A common human epithelial sodium channel (ENaC) polymorphism, αT663A, is present in the cytoplasmic C terminus of the α-subunit, although it is unclear whether this polymorphism segregates with blood pressure. We examined whether this polymorphism was associated with differences in functional Na\(^+\) channel expression. Whole cell amiloride-sensitive currents in *Xenopus* oocytes expressing wild type channels (αT663\(\beta\)γ) were significantly 1.3–2.0-fold higher than currents measured in oocytes expressing channels with an Ala, Gly or Leu, or Lys at position α663. In contrast, differences in functional human ENaC expression were not observed with oocytes expressing channels having Thr (wild type), Ser, or Asp at this position. The surface expression of channels, measured using an epitope-tagged β-subunit, was significantly reduced in oocytes expressing αT663A\(\beta\)γ when compared with oocytes expressing αT663\(\beta\)γ. The corresponding polymorphism was generated in the mouse α-subunit (mαA692T) and was not associated with differences in functional βγ-mouse ENaC expression. The polymorphism is present in a region that is not well conserved between human and mouse. We generated a mouse/human chimera by replacing the distal C terminus of the mouse α-subunit with the distal C terminus of the human α-subunit. Co-expression of this m(1-678)/h(650–669)T663A chimera with mouse \(\beta\)γ led to a significant reduction in whole cell Na\(^+\) currents and surface expression when compared with m(1-678)/h(650–669)T663-m\(\beta\)γ. Our results suggest that hoT663A is a functional polymorphism that affects human ENaC surface expression.

Functional Polymorphism in the Carboxyl Terminus of the α-Subunit of the Human Epithelial Sodium Channel*  

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Epithelial sodium channels (ENaC)\(^3\) are expressed in principal cells in the late distal convoluted tubule and collecting tubule, where they serve as a final site for reabsorption of Na\(^+\) from the glomerular ultrafiltrate. Volume regulatory hormones, such as aldosterone, have a key role in modifying rates of renal tubular Na\(^+\) reabsorption through regulation of functional ENaC expression at the apical plasma membrane (1). Epithelial Na\(^+\) channels are composed of three structurally related subunits, termed α-, β-, and γ-ENaC that likely assemble as a αβ1γ1 tetramer (2, 3), although an alternative subunit stoichiometry has been proposed (4). The three subunits share limited (30–40%) sequence identity but share a common topology of two membrane-spanning domains and intracellular N and C termini (5–7).

Changes in ENaC functional expression are associated with alterations in blood pressure (8, 9). Na\(^+\) channel gain-of-function mutations have been identified in patients with Liddle’s syndrome, a disorder characterized by volume expansion, hypokalemia, and hypertension (10, 11). ENaC loss-of-function mutations have been identified in patients with type I pseudohypoaldosteronism, a disorder characterized by volume depletion, hypotension, and hyperkalemia (12, 13). Some common human ENaC polymorphisms may segregate with blood pressure (i.e. βT594M) (14), suggesting that ENaC polymorphisms that alter functional channel expression may contribute to the development of hypertension in the general population.

A common polymorphism has been described in C terminus of the α-subunit of human ENaC (hENaC), αT663A (15, 16). The question of whether this polymorphism segregates with blood pressure has been addressed by several groups, and conflicting results have been reported (15, 16). We examined whether this polymorphism was associated with altered Na\(^+\) channel activity when expressed in *Xenopus* oocytes. We observed that oocytes expressing wild type αT663\(\beta\)γ channels had significantly higher currents than oocytes expressing αT663A\(\beta\)γ. Furthermore, the higher currents observed with αT663\(\beta\)γ channels were associated with higher levels of cell surface expression of channels, suggesting that this polymorphism altered channel trafficking. This polymorphism is present in the distal C terminus of the α-subunit, a region that is not well conserved between the human and mouse α-subunit. Although the αA692T polymorphism in mouse ENaC (mENaC), corresponding to human αT663A, was not associated with differences in functional αβγ-mENaC expression, replacement of the distal C terminus of the mouse α-subunit with the distal C terminus of the human α-subunit restored the functional differences that were observed with the human αT663A polymorphism.
**EXPERIMENTAL PROCEDURES**

**Preparation of hENaC Mutants—**α-, β-, and γhENaC cDNAs were from M. J. Welsh (University of Iowa). Mouse ENaC cDNAs have been described and used by our group previously (17). All mutants and mouse/human chimeras were generated using a PCR-based mutagenesis technique as described previously (18). All sequences were confirmed by automated DNA sequence analyses performed at sequencing facilities at the University of Pennsylvania, the Children’s Hospital of Philadelphia, or the University of Pittsburgh.

