Received: 3 November 2023 | Revised: 7 February 2024 | Accepted: 11 February 2024

DOI: 10.1111/jch.14785

ORIGINAL ARTICLE

WILEY

ATP2B1 gene polymorphisms associated with resistant hypertension in the Japanese population

Yusuke Kobayashi MD, PhD[1,2] | Keisuke Yatsu MD, PhD[3] | Aiko Haruna MD[4] | Rina Kawano MD[4] | Moe Ozawa MD, PhD[2,5] | Tatsuya Haze MD, PhD[1,4] | Shiro Komiya MD, PhD[5] | Shota Suzuki MD[4] | Yuki Ohki MD, PhD[4] | Akira Fujiwara MD, PhD[4] | Sanae Saka MD[5] | Nobuhito Hirawa MD, PhD[4] | Yoshiyuki Toya MD, PhD[2] | Kouichi Tamura MD, PhD[2]

[1]Center for Novel and Exploratory Clinical Trials (Y-NEXT), Yokohama City University, Yokohama, Japan

[2]Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[3]Minna no Naika Clinic Ningyocho-Suitengu, Tokyo, Japan

[4]Department of Nephrology and Hypertension, Yokohama City University Medical Center, Yokohama, Japan

[5]Department of Nephrology and Hypertension, Saiseikai Yokohamashi Nanbu Hospital, Yokohama, Japan

Correspondence
Yusuke Kobayashi MD PhD, Yokohama City University Center for Novel and Exploratory Clinical Trials (Y-NEXT), 1-1-1, Fukuura, Kanazawa, Yokohama, Kanagawa 236-0004, Japan.
Email: yusuke@yokohama-cu.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Numbers: 16K09477, 19K17610

Abstract

Single-nucleotide polymorphisms (SNP) of ATP2B1 gene are associated with essential hypertension but their association with resistant hypertension (RHT) remains unexplored. The authors examined the relationship between ATP2B1 SNPs and RHT by genotyping 12 SNPs in ATP2B1 gene of 1124 Japanese individuals with lifestyle-related diseases. Patients with RHT had inadequate blood pressure (BP) control using three antihypertensive drugs or used ≥4 antihypertensive drugs. Patients with controlled hypertension had BP controlled using ≤3 antihypertensive drugs. The association between each SNP and RHT was analyzed by logistic regression. The final cohort had 888 (79.0%) and 43 (3.8%) patients with controlled hypertension and RHT, respectively. Compared with patients homozygous for the minor allele of each SNP in ATP2B1, a significantly higher number of patients carrying the major allele at 10 SNPs exhibited RHT (most significant at rs1401982: 5.8% vs. 0.8%, $p = .014$; least significant at rs11105378: 5.7% vs. 0.9%, $p = .035$; most nonsignificant at rs12817819: 5.1% vs. 10%, $p = .413$). After multivariate adjustment for age, sex, systolic BP, and other confounders, the association remained significant for rs2681472 and rs1401982 (OR: 7.60, $p < .05$ and OR: 7.62, $p = .049$, respectively). Additionally, rs2681472 and rs1401982 were in linkage disequilibrium with rs11105378. This study identified two ATP2B1 SNPs associated with RHT in the Japanese population. rs1401982 was most closely associated with RHT, and major allele carriers of rs1401982 required significantly more antihypertensive medications. Analysis of ATP2B1 SNPs in patients with hypertension can help in early prediction of RHT and identification of high-risk patients who are more likely to require more antihypertensive medications.

KEYWORDS
ATP2B1 gene polymorphisms, blood pressure, genotyping, Japanese, resistant hypertension

1 | INTRODUCTION

Hypertension is a primary risk factor of cardiovascular events.[1] Although hypertension is highly prevalent globally, a large percentage of individuals with hypertension do not have adequate blood pressure control despite the use of multiple antihypertensive medications. Approximately 10%–15% of patients with hypertension live with resistant hypertension (RHT), which makes them vulnerable to numerous cardiovascular ailments, including coronary artery disease (CAD), heart failure, renal disease, and stroke. It is caused by various factors, including high salt intake, heavy alcohol consumption, obesity, diabetes, kidney disease, and heart failure and is associated with a high risk of cardiovascular disease and death.[2] However, despite various studies on the genetic aspects of RHT, reports are inconsistent, and the etiopathogenesis of the disease remains poorly understood.[3–7]

Several genome-wide association analyses have been performed in European,[8] Korean,[9,10] and Japanese[11] populations. ATP2B1, which is located on chromosome 12q21.3, is one of the most studied genes associated with the development of hypertension.[12] This gene encodes the plasma membrane Ca^{2+}-ATPase isoform 1 (PMCA1), a protein involved in Ca^{2+} transportation and homeostasis in cells.[13,14] Systemic knockout of ATP2B1 results in embryonic lethality. In our previous study, ATP2B1-specific knockout mice showed elevated intracellular calcium concentrations in vascular smooth muscles and increased blood pressure due to enhanced vasoconstriction.[15] Mice with systemic ATP2B1 knockout showed increased blood pressure owing to decreased endothelial nitric oxide synthase activity in vascular endothelial cells and decreased nitric oxide (NO) production in vascular endothelial cells and the aorta.[16] Additionally, deletion of PMCA1 in the intestines of mice was found to affect bone mineral density.[17] This indicates that ATP2B1 knockout affects various tissues and increases blood pressure.

