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Epithelial sodium channel (ENaC) family: Phylogeny, structure–function, tissue distribution, and associated inherited diseases

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ABSTRACT

The epithelial sodium channel (ENaC) is composed of three homologous subunits and allows the flow of Na⁺ ions across high resistance epithelia, maintaining body salt and water homeostasis. ENaC dependent reabsorption of Na⁺ in the kidney tubules regulates extracellular fluid (ECF) volume and blood pressure by modulating osmolarity. In multi-ciliated cells, ENaC is located in cilia and plays an essential role in the regulation of epithelial surface liquid volume necessary for cilial transport of mucus and gametes in the respiratory and reproductive tracts respectively.

The subunits that form ENaC (named as alpha, beta, gamma and delta, encoded by genes SCNN1A, SCNN1B, SCNN1G, and SCNN1D) are members of the ENaC/Degenerin superfamily. The earliest appearance of ENaC orthologs is in the genomes of the most ancient vertebrate taxon, Cyclostomata (jawless vertebrates) including lampreys, followed by earliest representatives of Gnathostomata (jawed vertebrates) including cartilaginous sharks. Among Euteleostomi (bony vertebrates), Actinopterygii (ray finned-fishes) branch has lost ENaC genes. Yet, most animals in the Sarcopterygii (lobe-finned fish) branch including Tetrapoda, amphibians and amniotes (lizards, crocodiles, birds, and mammals), have four ENaC paralogs. We compared the sequences of ENaC orthologs from 20 species and established criteria for the identification of ENaC orthologs and paralogs, and their distinction from other members of the ENaC/Degenerin superfamily, especially ASIC family. Differences between ENaCs and ASICs are summarized in view of their physiological functions and tissue distributions. Structural motifs that are conserved throughout vertebrate ENaCs are highlighted. We also present a comparative overview of the genotype–phenotype relationships in inherited diseases associated with ENaC mutations, including multisystem pseudohypoaldosteronism (PHA1B), Liddle syndrome, cystic fibrosis-like disease and essential hypertension.

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Abbreviations: ASIC, acid-sensing ion channel; ASL, airway surface liquid; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial sodium channel; ECF, extracellular fluid; ICF, intracellular fluid; PHA, pseudohypoaldosteronism; PRA, plasma renin activity; TM, transmembrane; TRCs, taste receptor cells.

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1. Introduction

As it is well known, 60–70% of the human body weight is water. About 2/3 of this water is within the cells (intracellular fluid, ICF) and the remaining 1/3 fills the extracellular spaces and the vascular bed in the circulatory system (extracellular fluid, ECF) (Ruth and Wassner, 2006). The cell membrane, as a semi-permeable barrier, is permeable to water molecules. Yet, the net movement of water between ECF and ICF depends on the relative osmolarity of these compartments and the permeability of the membranes (Fischbarg, 2010). In most vertebrates, the osmolarity of both the ECF and ICF is determined mainly by the concentration of electrolytes (dissolved salt ions carrying a net charge, mainly Na⁺, K⁺, Ca⁺², Mg⁺², Cl⁻, HCO₃⁻, PO₄³⁻, SO₄²⁻). In the ECF, Na⁺ is the electrolyte with the highest concentration and thus it is the major determinant of the osmolarity of the ECF (Takei, 2000). Osmolarity-dependent volume changes may lead to shrinking or swelling of cells. To prevent damage from such changes and to protect the nervous system, mammals maintain a common osmotic set-point near 300 mosmol/L (Bourgue, 2008). Thus, in vertebrates, the regulation of water and electrolyte homeostasis is highly interdependent (Ruth and Wassner, 2006).

The processes of absorption, secretion and excretion of water and solutes take place in epithelial cell layers that cover the internal and external surfaces of the body. In terms of permeability properties, epithelia are classified into two groups as leaky- and tight-epithelia (Fischbarg, 2010; Reddy and Stutts, 2013). Leaky epithelia are located generally in an isoosmotic environment as in the small intestine and proximal kidney tubules and are highly permeable to water. In contrast to leaky epithelia, the cells in tight epithelia are connected by complex tight junctions that reduce the permeability of the epithelia (Capaldo et al., 2014; Reddy and Stutts, 2013).

The epithelial sodium channel (ENaC), that is the focus of this review, is located mostly in tight or high-resistance epithelia. As a constitutively active channel, ENaC allows the flow of Na⁺ ions from the lumen into the epithelial cell, across the apical cell membrane (Garty and Palmer, 1997; Kashlan and Kleyman, 2011; Kellenberger and Schild, 2015) (Fig. 1). The absorbed Na⁺ ions are then pumped out of the cell into the interstitial fluid by the action of Na⁺/K⁺ ATPase located on the basolateral membrane (Fig. 1). As ENaC modulates the amount of Na⁺ in the ECF, it has a central role in the regulation of ECF volume and blood pressure (Büsst, 2013; Rossier et al., 2015). The activity of ENaC is regulated by the renin–angiotensin–aldosterone system (Asher et al., 1996; Bhalla and Hallows, 2008; Büsst, 2013; Rossier et al., 2015) and a complex variety of extracellular factors including Na⁺, Cl⁻, protons, shear stress and proteases (Bhalla and Hallows, 2008; Kashlan and Kleyman, 2012, 2011; Kellenberger and Schild, 2015).

The subunits that form ENaC constitute a family within the ENaC/ Degenerin superfamily. In addition to ENaC, this superfamily includes acid-sensing ion channels (ASICs) (Deval and Lingueglia, 2015; Kellenberger and Schild, 2015, 2002; Lin et al., 2015; Omerbašić et al., 2014; Waldmann and Lazdunski, 1998), pickpocket genes in the Diptera order including *Drosophila* and mosquitoes (Zelle et al., 2013),



Fig. 1. Schematic illustration of the location and function of ENaC in epithelia.

Degenerin subunits involved in sensory transduction in nematodes such as *Caenorhabditis elegans* (Eastwood and Goodman, 2012; Liddle et al., 1963), and peptide-gated *Hydra* Na⁺ channels (HyNaC) in hydrozoans (Gründer and Assmann, 2015).

The first sequences of ENaC subunits were based on cDNAs cloned from mRNAs isolated from rat and human tissues (Canessa et al., 1994b; Lingueglia et al., 1993; McDonald et al., 1995, 1994; Voilley et al., 1995, 1994; Waldmann et al., 1995). Later development of rapid genome sequencing techniques has led to the determination of the sequences of ENaC/Degenerin superfamily members in a growing number of species. This review concentrates on the sequences and phylogenetic relationships of ENaC paralogs and orthologs across species and with other homologous proteins that have been mostly revealed by genome sequences of many species.

In biology, the word "homology" is also used to describe functional equivalence and not just sequence and structural similarity. Thus, after inter-species sequence comparisons, we shall also present the physiological implications of the currently available information about ENaC phylogenetic distribution and function.

2. Nomenclature of ENaC homologs

2.1. Definitions: homolog, paralog, ortholog

In studies of protein evolution, the word "homologous" is used to describe proteins that share significant sequence similarity that is assumed to derive from a common ancestral origin. This concept of homology covers both proteins that are homologous across species as well as proteins that are present in multiple copies in the genome of a single species. To distinguish between these two types of homologous proteins, two separate terms were coined by Walter Fitch (Fitch, 1970): orthologous and paralogous. Within the genome of a single species, there are many genes that represent duplicate copies encoding isoforms of proteins with similar functions. The most common example is the globin family that includes α -globin, β -globin, and myoglobin. Homologous proteins that exist "in parallel" within one species are called "paralogs", a hybrid word combining "parallel" with "homolog". The word "ortholog" is used for homologous proteins that originate from a single ancestral gene in the last common ancestor of the compared species. Continuing the globin example, the ortholog of human α -globin is any of the α -globins in related primates. Further examples of these terms are provided by (Koonin, 2005).

2.2. ENaC paralogs

In the human genome there are nine genes that encode for ENaC paralogs. These paralogs are grouped into two families based on their homology: 1. non-voltage gated sodium channel family that is composed of four genes encoding ENaC homologs and 2. acid-sensing (proton-gated) ion channels (ASIC) family that is composed of five homologous genes. The four ENaC genes have been assigned abbreviations as SCNN1A, SCNN1B, SCNN1G, and SCNN1D by the Human Genome Organization (HUGO) Gene Nomenclature Committee (http://www. genenames.org/) following the Greek letters assigned to the four ENaC subunits α , β , γ , and δ (Tables 1 and 2). The second "N" in "SCNN1" was added to distinguish between the NON-voltage gated ENaC and the SCN1 symbol assigned to the "sodium channel, voltage-gated, type I" that is expressed in neurons and muscle. The UniProt protein database (UniProt, 2014) uses an abbreviated code for ENaC subunits (SCNNA, SCNNB, SCNNG and SCNND) to which the abbreviated species name is appended (Table 2). For the mouse genome, the convention for gene nomenclature starts with an uppercase letter, followed by all lowercase letters as shown in Table 1. For mouse, the gene for SCNN1D is not listed as it was not found in the mouse genome (Giraldez et al., 2012). Another common name for ENaC subunits is "amiloride-sensitive sodium channel" as ENaC is inhibited by amiloride (Garty and Palmer, 1997; Kashlan and Kleyman, 2011).

 Table 1

 Characteristics of the genes and transcripts encoding for ENaC subunits.^a

Species	Gene	Chro.	CCDS code	Ensembl Transcript ID	Pre-spliced (nt)	Exons	Coding exons
Human	SCNN1A	12p	8543.1	ENST00000228916	28,703	13	12
	SCNN1B	16p	10609.1	ENST00000343070	79,030	13	12
	SCNN1G	16p	10608.1	ENST00000300061	34,169	13	12
	SCNN1D	1p	-	ENST00000400928	10,806	16	13
Mouse	Scnn1a	6	39641.2	ENSMUST0000081440	23,603	12	12
	Scnn1b	7	21804.1	ENSMUST0000033161	53,691	13	12
	Scnn1g	7	21803.1	ENSMUST0000000221	33,971	13	12
Rat	Scnn1a	4	-	ENSRNOT0000067271	23,137	12	12
	Scnn1b	1	-	ENSRNOT0000067138	54,743	13	12
	Scnn1g	1	-	ENSRNOT0000024057	33,957	13	12

^a Based on the NCBI CCDS, and Ensembl databases.

As detailed below, the HUGO nomenclature appears to be sufficient for naming ENaC orthologs in other vertebrate species whose genomes have been sequenced.

2.3. ASICs and other homologs

The five genes that code for the five Acid-Sensing Ion Channel (ASIC) subunits in the human genome have been numbered as ASIC1, ASIC2, ASIC3, ASIC4 and ASIC5 by the HUGO Gene Nomenclature. The same abbreviation is used by the UniProt database (e.g. ASIC1_HUMAN). These channels were previously called as ACCN and BNaC (García-Añoveros et al., 1997). One example of the proliferation of names is ASIC5. The product of this gene was initially named "brain, liver, intestine Na⁺ channel" (BLINaC) in mouse and rat. The homologous protein in humans was found to be expressed in the intestine. Therefore, it was named "intestine Na⁺ channel (INaC)" in humans (Schaefer et al., 2000). A more recent study renamed the same protein as "bile acid-sensitive ion channel" (BASIC) (Lefèvre et al., 2014). Although referred to as ASIC5, it is not an acid-activated ion channel. The multiplicity of names for one protein emphasizes the need to adhere to names stan-dardized by international nomenclature.

Many of the ENaC homologs were named based on the protein characteristics such as, sites of expression (e.g. "INaC", "BLINaC"), physiologic consequences of activating mutations (e.g. "degenerin"), ligand interactions (e.g. "FMRFamide-activated", "amiloride-sensitive", "acidsensing"), organism (e.g. HyNaC for channels in *Hydra*) and original gene name (e.g. pickpocket in *Drosophila*). As noted with ASIC5, the use of different terms to name homologous proteins results in unrelated names for proteins that are highly homologous or orthologous. Moreover, homologous proteins may be expressed in different cell types and fulfill multiple functions in different species, as observed with ENaC/Degenerin superfamily members. Thus, assignment of one name for a protein may not be relevant for an orthologous protein in a different species.

As an alternative to naming proteins based on functional characteristics, HUGO has taken the approach of a serial numbering system based on homologous groupings (e.g. ASIC1...ASIC5, SCNN1A...SCNN1D). In

Table 2

Length and mass of human and n	nouse ENaC subunits.
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Species	Subunit	Gene	CCDS code	Uniprot name	Length ^a (aa)	Mass ^a (Da)
Human	Alpha	SCNN1A	8543.1	SCNNA_HUMAN	669	75,704
	Beta	SCNN1B	10609.1	SCNNB_HUMAN	640	72,659
	Gamma	SCNN1G	10608.1	SCNNG_HUMAN	649	74,270
	Delta	SCNN1D	-	SCNND_HUMAN	638	70,215
Mouse	Alpha	Scnn1a	39641.2	SCNNA_MOUSE	699	78,893
	Beta	Scnn1b	21804.1	SCNNB_MOUSE	638	72,197
	Gamma	Scnn1g	21803.1	SCNNG_MOUSE	655	74,635
Rat	Alpha	Scnn1a	-	SCNNA_RAT	698	78,888
	Beta	Scnn1b	-	SCNNB_RAT	638	71,995
	Gamma	Scnn1g	-	SCNNG_RAT	650	74,066

^a Based on Uniprot database.

our view, this is a better approach for the nomenclature of ENaC/ Degenerin superfamily, as it provides identical names to orthologs across species. In the current genomic era, protein sequences are predicted based on genomic sequence analysis that includes comparisons between predicted and known protein sequences. This approach of naming proteins based on sequence homology avoids the problems of names associated with protein characteristics.

In numerous invertebrate Metazoan species there is a multitude of highly divergent proteins that show sequences homologous to ENaC/ Degenerin superfamily members, but clearly represent different families based on low sequence similarity. As there is no standardized nomenclature for these proteins, in this review we used the names as in the original database records.

3. Chromosomal location and intron-exon organization of ENaC genes

In the human genome, SCNN1A encoding the α subunit is located on the short arm of chromosome 12 (12p) (Voilley et al., 1994). The genes SCNN1B and SCNN1G encoding the β and the γ subunits are located side by side on the short arm of chromosome 16 (16p) (Shimkets et al., 1994; Voilley et al., 1995). The SCNN1D gene encoding the δ subunit is located in chromosome 1p (Table 1).

In the mouse genome, the gene Scnn1a is located on chromosome 6, and Scnn1b and Scnn1g are juxtaposed at a region of chromosome 7 that shares synteny with the human chromosome 16 (Brooker et al., 1995; Pathak et al., 1996) (Table 1). Mouse genome appears to have lost the gene for the delta subunit (Giraldez et al., 2012). Yet, as detailed in Section 6, most vertebrate genomes have a gene that encodes for the delta subunit.

Sequencing of the α , β , and γ genes of the human genome revealed that all three genes include 13 exons but only 12 of these contain translated sequence (Fig. 2) (Table 1) (Ludwig et al., 1998; Saxena et al., 2002, 1998; Thomas et al., 1996). In the human somatic chromosomes, the average number of exons per coding gene ranges from 8.5 to 13.5 (Hubé and Francastel, 2015).

In all three genes, SCNN1A, SCNN1B and SCNN1G, the introns are located at identical positions in the coding sequence (Saxena et al., 1998). The SCNN1D gene structure, revealed by the human genome sequencing project, includes at least 16 exons 13 of which are protein coding (Table 1). Despite the conservation of the intron positions within the coding sequence, the sizes of the introns have diverged greatly resulting in significant differences between gene lengths (Fig. 2). The sizes of the primary transcripts prior to splicing range from 10,806 bp (for SCNN1D) to 79,030 (for SCNN1B) (Table 1) (Fig. 2). Among the four genes, the longest intron is intron #1 of SCNN1B (Fig. 2, note that there is a break in the x-axis of nucleotide position). In both SCNN1A and SCNN1B genes, the longest introns are intron #1 or #2 closest to the 5'-end of the transcription initiation site (Fig. 2). This represents a general trend that in genomes the longest introns appear at the 5'-end of the gene (Zhu et al., 2009).



Fig. 2. Intron–exon organization of the human ENaC genes, SCNN1A, SCNN1B, SCNN1G and SCNN1D and their primary transcripts based on the NCBI *Homo sapiens* Annotation Release 107 (2015-03-13). The name of each gene and its chromosomal location are noted at the left-edge of the diagrams. Under each exon–intron map, there are two coordinates: the upper one specifies the chromosomal coordinates, and the lower one specifies the position of the nucleotide (in kb) starting at the 5'-end of the RNA transcript (marked as 0). The codes above the diagrams represent the ID numbers of the RNA transcript (starting with NM_) and the encoded protein (starting with NP_) in the NCBI Gene database. For SCNN1A, two transcripts are shown as examples of alternative splicing products. Notes: 1) SCNN1A coordinates are given in a scale that descends from left-to-right because the gene is located in the reverse strand of the chromosome. 2) The x-axis for SCNN1B intron #1 includes a break between 5 kb and 45 kb marks. Display of the full sequence (i.e., without a break) would lead to the visible merger of exons 9 and 10 and hence disappearance of the intron 9 because of the short size of intron 9. Additional information about the genes and their products is provided in Tables 1 and 2.



Fig. 3. Schematic illustration of the transmembrane localization of an ENaC subunit. The sequence shown is of human α subunit (see Table 1). All homologous ENaC subunits have two transmembrane segments. The TM segments for this figure was predicted by the Phobius program (see Table 3) and drawn using Protter (Omasits et al., 2014). The extracellular domain includes about 70% of the sequence of amino acids of an ENaC subunit.

Analyses of the RNA transcripts of the genes encoding ENaC subunits have provided evidence for alternative RNA splicing products and multiple translation initiation sites (see Ensembl records listed in Table 1) (Berman et al., 2015; Bremner et al., 2002; Thomas et al., 2002). Alternative splicing is common in vertebrates and is thought to contribute to a higher level of phenotypic complexity in mammals (Kim et al., 2007). In cases where there was more than one isoform sequence for a gene, we used the UniProt Canonical Sequence or an NCBI Consensus CDS (CCDS) as the representative sequence for the gene in homology analyses for paralogs and orthologs.

4. Assembly of ENaC with paralogs

Previous studies have established that ENaC paralogs serve as subunits that form the channel (Canessa et al., 1994b; Kashlan and Kleyman, 2011). The most salient common feature of ENaC paralogs is the presence of two segments that function as two transmembrane (TM) segments embedded in the membrane, referred to as TM1 and TM2 (Fig. 3). In membrane-bound form, the amino (N) and the carboxy (C) termini of ENaC are intracellular, and a large extracellular segment, comprising about 70% of the amino acids of each subunit, connects the TM segments.

Although the structure of ENaC is not known, the strong hydrophobicity of the TM segments and homology with the resolved ASIC1 structure (Jasti et al., 2007) allows prediction of the TM segments (Table 3) (Fig. 3). In humans, the four ENaC subunits show significant sequence similarity in large segments of the extracellular region (Fig. 4). The most divergent parts of the ENaC paralogs are the N- and C-termini (Fig. 4).

The resolved structures of chicken ASIC1 revealed a homotrimer composed of three identical subunits (Baconguis et al., 2014; Jasti et al., 2007) (Fig. 5). In contrast to ASIC1 structure, independent lines of evidence indicate that ENaC is assembled as a heterotrimer composed of α (or δ), β and γ subunits:

- 1. Specific mutations in any one of the three genes coding for the α , β , and γ -ENaC were shown to result in an autosomal recessive disorder termed multi-system pseudohypoaldosteronism type I (PHA) (Chang et al., 1996; Hanukoglu, 1991). The underlying mechanism of multi-system PHA is the unresponsiveness to aldosterone in target organs expressing ENaC including kidney, sweat and salivary glands, reproductive and respiratory tracts (Enuka et al., 2012; Hanukoglu, 1991). In affected patients the disease is characterized by severe hyponatremia, hyperkalemia, dehydration and acidosis that starts in infancy and continues later in life with varying severity (Belot et al., 2008; Chang et al., 1996; Edelheit et al., 2010, 2005; Hanukoglu and Hanukoglu, 2010; Hanukoglu, 1991; Strautnieks et al., 1996). So far, no case of PHA has been identified that is caused by a mutation in the SCNN1D gene encoding δ -ENaC.
- Gene knockout studies inactivating the genes coding for the α, β, and γ subunits in mice showed that all three subunits are essential for survival. All gene knockout mice without either α, β, or γ subunits (genotype: -/-) die within <50 h after birth, with respiratory insuf-ficiency or kidney dysfunction leading to hyperkalemia, metabolic

Table 3

Intracellular, extracellular and transmembrane (TM) segments of human ENaC subunits. The position 1 of TM1 was predicted using Phobius software (Käll et al., 2004). The position of TM2 is based on homology with the ASIC1 structure (Jasti et al., 2007).

	Cytoplasmic N-ter	TM1	Extracellular	TM2	Cytoplasmic C-ter
Alpha	1-84	85-106	107-543	544-575	576-669
Beta	1-49	50-70	71-514	515-546	547-640
Gamma	1-53	54-79	80-523	524-555	556-649
Delta	1–87	88-107	108-520	521-552	553-638

acidosis and severe dehydration (Barker et al., 1998; Bonny and Hummler, 2000; Hummler et al., 1996).

- 3. Robust expression of ENaC activity in *Xenopus* oocytes requires all three subunits (α , β , and γ) (Canessa et al., 1994b; Edelheit et al., 2014, 2011; Giraldez et al., 2007). Expression of one or two ENaC subunits in *Xenopus* oocytes yields either minimal or no detectable channel activity (Canessa et al., 1994b; Edelheit et al., 2011; Giraldez et al., 2007).
- 4. Assessment of the stoichiometry of ENaC subunits using fluorescently labeled subunits, and imaging of ENaC-antibody complexes by atomic force microscopy indicated that the subunits are assembled as heterotrimers with a ratio of 1:1:1 (Staruschenko et al., 2005; Stewart et al., 2011).
- 5. Post-translation processing of the channel, including N-glycan maturation and furin-dependent cleavage, requires expression of all three subunits (Hughey et al., 2004).

Studies examining the structure of ENaC by molecular modeling and site-directed mutagenesis of conserved residues support the concept that ENaC structure is homologous to ASIC1 channel. In contrast to ASIC1 that functions as a homotrimer, ENaC is an obligate heterotrimer (Edelheit et al., 2014; Kashlan and Kleyman, 2011; Stockand et al., 2008). A study based on mutagenesis of Cl⁻ inhibitory sites suggests that the clockwise orientation of the subunits is $\alpha\gamma\beta$, when viewed from the top of the channel (Collier and Snyder, 2011).

In summary, the three paralogs encoding the α (or δ), β and γ subunits are essential for the assembly of functional channels. As summarized below, these three paralogs are highly conserved in all vertebrates. The evolutionary conservation of these genes provides further evidence that the subunits encoded by these genes are essential for the assembly of the heterotrimeric channel. The tissue distribution of the δ subunit is different from that of other subunits and its activity has been studied less. Excellent reviews by Giraldez et al. and Ji et al. summarize the characteristics of δ -ENAC (Giraldez et al., 2012; Ji et al., 2012).

4.1. Trimeric structure and channel pore

In the trimeric structure of ASIC1, one of the issues that have been intensively studied is the location of the channel pore through which ions flow across the membrane. ASIC1 has six transmembrane segments - three of each of TM1 and TM2. The structure of ASIC1 revealed that the TM1 and TM2 helices are organized in two separate concentric triads. The central pore is formed by the triad of TM2s. TM1s form a triad around the TM2 triad (Baconguis et al., 2014; Gonzales et al., 2009; Li et al., 2011). Most studies on ENaC suggest a similar organization of the TM segments in ENaC as well (Tolino et al., 2011). Section 11 on conserved motifs presents the properties of these segments in detail.

One of the major unresolved questions in ENaC function is the path(s) of ions into the channel pore in the membrane as described above. On top of the channel pore, the extracellular regions of the three subunits form a tripartite funnel with rotational symmetry (Fig. 5) (Baconguis et al., 2014; Jasti et al., 2007). However, the three subunits are not completely tightly juxtaposed along their entire lengths and there are fenestrations between the subunits above the pore around the region called "extracellular vestibule" (Baconguis et al., 2014). The hollow space along the central axis of rotational symmetry of this channel has been called a "vestibule". This vestibule leads from the top opening in the lumen to the channel pore embedded in the membrane. Under different crystallization conditions, segments of this vestibule may be constricted or expanded (Baconguis et al., 2014). These different states suggest that dynamic vestibule constriction and expansion may regulate ion flow into the channel pore.

The extracellular and central segments of the vestibule are surrounded by beta-strands of the palm domain two of which are connected to the TM helices (β 1 to TM1 and β 12 to TM2) (Fig. 5). Thus, changes in the angles of TM helices may effect constriction of the



Fig. 4. Aligned sequences of human α , β , γ and δ -ENaC subunits and conserved positions of introns in all four subunits. Residues that are identical in all four subunits are shaded. The numbers (2 to 12) below the sequences mark the position and number of the intron located in or at the end of the codon of the specific residue above the number. In the 5' portion of the gene encoding δ -ENaC subunit there are additional introns that are not shown here. The sequences were aligned using the ClustalW2 program, and the alignment of some residues in the amino and carboxy termini were manually edited to eliminate some gaps without affecting percent identity score. TM1 and TM2 mark the predicted transmembrane segments of the proteins.



Fig. 5. A. Ribbon structure model of subunit A of chicken ASIC1 (PDB ID: 2QTS). Segments in helical conformation are red colored and segments in sheet conformation are blue colored. B. The surface structure of subunits A and B of ASIC1. The four hydrophobic helices of the A and B subunits are embedded in the lipid bilayer marked by light-blue shading. The third subunit (C) surface is not shown to allow visibility of the central pore predicted by the Porewalker software (Edelheit et al., 2014). Red colored small spheres represent water molecules placed at the center of the predicted pore and extracellular vestibule in each 3 Angstrom slice of 2QTS calculated by Porewalker.

vestibule. Conversely, movement of the β 1 and β 12 strands may effect opening or closing of the channel gate by modulating the position of the TM helices. For ASIC1, there is evidence that the movement of the coiled linker region immediately prior to β 1 and β 12 strands may effect channel opening and closing (Li et al., 2010; Springauf et al., 2011). The dynamics of these parts are also affected by the interactions between the thumb and finger domains (Gwiazda et al., 2015; Yang et al., 2009). There is a variety of intracellular and extracellular factors that can affect the dynamics of these segments, e.g. cytoplasmic Ca²⁺ (Gu, 2008), binding to actin and other cytoskeletal proteins (Ilatovskaya et al., 2012; Sasaki et al., 2014), phosphoinositides that serve as second messengers in intracellular signaling cascades (Hille et al., 2015; Pochynyuk et al., 2008), extracellular ions, including Na⁺ and Cl⁻, pH and cleavage by extracellular proteases (Kashlan and Kleyman, 2012; Kellenberger and Schild, 2015).

5. Homology between ENaC and ASIC paralogs

To assess the similarity of the ENaC and ASIC sequences, Fasta format of the selected sequences were downloaded from the Uniprot database. Multiple sequence alignments were carried out by the CLUSTALW software (version 2.1) with default parameters (http://www.genome.jp/ tools/clustalw/) (Chenna et al., 2003). Percent identity figures were calculated using GeneDoc (Nicholas and Deerfield, 1997). Sequence alignments for the figures were generated using the Jalview program (Waterhouse et al., 2009).

Among the four human ENaC subunits, greatest similarity exists between the α and δ subunits (34% identity) and the β and γ subunits (34% identity) (Table 4). The percent identity between other pairs (e.g. α vs. β or γ) is between 23 and 27% (Table 4). Since the N- and C-termini of ENaC subunits show divergence, we also determined the

Table 4

Percent identity between human ENaC and ASIC subunits along their entire sequences.

-		-	-					
	SCNNA	SCNNB	SCNNG	SCNND	ASIC1	ASIC2	ASIC3	ASIC4
SCNNB_HUMAN	26							
SCNNG_HUMAN	27	34						
SCNND_HUMAN	34	23	23					
ASIC1_HUMAN	13	16	15	16				
ASIC2_HUMAN	13	15	15	13	64			
ASIC3_HUMAN	14	14	15	14	46	45		
ASIC4_HUMAN	12	12	11	14	35	31	32	
ASIC5_HUMAN	12	14	13	13	22	22	21	17

The sequences were aligned using ClustalW2 program (version 2.1).

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Percent identity between human ENaC and ASIC subunits in the conserved central segment including TM1 + extracellular domain + TM2 (see Figs. 3 and 4).

	SCNNA	SCNNB	SCNNG	SCNND	ASIC1	ASIC2	ASIC3	ASIC4
SCNNB_HUMAN	31							
SCNNG_HUMAN	33	36						
SCNND_HUMAN	37	28	28					
ASIC1_HUMAN	16	19	18	18				
ASIC2_HUMAN	16	18	17	17	74			
ASIC3_HUMAN	17	17	16	17	52	53		
ASIC4_HUMAN	15	17	15	17	49	47	46	
ASIC5_HUMAN	16	17	16	16	26	27	26	25

sequence identity in the extracellular regions of ENaC subunits. These values indicate a 2–6% higher sequence identity in the extracellular regions (Table 5), as compared to the full-length sequences of ENaC subunits (Table 4).

In contrast to ENaC subunits, the sequence identities between human ENaC and human ASIC subunits are much lower: in the range of 11 to 16% (Table 4). Thus, clearly ENaC and ASIC paralogs belong to distinct families as marked by the demarcation lines in Table 4. Percent sequence identity between ASIC subunits themselves ranges from 17 to 64% (Table 4). Similar to ENaC, the extracellular segments of ASIC subunits share higher identity than the whole sequences (compare



Fig. 6. Topology diagram of chicken ASIC1 structure. The cylinders represent helical segments, and the arrows represent β -strands. The transmembrane (TM), and secondary structural domains (palm, β -ball, finger, thumb and knuckle) were colored distinctly and named as in Jasti et al. (2007). Certain features of the diagram were adopted from previous diagrams (Eastwood and Goodman, 2012; Kashlan and Kleyman, 2011).

Fig. 7 vs. Fig. 6 for ASIC), reflecting divergence of N- and C-terminal sequences (see Section 11).

Comparisons of the sequences of all four ENaC paralogs from six species (in addition to human) indicate that the degree of sequence identity between the four paralogs within each species is quiet similar to that observed in the human genome (compare Tables 4 and 6).

In the CATH protein structural domain database (Sillitoe et al., 2015), ASIC and ENaC channels are listed as two separate families within the Superfamily number 2.60.470.10 titled "Acid-sensing ion channels like domains". CATH classification system is mostly based on specific local structural domains. The domain selected for the classification is mainly the "palm" domain based on the ASIC1 structure (2QTS). The palm domain is composed of a complex of β -sheets. Therefore within the CATH database, the channel is included under Class 2 for "Mainly beta" type domains. Since the ASIC1 structure is an intricate complex of α -helices and β -sheets this classification does not take into account the full structural view of the channels.

5.1. Sites of divergence among ENaC and ASIC paralogs

The divergence of N- and C-termini of ENaC/Degenerin superfamily members (noted above) represents a general trend in protein families. Previous studies on other proteins have shown that changes in protein domain architecture are most common in the N- and C-termini of proteins (Björklund et al., 2005; Forslund and Sonnhammer, 2012). In contrast to α - and δ -ENaC, the N- and C-termini of human β - and γ -ENaC are highly conserved. The structures of these terminal segments are currently not known, but there are studies indicating that these cytoplasmic domains interact, either directly or indirectly, with other cytoplasmic and cytoskeletal proteins such as syntaxin (Berdiev et al., 2004; Condliffe et al., 2003), actin (Copeland et al., 2001), ubiquitin

Table 6

Percent sequence identity between four paralogous ENaC subunits (α , β , γ and δ) in six species (for comparison of human paralogs see Fig. 4 and Table 4).

		α	β	γ
Rhesus	β	24		
	γ	27	31	
	δ	25	16	18
Bovine	β	28		
	γ	28	34	
	δ	38	23	25
Tasmanian d.	β	26		
	γ	27	32	
	δ	37	21	22
Xenopus	β	29		
	γ	31	30	
	δ	39	27	28
Alligator	β	27		
	γ	29	34	
	δ^{a}	39	25	27
Coelacanth	β	26		
	γ	29	29	
	δ	43	24	27

^a Named by us as the δ -subunit. Named as "alpha like" in the original report.

ligase Nedd4 and protein kinases (Asher et al., 2001; Bobby et al., 2013; Shi et al., 2002).

Since the structure of the extracellular region of ASIC1 has been resolved and in this region there is a significant homology between ASIC1 and ENaC subunits, we shall present the sites of divergence in this region in terms of the secondary structural segments of ASIC1. The original study on the crystal structure of ASIC1 noted that ASIC1 structure resembles a hand holding a ball (Jasti et al., 2007). Hence, domains within the extracellular regions are referred to as palm, thumb, knuckle, finger and β -ball (Jasti et al., 2007). The palm and β -ball domains are formed by non-contiguous β -strands and loops, and are in close proximity to the membrane (Fig. 6). More peripheral domains (thumb, knuckle and finger) are formed by contiguous α -helices and loops (Fig. 6).

To facilitate location of divergent regions in ENaC relative to the structural domains of ASIC1 in Fig. 7 we provide an alignment of the β -subunit with ASIC1 sequence including marking of the positions of the secondary structural elements according to the PDB ID 2QTS (Fig. 7).

In the extracellular region of ENaC subunits, there are several highly divergent segments where insertions/deletions are found (Figs. 4 and

7). One divergent area is in the finger domain in between helix #1 and β -strand #3 (Figs. 4, 6 and 7). This segment is divergent in four ENaC paralogs and is characterized by poorly aligned sequences including large insertions and deletions (Figs. 4 and 7). This "finger" domain shows the highest variability among ENaC/Degenerin superfamily members indicating that this region may have an important role in conferring functional specificity (Eastwood and Goodman, 2012; Kashlan and Kleyman, 2011). For example, the α and γ -subunit finger domains have inhibitory tracts that are released following proteolytic processing (Bruns et al., 2007; Carattino et al., 2008a, 2006; Kashlan et al., 2011; Passero et al., 2010).

Another divergent segment in ENaC starts at about residue 376 of the human β -ENaC and includes an insertion of three residues (Fig. 4). In alignment with ASIC1 this region is located in the region between β -9 and α -4 (Fig. 7). This is the region that connects the palm domain of ASIC1 to the thumb domain (Jasti et al., 2007). This region has been proposed to transmit conformational changes in the periphery of the extracellular region to the channel pore and gate (Jasti et al., 2007; Li et al., 2011; Shi et al., 2011). Other divergent areas include the knuckle domain and the loop connecting the β -6 and β -7 strands. Residues in



Fig. 7. Secondary structures in the sequence of chicken ASIC1. The positions of the structures were taken from the PDB file of 2QTS. The numbering of the structures is based on Jasti et al. (2007). Note that some short stretches of helix and β -strand are not numbered. For comparison of sequence conservation, human β -ENaC is globally aligned with the ASIC1 sequence and identical residues were gray color shadowed. Note that most but not all secondary structures are associated with conserved sequences.

the β -6- β -7 loop of the α subunit have been proposed to function as an extracellular Na⁺ binding site that is involved in Na⁺ self-inhibition (Kashlan et al., 2015).

In conclusion, it appears that areas of divergence that are seen in ENaC and ASIC1 comparisons are located in the connecting segments within the finger and thumb domains. In additions to these, there are a few other sequence differences but the sequence homology predominates especially in the β -strand segments in the palm and β ball domains (Figs. 6 and 7).

It is interesting that the most divergent areas within members of the ENaC/Degenerin family are in the periphery of the extracellular region. There is growing evidence that these divergent areas have sites of direct interaction with extracellular regulatory factors that modulate channel activity, such as proteases (Bruns et al., 2007; Vallet et al., 1997), inhibitory peptide released by proteases (Carattino et al., 2006; Kashlan et al., 2010), extracellular chloride (Cl⁻) ions (Collier and Snyder, 2011), extracellular Na⁺ (Chraibi and Horisberger, 2002; Edelheit et al., 2014; Winarski et al., 2010), protons (Collier et al., 2012; Krauson et al., 2013), and laminar shear stress induced by fluid flow (Shi et al., 2012). As the different ENaC/Degenerin family members are regulated by distinct factors, evolutionary divergence within the peripheral domains may have been a key factor in allowing this family to evolve with different functional properties.

