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Rare variants in genes encoding subunits of the epithelial Na^+ channel are associated with blood pressure and kidney function

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Abstract

Background: The epithelial Na^+ channel (ENaC) is intrinsically linked to fluid volume homeostasis and blood pressure. Specific rare mutations in *SCNN1A*, *SCNN1B*, and *SCNN1G,* genes encoding the α, β, and γ subunits of ENaC, respectively, are associated with extreme blood pressure phenotypes. No associations between blood pressure and *SCNN1D*, which encodes the δ subunit of ENaC, have been reported. A small number of sequence variants in ENaC subunits have

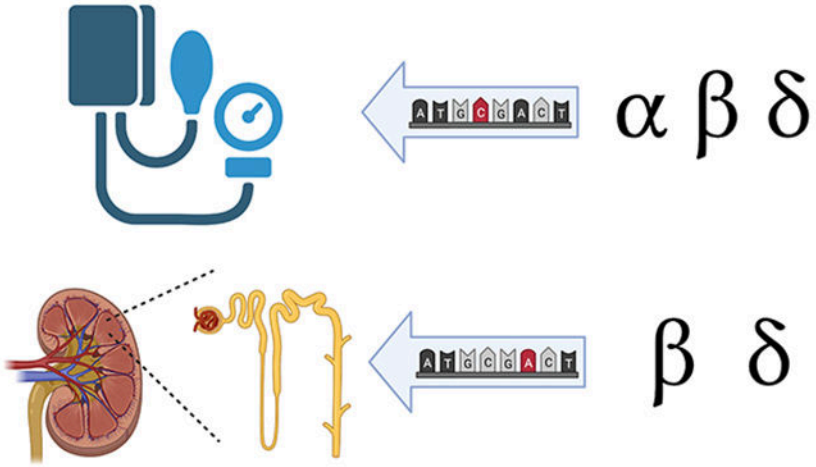
been reported to affect functional transport *in vitro* and/or blood pressure. The effects of the vast majority of rare and low frequency ENaC variants on blood pressure are not known.

Methods: We explored the association of low frequency and rare variants in the genes encoding ENaC subunits, with systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP). Using whole-genome sequencing data from fourteen studies participating in the TOPMed Whole-Genome Sequencing Program and sequence kernel association tests.

Results: We found that variants in *SCNN1A* and *SCNN1B* were associated with DBP and MAP ($p < 0.00625$). Although *SCNN1D* is poorly expressed in human kidney tissue, *SCNN1D* variants were associated with SBP, DBP, MAP, and PP ($p < 0.00625$). ENaC variants in two of the four subunits (*SCNN1B*, and *SCNN1D*) were also associated with estimated glomerular filtration rate ($p < 0.00625$), but not with stroke.

Conclusions: Our results suggest that variants in extrarenal ENaCs, in addition to ENaCs expressed in kidneys, influence blood pressure and kidney function.

Graphical Abstract



Keywords

ENaC; blood pressure; estimated glomerular filtration rate; stroke; gene variants

Introduction

The regulated absorption of filtered Na^+ along the nephron helps govern extracellular fluid volume and blood pressure. The aldosterone-sensitive distal nephron (ASDN) has a critical role in Na^+ absorption and is where key volume regulatory hormones and signaling pathways affect the absorption of filtered Na^+. The epithelial Na^+ channel (ENaC) is expressed in the distal aspects of the ASDN where it mediates the absorption of filtered Na^+ across the luminal membrane of principal cells[1,2]. In addition to its role in Na^+ absorption, ENaC function is required for K^+ secretion in the ASDN[1,3]. ENaCs are also found at other tissues that affect total body Na^+ and blood pressure. It functions as a salt sensor in lingual

epithelia and influences salt intake, whereas expression in the distal colon has a role, albeit minor, in absorption of ingested Na^+[4,5]. ENaC expression in antigen presenting immune cells has been proposed to facilitate release of cytokines in response to increased salt intake, which contribute to an increase in blood pressure[6,7]. ENaCs in vascular smooth muscle and endothelial cells may influence vascular tone[7,8]. Finally, ENaCs are expressed at specific sites in the central nervous system where they influence autonomic tone and blood pressure[9].

ENaCs are formed from structurally related subunits, termed α, β, γ, and δ, which are encoded by the genes *SCNN1A*, *SCNN1B*, *SCNN1G*, and *SCNN1D*, respectively. These are Na^+ selective channels that are primarily αβγ or δβγ heterotrimers, which exhibit differences in functional and regulatory properties[2]. ENaCs in human kidney are primarily a αβγ heterotrimer[10], while ENaCs containing the δ subunit are expressed in other tissues[11,12]. Each ENaC subunit has two transmembrane domains. The second transmembrane domain from each subunit within a channel complex contributes to the channel pore, cation selectivity filter, and gate. The transmembrane domains are connected by a large, structurally complex extracellular domain that functions as a sensor, where specific extracellular factors, including monovalent cations and anions (Na^+, Cl^-, H^+), peptides, proteases, and shear stress interact with the extracellular domain to regulate channel activity[2]. Short cytoplasmic amino (N)- and carboxyl (C)-termini also have key regulatory sites. For example, a Pro-Tyr (PY) motif in the cytoplasmic C-termini of ENaC subunits is a binding site for the ubiquitin ligase NEDD4–2 that facilitates channel ubiquitination at the cell surface and subsequent internalization[13]. The cytoplasmic N-termini of ENaC subunits have a His-Gly motif that affects channel gating[14].

