HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2024 December 01.

Published in final edited form as:

Hypertension. 2023 December ; 80(12): 2665–2673. doi:10.1161/HYPERTENSIONAHA.123.21229.

L-Type Calcium Channel Subunit Alpha1D (CACNA1D) Gene Polymorphisms Associate with Increased Blood Pressure and Salt Sensitivity of Blood Pressure in Caucasians

Ana Maria Stanton[1], **Mahyar Heydarpour**[1], **Jonathan S. Williams**[1], **Gordon H. Williams**[1], **Gail K. Adler**[1]

[1]Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Abstract

Background: Disease-causing mutations in CACNA1D gene occur in aldosterone(ALDO)-producing adenomas and familial hyperaldosteronism. We determined whether single nucleotide polymorphisms(SNPs) in CACNA1D gene associate with higher ALDO resulting in salt sensitivity of blood pressure(SSBP) and increased blood pressure(BP) in men and women.

Methods: Data were obtained from the HyperPATH cohort, where participants completed a cross-over intervention of liberal and restricted sodium diets. Multi-Ethnic Genotyping Array identified 104 CACNA1D SNPs that met quality control. SNP rs7612148 strongly associated with systolic BP and was selected for study in 521 Caucasians in 3 scenarios (A. Hypertensives; B. Normotensives; C. Total population=Hypertensives+Normotensives) using multivariate regression analysis.

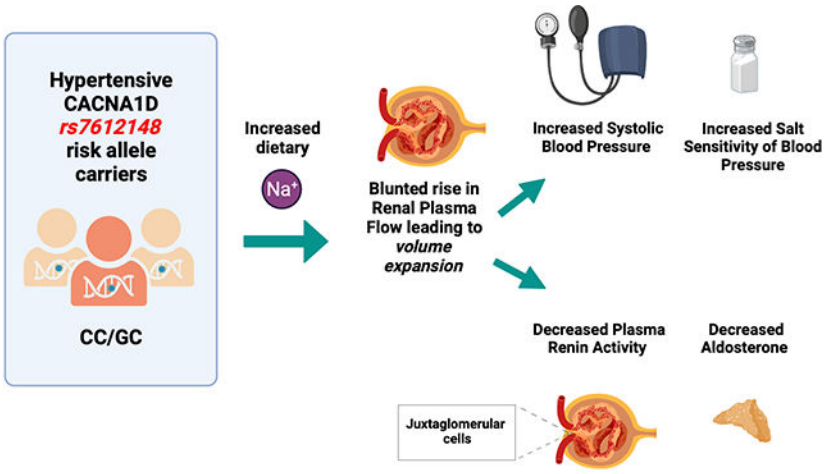
Results: In the total population and in hypertensives but not normotensives, risk allele carriers (CC, GC), as compared with nonrisk allele homozygotes (GG), exhibited higher SSBP and, on liberal sodium diet, higher systolic BP, lower baseline and angiotensin II-stimulated ALDO, and lower plasma renin activity. On restricted sodium diet, BP was similar across genotypes, suggesting sodium restriction corrected/neutralized the genotype effect on BP. Because increased ALDO did not appear to drive the increased SSBP and increased BP on liberal sodium diet, we assessed renal plasma flow(RPF). RPF increase from restricted to liberal sodium diets was blunted in risk allele homozygotes in the total population and in hypertensives. A replication study in another cohort (HyperPATH-B) confirmed BP-genotype associations.

Conclusions: CACNA1D rs7612148 risk allele associates with increased BP and SSBP, likely due to an impaired ability to increase RPF in response to a liberal sodium diet and not to excess ALDO.

Graphical Abstract

Authors and correspondence: Ana Maria Stanton, MD, amstanton@bwh.harvard.edu, Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, 221 Longwood Ave, Boston, MA 02115.

Disclosures: None



Keywords

aldosterone; allele; blood pressure; CACNA1D; renal plasma flow

INTRODUCTION

Primary aldosteronism (PA) is the most common cause of secondary hypertension, with a prevalence of approximately 6% in hypertensives seen in a primary care setting and up to 25% in those with resistant hypertension[1–6]. PA is caused by an excessive renin-independent production of aldosterone (ALDO). Unilateral PA is associated with somatic mutations in the adrenal cortex[3,4,7–9]. PA leads to increased risk of coronary artery disease, left ventricular hypertrophy, heart failure, atrial fibrillation, stroke, and type 2 diabetes as compared with those with similar blood pressure (BP) elevations due to essential hypertension [2–4,10].

Somatic and germline mutations encoding ion channels and ATPases have been identified in aldosterone-producing adenomas and in familial hyperaldosteronism, demonstrating the importance of calcium signaling in the development of PA[3,11–13]. CACNA1D encodes $Ca_V1.3$ channel, a long-lasting L-type, voltage-gated calcium channel expressed in multiple tissues, including adrenal zona glomerulosa [14,15]. Somatic mutations in CACNA1D gene have been found in aldosterone-producing adenomas, aldosterone-producing nodules, and aldosterone-producing cell clusters, also known as aldosterone-producing micronodules, and are the most frequent genetic alterations in young-onset aldosterone-producing nodules [9].

Since gain of function mutations in CACNA1D lead to ALDO overproduction and increased BP [11,16,17], we hypothesized that germline polymorphic variants in CACNA1D would associate with increased ALDO, salt sensitivity of blood pressure (SSBP), and increased BP. We tested this hypothesis in hypertensive and normotensive individuals from the International Hypertension Pathotypes (HyperPATH) cohort A.

METHODS

Data to support this study findings are available from the corresponding author upon reasonable request.

