 genes, *FGF5* rs1458038, *CYP17A1*, rs1004467, and *CSK* rs1378942, odds ratio of the highest risk group was 2.27 (95% CI: 1.65 to 3.12; $P=4.6\times10^{-7}$) compared with the lower risk group. In summary, this study confirmed common genetic variation in *ATP2B1,* as well as *FGF5*, *CYP17A1*, and *CSK*, to be associated with blood pressure levels and risk of hypertension. **(*Hypertension*. 2010;56:973-980.)**

Key Words: hypertension ■ genetic variation ■ ATP2B1 ■ Millennium Genome Project ■ Global BPgen

Because of its large impact on a number of cardiovascular diseases, hypertension is a major contributor to global health burden. Because hypertension is one of the most prevalent complex genetic disorders, with a heritability of ≤60% based on the estimation by 24-hour blood pressure (BP) readings,[1] numerous studies, including recent genome-wide association studies (GWAS),[2–6] have attempted to identify genetic variation associated with human BP levels.

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Except for rare mendelian forms of hypertension,[7] the estimated effects of each genetic factor on BP levels have been found to be small in the general population (typically <1.0 mm Hg on systolic BP [SBP] and <0.5 mm Hg on diastolic BP [DBP] per risk allele). However, multiple risk alleles are known to have a cumulative impact on several complex traits, including BP and hypertension risk.[3] In addition, it is anticipated that identification of novel susceptibility genes would lead to further understanding of disease pathogenesis.

As a part of a series of nationally based cooperative projects, the Millennium Genome Project (Millennium GPJ), we conducted multiple candidate gene analyses to identify susceptible genes and polymorphisms for hypertension. In a previously reported study,[6] we focused on 307 genes, which were genes encoding components of signal transduction pathways potentially related to BP regulation, including receptors, soluble carrier proteins, binding proteins, channels, enzymes, and G proteins. That study identified 38 single nucleotide polymorphisms (SNPs) as suggestively associated with hypertension by analysis of 758 hypertensive patients and 726 normotensive controls.[6] To extend our previous study, we have now genotyped all 38 of the SNPs in a replication panel composed of 1929 hypertensives and 1993 normotensives and have taken forward validated SNPs with further genotyping in a large Japanese genetic epidemiological cohort sample (n=14 105). An in silico validation analysis of our most promising loci was performed using the Global Blood Pressure Genetics (Global BPgen) consortium data set, a large-scale GWAS of samples of European descent.[2] Furthermore, we also conducted a replication analysis of recent European GWAS-derived susceptible loci for hypertension from Global BPgen[2] and CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) GWAS[3] in a Japanese large-scale general population sample (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).

Methods

Case and Control Subjects (Screening Panel)

Details of the screening panel subjects have been described previously.[6] Briefly, hypertensive patients and normotensive controls were recruited in the Asahikawa, Tokyo, Osaka, and Hiroshima regions of Japan according to the following criteria. Hypertensive subjects (n=758) had a previous diagnosis of hypertension at between 30 and 59 years of age and were either being treated with antihypertensive medication or had a SBP >160 mm Hg and/or DBP >100 mm Hg. They had a family history of hypertension in their parents and/or siblings and were not obese (body mass index [BMI] <25 kg/m^2). Normotensive controls (n=726) aged >45 years were recruited from the same regions. These individuals have never been treated with antihypertensive medications, and their SBP was <120 mm Hg and DBP <80 mm Hg. They had no family history of hypertension. All of the subjects were unrelated and were native Japanese.

Cohort-Based Population Samples

Seven independent study cohorts for cardiovascular diseases and related risk factors were combined to compose a large-scale Japanese genetic epidemiological population sample of 14 105. The Ohasama, Shigaraki, Takashima, Suita, and Nomura studies are general population-based genetic epidemiological studies. The study subjects were recruited via a medical checkup process for community residents. The 2 other cohorts, Yokohama and Matsuyama, are derived from employees of large manufacturing industries. The clinical parameters used in this study were obtained from personal health records during annual medical checkups. Further details of the study cohorts are described in the online Data Supplement.

Nested Case and Control Subjects Derived From the Cohort-Based Sample (Replication Panel)

Hypertensive cases and normotensive controls were chosen from the cohort-based population samples described above (n=11 569; the Suita study was excluded because of ethical issues). The selection criteria of the hypertensive and normotensive subjects were as follows: hypertensive subjects (n=1929) aged ≤64 years and either treatment with antihypertensive medication and/or SBP >160 mm Hg and/or DBP >90 mm Hg; normotensive subjects (n=1993) aged ≥40 years and having SBP <120 mm Hg and DBP <80 mm Hg; and no current use of antihypertensive medication and free from any history of cardiovascular disease.

Global BPgen (In Silico) Analyses

To investigate cross-validation of the most promising SNPs, we obtained results for 4 SNPs in the *ATP2B1* gene from the Global BPgen consortium, a study that is composed of 17 GWAS studies with 34 433 individuals of European descent. A detailed description of the study design and phenotype measurement for all of the cohorts has been reported previously.[2]

Validation of Published BP Polymorphisms in the Japanese Millennium Cohort

Thirteen loci have been identified recently and robustly validated for association with BP and hypertension in recent large-scale GWAS of European samples, by the Global BPgen consortium[2] and the CHARGE consortium.[3] From the associated SNPs reported at these 13 loci, we selected SNPs expected to have minor allele frequencies in Japanese samples >0.10, based on the HapMap database (JPT only, Public Release No. 27)[8]: *FGF5* rs1458038, *CYP17A1* rs1004467, *CSK* rs1378942, *PLCD3* rs12946454, *PLEKHA7* rs381815, *ULK4* rs9815354, and *CSK-ULK3* rs6495122. These 7 SNPs were genotyped in the Japanese population-based cohort sample to test whether the same associations exist in samples of Japanese ancestry.

Genotyping

Genomic DNA was extracted from peripheral blood. All of the SNPs were analyzed by TaqMan probe assays (Applied Biosystems Co, Ltd) using commercially available primers and probes purchased from the Assay-on-Demand system. The fluorescence level of PCR products was measured using an ABI PRISM 7900HT sequence detector.

Ethical Considerations

All of the study procedures were approved by the ethics committee of each university or research institute. Written informed consent was obtained from all of the participating subjects.

Ex Vivo Expression Analysis of ATP2B1 mRNA

Umbilical artery smooth muscle cells were isolated from umbilical cords obtained at delivery (n=34). Expression levels of ATP2B1 mRNA were analyzed by RT-PCR using a relative quantification method. Further details of the ex vivo expression analysis are described in the online Data Supplement.

Statistical Analysis

At each SNP, frequency differences in each genotype among hypertensive and normotensive subjects were assessed using a χ^2 test. Linkage disequilibrium (LD) coefficients were calculated using the Haploview software (Broad Institute).[9] Adjusted odds ratios for hypertension, as well as coefficients and SEs for SBP and DBP, were calculated using logistic and linear multiple regression analysis,

Table 1. Association of ATP2B1 SNPs With Hypertension in the Screening and Replication Panels

			Screening Panel						Replication Panel						
SNP	Genotype		Genotype Frequency			HWE	Call Rate	Odds (*P*)	Genotype Frequency			HWE	Call Rate	Odds (*P*)	Overall Odds (*P*)
rs1401982	AA/AG/GG	HT	318	328	92	0.603	96.3	1.28 (0.001)	825	833	247	0.108	98.7	1.25 (3.0×10^{-6})	1.26 (1.5×10^{-8})
		NT	249	324	118	0.474			699	961	305	0.397			
rs2681472	AA/AG/GG	HT	335	321	90	0.334	97.8	1.26 (0.003)	846	832	242	0.095	99.5	1.26 (1.0×10^{-6})	1.26 (8.7×10^{-9})
		NT	267	328	111	0.539			715	966	303	0.431			
rs2070759	GG/GT/TT	HT	216	379	151	0.515	97.6	1.16 (0.045)	582	896	399	0.118	97.2	1.18 (4.4×10^{-4})	1.17 (5.3×10^{-5})
		NT	186	341	175	0.454			507	956	474	0.579			
rs11105364	TT/TG/GG	HT	335	322	88	0.432	97.2	1.29 (0.001)	846	834	236	0.171	99.3	1.25 (2.4×10^{-6})	1.26 (4.1×10^{-9})
		NT	261	323	113	0.438			729	947	303	0.874			
rs11105378	CC/CT/TT	HT	359	301	76	0.276	97.3	1.37 (6.3×10^{-5})	868	821	217	0.280	98.8	1.28 (1.4×10^{-7})	1.31 (4.1×10^{-11})
		NT	280	320	108	0.295			746	922	300	0.586			

The screening panel is composed of 758 middle age–onset severe hypertensive patients and 726 middle-aged to elderly evidently normotensive controls (Table S4). The replication panel consists of 1929 hypertensive cases, and 1993 normotensive controls selected from 11 569 cohort sample were enrolled (Table S2). ORs and *P* values for allelic model are shown.

adjusting for sex, age, age^2, BMI, and cohort variables, using additive (1 degree of freedom) and genotypic (2 degrees of freedom) genetic models. Adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15 mm Hg for SBP and +10 mm Hg for DBP).[10] The Global BPgen data and statistical methods have been described elsewhere.[2] Meta-analysis was performed assuming fixed effects and using inverse variance weights. An unweighted genetic risk score based on 4 SNPs (*ATP2B1* rs1105378, *FGF5* rs1458038, *CYP17A1* rs1004467, and *CSK* rs1378942) was calculated by adding the number of risk alleles showing higher BP values. Risk allele of each SNP was defined as follows: *ATP2B1*, C allele; *FGF5*, T allele; *CYP17A1*, A allele; and *CSK*, C allele. The *CSK-ULK3* SNP rs6495122 showing positive association with BP trait and hypertension was not included in the calculation of genetic risk score, because the strong LD with the *CSK* SNP rs1378942 ($D'=0.884$; $r^2=0.731$) is most parsimoniously explained by both SNPs tagging a single risk variant. Differences in mRNA expression levels among the *ATP2B1* rs1105378 genotype were assessed by ANOVA. The statistical analyses were performed using a commercially available statistical software package (JMP version 8, SAS Institute).

Results

Replication Genotyping

The clinical characteristics of the replication panel chosen from the cohort-based population samples (Table S1, available in the online Data Supplement) are shown in Table S2. Stringent case and control definitions, corresponding with the extreme upper ≈17% and lower ≈17% of the general population, were used to maximize power for fixed genotyping costs.[11] Thirty-six SNPs were successfully genotyped, and results for all of the SNPs are shown in Table S3. Significant association was observed for the *ATP2B1* rs2070759 polymorphism located in intron 8 ($P=4.4\times10^{-4}$; allele odds ratio [OR]: 1.18 [95% CI: 1.07 to 1.29]). Several other SNPs also showed marginally significant association; however, the *P* values did not reach statistical significance after application of Bonferroni correction for multiple comparisons (threshold: 0.05/36=0.0014; Table S3; we note that no other SNPs are significant if the less conservative Benjamini-Hochberg procedure is used to control the false discovery rate at 0.05). Although, the replication results in the less-strict nested case-control sample chosen from the same population sample have been reported in our previous article,[6] the association was recalculated to narrow down the SNPs to be applied to the following dense SNP analysis.

Dense SNP Analysis of the ATP2B1 Gene

To more precisely identify the SNP or SNPs increasing susceptibility for hypertension, we performed "de novo" genotyping of a dense SNP panel around marker rs2070759 in individuals from the original screening panel (Table S4).[6] Forty-one tag SNPs located in a 167-kb region around rs2070759 were selected using the HapMap database (Table S5).[8] Among the 27 SNPs polymorphic in our Japanese sample, the most significant association was observed with rs11105378; this yielded an allelic *P* value of 6.3×10^{-5} (OR: 1.37 [95% CI: 1.17 to 1.60]; Table 1 and Figure S2).

