Regulation of Epithelial Sodium Channel Trafficking by Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)*

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Background: The epithelial Na\(^{+}\) channel ENaC functions as a pathway for Na\(^{+}\) absorption across epithelia.

Results: PCSK9 reduced ENaC expression at the cell surface by enhancing its proteasomal degradation.

Conclusion: PCSK9 inhibits ENaC-mediated Na\(^{+}\) absorption.

Significance: These findings provide new insights into mechanisms that regulate Na\(^{+}\) homeostasis and blood pressure.

The epithelial Na\(^{+}\) channel (ENaC) is critical for Na\(^{+}\) homeostasis and blood pressure control. Defects in its regulation cause inherited forms of hypertension and hypotension. Previous work found that ENaC gating is regulated by proteases through cleavage of the extracellular domains of the α and γ subunits. Here we tested the hypothesis that ENaC is regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), a protease that modulates the risk of cardiovascular disease. PCSK9 reduced ENaC current in Xenopus oocytes and in epithelia. This occurred through a decrease in ENaC protein at the cell surface and in the total cellular pool, an effect that did not require the catalytic activity of PCSK9. PCSK9 interacted with all three ENaC subunits and decreased their trafficking to the cell surface by increasing proteasomal degradation. In contrast to its previously reported effects on the LDL receptor, PCSK9 did not alter ENaC endocytosis or degradation of the pool of ENaC at the cell surface. These results support a role for PCSK9 in the regulation of ENaC trafficking in the biosynthetic pathway, likely by increasing endoplasmic reticulum-associated degradation. By reducing ENaC channel number, PCSK9 could modulate epithelial Na\(^{+}\) absorption, a major contributor to blood pressure control.

The epithelial Na\(^{+}\) channel (ENaC)\(^2\) plays an important role in absorption of Na\(^{+}\) across epithelia, including the kidney, collecting duct and connecting tubule, lung, distal colon, and sweat duct (reviewed in Refs. 1, 2). A heterotrimer composed of three homologous subunits (α, β, and γ), ENaC is expressed at the apical membrane where it forms a pathway for Na\(^{+}\) to enter the cell. Na\(^{+}\) leaves the cell at the basolateral membrane via the Na\(^{+}\)-K\(^{+}\)-ATPase, which completes the pathway for Na\(^{+}\) absorption. This process is critical to control extracellular volume and to maintain the composition and quantity of epithelial surface liquid. This is illustrated by several diseases. For example, ENaC mutations that slow its retrieval from the cell surface cause an inherited form of hypertension (Liddle’s syndrome), resulting from excessive renal Na\(^{+}\) absorption (3–5). Defects in ENaC regulation are responsible for most of the known genetic forms of hypertension (6). Conversely, loss of function mutations cause pseudohypoaldosteronism type 1, a disorder of renal Na\(^{+}\) wasting (7). In the lung, defects in ENaC activity cause pulmonary edema and may contribute to the pathogenesis of cystic fibrosis (8).

Previous work indicates that ENaC is regulated by serine proteases (reviewed in Ref. 9). Furin, a member of the proprotein convertase family, cleaves the extracellular domain of αENaC at basic motifs, removing a 26-amino acid fragment (10). In γENaC, furin cleaves the extracellular domain at a single site and a second, more distal site is cleaved by additional proteases (e.g. CAPI/prostasin, plasmin, elastase), releasing a fragment of ~43 amino acids (11–15). Proteolytic cleavage of α- and γENaC converts quiescent channels into active Na\(^{+}\)-conducting channels. This activation occurs by relieving the channel from inhibition by extracellular Na\(^{+}\) (“Na\(^{+}\) self-inhibition”) (16). Proteolytic cleavage of ENaC is a regulated process. For example, cleavage is inhibited by increased intracellular Na\(^{+}\), providing a negative feedback mechanism to regulate Na\(^{+}\) absorption (17). Conversely, cleavage is enhanced by Na\(^{+}\) depletion and aldosterone infusion (18, 19). Cleavage is also disrupted in pathological states. In Liddle’s syndrome, cleavage is increased, likely through prolonged exposure of ENaC to proteases present at the cell surface (5). There is also evidence to suggest that ENaC cleavage is increased in nephrotic syndrome (15, 20) and cystic fibrosis (21, 22).

