Nonsynonymous single nucleotide polymorphisms of NHE3 differentially decrease NHE3 transporter activity

Xinjun Cindy Zhu, Rafiquel Sarker, John R. Horton, Molee Chakraborty, Tian-E Chen, C. Ming Tse, Boyoungh Cha, and Mark Donowitz

Department of Medicine, Division of Gastroenterology and Hepatology, Center of Cardiovascular Sciences, Albany Medical Center, Albany, New York; Departments of Physiology and Medicine, Division of Gastroenterology, Johns Hopkins University School of Medicine, Baltimore, Maryland; and Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia

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Chronic diarrhea remains a significant burden on the U.S. healthcare system (18). Abnormal function of intestinal ion transporters has long been known to be causative for the pathophysiology of diarrhea (15). Among apical membrane transport proteins, the Na+/H+ exchanger 3 (NHE3) (also known as solute carrier family 9 member 3; SLC9A3) is a major contributor to salt absorption in the gastrointestinal and renal systems, where it helps maintain body water and sodium homeostasis (3, 21, 39). Genetic evidence of its role in epithelial Na+ absorption includes that NHE3 knockout mice display modest diarrhea along with increased fluid in the small intestine and colon (17, 24). These mice also exhibit reduced fractional proximal renal reabsorption of Na+, resulting in significantly reduced blood pressure (37).

NHE3 is composed of two functionally distinct domains: 1) an NH2-terminal transmembrane domain that mediates Na+ and H+ ion exchange, and 2) a large cytoplasmic COOH-terminal domain that regulates transporter activity by affecting either NHE3 exocytosis or endocytosis (11, 22). The ~377 amino-acid COOH terminus of NHE3 interacts both directly and indirectly with an array of regulatory proteins, including cytoskeletal proteins, kinases, phosphatases, scaffold proteins, and others, by which the regulation of NHE3 activity is achieved (11–13, 43). This regulation involves both increased and reduced NHE3 activity, as occurs sequentially after meals. Mutations in this region lead to dysregulation of NHE3 to physiological activators or inhibitors (27, 38, 44).

Recent studies have identified genetic variations in genes that account for individual susceptibility to chronic diarrhea (16, 42). Consequently, we searched the National Center for Biotechnology Information dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP, 2008) and found a total of 458 SNPs in NHE3. All but nine of these SNPs are located in introns. Of the nine SNPs in exons, three are nonsynonymous mutations, each affecting different amino acid residues in the intracellular COOH terminus (Fig. 1 and Table 1). In the present study, we examined the effect of these three variants on NHE3 transporter activity under basal, inhibitory, and stimulatory conditions designed to mimic postprandial regulation of NHE3. We also evaluated the effect of these mutations on the interaction between NHE3 and calcineurin B homologous protein (CHP), an NHE3 binding protein that associates with the NHE3 COOH terminus under basal conditions and is necessary for NHE activity (10).

MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma (St. Louis, MO) except as follows: penicillin and streptomycin were from Whittaker MA Bioproducts (Walkersville, MD); culture media and G-418 (neomycin) were from Gibco-BRL (Grand Island, NY); polyclonal anti-vesicular stomatitis virus (anti-VSVG) and anti-HA antibodies were from Covance Research Products (Princeton, NJ); mouse monoclonal anti-VSVG antibody was kindly provided by Thomas Kreis via Daniel Louvard (Curie Institute, Paris, France). The BCECF-AM
[acetoxymethyl derivative of (2’7’)-bis(2-carboxyethyl)-5,6-carboxyfluorescein] was from Invitrogen (Carlsbad, CA).

