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Epithelial sodium channel (ENaC) in GtoPdb v.2025.3

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Abstract

Overview
The epithelial sodium channels (ENaC) are located on the apical membrane of epithelial cells in the kidney tubules, lung, respiratory tract, male and female reproductive tracts, sweat and salivary glands, placenta, colon, and some other organs [10, 49, 15, 24, 23]. In these epithelia, Na^+ ions flow from the extracellular fluid into the cytoplasm of epithelial cells *via* ENaC and are then pumped out of the cytoplasm into the interstitial fluid by the Na^+/K^+ ATPase located on the basolateral membrane [43]. As Na^+ is one of the major electrolytes in the extracellular fluid (ECF), osmolarity change initiated by the Na^+ flow is accompanied by a flow of water [7]. Thus, ENaC has a central role in regulating ECF volume and blood pressure, primarily *via* its function in the kidney [44]. The expression of ENaC subunits, hence its activity, is regulated by the renin-angiotensin-aldosterone system, and other factors involved in electrolyte homeostasis [44, 33].

The genetics of the hereditary systemic pseudohypoaldosteronism type-I revealed that the activity of ENaC is dependent on three subunits encoded by three genes [24, 12]. Within the protein superfamily that includes ENaC, the crystal structure of ASIC was determined first, revealing a trimeric structure with a large extracellular domain anchored in the membrane with a bundle of six TM helices (two TM helices/subunit) [3, 27]. The first 3D structure of human ENaC was determined by single-particle cryo-electron microscopy at a resolution of 3.7 Å [39]. A recent study improved the resolution to 3 Å [40]. These structures confirmed that ENaC has a 3D quaternary structure similar to ASIC. ENaC is assembled as a hetero-trimer with a clockwise order of α-γ-β subunit viewed from the top, as shown previously [13]. In contrast to ASIC1 which can assemble into a functional homotrimer, ENaC activity can be reconstituted fully only as a heterotrimer with an αβγ or a δβγ composition [30].

In the respiratory tract and female reproductive tract, large segments of the epithelia are composed of multi-ciliated cells. In these cells, ENaC is located along the entire length of the cilia that cover the cell surface [17]. Cilial location greatly increases ENaC density per cell surface and allows ENaC to serve as a sensitive regulator of osmolarity of the periciliary fluid throughout the whole depth of the fluid bathing the cilia [17]. In contrast to ENaC, CFTR (ion transporter defective in cystic fibrosis) is located on the non-cilial cell surface [17]. In the *vas deferens* segment of the male reproductive tract, the luminal surface is covered by microvilli and stereocilia projections with backbones composed of actin filament bundles [49]. In these cells, both ENaC and the water channel aquaporin AQP9 are localized on these projections and also in the basal and smooth muscle layers [49]. Thus, ENaC function regulates the volume of fluid lining epithelia essential for mucociliary clearance of respiratory airways, transport of germ cells, fertilization, implantation, and cell migration [38, 17, 24].

Genes and Phylogeny
In the human genome, there are four homologous genes (*SCNN1A*, *SCNN1B*, *SCNN1D*, and *SCNN1G*) that encode four proteins, α-, β-, γ-, and δ-ENaC that may be involved in the assembly of ENaC [11, 35, 48, 54]. These four subunits share 23-34% sequence identity and <20% identity with ASIC subunits [24]. The genes coding for all four ENaC subunits are present in all bony vertebrates with the exception of ray-finned fish genomes that have lost all ENaC genes. The mouse genome has lost the gene *SCNN1D* that codes for δ-ENaC [19, 24, 24]. The α-, β-, and γ-ENaC genes are also present in jawless vertebrates (*e.g.*, lampreys) and cartilaginous fishes (*e.g.*, sharks) [24]. Examination of the methylation patterns of the 5'-flanking region of *SCNN1A, SCNN1B*, and *SCNN1G* genes in human cells showed an inverse correlation between gene expression and DNA

methylation, suggesting epigenetic transcriptional control of ENaC genes [42].

Channel biogenesis, assembly and function
The expression of ENaC subunits is regulated primarily by aldosterone and many additional extracellular and intracellular factors [44, 32, 41]. Most of the studies indicate that the expression of the three subunits is not coordinated [9]. However, the transport of the subunits to the membrane is dependent on three intact subunits. Even a missense mutation in one subunit reduces the concentration of assembled channels on the cell surface [16].