**Expression of ENaC in Xenopus Oocytes—**cRNAs for wild type and mutant shENaC and mENaC, wild type βhENaC and βmENaC, and wild type γshENaC and γmENaC were synthesized from linearized plasmids containing the appropriate cDNAs using appropriate RNA polymerases (T7, T3, or SP6, mMessage mMachine, Ambion Inc., Austin, TX) and stored at −80°C. cRNA concentration was determined spectrophotometrically. Stage V-VI oocytes were surgically harvested from female Xenopus laevis (Nasco, Fort Atkinson, WI) and pretreated with 2 mg/ml collagenase (type IV) as described previously (18). Oocytes were injected with 2 ng/subunit of hENaC cRNAs or 0.33 ng/subunit of mENaC cRNAs in 50 nl of H2O. After injection, oocytes were incubated at 18°C in modified Barth’s saline (MBS) (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 15 mM HEPES, 0.3 mM Ca(NO3)2, 0.41 mM CaCl2, 0.82 mM MgSO4, pH 7.2) supplemented with 10 μg/ml sodium penicillin, 10 μg/ml streptomycin sulfate, and 100 μg/ml gentamicin sulfate. Animal protocols were approved by the University of Pennsylvania, the University of Pittsburgh, and the Children’s Hospital of Philadelphia Institutional Animal Care and Use Committees.

**Two-electrode Voltage Clamp—**A two-electrode voltage clamp (TEV) was performed 24–48 h after cRNA injection at room temperature using a DigiData 1320 interface and Axon Geneclamp 500B Amplifier (Axon Instruments, Foster City, CA). Data were acquired at 200 Hz, and analyses were performed using pClamp 8.0 or 8.1 software (Axon Instruments) on an 833-MHz Pentium III personal computer (Dell Computer, Austin, TX). Pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Inc., Sarasota, FL) with a micropipette fuller (Sutter Instrument Co., Novato, CA) and had resistances of 0.5–5 megohms when filled with 3 M KCl and inserted into the bath solution. Oocytes were maintained in a recording chamber with 1 ml of bath solution and continuously perfused with bath solution at a flow rate of 4–5 ml/min. The bath solution contained 100 mM sodium glucose, 2 mM KCl, 1.8 mM CaCl2, 3 mM BaCl2, 10 mM tetraethylammonium chloride, and 10 mM HEPES, pH 7.4. A series of voltage steps (1 s) from −140 to +60 mV (adjusted for resting membrane potential) in 20-mV increments were performed, and whole cell currents were recorded 750 ms after the initiation of the −100-mV step for data analysis. ENaC-mediated current was defined as the difference in whole oocyte current at a −100-mV holding potential (adjusted for resting membrane potential) before and after the addition of 10 μM amiloride-HCl (Sigma) to the bath solution.

**Cell Surface Expression—**Two approaches were used to examine the surface expression of ENaC in oocytes. A chemiluminescence-based assay was performed as described by Zerangue et al. (19) and modified by Yoo et al. (21). Oocytes were injected with 0.33 ng/subunit of m(1–678)/h(650–669)/T663-mβ-mFLM-mγ ENaC. 42–48 h after injection oocytes were incubated for 10 min at 4°C in 4% formaldehyde in MBS. Oocytes were subsequently washed three times with MBS containing 10 mg/ml of bovine serum albumin (MBS-BSA), incubated an additional 3 h with MBS-BSA, and then incubated overnight with MBS-BSA supplement with 1 μg/ml mouse monoclonal anti-FLAG antibody (M2, Sigma) at 4°C. Oocytes were then washed at 4°C for 1 h in MBS-BSA and incubated with MBS-BSA supplemented with 1 μg/ml horseradish peroxidase-coupled secondary antibody for 1–1.5 h at 4°C (peroxidase-conjugated AffiniPure F(abasha)) fragment goat anti-mouse IgG, Jackson Immunoresearch Laboratories, West Grove, PA). Cells were extensively washed (12 times over 2 h) at 4°C and transferred to MBS without BSA. Individual oocytes were placed in 100 μl of SuperSignal Elisa Femto (Pierce) and incubated at room temperature for 1 min. Chemiluminescence was quantitated in a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA).