Cell culture, plasmid construction, and transfection. PS120 fibroblasts do not express endogenous plasma membrane NHEs. We used these cells for stable expression of human NHE3 wild-type (26, 27) and mutants R474Q, R474A, V567M, and V567A, epitope-tagged with a COOH-terminal vesicular stomatitis virus glycoprotein (VSV-G) epitope (28). Rabbit NHE3 wild-type and mutant R799C and R799A with a triple-HA epitope tag at the NH2 terminus were also stably expressed in PS120 fibroblasts. All the NHE3 mutants were made using the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s protocol. The templates for mutagenesis were the pcDNA3.1/Neoycin (Invitrogen) containing human NHE3-VSVG or rabbit HA3-NHE3. NHE3 mutants were transfected into PS120 cells as described previously (28). G-418-resistant cells were selected by growth in 400 μg/ml G-418. Clonal cell lines were isolated by limiting dilution and screened by Western blot for maximal expression. All PS120 cell lines were grown in DMEM supplemented with 25 mM NaHCO3, 10 mM HEPES, 50 U/ml penicillin, 50 μg/ml streptomycin, 10% fetal bovine serum in a 5% CO2-95% O2 incubator at 37°C.

Measurement of Na+/H+ exchange activity. Na+/H+ exchange activity was determined fluorometrically using the intracellular pH-sensitive dye BCECF-AM (5 μM) in PS120 cells stably expressing wild-type NHE3 and mutants as described previously (28). Na+/H+ exchange activity was calculated as the product of the change in pHi (ΔpHi) times the intracellular buffering capacity at each of multiple pHi values, as described previously (8). Kinetics of Na+/H+ exchange were analyzed by Hill plot using Origin (Microcal Software) to estimate NHE3 transport activity (Vmax) and K’(H+), in individual experiments (28). K’(H+), is a complex term composed of the effective concentration or two H+ binding/transport sites, interaction factors, and dissociation constants, as described previously (28). Means ± SE were determined from at least three experiments. In the experiments to determine the effects of cAMP, dexamethasone, and serum on NHE3 transporter activity, PS120 cells were first serum starved for different time periods (cAMP 3 h, serum 6 h, and dexamethasone 48 h). Cells were then incubated with 100 μM cAMP for 30 min, 10 μM dexamethasone for 2 h, or 10% dialyzed fetal bovine serum for 3 h before Na+/H+ exchange measurement.

Measurement of surface NHE3. To measure surface levels of NHE3, PS120 cells stably expressing NHE3 wild-type, R474Q, V567M, or R799C were grown in 10-cm dishes to 90% confluency. Cells were incubated in serum-free medium for 3 h, followed by biotinylation assay with NHS-SS-biotin as described previously (22). Briefly, cells were washed twice in ice-cold PBS and once in borate buffer (154 mM NaCl, 10 mM boric acid, 7.2 mM KCl, and 1.8 mM CaCl2, pH 9.0). Plasma membrane proteins were biotinylated in 5 ml borate buffer containing 5 μg of sulfo-NHS-SS-biotin by gently shaking at 4°C for 30 min. Cells were then washed extensively with quenching buffer (120 mM NaCl and 20 mM Tris, pH 7.4) to remove excessive sulfo-NHS-SS-biotin, followed by two PBS rinses. Tissues were then scraped and lysed in 1 ml of N’ buffer (60 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM KCl, 5 mM EDTA trisodium, 3 mM EGTA, 0.05% SDS, and 1% Triton X-100) and sonicated on ice. An aliquot of 80 μl lysate was saved for total NHE3 measurement. The total cell lysate (T) (0.8 ml) was incubated with streptavidin-agarose beads (50 μl of bed volume) to pull down the surface biotinylated proteins (S) that were eluted from the beads with 80 μl of Laemmli sample buffer (1). The supernatant, after incubation with streptavidin-agarose beads, was saved as the intracellular fraction (C). As indicated, each sample with several dilutions (μl) from total, surface, and intracellular fractions was subjected to 12.5% SDS-PAGE. Western blot assay was employed to quantify NHE3 protein by anti-VSV-G or anti-HA antibodies, and GAPDH was used as a loading control. Protein concentrations were determined (intensity units/ul) with an Odyssey Infrared Imaging System. Multiple volumes for each total, surface, and intracellular sample were plotted using linear regression, and the intensity of the signal was used to obtain a single value for each sample, which was expressed as intensity units per microliter. The results were presented as the percentage of total NHE3 on the surface by considering the dilution of each fraction.