The most associated SNP and the 4 others from the dense SNP analyses were subsequently genotyped in the replication panel. Significant association of rs11105378 was confirmed in the replication panel with an allelic *P* value of 1.4×10^{-7} (OR: 1.28 [95% CI: 1.17 to 1.41]; Table 1). Meta-analysis of both study panels indicated significant association ($P=4.1\times10^{-11}$; OR: 1.31 [95% CI: 1.21 to 1.42]) and confirmed that the strongest association is seen for rs11105378. The D' and r^2 measures of LD between rs2070759 and rs11105378 were 0.92 and 0.48, respectively. Other SNPs, rs1401982 ($D'=0.99$; $r^2=0.64$), rs2681472 ($D'=0.99$; $r^2=0.61$), rs11105364 ($D'=0.97$; $r^2=0.59$), located within the same LD block, were also significantly associated with hypertension (Table 1). The strong LD between associated SNPs suggests a single true association signal in this region.

We examined for possible association of SNPs in the *ATP2B4* gene, a well-investigated isoform of the *ATP2B1* gene, with hypertension in the screening panel. We observed no significant correlation with the 17 SNPs analyzed, which were selected using the HapMap database (Table S6).

Population-Based Meta-Analyses of ATP2B1 SNPs

The complete Japanese population-based sample was subsequently genotyped for the 4 most significant SNPs in

Table 2. Meta-Analysis of ATP2B1 SNPs With BP Traits

		Millennium GPJ			Global BPgen			CHARGE*			Pooled	
SNP	Coded Allele	n (Frequency)	Coefficient (SE), mm Hg	*P*	n (Frequency)	Coefficient (SE), mm Hg	*P*	n (Frequency)	Coefficient (SE), mm Hg	*P*	Coefficient (95% CI), mm Hg	*P*
SBP												
rs1401982	G	13 944	−1.22	1.8×10^{-7}	33 885	−0.30	0.022				−0.52	3.9×10^{-6}
		(0.376)	(0.23)		(0.385)	(0.13)					(−0.74 to −0.30)	
rs2681472	G	14 032	−1.33	1.2×10^{-8}	33 803	−0.62	5.2×10^{-4}	0.17	−1.29	3.5×10^{-11}	−1.03	9.9×10^{-20}
		(0.373)	(0.23)		(0.158)	(0.18)			(0.19)		(−1.26 to −0.81)	
rs11105364	G	14 013	−1.34	8.9×10^{-9}	33 877	−0.60	7.4×10^{-4}	0.17	−1.30	4.8×10^{-11}	−1.03	1.2×10^{-19}
		(0.364)	(0.23)		(0.179)	(0.18)			(0.19)		(−1.25 to −0.81)	
rs11105378	T	13 948	−1.33	1.5×10^{-8}	33 171	−0.59	0.001	0.16	−1.31	9.1×10^{-11}	−1.02	1.4×10^{-18}
		(0.360)	(0.23)		(0.158)	(0.18)			(0.20)		(−1.24 to −0.79)	
DBP												
rs1401982	G	13 944	−0.72	2.0×10^{-7}	33 898	−0.18	0.041				−0.34	8.1×10^{-6}
		(0.376)	(0.14)		(0.392)	(0.09)					(−0.49 to −0.19)	
rs2681472	G	14 032	−0.65	2.7×10^{-6}	33 829	−0.35	0.003	0.17	−0.64	3.7×10^{-8}	−0.54	9.7×10^{-15}
		(0.373)	(0.14)		(0.157)	(0.12)			(0.11)		(−0.68 to −0.41)	
rs11105364	G	14 013	−0.70	4.5×10^{-7}	33 898	−0.34	0.004	0.17	−0.63	1.2×10^{-7}	−0.54	7.5×10^{-14}
		(0.364)	(0.14)		(0.158)	(0.12)			(0.12)		(−0.68 to −0.40)	
rs11105378	T	13 948	−0.70	5.4×10^{-7}	33 183	−0.33	0.005	0.16	−0.62	3.1×10^{-7}	−0.54	1.6×10^{-13}
		(0.360)	(0.14)		(0.158)	(0.12)			(0.12)		(−0.68 to −0.39)	

Coefficients and SE for SBP and DBP were calculated under the additive model using multiple regression analysis adjusted for age, age^2, sex, and BMI. In both Millennium GPJ and Global BPgen, adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15 mm Hg for SBP and +10 mm Hg for DBP).[2] In the Japanese Millennium GPJ and also for some cohorts within Global BPgen, cohort variables were also adjusted to avoid residual population stratification.

*Results of the CHARGE Study were obtained from the published article.[3]

ATP2B1. To further validate and get more precise effect size estimates in Japanese, for this analysis, hypertensive cases were defined as individuals with treatment with antihypertensive medication, SBP ≥140 mm Hg, or DBP ≥90 mm Hg. The ORs for the 4 SNPs were all extremely similar (ranging from 1.19 to 1.21 under the additive model adjusted for age, age^2, sex, BMI, and cohort variables; see Table S7). These associations were replicated in the Global BPgen subjects of European descent; the pooled analysis demonstrated increased significance (rs1105378: OR: 1.17 [95% CI: 1.11 to 1.23]; $P=7.0\times10^{-10}$), as expected for a larger total sample size (n=28 866; Table S7).

We next evaluated the effect of the most associated SNP, rs11105378, on BP levels in the Millennium GPJ cohort (Table 2). We adjusted for several covariates that are associated with BP phenotypes: age ($r=0.362$; $P<0.001$ for SBP), BMI ($r=0.275$; $P<0.001$), and sex (male: 131.7±18.2; female: 128.6±20.8 mm Hg; $P<0.001$). In multiple regression analysis for BP levels, including also cohort indicator variables as covariates, the results for a 2-degree-of-freedom test with the TT genotype as a reference identified both the TC genotype (coefficient=+1.66 mm Hg; $P=2.2\times10^{-4}$) and CC genotype (+2.47 mm Hg; $P=4.9\times10^{-8}$) as independent determinants for SBP after adjustment. The TC (+0.91 mm Hg; $P=8.0\times10^{-4}$) and CC genotypes (+1.32 mm Hg; $P=1.8\times10^{-6}$) were also independently associated with DBP levels. We depict the covariate adjusted mean BP levels by rs11105378 genotype in Figure S3. Results of each cohort separately are summarized in Table S8. We next performed a meta-analysis of data from the Millennium GPJ and 2 large epidemiological studies (Global BPgen and CHARGE; Table 2). Results show the per-allele differences in SBP and DBP to be ≈1.0 and 0.5 mm Hg, respectively.

Genotype-Specific Differences in Ex Vivo Expression of ATP2B1 mRNA

Differences in *ATP2B1* mRNA expression in umbilical artery smooth muscle cells among rs11105738 genotype are shown in Figure 1. Assuming a recessive genetic model, cells homozygous for T allele showed significantly higher levels of

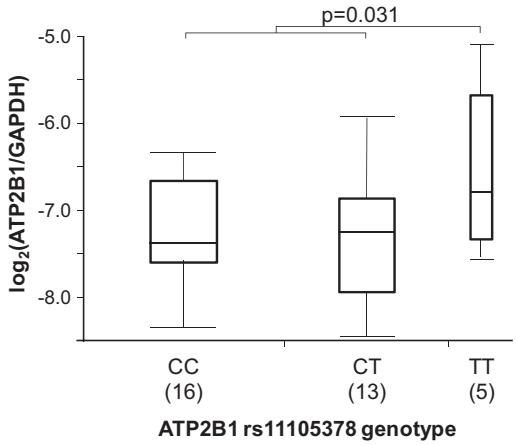

Figure 1. Ex vivo expression analysis of *ATP2B1* mRNA. Graphs depict the log^2 relative expression levels of the *ATP2B1* mRNA in umbilical artery smooth muscle cells obtained by normalizing to GAPDH. Genotype of *ATP2B1* rs11105378 of each sample was analyzed by direct sequencing using isolated genomic DNA from umbilical artery smooth muscle cells.

Table 3. Meta-Analysis of SNPs With BP Traits

SNP	Coded Allele	Millennium GPJ n (Frequency)	Millennium GPJ Coefficient (SE), mm Hg	Millennium GPJ *P*	Global BPgen n (Frequency)	Global BPgen Coefficient (SE), mm Hg	Global BPgen *P*	Pooled Coefficient (95% CI), mm Hg	Pooled *P*
Systolic BP									
FGF5	T	13 826	1.33	1.6×10^{-8}	30 850	0.62	1.6×10^{-6}	0.81	1.1×10^{-11}
rs1458038		(0.343)	(0.23)		(0.275)	(0.14)		(0.58 to 1.05)	
CYP17A1	A	14 007	0.89	2.3×10^{-4}	33 735	0.94	1.0×10^{-5}	0.92	6.2×10^{-9}
rs1004467		(0.680)	(0.24)		(0.901)	(0.21)		(0.61 to 1.23)	
CSK	C	13 920	0.77	0.007	34 126	0.62	2.4×10^{-6}	0.65	4.2×10^{-8}
rs1378942		(0.803)	(0.28)		(0.36)	(0.13)		(0.42 to 0.88)	
PLCD3	T	14 003	0.11	0.703	32 120	0.68	3.9×10^{-6}	0.57	2.5×10^{-5}
rs12946454		(0.831)	(0.30)		(0.28)	(0.15)		(0.30 to 0.83)	
PLEKHA7	T	14 030	0.11	0.687	33 706	0.52	2.6×10^{-4}	0.44	4.7×10^{-4}
rs381815		(0.199)	(0.28)		(0.26)	(0.14)		(0.19 to 0.68)	
CSK-ULK3	A	14 014	0.68	0.017	33 308	0.47	2.4×10^{-4}	0.51	1.7×10^{-5}
rs6495122		(0.812)	(0.28)		(0.45)	(0.13)		(0.28 to 0.74)	
ULK4	A	13 976	−0.67	0.059	32 034	0.17	0.297	0.01	0.950
rs9815354		(0.116)	(0.35)		(0.18)	(0.17)		(−0.29 to 0.31)	
DBP									
FGF5	T	13 826	0.73	1.8×10^{-7}	30 850	0.55	1.5×10^{-8}	0.61	6.1×10^{-14}
rs1458038		(0.343)	(0.14)		(0.275)	(0.10)		(0.45 to 0.77)	
CYP17A1	A	14 007	0.29	0.047	33 735	0.40	5.4×10^{-3}	0.35	4.9×10^{-4}
rs1004467		(0.680)	(0.14)		(0.901)	(0.14)		(0.15 to 0.54)	
CSK	C	13 920	0.41	0.015	34 126	0.48	5.9×10^{-8}	0.46	5.2×10^{-9}
rs1378942		(0.803)	(0.17)		(0.36)	(0.09)		(0.31 to 0.62)	
PLCD3	T	14 003	0.14	0.426	32 120	0.34	5.7×10^{-4}	0.30	1.9×10^{-4}
rs12946454		(0.831)	(0.18)		(0.28)	(0.09)		(0.14 to 0.46)	
PLEKHA7	T	14 030	0.13	0.437	33 706	0.23	0.014	0.20	0.018
rs381815		(0.199)	(0.17)		(0.26)	(0.10)		(0.04 to 0.37)	
CSK-ULK3	A	14 014	0.38	0.027	33 308	0.35	4.2×10^{-5}	0.36	7.4×10^{-6}
rs6495122		(0.812)	(0.17)		(0.45)	(0.09)		(0.20 to 0.51)	
ULK4	A	13 976	0.21	0.325	32 034	0.40	2.9×10^{-4}	0.36	2.3×10^{-4}
rs9815354		(0.116)	(0.21)		(0.18)	(0.11)		(0.17 to 0.55)	

ATP2B1 mRNA as compared with cells carrying 1 or 2 C alleles ($P=0.031$; see Figure 1). Under an additive genetic model, the overall *P* value was marginally significant ($P=0.091$).

