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Human epithelial Na^+ channel missense variants identified in the GenSalt study alter channel activity

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Ray EC, Chen J, Kelly TN, He J, Hamm LL, Gu D, Shimmin LC, Hixson JE, Rao DC, Sheng S, Kleyman TR. Human epithelial Na^+ channel missense variants identified in the GenSalt study alter channel activity. *Am J Physiol Renal Physiol* 311: F908–F914, 2016. First published August 31, 2016; doi:10.1152/ajprenal.00426.2016.—Mutations in genes encoding subunits of the epithelial Na^+ channel (ENaC) can cause early onset familial hypertension, demonstrating the importance of this channel in modulating blood pressure. It remains unclear whether other genetic variants resulting in subtler alterations of channel function result in hypertension or altered sensitivity of blood pressure to dietary salt. This study sought to identify functional human ENaC variants to examine how these variants alter channel activity and to explore whether these variants are associated with altered sensitivity of blood pressure to dietary salt. Six-hundred participants of the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study with salt-sensitive or salt-resistant blood pressure underwent sequencing of the genes encoding ENaC subunits. Functional effects of identified variants were examined in a *Xenopus* oocyte expression system. Variants that increased channel activity included three in the gene encoding the α-subunit (αS115N, αR476W, and αV481M), one in the β-subunit (βS635N), and one in the γ-subunit (γL438Q). One α-subunit variant (αA334T) and one γ-subunit variant (βD31N) decreased channel activity. Several α-subunit extracellular domain variants altered channel inhibition by extracellular Na^+ (Na^+ self-inhibition). One variant (αA334T) decreased and one (αV481M) increased cell surface expression. Association between these variants and salt sensitivity did not reach statistical significance. This study identifies novel functional human ENaC variants and demonstrates that some variants alter channel cell surface expression and/or Na^+ self-inhibition.

epithelial sodium channel; Genetic Epidemiology Network of Salt Sensitivity; salt-sensitive hypertension; sodium self-inhibition; two-electrode voltage clamp

HYPERTENSION IS A MAJOR CONTRIBUTOR to cardiovascular disease (25, 33) and is the leading risk factor for mortality globally, with 9.4 million attributable deaths in 2010 (26). Among modifiable determinants of hypertension, Na^+ consumption ranks among the most significant (11, 51). Elevated Na^+ consumption is associated with higher blood pressure (18, 21, 28, 30, 31) and contributed to 1.6 million cardiovascular deaths in 2010 (35). However, individuals vary in blood pressure sensitivity to dietary Na^+ (21, 32, 50), and genetic background likely plays a significant role in determining salt sensitivity (5). Almost all monogenetic hypertensive syndromes involve decreased urinary Na^+ excretion by the kidney (7, 49), highlighting the importance of the kidney in regulating blood pressure and suggesting that dysregulation of renal Na^+ handling likely contributes to non-Mendelian hypertension.

The epithelial Na^+ channel (ENaC) is expressed in the kidney's late distal convoluted tubule, connecting tubule, and collecting duct, where it plays a key role in the absorption of Na^+ from tubular fluid (20, 34). Liddle syndrome, characterized by early onset hypertension and hypokalemia, occurs as a consequence of mutations that reduce ENaC ubiquitination, increasing the number of channels at the cell surface and increasing channel open probability (P_o) (13, 23, 44, 45). Human ENaC is comprised of three structurally related subunits, α, β, and γ, encoded by SCNN1A (chromosome 12), SCNN1B (chromosome 16), and SCNN1G (chromosome 16), respectively. Sibling-pair studies have previously revealed linkage disequilibrium between blood pressure and microsatellite markers on chromosome 16, near SCNN1B and SCNN1G (37, 52). Several studies have shown that selected common ENaC variants are associated with increases in blood pressure or changes in the salt sensitivity of blood pressure (8, 17, 27, 38, 47, 54, 55).