A second approach to examine surface expression used cell surface biotinylation to identify the surface pool of channels as described previously (22). βhENaC with a C-terminal V5 epitope tag (β-V5) was generated in pCDNA6 as described previously (23). cRNAs for αβ-V5γ hENaC were co-injected into Xenopus oocytes. As a control, oocytes were co-injected only with cRNAs for β-V5 and γ. After 48 h, oocytes were stripped mechanically of their vitelline membranes in hypertonic saline (MBS) (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 15 mM HEPES, 0.3 mM Ca(NO3)2, 0.41 mM CaCl2, 0.82 mM MgSO4, pH 7.2) supplemented with 10 μg/ml sodium penicillin, 10 μg/ml streptomycin sulfate, and 100 μg/ml gentamicin sulfate. Animal protocols were approved by the University of Pennsylvania, the University of Pittsburgh, and the Children’s Hospital of Philadelphia Institutional Animal Care and Use Committees.

![Figure 1](http://www.jbc.org/)

**FIG. 1. Expression of human αβγENaC in Xenopus oocytes.** αT663βγ (A) or αT663A (B) βγ-hENaC were expressed in oocytes, and TEV was performed as described under “Experimental Procedures.” Whole cell currents were measured by voltage clamping oocytes in 20-mV steps between −140 and +60 mV adjusted for base line transmembrane potential. Shown are current/voltage relationships in the absence (closed circles) or presence (open circles) of 10 μM amiloride. Means ± S.E. are illustrated. Error bars contained within the symbols are not apparent.

![Figure 2](http://www.jbc.org/)

**FIG. 2. The αT663A polymorphism reduces hENaC functional expression in oocytes.** αT663βγ or αT663Aβγ hENaC was expressed in oocytes, and TEV was performed. Amiloride-sensitive whole cell currents were determined at a −100-mV holding potential (adjusted for resting membrane potential) and are expressed relative to the mean whole oocyte current for αT663βγ-ENaC-expressing oocytes. Data are presented as mean ± S.E. with the p value determined by a two-tailed t test as described under “Experimental Procedures.”
medium (300 mM sucrose in MBS without penicillin, streptomycin, and gentamycin), and surface proteins were labeled with N-hydroxysulfosuccinimidobiotin (Pierce) as described previously (22). Oocytes (10/group) were subsequently lysed in 0.15 M NaCl, 0.01 M Tris-Cl, pH 8.0, 0.01 M EDTA, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 1.0 mM phenylmethanesulfonyl fluoride, 0.1 mM N-α-p-tosyl-L-lysine chloromethyl ketone, and 2 μg/ml aprotinin for 1 h at 4°C and centrifuged at 13,000 × g for 15 min at 4°C. Biotinylated proteins were precipitated with streptavidin-agarose (Pierce) and subjected to SDS-PAGE.

Statistical Analyses—Whole cell amiloride-sensitive current data were expressed relative to that of wild type ENaC. To decrease the influence of batch-to-batch variability in ENaC expression, data were normalized by the mean amiloride-sensitive current for the control condition (usually αT663βγ hENaC) within a batch of oocytes prior to combining data of multiple independent batches for statistical analysis. These data are presented as mean ± S.E. Because a Poisson distribu-
tion, rather than a Gaussian distribution, best described these combined data, \( p \) values were determined by a two-tailed \( t \) test after a square root transformation to better approximate a Gaussian distribution (24). A \( p \) value of \( <0.05 \) was considered significant. We also independently analyzed the statistical significance of these data without normalization and transformation using the Wilcoxon rank sum/Mann-Whitney \( U \) test for non-parametric results and obtained similar \( p \) values to the method outlined above (data not shown). Other data that were normally distributed are expressed as mean \( \pm \) S.E. with \( p \) values determined by a two-tailed \( t \) test. All data analyses were performed with SigmaStat version 2.03.