Because proteolytic cleavage modulates ENaC gating, there has been considerable interest in identifying additional proteases that regulate ENaC. The proprotein convertase family has nine members, including furin (23). In this work, we investigated a potential role for another member of this family, proprotein convertase subtilisin/kexin type 9 (PCSK9) (24). Consistent with a potential role in ENaC regulation, PCSK9 is expressed in the kidney and lung (24). It is synthesized as a 72-kDa immature precursor that undergoes autocatalytic cleavage in the endoplasmic reticulum to generate a 63-kDa mature protein (25). The cleaved N-terminal fragment remains
associated with the mature protein and is necessary for its secretion, allowing it to circulate in the blood (26).

Previous work has focused on the role of PCSK9 in the regulation of the LDL receptor (LDLR). By reducing expression of the LDLR at the cell surface, PCSK9 increases serum levels of LDL cholesterol (25, 27, 28). Rare gain-of-function PCSK9 mutations cause hypercholesterolemia and increase the risk of coronary heart disease, whereas loss-of-function mutations cause hypcholesterolemia and protect against heart disease (29–33). The mechanisms by which PCSK9 alters LDLR surface expression are not completely understood. Secreted PCSK9 (or recombinant PCSK9 added to the extracellular medium) binds to the LDLR and undergoes endocytosis (26, 27, 34–36). In the endocytic pathway, PCSK9 increases lysosomal degradation of the LDLR. Although secreted PCSK9 regulates LDLR trafficking, additional evidence suggests that PCSK9 may also induce LDLR degradation through an intracellular route (37). Interestingly, although PCSK9 induces degradation of the LDLR, its protease activity is not required (33, 38). Thus, it has been proposed that PCSK9 regulates the LDLR through a chaperone mechanism, rather than through its function as a protease. Although it seems clear that the PCSK9 regulates the LDLR and two closely related receptors (very low density lipoprotein receptor and apolipoprotein E receptor 2 (38)), additional substrates for PCSK9 have not been identified. Here we show that PCSK9 regulates ENaC and we explore the mechanisms that underlie this regulation.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—Human αENaC, βENaC, and γENaC were cloned in pMT3 as described previously (39, 40). Mutations were generated by site-directed mutagenesis (QuikChange; Stratagene). αENaC-FLAG, βENaC-FLAG, and γENaC-FLAG were generated by insertion of a FLAG epitope (DYKDDDDK) at the C terminus (5, 41). Human PCSK9-V5 was a generous gift from Nabil Seidah (24), and Nedd4-2-HA was generated as described (42). Mutations were generated (QuikChange, Stratagene) in αENaC (Y644A, R175A, R177A, R178A, R181A, R190A, R192A, R201A, R204A), βENaC (Y620A), and γENaC (Y627A) (G536C) as described previously (3, 43, 44) and in PCSK9 (S386A). All cDNAs were sequenced in the University of Iowa DNA Core Facility.

**Electrophysiology in Xenopus Oocytes**—Oocytes were harvested from *Xenopus laevis* females. They were treated for 1 h with 0.75 mg/ml type IV collagenase (Sigma) in Ca2+-free ND-96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES (pH 7.4)) and manually defolliculated (45–47). The cell nucleus was injected with cDNAs encoding either human α-, β-, or γENaC (0.03 μg each) with or without PCSK9 (0–0.9 μg) using TFX50 (Promega) as described previously (44, 49). Total cDNA was kept constant using GFP cDNA (which does not alter ENaC current). Two days after transfection, the current was measured in Ussing chambers under short-circuit conditions using an EC-825 amplifier (Warner Instruments), digitized with a Powerlab interface (ADInstruments), and recorded and analyzed with Chart software (ADInstruments). The cells were bathed in 116 mM NaCl, 2 mM KCl, 0.4 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES (pH 7.4 or 5). The amiloride-sensitive ENaC current was measured by adding 10 μM amiloride to the bathing solution. ASIC1 currents were detected by addition of pH 5 to the bathing solution.