Several rare ENaC variants that have large effects on blood pressure with Mendelian inheritance have been identified in *SCNN1A*, *SCNN1B*, and *SCNN1G*. These disorders include (i) Liddle syndrome, an autosomal dominant disorder where specific gain-of-function mutations in *SCNN1B*, and *SCNN1G* are associated with hypertension and hypokalemia, and (ii) pseudohypoaldosteronism type I (PHA1), an autosomal recessive disorder where specific loss-of-function mutations in *SCNN1A*, *SCNN1B*, or *SCNN1G* are associated with hypotension and hyperkalemia[15]. Liddle syndrome mutations primarily result in disruption of the PY motif, significantly increasing channel residency time at the cell surface[13]. PHA1 mutations have been described that result in specific deletions or amino acid substitutions that cause a profound loss of function *in vitro*[14,15].

Our group and others have identified a growing number of sites, including rare single nucleotide variants (SNVs) within the extracellular domains, where mutations affect channel gating activity[2,12]. This is congruent with the extracellular domains' role in sensing extracellular factors and regulating channel gating in response to these factors. For most variants that alter ENaC function and are not associated with Liddle syndrome or PHA1, it is unclear whether they influence blood pressure, serum [K^+], or the prevalence of blood pressure-associated disorders including stroke, myocardial infarction, and chronic kidney disease in specific populations.

Using whole-genome sequencing (WGS) and phenotype data available through the Trans-Omics in Precision Medicine (TOPMed) Whole-Genome Sequencing Project, we examined

common functional human ENaC variants and arrays of low-frequency and rare ENaC variants for association with blood pressure levels and related traits and health outcomes.

Materials and Methods

WGS and harmonized blood pressure phenotype data were available for analysis from 28,058 participants in fourteen studies from TOPMed (selected from the > 142,000 individuals in forty-one studies in TOPMed) (Table 1). After excluding 300 individuals < 20 years old or > 90 years old and including 641 individuals from the Samoan Soifua Manuia Study that were not included in the TOPMed cohort, 28,399 individuals from the harmonized blood pressure phenotype dataset were included in our main (blood pressure) analyses. Subsets of this sample were included in our secondary analyses: 9,090 for total strokes (4,399 cases and 4,691 controls), and 14,557 for eGFR. The individuals included in our main analyses were from six self-reported ancestry groups (58.3% European, 29.9% African, 6.3% Asian, 4.1% Samoan, 0.2% Native American, and 1.2% other) (Table S2). To account for underlying population substructure, we used principal components of ancestry (PCAs) calculated by TOPMed. Based on the spaghetti plot (Figure S1), we concluded that PCAs 1–11 accounted for the vast majority of inter-ancestry variance in our population and should be included as covariates in our analyses. Relatedness in the analysis cohorts was controlled for using a genetic relatedness matrix (GRM) calculated by TOPMed.

We conducted single variant analyses for three common (minor allele frequency (MAF) ≥ 0.05) ENaC variants, two of which are functional variants[16,17]. We also performed sequence kernel association tests (SKAT) and burden tests for all low-frequency and rare variants (MAF < 0.05), as well as separate SKAT for rare (MAF < 0.01) and low-frequency (0.01 ≤ MAF < 0.05) SNVs within the genomic regions of *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G*. In each analysis, we have tested for association with systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), estimated glomerular filtration rate (eGFR), and stroke.

Finally, we assessed *SCNN1D* expression in monocytes from eleven healthy patients. Monocytes isolated from heparinized blood were cultured in media containing either 150 mM NaCl or 190 mM NaCl for 72 hours. Total RNA isolated from the monocytes was sequenced, and expression analysis was performed to determine whether *SCNN1D* was expressed in those cells.

Additional methods details are presented in the Supplemental Materials[18–27].

Results

Three common functional ENaC variants—αA334T, αT663A, and βG165R—are not associated with SBP, DBP, MAP, or PP

Two common ENaC α subunit variants, αA334T (rs11542844, MAF = 0.17) and αT663A (rs2228576, MAF = 0.27) alter channel function in heterologous expression systems[16,17], although previous studies largely suggest that these variants do not affect blood pressure phenotypes[28–30]. There is also a common ENaC variant in the β subunit, βG165R

(rs2303157, MAF = 0.24), although its effect on ENaC function has not been described. To account for the impact of these common variants in our analyses of low frequency variants, we tested whether any of these common variants were associated with blood pressure phenotypes. We conducted single-variant analyses for the association of each variant with SBP, DBP, MAP and PP using age, sex, BMI, study, and the first eleven PCAs as covariates and controlled for relatedness with a GRM. No association was found between any of the variants and SBP, DBP, MAP, or PP (Table 2). These results are largely consistent with previous findings for αA334T and αT663A[16,17,28–30].