Study Population and Protocol

Participants were part of the International Hypertension Pathotypes cohort (HyperPATH cohort A), and the analyses of this study are original. In brief, hypertensive and normotensive men and women 18–65 years old, were recruited at 5 international study centers: Brigham and Women's Hospital (Boston, MA), Vanderbilt University (Nashville, TN), University of Utah Medical enter (Salt Lake City, UT), Hospital Broussais (Paris, France), and University of La Sapienza (Rome, Italy). Individuals with secondary forms of hypertension were excluded based on the following: extensive history including previous laboratory or radiologic assessments, physical examination, plasma cortisol, electrolytes, BUN, creatinine, TSH, CBC, urinalysis, glucose, and 24-hour urine cortisol, catecholamines, and ALDO levels on a 200–250 mEq sodium intake with urine creatinine used to monitor for completeness of collection. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and mineralocorticoid receptor antagonists were stopped three months prior to study measurements. All other anti-hypertensives were stopped a month prior to data collection. In a few instances, if needed for BP control, amlodipine was continued until two weeks prior to data collection. The study protocol consisted of a randomized, cross-over intervention of liberal (LIB) (200 mEq/day) and restricted (RES) sodium (10 mEq/day) diets for 7 days each. On the last day of each diet, participants were admitted overnight to a Clinical Research Center. After being supine and fasted overnight, BP was assessed every 5 minutes for 20 minutes with an automated device (Dinamap; Critikon, Tampa, FL) and blood samples to evaluate adrenal physiology were collected. In addition, a 24-h urine was collected, and creatinine and sodium were determined to confirm sodium balance (urinary sodium ≥ 150 mmol/24 hours on liberal sodium diet and ≤ 30 mmol/24 hours on restricted sodium diet). HyperPATH samples were analyzed at a central laboratory. ALDO levels were measured using Coat-A-Count Radioimmunometric Assay (RIA) kit (SIEMENS, Los Angeles, CA); Plasma renin activity (PRA) by RIA assay (Diasorin, Stillwater, MN); Plasma renal flow was assessed by para-aminohippurate (PAH) clearance. PAH was measured using High Performance of Liquid Chromatography (HPLC)/ Mass Spectrometry (MS). The detailed protocol has been published previously [18].The protocol was approved by the institutional review board of each study site and informed consent was obtained from all participants. We included all men and women who self-identified as Caucasians. Participants had no medical problems except hypertension and were not taking medications other than antihypertensives, statins, and thyroid medications to normalize TSH.

Genotype Data

DNA was extracted from peripheral leukocytes as previously described [19]. CACNA1D genotyping with a Multi-Ethnic Genotyping Array (Illumina platform, San Diego, CA) yielded 264 single nucleotide polymorphisms (SNPs), out of which 104 were in Hardy-Weinberg equilibrium for both allele and genotype frequencies, had a completion genotype

rate above 95%, and a minor allele frequency (MAF) >1%. We performed association analyses with an additive linear regression model, using PLINK version 1.9. Among these, SNP rs7612148 strongly associated with systolic blood pressure (SBP) after Bonferroni correction and thus was selected for this study. Caucasian individuals with genotype data were analyzed in 3 groups: Hypertensives, Normotensives, and Total population=Hypertensives + Normotensives.

Replication Study

To confirm genotype-BP associations observed in HyperPATH cohort A, we performed association analysis in a replication study of HyperPATH cohort B. The study procedures performed in HyperPATH cohort B were similar to those performed in HyperPATH cohort A. Cohort B participants were recruited from Boston MA, Salt Lake City UT and Nashville TN. Cohort B participants were studied after cohort A participants. 238 individuals in cohort B dataset were Caucasian and had data on CACNA1D SNP rs7612148 as well as phenotyping data.

Statistical Analyses

Hardy-Weinberg Equilibrium was assessed using X^2 test for quality control of genotype data. Continuous variables are presented as mean and standard error of the mean (SEM). Categorical variables are presented as a percentage of the total sample. Baseline analyses included one-way ANOVA and X^2 test. Outcome variables such as SBP, Diastolic BP (DBP), SSBP, and ALDO are presented as least square means and SEM. We performed a multiple linear regression model on the association between CACNA1D rs7612148 genotype and outcome variables and adjusted the model for age, sex, body mass index (BMI), disease states (normotension and hypertension), and study sites (Boston, Nashville, Salt Lake City, Paris, Rome). The β-coefficient in Table 2 and supplemental table S2, S3, and S4 indicates the magnitude of difference between genotypes. Statistical significance was indicated by 2-sided $P < 0.05$. All statistical analyses were performed using Stata Statistical Software: Release 17 (StataCorp LLC, College Station, TX). Figures were done using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, CA) and BioRender.com.

RESULTS

714 Caucasian participants in HyperPath had genotype data that met quality control and SBP on a LIB Na^+ diet. CACNA1D rs7612148 minor allele “G” had a frequency of 45% in Caucasians. The beta coefficient was −3.174 mmHg indicating that the SBP is 3.174 mmHg lower for each G allele a person carries (Supplemental Table S1). 157 participants with diabetes and 36 participants without covariates were excluded from further analyses. 521 normotensive and hypertensive participants were analyzed in 3 scenarios (total population, hypertensives, and normotensives) as shown in Figure 1. In the total population age, sex, and BMI were similar among genotypes. 62% of participants were hypertensives. Baseline characteristics of participants classified by genotype are shown in Table 1. There were more women in the heterozygote risk allele genotype of the normotensive group as compared to other genotypes (P=0.034). 24-h urinary sodium excretion was assessed on the seventh day

of each fixed dietary sodium intake and was similar across genotypes on both LIB and RES sodium diets (Table 2 and Supplemental Table S2).

Blood Pressure and Genotype

CACNA1D SNP rs7612148 risk allele carriers (homozygotes [CC] and heterozygotes [GC]), as compared with nonrisk allele homozygotes (GG), exhibited higher SBP on LIB sodium diet in the total population (CC vs. GG: 132 ± 1 mmHg vs. 128 mmHg ± 1 mmHg, P=0.016; GC vs GG: 133 ± 1 mmHg vs. 128 mmHg ± 1 mmHg, P=0.001) and in the hypertensive group (CC vs. GG: 146 ± 2 mmHg vs. 138 mmHg ± 2 mmHg, P=0.003; CG vs GG: 146 ± 1 mmHg vs. 138 mmHg ± 2 mmHg, P=0.001) but not in normotensives as shown in Figure 2 and Table 2. In addition, in risk allele carriers as compared with nonrisk allele homozygotes, DBP trended higher on LIB sodium diet in the total population (CC vs. GG: 80 ± 1 mmHg vs. 78 mmHg ± 1 mmHg, P=0.071; GC vs GG: 80 ± 1 mmHg vs. 78 mmHg ± 1 mmHg, P=0.060) and was higher in the hypertensive group (CC vs. GG: 88 ± 1 mmHg vs. 85 mmHg ± 1 mmHg, P=0.027; GC vs GG: 88 ± 1 mmHg vs. 85 mmHg ± 1 mmHg, P=0.023) but did not differ significantly in normotensives (Table 2). There was no sex by genotype interaction. Data for men and women are displayed in supplemental Table S3.

On RES sodium diet, SBP and DBP were similar across genotypes in the total population, hypertensives, and normotensives. (Supplemental Table S2). We assessed the change in SBP in response to Angiotensin II (Ang II) on the two diets, separately. In the total population, there were no significant differences by genotype on either the LIB (CC vs. GG: 14 ± 1 mmHg vs. 14 mmHg ± 1 mmHg, P=0.946; GC vs GG: 14 ± 1 mmHg vs. 14 mmHg ± 1 mmHg, P=0.935) or RES (CC vs. GG: 17 ± 1 mmHg vs. 16 mmHg ± 1 mmHg, P=0.847; GC vs GG: 16 ± 1 mmHg vs. 16 mmHg ± 1 mmHg, P=0.888) sodium diets in the change in SBP in response to Ang II. Thus, BP responsiveness to angiotensin II was similar across genotypes.