**Electrophysiology in Epithelia**—To test the effect of PCSK9 on ENaC current in epithelia, Fischer rat thyroid (FRT) cells were cultured on permeable filter supports (Millicell PCF, 0.4-μm pore size, 12-mm diameter) in F-12 Coon’s medium (Sigma) with 5% fetal calf serum (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C. Cells were transfected with α-, β-, and γENaC (0.03 μg each) and with or without PCSK9 (0–0.9 μg) using TFX50 (Promega) as described previously (44, 49). Total cDNA was kept constant using GFP cDNA (which does not alter ENaC current). Two days after transfection, the current was measured in Ussing chambers under short-circuit conditions using an EC-825 amplifier (Warner Instruments), digitized with a Powerlab interface (ADInstruments), and recorded and analyzed with Chart software (ADInstruments). The apical and basolateral surfaces were bathed in 135 mM NaCl, 1.2 mM CaCl2, 1.2 mM MgCl2, 2.4 mM K2HPO4, 0.6 mM KH2PO4, and 10 mM HEPES (pH 7.4) at 37 °C. Amiloride (10 μM) was added to the apical solution to quantitate ENaC current.

For exocytosis experiments, FRT cells were transfected with αENaC, βENaC, and γENaC (0.167 μg each subunit) with PCSK9 or GFP cDNA (0.5 μg) (43, 49). Channels at the cell surface were irreversibly blocked by covalent modification of the introduced cysteine with 1 mM [2-(trimethylammonium)-ethyl]methanethiosulfonate bromide (MTSET). Following removal of MTSET, we measured the rate of current increase to quantitate exocytosis of unblocked channels. Time constants (τ) were determined by fitting the data to single-exponential equations using IGOR Pro 6.01 software.

**Coominunoprecipitation**—HEK 293T cells were cultured in Dulbecco’s modified Eagle’s medium. To test for interactions between ENaC and PCSK9, the cells were transfected with αENaC, βENaC, and γENaC (individually or together, 1 μg each) and PCSK9-V5 or GFP (1 or 3 μg) using Lipofectamine2000 (Invitrogen) (5, 41). One ENaC subunit contained a FLAG epitope. Two days after transfection, the cells were lysed in Nonidet P-40 lysis buffer (0.4% sodium deoxycholate, 1% Nonidet P-40, 63 mM EDTA, 50 mM Tris-HCl (pH 8), and protease inhibitor mixture (Sigma)). 500 μg of cellular protein was immunoprecipitated with anti-FLAG M2 affinity gel (Sigma) or anti-V5 antibody (Invitrogen) with immobilized protein A (Pierce) beads overnight at 4 °C. Following SDS-PAGE, ENaC and PCSK9 were detected by immunoblot analysis using anti-FLAG M2 monoclonal antibody-peroxidase conjugate (1:5000, Sigma) at 1:5000 dilution or anti-V5 antibody (1:5000) and enhanced chemiluminescence (ECL Plus, GE Healthcare).

**Biotinylation**—To quantitate ENaC expression at the cell surface, HEK 293 cells expressing α-, β-, and γENaC (FLAG epitope on one subunit) and PCSK9 or GFP were washed with
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4 °C PBS containing 1 mM CaCl₂ and 1 mM MgCl₂ (PBS-CM). Surface proteins were biotinylated with 0.5 mg/ml sulfo-NHS-biotin (Pierce) for 30 min at 4 °C (5). Excess biotin was quenched with 100 mM glycine in PBS-CM for 20 min at 4 °C. The cells were lysed in 1% Nonidet P-40, 150 mM NaCl, 50 mM Tris (pH 7.4), and protease inhibitors (Sigma) at 4 °C, and then centrifuged at 14,000 rpm for 10 min to remove insoluble material. Biotinylated proteins were isolated with NeutrAvidin-agarose beads (Pierce) overnight at 4 °C. Following extensive washing, biotinylated proteins were eluted with SDS sample buffer (4% SDS, 100 mM dithiothreitol, 20% glycerol, and 100 mM Tris-Cl (pH 6.8)) and separated by SDS-PAGE. Biotinylated ENaC and ENaC in the total cellular lysate were detected by immunoblot using anti-FLAG M2-peroxidase-conjugated antibody (1:5000, Sigma) and enhanced chemiluminescence (ECL Plus, GE Healthcare) and quantitated by densitometry (ImageJ) using non-saturated exposures.

Degradation—To measure the rate of ENaC degradation, HEK 293 cells transfected with αENaC-FLAG, βENaC, and γENaC with PCSK9 or GFP were incubated with cycloheximide (10 μg/ml) for 0–120 min. Remaining αENaC-FLAG at each time point was detected by immunoblot (anti-FLAG M2-peroxidase-conjugated antibody) and quantitated by densitometry. To identify the location of degradation, cells were treated with 10 μM N-acetyl-Leu-Leu-norleucinal or 5 mM NH₄Cl.