Low frequency and rare ENaC variation in specific subunits is, in aggregate, associated with SBP, DBP, MAP or PP

We performed SKAT to determine the impact of aggregated low frequency (MAF < 0.05) variation within the genomic regions of *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* on four blood pressure measures (SBP, DBP, PP, and MAP) using age, sex, BMI, and PCAs 1–11 as covariates, and controlled for relatedness using a GRM. The threshold for significance, based on the number of genes tested, was 0.00625 (see Methods). There were 3,972 *SCNN1A*, 12,334 *SCNN1B*, 2,405 *SCNN1D*, and 4,093 *SCNN1G* variants with a MAF < 0.05 within the promoter regions and gene boundaries of each gene that also had genotype missingness rates < 0.15. These variants present across the 14 studies were included in the analyses. There was a significant association of *SCNN1A* aggregate variants with DBP ($p = 0.00004$) and MAP ($p = 0.0002$), but not with SBP or PP (Table 4). *SCNN1B* variants were significantly associated with DBP ($p = 0.002$) and MAP ($p = 0.003$). *SCNN1D* variants were significantly associated with all of four blood pressure phenotypes: SBP ($p = 0.0008$), DBP ($p = 0.002$), PP ($p = 0.003$), and MAP ($p = 0.0008$). Finally, those at *SCNN1G* were not significantly associated with any of the four blood pressure phenotypes. As a negative control, DBP was permuted by reassignment at random in the population and no associations were observed between any of the subunit genes and the permuted phenotype values. We also performed SKAT with the combined variants from *SCNN1A*, *SCNN1B*, and *SCNN1G* (20,399 in total) and each of the four blood pressure measures. Because of the associations with SBP, DBP, PP, and MAP, *SCNN1D* variants were not included in the combined analyses of blood pressure phenotypes to avoid biasing the results. The combined analysis showed association with both DBP ($p = 0.0002$) and MAP ($p = 0.0006$, see Table 3).

We repeated the analyses with rare (MAF < 0.01) and low frequency (0.01 ≤ MAF < 0.05) variants separately to determine if one class of variants was driving the significance of our findings. In our analyses of rare variants: *SCNN1A* (3,859 variants) were significantly associated with DBP ($p = 0.0004$) and MAP ($p = 0.002$); *SCNN1B* (11,925 variants) were also associated with DBP ($p = 0.003$) and MAP ($p = 0.006$); the combined analysis of *SCNN1A*, *SCNN1B*, and *SCNN1G* (19,806 variants) was associated with DBP ($p = 0.0004$) and MAP ($p = 0.001$); and the combined analysis of *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* (22,099 variants) was associated with DBP ($p = 0.0002$) and MAP ($p = 0.0009$). Neither *SCNN1D* (2,293 variants) nor *SCNN1G* (4,022 variants) were associated with any of the four phenotypes (See Table 3).

In our analyses of low frequency variants: *SCNN1A* (113 variants) were significantly associated with DBP ($p = 0.0007$) and MAP ($p = 0.002$); *SCNN1D* (112 variants) were associated with SBP ($p = 0.0005$), DBP ($p = 0.003$), PP ($p = 0.005$), and MAP ($p = 0.0007$); the combined analysis of *SCNN1A*, *SCNN1B*, and *SCNN1G* (593 variants) were associated with DBP ($p = 0.004$) and MAP ($p = 0.005$); and the combined analysis of *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* (705 variants) were associated with SBP ($p = 0.006$), DBP ($p = 0.001$), and MAP ($p = 0.001$). Neither *SCNN1B* (409 variants) nor *SCNN1G* (71 variants) were associated with any of the four phenotypes (See Table 3).

We repeated these analyses using another gene-based test, the burden test, and found no association between *SCNN1A*, *SCNN1B*, *SCNN1D*, or *SCNN1G* and SBP, DBP, MAP, or PP, suggesting that our analyses include variants that are increasing blood pressure as well as variants that are decreasing blood pressure (Table S3). We also performed SKAT analyses on *SCNN1A* (MAF < 0.05), *SCNN1*B (MAF < 0.05), and *SCNN1D* (0.01 ≤ MAF 0.05) upstream, downstream, missense, synonymous, and intronic variants, separately, with DBP to determine if any class of variant was contributing to our results. We found no association between DBP and *SCNN1A*, *SCNN1B*, or *SCNN1D* upstream, downstream, missense, synonymous, and intronic variants (Table S4).