Immunoprecipitation of NHE3 by CHP protein. PS120 cells expressing NHE3 or its mutants were grown on 10-cm Petri dishes to 70–80% confluency, and cell lysates were prepared and incubated
Table 1. Summary of total 43 nonsynonymous SNPs at the COOH terminus of NHE3 present in the database

<table>
<thead>
<tr>
<th>NHE3 COOH Terminus</th>
<th>Nonsynonymous SNP</th>
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<td>α0 (aa 458–471)</td>
<td>rs368029489 Arg471Trp</td>
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<tr>
<td>Loop (aa472–474)</td>
<td>rs144153604 Gln472Asp; rs13154302 Arg474Gln</td>
</tr>
<tr>
<td>α1 (aa 475–494)</td>
<td>rs372558316 Gly481Arg</td>
</tr>
<tr>
<td>Loop (aa 495–503)</td>
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<tr>
<td>α2 (aa 504–513)</td>
<td>rs143487075 Asp505Asn</td>
</tr>
<tr>
<td>α3 (aa 518–560)</td>
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</tr>
<tr>
<td>α4 (aa 562–593)</td>
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</tr>
<tr>
<td>α5 (aa 599–605)</td>
<td>rs115689317 Arg604Gln; rs371725135 Arg605Trp; rs146547322 Arg605Gln</td>
</tr>
<tr>
<td>Loop (aa 606–613)</td>
<td>rs145653595 Ala611Val</td>
</tr>
<tr>
<td>α6 (aa 614–637)</td>
<td>rs140444535 Thr619Met; rs202223784 Gln621Arg</td>
</tr>
<tr>
<td>Loop (aa 638–645)</td>
<td>rs372661265 Thr641Met; rs105976302 Thr643Met; rs377342139 Asp645Asn</td>
</tr>
<tr>
<td>α7 (aa 646–667)</td>
<td>rs145136022 Lys647Glu; rs373185294 Arg650Pro; rs199498162 Arg650Gly; rs377627891 Arg655Lys; rs144798475 Arg658Gln; rs201741190 Lys659Asn; rs199602995 Glu662Gln</td>
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<td>α8 (aa 675–691)</td>
<td>rs327062509 Asn686Ser; rs368610931 Ser703Gly; rs199817159 Gln706Arg; rs374243437 Glu712Lys; rs377080845 Asp719Glu; rs145183553 Thr720Ala</td>
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<td>rs20197670 Ala762Asp; rs200138066 Gln790His; rs368047255 Pro792Ser; rs58623748 Cys799Arg</td>
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</tbody>
</table>

Single nucleotide polymorphisms (SNPs) that are located near R474, V567, and R799 are highlighted by an underline. Database was accessed in May 2014. NHE3, Na+/H+ exchanger 3.

with agarose beads conjugated to anti-HA antibody. The agarose beads were then washed and bound proteins were eluted with Laemmli sample buffer. Protein samples were analyzed by SDS-PAGE and detected by Western blotting with anti-NHE3 and anti-CHP antibodies. Protein bands in the Western blots were quantified using an Odyssey Infrared Imaging System.

**RESULTS**

Effect of nonsynonymous SNPs on Na+/H+ exchange activity under basal conditions. We searched the 2008 dbSNP (build 129) and Ensembl databases and found 458 SNPs for human NHE3, with 9 SNPs in exons and the rest in introns. Of these nine SNPs, six are synonymous silent substitutions giving the same amino acid sequence as wild type and three are missense mutations, R477Q, V567M, and R799C (Fig. 1 and Table 1). These three SNPs all affect amino acid residues in the cytoplasmic COOH-terminal domain of NHE3. To determine whether these affect NHE3 transporter activity, we introduced the same three point mutations and also separately replaced each amino acid with Ala, by site-directed mutagenesis into human and/or rabbit NHE3 and stably transfected the expression constructs into PS120 fibroblasts, a cell line that does not express any endogenous plasma membrane NHE. This cell line also lacks the NHE3 regulatory protein, NHERF2, which is required for many aspects of NHE3 activity (22). Accordingly, NHERF2 and each NHE3 variant were stably cotransfected into PS120 cells. Ala substitution tested the hypothesis that any mutation other than the usual polymorphic amino acid would similarly affect NHE3 activity. We monitored Na+/H+ exchange activity using BCECF/fluorometry (28). Basal NHE3 transport activity (V_{max}) was reduced in all three NHE3 variants (Fig. 2A). In all cases, K'(H^+) was significantly increased compared with wild type (Fig. 2A, inset).