Replication Analysis of European GWAS-Derived Susceptible SNPs in Japanese

We next conducted a replication analysis in the Millennium GPJ, in which we tested associated SNPs identified in recent large-scale European GWAS by the Global BPgen[2] and the CHARGE consortia.[3] From the 7 most promising SNPs of which the minor allele frequency in Japanese was >0.10 based on the HapMap database, 4 SNPs, namely, *FGF5* rs1458038, *CYP17A1* rs1004467, *CSK* rs1378942, and *CSK-ULK3* rs6495122, showed significant association in either binary trait analyses (Tables S9) or quantitative trait analysis (Table 3 and S10). The most significant association was observed with *FGF5* rs1458038; this yielded a *P* value of 1.6×10^{-8} (+1.33 mm Hg) with SBP and 1.8×10^{-7} (+0.73 mm Hg) with DBP in the Millennium GPJ cohort, and the effect size was greater than that of Europeans (Table 3). Meta-analysis of both study panels with data from Global BPgen indicated further significant associations.

Multiple Regression Analysis for BP Trait and Hypertension in Japanese

To clarify whether the 4 susceptibility SNPs (*ATP2B1*, *FGF5*, *CYP17A1,* and *CSK*) were independently associated with BP traits and hypertension, multiple regression analysis was performed with possible covariates (Table S11). After adjustment for age, age^2, sex, BMI, and drinking habits, this analysis confirmed that all 4 of the SNPs were independent determinants for both BP traits and hypertension.

Combined Effect of Risk Genotypes on Hypertension

A risk score for 4 susceptible genotypes was calculated to evaluate their combined effects on hypertension. ORs asso-

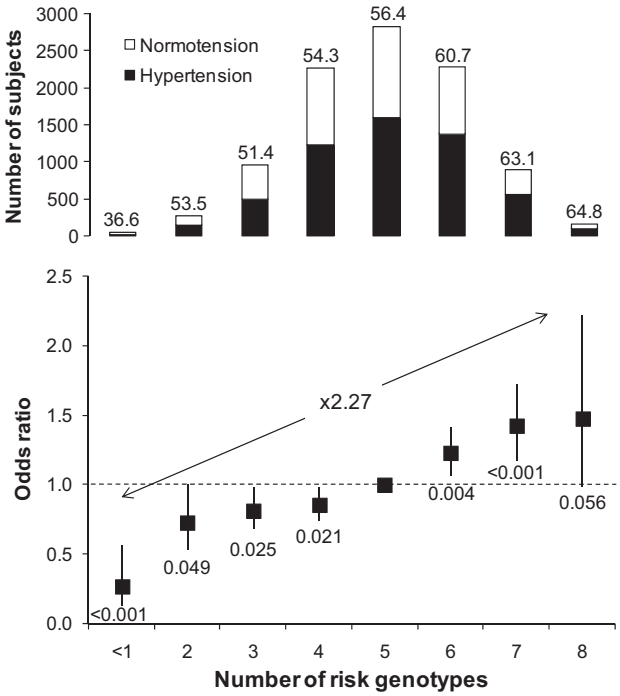

Figure 2. ORs for hypertension according to the number of risk genotypes Number of risk genotype was calculated by the following 4 SNPs: *ATP2B1* rs1105378, *FGF5* rs1458038, *CYP17A1*, rs1004467, and *CSK* rs1378942. Hypertensive subjects were defined as being treated with antihypertensive medication, SBP ≥140 mm Hg, or DBP ≥90 mm Hg; normotensive subjects were defined as all not treated with antihypertensive medication, SBP ≤120 mm Hg, and DBP ≤85 mm Hg.[2] Adjusted OR for hypertension and BP levels were calculated using logistic and linear multiple regression analysis, adjusting for sex, age, age^2, BMI, and cohort variables. Frequency of hypertension and *P* values for the hypertension odds are shown in the top of column and the bottom of square, respectively.

ciated with increasing number of risk genotypes in a covariates adjusted logistic regression model are depicted in Figure 2 (overall P value was 5.4×10^{-5}). Compared with the reference group (5 risk genotypes), individuals carrying 7 or 8 risk genotypes had higher risk (OR: 1.43 [95% CI: 1.20 to 1.72]; $P=1.0\times10^{-4}$) in contrast to the lower OR of individuals with ≤2 risk genotypes (OR: 0.63 [95% CI: 0.47 to 0.85]; $P=0.020$). The OR of the high-risk group was raised to 2.27 (95% CI: 1.65 to 3.12; $P=4.6\times10^{-7}$) compared with the lowest risk group. Adjusted per-allele OR for hypertension was 1.17 (95% CI: 1.12 to 1.21; $P=4.0\times10^{-15}$). The distribution of the Japanese population sample among the number of risk genotypes is shown in Figure S4.

Discussion

The present study has identified SNPs located upstream or within the *ATP2B1* gene as strong susceptibility polymorphisms for hypertension in Japanese. These are findings that have also been reported recently in individuals of European descent[3] and in Koreans.[4] Although numerous studies have attempted to identify genetic markers for hypertension over the past 2 decades, there has been little cross-validation of loci in different ethnic groups so far except for mendelian forms of hypertension. The SNPs in *ATP2B1* identified in this study showed significant association in large-scale studies in populations with different ancestries and using different discovery approaches, including GWAS in the CHARGE consortium and the Korean study and an independent candidate gene analysis in our present study. Similar findings in different ethnic groups with different methods further strengthen these findings and indicate the *ATP2B1* gene region as a susceptibility locus of likely global significance for BP variation and development of hypertension. Two replication results very recently reported by another Japanese group[12] and a Korean group[13] also indicated the disease susceptibility of *ATP2B1* SNPs located in the same LD block.

No biological data have been provided whether SNP rs1105378 or other SNPs in strong LD have any effect on the transcriptional activity or transcriptional regulation of the *ATP2B1* gene. Furthermore, although alternative splicing has been found to generate several variants of *ATP2B1* mRNA,[14] the SNP associations that we have observed do not shed light on whether this is a potential mechanism for affecting BP. Our data first showed that the effect of SNPs on ATP2B1 gene expression levels is a potential mechanism by which disease-associated SNP alleles cause the phenotypic changes. Changes in the *ATP2B1* gene product levels are involved in BP regulation. We found no microRNA harboring rs11105378 in the miRBase database.[15]

The *ATP2B1* (so-called *PMAC1*) gene encodes the plasma membrane calcium ATPase isoform 1, which removes bivalent calcium ions from eukaryotic cells against very large concentration gradients and plays a critical role in intracellular calcium homeostasis. Although pathophysiological implications of *ATP2B1* gene products on the development of hypertension are uncertain, it has been reported that inhibition of *ATP2B1* by the selective inhibitor caloxin 2A1 showed endothelium-dependent relaxation of rat aorta by increasing cytosolic Ca^{2+} concentration and consequent activation of endothelial NO synthase.[16] Other information on the role of *ATP2B1* has been obtained from experiments using bladder smooth muscle cells: contractility measurements on these cells have documented the important role of *ATP2B1* in the extrusion of Ca^{2+} after carbachol stimulation or depolarization with potassium chloride.[17] These reports suggest altered vascular reactivity as a plausible explanation for disease susceptibility of *ATP2B1* gene.

In mammals, calcium ATPase isoforms are encoded by ≥4 separate genes (*ATP2B1* to *ATP2B4*).[18] It has been reported that overexpression of the human *ATP2B4* gene in arterial smooth muscle cells in mice increases vascular reactivity and BP partly because of negative regulation of neuronal NO synthase.[19] We, therefore, examined the possible association of *ATP2B4* gene polymorphisms with hypertension by using the screening panel. However, no significant correlation was observed in the 17 SNPs analyzed, which were selected by reference to the HapMap database. The pathophysiological association of plasma membrane Ca^{2+} pump with BP regulation may be isoform specific.

Numerous studies, including the recent GWAS,[3–6] have attempted to identify genetic variations associated with human BP levels. At present, it is not clear to what extent findings from GWAS in one population can be extrapolated

to other populations with different lifestyles and genetic background. However, the present study provides a cross-validation of 4 of 7 SNPs (most likely representing 3 of 6 independent signals) derived from European GWAS. Replication studies in other Japanese[12] and Korean[13] populations also reported the cross-validation of European GWAS-derived SNP. Conservation of susceptible loci for hypertension was independent of ethnic background. This finding suggests an existence of unidentified common etiology of essential hypertension in relation to the susceptible genes and their physiological pathways.

Although individual common genetic variants confer a modest risk of hypertension, their combination showed a large impact on hypertension. The genetic risk score was associated with ≤2.27-times greater odds for hypertension. Similar observations have been found in other common diseases and multifactorial phenotypes, including, for example, type 2 diabetes mellitus,[20] serum lipid levels,[21] and serum uric acid levels.[22] We reported previously that the findings of the cross-sectional analysis revealed a similar association in the longitudinal analysis[23]; the fat mass and obesity-associated gene polymorphism was an independent risk factor for the future development of obesity after adjustment for possible confounding factors. The present cross-sectional study cannot address the question of whether the *ATP2B1* polymorphism and other susceptible variants predict future development of hypertension. However, recent articles investigating a prognostic significance of susceptible variants for type 2 diabetes mellitus[24] and cardiovascular disease[25] showed poor predictive performance of common variants in spite of the high OR observed in subjects carrying multiple risk alleles. A small proportion of the genetically high-risk persons attributed to independent inheritance of risk alleles may make it difficult to discriminate intermediate-risk persons. Genetic information may be most useful to identify a high-risk individual's need for early intervention.

Several definitions of hypertension were used in this study to explore susceptible SNPs with modest effects and to further validate the susceptibility. Since it was expected to be underpowered to detect the effects of common variants in a dichotomized analysis with slightly elevated BP, subjects with high normal BP were excluded from the 65 347 case-control analyses. All of the alleles associated with hypertension in a dichotomized analysis (Table S7) were also associated with BP levels (Table 2). Our methodology may, thus, be appropriate to identify susceptible variants for hypertension.

Perspectives

We have identified SNPs located in the *ATP2B1* gene region as susceptibility loci for hypertension in Japanese using a multistage association study, an association that has now been confirmed across different ethnic groups. Differences in the ex vivo *ATP2B1* mRNA expression levels further supported the disease susceptibility of SNP rs1110578. We also replicated the susceptibility of the European GWAS-derived SNPs in Japanese. Because hypertension is a trait that is preventable by dietary and exercise interventions, early detection of at-risk populations using genetic information may be useful in preventing future hypertension-related diseases.