Low frequency and rare ENaC variation in specific subunits is, in aggregate, associated with eGFR

High blood pressure is associated with chronic kidney disease (CKD) and stroke[31,32]. ENaC variants associated with altered blood pressure may also be associated with health outcomes such as stroke, eGFR and CKD. We hypothesized that ENaC variants are associated with eGFR and that SBP and DBP mediate these associations. Stepwise regression indicated that DBP was contributing to the variance of the null model for eGFR and therefore, we included DBP as a covariate in our eGFR analyses. We performed SKAT analyses using data from 14,557 individuals from seven TOPMed studies (Tables S5 and S6) to test for associations between eGFR (from which CKD is diagnosed) and variants with MAF < 0.05 in *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G*. In addition to DBP, models were adjusted for age, sex, BMI, and PCs 1–11 as covariates. We identified associations between eGFR and *SCNN1B* (n = 8,770) and *SCNN1D* (n = 1,758) variants ($p = 0.005$, $p = 0.002$, respectively), but not the *SCNN1A* (n = 2,739) and *SCNN1G* (n = 2,868) variants included in the analyses ($p = 0.01$, and $p = 0.1$, respectively) (Table 4). When combining ENaC variants (MAF < 0.05) from all four subunits (16,135 variants), we observed a significant association between the variants and eGFR (p=0.0003). As a negative control, eGFR phenotypes were permuted to randomize eGFR across samples and the analyses were repeated. There were no associations with permuted eGFR.

We repeated these analyses separately with rare and low frequency variants. Only low frequency variants in *SCNN1D* (110 variants) were significantly associated with eGFR ($p = 0.001$). There was no association between eGFR and rare variants in individual subunits, *SCNN1A* (2,626 variants), *SCNN1B* (8,365 variants), *SCNN1D* (1,648 variants), and *SCNN1G* (2,797 variants) ($p = 0.008$, $p = 0.007$, $p = 0.2$, and $p = 0.01$, respectively, see Table 4). However, when combining ENaC variants of all four subunits (15,436 variants)

we observed significant associations between rare variants and low frequency variants and eGFR ($p = 0.002$ and 0.003, respectively).

These analyses were repeated using burden tests. Of the conditions tested, only *SCNN1D* low frequency variants ($0.01 \leq MAF < 0.05$) were significantly associated with eGFR, suggesting strong directionality of effect for these variants (Table S7).

Low-frequency and rare variation in SCNN1A, SCNN1B, SCNN1D, and SCNN1G is not associated with stroke

We also performed SKAT analyses using data from a subset of individuals from the blood pressure analyses consisting of 9,090 individuals (4,399 cases and 4,691 controls) from the Women's Health initiative (Tables S8 and S9) to test for associations between stroke and low frequency and rare variation ($MAF < 0.05$) in *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* using age, sex, BMI, and PCAs 1–11 as covariates. After removing variants monomorphic in this subset, 2,330 S*CNN1A* variants 6,965 *SCNN1B* variants, 1,621 SCNN1D, and 2,208 *SCNN1G* variants remained in the analyses. There was no significant association of combined variants with $MAF < 0.05$ in *SCNN1A*, *SCNN1B*, *SCNN1D*, or *SCNN1G* with overall stroke ($p = 0.08$, $p = 0.5$, $p = 0.5$, and $p = 0.2$, respectively) (Table 5). Additionally, we performed SKAT with 13,124 variants from *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* combined. The combined ENaC variation SKAT was not associated with stroke ($p = 0.3$). We repeated these analyses with rare and low frequency variants separately. There were no associations between *SCNN1A* (2,216 and 114 variants, respectively), *SCNN1B* (6,556 and 409 variants, respectively), *SCNN1D* (1,497 and 124 variants, respectively), *SCNN1G* (2,137 and 71 variants, respectively), or all four subunits combined (12,406 and 718 variants, respectively) (See Table 5). We repeated these analyses using burden tests, and found no association between *SCNN1A*, *SCNN1B*, *SCNN1D*, or *SCNN1G* and stroke (Table S10).

SCNN1D is expressed in human monocytes

The ENaC δ subunit is poorly expressed in transporting epithelia that regulate total body Na^+ (kidney and colon)[10,33], in contrast to the α, β, and γ subunits. Our results showing association of *SCNN1D* low-frequency and rare variants with blood pressure phenotypes, and not *SCNN1G*, suggest that ENaC function in Na^+ sensing cells influence blood pressure variation in the general population. *SCNN1D* is expressed in the central nervous system, where rare variants could influence blood pressure parameters. Antigen-presenting cells have recently been reported to have an ENaC-dependent response to increased Na^+ intake, leading to an increase in blood pressure[6]. We previously showed that *SCNN1A* and *SCNN1G*, but not *SCNN1B*, are expressed in monocyte-derived dendritic cells in mice[6]. To determine whether *SCNN1D* may have a role in Na^+ sensing in human monocytes and monocyte-derived cells, we performed RNA sequencing on human monocytes isolated from 11 volunteers and exposed to either normal or elevated sodium. We detected *SCNN1D* expression in cells from each of the volunteers (Figure 1). We did not detect an effect of elevated sodium exposure on *SCNN1D* expression in these cells. Nonetheless, expression of *SCNN1D* suggests the δ subunit of ENaC may be involved in Na^+ sensing in these cells.