ENaC is a constitutively active channel, *i.e.*, the flow of Na^+ ions is not dependent on an activating factor. Hence, heterologous cells expressing ENaC (*e.g.*, *Xenopus* oocytes), must be maintained in a solution that contains amiloride to keep ENaC inhibited. To measure ENaC activity, the bath solution is switched to a solution without amiloride. ENaC has two major states: 1) Open, and 2) Closed. The probability of ENaC being in the open state is called ENaC open probability (Po). ENaC activity is regulated by a diverse array of factors that exert their effects by modifying, directly or indirectly, two major parameters: 1) The density of ENaC in the membrane; and 2) The channel open probability [28, 30]. The Po of ENaC is greatly decreased by external Na^+ and this response is called Na^+ self-inhibition [50, 4, 26].

An important aspect of ENaC regulation is that the α and the γ subunits have conserved serine protease cleavage sites in the extracellular segment [24]. Cleavage of these subunits by proteases such as furin and plasmin leads to the activation of ENaC [45, 31, 1].

Diseases associated with ENaC mutations
Mutations in any of the three genes (*SCNN1A, SCNN1B*, and *SCNN1G*) may cause partial or complete loss of ENaC activity, depending on the mutation [12, 21]. Such loss-of-function mutations are associated with a syndrome named "systemic" or "multi-system" autosomal recessive pseudohypoaldosteronism type I (PHA1B) [20, 12, 24, 17, 56, 47]. So far, no mutation has been found in the *SCNN1D* gene that causes PHA. PHA patients suffer from severe salt loss from all aldosterone target organs expressing ENaC, including kidney, sweat and salivary glands and respiratory tract. During infancy and early childhood, the severe electrolyte disturbances, dehydration and acidosis may require recurrent hospitalizations. The severity and frequency of salt-wasting episodes improve with age [22]. PHA1B is also associated with a dysfunctional female reproductive system [17, 6].

The carboxy-terminal of ENaC includes a short consensus sequence called the PY motif. Mutations in this motif in *SCNN1B* and *SCNN1G* are associated with Liddle syndrome, which is characterized by early-onset hypertension [5, 51]. The PY motif is recognized by Nedd4-2 that is a ubiquitin ligase. Thus, mutations in the PY motif reduce ubiquitylation of ENaC leading to the accumulation of ENaC in the membrane, consequently enhance the activity of ENaC [46].

ENaC expression in tumors
The observation that $[Na^+]$ is higher in many cancerous cells as compared to non-cancerous cells has led to the suggestion that enhanced expression of ENaC may be responsible for increased metastasis [34]. However, analysis of RNA sequencing data of ENaC-encoding genes, and clinical data of cervical cancer patients from The Cancer Genome Atlas showed a negative correlation with histologic grades of tumor [52]. Similarly, studies on breast cancer cells that altered α-ENaC levels by over-expression or siRNA-mediated knockdown showed that increased α-ENaC expression was associated with decreased breast cancer cell proliferation [55]. In contrast, analysis of RNA sequencing data from The Cancer Genome Atlas showed that high expression of *SCNN1A* was correlated with poor prognosis in patients with ovarian cancer [36]. These findings indicate that the association of ENaC levels with tumorigenesis varies depending on the tissue.

COVID-19
The surface of SARS-CoV-2 virions that cause COVID-19 is covered by many glycosylated S (spike) proteins. These S proteins bind to the membrane-bound angiotensin-converting enzyme 2 (ACE2) as a first step in the entry of the virion into the host cell. Viral entry into the cell is dependent on the cleavage of the S protein (at Arg-667/Ser-668) by a serine-protease. Anand *et al*. showed that this cleavage site has a sequence motif that is homologous to the furin cleavage site in α-ENaC [2]. A comprehensive review on the pathological consequences of COVID-19 suggests a role for ENaC in the early phases of COVID-19 infection in the respiratory tract epithelia [18].