The effect of nonsynonymous SNPs on surface expression of NHE3. Previous studies have shown that NHE3 resides in both the plasma membrane and endosomal compartments and traffics between these pools by exocytosis and endocytosis under both basal and acutely regulated conditions (1, 6, 9, 23, 26, 29, 40, 41). To determine whether the decreased basal NHE3 activity in these NHE3 mutations was due to reduced total or surface expression of NHE3, we performed cell surface biotinylation assays to measure the total amount of NHE3 protein expressed in the cells and on the plasma membrane surface (Fig. 2B). There were reduced levels of NHE3 in the surface fraction in the V567M and R799C mutants but not in R474Q (Fig. 2, B and C). Normalization of NHE3 in the surface fraction to total NHE3 compared with wild-type total set as 100% revealed that the percentage of surface expression of NHE3 was reduced in both the V567M and R799C mutants, whereas R474Q had a surface expression level similar to that of wild-type NHE3 (Fig. 2C).

Impaired intrinsic transporter activity in R474Q and R799C variants. The intrinsic activities of the wild-type and mutant NHE3 molecules were compared by normalizing NHE3 activity (V_{max}) to the relative amount of plasma membrane NHE3 (Fig. 2C) again normalized to that of wild-type NHE3. There was a significant reduction in the transporter activity/surface expression in variants R474Q and R799C, indicating that there are defects in intrinsic protein function (turnover number of Na+ and H+). In contrast, the intrinsic activity/surface molecule of variant V567M was increased compared with wild type (Fig. 2C, 5th column).

Alanine replacement at the polymorphic sites impairs NHE3 activity. Whether the altered Na+/H+ exchange activity observed in the three NHE3 SNPs was due to substitution by a particular amino acid residue used or to the specific location of the polymorphic sites was further determined. Mutations at these three SNP sites were engineered, converting each mutated amino acid into alanine. The NHE3 mutant R474A displayed reduced transporter activity without a change in its surface expression. The V567A variant exhibited reduced transport activity and surface expression. Therefore, these two mutants behaved similarly to the corresponding SNP variants (compare Figs. 2 and 3). The R799A mutant appeared to be unstable with too low protein expression to perform the assays (data not shown); therefore, this alanine mutant was excluded from further study.