6. Phylogenetic distribution of ENaC orthologs

Determination of genomic sequences of many eukaryotic species has provided ENaC gene sequences from a broad spectrum of vertebrates. Comparison of ENaC gene and protein sequences across species is useful from several perspectives. Knowledge about ENaC orthologs across species can contribute to our understanding of the significance and function of ENaC subunits. Conservation of a gene across species suggests an important physiological function for the organism (see for example Studer et al., 2011). Secondly, comparisons of the sequences of the ENaC subunits enhance our understanding of the structural and functional importance of conserved sequence segments. Thirdly, the absence of an ENaC gene in a species is important information as the species may use alternative subunits or channels to fulfill the homeostatic functions of ENaC.

The Ensembl genome database (release 79) of vertebrate and eukaryotic species currently includes 540 genes homologous to ENaC family members, 188 of which encode one of the four ENaC subunits. The remainder represents ASICs or other family members from different species. A BLAST search of the UniProt protein database shows that ENaC subunits are found in vertebrates. BLAST search of UniProt bacteria, fungi and plant protein sequence databases did not reveal orthologs of human ENaC subunits. Here we provide a summary of the appearance of ENaC genes in Metazoan species.

6.1. Cyclostomata and Chondrichthyes (cartilaginous fishes)

In the phylogeny of vertebrates, the most ancient taxon is Cyclostomata, i.e. jawless vertebrates. Lampreys and hagfishes are common extant species that represent this taxon. These fishes have only cartilaginous elements as a primitive skeleton that supports their body parts (Shimeld and Donoghue, 2012). The genome of sea lamprey includes three genes that code for the orthologs of α , β and γ -ENaC, but apparently does not include a gene for the delta subunit (Table 7) (Smith et al., 2013). The sequence of lamprey α subunit is not complete (S4RTA3_PETMA).

Next steps in the evolution of vertebrates include the development of jaw and skeleton leading to the formation of Gnathostomata (jawed vertebrates) (Donoghue et al., 2006; Kawasaki and Weiss, 2006; Kuratani, 2012). The earliest representatives of this branch include cartilaginous fish species, including rays and sharks. The genome of the cartilaginous elephant shark has been determined and it includes three orthologous ENaC genes (Venkatesh et al., 2007) (Table 7).

Table 7

Presence (+) or absence	(-) of	genes encoding SCNN1A	, SCNN1B, SCNN1G,	, and SCNN1D in no	n-mammalian vertebrates.
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Taxon	Example species	А	В	G	D	Reference
Cyclostomata (jawless vertebrates) Petromyzontidae (lampreys)	Petromyzon marinus (sea lamprey)	+	+	+	_	Smith et al. (2013)
Gnathostomata (jawed vertebrates) Chondrichthyes (cartilaginous fishes)	Callorhinchus milii (elephant shark)	+	+	+	_	Venkatesh et al. (2007)
Euteleostomi (bony vertebrates) Actinopterygii (ray-finned fishes) Coelacanthiformes (lobe-finned fishes) Dinnoi (lungfishes)	Danio rerio (zebrafish) Latimeria chalumnae (coelacanth) Neoceratodus forsteri (lungfish)	- + +	- + +	- + +	- + 2	Venkatesh et al. (2007) Amemiya et al. (2013) Uchiyama et al. (2012)
Euteleostomi: Tetrapoda: Amphibia: Batrachia: Pipidae (tongueless frogs)	Anura (frogs and toads) Xenopus tropicalis (frog)	+	+	+	+	Hellsten et al. (2010)
Euteleostomi: Tetrapoda: Amniota: Sauropsida: Alligatorinae (alligators)	Sauria: Archelosauria: Archosauria: Crocodylia Alligator mississippiensis (American alligator)	+	+	+	+	Green et al. (2014)
Euteleostomi: Tetrapoda: Amniota: Sauropsida:	Sauria: Archelosauria: Archosauria: Dinosauria: Aves (birds)				
Galliformes (fowls)	Gallus gallus (chicken)	+	+	+	+	Chicken-Genome (2004)
Galliformes	Meleagris gallopavo (turkey)	+	+	+	+	Dalloul et al. (2010)
Gruiformes	Eurypyga helias (sunbittern)	+	+	+	+	Zhang et al. (2014)
Passeriformes (perching birds)	Taeniopygia guttata (zebra finch)	+	+	+	+	Warren et al. (2010)
Passeriformes	Ficedula albicollis (flycatcher)	+	+	+	+	Ellegren et al. (2012)
Piciformes	Picoides pubescens (downy woodpecker)	+	+	+	+	Zhang et al. (2014)
Spheniscidae (penguins)	Aptenodytes forsteri (emperor penguin)	+	+	+	+	Zhang et al. (2014)
Futeleostomi: Tetrapoda: Amniota: Sauropsida:	Sauria: Archelosauria: Testudines (turtles)					
Trionychidae (soft-shelled turtles)	Pelodiscus sinensis (soft-shelled turtle)	+	+	+	+	Wang et al. (2013b)
Euteleostomi: Tetrapoda: Amniota: Sauropsida:	Sauria: Lepidosauria					
Squamata (lizards and snakes)	Anolis carolinensis (green anole lizard)	+	+	+	+	Alföldi et al. (2011)

6.2. Euteleostomi (bony vertebrates)

In evolution, the development of jaw is followed by the development of bony fishes. The clade of Euteleostomi (bony vertebrates) includes two branches:

1. Actinopterygii (ray-finned fishes): The "ray-finned" description is based on spiny projections in the fins of these fishes.

Comparison of shark, human and teleost ray-finned fish genomes has revealed that 154 genes (including ENaC paralogs) that have orthologs in the shark genome are not present in ray-finned fish genomes (Venkatesh et al., 2007). Thus, the whole clade of Actinopterygii (ray finned-fishes), which includes Zebrafish, do not have ENaC genes. However, they have ASIC genes. During the course of evolution, ENaC genes may have been lost at the onset of the branch of ray-finned fishes for lack of a functional need or were replaced functionally by alternative genes and proteins (Uchiyama et al., 2014; Venkatesh et al., 2007).

The EnsemblCompara GeneTree shows one "SCNN1A" gene for *Lepisosteus oculatus* (spotted gar) that is a freshwater ray-finned fish. Our comparison of this protein with human ASIC and ENaC paralogs showed that it is more homologous to ASIC than ENaC paralogs. UniProt database includes 9 protein fragments from the spotted-gar genome that show homology to "amiloride-sensitive so-dium channel family". Our comparison of 4 partial sequences (with lengths >400 residues) from the UniProt database with human ASIC and ENaC paralogs showed that all four sequences share 49–61% sequence identity with human ASIC1, while they share 13–15% with human ENaC paralogs. Therefore, the naming of the single ray-finned fish spotted gar protein (ENSLOCP00000013400) as "SCNN1A" appears to be in error. Thus with the elimination of this case, so far ray-finned fish genomes do not appear to have ENaC orthologs as noted above.

 Sarcopterygii (lobe-finned fish): The "lobe-finned" description was given because of their fleshy paired fins which are considered an early form of limb development in tetrapod vertebrates with four limbs. Therefore, this clade also includes all Tetrapoda species. Sarcopterygii includes two ancient taxa with extant fishes: Coelacanthiformes (lobe finned fishes, coelacanth) and Dipnoi (lungfishes) (Table 7). The three ENaC genes are present in the genomes of these fish (Amemiya et al., 2013; Uchiyama et al., 2014, 2012). Tetrapoda is considered a branch that emerged in parallel to Dipnoi.

6.3. Amphibia

In the evolutionary ladder, the development of bony vertebrates was followed by the emergence of tetrapods with four limbs. Amphibians (frogs, toads and salamanders) represent the first class of tetrapods. *Xenopus tropicalis* (frog) genome includes genes encoding the four ENaC paralogs (Hellsten et al., 2010) (Table 7).

6.4. Sauropsida

The second group of tetrapods is Amniota (amniotes) characterized by having an egg or embryo covered with an amniotic membrane. Amniotes include two clades: Sauropsida that includes birds and reptiles, and Mammalia (mammals).

The genome sequences of three crocodilians have been recently reported (Green et al., 2014). Currently, NCBI Genome database Genome Assembly and Annotation report (including a list of predicted proteins) is available only for *Alligator mississippiensis* (American alligator). Search of this database for amiloride-sensitive sodium channel yielded four sequences (XP_006258424.1, XP_006268483.1, XP_006268484.1, and XP_006277862.1). In this report, the first and the fourth sequences were named as "amiloride-sensitive sodium

channel subunit alpha-like", while the second and the third sequences were named as "...subunit beta" and "...subunit gamma". Percent identities of these four sequences are shown on Table 6. These results show that the second sequence (XP_006277862.1) that was labeled as "alpha-like", matches other delta-ENaC sequences in terms of its percent identity with the other alligator ENaC subunits (Table 6) and human ENaC subunits (results not shown). Thus, we conclude that this alligator has four ENaC paralogs including one gene coding for the delta subunit.

The Ensembl (release 79) Gene Tree view includes two reptiles: softshell turtle and green anole lizard (Table 7). Both of these genome sequences also include four genes coding for the four ENaC paralogs (Table 7).

Bird genomes that are listed in Ensembl (release 79) Gene Tree view, include four genes coding for ENaC subunits. The recently determined genome of sunbittern (*Eurypyga helias*) (Zhang et al., 2014) is not yet included in the Ensembl database. Similar to the case of alligator genome noted above, NCBI Genome database Genome Assembly and Annotation report includes four amiloride-sensitive sodium channel entries one of which was listed as "...alpha-like". Our sequence identity analysis unequivocally classifies this "alpha-like" as the δ subunit. Therefore, this genome also includes four ENaC heterologs (Table 7). Since birds and crocodilians are considered evolutionary descendants of dinosaurs (Green et al., 2014), it is likely that dinosaurs also had four genes coding for ENaC subunits.

6.5. Mammalia

The class of Mammalia includes three taxa: egg-laying mammals (Monotremata), marsupials (Metatheria) and placental mammals (Eutheria). In nearly all mammals in these three clades, there are four ENaC genes (Table 8). Ensembl genome database (release 79) includes 38 mammalian species, including 34 placental mammals, 3 marsupials (opossum, Tasmanian devil, wallaby) and egg-laying platypus. All of these species have four paralogs of ENaC with the exception of the mouse genome that appears to have lost the gene for the delta subunit (Ensembl Gene Tree for ENaC homologs). The rat genome, that is a very close phylogenetic relative of the mouse, includes four ENaC paralogs, but the δ subunit sequence is presently available only as a fragment (NCBI Accession: NC_005104.4).

In the Ensembl (release 79) Gene Tree view, there are only one to three ENaC paralogs for some mammalian species. Our examination of the genome in each of these cases showed that in most cases the genome sequence does include the missing paralog(s); in other cases the genome sequence is incomplete.

6.6. Summary for Tetrapoda

For the genomes of tetrapods where sequence information is available, including amphibians and amniotes (lizards, crocodiles, birds, and mammals) there are four paralogs of ENaC with the exception of mouse that has lost the gene for the delta subunit (Tables 7 and 8) (Giraldez et al., 2012).

7. Homologs in invertebrates

As noted in the introduction, invertebrate species have many genes encoding polypeptides homologous to ASIC/ENaC such as mec and deg genes in *C. elegans*, and pickpocket genes in *Drosophila* (Table 9). In global (end-to-end) sequence alignment, homologous *C. elegans* (CAEEL) proteins share up to 16% sequence identity with ENaC subunits from 18 vertebrate species (Table 10). In contrast, among ENaC subunits, percent identities are 40–95% depending on the taxonomic distance (Tables 11 and 12).

Table 10 includes only comparisons with the α ENaC subunit. Comparisons with the β and γ sequences from the same 18 species show a highly similar range of identity (data not shown), i.e., there is

Table 8

Presence (+) or absence (-) of genes encoding SCNN1A, SCNN1B, SCNN1G, and SCNN1D in mammals.

Taxon	Example species	А	В	G	D	Reference
Monotremata (egg-laying mammals)	Ornithorhynchus anatinus (platypus)	+	+	+	+	Warren et al. (2008)
Matathania (manuniala)						
Diprotodontia	Macronus eugenii (tammar wallahy)	+	+	+	+	Renfree et al (2011)
Didelphimorphia	Manadelphis domestica (apossum)			- -		Mikkelsen et al. (2007)
Dacuuridae	Sarconhilus harricii (Təsməniən dovil)	т 1	- T	- T	т 1	Millor et al. (2011)
Dasyulluae	Surcophilus hurrisii (Tasinanian devii)	Ŧ	Ŧ	Ŧ	Ŧ	
Eutheria (placental mammals) Afrotheria						
Elephantidae (elephants)	Loxodonta africana (African elephant)	+	+	+	+	Elephant genome project
Tenrecidae (tenrecs)	Echinops telfairi (hedgehog)	+	+	+	+	
Boreoeutheria: Laurasiatheria: Carnivora (ca	rnivores)					
Canidae (dog, coyote, wolf, fox)	Canis lupus familiaris (dog)	+	+	+	+	Lindblad-Toh et al. (2005)
Felidae (cat family)	Felis catus (domestic cat)	+	+	+	+	Pontius et al. (2007)
Denote the interview of						
Boreoeutneria: Laurasiatneria: Cetartioaactyi	a (whates, hippos, ruminants, pigs, cameis etc.)					Maria and a second s
Cetacea (whales)	Orcinus orca (killer whale)	+	+	+	+	Marine mammal genomics
						Ensembl
Boreoeutheria: Laurasiatheria: Cetartiodacty	a: Ruminantia					
Bovinae	Bos taurus (cow)	+	+	+	+	Flsik et al. (2009)
Caprinae	Ovis aries (sheen)	+	+	+	+	Sheen Cenomics Consortium
capiniac	ovis unes (sneep)			1	1	Sheep denomies consortium
Boreoeutheria: Laurasiatheria: Perissodactyla	ı (odd-toed ungulates)					
Equidae (horses)	Equus caballus (horse)	+	+	+	+	Wade et al. (2009)
Boreoeutheria: Euarchontoglires: Rodentia						
Muridae	Mus musculus (mouse)	+	+	+	—	Takada et al. (2013)
Muridae	Rattus norvegicus (rat)	+	+	+	+	Saar et al. (2008)
Porooguthoria: Eugrebontogliras: Drimatas						
Hominidae	Pan tradadutas (chimpanzoo)			1		Chimpanzoo Sequencing and Analysis Consortium
Hominidae	Corilla gorilla	- -	- -	- -	- -	Scally et al. (2012)
Hominidae	Goriniu goriniu Dongo ghaliji (Sumatran orangutan)	+	+	+	+	Scally et al. (2012)
Correspitheridae (Old World mortress)	Masaga mulatta (rhosus monkou)	т	- -	- -	T .	$\frac{1}{2014}$
Cercoprinectuae (Old world monkeys)	Callithain incoluse (mesus monkey)	+	+	+	+	$\sum_{i=1}^{n} i \in I_{i} (2014)$
Platyrrnini (New World monkeys)	<i>Califithrix Jacchus</i> (marmoset)	+	+	+	+	vvoriey et al. (2014)

no significant difference in the similarity of any CAEEL homolog to any of the three ENaC subunits. Similarly, *C. elegans* homologs share only a low (<15%) sequence identity with the human ASIC isoforms. Thus, these homologs represent a family(s) separate from the ENaC as well as ASIC families. In Table 9 we note only a few references for the Deg family of proteins in nematodes. The UniProt protein database includes many polypeptides that belong to this family in various worms. As these

are outside the scope of this review we will not further relate to these sequences.

In addition to the nematode and arthropod species, BLAST search of UniProt protein database shows significant sequence identity in the range of 13–22% between predicted protein sequences from the genome of *Strongylocentrotus purpuratus* (purple sea urchin) (unpublished yet; available at Ensembl database) (Table 9) and

Table 9

Sodium channel families within the DEG/ENaC superfamily.

	Channel/gene name ^a	Phylum	Genus/species	Reference
Invertebrates		Annelida (annelid worms)	Helobdella (leech)	Simakov et al. (2013)
	Pickpocket (ppk)	Arthropoda	Drosophila	Zelle et al. (2013)
			Anopheles	Holt et al. (2002)
			Tribolium castaneum	Kim et al. (2009)
	Hydra Na ⁺ channel (HyNaC)	Cnidaria	Hydra	Gründer and Assmann (2015)
	Sp-Scnn1a	Echinodermata	Strongylocentrotus (sea urchin)	Ensembl database
	Sp-Scnn1g			
	FMRFamide-activated amiloride-sensitive	Mollusca	Aplysia (sea hare)	Furukawa et al. (2006)
	sodium channel (FaNaC)		Crassostrea (oyster)	Zhang et al. (2012)
			Helix aspersa (snail)	Lingueglia et al. (2006)
			Planorbella trivolvis	
	Degenerin (deg) (mec) (unc)	Nematoda	C. elegans	Eastwood and Goodman (2012)
			Toxocara canis	Zhu et al. (2015)
			Trichuris suis	Jex et al. (2014)
	Putative FMRFamide-gated Na ⁺ channel	Platyhelminthes (flatworms)	Schistosoma mansoni	Protasio et al. (2012)
			Echinococcus	Zheng et al. (2013)
	C3Y149_BRAFL	Chordata	Branchiostoma floridae (Florida lancelet)	Putnam et al. (2008)
	C3ZNH4_BRAFL			
Vertebrates	Acid-sensing ion channel (ASIC)	Chordata	Wide distribution	Deval and Lingueglia (2015)
	Epithelial Na Channel (ENaC)	Chordata	Wide distribution	This review

^a Names for the retrieval of sequence records from the Uniprot database.

Table 10 Percent identity betv	veen globally	' aligned amii	no acid sequ	iences of sele	ected metazo	an ENaC l	homologs aı	nd α ENaC s	ubunit seq	luences from	18 vertebrate	species (se	e Tables 7 a	nd 8 for the fu	ll names of	the species).		
	Human	Chimp.	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish	Coelacanth
CAEEL-deg1	12	12	12	13	13	13	12	13	13	13	12	13	12	13	13	12	13	12
CAEEL-del1	16	15	15	15	15	15	16	16	15	16	15	14	14	15	15	15	15	13
CAEEL-mec4	14	14	13	13	13	13	13	13	13	14	12	14	14	14	14	13	13	12
CAEEL-mec10	14	14	13	14	13	14	14	14	14	14	13	13	14	14	13	12	13	12
CAEEL-unc8	15	15	15	15	14	15	15	14	15	15	14	14	14	14	14	14	13	13
CAEEL-unc105	14	14	14	13	12	13	13	13	13	12	11	14	13	13	13	12	12	12
CAEEL-asic1	11	11	10	11	11	10	10	11	11	11	12	11	11	11	11	12	11	11
STRPU-Scnn1a	18	17	17	17	18	17	17	18	18	16	15	16	17	17	17	17	15	15
STRPU-Scnn1bL	15	14	14	14	15	14	15	15	15	16	15	14	14	14	13	15	15	15
STRPU-Scnn1g	16	15	14	16	16	15	15	15	16	15	16	14	14	14	15	16	15	14

sequences were selected from the Uniprot database. Species abbreviation is followed by the gene symbol. Species: Caenorhabditis elegans (CAEEL): S. purpuratus (STRPU, sea urchin)

vertebrate ENaC subunit sequences. Sea urchins belong to the phylum Echinodermata (echinoderms). In the records of this genome, some of these homologs have been assigned names as such as "amiloride-sensitive sodium channel subunit alpha", "... beta" and "...gamma". Multi-sequence comparisons of these proteins with vertebrate ENaC sequences show up to 18% partial sequence identity and reveal large areas of sequence insertions. These echinoderm sequences likely constitute an additional family within the ENaC/Degenerin superfamily. The functional characteristics of these proteins have not been determined, and we believe that it is premature yet to call these proteins with names that imply a direct orthologous relationship with vertebrate ENaC subunits. Moreover, sea urchin homologs show greater sequence identity with vertebrate ASIC paralogs than with ENaC. Gene Tree display in Ensembl Metazoa Genome database links between these sea urchin proteins and Deg type proteins from invertebrate species listed in Table 9.

Among invertebrates the taxon that is closest to vertebrates is Cephalochordata that includes lancelets. Cephalochordata and Vertebrata are two of the subphyla of Chordata (Table 9). The genome sequence of Florida lancelet (amphioxus) has been determined (Putnam et al., 2008). BLAST search of the predicted lancelet proteins using ENaC sequences yields many homologous fragments. Most of these lancelet sequences share greater homology with human ASIC (7–24%) than with ENaC paralogs. As many of these sequences are in the status of homology predicted proteins, it is too early to make definitive statements regarding phylogenetic relationships. Nonetheless, the lancelet sequences do not appear to be direct orthologs of human ENaC paralogs.

In summary, among invertebrate species, there are many members of the ENaC/Degenerin superfamily that clearly differ from ENaC. Thus, these homologs do not appear to be direct orthologs or ancestors of ENaC. As we discuss below, the ancestors of ENaC apparently emerged prior to the branching of the first vertebrates but there is not an apparent direct ancestor of ENaC among the invertebrate sequences available at present. The total number of eukaryotic species is estimated as ~8.7 million (Mora et al., 2011) and only a few percent are vertebrate species. Hence, determination of more invertebrate genomes may lead to the findings of new families within the ENaC/Degenerin superfamily.

The multiplicity and divergence of invertebrate sequences that show homology to "amiloride-sensitive sodium channels" require extended efforts to classify these proteins into families based on their homology and phylogenetic distance among other metazoan sequences.

8. Homology between ENaC orthologs

To determine the degree of conservation and sites of divergence of ENaC orthologs, we examined in more detail 20 species for which full sequence of the three ENaC subunits are available. Tables 11 and 12 show results for 18 species rather than 20 we analyzed (omitted gorilla and rat) because of page and font size limitations. The species selected included representatives of primates (rhesus, chimpanzee, and human), elephant, ruminants (cow), carnivores (dog), rodents (mouse), leporids (rabbit), whales (killer whale Orca), marsupials (Tasmanian devil), egg-laying mammals (platypus), birds (chicken and flycatcher), reptiles (alligator and turtle), amphibians (*Xenopus*), lobe finned fishes (coelacanth), and lungfish.

Table 11 presents the percent sequence identity for α subunit orthologs from 18 species with headers that mark taxonomic classification. Each cell of the table gives percent identity between two sequences from the species listed in the respective header and the first column. To determine the percent identity in the conserved extracellular domain of α subunit orthologs, we also compared the sequences of the extracellular domain (Fig. 3). On the average across species, sequence identity is ~9% higher in this central segment, than the sequence identity along the entire length of the orthologous proteins (data not shown).

Table 11

Percent identity between globally aligned amino acid sequences of α-ENaC orthologs from 18 species of Vertebrata (see Tables 7 and 8 for the full names of the species).

	Tetrapod	a															Dipnoi
	Amniota															Amphibia	
	Mammal	ia										Saurop	osida				
	Placental	mammal	s							Marsupial	Egg laying						
	Primates											Birds		Reptiles			
	Human ^a	Chimp.	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycatc.	Alligator	Turtle	Xenopus	Lungfish
Chimpanzee	95																
Rhesus	86	89															
Elephant	84	81	76														
Bovine	81	79	74	83													
Dog	82	82	78	80	79												
Mouse	78	79	76	77	74	77											
Rabbit	78	76	71	79	78	75	75										
Orca	80	77	73	81	85	77	73	77									
Tasmanian	66	64	61	69	67	64	63	66	67								
Platypus	62	60	56	62	62	59	57	62	61	62							
Chick	55	53	52	55	53	53	53	55	54	56	50						
Flycatcher	54	52	51	55	54	53	52	56	55	57	50	89					
Alligator	54	53	51	55	53	53	53	55	54	57	48	80	78				
Turtle	56	54	51	53	53	53	53	54	54	55	49	79	78	81			
Xenopus	51	50	48	52	52	50	48	52	52	51	48	49	51	49	50		
Lungfish	43	41	39	44	44	41	41	44	44	42	40	44	45	44	44	45	
Coelacanth	43	41	39	44	43	42	41	45	44	44	42	45	46	47	46	46	48

^a The first species (human) listed on the header row is not listed in the first column to avoid including self-comparisons (e.g. human vs. human) that obviously equal 100%.

Table 12 shows the percent identity between the entire sequences of β -(upper table) and γ subunit orthologs (lower table) in 18 species.

show high divergence at their N- and C-termini. In contrast, the N- and C-termini of the β and γ subunits are well conserved (see Section 11).

Global alignment of α sequences from 20 species showed that Nand C-termini of orthologous α subunits are divergent across species (see Section 11). Similar to the α subunit, δ subunit orthologs also

By the comparisons presented here we also wanted to examine if the rate of evolutionary change of ENaC orthologs among different species is

Table 12

Percent identity between globally aligned amino acid sequences of β - and γ -ENaC orthologs from 18 species.

SCNN1B	Human	Chimp.	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
Chimpanzee	87																
Rhesus	97	85															
Elephant	82	72	82														
Bovine	85	75	84	81													
Dog	87	77	87	84	86												
Mouse	83	73	83	80	80	82											
Rabbit	85	75	85	80	83	85	81										
Orca	83	73	83	81	89	86	80	81									
Tasmanian	75	65	75	74	75	75	74	75	73								
Platypus	75	66	74	74	74	75	75	73	75	79							
Chick	61	54	61	59	61	62	60	59	61	63	65						
Flycatcher	64	56	64	62	64	64	62	63	63	66	67	82					
Alligator	63	56	63	62	63	64	61	62	63	66	67	78	78				
Turtle	61	53	61	58	60	62	59	61	60	61	63	71	70	74			
Xenopus	57	51	58	57	57	58	56	57	56	59	60	59	58	59	56		
Lungfish	52	46	52	52	51	53	54	52	52	51	53	52	54	53	49	54	
Coelacanth	48	43	48	47	49	48	49	48	49	49	51	49	49	50	45	47	49
SCNN1G	Human	Chimp.	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee	Human 89	Chimp.	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus	Human 89 92	Chimp. 83	Rhesus	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant	Human 89 92 83	Chimp. 83 73	Rhesus 78	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine	Human 89 92 83 85	Chimp. 83 73 76	Rhesus 78 80	Eleph.	Bovine	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog	Human 89 92 83 85 88	Chimp. 83 73 76 79	Rhesus 78 80 83	Eleph. 81 84	Bovine 85	Dog	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse	Human 89 92 83 85 88 85 88 85	Chimp. 83 73 76 79 75	Rhesus 78 80 83 80	Eleph. 81 84 79	Bovine 85 82	Dog 85	Mouse	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit	Human 89 92 83 85 88 85 88 85 86	Chimp. 83 73 76 79 75 77	Rhesus 78 80 83 80 81	Eleph. 81 84 79 81	Bovine 85 82 82	Dog 85 87	Mouse 86	Rabbit	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca	Human 89 92 83 85 88 85 88 85 86 78	Chimp. 83 73 76 79 75 77 70	Rhesus 78 80 83 80 81 74	Eleph. 81 84 79 81 77	Bovine 85 82 82 82 84	Dog 85 87 81	Mouse 86 77	Rabbit 78	Orca	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian	Human 89 92 83 85 88 85 86 78 78 72	Chimp. 83 73 76 79 75 77 70 64	Rhesus 78 80 83 80 81 74 68	Eleph. 81 84 79 81 77 74	Bovine 85 82 82 82 84 72	Dog 85 87 81 73	Mouse 86 77 71	Rabbit 78 73	Orca 68	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus	Human 89 92 83 85 88 85 86 78 72 75	Chimp. 83 73 76 79 75 77 70 64 67	Rhesus 78 80 83 80 81 74 68 71	Eleph. 81 84 79 81 77 74 76	Bovine 85 82 82 84 72 75	Dog 85 87 81 73 75	Mouse 86 77 71 75	Rabbit 78 73 74	Orca 68 70	Tasman.	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus Chick	Human 89 92 83 85 88 85 86 78 72 72 75 63	Chimp. 83 73 76 79 75 77 70 64 64 67 56	Rhesus 78 80 83 80 81 74 68 71 59	Eleph. 81 84 79 81 77 74 76 63	Bovine 85 82 82 84 72 75 62	Dog 85 87 81 73 75 63	Mouse 86 77 71 75 62	Rabbit 78 73 74 62	Orca 68 70 60	Tasman. 76 61	Platypus	Chick	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Dog Mouse Rabbit Orca Tasmanian Platypus Chick Flycatcher	Human 89 92 83 85 88 85 88 85 78 72 72 75 63 59	Chimp. 83 73 76 79 75 77 70 64 67 56 52	Rhesus 78 80 83 80 81 74 68 71 59 56	Eleph. 81 84 79 81 77 74 76 63 60	Bovine 85 82 82 84 72 75 62 59	Dog 85 87 81 73 75 63 60	Mouse 86 77 71 75 62 59	Rabbit 78 73 74 62 59	Orca 68 70 60 57	Tasman. 76 61 58	Platypus 66 62	Chick 86	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus Chick Flycatcher Alligator	Human 89 92 83 85 88 85 86 78 72 75 63 59 64	Chimp. 83 73 76 79 75 77 70 64 67 56 52 52 56	Rhesus 78 80 83 80 81 74 68 71 59 56 60	Eleph. 81 84 79 81 77 74 76 63 60 64	Bovine 85 82 82 84 72 75 62 59 63	Dog 85 87 81 73 75 63 60 63	Mouse 86 77 71 75 62 59 63	Rabbit 78 73 74 62 59 64	Orca 68 70 60 57 59	Tasman. 76 61 58 63	Platypus 66 62 66	Chick 86 81	Flycat.	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus Chick Flycatcher Alligator Turtle	Human 89 92 83 85 88 85 86 78 72 75 63 59 64 62	Chimp. 83 73 76 79 75 77 70 64 67 56 52 56 55	Rhesus 78 80 83 80 81 74 68 71 59 56 60 60 60	Eleph. 81 84 79 81 77 74 76 63 60 64 64 62	Bovine 85 82 82 84 72 75 62 59 63 63 62	Dog 85 87 81 73 75 63 60 63 62	Mouse 86 77 71 75 62 59 63 63 62	Rabbit 78 73 74 62 59 64 62	Orca 68 70 60 57 59 59	Tasman. 76 61 58 63 62	Platypus 66 62 66 65	Chick 86 81 79	Flycat. 76 74	Alligator	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus Chick Flycatcher Alligator Turtle Xenopus	Human 89 92 83 85 88 85 88 85 88 78 72 75 63 59 64 64 62 55	Chimp. 83 73 76 79 75 77 70 64 67 56 52 56 55 48	Rhesus 78 80 83 80 81 74 68 71 59 56 60 60 52	Eleph. 81 84 79 81 77 74 76 63 60 64 62 55	Bovine 85 82 82 84 72 75 62 59 63 63 62 55	Dog 85 87 81 73 75 63 60 63 62 55	Mouse 86 77 71 75 62 59 63 62 55	Rabbit 78 73 74 62 59 64 62 55	Orca 68 70 60 57 59 59 59 52	Tasman. 76 61 58 63 63 62 54	Platypus 66 62 66 65 55	Chick 86 81 79 57	Flycat. 76 74 54	Alligator 79 58	Turtle	Xenopus	Lungfish
SCNN1G Chimpanzee Rhesus Elephant Bovine Dog Mouse Rabbit Orca Tasmanian Platypus Chick Flycatcher Alligator Turtle Xenopus Lungfish	Human 89 92 83 85 88 85 88 85 88 78 72 75 63 59 64 62 55 50	Chimp. 83 73 76 79 75 77 70 64 64 67 56 52 56 55 48 44	Rhesus 78 80 83 80 81 74 68 71 59 56 60 60 52 48	Eleph. 81 84 79 81 77 74 76 63 60 64 62 55 51	Bovine 85 82 82 84 72 75 62 59 63 62 55 55 51	Dog 85 87 81 73 63 60 63 62 55 50	Mouse 86 77 71 75 62 59 63 62 55 55 51	Rabbit 78 73 74 62 59 64 62 55 49	Orca 68 70 60 57 59 59 59 52 48	Tasman. 76 61 58 63 62 54 50	Platypus 66 62 66 65 55 51	Chick 86 81 79 57 51	Flycat. 76 74 54 50	Alligator 79 58 52	57 52	Xenopus 52	Lungfish



Fig. 8. Correlation between the sequence identities among α , β and γ subunits of ENaC for 20 species relative to human ENaC. A) Correlation of the extent of identity of α and β subunits with their human counterparts. B) Correlation of the extent of identity of β and γ subunits with their human counterparts. The x, y coordinates of each point are percent identities between human sequence and the sequence of another species for the subunit indicated in the x and y axes. The sequences were from human, chimpanzee, gorilla, rhesus, elephant, bovine, dog, mouse, rat, rabbit, orca, Tasmanian devil, platypus, chicken, flycatcher, alligator, turtle, *Xenopus*, lungfish, and coelacanth.

similar for the three subunits. Previous studies have indicated that interacting proteins show similar patterns and dynamics of evolution (Lemos et al., 2005). Since the three subunits (α , β and γ) assemble to form a tight complex of a functional channel, we hypothesized that the rate of divergence as measured by the sequence identity would be similar across species for all three subunits.

A cursory comparison of the figures in Tables 11 and 12 shows that for each pair of species the percent identity for all three subunits are similar. For example, percent identity between human and turtle α , β and γ sequences is 56, 61 and 62% respectively. The correlation between the sequence identities among α and β subunits and β and γ subunits was r = 0.96 and r = 0.97 respectively (Fig. 8). Thus, as measured by the percent identity, the divergence of the three subunits has proceeded at similar levels during the species evolution.

For all three ENaC subunits (α , β , and γ), sequence identity between orthologs is consistent with the phylogenetic distance between species.

The following list represents some highlights of this phylogeny related homology:

- 1. ENaC subunits of extant species within the same taxonomic family share generally >87–96% sequence identity. Example: Human and chimpanzee (family Hominidae) (Tables 11 and 12).
- 2. All placental mammal sequences, including marine mammal *Orcinus orca* (killer whale), share >70% identity (Tables 10 and 11).
- 3. Birds and reptiles share a common ancestor (Green et al., 2014). Consistent with this phylogenetic relationship, chicken, and flycatcher ENaC sequences share the highest identity (70–81%) with alligator and turtle (Tables 11 and 12). In contrast, sequence identity between ENaC sequences from birds versus mammalian species is lower, ranging between 50 and 66% (Tables 11 and 12).
- 4. ENaC orthologs in the amphibian *Xenopus*, share 47–59% identity with the sequences from amniotic animals (Tables 11 and 12).
- 5. The orthologs in coelacanth that are descendants of the earliest forms of vertebrates share about 39–55% identity with the ENaC sequences from other Vertebrata species (Tables 11 and 12).
- Lungfish (Table 8), considered a species closest to tetrapods, share 49–57% identity with coelacanth sequence and 39–54% identity with other vertebrates (Tables 11 and 12).

8.1. Insertions and deletions in orthologs

In phylogenetic comparisons above, we noted that in some ENaC/ Degenerin homologs, in addition to sequence divergence, there are major insertions/deletions (extending for tens to hundreds of residues) relative to ENaC. Thus, we concluded that such proteins belong to different families within the ENaC/Degenerin superfamily. The major differences in the functions of these families of proteins are associated with specific structural features built upon the major common scaffold of these channels. Whereas ENaC is constitutively active and functions in transport of Na⁺ across epithelia and consequently regulates extracellular fluid volume, ASIC and degenerin type channels fulfill mainly sensory functions (Ben-Shahar, 2011) (see Section 14). The large insertions in the finger domain (Fig. 6) of DEG family of proteins are apparently part of the complex of mechano-sensitivity of these channels (Eastwood and Goodman, 2012).

In the alignment of α subunit sequences from tetrapod species, it can be seen that the N- and C-termini show divergence (see Section 11). However, the extracellular regions do not have major insertions and deletions. Several sequences have deletion/insertion of 2–6 residues relative to the human ortholog. Nearly all of these are located at sites of sequence divergence when compared with ASIC1 (see Section 5.1).

Alignments of β -ENaC sequences also show no major insertions/deletions for 20 species. The anole lizard β -ENaC has a 16-residue insert starting at residue 406. The status of this protein is currently "uncharacterized protein" implying it may have errors.

Alignment of γ subunit sequences from 20 species shows high homology in the extracellular region, with the exception of the chimpanzee sequence that has an ~65 residue deletion. Such a deletion is not found in other mammalian species, and could reflect an error.

Overall, ENaC family orthologs are highly conserved throughout the spectrum of vertebrate species. The degree of their sequence identity is related to their phylogenetic/taxonomic distance. ENaC orthologs do not have major insertions/deletions and can be readily distinguished from members of other families within the ENaC/Degenerin superfamily by their high percent of sequence identity.