The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study searched for genetic polymorphisms associated with changes in sensitivity of blood pressure to dietary sodium (16, 55). This study examined subjects living in six rural villages in northern China. This region was selected due to its minimal ethnic and environmental heterogeneity and because individuals in this region have a habitually high dietary Na^+ intake, approaching 5.2 g/day (16). Study subjects underwent a dietary intervention that included measurements of blood pressure on a low- and high-salt diet. Subjects with blood pressure that was unusually salt sensitive or unusually salt resistant were identified for comparative sequencing of SCNN1A, SCNN1B, and SCNN1G. We asked whether identified ENaC variants have functional effects by expressing

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them in *Xenopus* oocytes and comparing amiloride-sensitive current amplitudes. For variants that alter channel activity, we further asked whether these changes occur as a consequence of changes in cell surface expression or of changes in Na^+ self-inhibition, a process wherein extracellular Na^+ binds the channel and decreases channel P_o (12, 14, 29, 42). Finally, we examined whether gain-of-function variants were significantly more common in salt-sensitive study subjects.

MATERIALS AND METHODS

Identification of ENaC variants. Study participants of Han ancestry (1,906 subjects) were selected for a dietary intervention that was approved by Institutional Review Boards at relevant institutions and by the National Human Genomic Resource Administration of China, as previously described (16). Briefly, individuals were excluded from the study if they had stage 2 hypertension, were receiving antihypertensive medications, or if they had secondary hypertension, cardiovascular, or chronic kidney disease. During a 3-day baseline assessment, blood pressures were measured while participants consumed their usual diet. Subjects were then administered a low-salt diet (51.3 mmol/day Na^+) for 7 days followed by a high-salt diet (307.8 mmol/day) for 7 days, with continued monitoring of blood pressures. The 300 subjects with the highest and 300 subjects with the lowest mean arterial blood pressure responses were designated as salt sensitive and salt resistant, respectively, and were selected for targeted gene sequencing. SCNN1A, SCNN1B, and SCNN1G were sequenced using the VariantSEQr system (Applied Biosystems). Nonsynonymous exonic variants were individually tested for association with salt sensitivity using either a Chi square or Fisher's exact test (when an expected cell count was <5). Due to the low power of single marker analyses to identify rare and low-frequency variants influencing salt sensitivity, aggregate rare variant analysis was conducted using the optimal unified sequence kernel association test (SKAT-O). SKAT-O encompasses burden tests and SKAT by deriving an optimal test statistic under a range of scenarios in which variants may be protective or deleterious (24). Before SKAT-O testing, low-frequency and rare variants (minor allele frequency <5%) were aggregated according to gene and predicted gain-of-function or loss-of-function status.

Site-directed mutagenesis and expression of human ENaC in Xenopus oocytes. cDNAs encoding human α-, β-, and γ-ENaC subunits were mutated using the QuickChange II XL mutagenesis kit (Agilent Technologies, Santa Clara, CA) to introduce selected variants. cDNAs were transcribed in vitro using the T7 or SP6 mMessage mMachine kit (Life Technologies, Grand Island, NY). The synthesized cRNAs (2 ng/subunit) were injected into stage V–VI *Xenopus laevis* oocytes. Oocytes were incubated at 18°C in modified Barth's saline [MBS: 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 15 mM HEPES, 10 μg/ml streptomycin sulfate and sodium penicillin, and 100 μg/ml gentamycin sulfate, pH 7.4] for 24–48 h before electrophysiological recording at room temperature. The protocol for harvesting oocytes from *X. laevis* was approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

Recording and analysis of currents in Xenopus oocytes. Whole cell currents were recorded using standard two-electrode voltage-clamp techniques with an Axoclamp 900A microelectrode amplifier with a Digidata 1440A analog-to-digital converter (Molecular Devices, Sunnyvale, CA). Currents were recorded at room temperature (20–24°C), using the Clampex 10 Data Acquisition Module (Molecular Devices), with a sampling frequency of 1 KHz, a transmembrane electrical potential of −100 mV, and filtered at 20 Hz. Oocytes were perfused via gravity perfusion, with solution changes controlled via an eight-channel ValveLink 8.2 perfusion controller (AutoMate Scientific, Berkeley, CA). Standard 110 mM Na^+ recording solution was 110 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, and 10 mM HEPES, pH 7.4. Perfusion with 10 μM amiloride allowed subtraction of baseline (non-ENaC-mediated) currents. For examination of relative current amplitudes, currents were normalized to currents from a similar number of wild-type channels recorded from the same batch of oocytes on the same day. Normalized current amplitudes from multiple recording days were then averaged for comparison. Statistical significance was examined using a two-tailed Student's *t*-test.