Altered response to dexamethasone stimulation but normal serum stimulated and cAMP inhibited activity in the NHE3 SNPs. Previous studies have shown that dexamethasone acutely (short times of exposure) stimulates NHE3 activity (33–35) and stimulated by 10% dialyzed serum simulated and cAMP inhibited activity in the NHE3 (1, 6, 9, 23, 26, 29, 40, 41). To determine whether dexamethasone stimulation of these NHE3 mutants was affected with NHERF2 and each NHE3 variants. Dexamethasone (4 h) on NHE3 activity in PS120 cells stably cotransfected with NHERF2 and NHE3 mutants. Dexamethasone increased NHE3 V_{max} and reduced K'(H^+), in wild-type NHE3 (Fig. 4, A, C, and D) and NHE3 V567M but had no effect on either V_{max} or K'(H^+), of R474Q (Fig. 4, B, C, and D) and R799C (Fig. 4, C and D). Wild-type NHE3 activity was inhibited by cAMP (33–35) and stimulated by 10% dialyzed serum.
Fig. 2. The 3 NHE3 variants have decreased basal Na\(^+/\)H\(^+/\) exchange activity. A: Na\(^+/\)H\(^+/\) transporter activity was measured in PS120/NHERF2 cells transfected with wild-type (WT) NHE3 or its mutants R474Q, V567M, and R799C. Data are presented as means ± SE from at least 3 independent experiments. *P < 0.05, comparison between wild-type NHE3 and the individual mutants (Student’s t-test). Inset: K’(H\(^+\)) obtained by kinetic analysis of the same experiments used for the NHE3 transport activity (V\(_{\text{max}}\)) calculations. P\(_{\text{i}}\) < 0.05. B: surface expression of NHE3 (either epitope tagged with VSVG (NHE3V) or HA (NHE3HA)) and its polymorphic variants R474Q, V567M, and R799C was determined by cell surface biotinylation assay. NHE3 proteins in total lysates (T), surface fractions (S), and intracellular fractions (C) are labeled. A representative experiment, repeated 4 times is shown. Molecular mass standards are shown at right. GAPDH is only shown for WT. C: table shows results are means of 3–4 experiments ± SE. Column 1: relative Na\(^+/\)H\(^+/\) transporter activity in the variants after normalizing to WT NHE3 in each experiment. Column 2: total expression of each polymorphism normalized to WT NHE3. Column 3: percentage of total NHE3 on the plasma membrane. Column 4: normalized surface NHE3 per cell, which was obtained as the product of the normalized surface NHE3 (column 2) times the percent surface expression of each construct (column 3). Rounding causes some variation. In columns 1–4, *P < 0.05. Column 5 is column 1/column 4; however, since experiments in columns 1 and 4 did not entirely overlap, statistical evaluation is not provided.
SNPs in the NHE3 gene. We focused on three nonsynonymous SNPs that result in change in three amino acids within the cytoplasmic, COOH-terminal domain of NHE3. However, we cannot conclude that other SNPs in the NHE3 gene are less important in the setting of NHE3 activity. For instance, SNPs could alter NHE3 activity by influencing expression of the NHE3 gene by altering transcription and/or translation of NHE3 or affecting mRNA stability or microRNA effects. The SNP database searched included all SNPs identified up to 2008. Since that time, many new SNPs have been identified in

R799C also failed to respond to acute stimulation with dexamethasone, while responding normally to inhibition by cAMP and stimulation by serum. These results suggest that there could be a defect in NHE3 function during some postprandial stimulation and under stress conditions in patients expressing these SNPs. In addition, we demonstrated that the R474Q variant had reduced interaction with CHP, providing a mechanism by which the compromised function might occur since CHP binding has been shown to be necessary for Na+/H+ exchange activity by the homologous NHE1 protein (34, 35).

Fig. 3. Alanine substitutions at two polymorphic sites of NHE3 also decreased basal NHE3 activity. Na+/H+ basal activity was measured in PS120/NHERF2 cells stably expressing WT, R474A, or V567A. Data are presented as means ± SE from at least 3 independent experiments. *P < 0.05, comparison between WT NHE3 and R474A and V567A (Student’s t-test). B: surface expression of NHE3 and the mutants R474A and V567A determined by surface biotinylation assay. Studies as described in the legend of Fig 2B. Representative experiment is shown that was repeated at least 3 times. C: table shows results of 4–6 experiments and statistical evaluation as described in Fig. 2C. ↑ P < 0.05.

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Fig. 4. Loss of response to dexamethasone stimulation in NHE3 variants. NHE3 activity was measured in PS120 cells stably expressing WT (A) and R474Q (B) in the presence (●) or absence (○) of dexamethasone (Dexa). C: data are presented as means ± SE of Vmax (C) and K’(H+); (D) for NHE3 activity measured in PS120/NHERF2 cells stably expressing WT, R474Q, V567M, and R799C in the presence (filled bars) and absence (open bars) of dexamethasone from at least 3 independent experiments. *P < 0.05, comparison between controls and dexamethasone-treated groups (paired Student’s t-test).
the exons of NHE3, including 40 additional nonsynonymous SNPs that affect residues in the NHE3 COOH-terminal domain. These are summarized in Table 1. Notably, two SNPs affect residues close to R474, seven affect residues near V567, and two affect residues near R799. Whether these SNPs impact NHE3 activity in a similar way to the SNPs characterized in the present study is not known. Our targeted sequencing of the three SNPs in DNA samples from six patients with congenital diarrhea did not detect mutations at these residues (data not shown), which suggests that other mutations in NHE3 (and/or in other genes) might be contributing to that form of diarrhea.