9. Identifying ENaC family members within the ENaC/Degenerin superfamily

Members of the ENaC/Degenerin superfamily are readily identified by their common structural features: a large extracellular region connecting two transmembrane domains, and relatively short intracellular N- and C-termini (Fig. 3). Beyond these common structural features, the proteins share sequence homology of varying degrees, depending on their subfamily and the phylogenetic distance between species. Among vertebrates, there are two subfamilies: ASIC and ENaC. Analyses presented above show that ENaC paralogs in vertebrate species can be readily distinguished from ASIC paralogs.

In phylogenetic comparisons, we noted that some homologs are marked as ENaC orthologs in genome analysis. However, our analyses indicate that these are ASIC rather than ENaC orthologs. As more genome sequences are determined, misclassification of orthologs may occur. To avoid this problem, we formulated thresholds of sequence identity that can clearly distinguish ENaC orthologs from other members of ENaC/Degenerin superfamily.

9.1. Threshold for orthologs

The sequences of ENaC orthologs across species show a high degree of conservation with the lowest sequence identity of 39% between tetrapod species and lobe-finned fish coelacanth in global alignment (Tables 10 and 11). The termini of α subunit orthologs are more divergent, while the sequences of the extracellular region have about 10% higher sequence identity. Thus, in a case where the classification of a sequence is unclear, extracellular regions should be compared. Secondly, insertion/deletion of a large segment (>10 residues) should raise concerns regarding subfamily classification (see Section 8.1).

Protein structure database SCOP employed a minimal criteria of 30% sequence identity for assignment of proteins into the same protein family (Murzin et al., 1995). CATH database uses >35% sequence similarity as the criteria for classification as members of a family (Sillitoe et al., 2015). The observation that among ENaC orthologs sequence identity is >38%, matches the requirements of these two databases for the classification of these proteins as members of the same family of ENaC. As sequence identity with ASIC homologs (see Table 4) and other Degenerin type proteins are generally less than 20%, these proteins represent members of families different from ENaC.

9.2. Threshold for paralogs

Multisequence comparisons presented here show a consistent picture. Global alignments within species show that ENaC paralogs generally share >20% sequence identity with one another (Tables 4 and 6). In contrast, all four ENaC subunits share less than 20% sequence identity with ASIC. This also extends to other homologs, such as Degenerins. Thus within species, 20% sequence identity appears as the cut-off point for the ENaC family as opposed to membership in the ASIC family among vertebrates.

10. Pedigree of ENaC family members

By definition, paralogous proteins emerge as a result of a duplication of a gene in a genome and then diverge as a result of accumulation of mutations in duplicate copies at evolutionary time scale. There are several strong lines of evidence that the four ENaC subunits share a common ancestor:

- 1. All four ENaC subunits share the highest homology among themselves as compared to other families.
- The genes for all four ENaC subunits have introns in the same locations (Fig. 4) (Saxena et al., 1998) while many introns of other homologs are at different positions.

Within the ENaC family, two pairs appear to have distinct ancestors: 1) the α and δ subunits, and 2) the β and γ subunits. Apparently, an ancestral ENaC sequence underwent a gene duplication that resulted in the formation of two ancestral genes that again underwent independent duplication events. The result is four paralogous genes coding for the four ENaC subunits. The evidence for two duplication events includes the following:

- Within each pair of subunits (α and δ; β and γ), there is higher sequence identity than with the other pair of subunits (Tables 4 and 6).
- 2. The genes encoding the β and γ subunits are in adjacent locations on the same chromosome (Brooker et al., 1995), providing evidence that they resulted from a local gene duplication event.

The information provided above on the human genome and other species represents a picture that is true for vertebrates in general. The Ensembl genome database (release 79) of vertebrate and eukaryotic species currently includes 540 homologs of ENaC. A phylogenetic "Gene Tree" constructed for these 540 ENaC homologs using EnsemblCompara GeneTrees paralogy prediction method (Vilella et al., 2009) presents a picture that is consistent with the information provided above.

In Fig. 9 we present a hypothetical "pedigree" for the ENAC paralogous genes based on the Ensembl Gene Tree. A phylogenetic tree is analogous to a pedigree. But, phylogeny differs from pedigree in that while in a pedigree ancestor is known, in phylogeny the ancestor is deduced based on homology relationships. The Ensembl Gene Tree predicts a common ancestral gene for all the ENAC homologs that was duplicated. These duplicate genes were once again duplicated to generate the ancestral genes from which the four ENAC genes derive (Fig. 9).

As noted in Section 6, the genes coding for the α , β and γ subunits are present in all vertebrates, except ray-finned fishes, starting with the most ancient jawless vertebrate species such as lamprey (Table 7). SCNN1D gene coding for the δ subunit appears only in Euteleostomi (bony vertebrates) (Table 7).

The widespread phylogenetic spread of the four ENaC subunits provides evidence that the gene duplications that resulted in the formation of these subunits represent an ancient event that preceded the evolution of vertebrates. There is strong evidence for two rounds of whole-genome duplication (2R-WGD) prior to the diversification of the vertebrates (Cañestro et al., 2013; Putnam et al., 2008). 2R-WGD could result in the generation of four copies of duplicated genes. Yet, it is assumed that duplicate copies of many genes were lost after initial duplication. Currently, we do not know whether all paralogs of ENaC are descendants of this 2R-WGD event. Duplicated genes may also originate as a result of local gene duplication events, independent of whole genome duplication (Cañestro et al., 2013). The SCNN1B and SCNN1G genes coding for the β and γ subunits most likely represent products of a local duplication event as they are immediate chromosomal neighbors. A recent review provides a general overview of the evolution of ENaC and other functionally related proteins such as Na⁺-K⁺-ATPase and renin-angiotensin-aldosterone system proteins and enzymes (Rossier et al., 2015).

11. Conserved sequence motifs and their functions

Alignments of ENaC orthologs from different species reveal many segments as well as single isolated residues that are conserved in all species. The conservation of these residues and sequence segments suggests that these residues fulfill important functional roles. In this section, we shall summarize conserved sequence motifs and their functions as well as other important functional sites.

11.1. Cytoplasmic amino terminus

As can be seen in the alignments of the human ENaC sequences, the N-termini of α and δ subunits show heterogeneity in both their sequence and length (Fig. 4). A similar pattern of heterogeneity is observed in the alignment of the N-terminal sequences from 20 species (Fig. 10). In contrast to the α and δ subunits, the β and γ subunits from 20 species are highly conserved and most are of similar length (Fig. 10).

Chalfant et al. examined activities of rat ENaC subunits with Nterminal deletions. They found that deletion of residues 2–67 in the Nterminus of the α subunit reduced endocytosis of ENaC and increased the half-life of the channel in the membrane, suggesting that the Nterminus contains an endocytotic motif (Chalfant et al., 1999). Deletion of longer segments of α , β , and γ N-terminus (94, 50, and 94 residues respectively), drastically reduce ENaC activity (Bachhuber et al., 2005).



Fig. 9. A hypothetical phylogenetic tree for paralogs of ENAC. "Anc." is used as an abbreviation for "Ancestor". A "duplication node" represents a gene duplication event that yields two genes within one genome. A "speciation node" represents the formation of a new species that carries the gene of interest. By the convention of Ensembl Gene Tree, collapsed trees for paralogs are shown in blue color. The figure is based on a Gene Tree constructed for 540 ENAC homologs in the Ensembl genome database (release 79) of vertebrate and eukaryotic species using EnsemblCompara GeneTrees paralogy prediction method. The figure includes several modifications from the Gene Tree: The nodes for *C. elegans* degenerins and one homolog from a fish were omitted from the figure, and the positions of the nodes were modified to show branches in parallel. The number of homologs in each collapsed branch is written on the right side of the collapsed tree marking.

Yue et al. noted that the N-terminal segments of β and γ subunits contain a stretch of basic residues that is characteristic of Phosphatidylinositol 4,5-bisphosphate (PIP2) binding sites in other proteins (Yue et al., 2002). Mutation of 4 of these basic residues to nonpolar residues in the β but not in the γ subunit drastically reduced ENaC currents in the *Xenopus* oocyte expression system (Kunzelmann et al., 2005).

In a stretch of about 30 to 40 residues prior to the start of TM1, all four human ENaC paralogs have some strictly conserved residues (Figs. 4 and 10). These residues are conserved in the four human ENaC paralogs (Fig. 4) as well as in all 20 species examined (Fig. 10). The conservation of these sequences across species suggests that this region has an important functional role. A Gly37Ser missense mutation in this region of the β subunit causes multi-system PHA and this mutation reduces the open probability (P_0) of ENaC (Gründer et al., 1997). Mutations of the corresponding residue in the α and γ subunits also reduced channel activity, suggesting that this site has an important role in regulating channel gating (Gründer et al., 1997). Gründer et al. also examined the roles of the residues flanking the key Gly residue by systematic alanine mutagenesis of 28 residues (H77 to H104 in rat α subunit) (Gründer et al., 1999). The expression of ENaC with these mutant subunits in Xenopus oocytes showed that most mutants decreased channel activity, likely due to a reduction in channel Po (Gründer et al., 1999). The stretch of ten residues from T92 to C101 showed the highest sensitivity to alanine mutagenesis, with G95, H94 and R98 mutants showing the strongest reduction (Gründer et al., 1999). These residues are conserved in all 20 species with the exception of the platypus, which has an exceptionally short N-terminus (Uniprot ID: F7F7U2_ORNAN) (Fig. 10). Since the status of this sequence is marked as an "uncharacterized protein" it may have an error.

11.2. TM1

The resolved structure of ASIC1, and sequence similarities between ASICs and ENaCs provide important clues about the stretch of ENaC residues that form TM1. We have also used algorithms to predict the location of the TM1 (Fig. 4 and Table 3). Relative to the alignment with ASIC1 sequence, predicted TM1 segment starts three residues (KKK in human β subunit) after the start of the ASIC1 TM1 (cf. Figs. 4, 7 and 10). According to this prediction, TM1 is preceded by 2-3 Arg/Lys residues that are conserved in all ENaC orthologs (Figs. 3, and 10). Studies on the distributions of charged residues in α -helical TM segments indicated that positively charged residues Arg and Lys are present at much higher proportions on the cytoplasmic side of the TM segment of proteins. This trend was named the "positive-inside" rule (von Heijne, 1992). A recent study has shown that in 191 transmembrane α -helical segments, the residues Arg and Lys are present at highest proportion just before the start of the lipid bilayer (Pogozheva et al., 2014). At this location, the positively charged residues interact with the polar head groups of membrane lipids and contribute to the strength of membrane anchoring of ENaC. The conserved appearance of Arg and Lys just before the predicted TM1 provide support for the predicted location of the TM1 (Table 3, Figs. 3, and 10).

In the predicted TM1 location, a tryptophan (W) appears as one of the first three residues conserved in all 20 ENaC orthologs (Fig. 10). In ASIC1, a Trp appears as the third residue from the beginning of the first helix (Fig. 7). Analysis of 191 α -helical TM proteins showed that aromatic residues Trp and Tyr are predominantly located at the membrane-water interface (Pogozheva et al., 2014). These aromatic amino acids are known to partition into the interface region of

Fig. 10. Comparison of α , β , and γ sequences in the N-terminal, pre-TM1, and TM1 segments from twenty species. For each subunit, residues that are identical in at least 19 out of 20 species (95% identity) are shaded. The location of the predicted TM1 is shown above the sequences. The α subunits have N-termini of highly variable lengths (the numbers at the beginning of each sequence marks the number of additional residues that did not fit into the page), with little or no sequence conservation in this variable region. In contrast, the β and γ subunit N-termini are mostly of similar length and show a high degree of conservation within an ~40 residues-long segment prior to the TM1. The row of red letters, in between the β and γ sequence groups, mark the residues that are identical in both β and γ subunits. In four sequences (β : chicken; γ : gorilla, chicken and coelacanth) 2–5 residues prior to the first methionine were deleted to be consistent with other Uniprot sequences that start with Met as the first translated codon. There may be also sequencing errors in the unusually short platypus α sequence, and flycatcher γ sequence.

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α		-		_	_	_			_	_		_	_	_	_	_	_	_	_	_	_	_							_					_	_	_		_	_	_															—
Human	29	C)G	G	ΡF	= P	A	ΑP	0	01	ъτ	А	FF	F	А		E	F	ня	2 5	Y	RI	FΙ	F	F	FF	c c	NN	л	т	ĽН	IG	A	R	1	v c	SI	он	N	R <mark>N</mark>	кт	A	= w	AV		ΝI	ст	F	зм	MY	wc	F	G L L	F	GE
Chimpanzee	52	0	G	G	PE	ΞP	A	A P	0	01	ът	A	EE	E	A	LI	E	F	н	R S	Ŷ	RI	EL	F	E	F F	: c	NN	JТ	Ť.	i F	IG	A	R	Ľ	v c	S	ОН	N	RN	кт	A	= w	AV		NL.	СТ	F	SМ	MY	wc	F	GL	LF	GE
Gorilla	9	10	G	G	PE	ΞP	A	A P	0	01	ът	A	EE	E	A	LI	E	F	н	R S	Ŷ	RI	EL	F	E	FF	: c	NN	JТ	т	i F	IG	A	R	Ē	v c	S	он	N	RN	кт	A	= w	AV		NL	СТ	F	SМ	MY	wc	F	GL	F	GE
Bhosus	86	0	G	G	PE	ΞP	A	A P	o	01	ът	A	EE	E	A		/ E	F	ни	2 S	Ŷ	RI	EL	F	E	F F	: c	NN	JТ	Ť.	i F	IG	A	R		v c	S	ОН	N	RN	кт	A	= w	AV		NL	СТ	F	SМ	MY	wc		GL	LF	GE
Elephant	9	10	G	G	PE	ΕP	A	AP	E	01	ът	E	EE	E	A	LI	E	F	н	2 S	Ŷ	RI	ΕL	E	0	FF	: c	SN	JТ	Ť	i F	IG	A	R	Ľ	v c	S	он	N	RN	кт	A	= w	AV		NL	СТ	F	ЗM	MY	wc	F	GL	F	GE
Bovine	9	F	G	PG	PE	ΕP	SC	G P	P	PI	рт	E	EE	E	A	LL	E	F	н	R S	Y	RI	ΕL	F	E	FF	: c	NN	JТ	т	I F	IG	A	R	Ē	v c	S	он	N	RN	кт	V	= W	AV		NL	СТ	FO	ЗM	MY	wc	F	GO	F	GE
Dog	50	E	G	G	PK	(P	A	A P	00	01	ът	E	EE	E	A	LI	E	F	н	R S	Y	RI		E	0	FF	: c	NN	JТ	т	I F	IG	A	R	Ē	v c	s	кн	N	RN	кт	A	= W	AV		NL	CA	F	IM	MY	wc	F	GO	F	GE
Mouse	54	. (A	G	PE	ΕP	SI	ΕP	R	OF	ът	E	EE	E	A	LI	E	F	н	R S	Y	RI	ΕL	F		FF	: c	NN	I T	т	I H	IG	A	R	Ľ	v c	s	кн	N	RN	кт	A	= W	AV		WL	СТ	F	SМ	MY	wc	F	AL	LF	EE
Rat	54	C	G	G	ΡE	ΕP	s	A P	R	01	РΤ	Е	EE	E	A	LI	E	F	н	R S	Y	RI	ΕL	F	0	FF	c :	NN	I T	т	ГH	IG	A	R	L	v c	s	кн	N	RN	кт	A	= W	AV		NL	СТ	F	зм	MΥ	wc	F	AL	LF	ΕE
Rabbit	9	0	G	۶G	PE	Т	v	AP	00	01	РΤ	EI	DE	E	A	LI	E	F	н	R S	Y	RI	ΕL	F	o	FF	c :	NN	I T	т	LН	IG	A	R	L	v c	s	кн	N	RN	кт	A	= W	AV		NL	СТ	F	зм	MY	wc	F	GL	LF	GE
Orca	9	F	E	PG	PE	ΕP	A	AP	P	PS	5 Т	D	EE	E	Ρ	LL	E	F	нн	+ S	Y	RI	ΕL	F	0	FF	c :	NN	I T	т	I H	IG	A	R	L	v c	S	он	N	RN	КТ	A	= W	AV		NL	СТ	F	зм	MY	WC	F	GL	LF	GE
Tasmanian	9	0	iG	RН	ΡE	EP	G	GΡ	0	ĸ		Е	E١	κE	G	LI	E	F	۲ŀ	+ s	Y	RI	ΕL	E	ò	FF	c :	SN	I T	т	ĿН	IG	A	R	L	v c	s	кн	N	RN	кт	A	= w	AV		NL	СТ	F	зм	MY	wc	F	GL	L F	GΕ
Platyous	1						_		-			-			-			-			-	MI	LN	IT.	TI	RF	R L	RF	R	R	ΥF	RΡ	A	δP	R	СТ	C	ОН	N	RN	КТ	V	= W	AA		CL	CS	F	ЗM	MY	WC	F	GL	F	GD
Chick	29		-			E	00	00	-	- ł	ΚE	D	EF	RΕ	G	LI	E	F	ΥC	s s	Y	0	DV	F	0	FF	c	SN	I T	т	I F	IG	A	R	L	v c	S	кк	N	κ <mark>Ν</mark>	кт	A	= W	SV		FΙ	LT	F	G L	MY	wc	F	GI	LY	RE
Elycatcher	15					E	00	20	0	01	< F	F	E F	R F	G		E	F	Y	ss	Ŷ	0	FL	E	0	FF	: c	SN	JТ	т	I F	IG	A	R	Ē	v c	S	кк	N	ĸN	кт	A	= w	SV		FF	LT	F C	31	MY	wc	F	GI	Ý	RE
Alligator	24	. (н	00	к⊦	+ E	E	VP	E	ĸ/	A E	E	κ.	I E	G		E	F	HN	I S	Ŷ	RI	ΕL	E	0	FF	: c	SN	JТ	Ť	i F	IG	A	R	Ē	v c	S	кк	N	ĸN	кт	A	= w	SV		FF	LT	FO	G L	MY	wc	F	GI	Y	RE
Turtlo	26	E	N	00	κv	/ E	E١	V A	E	ĸı	E	ĸ	Εŀ	I E	G	L 1	E	F	н	s	Ŷ	н	E L	E	0	FF	: c	NN	JТ	Ť	ι. Η	IG	A	R	Ē	v c	S	KR	N	ĸN	кт	A	= w	IS V		FF	LT	FO	31	MY	wc	F	GI	Ý	RE
Yepopus	1						- 1	ит	ĸ	EF		N	E F	(E	A		E	F	F	ss	Ŷ	RI	E L	F	F	FF	: c	SN	JТ	Ť	i F	IG	A	R	. ·	v c	S	RR	N	RN	кт	A	= w			FL	VT	FO	31	MY	wc	F	GL	F	GO
Lungfich	1			- M	тс	эκ	EI	ΕE	A	EC	G	ĸ	с н К н	(E	P	MI	G	F	YE	s	Ŷ	01	ΕL	F	E	FF	: c	N N	JТ	Ť	i F	IG	т	R	M	v c	S	кн	N		кт	v	5 w	TI	L	F I	тт	FO	ι	MY	wc		GL		GO
Coelacanth	1						- 1	MS	E	KI	K E	E	KS	5 K	G	LI	E	F	Y	ss	Y	SI		F	0	FF	: c	ST	T	т	LE	IG	A	R	L	vc	Т	ER	N	ĸN	кт	A	w	SN	1	FV	AS	F	a L	MY	wc	F	GI	IF	GH
Coelacaliti									-			-													-			-							-				.						_										

β		10	20	30	40	50	60	70
Human	1	MH V K K Y L L H	k g <mark>l h r l q k g p g y</mark>	T <mark>Y K E L L V WY</mark>	C D N T N T H G P K R	IIC- <mark>EGPKK</mark>	K A M <mark>WF</mark> LL <mark>TL</mark>	L <mark>F</mark> A A <mark>L V</mark> C <mark>WQWG</mark> I F <mark>I</mark> R T
Chimpanzee	40 H R E G A T	⁻ MH V <mark>K</mark> K Y L L F	kg <mark>l h r l q kg pg y</mark>	T <mark>Y K E L L V WY</mark>	C D N T N T H G P K R	IIC- <mark>EGPKK</mark>	KAM <mark>WF</mark> LL <mark>TL</mark>	L F T A L V C WQ WG I F I R T
Gorilla	40 LSPGAT	⁻ MH V <mark>K</mark> K Y L L F	kg <mark>l h r l q kg pg y</mark>	T <mark>Y K E L L V WY</mark>	C D N T N T H G P K R	IIC- <mark>EGPKK</mark>	K A M <mark>W F</mark> L L <mark>T L</mark>	L F A A L V C WQ WG I F I R T
Rhesus	1	MHVKKYLLI	k c <mark>l h r l q kg pg y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIC- <mark>EGPKK</mark>	KAV <mark>WF</mark> LL <mark>TL</mark>	L F T A L V C WQ WG I F I R T
Elephant	1	MQVKKYLLI	K C <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	CNNTNTHGPKR	IIC- <mark>EGPKK</mark>	кам <mark>wf</mark> vl <mark>tl</mark>	L F T S L V C WQ WG V F I K T
Bovine	1	MHVKKYLLI	kg <mark>l h r l q kg pg y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIC- <mark>EGPKK</mark>	кам <mark>wf</mark> vl <mark>tl</mark>	L F T S L V C WQ WG L F I K T
Dog	1	MHLKKYLLI	K G <mark>L H R L Q K G P G Y</mark>	S <mark>Y K E L L V WY</mark>	CNNTNTHGPKR	IIC- <mark>EGPKK</mark>	KAM <mark>WF</mark> LI <mark>TL</mark>	L F T S L V C WQ WG V F I R T
Mouse	1	MPVKKYLLI	K C <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	CNNTNTHGPKR	IIC- <mark>EGPKK</mark>	KAM <mark>WF</mark> LL <mark>TL</mark>	L F A C L V C WQ WG V F I Q T
Rat	1	MPV <mark>K</mark> KYLLI	K C <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	CNNTNTHGPKR	IIC- <mark>EGPKK</mark>	KAM <mark>WF</mark> LL <mark>TL</mark>	L F A C L V C WQ WG V F I Q T
Rabbit	1	MHVKKYLLI	k c <mark>l h r l q kg pg y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIR- <mark>EGPKK</mark>	KAM <mark>WF</mark> LI <mark>TL</mark>	L F A S L V C WQ WG V F I K T
Orca	1	MHIKKYLLI	K C <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIC-EGPKK	KAM <mark>WF</mark> VL <mark>TL</mark>	L F T S L V C WQ WG V F I R T
Tasmanian	1	MHLKKYLVI	k c <mark>l h r l q kg pg y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIC-EGPKK	Q V M <mark>W F</mark> I L <mark>T L</mark>	L F A S L V L WQ W S I F I Q T
Platypus	1	MN V K K Y L V H	k c <mark>l h r l q kg pg y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIC- <mark>EGPKK</mark>	ĸ v m <mark>wf</mark> v l <mark>t l</mark>	V F A C V V F WE WG L F I Q T
Chick	1	MNLKRYFVF	R A <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIK- <mark>EGPKK</mark>	K V M W F F L T L	L F A S L V F WQ WG I L I N T
Flycatcher	1	MRLKKYLVF	R A <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIK- <mark>EGPKK</mark>	KFI <mark>WF</mark> FL <mark>TL</mark>	L F A S L V F WQ WG I L I N T
Alligator	1	MNLKRYFIF	R A <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIR-EGPKK	K F V <mark>W F</mark> F L <mark>T L</mark>	L F A S L V F WQ WG I L I N T
Turtle	1	MNLKKYFIF	R V <mark>L H R L Q K G P G Y</mark>	T <mark>YKELLVWY</mark>	C DNTNTHGPKR	IIR-EGPKK	KFI <mark>WF</mark> FL <mark>TL</mark>	L F A S L V F WQ WG I L I T T
Xenopus	1 MIHGKM	IKRL <mark>K</mark> R <mark>Y</mark> FTF	RA <mark>LHRIQKGPGY</mark>	T <mark>Y K E L L V W F</mark>	C DNTNTHGPKR	IIK- <mark>EGPKK</mark>	R V M W F I L T L	V F A G L V F WQ WG V L I L T
Lungfish	1	MFLKRWFIF	R A <mark>L H R L Q K G P G Y</mark>	G <mark>YSELFVWY</mark>	CNNTNTHGPKR	LII- <mark>EGPKK</mark>	кт L <mark>W</mark> S L F <mark>T V</mark>	TFACLVFWQWGLLIQT
Coelacanth	1	MS V R K Y F T F	RA <mark>LHRLQKGPGY</mark>	T <mark>Y K E L L V WY</mark>	C DNTNTHGPKR	IIK-EGPKK	Q V L <mark>W F</mark> I L <mark>T L</mark>	TFTALIFWQWGLLIQT
У				L WY	C NTNTHG R	I G	W TL	W
Human	1 MAP	GEKIKAKI <mark>ł</mark>	<mark>KNLPVTGPQA</mark> P	TIKE <mark>LM</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> GF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> LWQCAL <mark>L</mark> VFS
Chimpanzee	1 MAP	GEKIKAKI <mark>k</mark>	<mark>(K N L P V T G P Q A</mark> P [·]	T I KE <mark>LM</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> GF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> LWQCAL <mark>L</mark> VFS
Gorilla	1 MAP	G E K I K A K I <mark>I</mark>	<mark>(K N L P V T G P Q A</mark> P [·]	T I KE <mark>LM</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> GF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> LWQCAL <mark>L</mark> VFS
Rhesus	39 ILAMAP	GEKIKAKI <mark>ł</mark>	<mark>KNLPVTGPQA</mark> P	TIKE <mark>LM</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> GF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> LWQCAL <mark>L</mark> VFS
Elephant	1 MAP	G E K I K A Q I <mark>I</mark>	<mark>KNLPVTGPQA</mark> P	TIKE <mark>LM</mark> R <mark>WY</mark>	CLNTNTHGCRR	I V V S R G R L R	R L L <mark>W I</mark> L F <mark>T L</mark>	T <mark>AV</mark> AL <mark>I</mark> F <mark>WQCA</mark> L <mark>L</mark> VLS
Bovine	1 MAP	G E K I K A K I <mark>I</mark>	<mark>K K N L P V</mark> T <mark>G P</mark> Q <mark>A</mark> PI	N I K E <mark>L M</mark> Q <mark>WY</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> LF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> F <mark>WQCA</mark> L <mark>L</mark> ISS
Dog	1 MAP	GEKIKAKI <mark>ł</mark>	<mark>(K N L P V R G P Q A</mark> P [·]	T I K E <mark>L M</mark> R <mark>W Y</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> LF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> F <mark>WQCA</mark> L <mark>L</mark> VAS
Mouse	1 MAP	GEKIKAKI <mark>ł</mark>	<mark>(K N L P V R G P Q A</mark> P	T I KD <mark>L M</mark> H WY	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> AF <mark>TL</mark>	T <mark>AV</mark> AL <mark>IIWQCA</mark> L <mark>L</mark> VFS
Rat	1 MAP	GEKIKAKI <mark>ł</mark>	<mark>KNLPVRGPQA</mark> P	T I KD <mark>L M</mark> H WY	C M N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> AF <mark>TL</mark>	T <mark>AV</mark> AL <mark>IIWQCA</mark> L <mark>L</mark> VFS
Rabbit	1 MAP	GEKIKAKI <mark>I</mark>	<mark>(K N L P V K G P Q A</mark> P '	T I KE <mark>LM</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S P G R L R	<mark>R</mark> LL <mark>WI</mark> AF <mark>TL</mark>	T <mark>A V</mark> G L <mark>I</mark> F <mark>W Q C A</mark> L <mark>L</mark> V F S
Orca	1 MAA	GEKIKAKI <mark>k</mark>	<mark>(K N L P V R G P Q A</mark> P [·]	T I K E <mark>L M</mark> R <mark>W Y</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> LF <mark>TL</mark>	T <mark>AV</mark> AL <mark>I</mark> F <mark>WQCA</mark> L <mark>L</mark> ISS
Tasmanian	1 MAP	G E K I K A K I <mark>I</mark>	KNLT <mark>V</mark> TGPQ <mark>A</mark> P∶	S I K E <mark>L M</mark> K <mark>W Y</mark>	C L N T N T H G C R R	I V V S R <mark>G R L R</mark>	<mark>R</mark> LI <mark>WV</mark> VF <mark>TL</mark>	T <mark>A V</mark> G L <mark>I</mark> L <mark>W Q C A</mark> L <mark>L</mark> V L S
Platypus	1 MAP	GEKITAKI <mark>I</mark>	<mark>KNLPVTGPQA</mark> P	T I KE <mark>LM</mark> K <mark>WY</mark>	C L N T N T H G C R R	I V V S R <mark>G R L R</mark>	<mark>R</mark> LI <mark>WI</mark> IF <mark>TL</mark>	T <mark>AV</mark> GL <mark>IIWQCA</mark> L <mark>L</mark> ILS
Chick	1 MA	∧PGKITARI <mark></mark> ł	<mark>(</mark> K T <mark>L</mark> P <mark>V</mark> R <mark>G P</mark> Q <mark>A</mark> P	T L R E <mark>L M</mark> R <mark>W Y</mark>	C L N T N T H G C R R	I V V S R <mark>G R L R</mark>	<mark>R</mark> FI <mark>WI</mark> LL <mark>TL</mark>	S A V G L I L WQ C A E L L L N
Flycatcher	1			<mark>- M</mark> R <mark>WY</mark>	C L N T N T H G C R R	I V V S R <mark>G R L R</mark>	<mark>R</mark> LL <mark>WI</mark> LL <mark>TL</mark>	S A V G L I L WQ C A E L L MN
Alligator	1 MAA	GRKITAKI <mark></mark>	<mark>(K T L P V</mark> T <mark>G P</mark> Q <mark>A</mark> P [·]	T L G E <mark>L M</mark> R <mark>W Y</mark>	C L N T N T H G C R R	I V V S R G R L R	<mark>R</mark> LL <mark>WI</mark> AL <mark>TL</mark>	S <mark>AV</mark> AL <mark>ILWQCA</mark> E <mark>L</mark> VLG
Turtle	11 EFSMAP	• AKKIKAKI <mark>I</mark>	<mark>(ΚΤ<mark>ΙΡ</mark>ΥΤ<mark>GΡ</mark>Q<mark>Α</mark>Ρ΄</mark>	T V S E <mark>L M</mark> H <mark>WY</mark>	C M N T N T H G C R R	I V V S R <mark>G R L R</mark>	<mark>K</mark> FI <mark>WI</mark> LL <mark>TL</mark>	S <mark>AV</mark> GL <mark>ILWQCA</mark> E <mark>L</mark> IMS
Xenopus	1 MSKS	GKKLTQKL <mark>I</mark>	<mark>(K N L P V T G P Q A</mark> P [·]	Т L Y E <mark>L M</mark> Q <mark>WY</mark>	C L N T N T H G C R R	I V V S K <mark>G R L R</mark>	<mark>R</mark> WI <mark>WI</mark> SL <mark>TL</mark>	C <mark>AV</mark> AV <mark>I</mark> F <mark>WQCALL</mark> IMS
Lunfish	1 MGH	IG R R I S E S I <mark>I</mark>	K Q L P <mark>V T G P</mark> E <mark>A</mark> P	T V K N <mark>L M</mark> D <mark>WY</mark>	L N <mark>N T N T H G C R R</mark>	I A V S R G Y L R	<mark>R</mark> WI <mark>WI</mark> CF <mark>TV</mark>	S S <mark>V</mark> G M I F WQ WT L L L M S
Coelacanth	1 MT S R	KKSLPEKI <mark>ł</mark>	<mark>K E N L</mark> P <mark>V</mark> T <mark>G P</mark> Q <mark>A</mark> L 1	S I S E <mark>L M</mark> R <mark>W Y</mark>	C Y N T N T H G C L R	I V A <mark>S</mark> R <mark>G R L R</mark>	<mark>R</mark> WI <mark>WI</mark> LL <mark>TL</mark>	S <mark>AV</mark> AL <mark>I</mark> FWQCALLIIS

membranes. Hence, it has been suggested that they contribute strongly to the anchoring and precise positioning of TM segments in the lipid bilayer (Hong et al., 2007). The appearance of Trp at the beginning of the TM1 in all ENaC orthologs in 20 species provides further support for the predicted location of the TM1 (Figs. 3, 4 and 10). Analyses of TM1 of the α subunit of ENaC by tryptophan-scanning mutagenesis suggested two functionally different regions. N-terminal tryptophan residues altered both channel activity and cation selectivity, with a periodicity consistent with a helical structure. While C-terminal tryptophan residues also affected activity and selectivity, there was no apparent periodicity (Kashlan et al., 2006).

11.3. Extracellular region

The extracellular region, as revealed in the resolved structures of ASIC1, has a complex structure that resembles an outstretched hand holding a ball (Figs. 3 and 4). Hence, domains within the extracellular regions are referred to as palm, thumb, knuckle, finger and β ball (Jasti et al., 2007) (Fig. 7). The palm and β ball domains are formed by non-contiguous β strands and loops, and are in close proximity to the membrane (Figs. 3 and 4). More peripheral domains (thumb, knuckle and finger) are formed by contiguous α helices and loops, and are poorly conserved among ENaC/Degenerin family members, when compared with other parts of the extracellular region. Based on sequence homology and predicted secondary structure, it is likely that the structural features of the extracellular region of ASIC1 is shared among all members of the ENaC/Degenerin superfamily. This is one of the key defining features of this ion channel family. Below we present some of these conserved segments the functions of which have been examined.

11.3.1. Protease cleavage sites

Proteases activate ENaC by cleaving the α and γ subunits at multiple sites within their extracellular finger domains, releasing imbedded inhibitory tracts (Kleyman et al., 2009; Rossier and Stutts, 2009; Vuagniaux et al., 2002). Serine proteases represent one of the largest gene families with 175 predicted genes in the human genome (Szabo and Bugge, 2011). Despite variations in the cleavage sequence specificity of these enzymes, there is a common denominator of one or more Arg or Lys residue immediately preceding the cleavage site (Antalis et al., 2010).

Furin, a member of the proprotein convertase family of serine proteases, cleaves the α subunit twice at sites (RSTR (proximal) and RSAR (distal)) flanking an inhibitory tract (LPHPLQRL) (mouse sequences) (Carattino et al., 2008b, 2006; Hughey et al., 2004; Sheng et al., 2006). Furin cleaves the γ subunit once (RKRR), and cleavage by a second protease at a distal site releases another inhibitory tract (RFLNLIPLLVF) (Bruns et al., 2007; Passero et al., 2010). A polybasic RKRK sequence is one of the distal sites targeted by some of the nonfurin proteases that cleave the γ subunit and activate ENaC (Bruns et al., 2007; Passero et al., 2011; Patel et al., 2012). The sequences of the inhibitory tracts in both α and γ subunits are conserved in mammals (marked with blue shading in Fig. 11). While there is a divergence of the sequence of the homologous segment in Sauropsida, Amphibia, lungfish and coelacanth, the fact that amphibian ENaC is activated by proteases (Alli et al., 2012) suggests that this intrinsic inhibitory tract has evolved over time. At present, we are not aware of evidence that other members of the ENaC/Degenerin family are activated by proteases.

The α and γ subunit sequences from 20 species indicate that the key protease cleavage sites are strongly conserved in all species with the

exception of lungfish and coelacanth (Fig. 11). In Fig. 11, β and γ subunit sequences have been aligned together as these two proteins are products of paralogous duplicated genes. It is noteworthy that in the β sequences the protease cleavage motifs are missing (Fig. 11). In mammals and marsupials, there is a gap instead of the protease cleavage motifs (Fig. 11). Considering that both genes are apparently the descendants of the same gene, either the protease cleavage motifs were deleted from the SCNN1B gene or were later added to the SCNN1G gene.

11.3.2. Disulfide bonds

Within the extracellular regions of ENaC family members, there are 16 highly conserved cysteine residues that likely form eight disulfide bonds (Firsov et al., 1999; Jasti et al., 2007; Sheng et al., 2007). Based on the disulfide bonds in the resolved ASIC1 structure, these include five disulfide bonds in the thumb domain (Sherwood et al., 2012). The 16 extracellular cysteine residues are conserved in all 20 species examined with the exception of the γ subunit of chimpanzee and the β subunit of coelacanth. As noted above, there are two large gaps in the chimpanzee γ subunit sequence. These gaps probably reflect an error in sequence. The structural importance of the conserved cysteines has been demonstrated by site-directed mutagenesis experiments (Firsov et al., 1999; Sheng et al., 2007). There are additional Cys residues in the finger domain of family members in *C. elegans*, which may form additional disulfide bonds.