Measurement of Na^+ self-inhibition. To assess Na^+ self-inhibition, currents were recorded in 1 mM Na^+ solution (1 mM NaCl, 109 mM *N*-methyl-D-glucamine, 2 mM KCl, 2 mM $CaCl_2$, and 10 mM HEPES, pH 7.4), with abrupt transition to 110 mM Na^+ solution. The ratio of steady-state current in 110 mM Na^+ solution to the peak current that

Table 1. *Nonsynonymous ENaC variants identified in GenSalt*

Gene Exon*	Location	rs Number	Nucleotide Substitution	Amino Acid Substitution*	Minor Allele Frequency	Single Marker *P* Value†
SCNN1A	Chr 12p13					
Exon 2	6483972	rs377074479	C>G	P52R	0.002	0.25
Exon 2	6483606		G>A	S115N	0.001	0.49
Exon 3	6472753	rs55859427	G>C	G173R	0.001	1.00
Exon 4	6471244	rs368942111	G>A	R283H	0.001	1.00
Exon 6	6464581	rs11542844	G>A	A334T	0.181	0.32
Exon 11	6458506	rs113622727	C>T	R476W	0.001	1.00
Exon 12	6458386	rs201693951	G>A	V481M	0.004	0.22
Exon 13	6457062	rs2228576	G>A	T663A	0.456	0.91
SCNN1B	Chr 16p12					
Exon 2	23360011	rs370777535	G>A	D31N	0.001	1.00
Exon 5	23379257	rs142531781	C>T	S286L	0.002	1.00
Exon 13	23392103	rs13306629	G>A	S635N	0.003	0.50
SCNN1G	Chr 16p12					
Exon 3	23200921	rs5736	G>A	G183S	0.008	0.34
Exon 4	23203811		G>T	A253S	0.001	0.50
Exon 6	23208689	rs774394259	G>A	V340I	0.001	1.00
Exon 9	23224017	rs756463117	T>A	L438Q	0.003	1.00
Exon 13	23226779	rs72647543	G>T	D647Y	0.001	1.00

ENaC, epithelial Na^+ channel; GenSalt, Genetic Epidemiology Network of Salt Sensitivity; rs number, reference SNP identification number. *Exon and residue numbering as per common mRNA isoforms: for SCNN1A, NM_001038.5, encoding a 669-amino acid α−subunit; for SCNN1B, NM_000336.2, encoding a 640-amino acid β−subunit; for SCNN1G NM_001039.3 encoding a 649-amino acid γ subunit. †*P* value for single marker test (Chi square or Fisher's exact for sparse data). Includes heterozygous and homozygous participants.

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occurred immediately following transition to high-Na^+ solution was used to represent the magnitude of Na^+ self-inhibition. Kinetics of current decay were examined by fitting with a single exponential equation using Clampfit 10.4.

ENaC cell surface expression. ENaC surface expression in oocytes was examined by coexpressing α- and γ-subunits with a FLAG epitope-tagged β-subunit, as previously described (9, 10). One day following cRNA injection of oocytes, amiloride-sensitive currents were assessed using two-electrode voltage clamp, as above. Oocytes were then incubated for an additional day at 18°C. Subsequent steps were performed on ice, except for measurement of chemiluminescence, which was performed at room temperature (20–24°C). Oocytes were incubated for 30 min in antibiotic-free MBS and 1% bovine serum albumin (MBS/BSA) and then for 1 h with MBS/BSA with 1 μg/ml mouse anti-FLAG antibody (M2; Sigma). After being washed in cold MBS/BSA, oocytes were incubated for 1 h in MBS/BSA with 1 μg/ml horseradish peroxidase-coupled goat anti-mouse IgG (Jackson ImmunoResearch, WestGrove, PA). Oocytes were washed in cold MBS/BSA and then BSA-free MBS and transferred to a 96-well plate. SuperSignal ELISA Femto Maximum Sensitivity Substrate (100 μl; Thermo Scientific, Rockford, IL) was added to each well. Relative light units (RLU) were quantified using a GloMax-Multi+ Detection System (Promega, Madison, WI). The background mean RLU from wells with no cells was subtracted from the RLU for ENaC-injected oocytes.