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Disclosures

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Common Variants in the ATP2B1 Gene Are Associated With Susceptibility to Hypertension: The Japanese Millennium Genome Project

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ONLINE SUPPLEMENT

Common variants in the ATP2B1 gene are associated with susceptibility to hypertension
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SUPPLEMENTAL METHODS

ex vivo expression analysis of ATP2B1 mRNA

We obtained 34 umbilical cords at delivery (Kosei General Hospital). Umbilical arteries were excised from the cords and cut into small pieces. Umbilical artery smooth muscle cells (UASMCs) were separated using Hanks buffer containing 2 mg/ml collagenase and cultured in HuMedia-SG (Kurabo, Osaka, Japan) supplemented with epithelial growth factor (0.5 ng/ml), basic fibroblast growth factor (2 ng/ml), insulin (5 μg/ml), antibiotics and 5% fetal bovine serum. Total RNAs was extracted from UASMCs during early passages using TRIzol reagent according to manufacturer's instructions (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from 500 ng of the total RNA using a PrimeScript 1st strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan), and then diluted five times for subsequent real-time PCR (RT-PCR). RT-PCR was performed using TaqMan Gene Expression Assays on a 7900HT Sequence Detection System (Applied Biosystems). A relative quantification method [1] was used to measure the amounts of ATP2B1 (TaqMan assay ID, Hs00155949_m1) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1) as an internal control. Genotype of ATP2B1 rs11105378 of each sample was analyzed by direct sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit on a 3730xl GeneticAnalyzer, Applied Biosystems) using isolated genomic DNA from UASMCs (QIAamp DNA Mini Kit, QIAGEN GmbH, Hilden, Germany). The direct sequencing was performed with the following primers; forward 5'-TTCATAGCCCTTTTCATCTCTTTC-3', reverse 5'-AGAATCTCGGGAAAACAGCA-3'.

Table S1 Clinical characteristics of the cohort-based population sample

Parameters	Total (14,105)	Community-based general population					Company employee	
		Ohasama (1,592)	Shigaraki (2,273)	Takashima (1,730)	Suita (2,536)	Nomura (2,876)	Yokohama (2,290)	Matsuyama (808)
Age (years)	57.8±14.0	57.5±11.2	57.2±15.5	59.7±14.1	65.6±10.9	61.1±14.0	45.7±10.2	54.2±5.8
Sex (male/female)	6931/7174	601/991	862/1411	633/1097	1160/1376	1247/1629	1659/631	769/39
Body mass index (kg/m^2)	23.0±3.1	23.7±3.2	22.6±3.1	22.9±3.0	22.9±3.1	23.4±3.2	22.4±3.1	23.4±2.9
History of CVD	7.1	11.9	12.1	4.0	7.5	8.1	0.4	4.3
Systolic BP (mmHg)	130.1±19.6	131.7±14.2	130.1±19.5	130.6±21.3	124.5±18.9	137.7±22.1	123.8±14.9	134.3±19.1
Diastolic BP (mmHg)	77.9±11.5	74.4±9.4	76.7±11.7	76.8±12.0	75.6±10.5	81.0±11.8	78.3±10.3	85.1±12.2
Hypertension (%)	40.7	43.2	44.4	39.5	38.2	53.3	22.9	46.2
Antihypertensive treatment (%)	20.5	26.5	23.5	16.4	26.4	25.7	6.5	12.4

Values are mean±SD. Cardiovascular disease (CVD); stroke, myocardial infarction, and angina pectoris. Hypertension; any or all of systolic blood pressure more than 140 mmHg, diastolic blood pressure more than 90 mmHg, and current use of antihypertensive agents. The Ohasama study conducted by Tohoku University is a population-based longitudinal epidemiological study focusing on the clinical implications of home BP measurement [2]. Ohasama Town is a rural community located in the northern part of Japan (Iwate Prefecture). Subjects were recruited through a community-based annual medical check-up process. The Shigaraki [3] and Takashima [4]| studies of Shiga University of Medical Science are general population-based longitudinal studies. Both towns are located in central Japan (Shiga Prefecture). Subjects were recruited through a community-based annual medical check-up process. The Suita study conducted by the National Cardiovascular Center is based on the residents of Suita city, an urban city located in the second largest area Osaka, Japan [5]. Subjects were recruited through a biennial medical check-up process of the National Cardiovascular Center. The Nomura study of Ehime University is a longitudinal epidemiological study based on the Nomura Town residents, a largely rural community located in Ehime Prefecture [6]. Subjects were recruited through a community-based annual medical check-up process. The Yokohama (Yokohama City University) and Matsuyama (Ehime University) cohorts are derived from employees of large manufacturing industries located in Kanagawa and Matuyama City, Ehime Prefecture (western part of Japan) [7] respectively. In all cohorts, clinical parameters were obtained from personal health records during the annual or biennial medical check-up process. All study procedures were approved by the ethics committee of each University or Institution. Singed informed consent was obtained from all participating subjects.

Table S2 Clinical characteristics of the replication panel

Parameters	Hypertensive cases (1,929)	Normotensive controls (1,993)	p
Age (years)	55.1±7.1	55.2±9.5	0.680
Sex (male/female)	1,200/729	829/1,164	<0.001
Body mass index (kg/m^2)	24.4±3.1	21.9±2.7	<0.001
History of CVD (%)	5.4	0	<0.001
Systolic blood pressure (mmHg)	146.3±15.9	109.5±7.5	<0.001
Diastolic blood pressure (mmHg)	91.0±10.1	67.7±6.5	<0.001
Antihypertensive treatment (%)	47.5	0	<0.001

Values are mean±SD. Nested hypertensive cases and normotensive control subjects were chosen from the cohort-based population sample according to the following criteria: hypertensive subjects aged 64 years or younger, and were either being treated with antihypertensive medication or had a SBP more than 160 mmHg and/or DBP more than 90 mmHg; normotensive subjects aged 40 years or older, and all of SBP less than 120 mmHg, and DBP less than 80 mmHg, no current use of antihypertensive medication, and free from any history of cardiovascular disease. Cardiovascular disease (CVD) includes stroke, myocardial infarction, and angina pectoris.

Table S3 Association of 36 candidate SNPs with hypertension (replication panel)

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ACCN1	rs28933	AA/GA/GG	HT	464	974	449	0.159	97.6	1.03 (0.479)	1.07 (0.385)	1.02 (0.766)	(0.686)
			NT	469	986	485	0.466					
ADORA1	rs3766554	AA/GA/GG	HT	424	923	557	0.262	98.6	1.03 (0.548)	1.00 (0.977)	1.09 (0.289)	(0.523)
			NT	410	981	574	0.808					
ATP10A	rs3736186	GG/AG/AA	HT	791	868	263	0.312	99.4	**1.10 (0.040)**	1.04 (0.666)	**1.18 (0.010)**	**(0.033)**
			NT	734	963	280	0.206					
ATP10D	rs1058793	AA/GA/GG	HT	675	894	325	0.326	98.2	1.07 (0.147)	1.17 (0.060)	1.04 (0.555)	(0.169)
			NT	680	896	382	0.005					
ATP2A3	rs887387	TT/TC/CC	HT	936	775	189	0.126	98.7	1.05 (0.342)	1.02 (0.840)	1.07 (0.263)	(0.527)
			NT	936	836	200	0.508					
ATP2B1	rs2070759	GG/GT/TT	HT	582	896	399	0.118	97.2	**1.18 ($4.0*10^{-4}$)**	**1.2 (0.018)**	**1.27 (0.001)**	**(0.002)**
			NT	507	956	474	0.579					
CACNA1E	rs2293990	AA/TA/TT	HT	568	911	412	0.194	98.2	1.03 (0.532)	1.07 (0.372)	1.01 (0.881)	(0.661)
			NT	585	926	451	0.022					
CACNA2D2	rs2236957	GG/GA/AA	HT	459	925	496	0.499	97.3	1.00 (0.948)	1.00 (0.972)	1.01 (0.943)	(0.997)
			NT	471	954	512	0.523					
CAST	rs967591	AA/AG/GG	HT	442	916	552	0.100	99.1	1.00 (0.932)	0.98 (0.725)	1.02 (0.814)	(0.875)
			NT	451	964	561	0.345					
CHGA	rs3759717	CC/TC/TT	HT	744	877	288	0.263	99.1	1.00 (0.977)	0.93 (0.434)	1.04 (0.598)	(0.522)
			NT	755	943	281	0.624					
COL4A1	rs2305080	GG/GA/AA	HT	485	908	523	0.023	99.2	1.02 (0.723)	0.97 (0.707)	1.07 (0.332)	(0.468)
			NT	473	972	528	0.536					

Table S3 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
DLGAP2	rs2301963	CC/CA/AA	HT	510	904	493	0.024	98.6	1.05 (0.239)	1.07 (0.368)	1.08 (0.321)	(0.516)
			NT	497	932	532	0.029					
ERCC1	rs2298881	CC/CA/AA	HT	595	899	387	0.161	97.5	1.00 (0.948)	0.96 (0.642)	1.04 (0.616)	(0.702)
			NT	600	955	388	0.821					
EXOSC3	rs7158	AA/AG/GG	HT	511	967	418	0.327	97.9	1.01 (0.850)	1.09 (0.264)	0.95 (0.452)	(0.262)
			NT	545	941	458	0.187					
FGF2	rs3747676	GG/GA/AA	HT	415	937	519	0.839	96.4	1.01 (0.892)	1.07 (0.340)	0.94 (0.424)	(0.309)
			NT	444	908	556	0.050					
GIPC1	rs3815715	GG/GA/AA	HT	734	863	309	0.040	98.8	1.03 (0.585)	0.98 (0.794)	1.07 (0.330)	(0.510)
			NT	728	927	313	0.532					
GNA14	rs1801258	TT/TC/CC	HT	317	919	675	0.888	99.0	1.05 (0.321)	1.11 (0.128)	0.90 (0.903)	(0.249)
			NT	330	899	743	0.039					
GNAI2	rs2236943	GG/GA/AA	HT	556	912	429	0.137	97.9	1.04 (0.427)	1.02 (0.751)	1.07 (0.345)	(0.640)
			NT	543	953	448	0.448					
GUCA1C	rs2715709	AA/GA/GG	HT	225	886	767	0.204	97.1	1.06 (0.242)	1.12 (0.081)	0.98 (0.824)	(0.156)
			NT	236	853	843	0.373					
HCN4	rs3743496	GG/TG/TT	HT	431	877	594	0.002	98.2	1.01 (0.859)	0.94 (0.369)	1.11 (0.192)	(0.150)
			NT	408	959	583	0.710					
HLA-DMB	rs2071556	CC/CA/AA	HT	511	932	450	0.534	98.0	1.09 (0.060)	**1.17 (0.035)**	1.07 (0.346)	(0.105)
			NT	500	928	521	0.036					
KCNIP2	rs755381	TT/TC/CC	HT	453	904	543	0.044	98.2	1.05 (0.245)	1.03 (0.688)	1.12 (0.128)	(0.311)
			NT	425	957	569	0.548					

Table S3 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
KCNMB4	rs710652	CC/AC/AA	HT	660	953	298	0.131	99.2	1.09 (0.056)	**1.28 (0.003)**	1.03 (0.638)	(**0.012)**
			NT	669	930	379	0.083					
KCNN1	rs2278993	TT/TC/CC	HT	189	805	919	0.513	99.2	1.07 (0.152)	1.08 (0.259)	1.15 (0.207)	(0.335)
			NT	172	819	985	0.924					
PPP1R1B	rs3764352	TT/TC/CC	HT	547	940	374	0.412	96.0	1.07 (0.156)	1.16 (0.059)	1.04 (0.621)	(0.165)
			NT	546	928	431	0.333					
PTHR1	rs1138518	TT/TC/CC	HT	381	931	595	0.626	98.5	1.01 (0.814)	1.04 (0.599)	0.98 (0.843)	(0.803)
			NT	396	935	626	0.169					
PTPRT	rs3746539	AA/AG/GG	HT	495	991	430	0.119	99.1	1.04 (0.435)	1.12 (0.139)	0.99 (0.863)	(0.262)
			NT	514	975	482	0.644					
RAC2	rs929023	TT/TC/CC	HT	387	921	588	0.448	98.2	1.06 (0.200)	1.06 (0.438)	1.12 (0.173)	(0.373)
			NT	365	961	629	0.951					
RGS2	rs3767489	AA/GA/GG	HT	635	892	370	0.075	98.0	1.03 (0.483)	0.94 (0.476)	1.12 (0.099)	(0.104)
			NT	603	981	362	0.291					
RGS20	rs3816772	CC/CG/GG	HT	268	924	695	0.162	97.6	1.03 (0.543)	1.11 (0.132)	0.92 (0.377)	(0.112)
			NT	295	884	760	0.152					
SLC13A1	rs2140516	GG/GA/AA	HT	341	917	662	0.448	99.4	1.06 (0.225)	1.11 (0.135)	1.03 (0.736)	(0.322)
			NT	343	907	727	0.039					
SLC22A7	rs2270860	AA/GA/GG	HT	233	868	788	0.800	97.8	**1.1 (0.048)**	**1.15 (0.032)**	1.09 (0.406)	(0.100)
			NT	223	844	878	0.352					
SLC26A8	rs2295852	TT/TC/CC	HT	994	747	154	0.413	97.6	1.01 (0.835)	0.97 (0.806)	1.03 (0.690)	(0.857)
			NT	1002	779	153	0.926					