In humans, there are reports of decreased mRNA expression in the umbilical artery smooth muscle in rs11105378 alleles associated with elevated blood pressure.[18] Further, allele carriers with highly RHT had decreased mRNA levels in whole blood.[19] Many studies have also reported that decreased ATP2B1 mRNA expression is associated with increased blood pressure.[13] A recent systematic review and meta-analysis underscored the involvement of the ATP2B1 rs17249754 polymorphism (G/A) and the rs2681472 polymorphism (A/G) in the risk of blood pressure and hypertension, highlighting the possibility of new genetic biomarkers for hypertension.[20] Although ATP2B1 has been identified as a major candidate gene for hypertension, evidence regarding its genetic association with RHT is still limited. A previous study reported that an intronic single nucleotide polymorphism (SNP) (rs12817819) in ATP2B1 is associated with RHT in European American and Hispanic populations.[19] However, this association remains unexplored in the Japanese population.

The results of previous experiments in mice support that ATP2B1 may be involved in treatment resistance through various mechanisms such as vasoconstriction[15] and vasodilation.[16] The association with RHT has only been observed in one study in European American and Hispanic populations,[19] wherein, the ATP2B1 rs12817819 allele was associated with increased risk for RHT in hypertensive participants with documented CAD or suspected ischemic heart disease. Therefore, this study aimed to establish whether the major SNPs in ATP2B1 were associated with RHT in Japanese patients. We hypothesized that the SNP of ATP2B1, if potent, may have a stronger association with RHT.

2 | METHODS

2.1 | Study design and setting

This prospective cross-sectional study was approved by the ethics committee of Yokohama City University Hospital (protocol codes: A201126001 and A130725005) and conducted in accordance with the Declaration of Helsinki. Written informed consent to publish the data was obtained from each patient.

This study was conducted at Yokohama City University Hospital and Kobayashi Medical Clinic between August 2013 and March 2018. The patients were recruited between August 2013 and February 2015. We did not perform any power analysis; however, patients were recruited based on defined inclusion and exclusion criteria.

2.2 | Patient selection

The patients undergoing treatment for lifestyle-related diseases (e.g., hypertension, dyslipidemia, or diabetes) were evaluated. The inclusion criteria were patients of Japanese ethnicity who were undergoing treatment for lifestyle-related diseases and age ≥20 years at baseline. The exclusion criteria were as follows: endocrine hypertension (primary aldosteronism, pheochromocytoma) and renal vascular hypertension, insufficient data on office blood pressure and blood biochemistry, and ineligible for inclusion as research patients as deemed by the attending physician. We performed blood tests for endocrine factors and ultrasound examinations to evaluate the patients. Patients who were deemed eligible for the study underwent further thorough examinations at specialized institutions before being included in the study.

Patients with RHT were defined as those who could not achieve the target blood pressure (office blood pressure < 140/90 mm Hg) with three or more antihypertensive drugs (administered at optimal doses), including diuretics, or those who required four or more antihypertensive drugs for blood pressure control. Antihypertensive drugs included angiotensin-converting enzyme inhibitors, aldosterone receptor blockers, alpha and beta blockers, angiotensin receptor blockers, calcium channel blockers, central-acting agents, diuretics, and direct vasodilators. We followed standard guidelines for the treatment of hypertension[21] and administered drugs when it was possible to control the patients' blood pressure within the scope of routine practice. We included patients who had been treated with the same drug for at least 3 months and did not need a change of drugs. Patients with poorly controlled hypertension or those who did not achieve the target blood pressure with three antihypertensive medications, excluding diuretics,

TABLE 1 SNPs in ATP2B1 associated with hypertension, atherosclerosis, and cardiovascular disease.

SNPs	Associated with	References
rs17249754	Hypertension, atherosclerosis, metabolic syndrome, salt-sensitive hypertension	Qi et al.[24] ($p = .017$ for hypertension) Kelly et al.[25] ($p = 7.5\times10^{-15}$ for mean arterial pressure) Heo et al.[26] ($p = 2.11\times10^{-5}$ for hypertension; $p = .005$ for hyperlipidemia) Wang et al.[27] ($p = .002–.004$ for arterial stiffness; $p = 4.6E-05$ for hypertension) Lee et al.[28] ($p = .006$ for salt-sensitive hypertension)
rs2681492	Hypertension, mean arterial pressure	Kelly et al.[25] ($p = 3.4\times10^{-7}$ for mean arterial pressure) Kayima et al.[29] ($p = .01$ for blood pressure)
rs2681472	Hypertension, salt-sensitive hypertension, coronary artery disease	Wang et al.[27] ($p = .015$ for arterial stiffness, hypertension) Xi et al.[30] ($p = .000$ for hypertension) Rhee et al.[31] ($p = .040$ for salt-sensitive hypertension)
rs11105354	Hypertension, myocardial infarction, coronary atherosclerosis	Kulkarni et al.[32] ($p = .01$ for cardiovascular disease in patients with chronic kidney disease) Pike et al.[33] ($p = 1.4\times10^{-4}$ for blood pressure)
rs1401982	Hypertension, arterial stiffness	Wang et al.[25] ($p = .002–.004$ for arterial stiffness, hypertension) An et al.[34] ($p = .0007$ for decreased susceptibility of concurrent extra and intracranial stenosis)
rs7136259	Cardiovascular disease	Lu et al.[35] ($p = 5.68 \times 10^{-10}$ for coronary artery disease)
rs11105364	Hypertension	Levy et al.[8] ($p = 2.1E–08$ for hypertension)
rs11105378	Hypertension	Tabara et al.[18] ($p = 4.1 \times 10^{-11}$ for hypertension)
rs11105368	Hypertension	Levy et al.[8] ($p = 2.2E–08$ for hypertension)
rs2070759	Hypertension	Tabara et al.[18] ($p = 5.3 \times 10^{-5}$ for hypertension)
rs12579302	Hypertension	Levy et al.[8] ($p = 2.2E–08$ for hypertension)
rs12817819	Resistant-hypertension	Fontana et al.[19] ($p = 7.69 \times 10^{-4}$ for resistant-hypertension in Hispanics; $p = 2.44 \times 10^{-3}$ for European Americans)

Abbreviation: SNP, single nucleotide polymorphism.

were excluded from the study. Blood pressure was measured at least thrice while recording readings once a month.