Contents

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    - ENaC δ
      https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=740

References

1. Anand D, Hummler E and Rickman OJ. (2022) ENaC activation by proteases. *Acta Physiol (Oxf)* **235**: e13811 [PMID:35276025]
2. Anand P, Puranik A, Aravamudan M, Venkatakrishnan AJ and Soundararajan V. (2020) SARS-CoV-2 strategically mimics proteolytic activation of human ENaC. *Elife* **9** [PMID:32452762]
3. Baconguis I, Bohlen CJ, Goehring A, Julius D and Gouaux E. (2014) X-ray structure of acid-sensing ion channel 1-snake toxin complex reveals open state of a Na(+)-selective channel. *Cell* **156**: 717-29 [PMID:24507937]
4. Bize V and Horisberger JD. (2007) Sodium self-inhibition of human epithelial sodium channel: selectivity and affinity of the extracellular sodium sensing site. *Am J Physiol Renal Physiol* **293**: F1137-46 [PMID:17670907]
5. Bogdanović R, Kuburović V, Stajić N, Mughal SS, Hilger A, Ninić S, Prijić S and Ludwig M. (2012) Liddle syndrome in a Serbian family and literature review of underlying mutations. *Eur J Pediatr* **171**: 471-8 [PMID:21956615]
6. Boggula VR, Hanukoglu I, Sagiv R, Enuka Y and Hanukoglu A. (2018) Expression of the epithelial sodium channel (ENaC) in the endometrium - Implications for fertility in a patient with pseudohypoaldosteronism. *J Steroid Biochem Mol Biol* **183**: 137-141 [PMID:29885352]
7. Bourque CW. (2008) Central mechanisms of osmosensation and systemic osmoregulation. *Nat Rev Neurosci* **9**: 519-31 [PMID:18509340]
8. Buneman P, Christie G, Davies JA, Dimitrellou R, Harding SD, Pawson AJ, Sharman JL and Wu Y. (2020) Why data citation isn't working, and what to do about it *Database* **2020** [PMID:32367113]
9. Butterworth MB, Weisz OA and Johnson JP. (2008) Some assembly required: putting the epithelial sodium channel together. *J Biol Chem* **283**: 35305-9 [PMID:18713729]
10. Canessa CM, Merillat AM and Rossier BC. (1994) Membrane topology of the epithelial sodium channel in intact cells. *Am J Physiol* **267**: C1682-90 [PMID:7810611]
11. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD and Rossier BC. (1994) Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. *Nature* **367**: 463-7 [PMID:8107805]
12. Chang SS, Grunder S, Hanukoglu A, Rösler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA and Nelson-Williams C *et al.*. (1996) Mutations in subunits of the epithelial sodium

channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat Genet* **12**: 248-53 [PMID:8589714]
13. Collier DM and Snyder PM. (2011) Identification of epithelial Na+ channel (ENaC) intersubunit Cl- inhibitory residues suggests a trimeric alpha gamma beta channel architecture. *J Biol Chem* **286**: 6027-32 [PMID:21149458]
14. Danahay H, Gosling M, Fox R, Lilley S, Charlton H, Hargrave JD, Schofield TB, Hay DA, Went N and McMahon P *et al*.. (2025) Optimisation of a novel series of ENaC inhibitors, leading to the selection of the long-acting inhaled clinical candidate ETD001, a potential new treatment for cystic fibrosis. *Eur J Med Chem* **282**: 117040 [PMID:39561495]
15. Duc C, Farman N, Canessa CM, Bonvalet JP and Rossier BC. (1994) Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: localization by in situ hybridization and immunocytochemistry. *J Cell Biol* **127**: 1907-21 [PMID:7806569]
16. Edelheit O, Ben-Shahar R, Dascal N, Hanukoglu A and Hanukoglu I. (2014) Conserved charged residues at the surface and interface of epithelial sodium channel subunits--roles in cell surface expression and the sodium self-inhibition response. *FEBS J* **281**: 2097-111 [PMID:24571549]
17. Enuka Y, Hanukoglu I, Edelheit O, Vaknine H and Hanukoglu A. (2012) Epithelial sodium channels (ENaC) are uniformly distributed on motile cilia in the oviduct and the respiratory airways. *Histochem Cell Biol* **137**: 339-53 [PMID:22207244]
18. Gentzsch M and Rossier BC. (2020) A Pathophysiological Model for COVID-19: Critical Importance of Transepithelial Sodium Transport upon Airway Infection. *Function (Oxf)* **1**: zqaa024 [PMID:33201937]
19. Giraldez T, Rojas P, Jou J, Flores C and Alvarez de la Rosa D. (2012) The epithelial sodium channel δ-subunit: new notes for an old song. *Am J Physiol Renal Physiol* **303**: F328-38 [PMID:22573384]
20. Hanukoglu A. (1991) Type I pseudohypoaldosteronism includes two clinically and genetically distinct entities with either renal or multiple target organ defects. *J Clin Endocrinol Metab* **73**: 936-44 [PMID:1939532]
21. Hanukoglu A, Edelheit O, Shriki Y, Gizewska M, Dascal N and Hanukoglu I. (2008) Renin-aldosterone response, urinary Na/K ratio and growth in pseudohypoaldosteronism patients with mutations in epithelial sodium channel (ENaC) subunit genes. *J Steroid Biochem Mol Biol* **111**: 268-74 [PMID:18634878]
22. Hanukoglu A and Hanukoglu I. (2018) In systemic pseudohypoaldosteronism type 1 skin manifestations are not rare and the disease is not transient. *Clin Endocrinol (Oxf)* **89**: 240-241 [PMID:29702750]
23. Hanukoglu I, Boggula VR, Vaknine H, Sharma S, Kleyman T and Hanukoglu A. (2017) Expression of epithelial sodium channel (ENaC) and CFTR in the human epidermis and epidermal appendages. *Histochem Cell Biol* **147**: 733-748 [PMID:28130590]
24. Hanukoglu I and Hanukoglu A. (2016) Epithelial sodium channel (ENaC) family: Phylogeny, structure-function, tissue distribution, and associated inherited diseases. *Gene* **579**: 95-132 [PMID:26772908]
25. Heckel A, Frattini S, Hamprecht D, Kley J and Wiedenmayer D. (2017) Novel annelated phenoxyacetamides Patent number: WO2017028927A1. Assignee: Boehringer Ingelheim International Gmbh. Priority date: 20/08/2015. Publication date: 23/02/2017.
26. Horisberger JD and Chraïbi A. (2004) Epithelial sodium channel: a ligand-gated channel? *Nephron Physiol* **96**: p37-41 [PMID:14988660]
27. Jasti J, Furukawa H, Gonzales EB and Gouaux E. (2007) Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. *Nature* **449**: 316-23 [PMID:17882215]
28. Kashlan OB and Kleyman TR. (2012) Epithelial Na(+) channel regulation by cytoplasmic and extracellular factors. *Exp Cell Res* **318**: 1011-9 [PMID:22405998]
29. Kellenberger S, Gautschi I and Schild L. (2003) Mutations in the epithelial Na+ channel ENaC outer pore disrupt amiloride block by increasing its dissociation rate. *Mol Pharmacol* **64**: 848-56 [PMID:14500741]
30. Kellenberger S and Schild L. (2015) International Union of Basic and Clinical Pharmacology. XCI. structure, function, and pharmacology of acid-sensing ion channels and the epithelial Na+ channel. *Pharmacol Rev* **67**: 1-35 [PMID:25287517]
31. Kleyman TR and Eaton DC. (2020) Regulating ENaC's gate. *Am J Physiol Cell Physiol* **318**: C150-C162 [PMID:31721612]
32. Kleyman TR, Kashlan OB and Hughey RP. (2018) Epithelial Na^+ Channel Regulation by Extracellular and Intracellular Factors. *Annu Rev Physiol* **80**: 263-281 [PMID:29120692]
33. Knepper MA, Kwon TH and Nielsen S. (2015) Molecular physiology of water balance. *N Engl J Med* **372**: 1349-58 [PMID:25830425]
34. Leslie TK and Brackenbury WJ. (2023) Sodium channels and the ionic microenvironment of breast tumours. *J Physiol* **601**: 1543-1553 [PMID:36183245]
35. Lingueglia E, Voilley N, Waldmann R, Lazdunski M and Barbry P. (1993) Expression cloning of