Table S3 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
SLC2A11	rs2236620	AA/AG/GG	HT	308	890	715	0.266	99.0	1.04 (0.360)	1.00 (0.956)	1.16 (0.092)	(0.211)
			NT	279	953	738	0.306					
SLCO1B1	rs2291075	GG/GA/AA	HT	719	868	319	0.039	98.7	1.01 (0.866)	0.95 (0.524)	1.05 (0.466)	(0.493)
			NT	719	932	314	0.680					
WNK1	rs2255390	GG/GA/AA	HT	490	925	475	0.359	97.4	1.07 (0.139)	1.09 (0.262)	1.10 (0.201)	(0.339)
			NT	466	949	516	0.470					

The replication panel consists of 1,929 hypertensive cases and 1,993 normotensives controls selected from a 11,569 cohort sample (Table S2).

Table S4 Clinical characteristics of the screening panel

Parameters	Hypertensive cases (758)	Normotensive controls (726)
Male (n (%))	564 (74.4)	550 (75.8)
Age (years)	59.0±11.0	62.8±9.4
Body mass index (kg/m^2)	23.6±3.0	22.7±2.9
Systolic BP (mmHg)	163.5±24.6	115.9±12.0
Diastolic BP (mmHg)	100.3±15.7	72.0±7.6
Antihypertensive medication (n (%))	499 (65.8)	-

Values are mean±standard deviation. Hypertensive cases: non-obese hypertensive patients, who had a previous diagnosis of hypertension at between 30 and 59 years of age, were either being treated with antihypertensive medication or had a SBP more than 160 mmHg and/or DBP more than 100 mmHg, had a family history of hypertension in their parents and/or siblings. Normotensive controls: middle-aged to elderly subjects (aged more than 45 years), who had never been treated with antihypertensive medications, had a SBP less than 120 mmHg and DBP less than 80 mmHg, and had no family history of hypertension.

Table S5 Dense SNP analysis of the *ATP2B1* gene (screening panel)

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B1	rs3920010 (88464519)	GG/GA/AA	HT	17	191	542	0.971	97.9	0.95 (0.591)	0.72 (0.311)	0.97 (0.808)	(0.596)
			NT	22	177	504	0.187					
	rs3900133 (88512561)	CC/CA/AA	HT					NF				
			NT									
	rs1401982 (88513730)	AA/AG/GG	HT	318	328	92	0.603	96.3	1.28 (0.001)	1.34 (0.007)	1.45 (0.014)	(0.006)
			NT	249	324	118	0.474					
	rs988111 (88515650)	TT/TC/CC	HT					NF				
			NT									
	rs10858912 (88515998)	GG/GA/AA	HT					NF				
			NT									
	rs4516026 (88518251)	TT/TG/GG	HT					NF				
			NT									
	rs2854371 (88519597)	GG/GA/AA	HT	23	208	520	0.692	98.7	1.32 (0.008)	1.38 (0.333)	1.37 (0.008)	(0.028)
			NT	16	159	538	0.300					
	rs1520184 (88520698)	GG/GA/AA	HT					NF				
			NT									
	rs1356819 (88524892)	AA/AC/CC	HT	743	5	0	0.927	98.6	1.26 (0.707)	1.26 (0.706)		
			NT	709	6	0	0.910					
	rs957525 (88524946)	TT/TC/CC	HT	414	264	62	0.034	97.6	1.05 (0.554)	1.11 (0.303)	0.90 (0.599)	(0.389)
			NT	377	277	54	0.753					
	rs17017109 (88528238)	TT/TG/GG	HT	591	144	7	0.586	97.8	0.81 (0.094)	0.79 (0.079)	0.89 (0.842)	(0.211)
			NT	591	113	6	0.816					

Table S5 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B1	rs1520183 (88532742)	CC/CT/TT	HT					NF				
			NT									
	rs2681472 (88533090)	GG/GA/AA	HT	90	321	335	0.334	97.8	0.79 (0.003)	0.74 (0.044)	0.75 (0.006)	(0.012)
			NT	111	328	267	0.539					
	rs11614886 (88535251)	GG/GC/CC	HT					NF				
			NT									
	rs2070759 (88541867)	GG/GT/TT	HT	216	379	151	0.515	97.6	1.15 (0.054)	1.13 (0.297)	1.31 (0.033)	(0.096)
			NT	186	341	175	0.454					
	rs2070758 (88545352)	AA/AC/CC	HT	638	103	10	0.016	98.4	1.23 (0.113)	1.32 (0.050)	0.63 (0.377)	(0.056)
			NT	575	128	6	0.701					
	rs1050395 (88553032)	TT/TC/CC	HT	730	17	0	0.753	97.9	1.38 (0.327)	1.32 (0.406)		(0.468)
			NT	685	20	1	0.042					
	rs1050396 (88553110)	CC/CA/AA	HT					NF				
			NT									
	rs2056327 (88562685)	CC/CT/TT	HT					NF				
			NT									
	rs939329 (88564015)	AA/AG/GG	HT	196	382	168	0.485	97.4	1.08 (0.313)	1.04 (0.726)	1.18 (0.190)	(0.422)
			NT	178	343	178	0.623					
	rs7975689 (88571125)	AA/AG/GG	HT					NF				
			NT									
	rs7138016 (88572551)	TT/TA/AA	HT					NF				
			NT									

Table S5 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B1	rs12579302 (88574634)	GG/GA/AA	HT	105	310	333	0.018	98.9	0.80 (0.004)	0.76 (0.058)	0.76 (0.011)	(0.023)
			NT	127	319	273	0.046					
	rs11105359 (88575212)	TT/TG/GG	HT					NF				
			NT									
	rs11105360 (88575303)	TT/TC/CC	HT					NF				
			NT									
	rs11105361 (88576810)	CC/CA/AA	HT					NF				
			NT									
	rs7131965 (88590466)	TT/TC/CC	HT	731	15	0	0.025	98.7	0.90 (0.778)	0.83 (0.627)		(0.468)
			NT	707	11	1	0.990					
	rs11105364 (88593407)	TT/TG/GG	HT	335	322	88	0.276	97.2	1.29 (0.001)	1.36 (0.004)	1.44 (0.016)	(0.005)
			NT	261	323	113	0.295					
	rs11105368 (88598572)	GG/GC/CC	HT	349	284	89	0.883	94.0	1.25 (0.005)	1.21 (0.082)	1.53 (0.005)	(0.015)
			NT	294	260	119	0.212					
	rs7136259 (88605319)	TT/TC/CC	HT	323	325	87	0.348	97.2	1.24 (0.006)	1.22 (0.063)	1.50 (0.007)	(0.016)
			NT	277	312	119	0.389					
	rs17836871 (88606297)	TT/TC/CC	HT	419	260	61	0.025	97.8	1.08 (0.368)	1.16 (0.153)	0.90 (0.577)	(0.202)
			NT	376	282	53	0.990					
	rs11105378 (88614872)	TT/TC/CC	HT	76	301	359	0.276	97.3	0.73 ($6.3*10^{-5}$)	0.64 (0.005)	0.69 ($4.2*10^{-4}$)	($4.6*10^{-4}$)
			NT	108	320	280	0.295					
	rs12230074 (88614998)	GG/GA/AA	HT	83	328	332	0.883	97.6	0.82 (0.013)	0.70 (0.021)	0.82 (0.068)	(0.036)
			NT	108	316	282	0.212					

Table S5 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B1	rs11105379 (88619304)	TT/TC/CC	HT	450	240	39	0.348	96.3	1.11 (0.261)	1.12 (0.292)	1.16 (0.520)	(0.542)
			NT	413	244	43	0.389					
	rs10858918 (88620476)	TT/TC/CC	HT	40	266	442	0.998	98.6	0.90 (0.212)	0.82 (0.378)	0.89 (0.267)	(0.456)
			NT	46	267	402	0.852					
	rs2113894 (88623528)	AA/AT/TT	HT	459	232	43	0.063	96.3	1.12 (0.200)	1.14 (0.228)	1.17 (0.482)	(0.458)
			NT	413	235	47	0.090					
	rs1358350 (88626023)	TT/TA/AA	HT	49	202	445	<0.001	91.8	0.85 (0.085)	0.82 (0.345)	0.84 (0.113)	(0.263)
			NT	56	212	398	<0.001					
	rs12369944 (88626925)	CC/CA/AA	HT	617	97	15	<0.001	94.5	1.27 (0.066)	1.33 (0.043)	1.01 (0.976)	(0.104)
			NT	542	117	14	0.013					
	rs2280715 (88627833)	CC/CG/GG	HT	463	223	54	<0.001	97.0	1.14 (0.137)	1.16 (0.166)	1.17 (0.425)	(0.364)
			NT	413	228	59	0.001					
	rs11105381 (88630966)	GG/GA/AA	HT	452	259	37	0.990	98.2	1.09 (0.334)	1.09 (0.398)	1.18 (0.479)	(0.621)
			NT	413	255	41	0.843					
	rs1590008 (88631856)	TT/TC/CC	HT	438	265	42	0.818	98.2	1.11 (0.243)	1.12 (0.288)	1.18 (0.443)	(0.508)
			NT	399	266	47	0.767					

The screening panel is comprised of 758 middle age-onset severe hypertensive patients and 726 middle-aged to elderly evidently normotensive controls (Table S4). NF; no genotype frequency

Table S6 Association of 17 ATP2B4 SNPs with hypertension (screening panel)

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B4	rs4245719	GG/GA/AA	HT	287	343	117	0.389	98.5	0.90 (0.153)	0.90 (0.307)	0.82 (0.175)	(0.332)
			NT	293	327	94	0.854					
	rs4600103	GG/GA/AA	HT	286	312	129	0.007	94.3	1.03 (0.678)	1.08 (0.466)	0.98 (0.860)	(0.685)
			NT	252	304	117	0.128					
	rs17537593	TT/TA/AA	HT	64	237	432	<0.001	96.6	1.03 (0.704)	1.33 (0.154)	0.97 (0.761)	(0.252)
			NT	47	246	407	0.240					
	rs4951273	GG/GC/CC	HT	114	339	289	0.377	97.9	1.11 (0.178)	1.21 (0.214)	1.11 (0.323)	(0.383)
			NT	93	323	295	0.756					
	rs12749310	GG/GA/AA	HT	427	245	56	0.014	96.1	1.03 (0.766)	1.10 (0.370)	0.81 (0.305)	(0.256)
			NT	393	261	44	0.940					
	rs4297354	GG/GA/AA	HT	462	227	40	0.087	96.1	**1.20 (0.047)**	**1.27 (0.028)**	1.11 (0.662)	(0.086)
			NT	402	253	42	0.794					
	rs11576343	TT/TC/CC	HT	53	251	432	0.051	97.3	0.92 (0.323)	1.02 (0.918)	0.87 (0.202)	(0.382)
			NT	50	266	392	0.597					
	rs6594013	TT/TA/AA	HT	163	348	231	0.141	97.9	0.95 (0.443)	0.98 (0.856)	0.89 (0.310)	(0.587)
			NT	159	348	204	0.647					
	rs16852152	GG/GA/AA	HT	437	252	38	0.831	95.9	0.92 (0.354)	0.92 (0.449)	0.82 (0.418)	(0.618)
			NT	432	234	30	0.812					
	rs3766752	GG/GA/AA	HT	210	367	167	0.782	97.8	1.09 (0.225)	1.15 (0.235)	1.10 (0.433)	(0.454)
			NT	180	356	171	0.847					
	rs11808688	GG/GA/AA	HT	197	372	169	0.795	96.9	0.94 (0.389)	0.86 (0.183)	1.00 (0.985)	(0.370)
			NT	209	331	160	0.189					