2.3 | Sample preparation and genotyping

Patients were asked to gargle and scrub their oral cavity with a special brush, and oral mucosal cells were collected.[20] DNA was extracted using a QIAGEN DNA extraction kit (QUIQGEN, Venlo, Netherland). Real-time polymerase chain reaction (PCR) of the sample DNA was performed using human GAPDH as the positive control. We performed PCR of the oral mucosa using Takara Taq DNA polymerase and PCR components (Takara Bio, Shiga, Japan), following the method by Andrisin and coworkers[22] The following human GAPDH primers were used[23]: Forward- CCACCCATGGGCAAATTCCATGGCA; %GC54, TM (1MNa+) 76, TM (50mMNa+) 54; Reverse-TCTAGACGGGCAGGTCAGGTCCACC; and %GC63, TM (1MNa+) 79, TM (50mMNa+) 57. The thermal cycler conditions were as follows: 94°C for 1 min; 94°C for 15 s; 68°C for 15 s, 33 times; 72°C for 30 s; and final extension at 72°C for 5 min.

2.4 | Selection of SNPs

The 12 most frequently reported SNPs in ATP2B1 that were associated with hypertension, atherosclerosis, and cardiovascular disease were selected. Table 1 lists the selected SNPs with their background information. DNA samples were genotyped using an Illumina Infinium bead chip (RIKEN, Tokyo, Japan), according to the manufacturer's instructions. Loci-specific primers for each SNP were designed using the RIKEN Genesis. The linkage disequilibrium block structure and pattern were determined using Haploview version 4.2 (Broad Institute, Boston, USA.)

2.5 | Statistical analysis

The primary outcome of the study was the diagnosis of RHT. The secondary outcomes were systolic and diastolic blood pressure, diagnosis of hypertension, and the number of antihypertensive drugs. Primary exposure was considered an SNP in the ATP2B1 gene. The following covariates were considered: sex, clinical and behavioral characteristics at baseline (age, body mass index [BMI], estimated glomerular filtration rate (eGFR), fasting glucose, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, antihypertensive medication use, and history of diabetes), and blood pressure. The Hardy–Weinberg equilibrium (HWE) was separately tested among the case and control groups using x2 goodness-of-fit tests with one degree of freedom. Almost all SNPs were in HWE. Logistic regression models were used for association analysis. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were compared using Student's *t* test. The number of antihypertensives was compared with that of

TABLE 2 Comparison of baseline patient characteristics between patients with controlled hypertension and with resistant hypertension ($n = 931$).

	Controlled hypertension ($n = 888$)	Resistant hypertension ($n = 43$)	*p* value
Age, years	70.9 ± 10.8	73.2 ± 8.7	.174
Women, *n* (%)	513 (56.3)	34 (50.7)	.376
Body mass index (kg/m^2)	23.8 ± 3.6	25.6 ± 4.7	.001
eGFR (mL/min/1.73m2)	69.4 ± 16.0	58.7 ± 19.0	<.001
Fasting glucose (mg/dL)	98.2 ± 22.6	104.1 ± 21.1	.006
LDL cholesterol (mg/dL)	117.5 ± 29.3	120.7 ± 22.8	.543
HDL cholesterol (mg/dL)	67.4 ± 17.5	62.8 ± 18.5	.399
Triglyceride (mg/dL)	111.6 ± 65.6	116.8 ± 58.1	.495
History of diabetes, *n* (%)	181 (19.9)	21 (31.3)	.029

Note: Baseline data are expressed as the means ± standard deviations or counts (percentages).
Age, sex, BMI, eGFR, FBS, LDL, HDL, and TG are compared using Student's *t*-test. History of diabetes is compared using chi-square test. A *p* value of <.05 is considered statistically significant.
Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 3 Comparison of blood pressure and antihypertensive drug use between patients with controlled hypertension and with resistant hypertension ($n = 931$).

	Controlled hypertension ($n = 888$)	Resistant hypertension ($n = 43$)	*p* value
SBP (mm Hg)	129.1 ± 7.8	139.4 ± 9.0	**<.001**
DBP (mm Hg)	75.8 ± 6.8	76.8 ± 7.4	.149
MAP (mm Hg)	93.6 ± 5.9	97.7 ± 6.6	**<.001**
Number of antihypertensive drugs (*n*)	1.4 ± 1.0	4.0 ± 0.7	**<.001**
Antihypertensive drug class			
Calcium channel blocker, *n* (%)	468 (51.4)	57 (85.1)	**<.001**
ARB, *n* (%)	414 (45.4)	57 (85.1)	**<.001**
ACE inhibitor, *n* (%)	26 (2.9)	4 (6.0)	.144
Diuretic, *n* (%)	33 (3.6)	54 (80.6)	**<.001**
β-blocker, *n* (%)	120 (13.2)	40 (59.7)	**<.001**
α-blocker, *n* (%)	26 (2.9)	32 (47.8)	**<.001**
Nitrate, *n* (%)	36 (4.0)	9 (13.4)	**.002**
Others, *n* (%)	37 (40.6)	8 (11.9)	**.009**