an epithelial amiloride-sensitive Na+ channel. A new channel type with homologies to Caenorhabditis elegans degenerins. *FEBS Lett* **318**: 95-9 [PMID:8382172]
36. Lou J, Wei L and Wang H. (2022) *SCNN1A* Overexpression Correlates with Poor Prognosis and Immune Infiltrates in Ovarian Cancer. *Int J Gen Med* **15**: 1743-1763 [PMID:35221714]
37. Lu M, Echeverri F, Kalabat D, Laita B, Dahan DS, Smith RD, Xu H, Staszewski L, Yamamoto J and Ling J *et al.*. (2008) Small molecule activator of the human epithelial sodium channel. *J Biol Chem* **283**: 11981-94 [PMID:18326490]
38. Matalon S, Bartoszewski R and Collawn JF. (2015) Role of epithelial sodium channels in the regulation of lung fluid homeostasis. *Am J Physiol Lung Cell Mol Physiol* **309**: L1229-38 [PMID:26432872]
39. Noreng S, Bharadwaj A, Posert R, Yoshioka C and Baconguis I. (2018) Structure of the human epithelial sodium channel by cryo-electron microscopy. *Elife* **7** [PMID:30251954]
40. Noreng S, Posert R, Bharadwaj A, Houser A and Baconguis I. (2020) Molecular principles of assembly, activation, and inhibition in epithelial sodium channel. *Elife* **9** [PMID:32729833]
41. Palmer LG, Patel A and Frindt G. (2012) Regulation and dysregulation of epithelial Na+ channels. *Clin Exp Nephrol* **16**: 35-43 [PMID:22038262]
42. Pierandrei S, Truglio G, Ceci F, Del Porto P, Bruno SM, Castellani S, Conese M, Ascenzioni F and Lucarelli M. (2021) DNA Methylation Patterns Correlate with the Expression of *SCNN1A*, *SCNN1B*, and *SCNN1G* (Epithelial Sodium Channel, ENaC) Genes. *Int J Mol Sci* **22** [PMID:33916525]
43. Pirahanchi Y, Jessu R and Aeddula NR. (2021) Physiology, Sodium Potassium Pump *StatPearls* [PMID:30725773]
44. Rossier BC, Baker ME and Studer RA. (2015) Epithelial sodium transport and its control by aldosterone: the story of our internal environment revisited. *Physiol Rev* **95**: 297-340 [PMID:25540145]
45. Rossier BC and Stutts MJ. (2009) Activation of the epithelial sodium channel (ENaC) by serine proteases. *Annu Rev Physiol* **71**: 361-79 [PMID:18928407]
46. Rotin D and Staub O. (2011) Role of the ubiquitin system in regulating ion transport. *Pflugers Arch* **461**: 1-21 [PMID:20972579]
47. Saxena A, Hanukoglu I, Saxena D, Thompson RJ, Gardiner RM and Hanukoglu A. (2002) Novel mutations responsible for autosomal recessive multisystem pseudohypoaldosteronism and sequence variants in epithelial sodium channel alpha-, beta-, and gamma-subunit genes. *J Clin Endocrinol Metab* **87**: 3344-50 [PMID:12107247]
48. Saxena A, Hanukoglu I, Strautnieks SS, Thompson RJ, Gardiner RM and Hanukoglu A. (1998) Gene structure of the human amiloride-sensitive epithelial sodium channel beta subunit. *Biochem Biophys Res Commun* **252**: 208-13 [PMID:9813171]
49. Sharma S, Kumaran GK and Hanukoglu I. (2020) High-resolution imaging of the actin cytoskeleton and epithelial sodium channel, CFTR, and aquaporin-9 localization in the vas deferens. *Mol Reprod Dev* **87**: 305-319 [PMID:31950584]
50. Sheng S, Maarouf AB, Bruns JB, Hughey RP and Kleyman TR. (2007) Functional role of extracellular loop cysteine residues of the epithelial Na+ channel in Na+ self-inhibition. *J Biol Chem* **282**: 20180-90 [PMID:17522058]
51. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill Jr JR, Ulick S, Milora RV and Findling JW *et al.*. (1994) Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* **79**: 407-14 [PMID:7954808]
52. Song C, Lee Y and Kim S. (2022) Bioinformatic Analysis for the Prognostic Implication of Genes Encoding Epithelial Sodium Channel in Cervical Cancer. *Int J Gen Med* **15**: 1777-1787 [PMID:35210842]
53. Voilley N, Bassilana F, Mignon C, Merscher S, Mattéi MG, Carle GF, Lazdunski M and Barbry P. (1995) Cloning, chromosomal localization, and physical linkage of the beta and gamma subunits (SCNN1B and SCNN1G) of the human epithelial amiloride-sensitive sodium channel. *Genomics* **28**: 560-5 [PMID:7490094]
54. Waldmann R, Champigny G, Bassilana F, Voilley N and Lazdunski M. (1995) Molecular cloning and functional expression of a novel amiloride-sensitive Na+ channel. *J Biol Chem* **270**: 27411-4 [PMID:7499195]
55. Ware AW, Harris JJ, Slatter TL, Cunliffe HE and McDonald FJ. (2021) The epithelial sodium channel has a role in breast cancer cell proliferation. *Breast Cancer Res Treat* **187**: 31-43 [PMID:33630195]
56. Zennaro MC, Hubert EL and Fernandes-Rosa FL. (2012) Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol* **350**: 206-15 [PMID:21664233]