**SNPs and NHE3 exchanger activity.** The three characterized SNPs affect residues in the COOH-terminal regulatory domain of NHE3. This domain is necessary for the physiologic function of NHE3 that includes inhibition in the immediate postprandial state, presumably to increase luminal water to spread the digestive enzymes over the absorptive surface, followed by stimulation later in the digestive period, presumably to prevent dehydration related to eating. This regulation appears to occur via the COOH terminus acting as a scaffold on which large, multiprotein regulatory complexes form (11, 13, 22). Models of the secondary structure of the NHE3 COOH terminus predict that the NH2-terminal component of the intracellular domain is highly structured and contains multiple α-helices, while the COOH-terminal half is largely disordered under basal conditions (11, 20). The R474 and V567 polymorphisms are present in the NHE3 COOH-terminal domains that take part in the formation or stabilization of these signaling complexes. R474Q impairs intrinsic transporter activity under basal conditions, prevents dexamethasone stimulation of NHE3 activity in response to growth factors and osmotic stress (35). The CHP binding domain is conserved in other NHE isoforms (34) and as shown in Fig. 5A, this area is highly homologous for NHE3. There is 90% amino acid similarity between NHE1 (amino acids 516–542) and NHE3 (amino acids 473–499). Based on the analysis of the cocrystal structure of CHP2 and NHE1 (2, 35) and sequence alignment (Fig. 5A), we predict that the Arg residue at amino acid 474 in NHE3 may be positioned at the entry of the hydrophobic cleft, where it would be expected to help retain and stabilize the long stretch of the NHE3 α-helix in the CHP cleft. Since the R474Q polymorphism reduced CHP coprecipitation with NHE3, it is likely that the disrupted CHP binding explains the phenotype we described of reduced basal NHE3 activity. However, since only a 13% reduction of CHP binding was demonstrated, cause and effect has not been established. In addition, this stimulatory signaling complex involves direct binding of ezrin to NHE3 amino acids 517, 521, and 528, which requires phosphorylation of amino acids 516 and 527 (human NHE3) by Akt and GSK-3, respectively, as well as CHP binding (38). This ezrin binding to NHE3 is required not only for basal NHE3 activity but for multiple examples of stimulated NHE3 activity, and it is possible that ezrin binding is disrupted by this polymorphism to explain the failure of dexamethasone to stimulate NHE3.

The V567M polymorphism has a different effect on NHE3 activity than the R474Q, reducing NHE3 activity by decreasing the percentage of transporter on the plasma membrane as well as having less made although the function of each NHE3 molecule on the plasma membrane had full or increased  

\[ V_{\text{max}} \]

activity but with an increased \[ K' \] (19). This suggests that this phenotype is due to abnormal NHE3 trafficking or stability on
the membrane but with some altered turnover number. Due to the level of expression being variable among clones from the same construct in NHE3 studies in general, we are reluctant to attribute a role for altered expression in patients with these polymorphisms. The amino acid V567 is just outside the beginning of what we have called the stimulatory/inhibitor regulatory complex (11) and is predicted to help stabilize this part of the NHE3 COOH terminus. This NHE3 complex involves the direct binding of two kinases, one of which stimulates basal NHE3 activity (CK2) (36) while the other inhibits basal NHE3 activity (CaM kinase II) (1), as well as the area that the NHERF family of multi-PDZ domain proteins binds. The NHERFs form a large percentage of the NHE3 complexes and fix it to the plasma membrane in a dynamic manner that is necessary for regulation by trafficking (7). Whether this V567M polymorphism disrupts this NHE3 regulatory complex is not known, although we did not find that V567M altered NHERF2 binding (data not shown).