Note: Baseline data are expressed as the means ± standard deviations or as counts (percentages).
SBP, DBP, and MAP are compared using Student's *t*-test. The number of antihypertensives is compared using the Mann–Whitney U test, while the classes of antihypertensives is compared using the chi-square test. A *p* value of <.05 is considered statistically significant.
Abbreviations: ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.

the main classes of antihypertensives by using the chi-square test. For each SNP, we compared the percentage of RHT in minor allele homozygotes and major allele carriers using the chi-square test. The associations between particular alleles (rs2681472 and rs1401982) and other ATP2B1 SNPs were assessed using Haploview 4.2.[36] For two-group comparisons, we used the Student's *t*-test for normally distributed continuous variables and the Mann–Whitney *U* test for non-normally distributed continuous variables. Categorical variables were not included in this study. Pearson's correlation analysis was used to evaluate the relationships between the pairs of parameters. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the effects of different alleles and haplotypes. Bonferroni corrections and permutation tests were used to correct the *p*-values of the alleles. All statistical analyses were performed using SPSS statistical software version 22.0 (IBM Corp., Armonk, NY, USA). All tests were two tailed, with statistical significance set at $p < .05$.

3 | RESULTS

Among the 1124 patients with lifestyle-related diseases (e.g., hypertension, dyslipidemia, and diabetes) screened, 193 patients were excluded because of inadequately controlled hypertension. Finally, 888 patients with controlled hypertension and 43 patients with RHT were included in the analysis. Table 2 presents the characteristics of patients with controlled and RHT. The RHT group was associated with a higher BMI, lower eGFR, higher fasting glucose levels, and a higher proportion of diabetes. In addition, in the RHT group, the average systolic blood pressure was approximately 140 mm Hg with an average of four antihypertensive medications. SBP and the number of antihypertensive drugs used were significantly different in patients with RHT (Table 3).

Table 4 shows the percentage of patients with RHT for each of the 12 ATP2B1 SNPs. For each SNP, we conducted logistic regression analysis using the chi-square test to compare the percentage of RHT in minor allele homozygotes and in major allele carriers. Among these SNPs, 10 SNPs, excluding rs2070759 and rs12817819, as major alleles, were more significantly associated with RHT. After adjusting for various factors such as age and sex, rs2681472 and rs1401982 was still significantly associated with RHT (Table 5). Among these two alleles, we focused on the differences in patient background by genotype for rs1401982, the genotype with the most significant association, and found no differences in the main patient background, including blood pressure (Table 6).

Although major allele carriers and minor allele homomorphic carriers showed no significant differences in blood pressure, the former group used significantly more antihypertensive medications than the latter. Furthermore, a large proportion of patients with major alleles used more than four antihypertensive medications (Figure 1). These findings suggest that major allele carriers of rs1401982 require a greater number of antihypertensive medications than minor allele homozygotes to achieve similar blood pressure levels. Figure 2 shows

TABLE 4 Frequency of resistant hypertension according to SNPs of *ATP2B1*.

SNPs	Minor allele	MAF	AA/Aa (%)	aa (%)	*p* value
rs17249754	A	0.340	5.8	0.8	.023
rs2681492	C	0.343	5.8	0.8	.015
rs2681472	G	0.346	5.8	0.8	.014
rs11105354	G	0.123	5.8	0.9	.022
rs1401982	G	0.349	5.8	0.8	.014
rs7136259	T	0.342	5.8	0.9	.023
rs11105364	G	0.341	5.8	0.8	.023
rs11105378	T	0.335	5.7	0.9	.035
rs11105368	C	0.342	5.8	0.8	.023
rs2070759	T	0.439	5.5	3.8	.362
rs12579302	G	0.340	5.8	0.8	.023
rs12817819	T	0.098	5.1	10	.413

Note: For each SNP, we compared the frequency of resistant hypertension between minor allele homozygote carriers and major allele carriers using a chi-square test.
Logistic regression analysis; $p < .05$ is considered statistically significant.
Abbreviations: AA/Aa, major allele carrier; aa, minor allele homozygote; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms.

TABLE 5 Adjusted odds ratios for the probability of resistant hypertension according to the SNPs of *ATP2B1*.

SNPs	Minor allele	MAF	Adjusted OR (95% CI)	*p* value
rs17249754	A	0.340	7.33 (0.97–55.64)	.054
rs2681492	C	0.343	7.42 (0.98–56.37)	.053
rs2681472	G	0.346	7.60 (1.00–57.65)	<.050
rs11105354	G	0.123	6.57 (0.87–49.76)	.069
rs1401982	G	0.349	7.62 (1.01–57.81)	.049
rs7136259	T	0.342	7.15 (0.94–54.25)	.057
rs11105364	G	0.341	7.33 (0.97–55.64)	.054
rs11105378	T	0.335	6.61 (0.87–50.43)	.069
rs11105368	C	0.342	7.33 (0.97–55.64)	.054
rs2070759	T	0.439	1.18 (0.49–2.86)	.715
rs12579302	G	0.340	7.33 (0.97–55.64)	.054
rs12817819	T	0.098	0.28 (0.03–2.77)	.277

Note: Logistic regression analysis is adjusted for age, sex, body mass index, systolic blood pressure, estimated glomerular filtration rate, fasting blood glucose, low-density lipoprotein, high-density lipoprotein, triglycerides, and history of diabetes.
Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNPs, single nucleotide polymorphisms.

the linkage disequilibrium structure of the SNPs in ATP1B2. Association analysis between rs2681472 and rs1401982 and other ATP2B1 SNPs showed that the two SNPs were in linkage disequilibrium with rs11105378 (Figure 2), which was previously associated with mRNA expression levels of ATP2B1.