Salt sensitivity of BP and Genotype

Salt sensitivity of BP (SSBP) (SBP on LIB sodium diet minus SBP on RES sodium diet) was higher in risk allele carriers as compared to nonrisk allele homozygotes in the total population (CC vs. GG: 14 ± 1 mmHg vs. 8 mmHg ± 1 mmHg, P<0.001; GC vs GG: 13 ± 1 mmHg vs. 8 mmHg ± 1 mmHg, P=0.004) and in hypertensives (CC vs. GG: 18 ± 1 mmHg vs. 10 mmHg ± 2 mmHg, P=0.001; GC vs GG: 17 ± 1 mmHg vs. 10 mmHg ± 2, P=0.003) (Table 2). SSBP was similar among genotypes in normotensives. There was no sex by genotype interaction. Data for men and women are displayed in supplemental Table S3.

Renin, Aldosterone, and Genotype

Three measures of RAAS activity – plasma renin activity (PRA), baseline ALDO, and Ang II ALDO – were assessed. Overall, risk allele carriers had lower RAAS activity on a LIB sodium diet and this was more apparent in hypertensive individuals (Table 2). Specifically, as compared with hypertensive nonrisk allele homozygotes (GG), PRA on LIB sodium diet was lower in hypertensive risk allele homozygotes (CC) by 0.15 ng/ml/h (95% CI, −0.29, −0.01, P=0.022) and hypertensive heterozygotes (GC) by 0.18 ng/ml/h (95% CI, −0.34, −0.03, P=0.039). PRA was similar in the total population and in normotensives.

As compared to nonrisk allele homozygotes (GG), baseline ALDO on LIB sodium diet was lower in risk allele homozygotes (CC) in the total population by 1.3 ng/dl (95% CI, −2.3, −0.4, *P*=0.006) and in hypertensives by 1.5 ng/dl (95% CI, −2.7, −0.3, *P*=0.017). Similarly, the ALDO response to angiotensin II infusion (Ang II ALDO), on a LIB sodium diet, was lower in risk allele carriers in the total population and in hypertensive risk allele homozygotes as shown in Table 2. Baseline and Ang II stimulated ALDO were similar in normotensives and did not differ significantly on RES sodium diet (Supplemental Table S2).

Renal Plasma Flow and Genotype

We assessed the changes in renal plasma flow (RPF) from restricted to liberal sodium diets in the 217 individuals in whom we had data. As compared to nonrisk allele homozygotes (GG), the increase in RPF was lower in risk allele homozygotes (CC) in the total population by 31.3 ml/min/1.73m^2 (95% CI, −79.7, −2.9, *P*=0.0031) and in hypertensives by 31.6 ml/min/1.73m^2 (95% CI, −61.2, −2.1, *P*=0.036). The absolute value for change in renal plasma flow from RES sodium to LIB sodium for GC was mid-way between GG and CC in both the total population and hypertensive group but did not reach statistical significance likely related to sample size (Table 2). The change in RPF with increased dietary sodium intake was similar in normotensives among genotypes.

We then assessed the change in RPF in response to Ang II on the two diets, separately. In the total population were no significant differences by genotype on either the LIB (CC vs. GG: −104.3 ± 5 ml/min/1.73m^2 vs. −113.6 ± 5 ml/min/1.73m^2, *P*=0.153; GC vs GG: −109.0 ± 3 ml/min/1.73m^2 vs. −113.6 ± 5 ml/min/1.73m^2, *P*=0.447) or RES (CC vs. GG: −76.1 ± 5 ml/min/1.73m^2 vs. −60.2 ± 7 ml/min/1.73m^2, *P*=0.064; GC vs GG: −71.9 ± 4 ml/min/1.73m^2 vs. −60.2 ± 7 ml/min/1.73m^2, *P*=0.149) sodium diets in the change RPF in response to Ang II. RPF responsiveness to angiotensin II was similar across genotypes. We assessed the change in serum creatinine from restricted to liberal Na diets in the 138 individuals with data; this change did not differ between genotypes (data not shown).

Replication Analyses in HyperPATH B

In our replication cohort, mean age, sex, and BMI were similar among genotypes (Supplemental Table 2). In contrast to the HyperPATH A cohort, 51% of participants in the replication cohort had diabetes, average hemoglobin A_{1c} of 7%. CACNA1D SNP rs7612148 risk allele homozygotes (CC), as compared with nonrisk allele homozygotes (GG), on LIB sodium diet, exhibited higher SBP in the total population (CC vs. GG: 125 ± 2 mmHg vs. 118 mmHg ± 2 mmHg, β=7.14, *P*=0.022) and DBP (CC vs. GG: 73 ± 1 mmHg vs. 68 mmHg ± 1 mmHg, β=4.39, *P*=0.020). SBP and DBP were not different on RES sodium diet (Supplemental Table S4).

DISCUSSION

In this study, we identified a novel CACNA1D polymorphic variant, rs7612148, that is associated with BP. Individuals carrying the risk allele C at rs7612148 had increased BP on LIB sodium diet and greater SSBP. PRA on LIB sodium diet was lower in risk allele carriers, consistent with the concept that individuals were volume expanded on a LIB

sodium diet. Since some aldosterone-producing adenomas have mutations in CACNA1D, we had hypothesized that risk allele carriers, as compared to nonrisk homozygotes, would have higher ALDO leading to volume expansion. We found the opposite - ALDO was decreased on a LIB sodium diet in risk allele carriers as compared to nonrisk homozygotes. Thus, increased ALDO was not driving the volume expansion on LIB sodium diet. Risk allele carriers did have an impaired ability to increase renal plasma flow with increased dietary sodium. Thus, our data suggest that CACNA1D risk allele carriers had higher BP on LIB sodium diet due to an inability to increase renal plasma flow and appropriately excrete sodium (Figure 3). Additionally, sodium restriction corrected the genotype abnormality as BP was similar across genotypes on the restricted sodium diet. We replicated the BP findings in HyperPATH B, a smaller cohort that included people with diabetes.

SSBP is an independent risk factor for cardiovascular death [20] and has a prevalence of 60% in individuals with essential hypertension[21]. This study implicates, for the first time, the CACNA1D gene as one of the genetic factors contributing to SSBP. The observed association between CACNA1D polymorphic variant rs7612148 and BP in our Caucasian cohort is of a magnitude (8 mmHg higher in CC vs. GG in hypertensives) likely to be clinically relevant. Five mmHg reduction of SBP has been shown to reduce the risk of major cardiovascular events by 10% irrespective of previous cardiovascular disease and even at normal or high-normal BP values [22]. Furthermore, metanalyses demonstrate that commonly used antihypertensive medications lower SBP by around 10 mmHg [23,24].