**RESULTS**

**aT663A Polymorphism Is Associated with Altered Functional Channel Expression in Xenopus Oocytes**—Functional hENaC expression was assessed by TEV in oocytes expressing either \( \alpha T663A \beta \gamma \) hENaC (\( n = 56 \)) or the \( \alpha T663A \beta \gamma \) \( \alpha T663A \gamma \) before and after the addition of 10 \( \mu \)M amiloride. The linear current/voltage relationships are characteristic of amiloride-sensitive Na\(^+\)/H\(^+\) currents/voltage relationships is characteristic of amiloride-sensitive Na\(^+\)/H\(^+\) expression was assessed by TEV in oocytes expressing either ENaC or incubated in MBS (17, 18). Oocytes expressing \( \alpha T663A \beta \gamma \) channels exhibited mean \( \pm \) S.E. whole cell amiloride-inhibited currents of \(-2.15 \pm 0.29 \mu A\) at a \(-100\)-mV holding potential that were significantly lower than mean Na\(^+\) currents measured in oocytes expressing wild type channels \((-4.65 \pm 0.67 \mu A, p < 0.001\) (Fig. 2 for normalized data)). Given the small difference in whole cell Na\(^+\) currents we observed with oocytes expressing \( \alpha T663A \beta \gamma \) and \( \alpha T663A \gamma \), experiments were performed with two different cRNA preparations and four different batches of oocytes to confirm our findings.

**Analyses of \( \alpha T663A \) Mutants**—A conservative substitution of Ser for the Thr residue at position \( \alpha 663 \) did not alter levels of amiloride-sensitive Na\(^+\) current (Fig. 3A, \( p = n. s.\)). Thr and Ser residues are potential targets of Ser/Thr kinases, and phosphorylation of this residue would alter the charge at this site. We examined whether an acidic residue (Asp) at position \( \alpha 663 \) affected functional expression of amiloride-sensitive Na\(^+\) current. Levels of amiloride-sensitive Na\(^+\) current expression in oocytes injected with either wild type (\( \alpha T663A \beta \gamma \) hENaC or \( \alpha T663D \beta \gamma \) hENaC were similar (Fig. 3B, \( p = n. s.\)). In contrast, amiloride-sensitive currents in oocytes expressing Na\(^+\) channels with a basic residue at position \( \alpha 663 \) (\( \alpha T663K \beta \gamma \)) were significantly lower than currents observed with wild type hENaC (Fig. 3C, \( p = 0.025\)). Non-polar amino acids were also introduced at position \( \alpha 663 \), and mutant or wild type channels were expressed in oocytes. Mean whole cell amiloride-sensitive currents in oocytes expressing \( \alpha T663A \gamma \) (Fig. 3D, \( p = 0.011\)) or \( \alpha T663L \beta \gamma \) (Fig. 3E, \( p < 0.001\)) were significantly lower than the currents measured in oocytes expressing wild type ENaC. These data are summarized in Table 1.

**Functional Changes Associated with the T663A Polymorphism Are Species-specific**—The alignments of the C termini of mouse and human \( \alpha \)-subunits are illustrated in Fig. 4A starting with the Tyr in the PY motif that is present within all three ENaC subunits and is conserved across species. T663 is present within a region of the \( \alpha \)-subunit C terminus that is not well conserved across species. An Ala residue is present at the equivalent site in the mouse \( \alpha \)-subunit (maA692). We mutated mouse maA692 to Thr. Oocytes expressing mouse maA692 \( \beta \gamma \) (i.e. wild type, \( n = 38 \)) or mouse maA692T \( \beta \gamma \) (\( n = 36 \)) showed similar levels of amiloride-sensitive currents (Fig. 4B, \( p = n. s.\)). In contrast, oocytes expressing human \( \alpha T663A \) with mouse \( \beta \gamma \) (\( n = 43 \)) had significantly higher amiloride-sensitive currents than oocytes expressing human \( \alpha T663A \) with mouse \( \beta \gamma \) (Fig. 4C, \( n = 44, p = 0.017\)).