R799 lies in the downstream part of the NHE3 COOH terminus that appears nonstructured under basal conditions (11, 20). While this part of NHE3 binds megalin and PP2A and is the domain phosphorylated by both CK2 and CaM kinase II (7, 47), how it contributes to NHE3 function is not known. The observation that R799C increases CHP binding to NHE3 (Fig. 5, B and C) demonstrates that there is an interaction among seemingly distant parts of the COOH terminus and that understanding the structure of the COOH terminus, probably in the presence of the components of the signaling complexes, will be needed to fully understand how regulation of NHE3 occurs. The K′(H+), was increased for all three polymorphisms, which supports that this complex characteristic of NHE3 activity is influenced by the regulatory domain as well as by the transport domain. Given that the K′(H+) was affected by changes in different parts of the regulatory domain along with different mechanisms explaining the changes in NHE3 function, conclusions cannot be made about how these changes in the NHE3 COOH terminus alter K′(H+).

Altered response to dexamethasone. Dexamethasone has been shown to acutely stimulate NHE3 activity through increased surface expression of NHE3 (40, 41, 45). Both R474Q and R799C, which exhibited decreased intrinsic activity, lost the response to dexamethasone, whereas V567M, which displayed impaired membrane trafficking or stability, maintained a normal stimulatory response. This suggests that short-term dexamethasone exposure may influence NHE3 activity via an additional unidentified mechanism. cAMP is a key second messenger that mediates the regulatory effect of hormones and growth factors on NHE3 (5, 46, 47). The normal extent of cAMP related inhibition of NHE3 that was present in all these polymorphisms indicates that the contribution to diarrhea of the reduced NHE3-related Na+ absorption is likely to occur as in the population with wild-type NHE3, while the compensatory increased Na+ absorption due to NHE3 is likely to be compromised in some patients with these polymorphisms.

Potential clinical significance of SNPs with reduced NHE3 activity. Although we have identified three SNPs that have reduced NHE3 activity, it is not known if individuals bearing these SNPs have a phenotype related to abnormal bowel function. It would be predicted that this could take several forms that include an increased susceptibility to or increased severity of diarrhea, for instance that associated with a high glucocorticoid environment, such as inflammatory bowel disease, acute infectious diarrhea or even irritable bowel syndrome-diarrhea predominant, and/or reduced response to drugs that target stimulation of NHE3 to treat diarrhea or target inhibition of NHE3 to treat constipation and perhaps treat hypertension by reducing total body Na+, as recently suggested (30). In our separate studies, severely reduced NHE3 activity in several cases of congenital Na+ diarrhea was shown to be due to nonfunctional NHE3 mutations in the NH2-terminal transport domain in both alleles of NHE3 presenting as an autosomal recessive condition (unpublished observations, Janecke et al.). Thus in searching for clinical relevance of the functionally compromised polymorphisms, the population at risk is likely to be that in which both alleles are affected.

While the three polymorphisms studied have variable allele frequency, studies in specific populations with increased prevalence offer the best chance of understanding whether phenotypes exist as consequences of altered NHE3 activity from these SNPs. For instance, the genotype frequencies for 474R/474Q and 567V/567M are 0.02 and 0.22, respectively (14, 33). In contrast, the R799C polymorphism is distributed widely in European, African, and Asian populations (33); in fact, the European populations have genotype frequencies of 0.014 for 799R/799R, 0.215 for 799C/799R, and 0.771 for 799C/799C alleles, whereas the Asian-Han populations have much higher genotype frequencies for 799R/799R (0.302) and 799C/799R (0.514) but less frequency for 799C/799C (0.278), likely conferring reduced NHE3 activity (14, 33). Needing to be examined in these populations is the effects of the presence of a Cys rather than an Arg at amino acid position 799 on intestinal Na+ absorption and predisposition to acute and chronic diarrhea.

These and other nonsynonymous polymorphisms of NHE3 exist in variable gene frequencies among populations and since they have variable transport activities should be evaluated for possible contributions to diseases associated with abnormal water and electrolyte homeostasis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: X.C.Z., R.S., M.C., T.-E.C., C.M.T., B.C., and M.D. approved the final version of manuscript.

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