4 | DISCUSSION

This study identified two ATP2B1 SNPs that were associated with RHT in the Japanese population. In addition, rs1401982 was most closely associated with RHT, and major allele carriers of rs1401982 required significantly more antihypertensive medications. To our best knowledge, this study is the first to identify ATP2B1 SNPs associated with RHT.

Previous genome-wide and gene-centric studies have established strong associations of the ATP2B1 locus with SBP and DBP, as well as with hypertension.[8,10,37,38] Among East Asians, ATP2B1 rs2681472 is associated with a risk of CAD.[39] Among the 29 000 participants from the CHARGE consortium, the odds of developing hypertension was higher by 17% for rs2681472 carriers.[8] Another meta-analysis highlighted a significant association of rs17249754(G/A) (OR = 1.19, 95% CI: 1.10–1.28) and rs2681472 (A/G) (OR = 1.15, 95% CI: 1.12–1.17) with the risk of hypertension, as well as with high SBP and DBP.[20] The ATP2B1 genotypes rs2070759 and rs2681472 were also strongly associated with the risk of hypertension in the Saudi population.[38] Another case-control study in West Africa that involved patients with essential hypertension and normotensive individuals reported a strong association between ATP2B1 rs17249754 and hypertension.[40]

TABLE 6 Patient characteristics by rs1401982 allele.

	AA/Aa ($n = 888$)	aa ($n = 43$)	*p* value
Age (years)	129.4 ± 8.3	130.6 ± 6.9	.120
Women, *n* (%)	75.9 ± 6.7	75.9 ± 7.5	.965
Body mass index (kg/m^2)	23.9 ± 3.7	23.5 ± 3.3	.239
eGFR (mL/min/1.73m2)	69.1 ± 16.4	67.2 ± 15.3	.218
Fasting glucose (mg/dL)	99.0 ± 23.4	95.4 ± 15.7	.099
LDL cholesterol (mg/dL)	117.7 ± 29.2	117.3 ± 27.9	.882
HDL cholesterol (mg/dL)	67.2 ± 17.8	66.9 ± 16.2	.847
Triglyceride (mg/dL)	112.6 ± 66.6	106.2 ± 55.6	.308
History of diabetes, *n* (%)	17.1	20.4	.468

Note: Baseline data are expressed as the means ± standard deviations or counts (percentages).
Age, sex, BMI, eGFR, FBS, LDL, HDL, and TG are compared using Student's *t*-test. History of diabetes is compared using chi-square test.
Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The ATP2B1 rs12817819 allele is associated with an increased risk of RHT in hypertension participants with CAD or suspected ischemic heart disease.[19] Fontana and coworkers[19] used a gene-centric array containing approximately 50 000 SNPs to identify polymorphisms associated with RHT in hypertensive participants with CAD from INVEST-GENES (the INnternational Verapamil-SR Trandolapril STudy—GENEtic Substudy). The authors used logistic regression analysis using an additive model adjusted for age, sex, randomized treatment assignment, BMI, principal components for ancestry, and

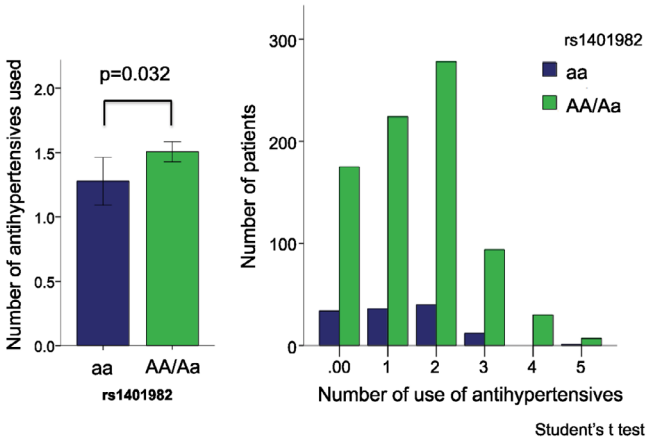

FIGURE 1 Major allele carriers of rs1401982 require more antihypertensive medications.

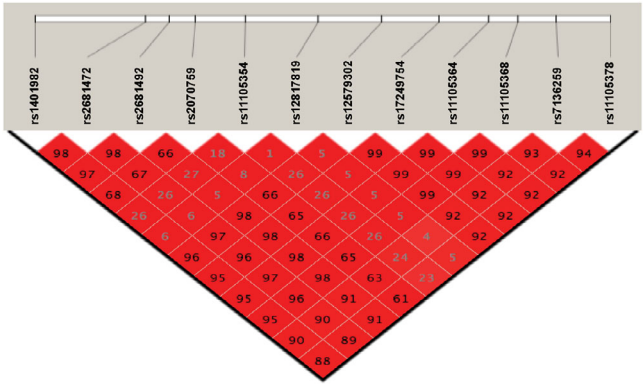

FIGURE 2 Haploview plot depicting pairwise linkage disequilibrium between the 12 studied SNPs with respect to Lewontin's coefficient (D¢) and Pearson's (r) statistics. SNPs rs2681472 and rs1401982 is in linkage disequilibrium with rs11105378. SNP, single nucleotide polymorphism.