To measure the rate of degradation of the cell surface fraction of ENaC, HEK 293 cells transfected with αENaC-FLAG, βENaC, and γENaC with PCSK9 or GFP were biotinylated on ice and then incubated at 37 °C for 0–120 min (5, 41). Biotinylated αENaC-FLAG was isolated using NeutrAvidin-agarose, detected by immunoblot (anti-FLAG M2-peroxidase-conjugated antibody), and quantitated by densitometry.

Endocytosis—To measure the rate of ENaC endocytosis, we used a previously described αENaC construct (αC₃₂) in which multiple arginines were simultaneously mutated to prevent proteolytic cleavage by furin but to retain the ability to be cleaved by trypsin (R175A, R177A, R178A, R181A, R190A, R192A, R201A, and R204A) (44). HEK 293 cells were transfected with αC₃₂-ENaC-FLAG, βENaC, and γENaC with PCSK9 or GFP were incubated with trypsin (5 μg/ml) for 5 min at 37 °C to generate a pool of cleaved channels at the cell surface (9)(44). The cells were washed three times with cold PBS-CM to remove trypsin, incubated at 37 °C for 0–60 min to allow endocytosis of cleaved channels, and then placed on ice. Cleaved channels remaining at the cell surface were labeled with biotin, isolated with NeutrAvidin-agarose, detected by immunoblot analysis (anti-FLAG M2-peroxidase-conjugated antibody), and quantitated by densitometry.

RESULTS

PCS9 Inhibits ENaC—We tested the effect of PCSK9 on ENaC current utilizing two expression systems. First, we injected Xenopus oocytes with α- , β- , and γENaC cDNA to generate amiloride-sensitive Na⁺ currents (Fig. 1A). We found that coexpression of PCSK9 decreased the Na⁺ current in a dose-dependent manner (Fig. 1A and B).

As a second strategy, we tested the effect of PCSK9 on the ENaC current in epithelia. Transfection of FRT epithelia with α-, β-, and γENaC resulted in amiloride-sensitive short-circuit currents (Fig. 1C). Cotransfection with PCSK9 produced a dose-dependent decrease in ENaC current (Fig. 1, C and D), similar to our results in oocytes. Thus, PCSK9 inhibited ENaC in two independent experimental systems.

We also tested the effect of PCSK9 on a related DEG/ENaC channel,ASIC1. PCSK9 reduced the proton-activated ASIC1 current by 24% in Xenopus oocytes (Fig. 1, E and F), less than its effect on ENaC.

PCS9 Interacts with ENaC—To begin to investigate the mechanism by which PCSK9 inhibits ENaC current, we tested whether PCSK9 and ENaC interact with one another. In Fig. 2A, we transfected HEK 293 cells with α- , β- , and γENaC (one of the subunits contained a FLAG epitope) along with PCSK9 (V5 epitope) and examined protein interactions using a coimmunoprecipitation assay. When we immunoprecipitated αENaC, we detected coprecipitated PCSK9 in cells cotransfected with ENaC and PCSK9 but not in cells transfected individually with either ENaC or PCSK9 (Fig. 2A, first panel). Likewise, we detected PCSK9 when we immunoprecipitated β- or
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PCSK9 Regulates ENaC Trafficking—We asked whether PCSK9 inhibits ENaC current through a change in ENaC surface expression. To test whether protease activity is needed for PCSK9 to reduce ENaC surface expression, we introduced a mutation that abolishes proteolytic activity (S386A) (33). PCSK9S386A decreased αENaC expressed at the cell surface and in the total cellular pool similar to wild-type ENaC (Fig. 3D). Thus, the catalytic activity is not required for PCSK9 to regulate ENaC, similar to its regulation of the LDLR.