Discussion

The consequences of variants that lead to extreme increases or decreases in ENaC activity, which manifest as Liddle syndrome and PHA1, respectively, are well known monogenic disorders. There are few studies examining the effects of other ENaC variants on complex traits such as blood pressure variation or hypertension-related health outcomes. We found that, in aggregate, low frequency and rare variants in *SCNN1A* and *SCNN1B* are associated with DBP and MAP, with both rare and low frequency variants contributing to the associations of *SCNN1A* with DBP and MAP and rare variants driving the associations of *SCNN1B* with MAP and DBP. Variants in *SCNN1D* are associated with SBP, DBP, PP, and MAP, and are driven by low-frequency variants. Variants in *SCNN1B* and *SCNN1D* are associated with eGFR, which, for SCNN1D, is, again, driven by low frequency variants. While there has been speculation about the impact of common and rare functional ENaC variants on blood pressure[7,34], we found no evidence that these common functional variants (αA334T, αT663A) are associated with SBP, DBP, PP, or MAP, consistent with the evidence provided by ClinVar[35]. These results are largely in agreement with previous findings[16,17,28–30]. We did not find an association of low frequency and rare variants in *SCNN1A, SCNN1B, SCNN1D*, and *SCNN1G*, assessed either separately or together, with stroke, despite the associations of hypertension with stroke[31,32]. Potential reasons for the lack of associations are small effect of these combined variants on blood pressure variation or heterogeneity of effects across ancestries that may have reduce power to detect effects, since these outcomes are likely mediated through increases in blood pressure. In addition, the SKAT test combines variants that increase and decrease blood pressure and therefore does not provide estimates for associations. As analyses of a large database may result in spurious results, and it will be important to confirm our key findings using other large databases.

ENaCs in human kidney are primarily αβγ channels[10]. We expected to find associations of low frequency and rare variants in *SCNN1A*, *SCNN1B*, and *SCNN1G* with blood pressure traits based on the known roles of ENaC in regulating the reabsorption of filtered Na^+ in the ASDN and whole-body Na^+ content, which are major determinants of extracellular fluid volume and blood pressure[1,2]. ENaC-dependent Na^+ absorption also has an important role in regulating serum [K^+], as it is tightly coupled to renal K^+ secretion mediated by the K^+ channels Kir1.1 and BK[1,3]. A lower serum [K^+] in the setting of increased in ENaC activity, coupled with increased K^+ secretion, is predicted to enhance activity of the Na^+–Cl^- cotransporter (NCC) in the DCT[36]. It was surprising that variants in *SCNN1G* were not associated with blood pressure traits, given what is known regarding the roles of specific sites in the γ subunit in regulating channel activity. We and others have identified sites in the γ subunit where specific mutations affect ENaC activity in heterologous expression systems[2,37,38]. Cleavage of the γ subunit at defined sites, with the release of an imbedded inhibitory tract, has a large effect on channel activity. Modification of specific γ subunit cytoplasmic residues by palmitoylation, and the interaction of specific acidic phospholipids with γ subunit residues also affect channel activity[2].

ENaC function in non-renal tissues may drive the blood pressure associations we observed. Among these are sites in humans that express channels with a δ subunit. We found by

RNA sequencing that human monocytes express ENaC δ subunits (Figure 2), which could contribute to the release of cytokines that affect blood pressure[6]. The δ subunit is expressed in human umbilical vein endothelial cells[39]. Previous studies have suggested that ENaC function in endothelial cells influence cellular stiffness and NO release[7,8], raising the possibility that *SCNN1D* variants could influence blood pressure. Recently, Paudel, et al. identified *SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G* mRNA and protein expression in human internal mammary artery and aorta and suggested that *SCNN1D* may be associated with hypertension[40]. ENaC δ subunits are also expressed in human taste buds and may have a role in mediating salt taste[4] and influence Na^+ intake. This could influence blood pressure by a number of mechanisms, including changes in extracellular and intravascular volume, release of cytokines from circulating dendritic cells[6] and monocytes, and through alterations in the gut microbiota and the subsequent induction of T_H17 cells[41]. Our observed association of low frequency and rare variants in *SCNN1D* with blood pressures suggest that the δ subunit has an unrecognized role in blood pressure regulation in human. This is consistent with a wider scope of *SCNN1D* expression as previously documented[12], based on the recently released encyclopedias of DNA elements (ENCODE 3) data[11]. Expression of *SCNN1D* in B cells, T cells and monocytes suggests a potential role of the δ subunit in immunity and immunity-mediated diseases.