CACNA1D risk allele carriers had suppressed renin on a LIB sodium diet and greater SSBP, suggesting that they are volume expanded on LIB sodium diet. We explored three potential etiologies for this volume expansion - excess ALDO production, altered renovascular responses to Ang II and altered renovascular responses to changes in dietary sodium. First, in contrast to our original hypothesis, ALDO was not elevated in risk allele carriers, suggesting rs7612148 is not associated with a primary overproduction of ALDO. Second, while SSBP has been associated with a blunted renovascular response to AngII with angiotensin converting enzyme inhibitors restoring responsiveness [25], we found that AngII-induced changes in RPF and BP were similar across rs7612148 genotypes. Thus, altered vascular responsiveness to Ang II is not contributing to SSBP associated with rs7612148 genotype. Third, risk allele carriers had a blunted rise in renal plasma flow when transitioning from restricted to liberal sodium diet, suggesting that CACNA1D regulates renal plasma flow and thereby BP. Importantly, removing the environmental burden of a liberal sodium diet corrects the genetic risk.

Consistent with our findings, genome-wide association studies have identified associations between CACNA1D SNPs and BP in East Asian and European cohorts (NHGRI-EBI Catalog of human genome-wide association studies). However, none identified a relationship between CACNA1D rs7612148 and BP, and none of the identified SNPs are in linkage disequilibrium (LD) with rs7612148. This may be because of differences in the investigated populations.

In Chinese cohorts, intronic variants of CACNA1D have been associated with BP, hypertension and the BP response to calcium channel blockers[26 27]. Follow up studies

identified 7 CACNA1D exon locus mutations that correlated with hypertension [28]. Wang and colleagues [29] selected 3 of these mutations and utilized CRISPR-Cas9 technology to generate Sprague-Dawley rats carrying mutations of the CACNA1D gene. Higher BP was observed in rats with double-site mutants versus those with single-site mutant and in rats with single-site mutations versus wild type rats. Increased endothelin-1 and increased vasoreactivity were potential factors in the pathophysiology of the elevated BP; plasma ALDO and potassium were not affected by the CACNA1D mutations. Additionally, rats with CACNA1D mutations exhibited target organ damage evidenced by arteriolar thickening in multiple organs, cardiac hypertrophy, glomerular atrophy. Taken together these data suggest that Cav1.3 is involved in BP regulation and that polymorphic variants in the gene may be associated with hypertension and with the BP response to calcium channel blockade.

A key strength of our study includes a rigorous protocol that controlled for multiple factors that influence BP and ALDO, including dietary sodium, medication use, body positioning, and diurnal variation. Limitations to our study are that the sample size is somewhat limited and restricted to Caucasians. Further, our replication cohort was smaller than the initial cohort, which is a likely explanation for why we were able to replicate the blood pressure findings but no other phenotypes. We do not know how the polymorphic variant at rs7612148 may affect CACNA1D function; however, analysis using HaploReg website (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) demonstrates that rs7612148 (location 3:53600270) is located within a 20 base-pair region (location 3:53600255 to 3:53600275) that has motif binding sites for eleven transcription factors. Future studies are needed to determine whether rs7612148 polymorphic variants alter CACNA1D transcription. Finally, while serum creatinine was similar across genotypes on the two diets, we cannot rule out an impact of genotype on glomerular filtration rate as our dataset does not have inulin clearance, an accurate measure of glomerular filtration rate. Finally, it is important to consider that associations do not provide causality. Additional studies are needed to further understand mechanisms behind the observed associations.

Perspectives

In summary, in Caucasians, CACNA1D gene polymorphisms are associated with greater systolic BP and SSBP. The associations appear to be driven by a blunted increase in renal plasma flow in response to liberal sodium diet, indicating an impaired ability to excrete a sodium load. Approximately 39 % of individuals carry this risk allele suggesting that CACNA1D may be an important contributor to the regulation of BP and SSBP in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

we gratefully acknowledge the support of the staff in the clinical research centers in which the studies were conducted.

Sources of Funding:

This study was conducted with support from the National Institutes of Health: K24 HL103845 (GKA), T32HL007609–34 (GKA and AMS).

Nonstandard Abbreviations

ALDO	Aldosterone
CACNA1D	L-Type Calcium Channel Subunit Alpha1D
BP	Blood Pressure
DBP	Diastolic Blood Pressure
LIB	Liberal
PA	Primary aldosteronism
PRA	Plasma renin activity
RES	Restricted
RPF	Renal plasma flow
SBP	Systolic Blood Pressure
SNP	Single nucleotide polymorphism
SSBP	Salt sensitivity of blood pressure

References

1. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G, Williams TA, Rabbia F, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. J Am Coll Cardiol. 2017;69:1811–1820. doi: 10.1016/j.jacc.2017.01.052 [PubMed: 28385310]
2. Mulatero P, Monticone S, Deinum J, Amar L, Prejbisz A, Zennaro MC, Beuschlein F, Rossi GP, Nishikawa T, Morganti A, et al. Genetics, prevalence, screening and confirmation of primary aldosteronism: a position statement and consensus of the Working Group on Endocrine Hypertension of The European Society of Hypertension. J Hypertens. 2020;38:1919–1928. doi: 10.1097/hjh.0000000000002510 [PubMed: 32890264]
3. Vaidya A, Hundemer GL, Nanba K, Parksook WW, Brown JM. Primary Aldosteronism: State-of-the-Art Review. Am J Hypertens. 2022. doi: 10.1093/ajh/hpac079
4. Zennaro MC, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and treatment of primary aldosteronism. Nat Rev Endocrinol. 2020;16:578–589. doi: 10.1038/s41574-020-0382-4 [PubMed: 32724183]
5. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, Papadopoulos N, Vogiatzis K, Zamboulis C. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. Lancet. 2008;371:1921–1926. doi: 10.1016/S0140-6736(08)60834-X [PubMed: 18539224]
6. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, Ganzaroli C, Giacchetti G, Letizia C, Maccario M, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. J Am Coll Cardiol. 2006;48:2293–2300. doi: 10.1016/j.jacc.2006.07.059 [PubMed: 17161262]

7. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, Strom TM, Monticone S, Amar L, Meatchi T, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. Hypertension. 2014;64:354–361. doi: 10.1161/HYPERTENSIONAHA.114.03419 [PubMed: 24866132]
8. Scholl UI, Goh G, Stölting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. Nat Genet. 2013;45:1050–1054. doi: 10.1038/ng.2695 [PubMed: 23913001]
9. Nanba K, Baker JE, Blinder AR, Bick NR, Liu CJ, Lim JS, Wachtel H, Cohen DL, Williams TA, Reincke M, et al. Histopathology and Genetic Causes of Primary Aldosteronism in Young Adults. J Clin Endocrinol Metab. 2022;107:2473–2482. doi: 10.1210/clinem/dgac408 [PubMed: 35779252]
10. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, Mulatero P. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2018;6:41–50. doi: 10.1016/s2213-8587(17)30319-4 [PubMed: 29129575]
11. Fernandes-Rosa FL, Boulkroun S, Zennaro MC. Genetic and Genomic Mechanisms of Primary Aldosteronism. Trends Mol Med. 2020;26:819–832. doi: 10.1016/j.molmed.2020.05.005 [PubMed: 32563556]
12. Scholl UI. Genetics of Primary Aldosteronism. Hypertension. 2022;79:887–897. doi: 10.1161/hypertensionaha.121.16498 [PubMed: 35139664]
13. Zennaro MC, Boulkroun S, Fernandes-Rosa F. Genetic Causes of Functional Adrenocortical Adenomas. Endocr Rev. 2017;38:516–537. doi: 10.1210/er.2017-00189 [PubMed: 28973103]
14. Feng T, Kalyaanamoorthy S, Barakat K. L-type calcium channels: structure and functions. Ion Channels in Health and Sickness. 2018;77305.
15. Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential. Pharmacol Rev. 2015;67:821–870. doi: 10.1124/pr.114.009654 [PubMed: 26362469]
16. Ortner NJ, Kaserer T, Copeland JN, Striessnig J. De novo CACNA1D Ca(2+) channelopathies: clinical phenotypes and molecular mechanism. Pflugers Arch. 2020;472:755–773. doi: 10.1007/s00424-020-02418-w [PubMed: 32583268]
17. Pinggera A, Mackenroth L, Rump A, Schallner J, Beleggia F, Wollnik B, Striessnig J. New gain-of-function mutation shows CACNA1D as recurrently mutated gene in autism spectrum disorders and epilepsy. Hum Mol Genet. 2017;26:2923–2932. doi: 10.1093/hmg/ddx175 [PubMed: 28472301]
18. Chamarthi B, Williams JS, Williams GH. A mechanism for salt-sensitive hypertension: abnormal dietary sodium-mediated vascular response to angiotensin-II. J Hypertens. 2010;28:1020–1026. doi: 10.1097/HJH.0b013e3283375974 [PubMed: 20216091]
19. Pojoga LH, Underwood PC, Goodarzi MO, Williams JS, Adler GK, Jeunemaitre X, Hopkins PN, Raby BA, Lasky-Su J, Sun B, et al. Variants of the caveolin-1 gene: a translational investigation linking insulin resistance and hypertension. J Clin Endocrinol Metab. 2011;96:E1288–1292. doi: 10.1210/jc.2010-2738 [PubMed: 21613355]
20. Elijovich F, Weinberger MH, Anderson CA, Appel LJ, Bursztyn M, Cook NR, Dart RA, Newton-Cheh CH, Sacks FM, Laffer CL, et al. Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association. Hypertension. 2016;68:e7–e46. doi: 10.1161/HYP.0000000000000047 [PubMed: 27443572]
21. Manosroi W, Williams GH. Genetics of Human Primary Hypertension: Focus on Hormonal Mechanisms. Endocr Rev. 2019;40:825–856. doi: 10.1210/er.2018-00071 [PubMed: 30590482]
22. Collaboration TBPLTT. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease across different levels of blood pressure: an individual participant-level data meta-analysis. Lancet. 2021;397:1625–1636. doi: 10.1016/s0140-6736(21)00590-0 [PubMed: 33933205]
23. Dimmitt SB, Stampfer HG, Martin JH, Ferner RE. Efficacy and toxicity of antihypertensive pharmacotherapy relative to effective dose 50. Br J Clin Pharmacol. 2019;85:2218–2227. doi: 10.1111/bcp.14033 [PubMed: 31219198]

24. Paz MA, de-La-Sierra A, Sáez M, Barceló MA, Rodríguez JJ, Castro S, Lagarón C, Garrido JM, Vera P, Coll-de-Tuero G. Treatment efficacy of anti-hypertensive drugs in monotherapy or combination: ATOM systematic review and meta-analysis of randomized clinical trials according to PRISMA statement. Medicine (Baltimore). 2016;95:e4071. doi: 10.1097/md.0000000000004071 [PubMed: 27472680]

25. Visser FW, Boonstra AH, Titia Lely A, Boomsma F, Navis G. Renal response to angiotensin II is blunted in sodium-sensitive normotensive men. Am J Hypertens. 2008;21:323–328. doi: 10.1038/ajh.2007.63 [PubMed: 18219299]

26. Lu X, Wang L, Lin X, Huang J, Charles Gu C, He M, Shen H, He J, Zhu J, Li H, et al. Genome-wide association study in Chinese identifies novel loci for blood pressure and hypertension. Hum Mol Genet. 2015;24:865–874. doi: 10.1093/hmg/ddu478 [PubMed: 25249183]

27. Kamide K, Yang J, Matayoshi T, Takiuchi S, Horio T, Yoshii M, Miwa Y, Yasuda H, Yoshihara F, Nakamura S, et al. Genetic polymorphisms of L-type calcium channel alpha1C and alpha1D subunit genes are associated with sensitivity to the antihypertensive effects of L-type dihydropyridine calcium-channel blockers. Circ J. 2009;73:732–740. doi: 10.1253/circj.cj-08-0761 [PubMed: 19225208]

28. Bao M, Li P, Li Q, Chen H, Zhong Y, Li S, Jin L, Wang W, Chen Z, Zhong J, et al. Genetic screening for monogenic hypertension in hypertensive individuals in a clinical setting. J Med Genet. 2020;57:571–580. doi: 10.1136/jmedgenet-2019-106145 [PubMed: 32561571]

29. Wang H, Zhu JK, Cheng L, Mao G, Chen H, Wu X, Hong H, Wang C, Lin P, Chen J, et al. Dominant role of CACNA1D exon mutations for blood pressure regulation. J Hypertens. 2022;40:819–834. doi: 10.1097/HJH.0000000000003085 [PubMed: 35142739]

Novelty and Relevance

What is New?

- CACNA1D rs7612148 is a novel gene polymorphism associated with increased blood pressure and salt sensitivity of blood pressure despite lower aldosterone concentrations.
- CACNA1D risk allele carriers exhibit a blunted increase in renal plasma flow in response to increases in dietary sodium.

What is Relevant?

- Our findings provide further understanding of the genetic basis of salt sensitivity of blood pressure, which is a risk factor for cardiovascular morbidity and mortality.

Clinical/Pathophysiological implications

In Caucasians, risk allele carriers of CACNA1D polymorphic variant have dysregulation of renal plasma flow leading on a liberal sodium diet to volume expansion, increased blood pressure and salt sensitivity of blood pressure. Future studies are warranted to determine whether calcium channel blockade would be an especially effective antihypertensive therapy in CACNA1D risk allele carriers.