11.3.3. Sites of N-linked glycosylation

During the process of translation of proteins that are membrane bound or secreted, oligosaccharides may be attached to the N4 of the asparagine residue at the start of a consensus sequence composed of three amino acids: Asn-Xaa-Ser/Thr. The extracellular region of rat α , β , and γ subunits were shown to have such sites that are glycosylated (Canessa et al., 1994a; Snyder et al., 1994). Alignment of the sequences of subunits from 20 species show that most of the sites identified in the rat sequences are conserved in mammals, but not in birds and lower species. Since the studies on glycosylated residues were carried out using rat subunits, we note here the conserved sites according to the rat subunit residue numbering. Thus, the homologs of the following rat residues are conserved as the first residue in N-glycosylation consensus sequence in most mammals: α subunit: N259, N320, N339, N424, and N538; β subunit: N135, N141, N146, N197, N205, N258, N362, N376, and N482; and γ subunit: N210, N249, N272, N292, and N498.

11.3.4. Knuckle domain

In the ASIC1 model, the knuckle domain is composed of two helices (α 6 and α 7) that are located at the top of each subunit (Figs. 3–5). The sequence of this region is conserved within each subunit, but shows divergence between ENaCs and ASICs (Fig. 7). In all three subunits there is a positively charged residue (Arg or Lys) at a position that corresponds to the end of the α 7 helix in ASIC1 (Fig. 7). Mutation of this residue (K498 in the human γ subunit) to alanine was shown significantly to reduce surface expression of ENaC (Edelheit et al., 2014). Recently Chen et al. showed that deletion of the entire knuckle domain (including the conserved Arg/Lys) in mouse β or γ subunit drastically reduced ENaC surface expression and consequently ENaC function (Chen et al., 2015). These consistent findings suggest that the conserved charged residues in this domain may be involved in binding to other proteins that are involved in the transport of ENaC subunits.

In contrast to effects in β or γ subunits, deletion of the knuckle domain in the α subunit resulted in channel activation as a result of a loss of Na⁺ self-inhibition (Chen et al., 2015).

Fig. 11. Serine protease cleavage sites in the extracellular domain of α , β and γ ENaC subunits from 20 species. Key basic amino acids (Arg (R) and Lys (K)) in the putative cleavage site are marked with yellow shading. The sequences of the respective subunits from 20 species were aligned by CLUSTALW. The conserved sequences of the inhibitory tracts located in between the two SP sites are marked light blue background. The residues of the substrate protein that are recognized by proteases are numbered based on their position relative to the cleaved peptide bond. P₁ marks the putative residue after which the peptide bond is cleaved by the SP (Antalis et al., 2010).

α		10	20	30	40	50	60	
Human	164	YSSFTTLVAGS	I RS <mark>RR</mark> DLRGT	I L PH PLQ RL	RVPPPPHGARR	A <mark>R</mark> SVAS	I SLRDNN PQVDW	219
Chimpanzee	187	YSSFTTLVAGS	RSRRDLRGT	L PH PLQ RL	RVPPPPHGARR	ARSVAS	SVRDNNPQVDW	242
Gorilla	144	YSSFTTLVAGS	RS <mark>RR</mark> DLRGT	LPHPLQRL	RVPPPPHGARR	a <mark>r</mark> svas	SVRDNNPQVDW	199
Rhesus	221	YDSSPTLVAG S	RG <mark>RR</mark> DLRGT	L PHLLQRL	RVPSPLHGA <mark>R</mark> Q	a <mark>r</mark> svas	SVRDNNPQVDW	276
Elephant	144	YNYSNTLGAH P	RSRRDLRGT	LPHPLQRL	QVPSPPHKARK	ARSADS	SVQDNNPQVNR	199
Bovine	144	YNSSKILVAH AI	C P P D I P E P		PVPAPPHAARG	APPAAGS	SMRDNNPQVNR	199
Mouse	189	YNSSYTROAG - GRR	STRDIRGA	I PHPLORI	RTPPPPNPARS	ARSASS	SVRDNNPQVDR	240
Rat	189	YN SSYTRQAG - ARRI	RSSRDLLGA	FPHPLQRL	RTPPPPYSGRT	ARSGSS	SVRDNNPQVDR	246
Rabbit	144	YNASTLEAQP	RHRRDVHPP	L PH PLQ RL	RVPPPRLEAR	A <mark>R</mark> SSAS	SVRDNSPEVGR	198
Orca	144	YN SSN SL VAH A	R <mark>GRR</mark> DLRES	LPHPLQRL	PVPAPPHAAS <mark>R</mark>	V <mark>R R</mark> S D S	SLSHSNPKVNR	199
Tasmanian	142	YN SSRSL SN K P	RL <mark>RR</mark> DISNQ	L PYPLVKI	PYP QSFSH	H <mark>R</mark> SGAA	G VQ ENN PQ VD K	194
Platypus	109	TTAALPSRP	RRGLGSP	L PH PLQ VL	RRPPPASQPRR	VRSGAR	GVRENGPRVGK	162
Elycatcher	130	YNMSLARSDGSAUF	TORRSTRS		PLRRQ		NIPENSPSVDK	195
Alligator	159	YNMSVTQSDWAAQY	THKRSSRS	L FHYVQRH	PLRRH	KRDNPA	IVEENNPPVDK	210
Turtle	161	YSMSQVQSNGAAQS	SQ <mark>KR</mark> SQRS	L SHH VQ RH	PLRRR	K R N E P V	SLKGNSPPVDK	212
Xenopus	128	YNSTGVQGWI PNNQ	RV <mark>KR</mark> D <mark>R</mark> AG	LPYLLELL	PPG SE	TH <mark>R</mark> VSR	SVIEEELQVKR	179
Lungfish	133	YNPLTSGNQSACNS	SSTAGTR	AFDESYMK	LEFLNDENTAY	SGPVKG	ATNSTSPVNHT	189
Coelacanth	128	YNTSQHGTSDDSMQ	SDI <mark>RR</mark> SN <mark>R</mark> IIL ▲	SAPDRVPLQV	LDEPAAEHART	ENQMAG	TDINNPALYKG	190
			Î			Î		
			P ₁			P ₁		
0		L	Eurip		 E	rin		
β			Funn		ru	nn T		
Human	129	PELSH	- ANATRNLNF	SIWNHTPLVLI	DERNPHHPMVL	DLFGDN	HNGLTSSS-AS	180
Chimpanzee	174	PELSH	- ANATRNLNF	SIWNHTPLVLI	DERNPHHPMVL	DLFGDN	HNGLTRSS-AS	225
Gorilla	174	PELSH	- ANATRNLNF	SIWNHTPLVLI	DERNPHHPMVL	DLFGDN	HNGLTSSS-AS	225
Rhesus	129	PELSH	- ANATRTLNS	SIWNHTPLVLI	DERNPHHPMVL	DLFGDN	HNGLTNSS-AS	180
Elephant	129	PESSN	- ASATRALNE				YAGSASSS-SA	180
Dog	129	PEHSD	- ANATRILNU		DEQNPHCPVVL	DLFGDI	HNGSASSSPAP	181
Mouse	129	PEASH	- SNTTRTLNF	TIWNHTPLVLI	DERNPDHPVVL	NLFGDS	HN SSNPAP	178
Rat	129	PKSSH	- TNTTSTLNF	TIWNHTPLVLI	DERNPDHPVVL	NLFGDS	HN SSNPAP	178
Rabbit	129	PESSQ	- ANATAAMNLS	SMWNYTPLVLI	DERDPHHPVVL	DLFAND	PTGSASSSPGP	181
Orca	129	PELSH	- VNATRALNL	TIWNHVPLVFI	DEQNPRHPVVL	DLFEDN	YNGSASSTPAP	181
lasmanian	129		HANYIENLNL SSNNTONINI			DLFAFG	HNNSKALSPSP	183
Chick	125		N SN A T EG L D L I		DEQDKDNPVIV	ELFETN	QSAAGN - QTAA	195
Flycatcher	129	PGN S	SDNVSPPLDL	ELWNQIPLVLI	DEHDKDNPVIL	DIFESN	Q N STA	175
Alligator	129	CGKQDPFSPLAVNS	SENDSQALDLO	RLWNQLPLVLI	DESHPDQ - PII	DIFTTN	RTGAGTQHNSS	190
Turtle	129	SKNRDATSALPLNS	SETPSQTLNL	RLWNQIPLVLI	DESDPERPVII	DLFETD	ESGSGAQPNNS	191
Xenopus	135	SSQNQGNTFTHN - NO	QTRQNVTLDP/	ALWNHIPLVVI	DETDPRNPIIH	NIFDNN	AVYSKNSSIRN	196
Lungtisn	129	KREPHSITP			UEIDADNPIVI	NVLGID	HILKIVVIPIA	190
V	129	KRERHSETF		LILFRIGUACU	TLQKFQFLILV	SFFIC	HILKIVULFLA	105
Y		<u> </u>		1	1	· · -	1	
Human	133	ESRKRREAES	MNSVSEGKQP	RFSHRIPLLIF	DQDEKGKARDF	FTG R	KRKVGGSIIHK	189
Chimpanzee	133		NSSISEGKOP	RESHRIPLLIF		FIG R	KRKVGGSTIHK	189
Rhesus	174	ESRKRREAES	NSSVLEGMQP	RFSHRIPLLIF	DQYEKGKARDF	FTG R	KRKVDGRIIHK	230
Elephant	131	FSEIKS <mark>RKRR</mark> EAES	MNSSWEGMRP	KFINSVPLLFF	DEDETSKVARD	FLTG - R	KRR <mark>VSGTIVHK</mark>	192
Bovine	133	EITS <mark>RKRR</mark> EAQS ¹	NSSVRKGTDP	KFLNLAPLMAF	EKGDTGKARDF	FTG R	KRK <mark>VNARIIHK</mark>	191
Dog	134	N TSRKRREAES	WSSAWEGTRP	KFLRLVPLMVF	SQDETSQARDF	LTG R	KRKFSGRIIHK	191
Mouse	134			RELNLIPLLVE		FIG R	KRKISGKIIHK	194
Rabbit	133		NSPAWEGVRP	KFLNLVPLLIF	NRDEKGKARDF	LSLG-R	KRKISGNIIHK	192
Orca	133	EITS <mark>RKRR</mark> EAES	NSSARKGTGS	KFLNLIPLLAF	EKGETSKARDF	RTG R	KRK VSGRIVHT	191
Tasmanian	131	L SN I KS <mark>RKRR</mark> EAD P	PQSSEGDSHS	KFLNILPLVVF	EPDDTTKEATD	LLTG - R	KRK <mark>VSGTIVHK</mark>	192
Platypus	134	LNEI KN <mark>RKRR</mark> QAD V	PNSSREDTSP	KFLNILPLLSF	KPNETGKKATE	FITG - <mark>R</mark>	KRKISANIIHK	195
Chick	132	FSEGKTKVRRAAGD	MNGT ESI	FFRHVPLLRF	ENSFR-AATDL	RSG R	KRKVEGSVEHK	187
Alligator	131	FSEGKPKVRRDAFD	RNAT) Y L E K V PI I R F	EDESR-TAIDL	н 5G Q Н SG Н	KRKLESNIEHK	198
Turtle	144	FSEGKSKVRRDAAD	MNSTGRNMQSI	KLLEKIPLLKF	DDLFKKTATEI	L SG H	KRKIEGSAFHQ	204
Xenopus	132	FTEPLIRSKRDV	GVNVENSTED	IFLKQIPLYRL	ESVKGSQLVVS	DLKT	KKRTRMSAKVI	190
Lungfish	136	SN <mark>RK</mark> L <mark>R</mark> SVLLN	EAPEEDSGVA	KLLQDMPLMKF	EVIKEDHVIVS	ELSSN <mark>R</mark>	QY <mark>R</mark> INNTFITR	195
Coelacanth	139	NESHVL <mark>R</mark> STET	SLNSESDKEVI	FSRSLPLLKI	EEMEQNYTIVS	DVFSDV	KQ RVNAPLMRK	198
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	cASIC1 homologs:	A413 E417	Q421		TM2		
		• •		_			
α		10	20	030 0	Sele.	50	60 70
Human	521 VAKVNIE	FKFINYKTN	IS <mark>ESP</mark> SVTA			-	I VIMELMIL RRERS
Chimpanzee	544 VAKVNIF	FKELNYKTN	ISESPSVTN	IVTLLSNLG SQW	LWFGSSVLSV	EMAELIFDLI	LVITFLMLLRRFRS
Gorilla	501 V <mark>AK</mark> V <mark>N</mark> IF	F K E L N Y K T N	IS <mark>ESP</mark> SVTN	IVT <mark>LLS</mark> N <mark>LG</mark> S <mark>QWS</mark>	<mark>SLWFGSSVLSV</mark> V	<mark>EMAELIFD</mark> LI	LVITFLMLLRRF
Rhesus	578 V <mark>AK</mark> V <mark>N</mark> IF	<mark>F</mark> K <mark>E</mark> LN <mark>YK</mark> TN	IS <mark>ESP</mark> SVTN	IVT <mark>LLS</mark> N <mark>LG</mark> S <mark>QW</mark>	<mark>SLWFGSSVLSV</mark> V	EMAEL IF <mark>d</mark> LI	LVITFLLLLRRFRS
Elephant	502 V <mark>ak</mark> l <mark>n</mark> if	F K E L N Y K T N	IS <mark>ESP</mark> SVTN	IVT <mark>LLS</mark> N <mark>LG</mark> S <mark>QWS</mark>	<mark>SLWFGSSVLSV</mark> V	 <td>LVITFLMLLRRFRS</td>	LVITFLMLLRRFRS
Bovine	502 V <mark>AK</mark> LNIF	<mark>F K</mark> ELN Y KSN	IS <mark>ESP</mark> SVTN	NVT <mark>LLS</mark> NLGSQW	<mark>SLWFGSSVLSV</mark> V	 EMAELIIDLI	LVITFLMLLRRFRS
Dog	542 VAKLNIF	F K E L K Y K T N	I S <mark>ESP</mark> SVTN	IVT <mark>LLSNLG</mark> SQW	<mark>SLWFGSSVLSV</mark> V	EMAELIFOLI	LVITFLMLLRRFRS
Mouse	548 VAKLNIF	FKELNYKTN	ISESPSVTN	IVSLLSNLGSQW	SLWFGSSVLSV	EMAELIFDLI	LVITLIMLLHRFRS
Rat	548 VAKLNIF	FKELNYKIN	ISESPSVIN	IVSLLSNLGSQW	SLWFGSSVLSV		
Orca	498 VAKLNIY	FRELNYKAN	ISESPSVIN				SVIIFLMLLRRFRS
Tasmanian	496 VAKINIE	FRELNYKTN	ISESPSVIN				
Platypus	464 VAKLNIY	FKELNYKTI	SESPSVIN	IVTLLSNLG SQW			FVITFLLLLRRLHR
Chick	507 VAKVNIF	FEEWNYKTN	IGESPAFT	VTLLSQLGNQW	LWFGSSVLSV		TVITFILAFRWFRS
Flycatcher	494 V <mark>AK</mark> VNIF	FEEWNYKTN	IG <mark>ETP</mark> AFP∖	/VT <mark>LLSQLGNQW</mark>	<mark>SLWFGSSVLSV</mark> M	I <mark>ELAELVLD</mark> F	IAITIILAFRWFRT
Alligator	510 V <mark>AK</mark> L <mark>N</mark> IF	FE <mark>E</mark> WK <mark>YK</mark> TN	IG <mark>ESP</mark> AFT∖	/VT <mark>LLS</mark> Q <mark>LG</mark> NQW	<mark>SLWFGSSVLSV</mark> A	ELA <mark>EL</mark> IL <mark>D</mark> F	VVITCILSFRWLRA
Turtle	512 V <mark>AK</mark> V <mark>N</mark> IF	FE <mark>E</mark> WK <mark>YK</mark> TN	IG <mark>ESP</mark> AFT∖	/VT <mark>LLS</mark> Q <mark>LG</mark> NQW	<mark>SLWFGSSVLSV</mark> V	^{elael} ildf	IAITIILSFKRFRS
Xenopus	479 I <mark>AK</mark> L <mark>N</mark> IY	FE <mark>E</mark> LN <mark>YK</mark> TI	L <mark>ESP</mark> TINN	MAM <mark>LLS</mark> L <mark>LG</mark> SQW	<mark>SLWFGSSVLSV</mark> V	′ <mark>E</mark> ML <mark>EL</mark> VI <mark>D</mark> F`	VIIGVMILLHRYYY
Lungfish	490 I <mark>AK</mark> L <mark>N</mark> VY	FYELN <mark>Y</mark> QTM	1G <mark>ESP</mark> SFT\	/VT <mark>LLS</mark> N <mark>MG</mark> SQW	<mark>SLWFGSSVLSV</mark> V	EMGELVFDL	IAVGVIVLRRRRRE
Coelacanth	493 VS <mark>K</mark> LTVY	F <mark>q</mark> Elhh <mark>k</mark> tv	/G <mark>ESP</mark> SINA	AAT <mark>LLS</mark> N <mark>LG</mark> SQW	SFWFGSSVLSV	EMVELLIDF	FVLSTILLFRHYCI
•					TM2		
β							
Human	492 IV <mark>KLNI</mark> Y	FQEF <mark>NYR</mark> T I	E <mark>ES</mark> A <mark>A</mark> NN I	VWLLS <mark>NLGGQF</mark> C	FWMGGSVLCLI	EFGELLID F	V <mark>wit</mark> i <mark>i</mark> klvalaks
Chimpanzee	502 IV <mark>KLNIY</mark>	<mark>FQE</mark> F <mark>NYR</mark> T I	E <mark>ES</mark> A <mark>A</mark> NN I	VWLLSNLGGQF0	FWMGGSVLCLI	EFGELLID F	V <mark>WIT</mark> I <mark>I</mark> KLVALAKS
Gorilla	537 IV <mark>KLNI</mark> Y	FQE FNYRT I	E <mark>ES</mark> A <mark>A</mark> NN I	VWLLSNLGGQF0	FWMGGSVLCLI	EFGEI I I D F Y	V <mark>WIT</mark> I <mark>I</mark> KLVALAKS
Rhesus	492 IVKLNIY	FQEFNYRTI	EESAANNL	. VWLLSNLGGQFC	FWMGGSVLCLI	EFGEIIIDF	WITIIKL VALAKS
Elephant	493 VVKLNIY	FQEFNYRTI	QESPANNI	VWLLSSLGGQFC	FWMGGSVLCLI	EFGEIIIDF	WITIKLVAFSKS
Bovine	493 I VKLNIY	FQEFNYRII	EESAANNI	VWLLSNLGGQFC	FWMGGSVLCLI	EFGELLIDE	WITITKLVALAKS
Dog					FWMGGSVLCLI	EFGELLIDE	WITIIKI VASCKG
Rat		FOFFNYRTI	EESPANNI		EWMGGSVLCL	EFGELLIDE	
Rabbit	493 VVKLNIY	FQEFNYRTI	EESAANNI	VWLLSNLGGQFC	FWMGGSVLCLI	EFAELLIDE	WITIKLVALAKG
Orca	493 VIKLNIY	FQEYNYRTI	EESAANNI	VWLLSNLGGQF	FWMGGSVLCLI	EFAELLID	WITIKLVALAKS
Tasmanian	495 IV <mark>KLNI</mark> Y	FQE F <mark>N F R</mark> T F	A <mark>ES</mark> A <mark>A</mark> NN V	VWLLS <mark>SLGGQFO</mark>	FWMGGSVLCII	EFGEIIID C	W <mark>WIT</mark> I <mark>I</mark> KLVAWVKG
Platypus	497 IV <mark>KLNI</mark> F	<mark>FQE</mark> F <mark>NYR</mark> T I	A <mark>ES</mark> A <mark>A</mark> HN I	VWLLS <mark>NLGGQF</mark>	FWMGGSVLCII	EFGEIIID F	I <mark>WIT</mark> I <mark>I</mark> KLVAWGKG
Chick	513 I I <mark>KLN</mark> I <mark>Y</mark>	FQEY <mark>NYR</mark> T I	S <mark>es</mark> a <mark>a</mark> tti	VWLLS <mark>SLGGQF</mark> C	FWMGGSVLCLI	<mark>EFGELLID</mark> SI	L <mark>WIT</mark> V <mark>I</mark> NIISWCKG
Flycatcher	496 II <mark>KLNI</mark> Y	FQEY <mark>NYRT I</mark>	S <mark>es</mark> a <mark>a</mark> tti	VWLLSSLGGQF0	FWMGGSVLCLI	EFGEI I I D SI	L <mark>WIT</mark> VIHLISWAKG
Alligator	507 IIKLNIY	FQEYNYRTI	SESAATTI	VWLLSSLGGQFC	FWMGGSVLCLI	EFGEIIIDFI	
Turtle	511 KLN Y	FQEYNYRTI	SESAATTI	VWLLSSLGGQFC	FWMGGSVLCLI	EFGEIIIDFI	LWITVIKISNWG KG
Xenopus	512 I VRLNI Y	FQEFNYRST	SESEATING	WILLSNLGGQFC	FWMGGSVLCII	EFGETTIDC	WWITIERFLAWSRN
Coelacanth		FREFNIRVI	SESAATDI		FWWGGSVLCII	EFGEVFIDC	
Coelacantin			S <mark>LOAA</mark> IDI				
Ŷ							
Human	501 <mark>LA</mark> KLLIF	YK <mark>DLNQR</mark> SI	M <mark>esp</mark> ansi	EM <mark>LLSN</mark> F <mark>GGQLC</mark>	<mark>ELWMISCS</mark> V <mark>VC</mark> VI	EI I EVFFIDI	FFS <mark>I</mark> IA <mark>R</mark> RQWQKAK
Chimpanzee	437 <mark>LA</mark> KLLIF	YK <mark>DLNQR</mark> SI	M <mark>esp</mark> ansi	EM <mark>LLSN</mark> F <mark>GGQLC</mark>	<mark>ELWMSCS</mark> V <mark>VC</mark> VI	EI I EVFFIDI	FFS <mark>I</mark> IA <mark>R</mark> RQWQKAK
Gorilla	503 <mark>LA</mark> KLLIF	Y K <mark>D L N Q R</mark> S I	M <mark>esp</mark> ansi	EM <mark>LLSN</mark> F <mark>GGQLC</mark>	<mark>ELWMSCS</mark> V <mark>VC</mark> VI	EI I EVFFIDI	FFS <mark>I</mark> IA <mark>R</mark> RQWQKAK
Rhesus	542 LAKLLIF	YKDLNQRSI	MESPANSI	EMLLSNFGGQLC	LWMSCS VVCVI	EIIEVFFIDI	FFSIIARRQWQKAK
Elephant	505 LAKLLIY	YKDLNHRSV	METPONTI			EIFEVEFID	SLSTITERQWQKAK
Bovine	504 LAKLLIF		MESDANSI			ETTEVETID:	
Mouse			MESPANSI				EESTIARROWOKAK
Rat		YKDINORSI	MESPANSI			ELLEVEELD	FESTIARROWHKAK
Rabbit	505 WAKLLIF	YKDLNQRSI	MESPANSI	EMLLSNFGGQLC		EILEVEFID	SLSIVTRRQWQKAK
Orca	503 LAKLLIF	YKDLNQRSI	VESPANSI	EMLLSNIGGQLC	LWMSCSIVCII	EIIEVFFID	SL S <mark>I</mark> VA <mark>R</mark> HQWHKAK
Tasmanian	507 LAKLLIF	YKDLNQRSI	IESPANSI	EILLSNFGGQLC	LWMSCSVVCVL	EIIEVFFVD	SLS <mark>I</mark> IT <mark>R</mark> RCWQKIK
Platypus	509 <mark>LANL</mark> MIY	Y K <mark>D L N</mark> Q <mark>R</mark> S I	M <mark>esp</mark> anri	EN <mark>LLSN</mark> F <mark>GGQLC</mark>	<mark>LWMSCS</mark> V <mark>VC</mark> VI	EI I EVF FIDI	FFS <mark>I</mark> IA <mark>R</mark> RRWQNAE
Chick	501 <mark>LANL</mark> MVF	Y K <mark>D L N</mark> E <mark>R</mark> F I	S <mark>EN P</mark> A <mark>N</mark> T L	. V I <mark>L L SN</mark> F <mark>GGQ L C</mark>	<mark>ELWMSCS</mark> V <mark>VC</mark> VI	EIIEVFFID:	SFS <mark>I</mark> VM <mark>R</mark> RQWQKAK
Flycatcher	473 <mark>LANL</mark> MVF	Y K <mark>D L N</mark> E <mark>R</mark> F I	S <mark>EN P</mark> A <mark>N</mark> T L	. V I <mark>L L SN</mark> F <mark>GGQ L C</mark>	<mark>LWMSCS</mark> V <mark>VC</mark> VI	EIVEVFLID:	SLS <mark>I</mark> VL <mark>R</mark> RQWQRAK
Alligator	499 LANLMVF	Y KD L N K R F I	SEN PANSI	VILLSNFGGQLC	<mark>EL WMSCS</mark> V <mark>V C</mark> V I	EIVEVEFID	SL S <mark>I</mark> I M <mark>R</mark> RRWQ KT K
Turtle	518 LANLVVF	KDLNERFI	SENPANNL	VILLSNFGGQLC		ELIEVEFID	SF ST VMR RRWQ KMK
Xenopus	504 LANLNIF	VODINI DO	SESPIYNI	AT LL SN F GGQLC		ENVEYELVE	SFWVVLRQRWR
Coelacanth	511 LVNLGIE		SESPANSI				
ooolaoantii		10	20	30	40	50	60 70
							10

Fig. 12. Comparison of α , β , and γ sequences in the pre-TM2 and TM2 segment from twenty species. For each subunit, residues that are identical in at least 19 out 20 species (95% identity) are shaded. The location of the TM2 based on homology to ASIC1 is shown above the sequences. In the preTM2 region, only three charged residues are conserved in all three subunits. The positions of these charged residues are marked at the top of the alignments by the corresponding cASIC1 homologs, Ala413, Glu417 and Gln421. Column headers: Deg: degenerin or "Deg" residue. Ami: amiloride binding residues. Sel.: selectivity filter.

11.3.5. Palm domain

A stretch of 15 residues prior to TM2 form the two β -strands (β 11 and β 12) that are a central component of the palm domain (Figs. 6 and 7). This region is highly conserved in all three subunits and includes three charged residues that are conserved in all three subunits in all 20 species examined (Fig. 12). These residues are homologous to A413, E417 and Q421 in cASIC1 (Fig. 12). In cASIC1, the R-group of E417 protrudes into the central vestibule (Fig. 13) and has been implicated in proton binding and functional conformational changes in ASIC (Ishikita, 2011). In all three ENaC subunits, the homologous residues are positively charged Lys or Arg (K534, R505 and R514 in human α , β and γ subunits respectively) in all 20 species (Fig. 12). An R514A mutant in human γ subunit significantly leads to a decrease in sodium feedback inhibition consistent with an increase in channel open probability (Edelheit et al., 2014). The other two residues (A413 and Q421) are located at the interface between subunits (Fig. 13) (Jasti et al., 2007). Because of the strict conservation of homologous residues in ENaC subunits, these residues are probably located at symmetrical sites in ENaC subunits as they are in ASIC1 (Fig. 13). Site-directed mutagenesis studies using human ENaC subunits showed that mutation of these residues to alanine also leads to a decrease in sodium feedback inhibition that controls channel open probability (Po) (Edelheit et al., 2014, 2011). A human γ subunit variant in the palm domain (L5110) is associated with an increase in channel open probability (Chen et al., 2013).

11.4. TM2

Prior to the report on ASIC1 structure, the location of the TM2 in ENaC subunits was predicted by various software based on hydrophobicity of this region (Canessa et al., 1994a; Saxena et al., 1998). After the publication of the ASIC1 structure in 2007, the helical TM2 segment of ASIC1 has been generally adopted as the location of TM2 in ENaC subunits as well, based on the strong sequence homology in this region and subsequent empirical studies (Kashlan and Kleyman, 2011). While there is overlap between earlier predictions and ASIC1 structure based segment, a segment identified as "pre-M2" (i.e. before the TM2) in earlier work (Kellenberger et al., 1999; Schild et al., 1997), resides within TM2. The terms used in earlier studies should be examined to avoid confusion about regions studied.

Fig. 12 presents alignments of α , β and γ subunits in the TM2 segments in 20 species where the TM2 was marked based on homology with ASIC1. These alignments show that TM2 is highly conserved in all subunits.

A number of key functional sites are within the TM2 region of ENAC subunits, including the channel gate, amiloride binding site and selectivity filter. Fig. 10 illustrates how these sites are conserved through evolution. Functional studies as well as resolved ASIC1 structures suggest that the channel gate is within the outer part of the second membrane spanning domain, within the region encompassing an LLSN motif that is conserved among ENAC subunits across species (Fig. 12). The Ser in this motif is a site where the introduction of large residues has been found to dramatically increase channel open probability (Kellenberger et al., 2002; Sheng et al., 2001a; Snyder et al., 1999). This site has been referred to as the degenerin or "Deg" site, as the introduction of large residues in mechanosensitive ENAC/Degenerin family member in *C. elegans* results in neurodegeneration, in association with an increase in channel open probability (Goodman et al., 2002; Sherwood et al., 2012).

One of the characteristics of $\alpha\beta\gamma$ ENaC is its inhibition by relatively low concentrations of amiloride (Kleyman and Cragoe, 1988). Other family members, including $\delta\beta\gamma$ and ASICs, are inhibited by amiloride or its derivatives at higher concentrations of these drugs (Table 13) (Diochot et al., 2007; Ji et al., 2012). An amiloride binding site has been described at a site in the second membrane-spanning domain, consisting of a Ser in the α subunit, and a Gly in the beta and gamma subunits (Fig. 12). The introduction of specific mutations at these sites led to a profound loss of the efficacy of amiloride (Kashlan et al., 2005; Schild et al., 1997).

Another defining characteristic of ENaC is its cation selectivity. With regard to its ability to discriminate Na⁺ and K⁺, ENaC is the most Na selective mammalian ion channel (Table 13). A three-residue selectivity filter, consisting of a G/S–X–S motif, is present in the TM2 of ENaC subunits (Fig. 12). The introduction of specific mutations in the first or third residue of this motif resulted in channels that allow for modest K⁺ permeation (Kellenberger et al., 1999; Sheng et al., 2000; Snyder et al., 1999).

At the distal end of TM2 there are three charged residues (within the stretch of EMAELVFD in human α subunit) that are conserved in all 20 species examined (Fig. 12). Mutation of these acidic residues reduced channel conductance (Langloh et al., 2000; Sheridan et al., 2005) and also affect ion selectivity (Sheng et al., 2001b).

11.5. Carboxy terminus

In general, the cytoplasmic C-terminus contains sites of interaction with other proteins, signal transduction molecules, and ions that regulate ENaC function.

11.5.1. Motifs involved in signal transduction

In accordance with the "positive inside" rule noted above (in Section 11.2), the region after TM2 is enriched in positively charged Arg and Lys in all three subunits in all 20 species examined (Fig. 12). The proximity of these residues to the cytoplasmic side of the membrane allows interactions of these residues with polar heads of membrane phospholipids and phosphoinositides concentrated at the cytosolic surface of membranes (Di Paolo and De Camilli, 2006). This region in the β and γ subunits has a role in the binding of phosphotidylinositol triphosphate which has an allosteric effect on ENaC open probability (Pochynyuk et al., 2007, 2005). It is noteworthy that positively charged residues at the same analogous region in P2X receptor channels fulfill similar roles (Bernier et al., 2012).

One of the common mechanisms of membrane protein regulation is phosphorylation/dephosphorylation of critical residues by intracellular signal transduction systems. It is likely that the residues in the Cterminus of ENaC subunits may be substrates for such reactions. For example, Volk et al. showed that the activity of ENaC expressed in oocytes could be enhanced by activation of protein kinase C (PKC) by phorbol ester and that this effect was dependent on the presence of an intact C-terminus of α subunit (Volk et al., 2000). The C-terminus also is phosphorylated by the kinases SGK, casein kinase 2, and ERK (Diakov and Korbmacher, 2004; Shi et al., 2002; Yang et al., 2006).

An important regulator of ENaC activity is chloride ions. Truncation of the C-terminus of the β but not of α and γ subunits reduced cytoplasmic Cl⁻ inhibition of ENaC, suggesting that this segment is essential for down-regulation of ENaC by CFTR (Bachhuber et al., 2005; Ji et al., 2000).

11.5.2. Interaction with cytoskeletal elements

Cytoskeletal elements such as microtubules, actin filaments, and associated proteins form an essential part of the complex network of proteins involved in intracellular transport of proteins including endocytosis (Anitei and Hoflack, 2012). As ENaC subunits are transported to the cell membrane via the trans-Golgi network (Butterworth, 2010), cytoplasmic termini of ENaC may interact with cytoskeletal elements during transport and in the cell membrane itself. Indeed, the C-terminus of the α subunit was shown to bind to spectrin that is located in the intracellular side of the membrane. The sequence responsible for binding to the SH3 domain of α -spectrin (PPLALTAPPPA in rat α subunit) (Rotin et al., 1994) starts prior to the PY motif (see below) and partially overlaps with it (Fig. 12). This motif is conserved only in mammals (Fig. 12). In addition to spectrin, there is also evidence for direct

interaction of F-actin with the carboxy terminus of the α subunit (Mazzochi et al., 2006; Sasaki et al., 2014).

11.5.3. PY motif

The most conserved motif in the C-terminus of ENaC subunits is the PY motif (Fig. 14). The consensus sequence for the PY motif is PPPXYXXL that is located 65–70 residues after the end of the TM2 in α , β and γ subunits. Delta ENaC orthologs do not have a conserved PY motif. In the 20 species we examined the PY motif is conserved strictly in nearly all species (Fig. 14). Turtle β subunit sequence has a short C-terminus that may be a genome sequencing error. In contrast to the β and γ subunits, in Sauropsida, Amphibia and fishes the PY motif in these species makes it very unlikely that the lack of the motif is a sequencing error.

The PY motif is recognized by the WW domains in Nedd4–2 that is an E3 ubiquitin-protein ligase (Rotin and Staub, 2011). WW domains are ~40 residue long segments that are characterized by a conserved sequence that includes two tryptophans (W). Such modules are found in many proteins and bind to proline-rich sequences such as PY motif (Macias et al., 2002). With its WW domains, Nedd4–2 catalyzes ligation of ubiquitin to the ENaC subunit leading to the internalization of ENaC and eventual degradation in a proteasome or lysosome (Rotin and Staub, 2011).

Missense mutations in the β or γ subunit PY motif or truncations that lead to a loss of this motif in these subunits, cause Liddle syndrome (see Section 15.2). Mutation or deletion of the PY motif reduces the rate of ENaC ubiquitylation and consequent internalization, leading to accumulation of ENaC in the membrane (Lu et al., 2007). This in turn leads to enhanced absorption of filtered Na⁺ and consequently increases blood volume and blood-pressure (Rotin, 2008).

12. Tissue distribution of ENaC

12.1. Alpha, beta and gamma subunits of ENaC

Most of the studies on tissue localization of ENaC subunits have been carried out by immunohistochemical studies using antibodies generated against small segments of expressed proteins or synthetic peptides that represent short segments of ENaC subunits (Brouard et al., 1999; Coric et al., 2004, 2003; Duc et al., 1994; Hager et al., 2001; Masilamani et al., 1999; Tousson et al., 1989; Wang et al., 2013a). These studies provided evidence for the localization of ENaC in kidney, lung, salivary glands, skin, placenta and the colon. Expression of ENaC subunits has been also examined by in situ hybridization of tissue sections using cDNA probes (Greig et al., 2003). But, this approach does not provide an image of the intracellular localization of the subunits themselves.

To enhance the immunofluorescence signal, we generated polyclonal antibodies against the complete extracellular region of ENaC subunits (Enuka et al., 2012). These antibodies allowed us to visualize ENaC expression in the bronchial epithelia of human lung and the female reproductive tract extending from the uterus to the fallopian tube at a high resolution by immunofluorescence and 3D confocal microscopy (Enuka et al., 2012).

The expression and sites of localization of ENaC in the kidney nephron tubules have been recently extensively reviewed (Rossier, 2014) and the complexities of this subject are beyond the scope of the present review.