Table S6 Continued

Gene	SNP (position)	Genotype		Screening Panel					Odds ratio (p-value)			
				Genotype frequency			HWE	Call rate	Allelic	Recessive	Dominant	Additive
ATP2B4	rs4951130	GG/GA/AA	HT	410	278	50	0.758	97.2	**1.21 (0.025)**	1.22 (0.058)	1.40 (0.086)	(0.082)
			NT	356	283	65	0.421					
	rs12095268	TT/TA/AA	HT	367	313	67	0.982	98.0	1.09 (0.303)	1.09 (0.439)	1.19 (0.335)	(0.556)
			NT	333	300	74	0.599					
	rs12410036	TT/TC/CC	HT	48	256	439	0.200	97.7	0.90 (0.232)	0.93 (0.720)	0.87 (0.196)	(0.434)
			NT	49	264	394	0.599					
	rs7547344	GG/GA/AA	HT	172	362	205	0.618	97.7	1.00 (0.977)	1.02 (0.875)	0.98 (0.846)	(0.954)
			NT	163	354	194	0.951					
	rs955865	GG/GA/AA	HT	208	368	173	0.677	98.6	0.95 (0.456)	0.96 (0.733)	0.89 (0.370)	(0.668)
			NT	204	359	151	0.765					
	rs955866	TT/TC/CC	HT	170	366	208	0.712	98.5	1.05 (0.489)	1.11 (0.401)	1.04 (0.756)	(0.702)
			NT	151	361	206	0.758					

The screening panel is comprised of 758 middle age-onset severe hypertensive patients and 726 middle-aged to elderly evidently normotensive controls (Table S4).

Table S7 Meta-analysis of ATP2B1 SNPs with hypertension

SNP	Coded Allele	Millennium GPJ			Global BPgen			Pooled		
		OR (95% CI)	P	N	OR (95% CI)	P	N	OR (95% CI)	P	N
rs1401982	A	1.19 (1.11-1.29)	$1.3*10^{-6}$	9,967	1.07 (1.02-1.12)	0.010	19126	1.10 (1.06-1.15)	$1.5*10^{-6}$	29,093
rs2681472	A	1.21 (1.13-1.30)	$1.8*10^{-7}$	10,039	1.14 (1.06-1.22)	$2.2*10^{-4}$	19055	1.17 (1.12-1.23)	$2.1*10^{-10}$	29,094
rs11105364	T	1.21 (1.13-1.30)	$1.5*10^{-7}$	10,014	1.13 (1.06-1.21)	$4.6*10^{-4}$	19151	1.17 (1.11-1.22)	$3.1*10^{-10}$	29,165
rs11105378	C	1.21 (1.13-1.30)	$1.5*10^{-7}$	9,972	1.13 (1.05-1.21)	$5.9*10^{-4}$	18894	1.17 (1.11-1.23)	$7.0*10^{-10}$	28,866

In both Japanese Millennium GPJ and Global BP gen, hypertensive subjects were defined as being treated with antihypertensive medication, or SBP greater or equal to 140 mmHg, or DBP greater or equal to 90 mmHg; normotensive subjects were defined as all of not treated with antihypertensive medication, and SBP less or equal to 120 mmHg, and DBP less or equal to 85 mmHg [8]. Adjusted odds ratio was calculated under additive model using multiple logistic regression analysis adjusted for age, age^2, sex, BMI, and cohort variables. Within Global BPgen, individual cohort results were combined using inverse variance weighted meta-analysis of the effects on a log-odds-ratio scale.

Table S8 Association of ATP2B1 SNPs and blood pressure traits in each Japanese cohort

SNP	coded allele		cohort				SBP			DBP		
	allele	%	name	n	HWE	CR	coefficient	SE	p	coefficient	SE	p
		61.9	Ohasama	1569	0.227	98.6	0.35	0.60	0.558	0.06	0.39	0.868
		62.3	Yokohama	2269	0.588	99.1	-1.51	0.43	**$4.2*10^{-4}$**	-0.75	0.29	**0.009**
		62.6	Shigaraki	2191	0.908	96.4	-1.72	0.56	**0.002**	-0.91	0.35	**0.010**
rs1401982	A	61.8	Takashima	1718	0.302	99.3	-1.95	0.72	**0.007**	-0.90	0.41	**0.028**
		61.7	Suita	2529	0.506	99.7	-0.80	0.57	0.160	-0.44	0.33	0.182
		62.0	Matsuyama	803	0.175	99.4	-1.27	0.97	0.194	-1.39	0.62	**0.026**
		63.8	Nomura	2865	0.611	99.6	-1.39	0.56	**0.020**	-0.67	0.33	**0.045**
		62.1	Ohasama	1587	0.226	99.7	0.38	0.60	0.522	0.06	0.39	0.887
		62.6	Yokohama	2278	0.321	99.5	-1.52	0.43	**$3.8*10^{-4}$**	-0.78	0.28	**0.006**
		63.5	Shigaraki	2254	0.701	99.2	-2.03	0.56	**$2.9*10^{-4}$**	-1.15	0.35	**0.001**
rs2681472	A	62.3	Takashima	1718	0.257	99.3	-2.25	0.72	**0.002**	-1.03	0.41	**0.013**
		62.1	Suita	2528	0.655	99.7	-0.97	0.57	0.089	-0.49	0.33	0.131
		62.1	Matsuyama	802	0.191	99.3	-1.13	0.98	0.248	-1.39	0.62	**0.026**
		64.3	Nomura	2865	0.907	99.6	-1.42	0.60	**0.018**	-0.69	0.34	**0.041**
		62.2	Ohasama	1589	0.203	99.8	0.42	0.60	**0.477**	0.12	0.39	**0.766**
		63.3	Yokohama	2277	0.414	99.4	-1.61	0.43	**$1.8*10^{-4}$**	-0.79	0.29	**0.006**
		64.3	Shigaraki	2234	0.410	98.3	-2.11	0.56	**$1.7*10^{-4}$**	-1.16	0.35	**0.001**
rs11105364	T	62.7	Takashima	1727	0.570	99.8	-2.25	0.71	**0.002**	-0.98	0.41	**0.017**
		62.4	Suita	2530	0.635	99.8	-1.08	0.57	**0.058**	-0.54	0.33	**0.096**
		62.8	Matsuyama	805	0.285	99.6	-1.05	0.98	**0.285**	-1.35	0.62	**0.031**
		64.4	Nomura	2851	0.495	99.1	-1.30	0.60	**0.030**	-0.60	0.34	**0.077**

Table S8 Continued

SNP	coded allele		cohort				SBP			DBP		
	allele	%	name	n	HWE	CR	coefficient	SE	p	coefficient	SE	p
		62.9	Ohasama	1566	0.478	98.4	0.31	0.60	**0.600**	-0.04	0.39	**0.914**
		63.4	Yokohama	2258	0.244	98.6	-1.32	0.43	**0.002**	-0.66	0.29	**0.022**
		65.2	Shigaraki	2213	0.141	97.4	-2.45	0.56	**$1.3*10^{-5}$**	-1.31	0.35	**$2.2*10^{-4}$**
rs11105378	C	63.2	Takashima	1722	0.237	99.5	-2.41	0.72	**$8.5*10^{-4}$**	-1.15	0.41	**0.006**
		63.0	Suita	2521	0.498	99.4	-1.00	0.58	**0.084**	-0.42	0.33	**0.207**
		63.2	Matsuyama	803	0.434	99.4	-1.14	0.99	**0.249**	-1.56	0.63	**0.014**
		65.7	Nomura	2865	0.468	99.6	-1.11	0.60	**0.065**	-0.47	0.34	**0.164**

Coefficients and standardized error for systolic and diastolic BP were calculated under additive model using multiple regression analysis adjusted for age, age2, sex, BMI. Adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15mmHg for SBP and +10mmHg for DBP). CR indicates call rate.

Table S9 Association of European GWAS-derived SNPs with hypertension in the Japanese screening and replication panels

SNP	Genotype		Screening panel						Replication panel						overall
			Genotype frequency			HWE	Call rate	Odds (p value)	Genotype frequency			HWE	Call rate	Odds (p value)	Odds (p value)
FGF5 rs1458038	TT/TC/CC	HT	92	338	315	0.928	98.0	**1.19 0.030**	271	838	788	0.047	97.9	**1.21 ($1.1*10^{-4}$)**	**1.20 ($9.9*10^{-6}$)**
		NT	81	281	347	0.039			225	801	918	0.014			
CYP17A1 rs1004467	AA/AG/GG	HT	380	299	66	0.514	98.6	**1.35 ($1.4*10^{-4}$)**	894	869	168	0.034	99.8	1.09 (0.079)	**1.16 ($4.9*10^{-4}$)**
		NT	309	308	101	0.089			877	901	205	0.236			
CSK rs1378942	CC/CA/AA	HT	483	236	25	0.557	98.0	1.09 0.340	1237	605	72	0.853	98.9	1.04 (0.536)	1.05 (0.305)
		NT	452	223	35	0.274			1259	621	85	0.449			
PLCD3 rs12946454	TT/TA/AA	HT	28	210	510	0.276	98.8	1.12 0.256	68	526	1339	0.070	99.7	0.99 (0.907)	1.03 (0.624)
		NT	13	207	499	0.107			68	545	1364	0.140			
PLEKHA7 rs381815	TT/TC/CC	HT	27	242	483	0.624	98.8	1.05 0.596	85	567	1273	0.033	99.4	0.99 (0.913)	1.01 (0.852)
		NT	31	208	475	0.181			93	574	1308	0.004			
CSK-ULK3 rs6495122	AA/AC/CC	HT	508	204	21	0.924	96.8	1.18 0.085	1289	561	72	0.263	99.2	1.10 (0.102)	**1.12 (0.021)**
		NT	458	221	25	0.793			1267	626	77	0.976			
ULK4 rs9815354	AA/AG/GG	HT	7	142	598	0.654	98.5	0.90 0.374	31	385	1507	0.265	98.9	1.05 (0.463)	1.01 (0.873)
		NT	10	144	561	0.826			26	382	1548	0.659			

The screening panel is comprised of 758 middle age-onset severe hypertensive patients and 726 middle-aged to elderly evidently normotensive controls (Table S4). The replication panel consists of 1,929 hypertensive cases and 1,993 normotensives controls selected from a 11,569 cohort sample were enrolled (Table S2). Odds ratios and p-values for allelic model are shown.