C-terminal sequences of human and mouse \( \alpha \)-subunits diverge following a conserved YATLGP tract (Fig. 4A) suggesting that residues within the 20 C-terminal amino acids, in addition to Thr or Ala in position \( \alpha 669 \), may influence levels of function channel expression in association with this polymorphism. A mouse/human \( \alpha \)-subunit chimera (m1–678/h650–669/T663A) was created by replacing the distal C terminus of the mouse \( \alpha \)-subunit (SASPPLSASYGSSACAPAMAL) with the distal C terminus of the human \( \alpha \)-subunit (RPSPGGSAASSSTCPLGGP). We also generated the corresponding m1–678/h650–669/T663A \( \alpha \)-subunit chimera. The co-expression of m1–678/h650–669/T663A with mouse \( \beta \gamma \) led to a significant reduction in the normalized mean whole cell Na\(^+\) currents at \(-100\) mV (\(-1.34 \pm 0.13 \mu A, n = 62\)) when compared with m1–678/h650–669/T663A-\( \beta \gamma \) (\(-2.04 \pm 0.20 \mu A, n = 62, p = 0.007\) (Fig. 4D for normalized data)).

**\( \alpha ENaC \) Polymorphism Affects Surface Expression of ENaC**—The reduced functional expression of \( \alpha T663A \beta \gamma \) channels in oocytes likely reflects changes in the numbers of channels at the plasma membrane or changes in channel open probability. Because these residues are not within the pore of the channel (1, 18), it is unlikely that this polymorphism affects single-channel conductance. Previous studies (25) have also suggested that it is difficult to assess differences in ENaC open probability because the channel has inherently large variability in open probability.

We therefore examined whether differences in functional expression of \( \alpha T663A \beta \gamma \) and \( \alpha T663A \beta \gamma \) hENaC were associated with altered levels of cell surface expression of channels. In these experiments, the \( \beta \)-subunit had a C-terminal V5 epitope tag to identify the protein on immunoblots. Cell surface proteins of oocytes expressing \( \alpha T663A \beta \gamma \) or \( \alpha T663A \beta \gamma \) were labeled by biotinylation and recovered with streptavidin beads. The biotinylated \( \beta \)-subunits were subsequently detected by immunoblots. As shown in Fig. 5A, expression of \( \alpha T663A \beta \gamma \) hENaC resulted in a relative recovery of biotinylated \( \beta \)-V5 of 0.64 \pm 0.08 compared with that observed when \( \alpha T663A \gamma \) hENaC was expressed (\( n = 5, p = 0.003\)). Whole oocyte \( \beta \)-V5 expression was similar in the two groups (Fig 5B). These data suggest that the increases in functional expression observed with \( \alpha T663A \beta \gamma \) when compared with \( \alpha T663A \beta \gamma \), are because of differences in surface expression.

Previous studies (25, 26) suggested that the \( \alpha \)-subunit has a

| \( \alpha T663 \) (No. of oocytes) | 57 | 52 | 85 | 58 | 53 | 46 |
|---|---|---|---|---|---|---|
| Relative current (mean) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| S.E. | 0.09 | 0.08 | 0.07 | 0.10 | 0.11 | 0.11 |
| Residue \( \alpha 663 \) (No. of oocytes) | Ala (56) | Gly (50) | Leu (82) | Lys (60) | Asp (53) | Ser (40) |
| Relative current (mean) | 0.52 | 0.75 | 0.66 | 0.75 | 1.09 | 0.90 |
| S.E. | 0.05 | 0.08 | 0.06 | 0.09 | 0.12 | 0.12 |
| \( p \) value vs. wild type | <0.001 | 0.11 | <0.001 | 0.025 | n.s. | n.s. |
key role in facilitating the trafficking of β- and γ-subunits to the plasma membrane in oocytes. In the absence of α-subunit expression, levels of surface expression of β- and γ-subunits in oocytes are reduced markedly (25, 26). We observed that the recovery of biotinylated β-V5 in oocytes expressing only αENaC was 0.18 ± 0.06 (n = 3) of that observed with oocytes expressing αγENaC (data not shown). These data suggest that oocytes that are stripped of their vitelline membrane have only a limited labeling of intracellular β-subunits with N-hydroxylysosuccinimimidobiotin.