Tissue specificity of expression of ENaC subunits has been also investigated by large-scale microarray and high-throughput RNA sequencing experiments. The results of these studies can be accessed via the EMBL-EBI Expression Atlas database of gene expression (Petryszak et al., 2014). Experiments included in the Expression Atlas (https://www.ebi.ac.uk/gxa/home) report many tissues wherein ENaC subunits are expressed at varying levels. Consistent with the



Fig. 13. Location of the cASIC1 E417 and Q421 in ASIC1 structure (PDB ID: 2QTS). The three ribbon structures shown represent the β 12-strand region (from L414 to K423) of all three subunits of chicken ASIC1, termed in order A, B, and C (PDB 2QTS). For each subunit, only two residues, E417, and Q421, are shown in CPK style. In 20 species examined, the residue homologous to E417 is an arginine or lysine (K534 in α , R505 in β and R514 in γ subunit of human ENaC). The space in the center of the figure is part of the vestibule along the three-fold axis of symmetry that is thought to be part of the in pathway.

immunohistochemical studies, these studies report highest levels of expression for the α , β and γ subunits in the kidney, lung, and colon. Other tissues reported include the fallopian tube, esophagus, placenta, prostate, skin, stomach, thyroid, tongue and vagina (Expression Atlas).

Some recent studies have reported immunolocalization of ENaC subunits in astrocytes in the brain (Miller and Loewy, 2013), human eye (Krueger et al., 2012), nasal mucosa (Jiang et al., 2015), mouse ear (Morris et al., 2012), rat muscle (Simon et al., 2010), vascular endothelium (Kusche-Vihrog et al., 2014), vascular smooth muscle cells (Drummond et al., 2008), lymphocytes (Ottaviani et al., 2002) and platelets (Cerecedo et al., 2014). There is also evidence for the expression of ENaC in mammary epithelia (Wang and Schultz, 2014).

The functional significance of ENaC in organs, such as kidney, lung and the respiratory tract, sweat and salivary glands and the reproductive tract where ENaC is expressed at relatively high levels, has been established, based on the facts that mutations in ENaC genes result in major dysfunction in these tissues in multi-system PHA (Chang et al., 1996; Enuka et al., 2012; Hanukoglu, 1991; Hanukoglu et al., 2008). However, even in most severe cases of multi-system PHA there does not appear to be an abnormal function in the eye, ear, muscle or neural tissue function. Thus, the physiological significance of the low-level expression of ENaC in other tissues remains to be established.

Expression of ENaC has also been detected in the lingual epithelium and in taste receptor cells (TRCs) (Chandrashekar et al., 2010; Kretz et al., 1999). Most mammals can sense five basic tastes, sweet, sour, bitter, umami and salt by TRCs specific for each taste. In fungiform taste buds of mice there are amiloride-sensitive TRCs that are responsive specifically to NaCl (Shigemura et al., 2008). In genetically engineered mice lacking the α subunit specifically in TRCs, the neural response to low NaCl (<120 mM) in a subpopulation of TRCs was abolished while response to high NaCl remained intact (Chandrashekar et al., 2010). These studies suggest that ENaC in TRCs plays an essential role in the salt-taste receptor system (Chandrashekar et al., 2010; Oka et al., 2013).

In the mammalian order Cetacea that includes whales and dolphins, taste receptor buds have atrophied and appear in degenerate form (Tinker, 1988). Sequencing of the various cetacean genes for the receptors of the five tastes revealed that the receptor genes for four tastes are non-functional pseudogenes because of accumulation of mutations

Table 13

Functional characteristics of ASIC and ENaC type channels.

^a Source: Gründer and Pusch (2015).

(Zhu et al., 2014). However, in contrast to these, the three ENaC subunits, α , β , and γ , that also serve as salt taste receptor have remained intact and functional. As the authors note, the conservation of ENaC genes in Cetacea is because of the significance of ENaC in osmoregulation and other physiological functions and it is still not known whether Cetacea are capable of sensing salty taste (Zhu et al., 2014).

12.2. Delta subunit of ENaC

The first report on the cloning of the human δ subunit also showed that in northern blots the highest levels of its expression are observed in brain, pancreas, testis and ovary with only low levels in the kidney and lung (Waldmann et al., 1995). Consistent with these initial results, the Expression Atlas database results show that the expression of the delta subunit gene (SCNN1D) is relatively much lower in the kidney and lung but highest in neural tissues, (including cerebral cortex, cerebellum, hippocampus, hypothalamus and pituitary gland) and testis (Expression Atlas, 53 GTEx). Expression of the δ subunit has been also detected in the human nasal epithelium (Bangel-Ruland et al., 2010) and eye (Krueger et al., 2012). Overall, the tissue distribution pattern of the δ subunit is distinctly different from that of α , β and γ subunits.

12.3. ASICs

ASIC subunits are expressed mainly in the central and peripheral nervous systems and the gastrointestinal tract. ASIC tissue distribution has been recently extensively reviewed (Holzer, 2015; Lin et al., 2015; Zha, 2013).

13. Subcellular and cilial localization

As an ion channel on the apical side of the epithelium, ENaC would be expected to be localized on the apical membrane of cells. Indeed, in human polarized epithelial cells in the female reproductive tract and the respiratory airways, immunohistochemical and immunofluorescence studies show that ENaC is localized at the apical surface of the cells (Enuka et al., 2012). Studies on subcellular localization of α , β and γ subunits in mice and rat kidney nephron and in cultured Madin–Darby Canine Kidney (MDCK) epithelial cell line have yielded varying results probably in part because of hormonal and Na⁺ treatment conditions (Ackermann et al., 2010; Bao et al., 2014; Chanoux et al., 2013; Hager et al., 2001).

13.1. ENaC on cilia

A cilium is a finger like protrusion on the cell surface that has a microtubular skeleton called axoneme. The ciliary membrane that covers the axoneme is continuous with the cell membrane (Satir and Christensen, 2007). Most cells in mammals contain a single cilium (primary cilium) that is built upon the microtubular structure of a centriole. In three major organs, the lung, the reproductive tract, and the central nervous system, the epithelial surface of many cells have multiple cilia (200–300 per cell). These cilia are motile and beat in concert at a predetermined direction generating waves that move the fluid, particles and cells in the lumen of the epithelium (Brooks and Wallingford, 2014).

Using antibodies we generated, we showed that in multi-ciliated cells in the human bronchus and the female reproductive tract (extending from the uterus to the fimbria of fallopian tube), ENaC is specifically located in cilia (Enuka et al., 2012). This was also confirmed by co-localization of ENaC immunofluorescence with that of cilia-specific β -tubulin IV (Enuka et al., 2012).

The discovery that ENaCs are highly expressed in multi-ciliated cells in the lung and the reproductive tract and that they are specifically located over the entire length of cilia has increased our understanding of the function of ENaC in epithelia with motile cilia (Enuka et al., 2012). The depth of the fluid that bathes the cilia in the lumen has to be precisely regulated for normal cilial function (Choi et al., 2015; Tilley et al., 2015). Since Na⁺ is the major solute in the ECF, regulation of ENaC activity directly affects osmolarity of the periciliary fluid and consequently the flow and volume of the fluid in the lumen. Cilial location allows ENaC to serve as a sensor and regulator of osmolarity of the periciliary fluid along the entire length of the cilia. Thus, ENaC mediated changes in osmolarity would then modulate the fluid volume on the epithelial surface (Enuka et al., 2012). Since all airway epithelia are Na⁺ absorptive, ENaC plays a key role in pulmonary epithelia, but it should be noted that in the airway epithelium there are additional ion channels and transporters that contribute to the regulation of the composition and volume of the periciliary liquid (Hollenhorst et al., 2011).

The functional importance of ENaC in the multi-ciliated cells of the respiratory airway is illustrated by the contrasting phenotypes of two diseases: 1) In multi-system pseudohypoaldosteronism (see Section 15.1), the loss-of-function of ENaC leads to an increase in the volume of airway surface liquid (ASL) (Kerem et al., 1999). 2) In cystic fibrosis (see Section 15.3), dysfunction of the chloride transporter CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) leads to reduced inhibition of ENaC by chloride ions. Enhanced activity of ENaC contributes to the drastic reduction of the airway surface liquid (ASL) volume observed in cystic fibrosis. Normally, the undulating movement

of the cilia rapidly moves the mucus gel on top of the cilial layer, and together with the mucus, it pushes inhaled particles and microbes in the respiratory airways for clearance via the mouth (Rogers, 2007;

α		10	20	30	40	50	
Human	625	SLSQPGPAPSPALTA	P P P A Y A T L G	G P R P S P G G S A G	ASSSTCPLGGP		669
Chimpanzee	648	SLSQPGPAPSPALTA	ΡΡΡΑΥΑΤΙΟ	G P R P S P G G S T G	AGSSACPLGGP		692
Gorilla							
Ahesus	682	YLSQLGPAPSPALTA	P P P A Y A T L O	G P C P S P G G S G G	ASSTAYPLGGP		726
Elephant	606	SPSQPGPAASPALSA	<mark>ΡΡΡΑΥΑΤΙ</mark> Ο	GPRPSPLGSAE	PSSSACTMGEP		650
Bovine	606	SSSPPDPAISPALSA	P P P A Y A T L O	S P H P A P S G L A E	ASTSAHAPGEP		650
Dog	646	SSSLPGPAISPALTA	P P P A Y A T L O	G P C P S P S S L AG	AHSSAYTLGKP		690
Mouse	654	SLPQQGTTPPLALTA	<mark>ΡΡΡΑΥΑΤΙ</mark> Ο	GPSASPLDSAV	PGSSACAPAMA	L	699
Rat	654	SLPQQGMTPPLALTA	<mark>ΡΡΡΑΥΑΤΙ</mark> Ο	GPSAPPLDSAA	PDCSACALAAL		698
Rabbit	602	SVPQPGPTLPPSLTA	P P P A Y A T L O	SPCL SQ SG SAC.	A P G E P		640
Orca	604	SSSAVGPATSLALSA	PPPAYATLO	GPRLAPSGSTE	ASSSAHTPGEP		648
Tasmanian							
Platypus	560	AQLTRPPLDSSPSTA	P P P A Y A T L O	GPPPPGPCL			591
Chick	613	VVTTLPSYNSLEPCG	P S K D G E <mark>T</mark> G L	. E			637
Flycatcher	600	VVTTLPSYNSLEPRG	P S R D G E V G H	1 E			624
Alligator	616	VVTTLPSYNSLEQCE	SNRDAEMGL	. E			640
Turtle	618	VVTTLPSYNSLEPCR	RDGEAVIGL	. E			642
Xenopus	585	RQISVVADITPPPAY	ESLDLRSVO	G T L S S R S S S M R	SNRSYYEENGG	R R N	632
Lungfish	601	EISPPPAYDTLQLDV	P V A C A P D C E	Ε C T Q H V S H A S VI	HSQAPCSSQPE	QEASEGPTVL	655
Coelacanth	604	DVSAPPAYETLDLDL	P P S A A Q C I T	GCKCVHCASF	ISHEVEDLMSD	L AG D P	653
0							
В							
Human	601	TGPYPSEQALPIPGT	P P P N Y D S L F	RLQPLDVIESD	SEGDAI		640
Chimpanzee	611	TGPYPNEQALPIPGT	P P P N Y D S L F	RLQPLDVIESD	SEGDAI		650
Gorilla	646	TGPYPNEQALPIPGT	P P P N Y D S L F	LQPLDVIESD	SEGDAI		685
Rhesus	601	TGPYPSEOALPIPGT	P P P N Y D S L F	RLOPLDVIESD	SEGDAI		640
Elephant	602	SGAYADGRTLPIPGT	P P P N Y D S L F	RLOPLDVIESD	SEGDAI		641
Bovine	602	VEAYPHEONPPIPGT	P P P N Y D S L F	RLOPLDVIESD	SEGDAI		641
Dog	602	PGTYPDEQTLPIPGT	P P P N Y D S L F	RLOPLDVIESD	SEGDAI		641
Mouse	599	GEVYPDOOTLPIPGT	P P P N Y D S L F	RLOPLDTMESD	SEVEAI		638
Rat	599	AEVYPDQQTLPIPGT	P P P N Y D S L F	RLQPLDTMESD	SEVEAI		638
Rabbit	602	AEAYPDEQALPIPGT	P P P N Y D S L F	RLQPLDVVESD	S E G D A V		641
Orca	602	AGPHPHEQTPPIPGT	P P P N Y D S L F	RLQPLDVIESDI	NEGDAI		641
Tasmanian	607	PEDYPDEQALPIPGT	P P P N Y D S L F	RLQPLDVIESDI	NEGEAI		646
Platypus	606	PVGYPVGPSTPIPGT	P P P N Y D S L F	RMOPLDVIESD	SEAENA		645
Chick	623	E A L P P E P G T	P P P N Y D S L F	RVQPSHNPGTD	SDIECEEQRPA	ANHHGDASVW	671
Flycatcher	612	PAGPGAPALPPEPGT	P P P N Y D S L F	RVQPPDTLSPD	SDTDTE		651
Alligator	622	G R L P P E P G T	P P P N Y D S L F	RVQPLDVLELG	Т Т Т Е Р Е		655
Turtle							
Xenopus	620	VD I PG T	P P P N Y D S L F	RVNTAEPVSSD	E E N		647
Lungfish	613	PPDLYLPTTLEIPGT	<mark>Р Р Р К </mark> У D S L Р	RVHPIDTEHHSI	D S E D L		651
Coelacanth	611	CNEAYIPPREPTPGT	P P P N Y D S L F	RVQPVENTEQI	S D S E E N		650
V							
Y							
Human	608	ALHLPPALGTQVPGT	PPPKYNTLF	RLERAFSNQLTI	DTQMLDEL		649
Chimpanzee	544	ALHLPPALGTQVPGT	P P P K Y N T L F	RLERAFSNQLTI	DTQMLDEL		585
Gorilla	610	ALHLPPALGTQVPGT	PPPKYNTLF	RLERAFSNQLTI	DTQMLDEL		651
Rhesus	649	ALHLPPALGTQVPGT	PPPKYNTLF	RLERAFSNQLTI	DTQILDEL		690
Elephant	612	ALRLPPAVGTQVPGT	PP-RYNTLF	RLERAFS QLTI	DTQVPDES		652
Bovine	611	ALSLPPAPGSQVPGT	PPPRYNTLE	RLERAFSSQLTI	DIQIIFPH		652
Dog	605	ALRLPPAPGIQVPGI	PPPRYNTLE	RLERAFSDQLTI	DIQEPAES		646
Mouse	614	AMRLPPAPEAPVPGT	PPPRYNTLE	RLDSAFSSQLTI	DIQLINEF		655
Rat	609	AMRLPPAPGSTVPGT	PPPRYNTLE	RLDRAFSSQLII	DIQLINEL		650
Rabbit	612	ALSLPPAPGAQVPGT	PPPRYNTLF	RLERTFSQQLAI	DTRLPDEP		653
Orca	610	ALCLPPAPAAQVPGT	PPPRYNTLF	RLERAFSRQLTO	GTETPYPHQWP	EPHQQASHGD	664
Tasmanian	617	ALQLPQAPGAHVPGT	PPPKYNTLF	KIERIFSNQLEI	DIQUSENV		658
Platypus	618	ALRLPQPQGTHVPGT	PPPRYNTLF	RLERAFATQLEI	DTQDVGKL		659
Chick	611	ALRLPLPQEGHPPRT	PPPNYSTLF	RLETAFTEQLPI	DILEAGQH		652
Hycatcher	582	ALKLPLPQDSPLPRT	PPPNYSTLF	KLEI AFSEQLPI	DILELGQH		623
Alligator	609	ALRLPLPQENHLPRT	PPPNYSTLF	KLDIIFTEQL PI	DILEIESR		650
Turtle	628	ALRLPLPQENHMPRT	PPPNYSTLC	2LNAAFTDQL PI	DILEGRSH		669
xenopus	618	ALQLPQSQDSHVPRT	PPPKYNTLF	RIQSAFQLETI	DSDEDVERL		660
Lungfish	615	AMQLPCVQTGPVPST	PPPQYNALF	RIQSVEDEQVSI	DIEVN		653
Coelacanth	621	AVHLPHSESCPVPKT	PPPIYDAL F	κιψιαγαέζιδι	DIEDNEY		661

Fig. 14. Conservation of the PY motif in the C-termini of α , β and γ ENaC subunits from 20 species.

Tilley et al., 2015). When ASL volume is reduced, clearance of foreign particles, dust, microbes and viruses is impaired contributing to the chronic infections characteristic of cystic fibrosis.

In epithelia with motile-cilia, the interaction between ENAC and CFTR in the respiratory airways has been extensively studied because of the involvement of these channels in cystic fibrosis (Althaus, 2013). There are conflicting views regarding the mechanism of CFTR and ENAC interactions (Nagel et al., 2005). Some studies suggested that CFTR interacts directly with ENAC (Berdiev et al., 2009). But, while ENAC is located on the cilial surface, CFTR is located on the apical membrane outside of cilial borders (Enuka et al., 2012). Thus, the mechanism of CFTR action in multi-ciliated cells cannot be via direct interaction with ENAC. There is evidence that chloride ions inhibit ENAC (Bachhuber et al., 2005). Thus, CFTR may regulate ENAC activity via its modulation of Cl⁻ levels.

14. Functional differences between ENaC and ASIC

As noted above, ENaCs and ASICs are the only families that are expressed in vertebrates. While ENaCs and ASICs share similar structures, there are major differences in their functional characteristics as observed in heterologous expression systems as well as differences in the physiologic roles in vertebrates (Gründer and Pusch, 2015; Kellenberger and Schild, 2015). The major differences are outlined in Table 13.

ENaCs are constitutively active and facilitate the bulk transport of Na⁺ across high resistance epithelia in many organs (see Section 12). Transport of sodium is accompanied by a flow of fluid as a result of osmolarity changes. The physiological consequences of these effects depend on the tissue where ENaC is expressed.

ENaC in the distal nephron has an important role in regulating extracellular fluid volume and renal K⁺ secretion (Rossier et al., 2002). In the respiratory airway and alveoli, ENaC has a major role in regulating the volume of the airway and alveolar fluids (Chambers et al., 2007; Eaton et al., 2009). In the female reproductive tract, ENaC modulates uterine fluid absorption during the reproductive cycles (Ruan et al., 2014; Salleh et al., 2005). Fertilization of the oocyte in the oviduct and fallopian tube requires transport of the oocyte to the ampulla region of the tube. This process is dependent on the ciliary beating along the oviduct in addition to smooth muscle contractions (Cov et al., 2012). Similarly, the transport of embryo to the uterus is also dependent on cilial motion (Lyons et al., 2006). As ENaC is richly expressed on cilia in multi-ciliated cells in the oviduct (see Section 13) and in the uterine glands in the endometrium, reproductive processes of ovum and embryo transport and implantation are dependent on ENaC function (Enuka et al., 2012). In the tongue, ENaC has been identified as the salt taste "receptor" (Chandrashekar et al., 2010; Oka et al., 2013) (see Section 12).

In contrast to ENaC, ASICs are H⁺-gated ion channels that are closed in the resting state, and rapidly desensitize following activation. They are expressed in mammalian central and peripheral nervous systems and have roles in nociception, mechanosensation, fear-related behavior and seizure termination (Chen et al., 1998; Deval and Lingueglia, 2015; Price et al., 2001, 2000; Waldmann and Lazdunski, 1998; Ziemann et al., 2009, 2008). ASICs also play an important role in synaptic function regulating neural plasticity in pathological conditions (Zha, 2013). ASICs are also expressed in sensory neurons in the gastrointestinal tract, and play a role in acid-sensing within the gastrointestinal tract and may be involved in human sour taste sensing (Holzer, 2015).

15. Diseases associated with ENaC mutations

In this section, we shall briefly describe hereditary diseases that have been associated with mutations in the genes coding for ENaC subunits. The major characteristics of these diseases are summarized in Table 14.

15.1. Multi-system pseudohypoaldosteronism type 1 (PHA1B)

Type I pseudohypoaldosteronism (PHA) is a syndrome of unresponsiveness (also called resistance) to mineralocorticoid hormone aldosterone. This disease was first described by Cheek and Perry as an aldosterone unresponsiveness syndrome resulting in salt wasting in a child (Cheek and Perry, 1958). Subsequently over 50 studies reported many cases of PHA with characteristics of aldosterone resistance with varying degrees of salt wasting. In 1991, Hanukoglu established that PHA includes two independent syndromes (called renal and multisystem forms of PHA) that differ in their pathogenesis, mode of inheritance, the involvement of aldosterone target organs and the severity of salt wasting (Hanukoglu, 1991). Later studies confirmed this distinction and these two forms have been assigned two separate entries in the OMIM database (http://omim.org/): 1) renal form (PHA1A): OMIM #177735, inherited as an autosomal-dominant disease; and 2) multisystem form (PHA1B): OMIM #264350, inherited as an autosomalrecessive disease.

As commonly observed for other steroid hormone resistance diseases, initially the cause of PHA was suspected to be a mutation(s) in the mineralocorticoid receptor (MR) gene (Armanini et al., 1985). However, analysis of the linkage between PHA and polymorphisms adjacent to the mineralocorticoid receptor gene on chromosome 4 in 10 consanguineous families excluded mutations in the MR gene in multisystem PHA patients (Chung et al., 1995). By further homozygosity mapping, multi-system PHA locus was mapped to two regions coding for SCNN1A and SCNN1B and SCNN1G in chromosomes 12p and 16p respectively (Strautnieks et al., 1996). Indeed, sequencing of the genes coding for the α , β and γ subunits revealed that the multi-system form results from mutations in these three genes (Chang et al., 1996). Two years later Geller et al. reported that the milder renal form (PHA1A) is caused by mutations in the mineralocorticoid receptor gene (Geller et al., 1998).

15.1.1. Clinical presentation

Patients affected by multi-system PHA lose salt from all aldosteroneresponsive target organs expressing ENaC including kidney, sweat and salivary glands and respiratory tract (Hanukoglu, 1991). This may necessitate frequent hospitalizations especially during infancy and childhood for severe hyponatremia, hyperkalemia, acidosis and dehydration (Edelheit et al., 2005; Hanukoglu, 1991). These patients also exhibit recurrent pulmonary symptoms such as congestion, wheezing, recurrent lower respiratory tract infections and chronic rhinorrhea due to impaired lung fluid absorption causing excessive airway surface liquid (Hanukoglu et al., 1994; Kerem et al., 1999; Schaedel et al., 1999). They may also fail to conceive due to impaired ENaC expression in cilia lining the fallopian tube and the endometrial mucosa (Enuka et al., 2012). This form carries a high mortality risk especially in infancy, although mortality has been observed even in older patients (Hanukoglu, 1991; Porter et al., 2003; Saxena et al., 2002). Disease manifestations are life long, yet the severity and frequency of salt wasting episodes improve with age (Adachi et al., 2010; Hanukoglu and Hanukoglu, 2010; Hanukoglu et al., 2008).

PHA1B patients require high amounts of sodium chloride (up to 45 g/day) life long, to prevent recurrent salt wasting episodes from multiple organs (Hanukoglu and Hanukoglu, 2010; Hanukoglu et al., 2008; Hogg et al., 1991). The severity of PHA manifestations improves with age, depending on the nature of the mutations, environmental factors such as ambient temperatures and degree of compliance with the therapy (Adachi et al., 2010; Hanukoglu et al., 2008).

15.1.2. Genotype-phenotype relationships

Multi-system PHA patients characterized to date carry homozygous or compound heterozygous mutations in the genes coding for the α , β and γ subunits (Belot et al., 2008; Bonny et al., 2002; Chang et al., 1996; Edelheit et al., 2005; Kellenberger et al., 1999; Saxena et al.,

Table 14

Characteristics of hereditary disorders that result from mutations or polymorphisms in ENaC subunits.

	Multisystem PHA	Liddle syndrome	Cystic fibrosis-like disease
Inheritance	Autosomal recessive	Autosomal dominant	Polygenetic mechanism. ENaC association in some cases
Mutations in	α,β and γ subunits	PY motif at the C-terminus of β and γ subunits	Heterozygous variants in α , β or γ subunit genes. ENAC/CFTR genotype (see Table 15).
ENaC activity Water and electrolyte metabolism	Loss of function Hypovolemia (dehydration), hyponatremia, hyperkalemia	Gain of function Volume expansion, hypokalemia (usually)	Increased or decreased activity Rarely dehydration
Acid-base balance	Metabolic acidosis during recurrent salt wasting episodes	Metabolic alkalosis	Rarely metabolic alkalosis
Blood pressure Renin-aldosterone system	Hypotension during recurrent salt wasting episodes Hyperreninemia, hyperaldosteronism	Hypertension Hyporeninemia, normal-high aldosterone	Mostly normal Mostly normal aldosterone and PRA
End organs involved	Kidneys, sweat and salivary glands, respiratory tract, reproductive system, colon	Kidney	Respiratory tract, sweat glands. Less frequent: pancreatic and gastrointestinal tract.
Kidneys Respiratory tract	Impaired Na ⁺ reabsorption resulting in severe salt loss Recurrent lower pulmonary tract infections, chronic rhinitis. No chronic lung disease	Increased Na ⁺ and fluid reabsorption Normal	Normal renal function Chronic, moderate to severe bronchitis/bronchiectasis/ sinusitis. <i>P. aeruginosa</i> infections
Sweat and salivary glands	Increased chloride (>60 mmol/L). Aggravates renal salt wasting specifically in hot environments.	Normal	Borderline to highly increased sweat chloride (40 to >60 mmol/L), rarely normal. Normal salivary chloride.
Reproductive tract	Impaired ciliary function in endometrium and fallopian tube, impaired fertility	Normal	Normal
Age of onset/presentation	Infancy	Childhood/young adulthood Rare in infancy	Infancy, childhood, young adulthood
Outcome	High mortality in infancy. Decreasing frequency and severity of salt wasting episodes with age.	Premature death in undiagnosed young adults	Variable. Depends on severity of pulmonary involvement. No long term data in most studies
Therapy	High NaCl supplementation lifelong	Diuretics that block ENaC (amiloride or triamterene) and low salt diet	Variable

2002; Strautnieks et al., 1996; Wang et al., 2013c; Welzel et al., 2013). Nonsense, frameshift, and abnormal splicing mutations are associated with a severe phenotype (Edelheit et al., 2005). Generally, missense mutations result in a milder phenotype (Hanukoglu et al., 2008). Functional expression of subunits with frameshift mutations showed these mutations reduce but do not necessarily eliminate ENaC activity (Edelheit et al., 2010).

Transient severe salt loss (severe hyponatremia and hyperkalemia) was reported in a premature baby with a homozygous missense mutation in the SCNN1A gene but not in his brother born at term who carried the same mutation (Dirlewanger et al., 2011). In the neonatal period, the human kidney is characterized by an impaired ability to regulate water and sodium homeostasis and premature babies are even more susceptible to blood volume and electrolyte changes when ENaC activity is partial (Martinerie et al., 2009).

In cases where the mutant subunit has been expressed together with the other two wild-type subunits, a correlation has been observed between the in vitro sodium conductance activity of the mutated channel and the clinical severity of the disease (Hanukoglu et al., 2008).

Subjects carrying the mutations in one allele (e.g. parents) are asymptomatic. Rarely, increased sweat sodium and chloride concentrations have been observed in carriers of a mutation in the α subunit without additional hormonal or clinical phenotypes (Riepe et al., 2009).

15.2. Liddle syndrome

Liddle syndrome (OMIM #177200) is an autosomal dominant disorder characterized by early-onset hypertension associated with hypokalemia, metabolic alkalosis, and low levels of plasma renin activity (PRA) and aldosterone (Bogdanović et al., 2012; Hansson et al., 1995; Liddle et al., 1963) (see Table 14). The degree of phenotypic expression may vary even within the same family (Bogdanović et al., 2012; Findling et al., 1997). Liddle syndrome is caused by missense mutations in the PY motif of β or γ subunit of ENaC (Fig. 14), and nonsense or frameshift mutations that result in truncation of the C-terminus leading to the loss of the PY motif in these subunits (Furuhashi et al., 2005; Hansson et al., 1995; Ma et al., 2008; Schild et al., 1996; Shimkets et al., 1994). Mutation or elimination of the PY motif disrupts ubiquitin ligase Nedd4-2 binding to the PY motif (Rotin and Staub, 2011). This leads to accumulation of active channels at the cell surface and increased Na⁺ reabsorption in the kidney, resulting in elevated blood volume and blood pressure. Although in the α subunit there is also a PY-motif (Fig. 14), so far no case of Liddle syndrome has been reported with a mutation in the α subunit.

In addition to mutations in the PY motif region, two mutations in the C-terminus (β subunit, R563Q) and the TM2 segment (γ subunit, N530S) have been reported to be associated with Liddle syndrome phenotype. In the β subunit, an R563Q mutation is associated with low plasma renin activity (PRA), low aldosterone and hypertension in a minority of the individuals carrying the variant (Rayner et al., 2003). In the γ subunit, Hiltunen et al. found an N530S mutation in a patient who developed Liddle syndrome like symptoms at the age of 25 years (Hiltunen et al., 2002). Yet, in the same study the N530S mutation was also found in a healthy person with normal blood pressure (Hiltunen et al., 2002). Thus, the authors raise the possibility that the γ N530S mutation may not be the sole cause of hypertension and that there may be additional factors (such as other genes or environmental factors) responsible for the phenotype observed (Hiltunen et al., 2002). Functional expression of ENaC carrying the N530S mutation showed that the mutation increases ENaC open probability two fold. The increased ENaC activity was observed without a change in the surface expression of ENaC relative to the wild type (Hiltunen et al., 2002). It is probable that more mutations will be found that increase ENaC activity and that are associated with Liddle syndrome like symptoms, similar to the two cases noted above.

Generally Liddle syndrome is a rare disease. However, an extensive study that included a sample of 330 Chinese young hypertensive patients revealed that 1.5% of the patients had mutations associated with a loss of the PY motif (Wang et al., 2015). In a retrospective study in a cohort of 149 hypertensive US veterans, 6% were found to have biochemical abnormalities compatible with Liddle syndrome (Tapolyai et al., 2010). Thus, Liddle syndrome may be a common cause of monogenic hypertension in some populations (Padmanabhan et al., 2015).

Undiagnosed and untreated individuals with Liddle syndrome are at high risk of premature cardiovascular morbidity and mortality. Diuretics that block ENaC activity (amiloride or triamterene) and low salt diet, are usually adequate to control hypertension.

15.3. Cystic fibrosis-like disease

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR dysfunction affects epithelial chloride transport in multiple organs including the digestive system, sweat glands, pancreas, and the reproductive tract, but, progressive lung disease continues to be the major cause of morbidity and mortality.

Respiratory tract infections result from dehydration of airway surfaces that reduces mucociliary clearance and creates an environment conducive to bacterial infections leading to progressive respiratory insufficiency and eventually respiratory failure.

In the lumen of the respiratory tract, ENaC function is essential for normal mucociliary clearance (Althaus, 2013; Hobbs et al., 2013). As noted in Section 13, ENaC is expressed in the respiratory tract, located on the surface of cilia and regulates the volume of luminal fluid (Enuka et al., 2012). PHA1B patients with ENaC mutations suffer frequently from lower respiratory tract infections (Hanukoglu et al., 1994; Kerem et al., 1999; Schaedel et al., 1999). Increased airwayspecific expression of the β subunit of ENaC results in CF-like symptoms in mice (Mall et al., 2004). This is thought to result in over-expression of channels composed of only α and β subunits, which have a high intrinsic open probability (Mall et al., 2010). Therefore, it has been suggested that mutations in ENaC genes may be involved in some forms of cystic fibrosis. To examine the hypothesis that ENaC mutations may be associated with the degree of severity of CF, a French group screened genomic DNA of 56 CF patients for the presence of variants in SCNN1B and SCNN1G genes (Viel et al., 2008). By using denaturing highperformance liquid chromatography (DHPLC), they found 4 missense mutations in three patients out of 56 (T313M and G589S in β , and L481Q and V546I in γ subunit). However, nasal potential difference measurements did not indicate a functional effect of these variants on Na⁺ transport, at least in the nasal epithelium of these patients. Thus, the authors concluded that variants in SCNN1B and SCNN1G genes are not associated with CF severity in the cohort examined (Viel et al., 2008).

Cases that present with classical features of cystic fibrosis (such as chronic lung infections with elevated sweat chloride concentration), but without CFTR mutation or a single allele CFTR mutation, have been referred to as cystic fibrosis-like (CF-like) disease (Table 14). These cases lack a genetic diagnosis, and a commonly suspected cause is mutations in ENaC genes (Collawn et al., 2012).

Screening 185 patients with non-classic CF, Sheridan at al. identified 20 patients who had elevated sweat chloride concentrations, and pulmonary disease but without CFTR mutations (Sheridan et al., 2005). Sequencing of the ENaC genes (SCNN1A, B or G) revealed that two of the patients carry compound heterozygous mutations in the SCNN1B gene: a missense mutation (P267L) with a splice site mutation in one patient and two missense mutations (G294S and E539K) in the other. Neither patient had abnormal renin or aldosterone levels. In functional expression studies, P267L and E539K mutants showed decreased activity and G294S mutant showed increased activity (Sheridan et al.,

2005). The authors concluded that the compound heterozygous mutations identified in the β ENaC genes that have a mild effect on ENaC activity are associated with CF-like disease without causing severe renal salt loss (Sheridan et al., 2005).

In a multi-center European study, 30 ENaC variants were found in 76 patients with CF-like disease (Azad et al., 2009). Only two (hypoactive F61L and hyperactive V114I in SCNN1A) of the 30 variants were found in patients but not in the control populations. ENaC subunit variants had a significantly higher frequency in the patients as compared to controls. The variant W493R in the α subunit showed the most significant difference, and in functional expression in *Xenopus* oocytes showed over four-fold higher ENaC activity. Thus, the authors concluded that these variants may be involved in CF-like disease by a polygenetic mechanism (Azad et al., 2009). In a study including 99 Italian patients with CFTR-related diseases, 12 ENaC variants were found, but the allele frequency of these variants was not significantly different from controls (Amato et al., 2012).

Fajac et al. screened a group of 55 patients with diffuse idiopathic bronchiectasis (permanent dilation of the airways as a result of chronic bronchial infection) by sequencing SCNN1B and SCNN1G exons and identified five heterozygous missense variants (S82C, P369T, N288S in β , and G183S, E197K in γ subunit) in eight patients (Fajac et al., 2008). The S82C mutation was found in three unrelated patients who were also heterozygous for a CFTR mutation. The authors thus concluded that trans-heterozygous mutations in ENaC and CFTR may be responsible for the CF-like symptoms.

In a study including 60 Rwandan children with CF-like symptoms, five patients were found to have a heterozygous CFTR mutation. Two of these patients had a missense ENaC variant (V573I in α , V348M and G442V in β subunit); of these only V348M was not found in the control group (Mutesa et al., 2009). Functional expression of the V348M mutant showed that the mutation enhances ENaC activity (Rauh et al., 2013). Since the full ENaC subunit gene sequences were not determined in 55 of the patients, the relationship between ENaC variants and CF-like disease cannot be determined for the whole group.

A recent study on CF-like phenotypes examined the sequences of five genes (CFTR, SCNN1A, SCNN1B, SCNN1G and SERPINA1) in six patients by whole exome sequencing (Ramos et al., 2014). The authors detected three missense variants in SCNN1A (R204W, A357T, C641F) and one missense (R563Q) in SCNN1B, and four additional nucleotide variants. Two of these mutants (C641F and R563Q) appeared also in two CF controls but not in healthy controls. The authors suggest that the variants that appear at a higher frequency in patients with CF-like phenotype than in controls may be responsible for this phenotype. By their family analysis they also stress the importance of genetic/environmental factors in the development of CF-like disease (Ramos et al., 2014).

Brenan et al. examined 33 nonwhite, non-Hispanic patients with a CF-like disease whose CFTR gene analysis was non-diagnostic (19 with no mutations in CFTR gene and 14 with a heterozygous change in the CFTR gene) (Brennan et al., 2015). Sequencing of the exons and introns of SCNN1A, SCNN1B, and SCNN1G in all patients revealed 21 variants. Since the variants found in conjunction with a CFTR mutation were common polymorphisms, the authors concluded that there is no conclusive association of ENAC genetic variants with CF in their cohort.

In Table 15 we listed all the missense mutations identified in the studies reviewed above. Among the three ENaC genes, most of the variants have been observed in SCNN1A and SCNN1B. Proportionately, the highest number of mutants is in SCNN1A. Two of the major studies examined only two genes: SCNN1B and SCNN1G, thus the number of variants in SCNN1A in Table 15 is underrepresented. In our analysis of PHA1B mutants, we observed a similar trend that most of the PHA1B causing mutants appear in SCNN1A and only a few in SCNN1G (Edelheit et al., 2005).