other significant predictors of RHT. Replication of the top SNP was conducted in 241 European American women from WISE (Women's Ischemia Syndrome Evaluation). mRNA expression of rs12817819 was measured in whole blood to investigate its functional effect. The authors found that ATP2B1 rs12817819 was associated with RHT in INVEST cohorts. A consistent trend was observed at rs12817819 in WISE. Expression analyses revealed significant differences in ATP2B1 expression by rs12817819 genotype. Our study showed that in the Japanese population, ATP2B1 was associated not only with hypertension, but also with its resistance to treatment. The ATP2B1 rs2681472 polymorphism is associated with salt sensitivity in the Korean population[31] and with blood pressure phenotypes in the Chinese and Japanese populations.[8,11,41]

Tabara and coworkers[18] reported a possible association between the rs1401982 minor allele and a higher risk of hypertension in the Japanese population. In contrast, a negative correlation between rs1401982 and hypertension was found in the Chinese population.[27] The ATP2B1 rs1401982G allele is found in 27.96%, 34.95%, and 39.05% of the Chinese population, respectively.[42] In the present study, the minor allele frequency of the ATP2B1 rs1401982G allele was 34.9%. Compared to non-RHT, RHT is associated with a higher risk of all-cause mortality and adverse health outcomes, especially cardiovascular events. In a study of 200 000 patients in the United States, RHT was associated with an increased risk of major cardiovascular events (myocardial infarction, congestive heart failure or stroke) (HR: 1.47, 95% CI: 1.33–1.62, $p < .001$) within a median follow-up of 3.8 years.[6] In another large cohort of 470 386 individuals with hypertension, RHT was significantly associated with congestive heart failure (HR: 1.46, 95% CI: 1.40–1.52), ischemic heart events (HR: 1.24, 95% CI: 1.20–1.28), cerebrovascular accident (HR: 1.14, 95% CI: 1.10–1.19), end-stage renal disease (HR: 1.32, 95% CI: 1.27–1.37), and all-cause mortality (HR: 1.06, 95% CI: 1.03–1.08).[43] Considering the morbid health outcomes of RHT, strategic approaches that promote early detection and monitoring of patients at risk are needed. Analysis of ATP2B1 SNPs in patients with hypertension can also lead to early prediction of RHT and screening of high-risk patients who are more likely to require more antihypertensive medications.

4.1 | Decreased ATP2B1 mRNA expression is involved in the mechanism of RHT

The current study showed that rs2681472 and rs1401982 were associated with treatment-resistant hypertension. The rs11105378 genotype is associated with *ATP2B1* mRNA expression in umbilical artery smooth muscle cells.[18] In our study, rs11105378 was in linkage disequilibrium with rs2681472 and rs1401982. A previous study involving Chinese participants found that rs1105378 was significantly associated with hypertension.[44] Alleles such as SNPs rs2681492, rs2681472, and rs17249754, which are in strong linkage disequilibrium with rs1105378, have been suggested as genetic markers for the development of hypertension. Further, rs1105378 might be functionally important. In our study, mRNA expression of ATP2B1 might have also been decreased in the major allele carriers of rs2681472 and rs1401982, which were in linkage disequilibrium. Importantly, the results suggest that decreased mRNA expression of ATP2B1 may be associated with RHT via the same mechanism as the previously reported increases in vascular tension and decreases in NO production in mice.

4.2 | Limitations

This study has some limitations. Information on smoking and medication adherence was not available. Furthermore, nutritional intake and physical activity were not considered as confounding factors in our analyses. Since nutritional intake and physical activity are known to reduce blood pressure[45–47] by improving the effects of drugs or promoting a reduction in blood pressure in situations where pharmacological treatment alone is not effective, these could have impacted the results. In addition, pseudo-treatment-resistant hypertension

cannot be completely ruled out. The small number of patients with RHT in this study and the small proportion of patients with RHT among minor homozygotes suggest that SNPs in ATP2B1 may be involved in decreased mRNA expression. However, the SNPs involved in mRNA expression remain unclear. Further large-scale studies or studies involving a large number of RHT patients are needed to confirm our findings.

5 | CONCLUSIONS

ATP2B1 is associated with RHT in Japanese patients with lifestyle-related diseases. Further studies are warranted to determine the underlying mechanisms. We anticipate that examining ATP2B1 through blood tests in clinical settings could lead to personalized treatments, including modifications in antihypertensive medications, in the future. However, oral mucosa is easier and safer to obtain DNA samples than blood. This test would lead to the identification of individuals at risk for treatment-resistant hypertension, enabling the estimation of the required tailored medications.