RESULTS

Sixteen nonsynonymous ENaC variants were identified in 600 GenSalt participants (Table 1). Of these, eight were in the α-subunit, three in the β-subunit, and five in the γ-subunit. We generated human ENaC cDNAs containing these variants and examined the activity of resulting channels in the *Xenopus* oocyte expression system. Whole cell amiloride-sensitive currents of wild-type or mutant channels were measured using two-electrode voltage clamp (Fig. 1). Within the α-subunit, three variants increased channel activity: S115N, R476W, and V481M by 1.67 ± 0.11-, 1.37 ± 0.10-, and 2.75 ± 0.22-fold, respectively ($P < 0.001$ for each variant vs. wild type). One variant, A334T, resulted in a reduction in amiloride-sensitive Na^+ currents to 0.57 ± 0.06 of wild type ($P < 0.001$ vs. wild type), similar to previous findings (2). Neither G173R nor R283H significantly altered currents compared with wild type, with normalized mean currents of 1.05 ± 0.08 and 1.00 ± 0.10, respectively ($P = 0.62$ and 0.98). We and others have previously examined T663A (1, 2, 36, 40, 48, 53), and we did not functionally characterize it further. Another α-subunit variant, P52R, is present in an mRNA splice variant that adds 59 amino acids to the NH_2-terminus of the protein. This residue is not present in the commonly studied isoform (NM_001038.5) used as our control and was not assayed (6, 46). We examined three β-subunit variants. Of these, S635N increased currents 1.33 ± 0.07-fold compared with wild type, and D31N reduced currents to 0.61 ± 0.04 of that observed for wild-type channels ($P < 0.001$ for both). S286L did not significantly affect whole cell Na^+ currents (0.85 ± 0.07; $P = 0.06$). Of five γ-subunit variants examined, only L438Q significantly changed channel activity, increasing relative amiloride-sensitive Na^+ currents 1.77 ± 0.15-fold ($P < 0.001$ vs. wild type). The γ-subunit variants G183S, A253S, V340I, and D647Y did not significantly alter whole cell Na^+ currents.

Changes in ENaC activity may reflect changes in cell surface number, P_o, and/or single channel conductance. To determine whether variants that altered ENaC activity were associated