As detailed in the studies cited, many of the variants are also present in control groups. Yet, some of the mutants that were shown to affect

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Missense	mutations	found in	patients	with a	CF-like	phenotype
						F

	Variant	Change in function	Ethnicity	References
SCNN1A	V14G		Spanish	Ramos et al. (2014)
	F61L	↓ ENaC activity	Caucasian	Azad et al. (2009)
	V114I	↑ ENaC activity	Caucasian	Azad et al. (2009)
	R181W	↑ ENaC activity	Caucasian	Azad et al. (2009), Sheridan et al. (2005)
	R204W		Spanish	Ramos et al. (2014)
	A304P		Spanish	Ramos et al. (2014)
	A334T	↓ ENaC activity	Caucasian, nonwhite	Amato et al. (2012), Azad et al. (2009), Brennan et al. (2015)
	A357T		Spanish	Ramos et al. (2014)
	W493R	↑ ENaC activity	Caucasian	Azad et al. (2009)
	V573I		African, nonwhite	Brennan et al. (2015), Mutesa et al. (2009)
	C618F	↑ ENaC activity	Nonwhite	Brennan et al. (2015)
	C641F		Spanish	Ramos et al. (2014)
	T663A	↓ ENaC activity	Caucasian, nonwhite	Amato et al. (2012), Azad et al. (2009), Brennan et al. (2015)
SCNN1B	S82C	None	Caucasian	Azad et al. (2009), Fajac et al. (2008), Sheridan et al. (2005)
	P267L	↓ ENaC activity		Sheridan et al. (2005)
	N288S		Caucasian	Fajac et al. (2008)
	G294S	↑ ENaC activity		Sheridan et al. (2005)
	T313M	No change in NPD ^a	French	Viel et al. (2008)
	V348M	↑ ENaC activity	African	Mutesa et al. (2009)
	P369T	High basal NPD	Caucasian	Fajac et al. (2008)
	R388C		Nonwhite	Brennan et al. (2015)
	G442V		African, nonwhite	Brennan et al. (2015), Mutesa et al. (2009)
	E539K	↓ ENaC activity		Sheridan et al. (2005)
	R563Q		Spanish	Ramos et al. (2014)
	G589S	None, normal NPD	Caucasian, French	Azad et al. (2009), Viel et al. (2008)
	T594M		Nonwhite	Brennan et al. (2015)
SCNN1G	G183S		African, nonwhite	Brennan et al. (2015), Fajac et al. (2008)
	E197K	None	Caucasian	Azad et al. (2009), Fajac et al. (2008)
	L481Q	Normal NPD	French	Viel et al. (2008)
	V546I	Normal NPD	French	Viel et al. (2008)

^a NPD: nasal potential difference.

adversely ENaC activity have been reported in independent studies (Table 15). The total number of cases is still too small to reach definitive conclusions about the role of these variants/mutants in causing CF-like disease. In any case, the number of variants that may be associated with CF-like disease represents a small percentage of the total patients in each cohort and differs between ethnic groups.

15.4. Hypertension

In modern industrial societies, hypertension has emerged as one of most widespread health problems (Toka et al., 2013). A minority of the cases of hypertension can be ascribed to monogenic conditions such as Liddle's syndrome or Gordon's syndrome (Padmanabhan et al., 2015). The remainder of the cases is generally grouped as "essential hypertension" with multifactorial etiology, including multiple genetic, humoral, environmental and dietary factors (Su and Menon, 2001). The major systems that are responsible for the regulation of blood pressure include the renin-angiotensin-aldosterone system and Na⁺ transporters in the kidney (Padmanabhan et al., 2015; Rossier, 2014; Soundararajan et al., 2010; Su and Menon, 2001). Moreover, the Liddle syndrome described above firmly established the importance of ENaC in blood pressure regulation. Therefore, several large-scale studies have examined the association of ENaC variants with essential hypertension. Liu et al. examined 2880 Chinese subjects (GenSalt study) and found an association of blood pressure with SCNN1B and SCNN1G SNPs and variants (Liu et al., 2015). Rayner et al. screened 139 South African black hypertensives for the R563Q variant of β subunit and found that the variant was significantly associated with hypertension (Rayner et al., 2003). In a study in a Finland, the authors sequenced only exon 13 (that codes for TM2 and the C-terminal segment) of β and γ subunits (see Fig. 7) in 27 hypertensive patients. The three identified variants were then screened in 347 hypertensives. The frequency of all variants in the hypertensives was significantly higher (~3 fold) than that in two control groups. Functional expression of one variant (β G589S) showed slightly enhanced activity in *Xenopus* oocytes (Hannila-Handelberg et al., 2005).

Ambrosius et al. reported a small but significant association between a common α T663A variant and normal blood pressure (Ambrosius et al., 1999). The α A663 variant reduces ENaC activity and functional expression in *Xenopus* oocytes (Samaha et al., 2004; Tong et al., 2006), but the change in activity was opposite of that predicted by the results of Ambrosius et al. Furthermore, no association was found between α T663A variant and blood pressure in a sample of 247 Japanese hypertensives (Sugiyama et al., 2001). Nonetheless, homozygous A663 allele appears to have a differential effect on lung function (Foxx-Lupo et al., 2011).

Another polymorphism with conflicting reports is β -T594M. In a sample of black hypertensives from London, 8.3% (17 out of 206) were heterozygous for the β T594M variant, but in the control group only 2.1% had the same variant (Baker et al., 1998). However, in a much larger sample, including 1666 Jamaican blacks, no association was found between the β T594M allele and hypertension (Hollier et al., 2006). This variant did not alter ENaC activity in a heterologous expression system (Persu et al., 1998).

Persu et al. identified seven variants in the β subunits of, mostly white, 525 probands of hypertensive families, but could not identify an association between a variant and essential hypertension (Persu et al., 1998).

In summary, currently there does not appear to be a clear association between ENaC variants and essential hypertension. Yet, this area of research is just at its beginnings and requires examination of larger sets of SNPs and variants in selected populations. Some of the past studies have examined only specific segments (such as exon 13) of subunits. This approach skews the results. The SNPs that are accumulating in whole genome sequencing will present larger and more comprehensive databases for future examination. It is likely that such studies will reveal new variants associated with hypertension similar to that found in the Chinese GenSalt study. Since the majority of the variants so far screened do not seem to be associated with hypertension, the proportion of ENaC variants associated with hypertension would be expected to be small.

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https://en.wikipedia.org/wiki/SCNN1A https://en.wikipedia.org/wiki/SCNN1B https://en.wikipedia.org/wiki/SCNN1D https://en.wikipedia.org/wiki/SCNN1G

References

- Ackermann, D., Gresko, N., Carrel, M., Loffing-Cueni, D., Habermehl, D., Gomez-Sanchez, C., Rossier, B.C., Loffing, J., 2010. In vivo nuclear translocation of mineralocorticoid and glucocorticoid receptors in rat kidney: differential effect of corticosteroids along the distal tubule. Am. J. Physiol. Ren. Physiol. 299, F1473–F1485. http://dx. doi.org/10.1152/ajprenal.00437.2010.
- Adachi, M., Asakura, Y., Muroya, K., Tajima, T., Fujieda, K., Kuribayashi, E., Uchida, S., 2010. Increased Na reabsorption via the Na–Cl cotransporter in autosomal recessive pseudohypoaldosteronism. Clin. Exp. Nephrol. 14, 228–232.
- Alföldi, J., Di Palma, F., Grabherr, M., Williams, C., Kong, L., Mauceli, E., Russell, P., Lowe, C.B., Glor, R.E., Jaffe, J.D., Ray, D.A., Boissinot, S., Shedlock, A.M., Botka, C., Castoe, T.A., Colbourne, J.K., Fujita, M.K., Moreno, R.G., ten Hallers, B.F., Haussler, D., Heger, A., Heiman, D., Janes, D.E., Johnson, J., de Jong, P.J., Koriabine, M.Y., Lara, M., Novick, P.A., Organ, C.L., Peach, S.E., Poe, S., Pollock, D.D., de Queiroz, K., Sanger, T., Searle, S., Smith, J.D., Smith, Z., Swofford, R., Turner-Maier, J., Wade, J., Young, S., Zadissa, A., Edwards, S.V., Glenn, T.C., Schneider, C.J., Losos, J.B., Lander, E.S., Breen, M., Ponting, C.P., Lindblad-Toh, K., 2011. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature 477, 587–591. http://dx.doi.org/10.1038/ nature10390.
- Alli, A.A., Song, J.Z., Al-Khalili, O., Bao, H.-F., Ma, H.-P., Alli, A.A., Eaton, D.C., 2012. Cathepsin B is secreted apically from *Xenopus* 2F3 cells and cleaves the epithelial sodium channel (ENaC) to increase its activity. J. Biol. Chem. 287, 30073–30083. http://dx.doi.org/ 10.1074/jbc.M111.338574.
- Althaus, M., 2013. ENaC inhibitors and airway re-hydration in cystic fibrosis: state of the art. Curr. Mol. Pharmacol. 6, 3–12.
- Amato, F., Bellia, C., Cardillo, G., Castaldo, G., Ciaccio, M., Elce, A., Lembo, F., Tomaiuolo, R., 2012. Extensive molecular analysis of patients bearing CFTR-related disorders. J. Mol. Diagn. 14, 81–89. http://dx.doi.org/10.1016/j.jmoldx.2011.09.001.
- Ambrosius, W.T., Bloem, L.J., Zhou, L., Rebhun, J.F., Snyder, P.M., Wagner, M.A., Guo, C., Pratt, J.H., 1999. Genetic variants in the epithelial sodium channel in relation to aldosterone and potassium excretion and risk for hypertension. Hypertension 34, 631–637.
- Amemiya, C.T., Alföldi, J., Lee, A.P., Fan, S., Philippe, H., Maccallum, I., Braasch, I., Manousaki, T., Schneider, I., Rohner, N., Organ, C., Chalopin, D., Smith, J.J., Robinson, M., Dorrington, R.A., Gerdol, M., Aken, B., Biscotti, M.A., Barucca, M., Baurain, D., Berlin, A.M., Blatch, G.L., Buonocore, F., Burmester, T., Campbell, M.S., Canapa, A., Cannon, J.P., Christoffels, A., De Moro, G., Edkins, A.L., Fan, L., Fausto, A.M., Feiner, N., Forconi, M., Gamieldien, J., Gnerre, S., Gnirke, A., Goldstone, J.V., Haerty, W., Hahn, M.E., Hesse, U., Hoffmann, S., Johnson, J., Karchner, S.I., Kuraku, S., Lara, M., Levin, J.Z., Litman, G.W., Mauceli, E., Miyake, T., Mueller, M.G., Nelson, D.R., Nitsche, A., Olmo, E., Ota, T., Pallavicini, A., Panji, S., Picone, B., Ponting, C.P., Prohaska, S.J., Przybylski, D., Saha, N.R., Ravi, V., Ribeiro, F.J., Sauka-Spengler, T., Scapigliati, G., Searle, S.M.J., Sharpe, T., Simakov, O., Stadler, P.F., Stegeman, J.J., Sumiyama, K., Tabbaa, D., Tafer, H., Turner-Maier, J., van Heusden, P., White, S., Williams, L., Yandell, M., Brinkmann, H., Volff, J.-N., Tabin, C.J., Shubin, N., Schartl, M., Jaffe, D.B., Postlethwait, J.H., Venkatesh, B., Di Palma, F., Lander, E.S., Meyer, A., Lindblad-Toh, K., 2013. The African coelacanth genome provides insights into tetrapod evolution. Nature 496, 311–316. http://dx.doi.org/10.1038/nature12027.
- Anitei, M., Hoflack, B., 2012. Bridging membrane and cytoskeleton dynamics in the secretory and endocytic pathways. Nat. Cell Biol. 14, 11–19. http://dx.doi.org/10.1038/ ncb2409.
- Antalis, T.M., Buzza, M.S., Hodge, K.M., Hooper, J.D., Netzel-Arnett, S., 2010. The cutting edge: membrane-anchored serine protease activities in the pericellular microenvironment. Biochem. J. 428, 325–346.

- Armanini, D., Kuhnle, U., Strasser, T., Dorr, H., Butenandt, I., Weber, P.C., Stockigt, J.R., Pearce, P., Funder, J.W., 1985. Aldosterone-receptor deficiency in pseudohypoaldosteronism. N. Engl. I. Med. 313, 1178–1181. http://dx.doi.org/10.1056/NEIM198511073131902.
- Asher, C., Wald, H., Rossier, B.C., Garty, H., 1996. Aldosterone-induced increase in the abundance of Na⁺ channel subunits. Am. J. Physiol. 271, C605–C611.
- Asher, C., Chigaev, A., Garty, H., 2001. Characterization of interactions between Nedd4 and beta and gammaENaC using surface plasmon resonance. Biochem. Biophys. Res. Commun. 286, 1228–1231.
- Azad, A.K., Rauh, R., Vermeulen, F., Jaspers, M., Korbmacher, J., Boissier, B., Bassinet, L., Fichou, Y., des Georges, M., Stanke, F., De Boeck, K., Dupont, L., Balascáková, M., Hjelte, L., Lebecque, P., Radojkovic, D., Castellani, C., Schwartz, M., Stuhrmann, M., Schwarz, M., Skalicka, V., de Monestrol, I., Girodon, E., Férec, C., Claustres, M., Tümmler, B., Cassiman, J.-J., Korbmacher, C., Cuppens, H., 2009. Mutations in the amiloride-sensitive epithelial sodium channel in patients with cystic fibrosis-like disease. Hum. Mutat. 30, 1093–1103. http://dx.doi.org/10.1002/humu.21011.
- Bachhuber, T., König, J., Voelcker, T., Mürle, B., Schreiber, R., Kurzelmann, K., 2005. Cl⁻ interference with the epithelial Na⁺ channel ENAC. J. Biol. Chem. 280, 31587–31594. http://dx.doi.org/10.1074/jbc.M504347200.
- Baconguis, I., Bohlen, C.J., Goehring, A., Julius, D., Gouaux, E., 2014. X-ray structure of acidsensing ion channel 1-snake toxin complex reveals open state of a Na⁺-selective channel. Cell 156, 717–729. http://dx.doi.org/10.1016/j.cell.2014.01.011.
- Baker, E.H., Dong, Y.B., Sagnella, G.A., Rothwell, M., Onipinla, A.K., Markandu, N.D., Cappuccio, F.P., Cook, D.G., Persu, A., Corvol, P., Jeunemaitre, X., Carter, N.D., MacGregor, G.A., 1998. Association of hypertension with T594M mutation in beta subunit of epithelial sodium channels in black people resident in London. Lancet 351, 1388–1392.
- Bangel-Ruland, N., Sobczak, K., Christmann, T., Kentrup, D., Langhorst, H., Kusche-Vihrog, K., Weber, W.-M., 2010. Characterization of the epithelial sodium channel deltasubunit in human nasal epithelium. Am. J. Respir. Cell Mol. Biol. 42, 498–505. http://dx.doi.org/10.1165/rcmb.2009-00530C.
- Bao, H.-F., Thai, T.L., Yue, Q., Ma, H.-P., Eaton, A.F., Cai, H., Klein, J.D., Sands, J.M., Eaton, D.C., 2014. ENaC activity is increased in isolated, split-open cortical collecting ducts from protein kinase Cα knockout mice. Am. J. Physiol. Ren. Physiol. 306, F309–F320. http://dx.doi.org/10.1152/ajprenal.00519.2013.
- Barker, P.M., Nguyen, M.S., Gatzy, J.T., Grubb, B., Norman, H., Hummler, E., Rossier, B., Boucher, R.C., Koller, B., 1998. Role of gammaENaC subunit in lung liquid clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. J. Clin. Invest. 102, 1634–1640. http://dx.doi.org/10. 1172/ICI3971.
- Belot, A., Ranchin, B., Fichtner, C., Pujo, L., Rossier, B.C., Liutkus, A., Morlat, C., Nicolino, M., Zennaro, M.C., Cochat, P., 2008. Pseudohypoaldosteronisms, report on a 10-patient series. Nephrol. Dial. Transplant. 23, 1636–1641.
- Ben-Shahar, Y., 2011. Sensory functions for degenerin/epithelial sodium channels (DEG/ ENaC). Adv. Genet. 76, 1–26. http://dx.doi.org/10.1016/B978-0-12-386481-9.00001-8.
- Berdiev, B.K., Jovov, B., Tucker, W.C., Naren, A.P., Fuller, C.M., Chapman, E.R., Benos, D.J., 2004. ENaC subunit-subunit interactions and inhibition by syntaxin 1A. Am. J. Physiol. Ren. Physiol. 286, F1100–F1106.
- Berdiev, B.K., Qadri, Y.J., Benos, D.J., 2009. Assessment of the CFTR and ENaC association. Mol. BioSyst. 5, 123–127. http://dx.doi.org/10.1039/b810471a.
- Berman, J.M., Brand, C., Awayda, M.S., 2015. A long isoform of the epithelial sodium channel alpha subunit forms a highly active channel. Channels (Austin) 9, 30–43. http:// dx.doi.org/10.4161/19336950.2014.985478.
- Bernier, L.-P., Blais, D., Boué-Grabot, É., Séguéla, P., 2012. A dual polybasic motif determines phosphoinositide binding and regulation in the P2X channel family. PLoS One 7, e40595. http://dx.doi.org/10.1371/journal.pone.0040595.
- Bhalla, V., Hallows, K.R., 2008. Mechanisms of ENaC regulation and clinical implications. J. Am. Soc. Nephrol. 19, 1845–1854. http://dx.doi.org/10.1681/ASN.2008020225.
- Björklund, A.K., Ekman, D., Light, S., Frey-Skött, J., Elofsson, A., 2005. Domain rearrangements in protein evolution. J. Mol. Biol. 353, 911–923. http://dx.doi.org/10.1016/j. jmb.2005.08.067.
- Bobby, R., Medini, K., Neudecker, P., Lee, T.V., Brimble, M.A., McDonald, F.J., Lott, J.S., Dingley, A.J., 2013. Structure and dynamics of human Nedd4-1 WW3 in complex with the αENaC PY motif. Biochim. Biophys. Acta 1834, 1632–1641. http://dx.doi. org/10.1016/j.bbapap.2013.04.031.
- Bogdanović, R., Kuburović, V., Stajić, N., Mughal, S.S., Hilger, A., Ninić, S., Prijić, S., Ludwig, M., 2012. Liddle syndrome in a Serbian family and literature review of underlying mutations. Eur. J. Pediatr. 171, 471–478. http://dx.doi.org/10.1007/s00431-011-1581-8.
- Bonny, O., Hummler, E., 2000. Dysfunction of epithelial sodium transport: from human to mouse. Kidney Int. 57, 1313–1318. http://dx.doi.org/10.1046/j.1523-1755.2000.00968.x.
- Bonny, O., Knoers, N., Monnens, L., Rossier, B.C., 2002. A novel mutation of the epithelial Na⁺ channel causes type 1 pseudohypoaldosteronism. Pediatr. Nephrol. 17, 804–808.
- Bourque, C.W., 2008. Central mechanisms of osmosensation and systemic osmoregulation. Nat. Rev. Neurosci. 9, 519–531.
- Bremner, H.R., Freywald, T., O'Brodovich, H.M., Otulakowski, G., 2002. Promoter analysis of the gene encoding the beta-subunit of the rat amiloride-sensitive epithelial sodium channel. Am. J. Physiol. Lung Cell. Mol. Physiol. 282, L124–L134.
- Brennan, M.-L., Pique, L.M., Schrijver, I., 2015. Assessment of epithelial sodium channel variants in nonwhite cystic fibrosis patients with non-diagnostic CFTR genotypes. J. Cyst. Fibros. http://dx.doi.org/10.1016/j.jcf.2015.04.001.
- Brooker, D.R., Kozak, C.A., Kleyman, T.R., 1995. Epithelial sodium channel genes Scnn1b and Scnn1g are closely linked on distal mouse chromosome 7. Genomics 29, 784–786. http://dx.doi.org/10.1006/geno.1995.9934.
- Brooks, E.R., Wallingford, J.B., 2014. Multiciliated cells. Curr. Biol. 24, R973–R982. http:// dx.doi.org/10.1016/j.cub.2014.08.047.
- Brouard, M., Casado, M., Djelidi, S., Barrandon, Y., Farman, N., 1999. Epithelial sodium channel in human epidermal keratinocytes: expression of its subunits and relation to sodium transport and differentiation. J. Cell Sci. 112 (Pt 1), 3343–3352.

- Bruns, J.B., Carattino, M.D., Sheng, S., Maarouf, A.B., Weisz, O.A., Pilewski, J.M., Hughey, R.P., Kleyman, T.R., 2007. Epithelial Na⁺ channels are fully activated by furin- and prostasin-dependent release of an inhibitory peptide from the gamma-subunit. J. Biol. Chem. 282, 6153–6160.
- Büsst, C.J., 2013. Blood pressure regulation via the epithelial sodium channel: from gene to kidney and beyond. Clin. Exp. Pharmacol. Physiol. 40, 495–503. http://dx.doi.org/ 10.1111/1440-1681.12124.
- Butterworth, M.B., 2010. Regulation of the epithelial sodium channel (ENaC) by membrane trafficking. Biochim. Biophys. Acta 1802, 1166–1177. http://dx.doi.org/10. 1016/j.bbadis.2010.03.010.
- Canessa, C.M., Merillat, A.M., Rossier, B.C., 1994a. Membrane topology of the epithelial sodium channel in intact cells. Am. J. Physiol. 267, C1682–C1690.
- Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D., Rossier, B.C., 1994b. Amiloride-sensitive epithelial Na⁺ channel is made of three homologous subunits. Nature 367, 463–467.
- Cañestro, C., Albalat, R., Irimia, M., Garcia-Fernàndez, J., 2013. Impact of gene gains, losses and duplication modes on the origin and diversification of vertebrates. Semin. Cell Dev. Biol. 24, 83–94. http://dx.doi.org/10.1016/j.semcdb.2012.12.008.
- Capaldo, C.T., Farkas, A.E., Nusrat, A., 2014. Epithelial adhesive junctions. F1000Prime Rep. 6, 1. http://dx.doi.org/10.12703/P6-1.
- Carattino, M.D., Sheng, S., Bruns, J.B., Pilewski, J.M., Hughey, R.P., Kleyman, T.R., 2006. The epithelial Na⁺ channel is inhibited by a peptide derived from proteolytic processing of its alpha subunit. J. Biol. Chem. 281, 18901–18907.
- Carattino, M.D., Hughey, R.P., Kleyman, T.R., 2008a. Proteolytic processing of the epithelial sodium channel gamma subunit has a dominant role in channel activation. J. Biol. Chem. 283, 25290–25295.
- Carattino, M.D., Passero, C.J., Steren, C.A., Maarouf, A.B., Pilewski, J.M., Myerburg, M.M., Hughey, R.P., Kleyman, T.R., 2008b. Defining an inhibitory domain in the alphasubunit of the epithelial sodium channel. Am. J. Physiol. Ren. Physiol. 294, F47–F52. http://dx.doi.org/10.1152/ajprenal.00399.2007.
- Cerecedo, D., Martínez-Vieyra, I., Alonso-Rangel, L., Benítez-Cardoza, C., Ortega, A., 2014. Epithelial sodium channel modulates platelet collagen activation. Eur. J. Cell Biol. 93, 127–136. http://dx.doi.org/10.1016/j.ejcb.2014.02.003.
- Chalfant, M.L., Denton, J.S., Langloh, A.L., Karlson, K.H., Loffing, J., Benos, D.J., Stanton, B.A., 1999. The NH₂ terminus of the epithelial sodium channel contains an endocytic motif. J. Biol. Chem. 274, 32889–32896.
- Chambers, L.A., Rollins, B.M., Tarran, R., 2007. Liquid movement across the surface epithelium of large airways. Respir. Physiol. Neurobiol. 159, 256–270. http://dx.doi. org/10.1016/j.resp.2007.06.005.
- Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D.A., Hummler, E., Ryba, N.J.P., Zuker, C.S., 2010. The cells and peripheral representation of sodium taste in mice. Nature 464, 297–301. http://dx.doi.org/10.1038/nature08783.
- Chang, S.S., Grunder, S., Hanukoglu, A., Rösler, A., Mathew, P.M., Hanukoglu, I., Schild, L., Lu, Y., Shimkets, R.A., Nelson-Williams, C., Rossier, B.C., Lifton, R.P., 1996. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. Nat. Genet. 12, 248–253. http://dx.doi. org/10.1038/ng0396-248.
- Chanoux, R.a., Shubin, C.B., Robay, A., Suaud, L., Rubenstein, R.C., 2013. Hsc70 negatively regulates epithelial sodium channel trafficking at multiple sites in epithelial cells. Am. J. Physiol. Cell Physiol. 305, C776–C787. http://dx.doi.org/10.1152/ajpcell. 00059.2013.
- Cheek, D.B., Perry, J.W., 1958. A salt wasting syndrome in infancy. Arch. Dis. Child. 33, 252–256.
- Chen, C.C., England, S., Akopian, A.N., Wood, J.N., 1998. A sensory neuron-specific, protongated ion channel. Proc. Natl. Acad. Sci. U. S. A. 95, 10240–10245.
- Chen, J., Kleyman, T.R., Sheng, S., 2013. Gain-of-function variant of the human epithelial sodium channel. Am. J. Physiol. Ren. Physiol. 304, F207–F213. http://dx.doi.org/10. 1152/ajprenal.00563.2012.
- Chen, J., Ray, E.C., Yates, M.E., Buck, T.M., Brodsky, J.L., Kinlough, C.L., Winarski, K.L., Hughey, R.P., Kleyman, T.R., Sheng, S., 2015. Functional roles of clusters of hydrophobic and polar residues in the epithelial Na⁺ channel knuckle domain. J. Biol. Chem. http://dx.doi.org/10.1074/jbc.M115.665398.
- Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., Thompson, J.D., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31, 3497–3500.
- Chicken-Genome, 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432, 695–716. http:// dx.doi.org/10.1038/nature03154.
- Choi, H.-C., Kim, C.S.K., Tarran, R., 2015. Automated acquisition and analysis of airway surface liquid height by confocal microscopy. Am. J. Physiol. Lung Cell. Mol. Physiol. 309, L109–L118. http://dx.doi.org/10.1152/ajplung.00027.2015.
- Chraibi, A., Horisberger, J.D., 2002. Na self inhibition of human epithelial Na channel: temperature dependence and effect of extracellular proteases. J. Gen. Physiol. 120, 133–145.
- Chung, E., Hanukoglu, A., Rees, M., Thompson, R., Dillon, M., Hanukoglu, I., Bistritzer, T., Kuhnle, U., Seckl, J., Gardiner, R.M., 1995. Exclusion of the locus for autosomal recessive pseudohypoaldosteronism type 1 from the mineralocorticoid receptor gene region on human chromosome 4q by linkage analysis. J. Clin. Endocrinol. Metab. 80, 3341–3345.
- Collawn, J.F., Lazrak, A., Bebok, Z., Matalon, S., 2012. The CFTR and ENaC debate: how important is ENaC in CF lung disease? Am. J. Physiol. Lung Cell Mol. Physiol. 302, L1141–L1146. http://dx.doi.org/10.1152/ajplung.00036.2012.
- Collier, D.M., Snyder, P.M., 2011. Identification of epithelial Na⁺ channel (ENaC) intersubunit Cl⁻ inhibitory residues suggests a trimeric alpha gamma beta channel architecture. J. Biol. Chem. 286, 6027–6032. http://dx.doi.org/10.1074/jbc.M110. 198127.

- Collier, D.M., Peterson, Z.J., Blokhin, I.O., Benson, C.J., Snyder, P.M., 2012. Identification of extracellular domain residues required for epithelial Na⁺ channel activation by acidic pH. J. Biol. Chem. 287, 40907–40914. http://dx.doi.org/10.1074/jbc.M112.417519.
- Condliffe, S.B., Carattino, M.D., Frizzell, R.A., Zhang, H., 2003. Syntaxin 1A regulates ENaC via domain-specific interactions. J. Biol. Chem. 278, 12796–12804.
- Copeland, S.J., Berdiev, B.K., Ji, H.L., Lockhart, J., Parker, S., Fuller, C.M., Benos, D.J., 2001. Regions in the carboxy terminus of alpha-bENaC involved in gating and functional effects of actin. Am. J. Physiol. Cell Physiol. 281, C231–C240.
- Coric, T., Zhang, P., Todorovic, N., Canessa, C.M., 2003. The extracellular domain determines the kinetics of desensitization in acid-sensitive ion channel 1. J. Biol. Chem. 278, 45240–45247.
- Coric, T., Hernandez, N., de la Rosa, D.A., Shao, D., Wang, T., Canessa, C.M., 2004. Expression of ENaC and serum- and glucocorticoid-induced kinase 1 in the rat intestinal epithelium. Am. J. Physiol. Gastrointest. Liver Physiol. 286, G663–G670.
- Coy, P., García-Vázquez, F.A., Visconti, P.E., Avilés, M., 2012. Roles of the oviduct in mammalian fertilization. Reproduction 144, 649–660. http://dx.doi.org/10.1530/ REP-12-0279.
- Dalloul, R.A., Long, J.A., Zimin, A.V., Aslam, L., Beal, K., Blomberg, L.A., Bouffard, P., Burt, D.W., Crasta, O., Crooijmans, R.P.M.A., Cooper, K., Coulombe, R.A., De, S., Delany, M.E., Dodgson, J.B., Dong, J.J., Evans, C., Frederickson, K.M., Flicek, P., Florea, L., Folkerts, O., Groenen, M.A.M., Harkins, T.T., Herrero, J., Hoffmann, S., Megens, H.-J., Jiang, A., de Jong, P., Kaiser, P., Kim, H., Kim, K.-W., Kim, S., Langenberger, D., Lee, M.-K., Lee, T., Mane, S., Marcais, G., Marz, M., McElroy, A.P., Modise, T., Nefedov, M., Notredame, C., Paton, I.R., Payne, W.S., Pertea, G., Prickett, D., Puiu, D., Qioa, D., Raineri, E., Ruffer, M., Salzberg, S.L., Schatz, M.C., Scheuring, C., Schmidt, C.J., Schroeder, S., Searle, S.M.J., Smith, E.J., Smith, J., Sonstegard, T.S., Stadler, P.F., Tafer, H., Tu, Z.J., Van Tassell, C.P., Vilella, A.J., Williams, K.P., Yorke, J.A., Zhang, L., Zhang, H.-B., Zhang, X., Zhang, Y., Reed, K.M., 2010. Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo*): genome assembly and analysis. PLoS Biol. 8. http://dx.doi.org/10.1371/journal.pbio.1000475.
- Deval, E., Lingueglia, E., 2015. Acid-Sensing Ion Channels and nociception in the peripheral and central nervous systems. Neuropharmacology http://dx.doi.org/10.1016/j. neuropharm.2015.02.009.
- Di Paolo, G., De Camilli, P., 2006. Phosphoinositides in cell regulation and membrane dynamics. Nature 443, 651–657. http://dx.doi.org/10.1038/nature05185.
- Diakov, A., Korbmacher, C., 2004. A novel pathway of epithelial sodium channel activation involves a serum- and glucocorticoid-inducible kinase consensus motif in the C terminus of the channel's alpha-subunit. J. Biol. Chem. 279, 38134–38142. http://dx. doi.org/10.1074/jbc.M403260200.
- Diochot, S., Salinas, M., Baron, A., Escoubas, P., Lazdunski, M., 2007. Peptides inhibitors of acid-sensing ion channels. Toxicon 49, 271–284. http://dx.doi.org/10.1016/j.toxicon. 2006.09.026.
- Dirlewanger, M., Huser, D., Zennaro, M.C., Girardin, E., Schild, L., Schwitzgebel, V.M., 2011. A homozygous missense mutation in SCNN1A is responsible for a transient neonatal form of pseudohypoaldosteronism type 1. Am. J. Physiol. Endocrinol. Metab. 301, E467–E473. http://dx.doi.org/10.1152/ajpendo.00022.2011.
- Donoghue, P.C.J., Sansom, I.J., Downs, J.P., 2006. Early evolution of vertebrate skeletal tissues and cellular interactions, and the canalization of skeletal development. J. Exp. Zool. B Mol. Dev. Evol. 306, 278–294. http://dx.doi.org/10.1002/jez.b.21090.
- Drummond, H.A., Grifoni, S.C., Jernigan, N.L., 2008. A new trick for an old dogma: ENAC proteins as mechanotransducers in vascular smooth muscle. Physiology (Bethesda) 23, 23–31. http://dx.doi.org/10.1152/physiol.00034.2007.
- Duc, C., Farman, N., Canessa, C.M., Bonvalet, J.P., Rossier, B.C., 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, 1907–1921.
- Eastwood, A.L., Goodman, M.B., 2012. Insight into DEG/ENaC channel gating from genetics and structure. Physiology (Bethesda) 27, 282–290. http://dx.doi.org/10.1152/physiol. 00006.2012.
- Eaton, D.C., Helms, M.N., Koval, M., Bao, H.F., Jain, L. 2009. The contribution of epithelial sodium channels to alveolar function in health and disease. Annu. Rev. Physiol. 71, 403–423. http://dx.doi.org/10.1146/annurev.physiol.010908.163250.
- Edelheit, O., Hanukoglu, I., Gizewska, M., Kandemir, N., Tenenbaum-Rakover, Y., Yurdakök, M., Zajaczek, S., Hanukoglu, A., 2005. Novel mutations in epithelial sodium channel (ENaC) subunit genes and phenotypic expression of multisystem pseudohypoaldosteronism. Clin. Endocrinol. 62, 547–553. http://dx.doi.org/10.1111/ j.1365-2265.2005.02255.x.
- Edelheit, O., Hanukoglu, I., Shriki, Y., Tfilin, M., Dascal, N., Gillis, D., Hanukoglu, A., 2010. Truncated beta epithelial sodium channel (ENaC) subunits responsible for multisystem pseudohypoaldosteronism support partial activity of ENaC. J. Steroid Biochem. Mol. Biol. 119, 84–88. http://dx.doi.org/10.1016/j.jsbmb.2010.01.002.
- Edelheit, O., Hanukoglu, I., Dascal, N., Hanukoglu, A., 2011. Identification of the roles of conserved charged residues in the extracellular domain of an epithelial sodium channel (ENaC) subunit by alanine mutagenesis. Am. J. Physiol. Ren. Physiol. 300, F887–F897. http://dx.doi.org/10.1152/ajprenal.00648.2010.
- Edelheit, O., Ben-Shahar, R., Dascal, N., Hanukoglu, A., 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, 2097–2111. http://dx.doi.org/10.1111/febs.12765.
- Ellegren, H., Smeds, L., Burri, R., Olason, P.I., Backström, N., Kawakami, T., Künstner, A., Mäkinen, H., Nadachowska-Brzyska, K., Qvarnström, A., Uebbing, S., Wolf, J.B.W., 2012. The genomic landscape of species divergence in Ficedula flycatchers. Nature 491, 756–760. http://dx.doi.org/10.1038/nature11584.
- Elsik, C.G., Tellam, R.L., Worley, K.C., Gibbs, R.A., Muzny, D.M., Weinstock, G.M., Adelson, D.L., Eichler, E.E., Elnitski, L., Guigo, R., Hamernik, D.L., Kappes, S.M., Lewin, H.A., Lynn, D.J., Nicholas, F.W., Reymond, A., Rijnkels, M., Skow, L.C., Zdobnov, E.M.,

Schook, L., Womack, J., Alioto, T., Antonarakis, S.E., Astashyn, A., Chapple, C.E., Chen, H.-C., Chrast, J., Camara, F., Ermolaeva, O., Henrichsen, C.N., Hlavina, W., Kapustin, Y., Kiryutin, B., Kitts, P., Kokocinski, F., Landrum, M., Maglott, D., Pruitt, K., Sapojnikov, V., Searle, S.M., Solovyev, V., Souvorov, A., Ucla, C., Wyss, C., Anzola, J.M., Gerlach, D., Elhaik, E., Graur, D., Reese, J.T., Edgar, R.C., McEwan, J.C., Payne, G.M., Raison, J.M., Junier, T., Kriventseva, E.V., Eyras, E., Plass, M., Donthu, R., Larkin, D.M., Reecy, J., Yang, M.Q., Chen, L., Cheng, Z., Chitko-McKown, C.G., Liu, G.E., Matukumalli, L.K., Song, J., Zhu, B., Bradley, D.G., Brinkman, F.S.L., Lau, L.P.L., Whiteside, M.D., Walker, A., Wheeler, T., Casey, T., German, J.B., Lemay, D.G., Maqbool, N.J., Molenaar, A.J., Seo, S., Stothard, P., Baldwin, C.L., Baxter, R., Brinkmeyer-Langford, C.L., Brown, W.C., Childers, C.P., Connelley, T., Ellis, S.A., Fritz, K., Glass, E.J., Herzig, C.T.A., livanainen, A., Lahmers, K.K., Bennett, A.K., Dickens, C.M., Gilbert, J.G.R., Hagen, D.E., Salih, H., Aerts, J., Caetano, A.R., Dalrymple, B., Garcia, J.F., Gill, C.A., Hiendleder, S.G., Memili, E., Spurlock, D., Williams, J.L., Alexander, L., Brownstein, M.J., Guan, L., Holt, R.A., Jones, S.J.M., Marra, M.A., Moore, R., Moore, S.S., Roberts, A., Taniguchi, M., Waterman, R.C., Chacko, J., Chandrabose, M.M., Cree, A., Dao, M.D., Dinh, H.H., Gabisi, R.A., Hines, S., Hume, J., Jhangiani, S.N., Joshi, V., Kovar, C.L., Lewis, L.R., Liu, Y.-s., Lopez, J., Morgan, M.B., Nguyen, N.B., Okwuonu, G.O., Ruiz, S.J., Santibanez, J., Wright, R.A., Buhay, C., Ding, Y., Dugan-Rocha, S., Herdandez, J., Holder, M., Sabo, A., Egan, A., Goodell, J., Wilczek-Boney, K., Fowler, G.R., Hitchens, M.E., Lozado, R.J., Moen, C., Steffen, D., Warren, J.T., Zhang, J., Chiu, R., Schein, J.E., Durbin, K.J., Havlak, P., Jiang, H., Liu, Y., Qin, X., Ren, Y., Shen, Y., Song, H., Bell, S.N., Davis, C., Johnson, A.J., Lee, S., Nazareth, L.V., Patel, B.M., Pu, L.-L., Vattathil, S., Williams, R.L., Curry, S., Hamilton, C., Sodergren, E., Wheeler, D.A., Barris, W., Bennett, G.L., Eggen, A., Green, R.D., Harhay, G.P., Hobbs, M., Jann, O., Keele, J.W., Kent, M.P., Lien, S., McKay, S.D., McWilliam, S., Ratnakumar, A., Schnabel, R.D., Smith, T., Snelling, W.M., Sonstegard, T.S., Stone, R.T., Sugimoto, Y., Takasuga, A., Taylor, J.F., Van Tassell, C.P., MacNeil, M.D., Abatepaulo, A.R.R., Abbey, C.A., Ahola, V., Almeida, I.G., Amadio, A.F., Anatriello, E., Bahadue, S.M., Biase, F.H., Boldt, C.R., Carroll, J.A., Carvalho, W.A., Cervelatti, E.P., Chacko, E., Chapin, J.E., Cheng, Y., Choi, J., Colley, A.J., de Campos, T.A., De Donato, M., Santos, I.K.F.d.M., de Oliveira, C.J.F., Deobald, H., Devinoy, E., Donohue, K.E., Dovc, P., Eberlein, A., Fitzsimmons, C.J., Franzin, A.M., Garcia, G.R., Genini, S., Gladney, C.J., Grant, J.R., Greaser, M.L., Green, J.A., Hadsell, D.L., Hakimov, H.A., Halgren, R., Harrow, J.L., Hart, E.A., Hastings, N., Hernandez, M., Hu, Z.-L., Ingham, A., Iso-Touru, T., Jamis, C., Jensen, K., Kapetis, D., Kerr, T., Khalil, S.S., Khatib, H., Kolbehdari, D., Kumar, C.G., Kumar, D., Leach, R., Lee, J.C.-M., Li, C., Logan, K.M., Malinverni, R., Marques, E., Martin, W.F., Martins, N.F., Maruyama, S.R., Mazza, R., McLean, K.L., Medrano, J.F., Moreno, B.T., More, D.D., Muntean, C.T., Nandakumar, H.P., Nogueira, M.F.G., Olsaker, I., Pant, S.D., Panzitta, F., Pastor, R.C.P., Poli, M.A., Poslusny, N., Rachagani, S., Ranganathan, S., Razpet, A., Riggs, P.K., Rincon, G., Rodriguez-Osorio, N., Rodriguez-Zas, S.L., Romero, N.E., Rosenwald, A., Sando, L., Schmutz, S.M., Shen, L., Sherman, L., Southey, B.R., Lutzow, Y.S., Sweedler, J.V., Tammen, I., Telugu, B.P.V.L., Urbanski, J.M., Utsunomiya, Y.T., Verschoor, C.P., Waardenberg, A.J., Wang, Z., Ward, R., Weikard, R., Welsh, T.H., White, S.N., Wilming, L.G., Wunderlich, K.R., Yang, J., Zhao, F.-Q., 2009. The genome sequence of taurine cattle: a window to ruminant biology and evolution. Science 324 (80-.), 522-528. http://dx.doi.org/10.1126/science.1169588.