AUTHOR CONTRIBUTIONS

Conceptualization: Yusuke Kobayashi, Keisuke Yatsu; Data Curation: Yusuke Kobayashi; Formal Analysis: Yusuke Kobayashi; Investigation: Yusuke Kobayashi, Keisuke Yatsu, Keisuke Yatsu, Rina Kawano, Moe Ozawa, Tatsuya Haze, Shiro Komiya, S. Suzuki, Yuki Ohki, Akira Fujiwara, S. Saka; Project Administration: Yusuke Kobayashi, Keisuke Yatsu, Aiko Haruna, Moe Ozawa, Tatsuya Haze, Shiro Komiya, S. Suzuki, Yuki Ohki, Akira Fujiwara, S. Saka, Nobuhito Hirawa, Yoshiyuki Toya, Kouichi Tamura; Supervision: Yusuke Kobayashi, Nobuhito Hirawa, Yoshiyuki Toya, Keisuke Yatsu; Writing—original draft: Yusuke Kobayashi; Writing—review and editing: Yusuke Kobayashi, Keisuke Yatsu, Aiko Haruna, Rina Kawano, Moe Ozawa, Tatsuya Haze, Shiro Komiya, S. Suzuki, Yuki Ohki, Akira Fujiwara, S. Saka, Nobuhito Hirawa, Yoshiyuki Toya, Kouichi Tamura. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

We thank the staff of the Kobayashi Medical Clinic. We are particularly grateful to Dr. Hideo Kobayashi for his dedication and contribution to our research. We believe that the exceptional trust he earned from his patients was the most fundamental reason this study was carried out. Editorial support, in the form of medical writing, assembling tables, creating high-resolution images based on authors' detailed directions, collating author comments, copyediting, fact checking, and referencing, was provided by Editage. The KAKENHI was granted by Japan Society for the Promotion of Science Grant 16K09477, 19K17610.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Yusuke Kobayashi MD, PhD https://orcid.org/0000-0001-7996-3720

REFERENCES

1. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: executive Summary: a Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension*. 2018;71:1269-1324. Erratum in: Hypertension e136-e139. 2018;71. Erratum in: Hypertension 2018;72:e33.
2. Noubiap JJ, Nansseu JR, Nyaga UF, Sime PS, Francis I, Bigna JJ. Global prevalence of resistant hypertension: a meta-analysis of data from 3.2 million patients. *Heart*. 2019;105:98-105.
3. El Rouby N, Cooper-DeHoff RM. Genetics of resistant hypertension: a novel pharmacogenomics phenotype. *Curr Hypertens Rep*. 2015;17:583.
4. Cuspidi C, Macca G, Sampieri L, et al. Target organ damage and non-dipping pattern defined by two sessions of ambulatory blood pressure monitoring in recently diagnosed essential hypertensive patients. *J Hypertens*. 2001;19:1539-1545.
5. Pierdomenico SD, Lapenna D, Bucci A, et al. Cardiovascular outcome in treated hypertensive patients with responder, masked, false resistant, and true resistant hypertension. *Am J Hypertens*. 2005;18:1422-1428.
6. Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation*. 2012;125:1635-1642.
7. Smith SM, Gong Y, Handberg E, et al. Predictors and outcomes of resistant hypertension among patients with coronary artery disease and hypertension. *J Hypertens*. 2014;32:635-643.
8. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet*. 2009;41:677-687.
9. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet*. 2009;41:527-534.
10. Hong KW, Go MJ, Jin HS, et al. Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *J Hum Hypertens*. 2010;24:367-372.
11. Takeuchi F, Isono M, Katsuya T, et al. Blood pressure and hypertension are associated with 7 loci in the Japanese population. *Circulation*. 2010;121:2302-2309.
12. Xi B, Shen Y, Zhao X, et al. Association of common variants in/near six genes (ATP2B1, CSK, MTHFR, CYP17A1, STK39 and FGF5) with blood pressure/hypertension risk in Chinese children. *J Hum Hypertens*. 2014;28:32-36.
13. Hirawa N, Fujiwara A, Umemura S. ATP2B1 and blood pressure: from associations to pathophysiology. *Curr Opin Nephrol Hypertens*. 2013;22:177-184.
14. Long Y, Xia JY, Chen SW, et al. ATP2B1 gene silencing increases insulin sensitivity through facilitating Akt activation via the Ca^{2+}/calmodulin signaling pathway and Ca^{2+}-associated eNOS activation in endothelial cells. *Int J Biol Sci*. 2017;13:1203-1212.
15. Kobayashi Y, Hirawa N, Tabara Y, et al. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. *Hypertension*. 2012;59:854-860.