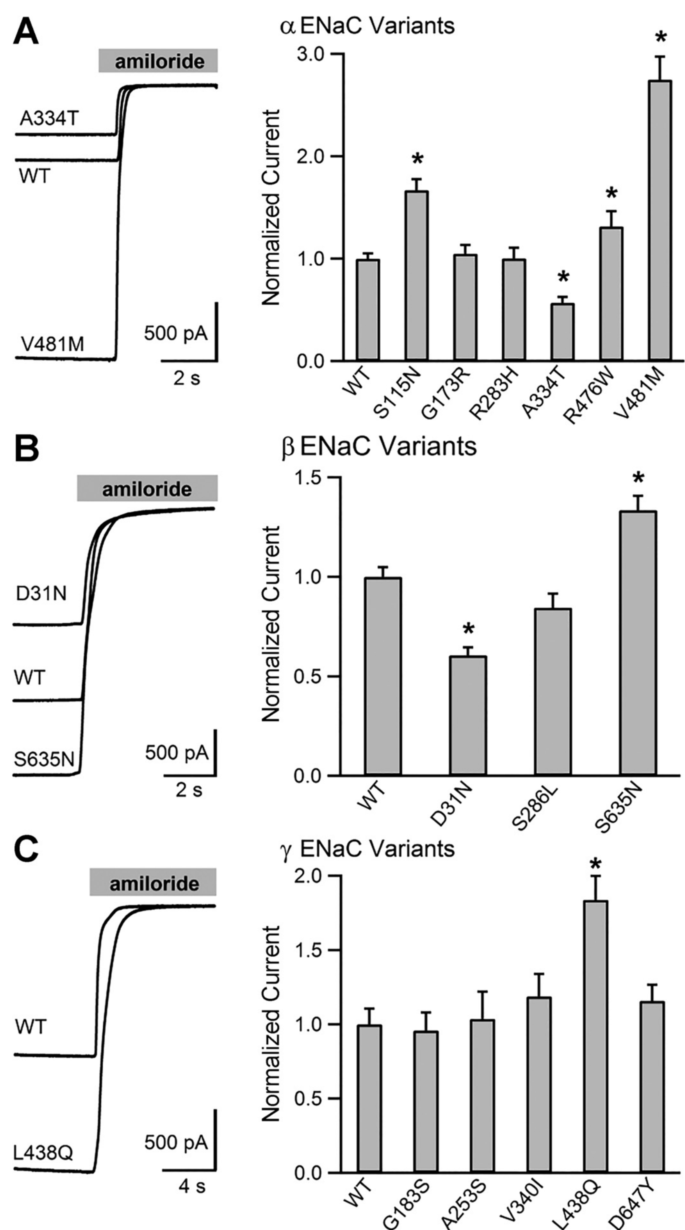

Fig. 1. Multiple human epithelial Na^+ channel (ENaC) variants alter channel activity. The effect of nonsynonymous variants in the α-, β-, and γ-subunits on amiloride-sensitive currents. *Left*, select representative current traces recorded in oocytes injected with cRNAs encoding the three ENaC subunits. By convention, inward currents are downward. Gray bar at the *top* represents perfusion with amiloride (10 μM). *Right*, bar graphs showing mean current amplitudes normalized to wild-type currents measured on the same day. $N \geq 37$ oocytes for each variant and wild type (WT). Error bars represent SE. *Statistically significant differences in current amplitude compared with wild type ($P < 0.05$) based on 2-tailed Student's *t*-test.

with changes in ENaC surface expression, we examined surface expression of selected mutant and wild-type channels using a β-subunit with an extracellular FLAG tag, in conjunction with a chemiluminescence-based assay. Of the five variants examined, a significant increase in ENaC surface expression was only seen with αV481M ($P < 0.01$ vs. wild-type), a variant associated with an increase in whole cell Na^+ currents (Fig. 2). A reduction in surface expression was seen with αA334T ($P < 0.01$ vs. wild type), a variant associated with a

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reduction in Na^+ currents. For three of the variants that were associated with an increase in ENaC functional expression (αS115N, αR476W, and γL438Q), levels of surface expression were similar to wild type ($P > 0.05$).

ENaC is not only permeable to Na^+ but also modulated by it. Na^+ binds the channel extracellular domain and impairs activity by promoting an allosteric reduction in channel P_o (12, 14, 19, 29, 39, 42). This process, referred to as Na^+ self-inhibition, can be examined experimentally by bathing ENaC-expressing oocytes in a low-concentration (1 mM) Na^+ bath to allow channels to adopt a higher P_o state, then abruptly increasing the extracellular Na^+ concentration. The resulting rise in chemical-driving force transiently increases inward Na^+ current. Channels then undergo Na^+ self-inhibition, with a decline in inward current amplitude reflecting a reduction in channel P_o. We assessed whether functional ENaC variants identified in GenSalt participants altered Na^+ self-inhibition (Fig. 3). Two variants exhibited reduced Na^+ self-inhibition (αR476W and αV481M), and one variant exhibited enhanced Na^+ self-inhibition (αA334T), compared with wild-type ENaC ($P < 0.01$). Other variants exhibited Na^+ self-inhibition that was similar to wild type.

The ENaC variants we studied were identified in the 300 subjects with the highest (salt-sensitive) and 300 subjects with the lowest (salt-resistant) responses to a high-Na^+ diet. We examined whether ENaC variants with enhanced activity were more likely to be found in the salt-sensitive individuals and whether variants with reduced activity were more likely to be found in the salt-resistant individuals. As noted in Table 2, neither of these associations reached statistical significance.