Table S10 Association of European GWAS-derived SNPs and blood pressure traits in each Japanese cohort

SNP	coded allele		cohort				SBP			DBP		
	allele	%	name	n	HWE	CR	coefficient	SE	p	coefficient	SE	p
FGF5 rs1458038	T	33.7	Ohasama	1557	0.174	97.8	1.58	0.60	**0.008**	0.44	0.39	0.260
		33.5	Yokohama	2223	0.005	97.1	0.84	0.44	0.055	0.46	0.29	0.115
		33.8	Shigaraki	2156	0.001	94.9	1.17	0.56	0.037	0.46	0.35	0.196
		31.4	Takashima	1714	0.163	99.1	2.43	0.73	**0.001**	1.62	0.42	**$1.0*10^{-4}$**
		33.6	Suita	2533	0.508	99.9	0.67	0.58	0.250	0.43	0.33	0.191
		33.4	Matsuyama	804	0.459	99.5	0.70	1.04	0.500	0.54	0.67	0.414
		38.2	Nomura	2841	0.105	98.8	1.85	0.58	**0.002**	1.09	0.33	**0.001**
CYP17A1 rs1004467	A	70.2	Ohasama	1579	0.254	99.2	1.41	0.45	**0.002**	0.48	0.30	0.110
		68.4	Yokohama	2276	0.812	99.4	1.05	0.57	0.065	0.03	0.36	0.938
		65.5	Shigaraki	2244	0.898	98.7	1.46	0.74	**0.050**	0.83	0.43	0.051
		67.8	Takashima	1714	0.573	99.1	-0.21	0.59	0.721	-0.34	0.34	0.308
		66.8	Suita	2533	0.865	99.9	0.12	1.05	0.911	-0.10	0.67	0.885
		67.4	Matsuyama	804	0.388	99.5	1.25	0.62	0.045	0.50	0.35	0.149
		69.7	Nomura	2859	0.475	99.4	1.41	0.45	**0.002**	0.48	0.30	0.110
CSK rs1378942	C	77.7	Ohasama	1575	0.821	98.9	-0.17	0.68	0.804	-0.53	0.45	0.241
		78.1	Yokohama	2245	0.152	98.0	0.73	0.52	0.157	0.48	0.35	0.167
		83.0	Shigaraki	2225	0.187	97.9	1.80	0.71	0.012	1.35	0.45	**0.003**
		80.7	Takashima	1703	0.808	98.4	-0.41	0.88	0.644	0.08	0.51	0.870
		80.5	Suita	2528	0.098	99.7	1.28	0.69	0.063	0.43	0.39	0.270
		79.7	Matsuyama	798	0.846	98.8	0.24	1.21	0.842	0.07	0.77	0.923
		81.0	Nomura	2848	0.075	99.0	1.18	0.72	0.103	0.63	0.41	0.121

Table S10 Continued

SNP	coded allele		cohort				SBP			DBP		
	allele	%	name	n	HWE	CR	coefficient	SE	p	coefficient	SE	p
PLCD3 rs12946454	T	81.6	Ohasama	1583	0.356	99.4	1.76	0.72	**0.015**	0.99	0.48	**0.038**
		83.0	Yokohama	2274	0.517	99.3	0.23	0.56	0.687	0.12	0.37	0.752
		83.3	Shigaraki	2242	0.966	98.6	0.46	0.72	0.524	0.76	0.46	0.094
		85.3	Takashima	1712	0.707	99.0	-1.37	0.98	0.163	-1.09	0.56	0.052
		83.2	Suita	2528	0.234	99.7	0.53	0.73	0.464	0.08	0.42	0.845
		82.4	Matsuyama	805	0.799	99.6	0.34	1.28	0.790	0.86	0.82	0.290
		82.4	Nomura	2861	0.142	99.5	-0.35	0.75	0.635	-0.05	0.42	0.899
PLEKHA7 rs381815	T	15.1	Ohasama	1590	0.566	99.9	0.22	0.79	0.778	0.23	0.52	0.657
		19.7	Yokohama	2281	0.457	99.6	-0.77	0.52	0.139	0.04	0.35	0.900
		19.3	Shigaraki	2248	0.587	98.9	-0.38	0.68	0.574	-0.90	0.43	**0.034**
		19.0	Takashima	1719	0.434	99.4	-0.196	0.87	0.271	-0.22	0.50	0.660
		20.2	Suita	2527	0.421	99.6	0.76	0.69	0.272	0.42	0.40	0.289
		20.2	Matsuyama	808	0.496	100.0	0.99	1.19	0.408	0.53	0.76	0.489
		23.2	Nomura	2859	0.007	99.4	0.88	0.66	0.187	0.73	0.37	0.052
CSK-ULK3 rs6495122	A	79.4	Ohasama	1581	0.050	99.3	-0.39	0.69	0.569	-0.46	0.45	0.308
		78.4	Yokohama	2288	0.157	99.9	0.88	0.51	0.086	0.66	0.34	0.055
		83.5	Shigaraki	2237	0.146	98.4	0.96	0.72	0.183	0.96	0.45	**0.034**
		80.6	Takashima	1720	0.221	99.4	0.03	0.86	0.969	0.06	0.49	0.907
		81.6	Suita	2529	0.004	99.7	0.87	0.69	0.211	0.18	0.40	0.654
		81.5	Matsuyama	806	0.734	99.8	1.35	1.24	0.276	0.68	0.79	0.391
		82.6	Nomura	2855	0.115	99.3	1.16	0.75	0.120	0.64	0.42	0.129

Table S10 Continued

SNP	coded allele		cohort				SBP			DBP		
	allele	%	name	n	HWE	CR	coefficient	SE	p	coefficient	SE	p
ULK4 rs9815354	A	14.9	Ohasama	1569	0.749	98.6	-0.08	0.80	0.918	0.32	0.53	0.543
		10.5	Yokohama	2269	0.122	99.1	-1.01	0.67	0.134	-0.44	0.45	0.331
		12.7	Shigaraki	2252	0.099	99.1	-1.58	0.80	**0.047**	-0.10	0.50	0.846
		12.0	Takashima	1710	0.201	98.8	-0.57	1.08	0.600	0.15	0.62	0.802
		11.9	Suita	2521	0.456	99.4	-1.03	0.86	0.232	-0.08	0.49	0.867
		11.4	Matsuyama	804	0.389	99.5	-0.91	1.50	0.547	0.70	0.96	0.467
		9.1	Nomura	2853	0.632	99.2	0.79	1.00	0.427	1.21	0.56	**0.030**

Coefficients and standardized error for systolic and diastolic BP were calculated under additive model using multiple regression analysis adjusted for age, age2, sex, BMI. Adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15mmHg for SBP and +10mmHg for DBP).

Table S11 Multiple linear regression analysis for BP trait and hypertension

Parameters	Coded allele	Systolic blood pressure			Diastolic blood pressure			Hypertension	
		Coefficient	Standardized coefficient	P	Coefficient	Standardized coefficient	P	Odds (95% C.I.)	p
Sex		2.38	0.05	<0.001	3.15	0.12	<0.001	1.33 (1.18-1.50)	<0.001
Age (years)		0.31	0.19	<0.001	0.96	1.03	<0.001	1.15 (1.12-1.19)	<0.001
Age^2		0.00	0.25	<0.001	-0.01	-0.74	<0.001	0.99 (0.99-0.99)	0.008
Body mass index (kg/m^2)		1.80	0.25	<0.001	1.12	0.27	<0.001	1.28 (1.26-1.30)	<0.001
Habitual drinking		0.79	0.02	0.035	0.93	0.04	<0.001	1.24 (1.11-1.40)	<0.001
ATP2B1 rs11105378	C	1.32	0.04	$4.4*10^{-8}$	0.71	0.04	$6.1*10^{-7}$	1.21 (1.12-1.30)	$4.0*10^{-7}$
FGF5 rs1458038	T	1.36	0.04	$1.5*10^{-8}$	0.77	0.04	$6.4*10^{-8}$	1.20 (1.11-1.29)	$1.4*10^{-6}$
CYP17A1 rs1004467	A	0.97	0.03	$8.9*10^{-5}$	0.35	0.02	0.017	1.14 (1.06-1.23)	$8.4*10^{-4}$
CSK rs1378942	C	0.71	0.02	0.014	0.36	0.02	0.036	1.09 (1.00-1.19)	0.046

Coefficients for systolic and diastolic BP were calculated using multiple linear regression analysis adjusted cohort variables. Adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15mmHg for SBP and +10mmHg for DBP). Hypertensive subjects were defined as being treated with antihypertensive medication, or SBP greater or equal to 140 mmHg, or DBP greater or equal to 90 mmHg; normotensive subjects were defined as all of not treated with antihypertensive medication, and SBP less or equal to 120 mmHg, and DBP less or equal to 85 mmHg [8].