PCSK9 Increases ENaC Degradation—To further investigate the mechanism by which PCSK9 reduced ENaC cell surface expression, we asked whether PCSK9 alters ENaC degradation using a cycloheximide chase assay. HEK 293 cells expressing ENaC and PCSK9 or GFP (control) were treated with cycloheximide for 0–120 min to inhibit protein synthesis. In Fig. 4, A and B, we detected and quantitated the remaining αENaC-FLAG at each time point by immunoblot analysis. In the absence of PCSK9, there was no significant decrease in αENaC over the 120-min time course of the experiment. In contrast, in

γENaC (Fig. 2A, first panel). There are two forms of PCSK9, full-length pro-PCSK9 (72 kDa) and autocatalytically cleaved PCSK9 (63 kDa) (Fig. 2A, third panel) (25). ENaC selectively coprecipitated the pro-PCSK9 form (Fig. 2A, first panel). Using a reciprocal strategy we found that α-, β-, and γENaC each coprecipitated when we immunoprecipitated PCSK9 (Fig. 2A, second panel). In the immunoblot analysis, we observed two bands for α- and γENaC, which correspond to the full-length (immature) and proteolytically cleaved (mature) forms, respectively (βENaC does not undergo cleavage). The bands that coprecipitated with PCSK9 correspond to the full-length forms of α- and γENaC.

Because α-, β-, and γENaC form a complex, we asked if each of the individual subunits could also bind to PCSK9. We cotransfected HEK 293 cells with one of the ENaC subunits, with or without PCSK9. When we immunoprecipitated each of the ENaC subunits, we detected pro-PCSK9 by immunoblot (Fig. 2B, top panel). Thus, the data indicate that PCSK9 interacts with each of the three ENaC subunits. Moreover, the interactions occur selectively between the uncleaved immature forms of PCSK9 and ENaC.

PCSK9 Reduces ENaC Cell Surface Expression—We asked whether PCSK9 inhibits ENaC current through a change in ENaC surface expression. In Fig. 3A, we used a biotinylation assay to detect the cell surface fraction of αENaC (coexpressed with β- and γENaC) in HEK 293 cells. Fig. 3B shows quantitative summary data. PCSK9 decreased both the full-length and proteolytically cleaved forms of αENaC at the cell surface. This decrease in surface expression corresponded to a decrease in αENaC in the total cellular pool, as detected by immunoblot analysis of cell lysates (Fig. 3A, bottom panel; and B; also see Fig. 2A). PCSK9 produced a similar decrease in expression of β- and γENaC at the cell surface and in βENaC in the total cellular pool (Fig. 3A and B). As negative controls, PCSK9 had no effect on the abundance of heterologously expressed Nedd4-2 or endogenous β-actin (Fig. 3C). These results indicate that PCSK9 inhibits ENaC current by reducing the number of channels at the cell surface.

To determine whether PCSK9 also regulates ENaC gating, we took advantage of a mutation that locks ENaC in the open state (“DEG” mutation, βS320K) (50). If PCSK9 inhibits ENaC in part through a change in gating, this mutation should blunt the effect. However, we found that PCSK9 inhibited mutant ENaC to the same extent as wild-type ENaC (Fig. 3D). This finding indicates that the changes we observed in ENaC surface expression are sufficient to explain PCSK9 inhibition of ENaC.

Prior work has shown that the PY motifs located in the C termini of ENaC subunits play an important role in trafficking (51). The PY motifs function as binding sites for Nedd4-2, an E3 ubiquitin ligase that catalyzes ENaC ubiquitination. This function as a signal to induce ENaC endocytosis and degradation in lysosomes. Importantly, mutations in the PY motifs cause Lidde’s syndrome, an inherited form of hypertension. To test whether the PY motifs are required for ENaC regulation by PCSK9, we mutated the conserved tyrosine residue within the motif of each ENaC subunit (αENaC:FLAG, βENaC-FLAG, or γENaC-FLAG, 1 µg) with or without PCSK9-V5 (1 µg). The proteins were immunoprecipitated and immunoblotted as in A. The data are representative of three experiments.
cells transfected with PCSK9, there was a time-dependent decrease in αENaC. This finding indicates that PCSK9 accelerates the rate of ENaC degradation.

To localize the site of the PCSK9-induced ENaC degradation, we incubated cells with inhibitors of the proteasome (N-acetyl-Leu-Leu-norleucinal) or lysosomes (NH₄Cl). We found that N-acetyl-Leu-Leu-norleucinal partially reversed the effect of PCSK9 on αENaC expression, whereas NH₄Cl had no effect (Fig. 4C). Together, the data indicate that PCSK9 reduces ENaC surface expression in part by enhancing its degradation in the proteasome.