- Enuka, Y., Hanukoglu, I., Edelheit, O., Vaknine, H., Hanukoglu, A., 2012. Epithelial sodium channels (ENaC) are uniformly distributed on motile cilia in the oviduct and the respiratory airways. Histochem. Cell Biol. 137, 339–353. http://dx.doi.org/10.1007/ s00418-011-0904-1.
- Fajac, I., Viel, M., Sublemontier, S., Hubert, D., Bienvenu, T., 2008. Could a defective epithelial sodium channel lead to bronchiectasis. Respir. Res. 9, 46. http://dx.doi.org/10. 1186/1465-9921-9-46.
- Findling, J.W., Raff, H., Hansson, J.H., Lifton, R.P., 1997. Liddle's syndrome: prospective genetic screening and suppressed aldosterone secretion in an extended kindred. J. Clin. Endocrinol. Metab. 82, 1071–1074. http://dx.doi.org/10.1210/ icem.82.4.3862.
- Firsov, D., Robert-Nicoud, M., Gruender, S., Schild, L., Rossier, B.C., 1999. Mutational analysis of cysteine-rich domains of the epithelium sodium channel (ENaC). Identification of cysteines essential for channel expression at the cell surface. J. Biol. Chem. 274, 2743–2749.
- Fischbarg, J., 2010. Fluid transport across leaky epithelia: central role of the tight junction and supporting role of aquaporins. Physiol. Rev. 90, 1271–1290. http://dx.doi.org/10. 1152/physrev.00025.2009.
- Fitch, W.M., 1970. Distinguishing homologous from analogous proteins. Syst. Zool. 19, 99–113.
- Forslund, K., Sonnhammer, E.L.L., 2012. Evolution of protein domain architectures. Methods Mol. Biol. 856, 187–216. http://dx.doi.org/10.1007/978-1-61779-585-5_8.
- Foxx-Lupo, W.T., Wheatley, C.M., Baker, S.E., Cassuto, N.A., Delamere, N.A., Snyder, E.M., 2011. Genetic variation of the alpha subunit of the epithelial Na⁺ channel influences exhaled Na⁺ in healthy humans. Respir. Physiol. Neurobiol. 179, 205–211. http://dx. doi.org/10.1016/j.resp.2011.08.008.
- Furuhashi, M., Kitamura, K., Adachi, M., Miyoshi, T., Wakida, N., Ura, N., Shikano, Y., Shinshi, Y., Sakamoto, K., Hayashi, M., Satoh, N., Nishitani, T., Tomita, K., Shimamoto, K., 2005. Liddle's syndrome caused by a novel mutation in the prolinerich PY motif of the epithelial sodium channel beta-subunit. J. Clin. Endocrinol. Metab. 90, 340–344. http://dx.doi.org/10.1210/jc.2004-1027.
- Furukawa, Y., Miyawaki, Y., Abe, G., 2006. Molecular cloning and functional characterization of the Aplysia FMRFamide-gated Na⁺ channel. Pflugers Arch. 451, 646–656. http://dx.doi.org/10.1007/s00424-005-1498-z.
- García-Añoveros, J., Derfler, B., Neville-Golden, J., Hyman, B.T., Corey, D.P., 1997. BNaC1 and BNaC2 constitute a new family of human neuronal sodium channels related to degenerins and epithelial sodium channels. Proc. Natl. Acad. Sci. U. S. A. 94, 1459–1464.

- Garty, H., Palmer, L.G., 1997. Epithelial sodium channels: function, structure, and regulation. Physiol. Rev. 77, 359–396.
- Geller, D.S., Rodriguez-Soriano, J., Vallo Boado, A., Schifter, S., Bayer, M., Chang, S.S., Lifton, R.P., 1998. Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. Nat. Genet. 19, 279–281.
- Giraldez, T., Afonso-Oramas, D., Cruz-Muros, I., Garcia-Marin, V., Pagel, P., González-Hernández, T., Alvarez de la Rosa, D., 2007. Cloning and functional expression of a new epithelial sodium channel delta subunit isoform differentially expressed in neurons of the human and monkey telencephalon. J. Neurochem. 102, 1304–1315. http:// dx.doi.org/10.1111/j.1471-4159.2007.04622.x.
- Giraldez, T., Rojas, P., Jou, J., Flores, C., Alvarez de la Rosa, D., 2012. The epithelial sodium channel δ-subunit: new notes for an old song. Am. J. Physiol. Ren. Physiol. 303, F328–F338. http://dx.doi.org/10.1152/ajprenal.00116.2012.
- Gonzales, E.B., Kawate, T., Gouaux, E., 2009. Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. Nature 460, 599–604. http://dx.doi.org/10.1038/ nature08218.
- Goodman, M.B., Ernstrom, G.G., Chelur, D.S., O'Hagan, R., Yao, C.A., Chalfie, M., 2002. MEC-2 regulates C. elegans DEG/ENaC channels needed for mechanosensation. Nature 415, 1039–1042.
- Green, R.E., Braun, E.L., Armstrong, J., Earl, D., Nguyen, N., Hickey, G., Vandewege, M.W., St. John, J.A., Capella-Gutierrez, S., Castoe, T.A., Kern, C., Fujita, M.K., Opazo, J.C., Jurka, J., Kojima, K.K., Caballero, J., Hubley, R.M., Smit, A.F., Platt, R.N., Lavoie, C.A., Ramakodi, M.P., Finger, J.W., Suh, A., Isberg, S.R., Miles, L., Chong, A.Y., Jaratlerdsiri, W., Gongora, J., Moran, C., Iriarte, A., McCormack, J., Burgess, S.C., Edwards, S.V., Lyons, E., Williams, C., Breen, M., Howard, J.T., Gresham, C.R., Peterson, D.G., Schmitz, J., Pollock, D.D., Haussler, D., Triplett, E.W., Zhang, G., Irie, N., Jarvis, E.D., Brochu, C.A., Schmidt, C.J., McCarthy, F.M., Faircloth, B.C., Hoffmann, F.G., Glenn, T.C., Gabaldon, T., Paten, B., Ray, D.A., 2014. Three crocodilian genomes reveal ancestral patterns of evolution among archosaurs. Science 346 (80-.), 1254449. http://dx.doi.org/10. 1126/science.1254449.
- Greig, E.R., Mathialahan, T., Boot-Handford, R.P., Sandle, G.I., 2003. Molecular and functional studies of electrogenic Na⁺ transport in the distal colon and rectum of young and elderly subjects. Gut 52, 1607–1615.
- Gründer, S., Assmann, M., 2015. Peptide-gated ion channels and the simple nervous system of *Hydra*. J. Exp. Biol. 218, 551–561. http://dx.doi.org/10.1242/jeb.111666.
- Gründer, S., Pusch, M., 2015. Biophysical properties of acid-sensing ion channels (ASICs). Neuropharmacology http://dx.doi.org/10.1016/j.neuropharm.2014.12.016.
- Gründer, S., Firsov, D., Chang, S.S., Jaeger, N.F., Gautschi, I., Schild, L., Lifton, R.P., Rossier, B.C., 1997. A mutation causing pseudohypoaldosteronism type 1 identifies a conserved glycine that is involved in the gating of the epithelial sodium channel. EMBO J. 16, 899–907. http://dx.doi.org/10.1093/emboj/16.5.899.
- Gründer, S., Jaeger, N.F., Gautschi, I., Schild, L., Rossier, B.C., 1999. Identification of a highly conserved sequence at the N-terminus of the epithelial Na⁺ channel alpha subunit involved in gating. Pflugers Arch. 438, 709–715.
 Gu, Y., 2008. Effects of [Ca²⁺]_i and pH on epithelial Na⁺ channel activity of cultured
- Gu, Y., 2008. Effects of [Ca²⁺]_i and pH on epithelial Na⁺ channel activity of cultured mouse cortical collecting ducts. J. Exp. Biol. 211, 3167–3173. http://dx.doi.org/10. 1242/jeb.019646.
- Gwiazda, K., Bonifacio, G., Vullo, S., Kellenberger, S., 2015. Extracellular subunit interactions control transitions between functional states of acid-sensing ion channel 1a. J. Biol. Chem. http://dx.doi.org/10.1074/jbc.M115.641688.
- Hager, H., Kwon, T.H., Vinnikova, A.K., Masilamani, S., Brooks, H.L., Frøkiaer, J., Knepper, M.A., Nielsen, S., 2001. Immunocytochemical and immunoelectron microscopic localization of alpha-, beta-, and gamma-ENaC in rat kidney. Am. J. Physiol. Ren. Physiol. 280, F1093–F1106.
- Hannila-Handelberg, T., Kontula, K., Tikkanen, I., Tikkanen, T., Fyhrquist, F., Helin, K., Fodstad, H., Piippo, K., Miettinen, H.E., Virtamo, J., Krusius, T., Sarna, S., Gautschi, I., Schild, L., Hiltunen, T.P., 2005. Common variants of the beta and gamma subunits of the epithelial sodium channel and their relation to plasma renin and aldosterone levels in essential hypertension. BMC Med. Genet. 6, 4 (BMC medical genetics).
- Hansson, J.H., Nelson-Williams, C., Suzuki, H., Schild, L., Shimkets, R., Lu, Y., Canessa, C., Iwasaki, T., Rossier, B., Lifton, R.P., 1995. Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. Nat. Genet. 11, 76–82.
- 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, 936–944. http://dx.doi.org/10.1210/jcem-73-5-936.
- Hanukoglu, A., Hanukoglu, I., 2010. Clinical improvement in patients with autosomal recessive pseudohypoaldosteronism and the necessity for salt supplementation. Clin. Exp. Nephrol. 14, 518–519. http://dx.doi.org/10.1007/s10157-010-0326-8.
- Hanukoglu, A., Bistritzer, T., Rakover, Y., Mandelberg, A., 1994. Pseudohypoaldosteronism with increased sweat and saliva electrolyte values and frequent lower respiratory tract infections mimicking cystic fibrosis. J. Pediatr. 125, 752–755.
- Hanukoglu, A., Edelheit, O., Shriki, Y., Gizewska, M., Dascal, N., Hanukoglu, I., 2008. Reninaldosterone 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, 268–274. http://dx.doi.org/10.1016/j.jsbmb.2008. 06.013.
- Hellsten, U., Harland, R.M., Gilchrist, M.J., Hendrix, D., Jurka, J., Kapitonov, V., Ovcharenko, I., Putnam, N.H., Shu, S., Taher, L., Blitz, I.L., Blumberg, B., Dichmann, D.S., Dubchak, I., Amaya, E., Detter, J.C., Fletcher, R., Gerhard, D.S., Goodstein, D., Graves, T., Grigoriev, I.V., Grimwood, J., Kawashima, T., Lindquist, E., Lucas, S.M., Mead, P.E., Mitros, T., Ogino, H., Ohta, Y., Poliakov, A.V., Pollet, N., Robert, J., Salamov, A., Sater, A.K., Schmutz, J., Terry, A., Vize, P.D., Warren, W.C., Wells, D., Wills, A., Wilson, R.K., Zimmerman, L.B., Zorn, A.M., Grainger, R., Grammer, T., Khokha, M.K., Richardson, P.M., Rokhsar, D.S., 2010. The genome of the Western clawed frog *Xenopus tropicalis*. Science 328 (80-), 633–636. http://dx.doi.org/10.1126/science.1183670.

Hille, B., Dickson, E.J., Kruse, M., Vivas, O., Suh, B.-C., 2015. Phosphoinositides regulate ion channels. Biochim. Biophys. Acta 1851, 844–856. http://dx.doi.org/10.1016/j.bbalip. 2014.09.010.

- Hiltunen, T.P., Hannila-Handelberg, T., Petäjäniemi, N., Kantola, I., Tikkanen, I., Virtamo, J., Gautschi, I., Schild, L., Kontula, K., 2002. Liddle's syndrome associated with a point mutation in the extracellular domain of the epithelial sodium channel gamma subunit. J. Hypertens. 20, 2383–2390. http://dx.doi.org/10.1097/01.hjh.0000042881. 24999.fc.
- Hobbs, C.A., Da Tan, C., Tarran, R., 2013. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? J. Physiol. 591, 4377–4387. http://dx.doi. org/10.1113/jphysiol.2012.240861.
- Hogg, R.J., Marks, J.F., Marver, D., Frolich, J.C., 1991. Long term observations in a patient with pseudohypoaldosteronism. Pediatr. Nephrol. 5, 205–210.
- Hollenhorst, M.I., Richter, K., Fronius, M., 2011. Ion transport by pulmonary epithelia. J. Biomed. Biotechnol. 2011, 174306. http://dx.doi.org/10.1155/2011/174306.
- Hollier, J.M., Martin, D.F., Bell, D.M., Li, J.-L., Chirachanchai, M.G., Menon, D.V., Leonard, D., Wu, X., Cooper, R.S., McKenzie, C., Victor, R.G., Auchus, R.J., 2006. Epithelial sodium channel allele T594M is not associated with blood pressure or blood pressure response to amiloride. Hypertension 47, 428–433. http://dx.doi.org/10.1161/01.HYP. 0000200704.45994.ff.
- Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., Clark, A.G., Ribeiro, J.M.C., Wides, R., Salzberg, S.L., Loftus, B., Yandell, M., Majoros, W.H., Rusch, D.B., Lai, Z., Kraft, C.L., Abril, J.F., Anthouard, V., Arensburger, P., Atkinson, P.W., Baden, H., de Berardinis, V., Baldwin, D., Benes, V., Biedler, J., Blass, C., Bolanos, R., Boscus, D., Barnstead, M., Cai, S., Center, A., Chaturverdi, K., Christophides, G.K., Chrystal, M.A., Clamp, M., Cravchik, A., Curwen, V., Dana, A., Delcher, A., Dew, I., Evans, C.A., Flanigan, M., Grundschober-Freimoser, A., Friedli, L., Gu, Z., Guan, P., Guigo, R., Hillenmeyer, M.E., Hladun, S.L., Hogan, J.R., Hong, Y.S., Hoover, J., Jaillon, O., Ke, Z., Kodira, C., Kokoza, E., Koutsos, A., Letunic, I., Levitsky, A., Liang, Y., Lin, J.-J., Lobo, N.F., Lopez, J.R., Malek, J.A., McIntosh, T.C., Meister, S., Miller, J., Mobarry, C., Mongin, E., Murphy, S.D., O'Brochta, D.A., Pfannkoch, C., Qi, R., Regier, M.A., Remington, K., Shao, H., Sharakhova, M.V., Sitter, C.D., Shetty, J., Smith, T.J., Strong, R., Sun, J., Thomasova, D., Ton, L.Q., Topalis, P., Tu, Z., Unger, M.F., Walenz, B., Wang, A., Wang, J., Wang, M., Wang, X., Woodford, K.J., Wortman, J.R., Wu, M., Yao, A., Zdobnov, E.M., Zhang, H., Zhao, Q., Zhao, S., Zhu, S.C., Zhimulev, I., Coluzzi, M., della Torre, A., Roth, C.W., Louis, C., Kalush, F., Mural, R.J., Myers, E.W., Adams, M.D., Smith, H.O., Broder, S., Gardner, M.J., Fraser, C.M., Birney, E., Bork, P., Brey, P.T., Venter, J.C., Weissenbach, J., Kafatos, F.C., Collins, F.H., Hoffman, S.L., 2002. The genome sequence of the malaria mosquito Anopheles gambiae. Science 298 (80-.), 129-149. http://dx.doi.org/10.1126/science.1076181.
- ogy 94, 72–79. http://dx.doi.org/10.1016/j.neuropharm.2014.12.009.
- Hong, H., Park, S., Jiménez, R.H.F., Kinehart, D., Tamm, L.K., 2007. Role of aromatic side chains in the folding and thermodynamic stability of integral membrane proteins. J. Am. Chem. Soc. 129, 8320–8327. http://dx.doi.org/10.1021/ja0688490.
- Hubé, F., Francastel, C., 2015. Mammalian introns: when the junk generates molecular diversity. Int. J. Mol. Sci. 16, 4429–4452. http://dx.doi.org/10.3390/ ijms16034429.
- Hughey, R.P., Bruns, J.B., Kinlough, C.L., Harkleroad, K.L., Tong, Q., Carattino, M.D., Johnson, J.P., Stockand, J.D., Kleyman, T.R., 2004. Epithelial sodium channels are activated by furin-dependent proteolysis. J. Biol. Chem. 279, 18111–18114.
- Hummler, E., Barker, P., Gatzy, J., Beermann, F., Verdumo, C., Schmidt, A., Boucher, R., Rossier, B.C., 1996. Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. Nat. Genet. 12, 325–328.
- Ilatovskaya, D.V., Pavlov, T.S., Neguliyaev, Y.A., Starushchenko, A., 2012. Mechanisms of epithelial sodium channel (ENaC) regulation by cortactin: involvement of dynamin. Cell Tissue Biol. 6, 52–59.
- Ishikita, H., 2011. Proton-binding sites of acid-sensing ion channel 1. PLoS One 6, e16920. http://dx.doi.org/10.1371/journal.pone.0016920.
- Jasti, J., Furukawa, H., Gonzales, E.B., Gouaux, E., 2007. Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. Nature 449, 316–323.
- Jex, A.R., Nejsum, P., Schwarz, E.M., Hu, L., Young, N.D., Hall, R.S., Korhonen, P.K., Liao, S., Thamsborg, S., Xia, J., Xu, P., Wang, S., Scheerlinck, J.-P.Y., Hofmann, A., Sternberg, P.W., Wang, J., Gasser, R.B., 2014. Genome and transcriptome of the porcine whipworm *Trichuris suis*. Nat. Genet. 46, 701–706. http://dx.doi.org/10. 1038/ng.3012.
- Ji, H.L., Chalfant, M.L., Jovov, B., Lockhart, J.P., Parker, S.B., Fuller, C.M., Stanton, B.A., Benos, D.J., 2000. The cytosolic termini of the beta- and gamma-ENAC subunits are involved in the functional interactions between cystic fibrosis transmembrane conductance regulator and epithelial sodium channel. J. Biol. Chem. 275, 27947–27956. http:// dx.doi.org/10.1074/jbc.M002848200.
- Ji, H.-L., Zhao, R.-Z., Chen, Z.-X., Shetty, S., Idell, S., Matalon, S., 2012. & ENAC: a novel divergent amiloride-inhibitable sodium channel. Am. J. Physiol. Lung Cell Mol. Physiol. 303, L1013–L1026. http://dx.doi.org/10.1152/ajplung.00206.2012.
- Jiang, Y., Xu, J., Chen, Y., Shi, J., Zhang, C., Li, J., 2015. Expression and distribution of epithelial sodium channel in nasal polyp and nasal mucosa. Eur. Arch. Otorhinolaryngol. http://dx.doi.org/10.1007/s00405-014-3477-5.
- Käll, L., Krogh, A., Sonnhammer, E.L.L., 2004. A combined transmembrane topology and signal peptide prediction method. J. Mol. Biol. 338, 1027–1036. http://dx.doi.org/10. 1016/j.jmb.2004.03.016.
- Kashlan, O.B., Kleyman, T.R., 2011. ENaC structure and function in the wake of a resolved structure of a family member. Am. J. Physiol. Ren. Physiol. 301, F684–F696.
- Kashlan, O.B., Kleyman, T.R., 2012. Epithelial Na⁺ channel regulation by cytoplasmic and extracellular factors. Exp. Cell Res. 318, 1011–1019. http://dx.doi.org/10.1016/j.yexcr. 2012.02.024.

- Kashlan, O.B., Sheng, S., Kleyman, T.R., 2005. On the interaction between amiloride and its putative alpha-subunit epithelial Na⁺ channel binding site. J. Biol. Chem. 280, 26206–26215.
- Kashlan, O.B., Maarouf, A.B., Kussius, C., Denshaw, R.M., Blumenthal, K.M., Kleyman, T.R., 2006. Distinct structural elements in the first membrane-spanning segment of the epithelial sodium channel. J. Biol. Chem. 281, 30455–30462.
- Kashlan, O.B., Boyd, C.R., Argyropoulos, C., Okumura, S., Hughey, R.P., Grabe, M., Kleyman, T.R., 2010. Allosteric inhibition of the epithelial Na⁺ channel through peptide binding at peripheral finger and thumb domains. J. Biol. Chem. 285, 35216–35223.
- Kashlan, O.B., Adelman, J.L., Okumura, S., Blobner, B.M., Zuzek, Z., Hughey, R.P., Kleyman, T.R., Grabe, M., 2011. Constraint-based, homology model of the extracellular domain of the epithelial Na⁺ channel alpha subunit reveals a mechanism of channel activation by proteases. J. Biol. Chem. 286, 649–660.
- Kashlan, O.B., Blobner, B.M., Zuzek, Z., Tolino, M., Kleyman, T.R., 2015. Na⁺ inhibits the epithelial Na⁺ channel by binding to a site in an extracellular acidic cleft. J Biol Chem 290, 568–576. http://dx.doi.org/10.1074/jbc.M114.606152.
- Kawasaki, K., Weiss, K.M., 2006. Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. J. Exp. Zool. B Mol. Dev. Evol. 306, 295–316. http://dx.doi.org/10.1002/jez.b.21088.
- Kellenberger, S., Schild, L., 2002. Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. Physiol. Rev. 82, 735–767.
- Kellenberger, S., 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. http://dx.doi.org/10.1124/pr.114. 009225.
- Kellenberger, S., Gautschi, I., Schild, L., 1999. A single point mutation in the pore region of the epithelial Na⁺ channel changes ion selectivity by modifying molecular sieving. Proc. Natl. Acad. Sci. U. S. A. 96, 4170–4175.
- Kellenberger, S., Gautschi, I., Schild, L., 2002. An external site controls closing of the epithelial Na⁺ channel ENaC. J. Physiol. 543, 413–424.
- Kerem, E., Bistritzer, T., Hanukoglu, A., Hofmann, T., Zhou, Z., Bennett, W., MacLaughlin, E., Barker, P., Nash, M., Quittell, L., Boucher, R., Knowles, M.R., 1999. Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. N. Engl. J. Med. 341, 156–162.
- Kim, E., Magen, A., Ast, G., 2007. Different levels of alternative splicing among eukaryotes. Nucleic Acids Res. 35, 125–131. http://dx.doi.org/10.1093/nar/gkl924.
- Kim, H.S., Murphy, T., Xia, J., Caragea, D., Park, Y., Beeman, R.W., Lorenzen, M.D., Butcher, S., Manak, J.R., Brown, S.J., 2009. BeetleBase in 2010: revisions to provide comprehensive genomic information for *Tribolium castaneum*. Nucleic Acids Res. 38, D437–D442. http://dx.doi.org/10.1093/nar/gkp807.
- Kleyman, T.R., Cragoe, E.J., 1988. Amiloride and its analogs as tools in the study of ion transport. J. Membr. Biol. 105, 1–21.
- Kleyman, T.R., Carattino, M.D., Hughey, R.P., 2009. ENAC at the cutting edge: regulation of epithelial sodium channels by proteases. J. Biol. Chem. 284, 20447–20451.
- Koonin, E.V., 2005. Orthologs, paralogs, and evolutionary genomics. Annu. Rev. Genet. 39, 309–338. http://dx.doi.org/10.1146/annurev.genet.39.073003.114725.
- Krauson, A.J., Rued, A.C., Carattino, M.D., 2013. Independent contribution of extracellular proton binding sites to ASIC1a activation. J. Biol. Chem. 288, 34375–34383. http:// dx.doi.org/10.1074/jbc.M113.504324.
- Kretz, O., Barbry, P., Bock, R., Lindemann, B., 1999. Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat. J. Histochem. Cytochem. 47, 51–64.
- Krueger, B., Schlötzer-Schrehardt, U., Haerteis, S., Zenkel, M., Chankiewitz, V.E., Amann, K.U., Kruse, F.E., Korbmacher, C., 2012. Four subunits (αβγδ) of the epithelial sodium channel (ENaC) are expressed in the human eye in various locations. Invest. Ophthalmol. Vis. Sci. 53, 596–604. http://dx.doi.org/10.1167/iovs.11-8581.
- Kunzelmann, K., Bachhuber, T., Regeer, R., Markovich, D., Sun, J., Schreiber, R., 2005. Purinergic inhibition of the epithelial Na⁺ transport via hydrolysis of PIP2. FASEB J. 19, 142–143. http://dx.doi.org/10.1096/fj.04-2314fje.
- Kuratani, S., 2012. Evolution of the vertebrate jaw from developmental perspectives. Evol. Dev. 14, 76–92. http://dx.doi.org/10.1111/j.1525-142X.2011.00523.x.
- Kusche-Vihrog, K., Tarjus, A., Fels, J., Jaisser, F., 2014. The epithelial Na⁺ channel: a new player in the vasculature. Curr. Opin. Nephrol. Hypertens. 23, 143–148. http://dx. doi.org/10.1097/01.mnh.0000441054.88962.2c.
- Langloh, A.L., Berdiev, B., Ji, H.L., Keyser, K., Stanton, B.A., Benos, D.J., 2000. Charged residues in the M2 region of alpha-hENaC play a role in channel conductance. Am. J. Physiol. Cell Physiol. 278, C277–C291.
- Lefèvre, C.M.T., Diakov, A., Haerteis, S., Korbmacher, C., Gründer, S., Wiemuth, D., 2014. Pharmacological and electrophysiological characterization of the human bile acidsensitive ion channel (hBASIC). Pflugers Arch. 466, 253–263. http://dx.doi.org/10. 1007/s00424-013-1310-4.
- Lemos, B., Bettencourt, B.R., Meiklejohn, C.D., Hartl, D.L., 2005. Evolution of proteins and gene expression levels are coupled in *Drosophila* and are independently associated with mRNA abundance, protein length, and number of protein–protein interactions. Mol. Biol. Evol. 22, 1345–1354. http://dx.doi.org/10.1093/molbev/msi122.
- Li, T., Yang, Y., Canessa, C.M., 2010. Asn415 in the beta11-beta12 linker decreases protondependent desensitization of ASIC1. J. Biol. Chem. 285, 31285–31291. http://dx.doi. org/10.1074/jbc.M110.160382.
- Li, T., Yang, Y., Canessa, C.M., 2011. Outlines of the pore in open and closed conformations describe the gating mechanism of ASIC1. Nat. Commun. 2, 399. http://dx.doi.org/10. 1038/ncomms1409.
- Liddle, G.W., Bledsoe, T., Coppage, W.S.J., 1963. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. Trans. Assoc. Am. Phys. 76, 199–213.
- Lin, S.-H., Sun, W.-H., Chen, C.-C., 2015. Genetic exploration of the role of acid-sensing ion channels. Neuropharmacology http://dx.doi.org/10.1016/j.neuropharm.2014.12.011.

- Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., Mauceli, E., Xie, X., Breen, M., Wayne, R.K., Ostrander, E.A., Ponting, C.P., Galibert, F., Smith, D.R., DeJong, P.J., Kirkness, E., Alvarez, P., Biagi, T., Brockman, W., Butler, J., Chin, C.-W., Cook, A., Cuff, J., Daly, M.J., DeCaprio, D., Gnerre, S., Grabherr, M., Kellis, M., Kleber, M., Bardeleben, C., Goodstadt, L., Heger, A., Hitte, C., Kim, L., Koepfli, K.-P., Parker, H.G., Pollinger, J.P., Searle, S.M.J., Sutter, N.B., Thomas, R., Webber, C., Baldwin, J., Abebe, A., Abouelleil, A., Aftuck, L., Ait-Zahra, M., Aldredge, T., Allen, N., An, P., Anderson, S., Antoine, C., Arachchi, H., Aslam, A., Ayotte, L., Bachantsang, P., Barry, A., Bayul, T., Benamara, M., Berlin, A., Bessette, D., Blitshteyn, B., Bloom, T., Blye, J., Boguslavskiy, L., Bonnet, C., Boukhgalter, B., Brown, A., Cahill, P., Calixte, N., Camarata, J., Cheshatsang, Y., Chu, J., Citroen, M., Collymore, A., Cooke, P., Dawoe, T., Daza, R., Decktor, K., DeGray, S., Dhargay, N., Dooley, K., Dooley, K., Dorje, P., Dorjee, K., Dorris, L., Duffey, N., Dupes, A., Egbiremolen, O., Elong, R., Falk, J., Farina, A., Faro, S., Ferguson, D., Ferreira, P., Fisher, S., FitzGerald, M., Foley, K., Foley, C., Franke, A., Friedrich, D., Gage, D., Garber, M., Gearin, G., Giannoukos, G., Goode, T., Goyette, A., Graham, J., Grandbois, E., Gyaltsen, K., Hafez, N., Hagopian, D., Hagos, B., Hall, J., Healy, C., Hegarty, R., Honan, T., Horn, A., Houde, N., Hughes, L., Hunnicutt, L., Husby, M., Jester, B., Jones, C., Kamat, A., Kanga, B., Kells, C., Khazanovich, D., Kieu, A.C., Kisner, P., Kumar, M., Lance, K., Landers, T., Lara, M., Lee, W., Leger, J.-P., Lennon, N., Leuper, L., LeVine, S., Liu, J., Liu, X., Lokyitsang, Y., Lokyitsang, T., Lui, A., Macdonald, J., Major, J., Marabella, R., Maru, K., Matthews, C., McDonough, S., Mehta, T., Meldrim, J., Melnikov, A., Meneus, L., Mihalev, A., Mihova, T., Miller, K., Mittelman, R., Mlenga, V., Mulrain, L., Munson, G., Navidi, A., Naylor, J., Nguyen, T., Nguyen, N., Nguyen, C., Nguyen, T., Nicol, R., Norbu, N., Norbu, C., Novod, N., Nyima, T., Olandt, P., O'Neill, B., O'Neill, K., Osman, S., Oyono, L., Patti, C., Perrin, D., Phunkhang, P., Pierre, F., Priest, M., Rachupka, A., Raghuraman, S., Rameau, R., Ray, V., Raymond, C., Rege, F., Rise, C., Rogers, J., Rogov, P., Sahalie, J., Settipalli, S., Sharpe, T., Shea, T., Sheehan, M., Sherpa, N., Shi, J., Shih, D., Sloan, J., Smith, C., Sparrow, T., Stalker, J., Stange-Thomann, N., Stavropoulos, S., Stone, C., Stone, S., Sykes, S., Tchuinga, P., Tenzing, P., Tesfaye, S., Thoulutsang, D., Thoulutsang, Y., Topham, K., Topping, I., Tsamla, T., Vassiliev, H., Venkataraman, V., Vo, A., Wangchuk, T., Wangdi, T., Weiand, M., Wilkinson, J., Wilson, A., Yadav, S., Yang, S., Yang, X., Young, G., Yu, Q., Zainoun, J., Zembek, L., Zimmer, A., Lander, E.S., 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438, 803-819. http://dx.doi.org/10.1038/ nature04338.
- Lingueglia, E., Voilley, N., Waldmann, R., Lazdunski, M., 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, 95–99.
- Lingueglia, E., Deval, E., Lazdunski, M., 2006. FMRFamide-gated sodium channel and ASIC channels: a new class of ionotropic receptors for FMRFamide and related peptides. Peptides 27, 1138–1152. http://dx.doi.org/10.1016/j.peptides.2005.06.037.
- Liu, F., Yang, X., Mo, X., Huang, J., Chen, J., Kelly, T.N., Hixson, J.E., Rao, D.C., Gu, C.C., Shimmin, L.C., Rice, T.K., Li, J., Schwander, K., He, J., Liu, D.-P., Gu, D., 2015. Associations of epithelial sodium channel genes with blood pressure: the GenSalt study. J. Hum. Hypertens. 29, 224–228. http://dx.doi.org/10.1038/jhh.2014.78.
- Locke, D.P., Hillier, L.W., Warren, W.C., Worley, K.C., Nazareth, L.V., Muzny, D.M., Yang, S.-P., Wang, Z., Chinwalla, A.T., Minx, P., Mitreva, M., Cook, L., Delehaunty, K.D., Fronick, C., Schmidt, H., Fulton, L.A., Fulton, R.S., Nelson, J.O., Magrini, V., Pohl, C., Graves, T.A., Markovic, C., Cree, A., Dinh, H.H., Hume, J., Kovar, C.L., Fowler, G.R., Lunter, G., Meader, S., Heger, A., Ponting, C.P., Marques-Bonet, T., Alkan, C., Chen, L., Cheng, Z., Kidd, J.M., Eichler, E.E., White, S., Searle, S., Vilella, A.J., Chen, Y., Flicek, P., Ma, J., Raney, B., Suh, B., Burhans, R., Herrero, J., Haussler, D., Faria, R., Fernando, O., Darré, F., Farré, D., Gazave, E., Oliva, M., Navarro, A., Roberto, R., Capozzi, O., Archidiacono, N., Della Valle, G., Purgato, S., Rocchi, M., Konkel, M.K., Walker, J.A., Ullmer, B., Batzer, M.A., Smit, A.F.A., Hubley, R., Casola, C., Schrider, D.R., Hahn, M.W., Quesada, V., Puente, X.S., Ordoñez, G.R., López-Otín, C., Vinar, T., Brejova, B., Ratan, A., Harris, R.S., Miller, W., Kosiol, C., Lawson, H.A., Taliwal, V., Martins, A.L., Siepel, A., Roychoudhury, A., Ma, X., Degenhardt, J., Bustamante, C.D., Gutenkunst, R.N., Mailund, T., Dutheil, J.Y., Hobolth, A., Schierup, M.H., Ryder, O.A., Yoshinaga, Y., de Jong, P.J., Weinstock, G.M., Rogers, J., Mardis, E.R., Gibbs, R.A., Wilson, R.K., 2011. Comparative and demographic analysis of orangutan genomes. Nature 469, 529-533. http://dx.doi.org/10.1038/ nature09687.
- Lu, C., Pribanic, S., Debonneville, A., Jiang, C., Rotin, D., 2007. The PY motif of ENaC, mutated in Liddle syndrome, regulates channel internalization, sorting and mobilization from subapical pool. Traffic 8, 1246–1264. http://dx.doi.org/10.1111/j.1600-0854.2007.00602.x.
- Ludwig, M., Bolkenius, U., Wickert, L., Marynen, P., Bidlingmaier, F., 1998. Structural organisation of the gene encoding the alpha-subunit of the human amiloride-sensitive epithelial sodium channel. Hum. Genet. 102, 576–581.
- Lyons, R.A., Saridogan, E., Djahanbakhch, O., 2006. The reproductive significance of human fallopian tube cilia. Hum. Reprod. Update 12, 363–372.
- Ma, B.-G., Chen, L., Ji, H.-F., Chen, Z.-H., Yang, F.-R., Wang, L., Qu, G., Jiang, Y.-Y., Ji, C., Zhang, H.-Y., 2008. Characters of very ancient proteins. Biochem. Biophys. Res. Commun. 366, 607–611. http://dx.doi.org/10.1016/j.bbrc.2007.12.014.
- Macias, M.J., Wiesner, S., Sudol, M., 2002. WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. FEBS Lett. 513, 30–37.
- Mall, M., Grubb, B.R., Harkema, J.R., O'Neal, W.K., Boucher, R.C., 2004. Increased airway epithelial Na⁺ absorption produces cystic fibrosis-like lung disease in mice. Nat. Med. 10, 487–493. http://dx.doi.org/10.1038/nm1028.
- Mall, M.A., Button, B., Johannesson, B., Zhou, Z., Livraghi, A., Caldwell, R.A., Schubert, S.C., Schultz, C., O'Neal, W.K., Pradervand, S., Hummler, E., Rossier, B.C., Grubb, B.R., Boucher, R.C., 2010. Airway surface liquid volume regulation determines different airway phenotypes in liddle compared with betaENaC-overexpressing mice. J. Biol. Chem. 285, 26945–26955. http://dx.doi.org/10.1074/jbc.M110. 151803.