FIGURE S1

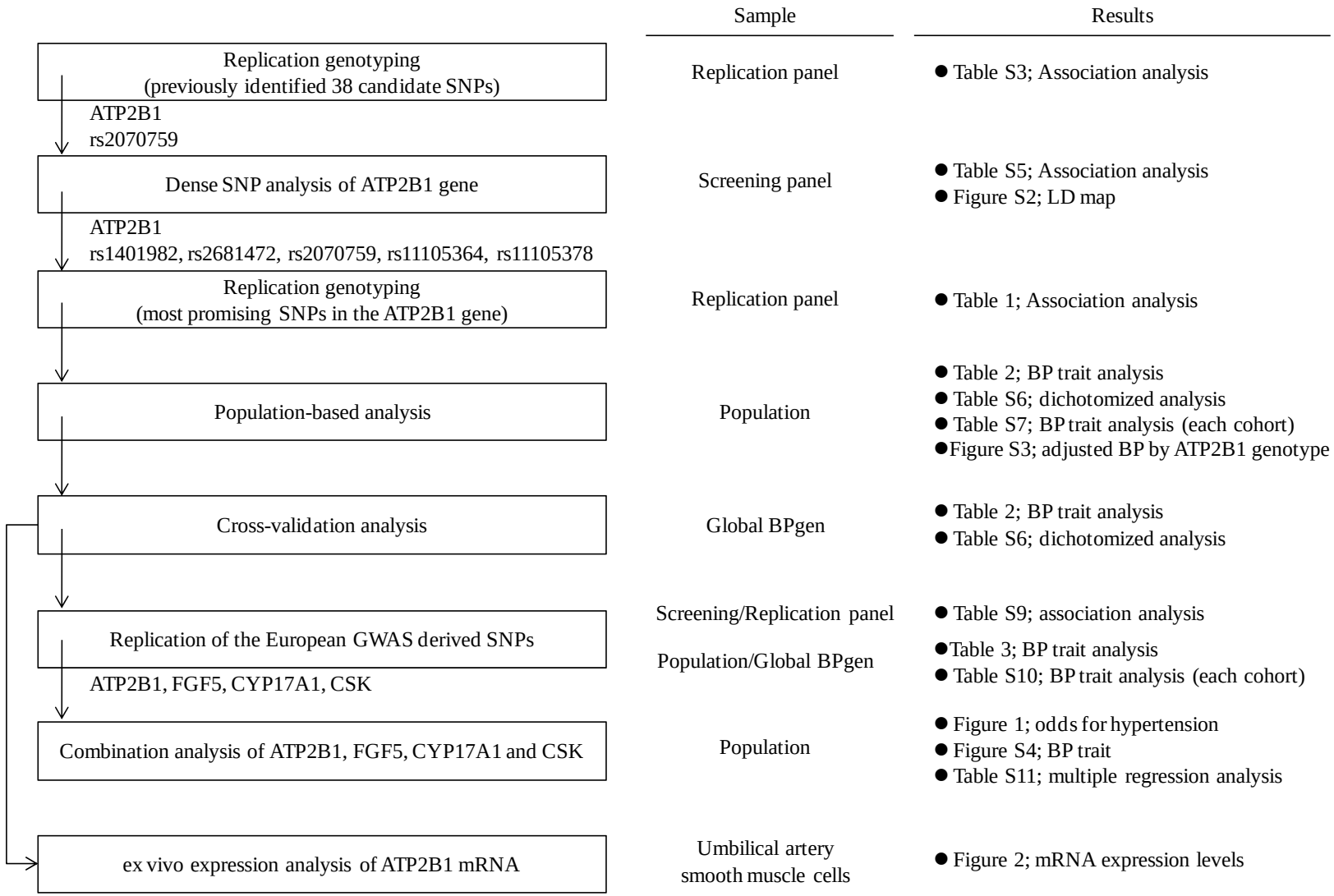

Figure S1 Study procedure and corresponding samples and results

FIGURE S2

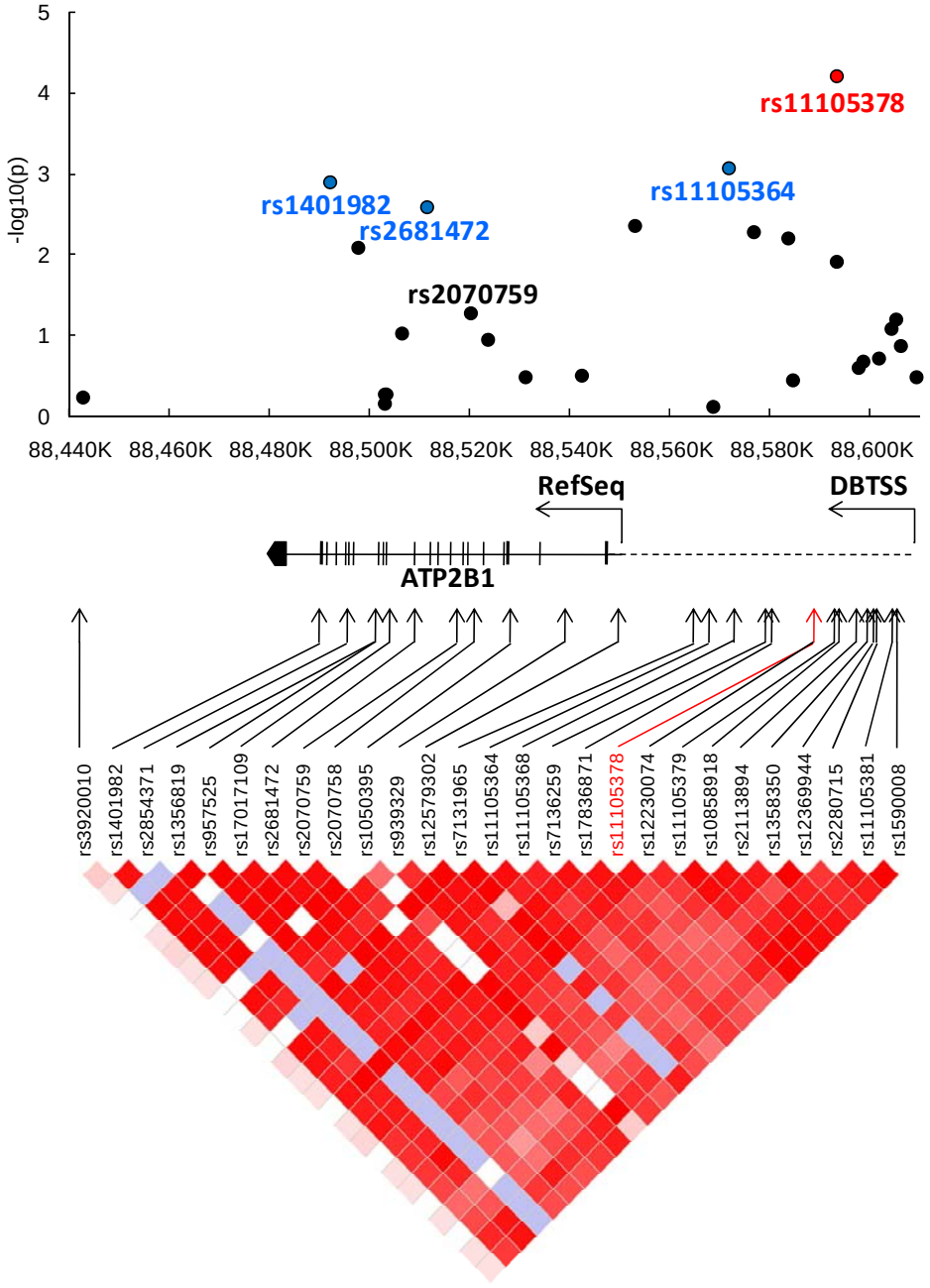

Figure S2 Dense SNP analysis of the ATP2B1 gene

The top graph shows p-values (-log10(P)) of association analyses using the screening panel (Table S4). The red circle (rs11105378) indicates the SNP showing the most significant association with hypertension. The lower panel shows a LD (D') map based on the genotype frequency of the control subjects

FIGURE S3

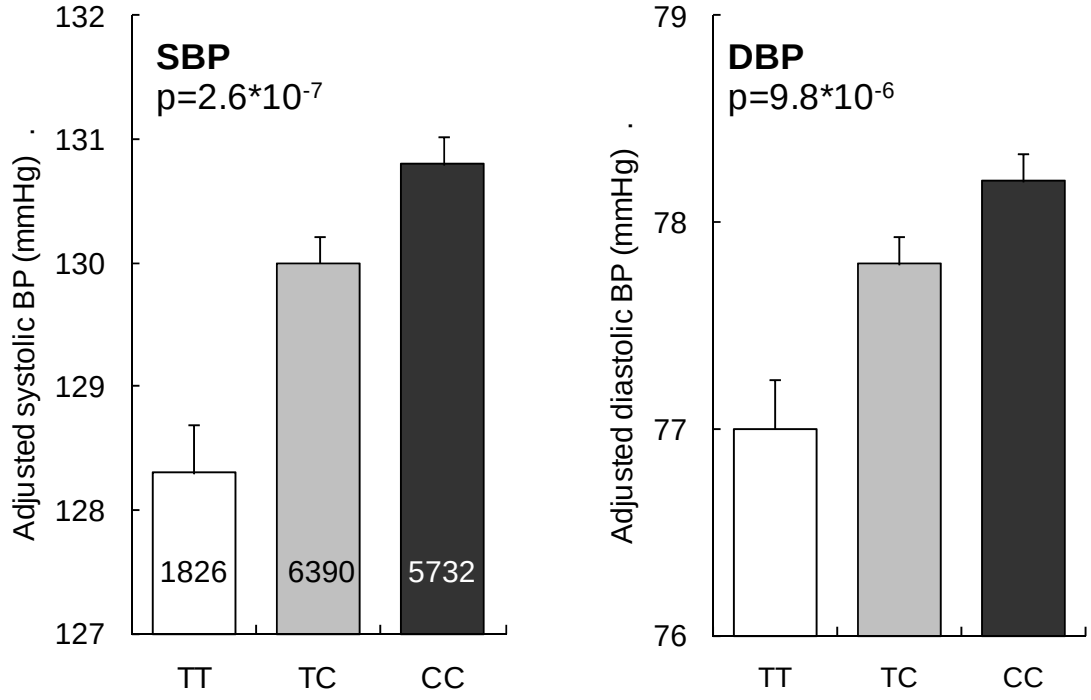

Figure S3 Adjusted systolic and diastolic BP among rs11105378 genotype

Values are mean±standard error adjusted for age, sex, body mass index, and cohort variables. Number of subjects in each genotype is represented in column.

FIGURE S4

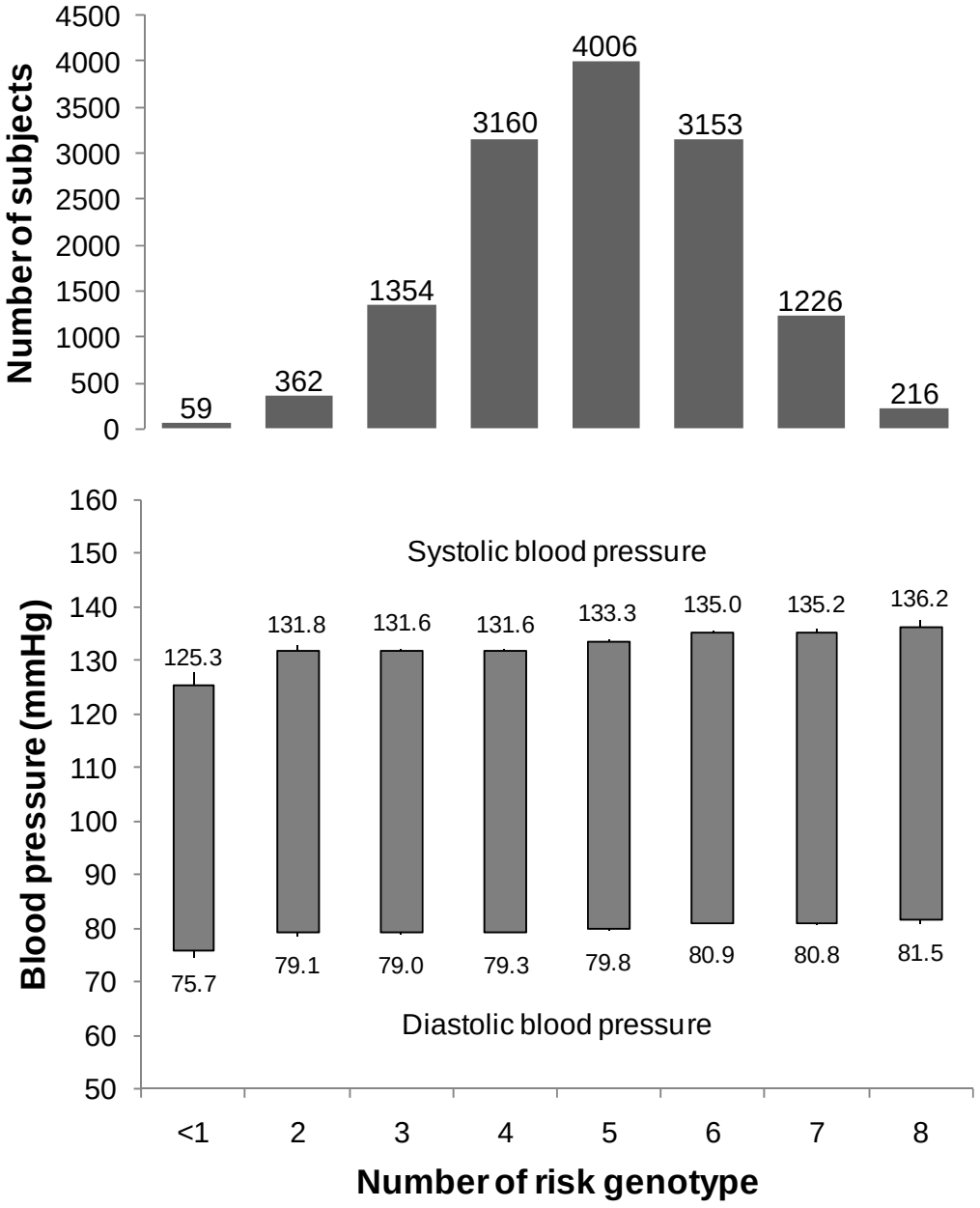

Figure S4 Adjusted blood pressure by the number of risk genotypes

Number of risk genotype was calculated by the following four SNPs; *ATP2B1* rs1105378, *FGF5* rs1458038, *CYP17A1*, rs1004467 and *CSK* rs1378942. Age, age2, sex, BMI and cohort variable adjusted systolic and diastolic BP is shown in the lower panel. Upper panel indicates the number of subjects in each group.

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