We also examined whether differences in the functional expression of mouse/human α-chimeras (m1–678/h650–669/γT663A) were associated with altered levels of cell surface expression of channels. Cell surface expression was determined using a β-subunit with an extracellular FLAG epitope tag and a chemi-

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**Fig. 4.** Residues in the vicinity of hoT663A have a role in conferring differences in function ENaC expression. A, alignments of the distal C termini of mouse and human α-subunits. Spaces were added to separate a conserved six-residue N-terminal tract (YATLGP) from the remainder of the C terminus and to highlight hoT663 and maA692 (in bold). The first and last amino acid residue numbers for each sequence are listed. Sequence alignments were performed with MacVector 7.2 (Accelrys) for the mouse and human α-subunits (GenBank™ Accession Numbers AF112185 and L29007). B, TEV was performed with oocytes expressing either mouse αA692/β or αA692Tβ. Amiloride-sensitive whole cell currents were determined at the −100-mV holding potential (adjusted for resting membrane potential) and are expressed relative to the mean whole cell amiloride-sensitive current for αA692/β. C, TEV was performed with oocytes expressing either human αT663/β or human αT663A/β. Amiloride-sensitive whole cell currents were determined at the −100-mV holding potential and expressed relative to the mean whole cell amiloride-sensitive current for human αT663/β. D, TEV was performed with oocytes expressing either m1–678/h650–669/γT663A or m1–678/h650–669/γT663. Amiloride-sensitive whole cell currents were determined at the −100-mV holding potential and expressed relative to the mean whole cell amiloride-sensitive current for m1–678/h650–669/γT663/β. p values were determined with a two-tailed t test as described under “Experimental Procedures.”
A common T663A polymorphism is present within the C terminus of the γ-subunit of human ENaC (15, 16). We observed a significant increase in functional hENaC expression in oocytes expressing γT663A, when compared with oocytes expressing γT663A (Fig. 1). One study (15) suggested previously that the human γT663A polymorphism was not associated with the changes in functional ENaC expression in Xenopus oocytes; however the number of oocytes analyzed was not reported. Given the inherent variability of the levels of luminescence-based assay that does not require stripping of the oocyte vitelline membrane. The mean relative cell surface expression of m(1–678)/h(650–669)T663A/H9252/H9253 was 0.59 ± 0.10 (n = 46) compared with that of m(1–678)/h(650–669)T663-mβγ (Fig. 6, 1.00 ± 0.18, n = 47, p = 0.016). Nonspecific labeling was examined in oocytes expressing m(1–678)/h(650–669)T663-mγ where the β-subunit did not contain an epitope tag. Mean nonspecific labeling was 0.18 ± 0.03 (n = 35) of the labeling observed in oocytes expressing epitope-tagged m(1–678)/h(650–669)T663-mβγ. We also performed separate control experiments to assess the labeling of intracellular β-subunits in this chemiluminescence-based assay. The labeling of oocytes expressing only human β-FLAG, which, as discussed above, has markedly reduced surface expression (25, 26), was indistinguishable from that of un.injected oocytes and was significantly less than that of oocytes expressing m(1–678)/h(650–669)T663A-FLAG/H9252/H9253 (25, 26) (data not shown).

**DISCUSSION**

A common T663A polymorphism is present within the C terminus of the α-subunit of human ENaC (15, 16). We observed a significant increase in functional hENaC expression in oocytes expressing αT663βγ, when compared with oocytes expressing αT663Aβγ (Fig. 1). One study (15) suggested previously that the human αT663A polymorphism was not associated with the changes in functional ENaC expression in Xenopus oocytes; however the number of oocytes analyzed was not reported. Given the inherent variability of the levels of
ENaC expression in oocytes, it may be difficult to detect small changes in the level of ENaC expression.