16. Fujiwara A, Hirawa N, Fujita M, et al. Impaired nitric oxide production and increased blood pressure in systemic heterozygous ATP2B1 null mice. *J Hypertens*. 2014;32:1415-1423. discussion 1423.
17. Ryan ZC, Craig TA, Filoteo AG, et al. Deletion of the intestinal plasma membrane calcium pump, isoform 1, Atp2b1, in mice is associated with decreased bone mineral density and impaired responsiveness to 1, 25-dihydroxyvitamin D3. *Biochem Biophys Res Commun*. 2015;467:152-156.
18. Tabara Y, Kohara K, Kita Y, et al. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese millennium genome project. *Hypertension*. 2010;56:973-980.
19. Fontana V, McDonough CW, Gong Y, et al. Large-scale gene-centric analysis identifies polymorphisms for resistant hypertension. *J Am Heart Assoc*. 2014;3:e001398.
20. Xie M, Yuan S, Zeng Y, et al. ATP2B1 gene polymorphisms rs2681472 and rs17249754 are associated with susceptibility to hypertension and blood pressure levels: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2021;100:e25530.
21. Shimamoto K, Ando K, Fujita T, et al. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2014). *Hypertens Res*. 2014;37:253-390.
22. Andrisin TE, Humma LM, Johnson JA. Collection of genomic DNA by the noninvasive mouthwash method for use in pharmacogenetic studies. *Pharmacotherapy*. 2002;22:954-960.
23. Ledingham MA, Denison FC, Kelly RW, Young A, Norman JE. Nitric oxide donors stimulate prostaglandin F(2alpha) and inhibit thromboxane B(2) production in the human cervix during the first trimester of pregnancy. *Mol Hum Reprod*. 1999;5:973-982.
24. Qi Y, Zhao H, Wang Y, et al. Replication of the top 10 most significant polymorphisms from a large blood pressure genome-wide association study of northeastern Han Chinese East Asians. *Hypertens Res*. 2014;37:134-138.
25. Kelly TN, Takeuchi F, Tabara Y, et al. Genome-wide association study meta-analysis reveals transethnic replication of mean arterial and pulse pressure loci. *Hypertension*. 2013;62:853-859.
26. Heo SG, Hwang JY, Uhmn S, et al. Male-specific genetic effect on hypertension and metabolic disorders. *Hum Genet*. 2014;133:311-319.
27. Wang Y, Zhang Y, Li Y, et al. Common variants in the ATP2B1 gene are associated with hypertension and arterial stiffness in Chinese population. *Mol Biol Rep*. 2013;40:1867-1873.
28. Lee S, Kim SH, Shin C. Interaction according to urinary sodium excretion level on the association between ATP2B1 rs17249754 and incident hypertension: the Korean genome epidemiology study. *Clin Exp Hypertens*. 2016;38:352-358.
29. Kayima J, Liang J, Natanzon Y, et al. Association of genetic variation with blood pressure traits among East Africans. *Clin Genet*. 2017;92:487-494.
30. Xi B, Tang W, Wang Q. Polymorphism near the ATP2B1 gene is associated with hypertension risk in East Asians: a meta-analysis involving 15 909 cases and 18 529 controls. *Blood Press*. 2012;21:134-138.
31. Rhee MY, Yang SJ, Oh SW, et al. Novel genetic variations associated with salt sensitivity in the Korean population. *Hypertens Res*. 2011;34:606-611.
32. Kulkarni S, Lenin M, Ramesh R, Delphine SCW, Velu K. Evaluation of single-nucleotide polymorphisms of transcription factor 7-like 2 and ATP2B1 genes as cardiovascular risk predictors in chronic kidney disease. *Int J Appl Basic Med Res*. 2019;9:221-225.
33. Pike MM, Schildcrout J, Baldwin S, Edwards T, Lipworth L, Robinson-Cohen C. Genetic variants associated with systolic blood pressure in children and adolescents. *J Am Heart Assoc*. 2023;12:e027993.
34. An D, Zhang J, Tang X, et al. Association of ATP2B1 common variants with asymptomatic intracranial and extracranial large artery stenosis in hypertension patients. *Clin Exp Hypertens*. 2019;41:323-329.
35. Lu X, Wang L, Chen S, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat Genet*. 2012;44:890-894.
36. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263-265.
37. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103-109.
38. Althwab SA, Ahmed AA, Rasheed Z, et al. ATP2B1 genotypes rs2070759 and rs2681472 polymorphisms and risk of hypertension in Saudi population. *Nucleosides Nucleotides Nucl Acids*. 2021;40:1075-1089.
39. Takeuchi F, Isono M, Yamamoto K, et al. Heterogeneous effects of association between blood pressure loci and coronary artery disease in east Asian individuals. *Circ J*. 2015;79:830-838.
40. Sombié HK, Kologo JK, Tchelougou D, et al. Positive association between ATP2B1 rs17249754 and essential hypertension: a case-control study in Burkina Faso, West Africa. *BMC Cardiovasc Disord*. 2019;19:155.
41. Liu C, Li H, Qi Q, et al. Common variants in or near FGF5, CYP17A1 and MTHFR genes are associated with blood pressure and hypertension in Chinese Hans. *J Hypertens*. 2011;29:70-75.
42. Wei BL, Yin RX, Liu CX, Deng GX, Guan YZ, Zheng PF. CYP17A1-ATP2B1 SNPs and Gene-Gene and gene-environment interactions on essential hypertension. *Front Cardiovasc Med*. 2021;8:720884.
43. Sim JJ, Bhandari SK, Shi J, et al. Comparative risk of renal, cardiovascular, and mortality outcomes in controlled, uncontrolled resistant, and nonresistant hypertension. *Kidney Int*. 2015;88:622-632.
44. Xu J, Qian HX, Hu SP, et al. Gender-specific association of ATP2B1 variants with susceptibility to essential hypertension in the Han Chinese population. *Biomed Res Int*. 2016;2016:1910565.
45. Jabbarzadeh Ganjeh B, Zeraattalab-Motlagh S, Jayedi A, et al. Effects of aerobic exercise on blood pressure in patients with hypertension: a systematic review and dose-response meta-analysis of randomized trials. *Hypertens Res*. 2024;47:385-398.
46. Chooi YC, Zhang QA, Magkos F, et al. Effect of an Asian-adapted Mediterranean diet and pentadecanoic acid on fatty liver disease: the TANGO randomized controlled trial. *Am J Clin Nutr*. 2023 Nov 29:S0002-9165(23)66285-9. doi:10.1016/j.ajcnut.2023.11.013. [Online ahead of print].
47. Currenti W, Losavio F, Quiete S, et al. Comparative evaluation of a low-carbohydrate diet and a Mediterranean diet in overweight/obese patients with type 2 diabetes mellitus: a 16-week intervention study. *Nutrients*. 2023;16:95.

How to cite this article: Kobayashi Y, Yatsu K, Haruna A, et al. ATP2B1 gene polymorphisms associated with resistant hypertension in the Japanese population. *J Clin Hypertens*. 2024;26:355–362. https://doi.org/10.1111/jch.14785