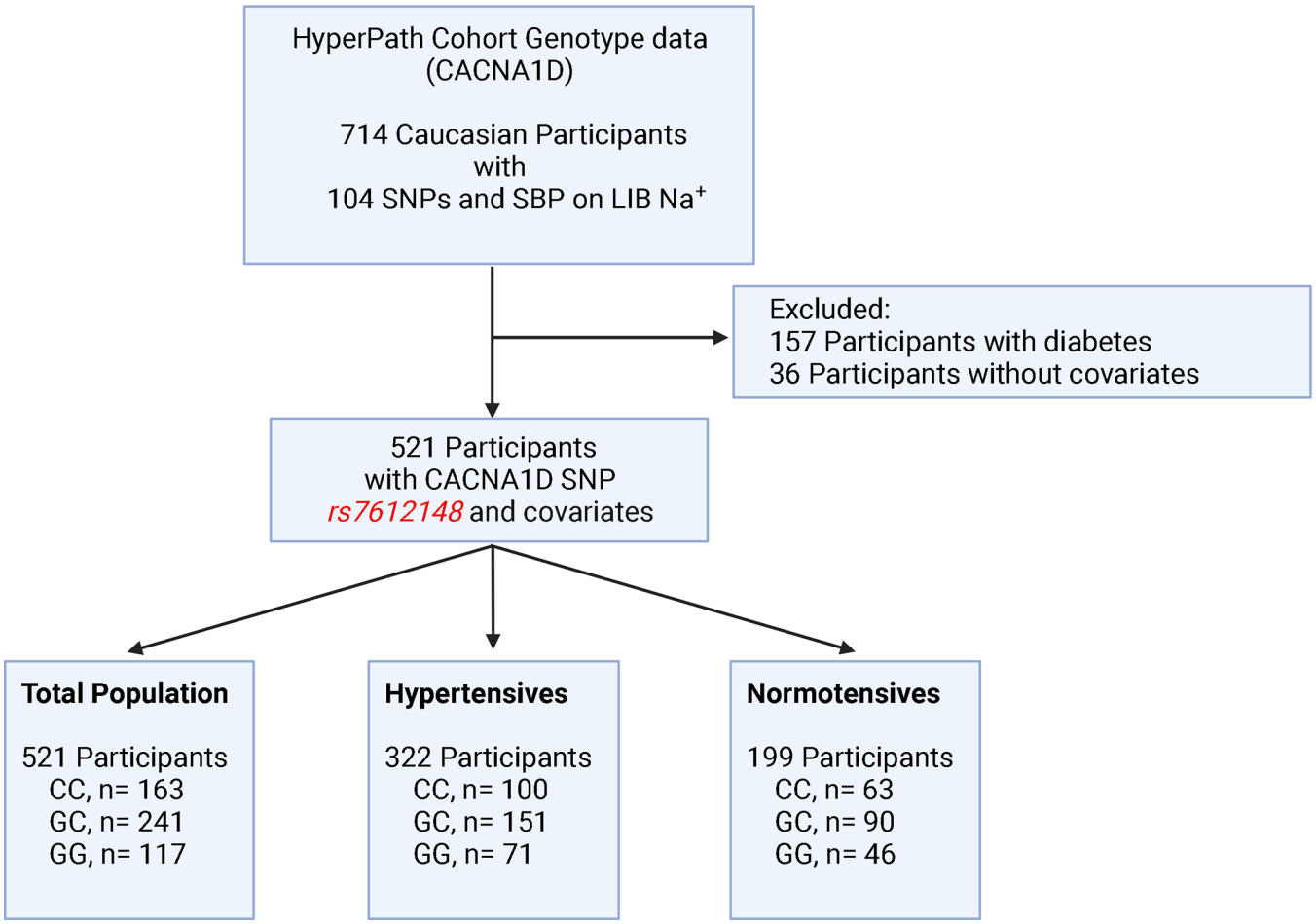


Figure 1. Consort Diagram
Figure was created with BioRender.