The most common variants resulted in a loss of function, including αA334T with a minor allele frequency of 18% and αT663A with a minor allele frequency of 45.6% in this cohort. We asked if these common loss-of-function variants influenced whether individuals with rare gain-of function ENaC variants exhibited the salt-sensitive phenotype. Even when accounting for these common variants, the segregation of the rare gain-of-function variants with salt sensitivity did not reach statistical significance.

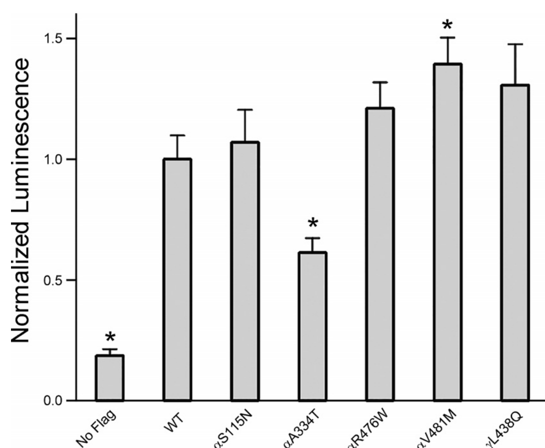

Fig. 2. Effects of functional ENaC variants on channel surface expression. Cell surface expression of ENaC in oocytes was determined by coexpressing Genetic Epidemiology Network of Salt Sensitivity (GenSalt) ENaC variants or wild type with a β-subunit containing a FLAG epitope tag. Oocytes were incubated with an antibody against FLAG, followed by a horseradish peroxidase-linked secondary antibody. Relative chemiluminescence was compared between wild type and the variants. No FLAG, background luminescence from oocytes expressing ENaC with no FLAG epitope tag ($N = 27$ oocytes for No FLAG control and >40 for wild type and variants). *Statistically significant difference from WT ($P < 0.01$).