Additional studies were performed to determine whether substitution of other amino acid residues at this site influenced functional ENaC expression. Whole cell amiloride-sensitive currents in oocytes expressing channels with a Thr, Ser, or Asp at position α663 were −1.3–2.0-fold higher than currents measured in oocytes expressing channels with an Ala, Gly or Leu, or Lys at this position (Table I and Figs. 2 and 3). Our observations that channels with Thr, Ser, or Asp at position α663 showed similar levels of functional expression in oocytes suggest that α663 may be a site of phosphorylation by Ser/Thr kinases. At present, we have no direct evidence that this site is phosphorylated in vivo nor whether phosphorylation of this site affects ENaC activity.

αT663 is present within a region of the α-subunit that is not well conserved between the human and mouse (Fig. 4A). An α663 residue is present at the homologous site in the mouse α-subunit (mouse A669), and we observed that oocytes expressing mouse αA669βγ and αA692βγ had similar levels of amiloride-sensitive currents (Fig. 4B). However, when the distal C terminus of the mouse α-subunit was replaced with the distal C terminus of the human α-subunit, creating a mouse/human chimera, oocytes co-expressing m1–678/h650–669/T663-mβγ had significantly higher whole cell Na+ currents than oocytes co-expressing m1–678/h650–669/T663A-mβγ (Fig. 4D). These results suggest that residues in the vicinity of αT663 have a role in conferring the observed differences in function expression between hαT663βγ and hαT663Aβγ. Furthermore, the differences in ENaC surface expression that we observed with the hαT663Aβγ and the m1–678/h650–669/T663A-mβγ polymorphisms (Figs. 5 and 6) were correlated with differences in function expression (Figs. 2 and 4D) and likely reflect differences in the delivery of channels to the plasma membrane and/or differences in the rate of retrieval from the plasma membrane. Our data suggest that the distal C terminus of the human α-subunit has a novel site that affects the intracellular trafficking of ENaC that is distinct from the PY motif in the β- and γ-subunits (25, 27, 28).

Increases in ENaC activity in renal collecting ducts may be associated with extracellular fluid volume expansion and hypertension, as has been reported in studies of patients with Liddle’s syndrome (8, 10, 29, 30). Whole cell Na+ currents measured in oocytes expressing mutations that are found in patients with Liddle’s syndrome were significantly higher than currents measured in oocytes expressing wild type ENaC (31, 32). However, correlations between increases in ENaC activity measured in oocytes and increases in blood pressure in humans with specific ENaC mutations or polymorphisms are not a consistent finding. For example, the βT594M polymorphism has been found to segregate with blood pressure in selected populations, suggesting that this polymorphism is associated with alterations in ENaC activity (14, 33). Lymphocytes obtained from patients homozygous for βT594 exhibited amiloride-sensitive whole cell Na+ currents that were enhanced by cAMP (34). Phorbol 12-myristate 13-acetate blocked the activation of Na+ currents by cAMP. In contrast, lymphocytes obtained from βT594 homozygotez exhibited cAMP-activated whole cell currents that were not blocked by phorbol 12-myristate 13-acetate, suggesting that this polymorphism was associated with altered ENaC activity (34). However, no differences in ENaC activity have been reported with the βT594M polymorphism in oocyte expression studies (35).

Although we have observed differences in hENaC activity with the αT663A polymorphism with oocyte expression, it is unclear whether the hαT663A polymorphism is associated with differences in blood pressure in humans. Arrang et al. (15, 36) reported that αA663 was more prevalent in African-American subjects than in Caucasian subjects, and its presence (αA663) was associated with a modest increase in blood pressure. However, other investigators have not observed differences in blood pressure with the αT663A polymorphism (16). Our functional studies in oocytes predict that αA663 would be associated with a reduced blood pressure. Certainly environmental and other genetic factors have an important role in the regulation of blood pressure in humans, and it may be difficult to demonstrate that specific ENaC polymorphisms contribute to essential hypertension in humans despite modest differences in functional activity when assayed in heterologous expression systems (37).

In summary, our results suggest that hαT663Aγ has increased activity in oocytes when compared with hαT663Aβγ. The increase in channel activity observed with hαT663γ reflects an increase in surface expression, suggesting that this polymorphism modifies the intracellular trafficking of ENaC. Furthermore, our studies using mouse/human α-subunit chimeras suggest that residues in the vicinity of αT663 have an important role in conferring differences in functional expression between hαT663βγ and hαT663Aβγ.
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