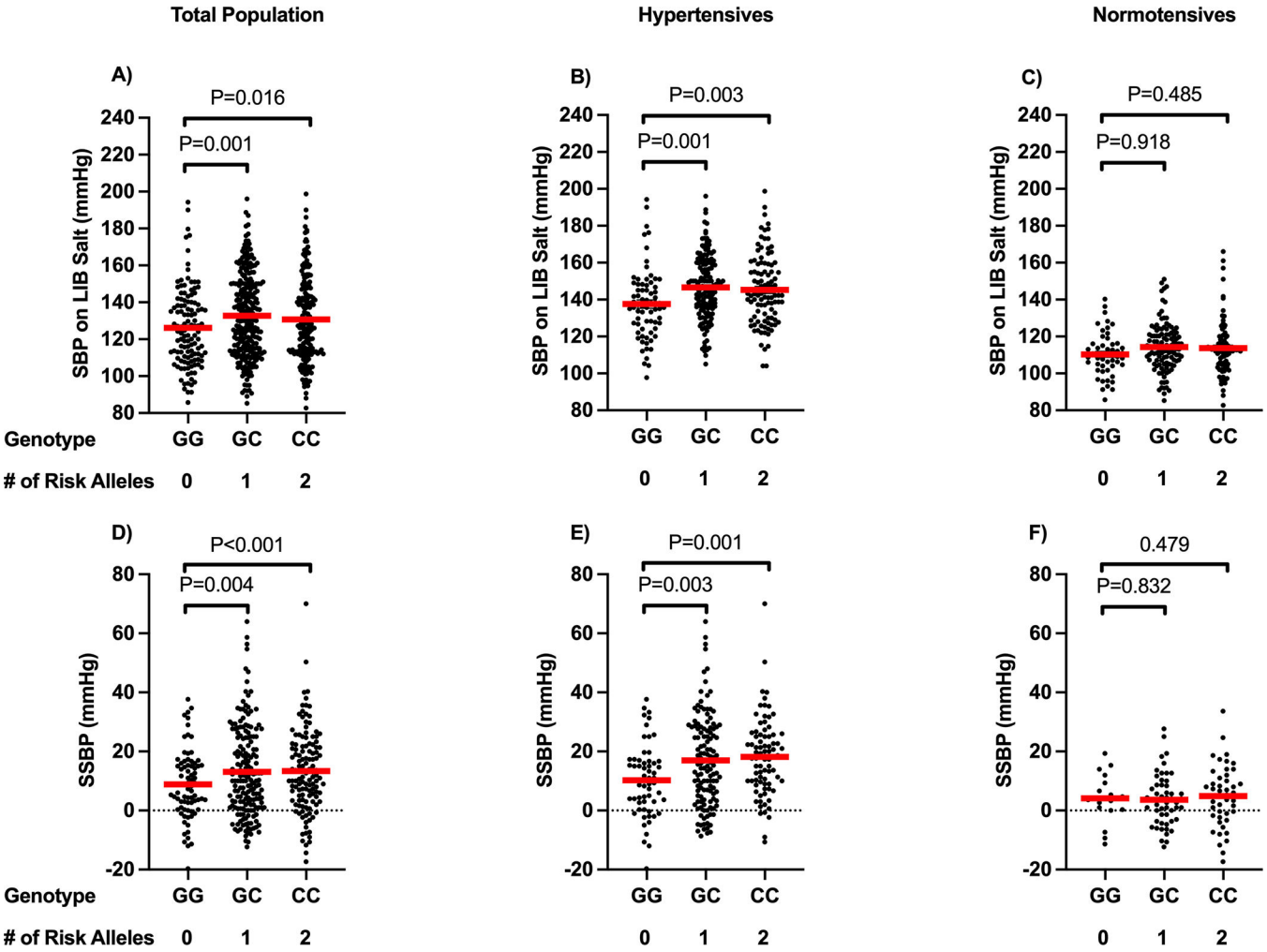


Figure 2. Systolic blood pressure and salt sensitivity of blood pressure in the 3 groups: Hypertensive + Normotensive (Total Population), Hypertensive, and Normotensive

SBP and SSBP are increased in CACNA1D risk allele carriers in the total population and in the hypertensive group, but not in the normotensive group. SBP indicates systolic blood pressure; SSBP, salt sensitivity of blood pressure.

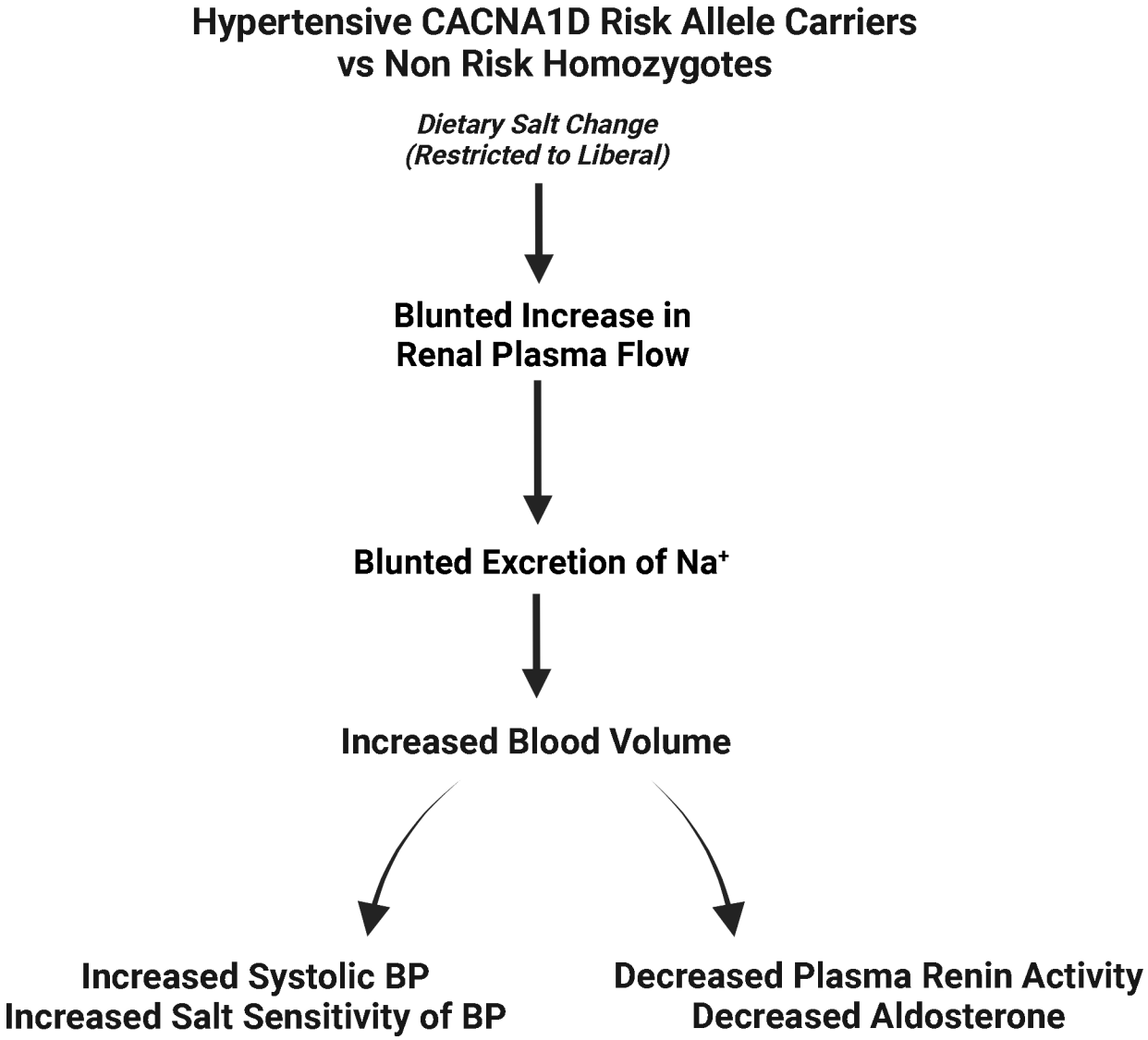


Figure 3. CACNA1D and blood pressure pathophysiology

When hypertensive CACNA1D risk allele carriers are exposed to an increase in dietary sodium, they exhibit a blunted increase in renal plasma flow. This leads to an impaired excretion of sodium, promoting an increase in blood volume, which results in a decrease in plasma renin activity and ALDO on a liberal sodium diet, an increase in systolic BP on a liberal sodium diet, and an increase in salt sensitivity of BP. *Figure was created with BioRender.*

Table 1.

Demographic Data for CACNA1D rs7612148

Characteristic	Total Population (n= 521)			P-Value
	CC	GC	GG	
n (%)	163 (31.29)	241 (46.26)	117 (22.46)	
Female sex, n (%)	65 (28.63)	107 (47.14)	55 (24.23)	0.464
Age, years	44 ± 0.86	45 ± 0.72	43 ± 1.12	0.355
BMI, Kg/m2	27 ± 0.38	28 ± 0.31	27 ± 0.43	0.625
	Hypertensives (n= 322)			
	CC	GC	GG	
n (%)	100 (31.06)	151 (46.89)	71 (22.05)	
Female sex, n (%)	35 (28.69)	64 (52.46)	23 (18.85)	0.278
Age, years	48 ± 0.87	48 ± 0.68	48 ± 1.03	0.904
BMI, Kg/m2	29 ± 0.42	28 ± 0.39	29 ± 0.47	0.996
	Normotensives (n=199)			
	CC	GC	GG	
n (%)	63 (31.66)	90 (45.23)	46 (23.12)	
Female sex, n (%)	30 (28.57)	43 (40.95)	32 (30.48)	0.034
Age, years	37 ± 1.33	39 ± 1.37	36 ± 1.98	0.318
BMI, Kg/m2	25 ± 0.62	26 ± 0.46	25 ± 0.70	0.427

Data are presented as mean ± SEM for continuous variables and n (%) for categorical variables. BMI indicates body mass index.