Aside from the well-defined Liddle syndrome variants, it is notable that few studies, to date, have found an association between low-frequency or rare ENaC variants and blood pressure measures. Five ENaC variants associated with increases or decreases in blood pressure salt-sensitivity were observed in the GenSalt study[42]. In addition, seven functional ENaC missense variants in the GenSalt study were identified by Ray et al.[37], although these were not associated with differences in salt-sensitivity. Other variants have been associated with hypertension in specific populations, including variants in *SCNN1B* and *SCNN1G*[43–45]. Additionally, an ENaC gain-of-function variant in *SCNN1A* was associated with a modest Liddle syndrome-like phenotype and a blunting of the inhibitory effect of extracellular Na^+ [2,46,47].

We noted that variants in *SCNN1B* and *SCNN1D* are associated with eGFR. While DBP is contributing to the variance of eGFR in our population, we controlled for DBP in our eGFR analyses and the associations of *SCNN1B* with eGFR are independent of DBP. The association with *SCNN1B* could reflect the effects of variants on ENaC function in the ASDN. Changes in extracellular fluid volume, blood pressure or volume regulatory hormones may influence glomerular filtration rate (GFR). Also, connecting tubule/collecting duct tubuloglomerular feedback, a phenomenon where ENaC dependent Na^+ transport in the ASDN influences renal afferent arterial tone[48] may be altered by ENaC variants. As mentioned above, associations with *SCNN1D* suggest that extrarenal ENaC influences GFR. Vascular ENaC influences renal vascular tone and blood flow, factors that will affect GFR[49]. ENaC-dependent release of cytokines from circulating dendritic cells[6] and monocytes may also affect glomerular function[50]. Low-frequency variation ($0.01 \leq MAF < 0.05$) in *SCNN1D* was also associated with eGFR via burden test. This may be caused by many of the variants exerting an effect in the same direction on eGFR, or one or several low-frequency variants with strong and similar directional effects on eGFR. These

possibilities are not mutually exclusive and will require future study to elucidate the impact of *SCNN1D* variants on eGFR.

Perspectives

Low frequency and rare variants in *SCNN1A*, *SCNN1B*, and *SCNN1D* are, in aggregate, associated with key blood pressure parameters and eGFR in TOPMed data. The association of *SCNN1D* variants with BP and eGFR highlight the importance of ENaCs outside of the nephron in regulating these physiologic parameters. These observations raise the possibility that humans with hypertension and specific non–Liddle ENaC variants, particularly variants with a gain-of function phenotype, may benefit from a trial of ENaC inhibitors, such as amiloride, to lower blood pressure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non standard abbreviations:

ASDN	aldosterone-sensitive distal nephron
DBP	diastolic blood pressure
ENaC	epithelial Na^+ channel
eGFR	estimated glomerular filtration rate
GRM	genetic relatedness matrix
MAP	mean arterial pressure
MAF	minor allele frequency
PCA	principal components of ancestry
PHA1	pseudohypoaldosteronism type I
PP	pulse pressure
SKAT	sequence kernel association tests
SNVs	single nucleotide variants

SBP	systolic blood pressure
TOPMed	Trans-Omics in Precision Medicine
WGS	whole-genome sequencing

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Novelty and Relevance

What is New?

We found associations between low-frequency and rare variation in *SCNN1A*, *SCNN1B*, and *SCNN1D*and blood pressure, and associations between *SCNN1B* and *SCNN1D* variation and eGFR.

What is Relevant?

Variation in *SCNN1D*, encoding the ENaC δ subunit that is poorly expressed in human kidney, is associated with blood pressure and eGFR.

Clinical/Pathophysiological Implications?

Humans with hypertension and specific non-Liddle ENaC variants, particularly variants with a gain-of function phenotype, may benefit from a trial of ENaC inhibitors, such as amiloride, to lower blood pressure.