Effect of PCSK9 on ENaC Exocytosis—ENaC surface expression is controlled through a balance between exocytosis of newly formed channels, endocytosis of cell surface channels, and recycling of channels in the endocytic pathway. Because PCSK9 increased ENaC degradation, it seemed likely that PCSK9 would reduce the pool of ENaC available for exocytosis. To test this possibility, we used a functional strategy we reported previously (49). We covalently modified the cell surface pool of ENaC and then measured the rate of appearance of unmodified channels at the cell surface. For these experiments, we placed a cysteine in the pore of αENaC (G536C) (43, 49).

When the mutant αENaC subunit was coexpressed in FRT epithelia with wild-type or mutant βENaC (0.2 ng each) with or without PCSK9 (0.8 ng) (mean ± S.E. relative to -PCSK9 group; n = 11–17; *, p < 0.004; n.s., p ≥ 0.05). E, immunoblot of biotinylated cell surface αENaC-FLAG coexpressed with βY620AK and γENaC (1 μg each) with PCSK9 or GFP (3 μg). F, immunoblot of biotinylated (top panel) and total (bottom panel) αENaC-FLAG coexpressed in HEK 293 cells with β- and γENaC (1 μg each) with GFP or PCSK9 (wild type or S386A, 3 μg). Irrelevant lanes were removed digitally.

FIGURE 3. PCSK9 reduces ENaC cell surface expression. A, immunoblot analyses of ENaC in the cell surface biotinylated fraction (top panel) and in the total cell lysate (bottom panel) from HEK 293 cells transfected with α-, β-, and γENaC (1 μg each, one subunit contained FLAG epitope) with or without PCSK9 (3 μg). Total cDNA was kept constant using GFP cDNA. ENaC protein in +PCSK9 group relative to -PCSK9 group is quantified by densitometry in B (mean ± S.E.; n = 3–5; *, p < 0.03). C, immunoblots of Nedd4-2-HA (anti-HA) and β-actin in HEK 293 cells transfected with Nedd4-2-HA (3 μg) with or without PCSK9 (3 μg). D, amiloride-sensitive current in Xenopus oocytes expressing human α- and γENaC with wild-type or mutant βENaC (0.2 ng each) with or without PCSK9 (0.8 ng) (mean ± S.E. relative to -PCSK9 group; n = 11–17; *, p < 0.004; n.s., p ≥ 0.05). E, immunoblot of biotinylated cell surface αENaC-FLAG coexpressed with βY620AK and γENaC (1 μg each) with PCSK9 or GFP (3 μg). F, immunoblot of biotinylated (top panel) and total (bottom panel) αENaC-FLAG coexpressed in HEK 293 cells with β- and γENaC (1 μg each) with GFP or PCSK9 (wild type or S386A, 3 μg). Irrelevant lanes were removed digitally.
The proprotein convertase furin regulates ENaC by proteolytic cleavage of the extracellular domains of α- and γENaC, which releases inhibitory peptides (9). In this manner, furin regulates ENaC gating, converting near-silent channels into active channels. However, there may be an additional level of complexity. Furin also proteolytically cleaves PCSK9, which inactivates it (53). Thus, through a decrease in PCSK9 activity, furin could increase ENaC cell surface expression. This raises the interesting possibility that furin regulates ENaC through dual effects on channel trafficking and gating.
Defects in ENaC regulation are responsible for the majority of the known genetic forms of hypertension, which is an important risk factor for coronary heart disease and other cardiovascular diseases. Thus, PCSK9 could modulate cardiovascular risk in part through its regulation of ENaC. We speculate that a decrease in PCSK9 activity would increase renal Na\(^{+}\)/H\(^{+}\) absorption and, therefore, raise the risk of hypertension and associated cardiovascular disease. However, such a mechanism would counter the previously reported effect of PCSK9 mutations on cardiovascular risk. Activating mutations were found to increase the risk, whereas loss-of-function mutations reduced the risk (29–33). These effects are thought to occur through changes in expression of the LDLR, which produce changes in serum levels of cholesterol. Thus, it is possible that PCSK9 regulation of ENaC and the LDLR have opposing effects on cardiovascular risk. On the other hand, our data suggest that PCSK9 regulates ENaC and the LDLR through different binding sites and different mechanisms. Thus, the PCSK9 mutations that disrupt LDLR regulation may have dissimilar effects on ENaC. Additional work will be required to test whether naturally occurring mutations in PCSK9 alter ENaC trafficking, renal Na\(^{+}\) homeostasis, and blood pressure.

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