“C”; risk allele for SBP

“G”; nonrisk allele for SBP

Table 2.

Systolic blood pressure, diastolic blood pressure, salt sensitivity of blood pressure, plasma renin activity, baseline aldosterone, angiotensin II stimulated aldosterone, and delta renal plasma flow on liberal sodium diet in CACNA1D groups.

Variable	Total Population (n=521)				
	Non-risk Allele Homozygotes (GG) n=117	**Risk Allele Heterozygotes (GC) n=241**	**Risk Allele Homozygotes (CC) n=163**	**β-Coefficient**	***P*-value**
SBP, mmHg (LIB Na^+)	128 ± 1	133 ± 1		5.7 (2.3, 9.1)	0.001
			132 ± 1	4.5 (0.9, 8.1)	0.016
DBP, mmHg (LIB Na^+)	78 ± 1	80 ± 1		2.0 (−0.8, 4.0)	0.060
			80 ± 1	2.0 (−0.2, 4.3)	0.071
SSBP, mmHg	8 ± 1	13 ± 1		5.0 (1.6, 8.3)	0.004
			14 ± 1	6.6 (3.1, 10.2)	<0.001
Plasma renin activity, ng/ml/h (LIB Na^+)	0.56 ± 0.05	0.47 ± 0.03		0.09 (−0.20, 0.03)	0.147
			0.43 ± 0.04	0.12 (−0.25, 0.00)	0.054
Baseline aldosterone, ng/dl (LIB Na^+)	6.0 ± 0.4	5.2 ± 0.3		−0.8 (−1.7, 0.1)	0.076
			4.6 ± 0.3	−1.3 (−2.3, −0.4)	0.006
Ang II stimulated aldosterone, ng/ml (LIB Na^+)	18.6 ± 1.0	15.7 ± 0.7		−2.9 (−5.4, −0.4)	0.024
			14.1 ± 0.9	−4.5 (−7.1, −1.8)	0.001
Delta renal plasma flow, ml/min/1.73m2	39.3 ± 11.7	24.6 ± 7.4		−14.7 (−42.0, 12.7)	0.291
			8.0 ± 8.5	−31.3 (−59.7, −2.9)	0.031
24h urinary Na^+ excretion (mEq/L)	223 ± 7	229 ± 5		5.8 (−10.6, 22.1)	0.488
			230 ± 6	6.3 (−11.3, 23.9)	0.481
	Hypertensives (n=322)				
	Non-risk Allele Homozygotes (GG) n=71	**Risk Allele Heterozygotes (GC) n=151**	**Risk Allele Homozygotes (CC) n=100**	**β-Coefficient**	***P*-value**
SBP, mmHg (LIB Na^+)	138 ± 2	146 ± 1		8.3 (3.5, 13.1)	0.001
			146 ± 2	7.7 (2.6, 12.9)	0.003
DBP, mmHg (LIB Na^+)	85 ± 1	88 ± 1		3.3 (0.5, 6.1)	0.023
			88 ± 1	3.5 (0.4, 6.5)	0.027
SSBP, mmHg	10 ± 2	17 ± 1		6.5 (2.3, 10.7)	0.003
			18 ± 1	7.8 (3.2, 12.4)	0.001
Plasma renin activity, ng/ml/h (LIB Na^+)	0.62 ± 0.06	0.47 ± 0.04		−0.15 (−0.29, −0.01)	0.039
			0.44 ± 0.05	−0.18 (−0.34, −0.03)	0.022
Baseline aldosterone, ng/dl (LIB Na+)	6.0 ± 0.5	5.4 ± 0.3		−0.6 (−1.7, 0.5)	0.269
			4.6 ± 0.4	−1.5 (−2.7, −0.3)	0.017
Ang II stimulated aldosterone, ng/ml (LIB Na^+)	17.9 ± 1.3	15.0 ± 0.9		−3.0 (−6.9, 0.1)	0.056
			13.7 ± 1.1	−4.0 (−7.5, −1.0)	0.010
Delta renal plasma flow, ml/min/1.73m2	35.7 ± 12.0	20.6 ± 7.3		−15.1 (−43.0, 12.8)	0.286
			4.05 ± 8.9	−31.6 (−61.2, −2.1)	0.036

24h urinary Na^+ excretion (mEq/L)	217 ± 8	229 ± 6		11.7 (−9.3, 32.8)	0.273
			229 ± 8	12.2 (−10.7, 35.1)	0.296
	Normotensives (n=199)				
	Non-risk Allele Homozygotes (GG) n=46	**Risk Allele Heterozygotes (GC) n=90**	**Risk Allele Homozygotes (CC) n=63**	**β-Coefficient**	***P*-value**
SBP, mmHg (LIB Na^+)	112 ± 2	112 ± 1		0.2 (−3.6, 4.0)	0.918
			111 ± 1	−1.4 (−5.5, 2.6)	0.485
DBP, mmHg (LIB Na^+)	67 ± 1	67 ± 1		0.1 (−2.7, 2.9)	0.954
			67 ± 1	0.5 (−2.5, 3.6)	0.730
SSBP, mmHg	4 ± 2	3 ± 1		−0.5 (−5.3, 4.3)	0.832
			5 ± 1	1.8 (−3.2, 6.7)	0.479
Plasma renin activity, ng/ml/h (LIB Na^+)	0.46 ± 0.08	0.45 ± 0.06		−0.01 (−0.21, 0.20)	0.952
			0.43 ± 0.07	−0.03 (−0.24, 0.19)	0.814
Baseline aldosterone, ng/dl (LIB Na^+)	5.7 ± 0.6	4.7 ± 0.4		−1.0 (−2.4, 0.4)	0.159
			4.9 ± 0.5	−0.9 (−2.4, 0.7)	0.265
Ang II stimulated aldosterone, ng/ml (LIB Na^+)	18.6 ± 1.7	16.6 ± 1.2		−2.0 (−6.2, 2.1)	0.335
			15.4 ± 1.5	−3.2 (−7.7, 1.2)	0.157
Delta renal plasma flow, ml/min/1.73m2	45.0 ± 23.5	30.7 ± 15.8		−14.3 (−70.5, 41.9)	0.614
			13.8 ± 17.0	−31.2 (−89.0, 26.7)	0.287
24h urinary Na^+ excretion (mEq/L)	235 ± 11	231 ± 8		−4.3 (−30.8, 22.2)	0.749
			228 ± 9	−7.4 (−35.7, 21.0)	0.608