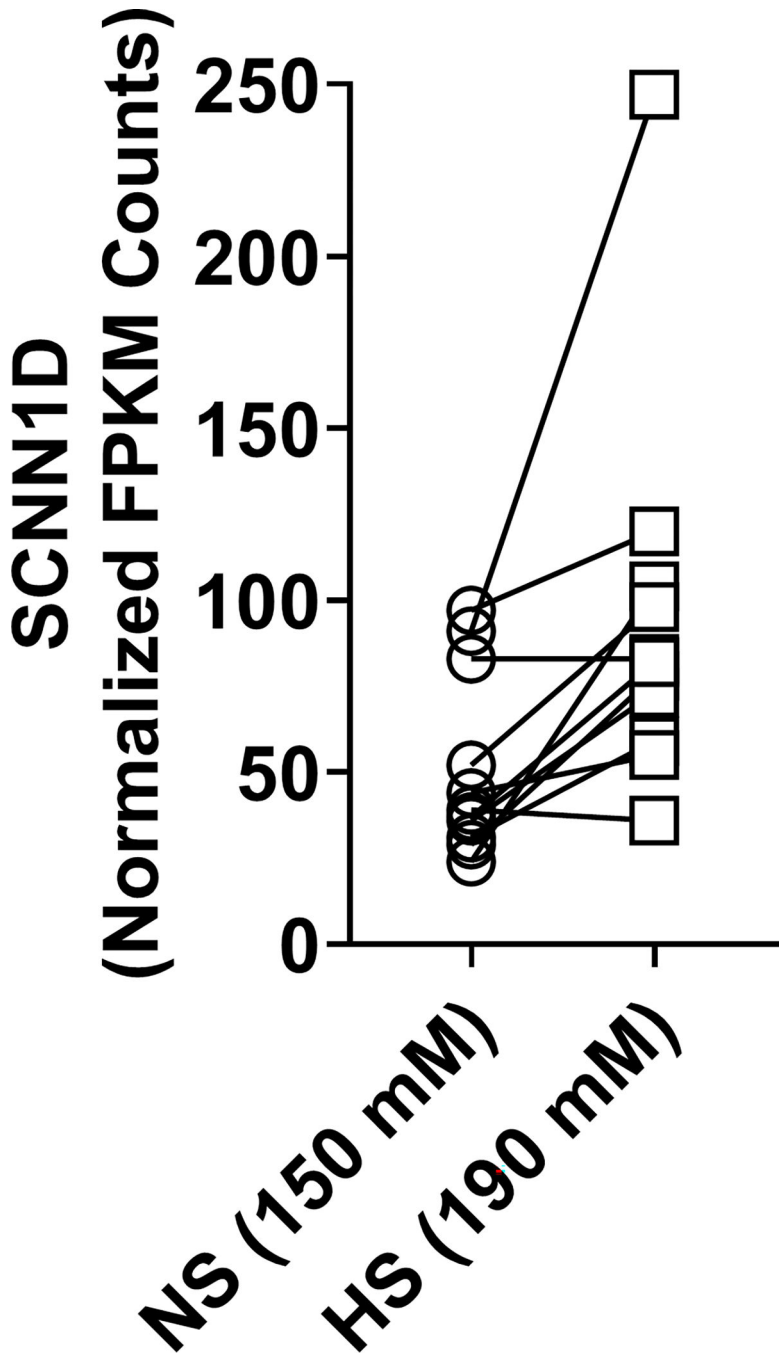

Figure 1. Expression of ENaC subunits in human monocytes.

Human monocytes were exposed to a normal (150 mM) or high (190 mM) NaCl solution for 72 h, and RNA was isolated and sequenced. Normalized fragments per kilobase of transcript per million mapped reads (FPKM) are shown. Similar levels of expression of δ ENaC were seen between the two exposures ($p = 0.06$, unpaired Student's t test).

Table 1.

Studies included in the blood pressure analyses.

The Genetics of Cardiometabolic Health in Amish and Women's Health Initiative (WHI) studies are subdivided according to the abbreviated study name provided in the harmonized dataset.

Study	*n*	Abbreviation
Genetics of Cardiometabolic Health in the Amish	732	Amish_FC13
	269	Amish_FC2
Atherosclerosis Risk in Communities Study VTE cohort	3,039	ARIC
Cleveland Family Study	744	CFS
Framingham Heart Study	3,270	FHS
Genetic Studies of Atherosclerosis Risk	1,626	GeneSTAR
Genetic Epidemiology Network of Arteriopathy	1,138	GENOA
Genetic Epidemiology Network of Salt Sensitivity	1,616	GenSalt
Hypertension Genetic Epidemiology Network	1,635	HyperGen
Jackson Heart Study	3,124	JHS
Samoan	1,167	Samoan
Women's Health Initiative	4,692	WHI_ctr
	4,403	WHI_stroke_case
	944	WHI_VTE_case
Total	28,399	

Table 2.

Single-variant association tests of common ENaC variants with SBP, DBP, and PP.

Results of single-variant analyses of two common functional ENaC α subunit variants and one common ENaC β subunit variant for associations with SBP, DBP, and PP.

Variant	rs ID	MAF	*p* value			
			SBP	DBP	MAP	PP
αA334T	rs11542844	0.18	0.9	0.9	0.9	0.7
αT663A	rs2228576	0.75	0.1	0.6	0.9	0.06
βG165R	rs2303157	0.24	0.3	0.9	0.6	0.07

Table 3.

Sequence kernel association tests of low frequency and rare ENaC variants with blood pressure measures.

SKAT was performed on variants with a MAF < 0.05 in the genomic regions of *SCNN1A, SCNN1B, SCNN1D,* and *SCNN1G* with SBP, DBP, PP, and MAP, separately, and then combined (*SCNN1A, SCNN1B* and *SCNN1G*). SKAT was separately performed on rare (MAF < 0.01) and low frequency (0.01 ≤ MAF < 0.05) variants. Significant *p* values are in bold.