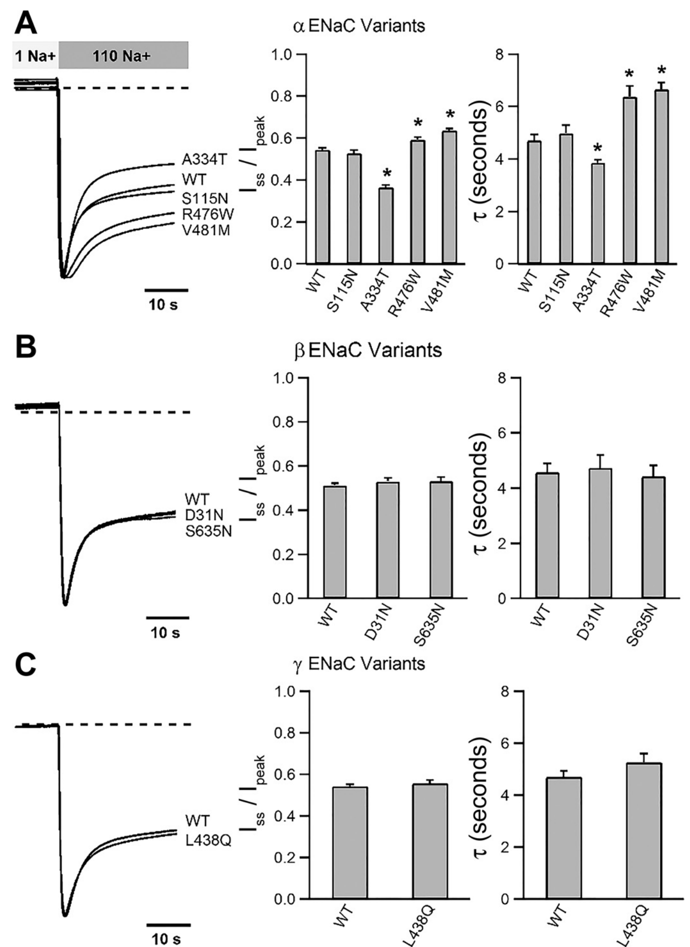

Fig. 3. Effects of ENaC variants on Na^+ self-inhibition. The Na^+ self-inhibition response was examined by rapidly transitioning ENaC-expressing oocytes from 1 to 110 mM extracellular Na^+ solution. *Left*, representative current traces showing an initial increase in inward current due to increased driving force, followed by a decay in current amplitude (Na^+ self-inhibition). Traces were normalized to that of wild type for ease of comparison. Broken lines indicate the zero current levels. Bars in *middle* show mean steady-state currents normalized to peak currents (I_{ss}/I_{peak}). Increased I_{ss}/I_{peak} reflects reduced Na^+ self-inhibition, whereas reduced I_{ss}/I_{peak} reflects enhanced Na^+ self-inhibition. *Right*, bars represent time constants (τ) based on a fit of curves using a single exponential decay function. $N = 6$ for all recordings. Error bars represent SE. *Statistically significant difference from wild-type channels ($P < 0.05$).

DISCUSSION

We examined whether ENaC variants in the GenSalt cohort altered ENaC activity. Of the 16 nonsynonymous ENaC variants identified in the 600 study participants, αT663A and αA334T are relatively common, with allele frequencies of 0.18 and 0.46, respectively. Consistent with previous findings (2), we found that αA334T reduces channel activity (2). We have previously examined the functional effects of the αT663A variant. We found that this variant decreases channel activity in the *Xenopus* oocyte expression system as a consequence of reduced cell surface expression (36, 40, 53). Although Ambrosius and colleagues did not observe the decrease in channel

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Table 2. *Comparison of functional variants and salt sensitivity*

Variants	Salt-Sensitive Carriers	Salt-Resistant Carriers	*P* Value (Burden)[a]	*P* Value (SKAT)[a]	*P* Value (SKAT-O)[a]
Gain of function			0.62	0.33	0.45
αS115N	1[b]	0			
αR476W	0	1[f]			
αV481M	4[c]	1[g]			
βS635N	3	1			
γL438Q	1[d]	2[h]			
Loss of function			0.17	0.40	0.18
αA334T	98	86			
αT663A	194	196			
βD31N	1[e]	0			

SKAT, sequence kernel association test. [a]Aggregate analysis of gain-of-function and loss-of-function variants, separately. [b]The carrier of αS115N is a heterozygous carrier of αA334T and homozygous for αT663A. [c]Among the 4 salt-sensitive carriers of αV481M, 3 are heterozygous carriers of αT663A, the other is heterozygous for αA334T and homozygous for αT663A. [d]The salt-sensitive carrier of γL438Q is heterozygous for αT663A. [e]The carrier of βD31N is homozygous for βT663A. [f]The salt-resistant carrier of αR476W is homozygous for αA334 and heterozygous for αT663A. [g]The salt-resistant carrier of αV481M is homozygous for αT663A. [h]Salt-resistant carriers of γL438Q are heterozygous for either αT663A or αA334T.

activity in *Xenopus* oocytes (1), Tong and colleagues observed similar effects in Chinese hamster ovary cells (48).

The present study identified novel relatively rare variants that altered ENaC activity. αV481M exhibited the most robust gain of function, increasing currents 2.7-fold. Other gain-of-function variants included αS115N, αR476W, βS635N, and γL438Q. A loss-of-function variant, βD31N, was also identified.

Three of these variants, αA334T, αR476W, and αV481M, altered the channel's Na^+ self-inhibition response. While αR476W and αV481M increased Na^+ currents in association with reducing Na^+ self-inhibition, αA334T reduced Na^+ currents and exhibited an enhanced Na^+ self-inhibition response. Each of these sites is located in the extracellular region of the α-subunit (Fig. 4). Based on sequence alignments and a homology model of the α-subunit (20), αA334 is located within a loop connecting β-strands (β6–β7) in the palm and β-ball domains, respectively. Mutations at selected sites within this loop of the mouse α-subunit significantly alter Na^+ self-inhibition (19). Because αA334T also reduced surface expression, a mutation at this site may affect ENaC stability and/or trafficking.