Gene. Chromosome:Start–End		Variants with MAF < 0.05		Variants with MAF < 0.01		Variants with 0.01 ≤ MAF < 0.05	
	Measure	*n*	*p* value	*n*	*p* value	*n*	*p* value
SCNN1A. 12:6346824–6379751	SBP	3,972	0.02	3,859	0.03	113	0.07
	DBP	3,972	**0.00004**	3,859	**0.0004**	113	**0.0007**
	PP	3,972	0.7	3,859	0.3	113	0.9
	MAP	3,972	**0.0002**	3,859	**0.002**	113	**0.002**
SCNN1B. 16:2329812–23381320	SBP	12,334	0.02	11,925	0.04	409	0.04
	DBP	12,334	**0.002**	11,925	**0.003**	409	0.01
	PP	12,334	0.3	11,925	0.3	409	0.04
	MAP	12,334	**0.003**	11,925	**0.006**	409	0.01
SCNN1D. 1:1280417–1292050	SBP	2,405	**0.0008**	2,293	0.2	112	**0.0005**
	DBP	2,405	**0.002**	2,293	0.03	112	**0.003**
	PP	2,405	**0.003**	2,293	0.04	112	**0.005**
	MAP	2,405	**0.0008**	2,293	0.1	112	**0.0007**
SCNN1G. 16:23184696–23216904	SBP	4,093	0.5	4,022	0.3	71	0.6
	DBP	4,093	0.2	4,022	0.1	71	0.4
	PP	4,093	0.06	4,022	0.1	71	0.09
	MAP	4,093	0.5	4,022	0.2	71	0.8
Combined (*SCNN1A, SCNN1B, SCNN1G*)	SBP	20,399	0.01	19,806	0.02	593	0.03
	DBP	20,399	**0.0002**	19,806	**0.0004**	593	**0.004**
	PP	20,399	0.3	19,806	0.1	593	0.4
	MAP	20,399	**0.0006**	19,806	**0.001**	593	**0.005**
Combined (*SCNN1A, SCNN1B, SCNN1D, SCNN1G*)	SBP			22,099	0.01	705	**0.006**
	DBP			22,099	**0.0002**	705	**0.001**
	PP			22,099	0.08	705	0.14
	MAP			22,099	**0.0009**	705	**0.001**

* *p* values < 0.00625 are significant

Table 4.

Sequence kernel association tests of low frequency and rare ENaC variants with eGFR.

SKAT was performed on variants with a MAF < 0.05 in the genomic regions of *SCNN1A, SCNN1B, SCNN1D,* and *SCNN1G* with eGFR, separately, and then combined. SKAT was separately performed on rare (MAF < 0.01) and low frequency (0.01 ≤ MAF < 0.05) variants. Significant *p* values are in bold.

Gene. Chromosome:Start–End	Variants with MAF < 0.05		Variants with MAF < 0.01		Variants with 0.01 ≤ MAF < 0.05	
	n	***p* value**	***n***	***p* value**	***n***	***p* value**
SCNN1A. 12:6346824–6379751	2,739	0.01	2,626	0.008	113	0.07
SCNN1B. 16:2329812–23381320	8,770	**0.005**	8,365	0.007	405	0.03
SCNN1D. 1:1280417–1292050	1,758	**0.002**	1,648	0.2	110	**0.001**
SCNN1G. 16:23184696–23216904	2,868	0.1	2,797	0.01	71	0.1
Combined (*SCNN1A*, *SCNN1B*, *SCNN1D*, *SCNN1G*)	16,135	**0.0003**	15,436	**0.002**	699	**0.003**

* *p* values < 0.00625 are significant

Table 5.

Sequence kernel association tests of low frequency and rare ENaC variants with stroke.

SKAT was performed on variants with a MAF < 0.05 in the genomic regions of *SCNN1A, SCNN1B, SCNN1D,* and *SCNN1G* with stroke, separately, and then combined. SKAT was separately performed on rare (MAF < 0.01) and low frequency (0.01 ≤ MAF < 0.05) variants.

Gene. Chromosome:Start–End	Variants with MAF < 0.05		Variants with MAF < 0.01		Variants with 0.01 ≤ MAF < 0.05	
	n	*p* value	*n*	*p* value	*n*	*p* value
SCNN1A. 12:6346824–6379751	2,330	0.08	2,216	0.05	114	0.2
SCNN1B. 16:2329812–23381320	6,965	0.5	6,556	0.7	409	0.4
SCNN1D. 1:1280417–1292050	1,621	0.5	1,497	0.6	124	0.4
SCNN1G. 16:23184696–23216904	2,208	0.2	2,137	0.1	71	0.3
Combined (*SCNN1A, SCNN1B, SCNN1D, SCNN1G*)	13,124	0.3	12,406	0.3	718	0.3