The αV481M variant led to a large increase in ENaC activity in association with suppressed Na^+ self-inhibition, suggesting that the variant increases the channel's P_o (29). We also found a modest increase in ENaC surface expression with this variant. αV481 is located at the start of β10, which is at the base of the palm domain (Fig. 4). Structural studies of ASIC1 suggest that this region undergoes a large conformational change during transition between closed and conducting states (3, 4). Given its location, it is not surprising that αV481M alters ENaC gating in response to extracellular Na^+. αR476 is likely located within a loop connecting an α-helix and β-strand (α5–β10) in the thumb and palm domains, respectively. We previously reported that mutations at the β9–α4 loop connecting the palm and thumb domains significantly altered the Na^+ self-inhibition and shear stress responses of mouse ENaC (43). Although it remains unclear whether variants modifying Na^+ self-inhibition alter predisposition to salt-sensitive hypertension, it is clear that individuals in the general population express ENaC with varied sensitivity to extracellular Na^+.

Seven variants did not show significant changes in ENaC activity in the current study. Genetic variations may affect protein function through multiple mechanisms, including altering the peptide sequence, mRNA levels, and translation efficiency. Our study focused on identifying functional human variants in the three ENaC subunits in the oocyte expression system. It is certainly possible that functional variants in other renal Na^+ transporters, or variants in proteins that regulate these transporters, have a role in modifying salt sensitivity in humans.

Genetic mechanisms underlying the salt sensitivity of blood pressure remain to be elucidated (22). We asked whether gain-of-function variants were associated with salt sensitivity and loss-of-function variants were associated with salt resistance. Single variant analyses did not reveal individual variants with statistically significant association with salt sensitivity. However, these data do not rule out the possibility that altered ENaC function modifies salt sensitivity, a correlation that may be difficult to detect in the context of modest functional effects, a relatively small cohort, and the likelihood that study subjects may harbor alleles both protecting against and predisposing to salt sensitivity. More definitive results may require larger cohorts or animal modeling to examine functionally relevant ENaC variants in a homogenous genetic background.

GRANTS

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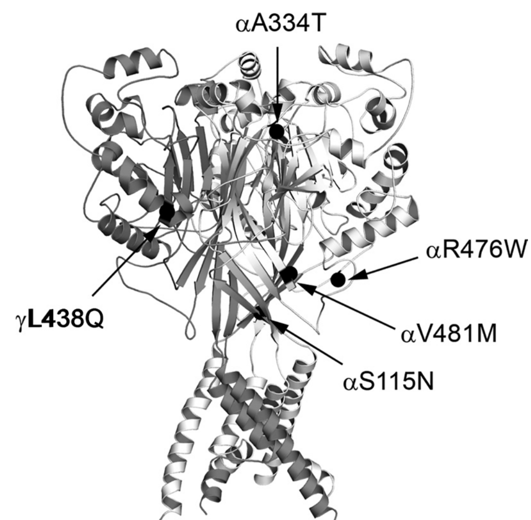

Fig. 4. Locations of functional variants on a structural model of ASIC1. Extracellular and transmembrane domains of 3 chicken ASIC1 subunits were rendered as white, light gray, and dark gray ribbons (PDB ID: 4NYK) (15, 41). α-Carbons of ASIC1 residues homologous to human ENaC residues bearing functional variants are shown as black spheres. Homologous ASIC1/ENaC residues are T76/αS115, I225/αA334, E363/αR476, V368/αV481, and F351/γL438.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.C.R., J.C., T.N.K., J.H., L.L.H., D.G., L.C.S., J.E.H., D.C.R., S.S., and T.R.K. analyzed data; E.C.R., J.C., T.N.K., J.H., L.L.H., D.G., L.C.S., J.E.H., D.C.R., S.S., and T.R.K. interpreted results of experiments; E.C.R. prepared figures; E.C.R. and T.R.K. drafted manuscript; E.C.R., T.N.K., J.H., L.L.H., D.G., J.E.H., D.C.R., S.S., and T.R.K. edited and revised manuscript; E.C.R., J.C., T.N.K., J.H., L.L.H., D.G., L.C.S., J.E.H., D.C.R., S.S., and T.R.K. approved final version of manuscript; J.C., L.L.H., L.C.S., and S.S. performed experiments; T.N.K., J.H., L.L.H., D.G., L.C.S., J.E.H., D.C.R., S.S., and T.R.K. conception and design of research.

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