- Martinerie, L., Pussard, E., Foix-L'Hélias, L., Petit, F., Cosson, C., Boileau, P., Lombès, M., 2009. Physiological partial aldosterone resistance in human newborns. Pediatr. Res. 66, 323–328. http://dx.doi.org/10.1203/PDR.0b013e3181b1bbec.
- Masilamani, S., Kim, G.H., Mitchell, C., Wade, J.B., Knepper, M.A., 1999. Aldosteronemediated regulation of ENaC alpha, beta, and gamma subunit proteins in rat kidney. J. Clin. Invest. 104, R19–R23.
- Mazzochi, C., Bubien, J.K., Smith, P.R., Benos, D.J., 2006. The carboxyl terminus of the alpha-subunit of the amiloride-sensitive epithelial sodium channel binds to F-actin. J. Biol. Chem. 281, 6528–6538. http://dx.doi.org/10.1074/jbc.M509386200.
- McDonald, F.J., Snyder, P.M., McCray, P.B., Welsh, M.J., 1994. Cloning, expression, and tissue distribution of a human amiloride-sensitive Na⁺ channel. Am. J. Physiol. 266, L728–L734.
- McDonald, F.J., Price, M.P., Snyder, P.M., Welsh, M.J., 1995. Cloning and expression of the beta- and gamma-subunits of the human epithelial sodium channel. Am. J. Physiol. 268, C1157–C1163.
- Mikkelsen, T.S., Wakefield, M.J., Aken, B., Amemiya, C.T., Chang, J.L., Duke, S., Garber, M., Gentles, A.J., Goodstadt, L., Heger, A., Jurka, J., Kamal, M., Mauceli, E., Searle, S.M.J., Sharpe, T., Baker, M.L., Batzer, M.A., Benos, P.V., Belov, K., Clamp, M., Cook, A., Cuff, J., Das, R., Davidow, L., Deakin, J.E., Fazzari, M.J., Glass, J.L., Grabherr, M., Greally, J.M., Gu, W., Hore, T.A., Huttley, G.A., Kleber, M., Jirtle, R.L., Koina, E., Lee, J.T., Mahony, S., Marra, M.A., Miller, R.D., Nicholls, R.D., Oda, M., Papenfuss, A.T., Parra, Z.E., Pollock, D.D., Ray, D.A., Schein, J.E., Speed, T.P., Thompson, K., VandeBerg, J.L., Wade, C.M., Walker, J.A., Waters, P.D., Webber, C., Weidman, J.R., Xie, X., Zody, M.C., Graves, J.A.M., Ponting, C.P., Breen, M., Samollow, P.B., Lander, E.S., Lindblad-Toh, K., 2007. Genome of the marsupial *Monodelphis domestica* reveals innovation in non-coding secuences. Nature 447, 167–177. http://dx.doi.org/10.1038/nature05805.
- Miller, R.L., Loewy, A.D., 2013. ENaC γ -expressing astrocytes in the circumventricular organs, white matter, and ventral medullary surface: sites for Na⁺ regulation by glial cells. J. Chem. Neuroanat. 53, 72–80. http://dx.doi.org/10.1016/j.jchemneu.2013.10. 002.
- Miller, W., Hayes, V.M., Ratan, A., Petersen, D.C., Wittekindt, N.E., Miller, J., Walenz, B., Knight, J., Qi, J., Zhao, F., Wang, Q., Bedoya-Reina, O.C., Katiyar, N., Tomsho, L.P., Kasson, L.M., Hardie, R.-A., Woodbridge, P., Tindall, E.A., Bertelsen, M.F., Dixon, D., Pyecroft, S., Helgen, K.M., Lesk, A.M., Pringle, T.H., Patterson, N., Zhang, Y., Kreiss, A., Woods, G.M., Jones, M.E., Schuster, S.C., 2011. Genetic diversity and population structure of the endangered marsupial *Sarcophilus harrisii* (Tasmanian devil). Proc. Natl. Acad. Sci. U. S. A. 108, 12348–12353. http://dx.doi.org/10.1073/pnas. 1102838108.
- Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., Worm, B., 2011. How many species are there on Earth and in the ocean? PLoS Biol. 9, e1001127. http://dx.doi.org/10.1371/ journal.pbio.1001127.
- Morris, L.M., DeGagne, J.M., Kempton, J.B., Hausman, F., Trune, D.R., 2012. Mouse middle ear ion homeostasis channels and intercellular junctions. PLoS One 7, e39004. http://dx.doi.org/10.1371/journal.pone.0039004.
- Murzin, A.G., Brenner, S.E., Hubbard, T., Chothia, C., 1995. SCOP: a structural classification of proteins database for the investigation of sequences and structures. J. Mol. Biol. 247, 536–540. http://dx.doi.org/10.1006/jmbi.1995.0159.
- Mutesa, L., Azad, A.K., Verhaeghe, C., Segers, K., Vanbellinghen, J.F., Ngendahayo, L., Rusingiza, E.K., Mutwa, P.R., Rulisa, S., Koulischer, L., Cassiman, J.J., Cuppens, H., Bours, V., 2009. Genetic analysis of Rwandan patients with cystic fibrosis-like symptoms: identification of novel cystic fibrosis transmembrane conductance regulator and epithelial sodium channel gene variants. Chest 135, 1233–1242.
- Nagel, G., Barbry, P., Chabot, H., Brochiero, E., Hartung, K., Grygorczyk, R., 2005. CFTR fails to inhibit the epithelial sodium channel ENAC expressed in *Xenopus laevis* oocytes. J. Physiol. 564, 671–682. http://dx.doi.org/10.1113/jphysiol.2004.079046.
- Nicholas, K.B., Deerfield, D.W.I.I., 1997. GeneDoc: analysis and visualization of genetic variation. EMBnew. News 4, 14.
- Oka, Y., Butnaru, M., von Buchholtz, L., Ryba, N.J.P., Zuker, C.S., 2013. High salt recruits aversive taste pathways. Nature 494, 472–475. http://dx.doi.org/10.1038/ nature11905.
- Omasits, U., Ahrens, C.H., Müller, S., Wollscheid, B., 2014. Protter: interactive protein feature visualization and integration with experimental proteomic data. Bioinformatics 30, 884–886. http://dx.doi.org/10.1093/bioinformatics/btt607.
- Omerbašić, D., Schuhmacher, L.-N., Bernal Sierra, Y.-A., Smith, E.S.J., Lewin, G.R., 2014. ASICs and mammalian mechanoreceptor function. Neuropharmacology http://dx. doi.org/10.1016/j.neuropharm.2014.12.007.
- Ottaviani, E., Franchini, A., Mandrioli, M., Saxena, A., Hanukoglu, A., Hanukoglu, I., 2002. Amiloride-sensitive epithelial sodium channel subunits are expressed in human and mussel immunocytes. Dev. Comp. Immunol. 26, 395–402.
- Padmanabhan, S., Caulfield, M., Dominiczak, A.F., 2015. Genetic and molecular aspects of hypertension. Circ. Res. 116, 937–959. http://dx.doi.org/10.1161/CIRCRESAHA.116. 303647.
- Passero, C.J., Carattino, M.D., Kashlan, O.B., Myerburg, M.M., Hughey, R.P., Kleyman, T.R., 2010. Defining an inhibitory domain in the gamma subunit of the epithelial sodium channel. Am. J. Physiol. Ren. Physiol. 299, F854–F861.
- Passero, C.J., Mueller, G.M., Myerburg, M.M., Carattino, M.D., Hughey, R.P., Kleyman, T.R., 2011. TMPRSS4-dependent activation of the epithelial sodium channel requires cleavage of the gamma subunit distal to the furin cleavage site. Am. J. Physiol. Ren. Physiol.
- Patel, Å.B., Chao, J., Palmer, L.G., 2012. Tissue kallikrein activation of the epithelial Na channel. Am. J. Physiol. Ren. Physiol. 303, F540–F550. http://dx.doi.org/10.1152/ ajprenal.00133.2012.
- Pathak, B.G., Shaughnessy, J.D., Meneton, P., Greeb, J., Shull, G.E., Jenkins, N.A., Copeland, N.G., 1996. Mouse chromosomal location of three epithelial sodium channel subunit genes and an apical sodium chloride cotransporter gene. Genomics 33, 124–127.

- Persu, A., Barbry, P., Bassilana, F., Houot, A.M., Mengual, R., Lazdunski, M., Corvol, P., Jeunemaitre, X., 1998. Genetic analysis of the beta subunit of the epithelial Na⁺ channel in essential hypertension. Hypertension 32, 129–137.
- Petryszak, R., Burdett, T., Fiorelli, B., Fonseca, N.A., Gonzalez-Porta, M., Hastings, E., Huber, W., Jupp, S., Keays, M., Kryvych, N., McMurry, J., Marioni, J.C., Malone, J., Megy, K., Rustici, G., Tang, A.Y., Taubert, J., Williams, E., Mannion, O., Parkinson, H.E., Brazma, A., 2014. Expression Atlas update—a database of gene and transcript expression from microarray- and sequencing-based functional genomics experiments. Nucleic Acids Res. 42, D926–D932. http://dx.doi.org/10.1093/nar/gkt1270.
- Pochynyuk, O., Staruschenko, A., Tong, Q., Medina, J., Stockand, J.D., 2005. Identification of a functional phosphatidylinositol 3,4,5-trisphosphate binding site in the epithelial Na⁺ channel. J. Biol. Chem. 280, 37565–37571. http://dx.doi.org/10.1074/jbc. M509071200.
- Pochynyuk, O., Tong, Q., Medina, J., Vandewalle, A., Staruschenko, A., Bugaj, V., Stockand, J.D., 2007. Molecular determinants of PI(4,5)P2 and PI(3,4,5)P3 regulation of the epithelial Na⁺ channel. J. Gen. Physiol. 130, 399–413. http://dx.doi.org/10.1085/jgp. 200709800.
- Pochynyuk, O., Bugaj, V., Stockand, J.D., 2008. Physiologic regulation of the epithelial sodium channel by phosphatidylinositides. Curr. Opin. Nephrol. Hypertens. 17, 533–540. http://dx.doi.org/10.1097/MNH.0b013e328308fff3.
- Pogozheva, I.D., Mosberg, H.I., Lomize, A.L., 2014. Life at the border: adaptation of proteins to anisotropic membrane environment. Protein Sci. 23, 1165–1196. http://dx.doi.org/ 10.1002/pro.2508.
- Pontius, J.U., Mullikin, J.C., Smith, D.R., Lindblad-Toh, K., Gnerre, S., Clamp, M., Chang, J., Stephens, R., Neelam, B., Volfovsky, N., Schäffer, A.A., Agarwala, R., Narfström, K., Murphy, W.J., Giger, U., Roca, A.L., Antunes, A., Menotti-Raymond, M., Yuhki, N., Pecon-Slattery, J., Johnson, W.E., Bourque, G., Tesler, G., O'Brien, S.J., 2007. Initial sequence and comparative analysis of the cat genome. Genome Res. 17, 1675–1689. http://dx.doi.org/10.1101/gr.6380007.
- Porter, J., Kershaw, M., Kirk, J., Trevelyan, N., Shaw, N.J., 2003. The use of sodium resonium in pseudohypoaldosteronism. Arch. Dis. Child. 88, 1138–1139.
- Price, M.P., Lewin, G.R., McIlwrath, S.L., Cheng, C., Xie, J., Heppenstall, P.A., Stucky, C.L., Mannsfeldt, A.G., Brennan, T.J., Drummond, H.A., Qiao, J., Benson, C.J., Tarr, D.E., Hrstka, R.F., Yang, B., Williamson, R.A., Welsh, M.J., 2000. The mammalian sodium channel BNC1 is required for normal touch sensation. Nature 407, 1007–1011. http://dx.doi.org/10.1038/35039512.
- Price, M.P., McIlwrath, S.L., Xie, J., Cheng, C., Qiao, J., Tarr, D.E., Sluka, K.A., Brennan, T.J., Lewin, G.R., Welsh, M.J., 2001. The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. Neuron 32, 1071–1083.
- Protasio, A.V., Tsai, I.J., Babbage, A., Nichol, S., Hunt, M., Aslett, M.A., De Silva, N., Velarde, G.S., Anderson, T.J.C., Clark, R.C., Davidson, C., Dillon, G.P., Holroyd, N.E., LoVerde, P.T., Lloyd, C., McQuillan, J., Oliveira, G., Otto, T.D., Parker-Manuel, S.J., Quail, M.A., Wilson, R.A., Zerlotini, A., Dunne, D.W., Berriman, M., 2012. A systematically improved high quality genome and transcriptome of the human blood fluke *Schistosoma mansoni*. PLoS Negl. Trop. Dis. 6, e1455. http://dx.doi.org/10.1371/ journal.pntd.0001455.
- Putnam, N.H., Butts, T., Ferrier, D.E.K., Furlong, R.F., Hellsten, U., Kawashima, T., Robinson-Rechavi, M., Shoguchi, E., Terry, A., Yu, J.-K., Benito-Gutiérrez, E.L., Dubchak, I., Garcia-Fernàndez, J., Gibson-Brown, J.J., Grigoriev, I.V., Horton, A.C., de Jong, P.J., Jurka, J., Kapitonov, V.V., Kohara, Y., Kuroki, Y., Lindquist, E., Lucas, S., Osoegawa, K., Pennacchio, L.A., Salamov, A.A., Satou, Y., Sauka-Spengler, T., Schmutz, J., Shin-I, T., Toyoda, A., Bronner-Fraser, M., Fujiyama, A., Holland, L.Z., Holland, P.W.H., Satoh, N., Rokhsar, D.S., 2008. The amphioxus genome and the evolution of the chordate karyotype. Nature 453, 1064–1071. http://dx.doi.org/10.1038/nature06967.
- Ramos, M.D., Trujillano, D., Olivar, R., Sotillo, F., Ossowski, S., Manzanares, J., Costa, J., Gartner, S., Oliva, C., Quintana, E., Gonzalez, M.I., Vazquez, C., Estivill, X., Casals, T., 2014. Extensive sequence analysis of CFTR, SCNN1A, SCNN1B, SCNN1G and SERPINA1 suggests an oligogenic basis for cystic fibrosis-like phenotypes. Clin. Genet. 86, 91–95. http://dx.doi.org/10.1111/cge.12234.
- Rauh, R., Soell, D., Haerteis, S., Diakov, A., Nesterov, V., Krueger, B., Sticht, H., Korbmacher, C., 2013. A mutation in the β-subunit of ENaC identified in a patient with cystic fibrosis-like symptoms has a gain-of-function effect. Am. J. Physiol. Lung Cell Mol. Physiol. 304, L43–L55. http://dx.doi.org/10.1152/ajplung.00093.2012.
- Rayner, B.L., Owen, E.P., King, J.A., Soule, S.G., Vreede, H., Opie, L.H., Marais, D., Davidson, J.S., 2003. A new mutation, R563Q, of the beta subunit of the epithelial sodium channel associated with low-renin, low-aldosterone hypertension. J. Hypertens. 21, 921–926. http://dx.doi.org/10.1097/01.hjh.0000059009.82022.9b.
- Reddy, M.M., Stutts, M.J., 2013. Status of fluid and electrolyte absorption in cystic fibrosis. Cold Spring Harb. Perspect. Med. 3, a009555. http://dx.doi.org/10.1101/cshperspect. a009555.
- Renfree, M.B., Papenfuss, A.T., Deakin, J.E., Lindsay, J., Heider, T., Belov, K., Rens, W., Waters, P.D., Pharo, E.A., Shaw, G., Wong, E.S.W., Lefèvre, C.M., Nicholas, K.R., Kuroki, Y., Wakefield, M.J., Zenger, K.R., Wang, C., Ferguson-Smith, M., Nicholas, F.W., Hickford, D., Yu, H., Short, K.R., Siddle, H.V., Frankenberg, S.R., Chew, K.Y., Menzies, B.R., Stringer, J.M., Suzuki, S., Hore, T.A., Delbridge, M.L., Patel, H.R., Mohammadi, A., Schneider, N.Y., Hu, Y., O'Hara, W., Al Nadaf, S., Wu, C., Feng, Z.-P., Cocks, B.G., Wang, J., Flicek, P., Searle, S.M.J., Fairley, S., Beal, K., Herrero, J., Carone, D.M., Suzuki, Y., Sugano, S., Toyoda, A., Sakaki, Y., Kondo, S., Nishida, Y., Tatsumoto, S., Mandiou, I., Hsu, A., McColl, K.A., Lansdell, B., Weinstock, G., Kuczek, E., McGrath, A., Wilson, P., Men, A., Hazar-Rethinam, M., Hall, A., Davis, J., Wood, D., Williams, S., Sundaravadanam, Y., Muzny, D.M., Jhangiani, S.N., Lewis, L.R., Morgan, M.B., Okwuonu, G.O., Ruiz, S.J., Santibanez, J., Nazareth, L., Cree, A., Fowler, G., Kovar, C.L., Dinh, H.H., Joshi, V., Jing, C., Lara, F., Thornton, R., Chen, L., Deng, J., Liu, Y., Shen, J.Y., Song, X.-Z., Edson, J., Troon, C., Thomas, D., Stephens, A., Yapa, L., Levchenko, T., Gibbs, R.A., Cooper, D.W., Speed, T.P., Fujiyama, A., Graves, J.A.M., O'Neill, R.J., Pask, A.J., Forrest, S.M., Worley, K.C., 2011. Genome sequence of an Australian kangaroo.

Macropus eugenii, provides insight into the evolution of mammalian reproduction and development. Genome Biol. 12, R81. http://dx.doi.org/10.1186/gb-2011-12-8-r81.

- Riepe, F.G., van Bemmelen, M.X., Cachat, F., Plendl, H., Gautschi, I., Krone, N., Holterhus, P.M., Theintz, G., Schild, L., 2009. Revealing a subclinical salt-losing phenotype in heterozygous carriers of the novel S562P mutation in the alpha subunit of the epithelial sodium channel. Clin. Endocrinol. 70, 252–258.
- Rogers, D.F., 2007. Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir. Care 52, 1134–1146 discussion 1146–9.
- Rossier, B.C., 2014. Epithelial sodium channel (ENaC) and the control of blood pressure. Curr. Opin. Pharmacol. 15, 33–46. http://dx.doi.org/10.1016/j.coph.2013.11.010.
- Rossier, B.C., Stutts, M.J., 2009. Activation of the epithelial sodium channel (ENaC) by serine proteases. Annu. Rev. Physiol. 71, 361–379.
- Rossier, B.C., Pradervand, S., Schild, L., Hummler, E., 2002. Epithelial sodium channel and the control of sodium balance: interaction between genetic and environmental factors. Annu. Rev. Physiol. 64, 877–897.
- Rossier, B.C., Baker, M.E., Studer, R.A., 2015. Epithelial sodium transport and its control by aldosterone: the story of our internal environment revisited. Physiol. Rev. 95, 297–340. http://dx.doi.org/10.1152/physrev.00011.2014.
- Rotin, D., 2008. Role of the UPS in Liddle syndrome. BMC Biochem. 9 (Suppl. 1).
- Rotin, D., Staub, O., 2011. Role of the ubiquitin system in regulating ion transport. Pflugers Arch. 461, 1–21. http://dx.doi.org/10.1007/s00424-010-0893-2.
- Rotin, D., Bar-Sagi, D., O'Brodovich, H., Merilainen, J., Lehto, V.P., Canessa, C.M., Rossier, B.C., Downey, G.P., 1994. An SH3 binding region in the epithelial Na⁺ channel (alpha rENaC) mediates its localization at the apical membrane. EMBO J. 13, 4440–4450.
- Ruan, Y.C., Chen, H., Chan, H.C., 2014. Ion channels in the endometrium: regulation of endometrial receptivity and embryo implantation. Hum. Reprod. Update 20, 517–529. http://dx.doi.org/10.1093/humupd/dmu006.
- Ruth, J.L., Wassner, S.J., 2006. Body composition: salt and water. Pediatr. Rev. 27, 181–187.
- Saar, K., Beck, A., Bihoreau, M.-T., Birney, E., Brocklebank, D., Chen, Y., Cuppen, E., Demonchy, S., Dopazo, J., Flicek, P., Foglio, M., Fujiyama, A., Gut, I.C., Gauguier, D., Guigo, R., Guryev, V., Heinig, M., Hummel, O., Jahn, N., Klages, S., Kren, V., Kube, M., Kuhl, H., Kuramoto, T., Kuroki, Y., Lechner, D., Lee, Y.-A., Lopez-Bigas, N., Lathrop, G.M., Mashimo, T., Medina, I., Mott, R., Patone, G., Perrier-Cornet, J.-A., Platzer, M., Pravenec, M., Reinhardt, R., Sakaki, Y., Schilhabel, M., Schulz, H., Serikawa, T., Shikhagaie, M., Tatsumoto, S., Taudien, S., Toyoda, A., Voigt, B., Zelenika, D., Zimdahl, H., Hubner, N., 2008. SNP and haplotype mapping for genetic analysis in the rat. Nat. Genet. 40, 560–566. http://dx.doi.org/10.1038/ng.124.
- Salleh, N., Baines, D.L., Naftalin, R.J., Milligan, S.R., 2005. The hormonal control of uterine luminal fluid secretion and absorption. J Membr Biol 206, 17–28.
- Samaha, F.F.F., Rubenstein, R.C.R.C., Yan, W., Ramkumar, M., Levy, D.I.D.I., Ahn, Y.J.Y.J., Sheng, S., Kleyman, T.R.T.R., 2004. Functional polymorphism in the carboxyl terminus of the alpha-subunit of the human epithelial sodium channel. J. Biol. Chem. 279, 23900–23907. http://dx.doi.org/10.1074/jbc.M401941200.
- Sasaki, S., Yui, N., Noda, Y., 2014. Actin directly interacts with different membrane channel proteins and influences channel activities: AQP2 as a model. Biochim. Biophys. Acta 1838, 514–520. http://dx.doi.org/10.1016/j.bbamem.2013.06.004.
- Satir, P., Christensen, S.T., 2007. Overview of structure and function of mammalian cilia. Annu. Rev. Physiol. 69, 377–400.
- Saxena, A., Hanukoglu, I., Strautnieks, S.S., Thompson, R.J., Gardiner, R.M., Hanukoglu, A., 1998. Gene structure of the human amiloride-sensitive epithelial sodium channel beta subunit. Biochem. Biophys. Res. Commun. 252, 208–213. http://dx.doi.org/10. 1006/bbrc.1998.9625.
- Saxena, A., Hanukoglu, I., Saxena, D., Thompson, R.J., Gardiner, R.M., 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, 3344–3350.
- Scally, A., Dutheil, J.Y., Hillier, L.W., Jordan, G.E., Goodhead, I., Herrero, J., Hobolth, A., Lappalainen, T., Mailund, T., Marques-Bonet, T., McCarthy, S., Montgomery, S.H., Schwalie, P.C., Tang, Y.A., Ward, M.C., Xue, Y., Yngvadottir, B., Alkan, C., Andersen, L.N., Ayub, Q., Ball, E.V., Beal, K., Bradley, B.J., Chen, Y., Clee, C.M., Fitzgerald, S., Graves, T.A., Gu, Y., Heath, P., Heger, A., Karakoc, E., Kolb-Kokocinski, A., Laird, G.K., Lunter, G., Meader, S., Mort, M., Mullikin, J.C., Munch, K., O'Connor, T.D., Phillips, A.D., Prado-Martinez, J., Rogers, A.S., Sajjadian, S., Schmidt, D., Shaw, K., Simpson, J.T., Stenson, P.D., Turner, D.J., Vigilant, L., Vilella, A.J., Whitener, W., Zhu, B., Cooper, D.N., de Jong, P., Dermitzakis, E.T., Eichler, E.E., Flicek, P., Goldman, N., Mundy, N.I., Ning, Z., Odom, D.T., Ponting, C.P., Quail, M.A., Ryder, O.A., Searle, S.M., Warren, W.C., Wilson, R.K., Schierup, M.H., Rogers, J., Tyler-Smith, C., Durbin, R., 2012. Insights into hominid evolution from the gorilla genome sequence. Nature 483, 169–175. http://dx.doi.org/ 10.1038/nature10842.
- Schaedel, C., Marthinsen, L., Kristoffersson, A.C., Kornfalt, R., Nilsson, K.O., Orlenius, B., Holmberg, L., 1999. Lung symptoms in pseudohypoaldosteronism type 1 are associated with deficiency of the alpha-subunit of the epithelial sodium channel. J. Pediatr. 135, 739–745.
- Schaefer, L., Sakai, H., Mattei, M., Lazdunski, M., Lingueglia, E., 2000. Molecular cloning, functional expression and chromosomal localization of an amiloride-sensitive Na⁺ channel from human small intestine. FEBS Lett. 471, 205–210.
- Schild, L., Lu, Y., Gautschi, I., Schneeberger, E., Lifton, R.P., Rossier, B.C., 1996. Identification of a PY motif in the epithelial Na channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. EMBO J. 15, 2381–2387.
- Schild, L., Schneeberger, E., Gautschi, I., Firsov, D., 1997. Identification of amino acid residues in the alpha, beta, and gamma subunits of the epithelial sodium channel (ENaC) involved in amiloride block and ion permeation. J. Gen. Physiol. 109, 15–26.

- Sheng, S., Li, J., McNulty, K.A., Avery, D., Kleyman, T.R., 2000. Characterization of the selectivity filter of the epithelial sodium channel. J. Biol. Chem. 275, 8572–8581.
- Sheng, S., Li, J., McNulty, K.A., Kieber-Emmons, T., Kleyman, T.R., 2001a. Epithelial sodium channel pore region. Structure and role in gating. J. Biol. Chem. 276, 1326–1334.
- Sheng, S., McNulty, K.A., Harvey, J.M., Kleyman, T.R., 2001b. Second transmembrane domains of ENaC subunits contribute to ion permeation and selectivity. J. Biol. Chem. 276, 44091–44098.
- Sheng, S., Carattino, M.D., Bruns, J.B., Hughey, R.P., Kleyman, T.R., 2006. Furin cleavage activates the epithelial Na⁺ channel by relieving Na⁺ self-inhibition. Am. J. Physiol. Ren. Physiol. 290, F1488–F1496.
- Sheng, S., Maarouf, A.B., Bruns, J.B., Hughey, R.P., Kleyman, T.R., 2007. Functional role of extracellular loop cysteine residues of the epithelial Na⁺ channel in Na⁺ self-inhibition. J. Biol. Chem. 282, 20180–20190.
- Sheridan, M.B., Fong, P., Groman, J.D., Conrad, C., Flume, P., Diaz, R., Harris, C., Knowles, M., Cutting, G.R., 2005. Mutations in the beta-subunit of the epithelial Na⁺ channel in patients with a cystic fibrosis-like syndrome. Hum. Mol. Genet. 14, 3493–3498.
- Sherwood, T.W., Frey, E.N., Askwith, C.C., 2012. Structure and activity of the acid-sensing ion channels. Am. J. Physiol. Cell Physiol. 303, C699–C710. http://dx.doi.org/10.1152/ ajpcell.00188.2012.
- Shi, H., Asher, C., Chigaev, A., Yung, Y., Reuveny, E., Seger, R., Garty, H., 2002. Interactions of beta and gamma ENaC with Nedd4 can be facilitated by an ERK-mediated phosphorylation. J. Biol. Chem. 277, 13539–13547.
- Shi, S., Ghosh, D.D., Okumura, S., Carattino, M.D., Kashlan, O.B., Sheng, S., Kleyman, T.R., 2011. Base of the thumb domain modulates epithelial sodium channel gating. J. Biol. Chem. 286, 14753–14761.
- Shi, S., Carattino, M.D., Kleyman, T.R., 2012. Role of the wrist domain in the response of the epithelial sodium channel to external stimuli. J. Biol. Chem. 287, 44027–44035. http://dx.doi.org/10.1074/jbc.M112.421743.
- Shigemura, N., Ohkuri, T., Sadamitsu, C., Yasumatsu, K., Yoshida, R., Beauchamp, G.K., Bachmanov, A.A., Ninomiya, Y., 2008. Amiloride-sensitive NaCl taste responses are associated with genetic variation of ENaC alpha-subunit in mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R66–R75. http://dx.doi.org/10.1152/ajpregu. 00420.2007.
- Shimeld, S.M., Donoghue, P.C.J., 2012. Evolutionary crossroads in developmental biology: cyclostomes (lamprey and hagfish). Development 139, 2091–2099. http://dx.doi.org/ 10.1242/dev.074716.
- Shimkets, R.A., Warnock, D.G., Bositis, C.M., Nelson-Williams, C., Hansson, J.H., Schambelan, M., Gill, J.R., Ulick, S., Milora, R.V., Findling, J.W., 1994. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 79, 407–414.
- Sillitoe, I., Lewis, T.E., Cuff, A., Das, S., Ashford, P., Dawson, N.L., Furnham, N., Laskowski, R.A., Lee, D., Lees, J.G., Lehtinen, S., Studer, R.A., Thornton, J., Orengo, C.A., 2015. CATH: comprehensive structural and functional annotations for genome sequences. Nucleic Acids Res. 43, D376–D381. http://dx.doi.org/10.1093/nar/gku947.
- Simakov, O., Marletaz, F., Cho, S.-J., Edsinger-Gonzales, E., Havlak, P., Hellsten, U., Kuo, D.-H., Larsson, T., Lv, J., Arendt, D., Savage, R., Osoegawa, K., de Jong, P., Grimwood, J., Chapman, J.A., Shapiro, H., Aerts, A., Otillar, R.P., Terry, A.Y., Boore, J.L., Grigoriev, I.V., Lindberg, D.R., Seaver, E.C., Weisblat, D.A., Putnam, N.H., Rokhsar, D.S., 2013. Insights into bilaterian evolution from three spiralian genomes. Nature 493, 526–531. http://dx.doi.org/10.1038/nature11696.
- Simon, A., Shenton, F., Hunter, I., Banks, R.W., Bewick, G.S., 2010. Amiloride-sensitive channels are a major contributor to mechanotransduction in mammalian muscle spindles. J. Physiol. 588, 171–185. http://dx.doi.org/10.1113/jphysiol.2009.182683.
- Smith, J.J., Kuraku, S., Holt, C., Sauka-Spengler, T., Jiang, N., Campbell, M.S., Yandell, M.D., Manousaki, T., Meyer, A., Bloom, O.E., Morgan, J.R., Buxbaum, J.D., Sachidanandam, R., Sims, C., Garruss, A.S., Cook, M., Krumlauf, R., Wiedemann, L.M., Sower, S.A., Decatur, W.A., Hall, J.A., Amemiya, C.T., Saha, N.R., Buckley, K.M., Rast, J.P., Das, S., Hirano, M., McCurley, N., Guo, P., Rohner, N., Tabin, C.J., Piccinelli, P., Elgar, G., Ruffier, M., Aken, B.L., Searle, S.M.J., Muffato, M., Pignatelli, M., Herrero, J., Jones, M., Brown, C.T., Chung-Davidson, Y.-W., Nanlohy, K.G., Libants, S.V., Yeh, C.-Y., McCauley, D.W., Langeland, J.A., Pancer, Z., Fritzsch, B., de Jong, P.J., Zhu, B., Fulton, LL, Theising, B., Flicek, P., Bronner, M.E., Warren, W.C., Clifton, S.W., Wilson, R.K., Li, W., 2013. Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. Nat. Genet. 45 (415–21), 421e1–421e2. http://dx. doi.org/10.1038/ng.2568.
- Snyder, P.M., McDonald, F.J., Stokes, J.B., Welsh, M.J., 1994. Membrane topology of the amiloride-sensitive epithelial sodium channel. J. Biol. Chem. 269, 24379–24383.
- Snyder, P.M., Olson, D.R., Bucher, D.B., 1999. A pore segment in DEG/ENaC Na⁺ channels. J. Biol. Chem. 274, 28484–28490.
- Soundararajan, R., Pearce, D., Hughey, R.P., Kleyman, T.R., 2010. Role of epithelial sodium channels and their regulators in hypertension. J. Biol. Chem. 285, 30363–30369.
- Springauf, A., Bresenitz, P., Gründer, S., 2011. The interaction between two extracellular linker regions controls sustained opening of acid-sensing ion channel 1. J. Biol. Chem. 286, 24374–24384. http://dx.doi.org/10.1074/jbc.M111.230797.
- Staruschenko, A., Adams, E., Booth, R.E., Stockand, J.D., 2005. Epithelial Na⁺ channel subunit stoichiometry. Biophys. J. 88, 3966–3975. http://dx.doi.org/10.1529/biophysj. 104.056804.
- Stewart, A.P., Haerteis, S., Diakov, A., Korbmacher, C., Edwardson, J.M., 2011. Atomic force microscopy reveals the architecture of the epithelial sodium channel (ENaC). J. Biol. 286, 31944–31952. http://dx.doi.org/10.1074/jbc.M111.275289.
- Stockand, J.D., Staruschenko, A., Pochynyuk, O., Booth, R.E., Silverthorn, D.U., 2008. Insight toward epithelial Na⁺ channel mechanism revealed by the acid-sensing ion channel 1 structure. IUBMB Life 60, 620–628.
- Strautnieks, S.S., Thompson, R.J., Hanukoglu, A., Dillon, M.J., Hanukoglu, I., Kuhnle, U., Seckl, J., Gardiner, R.M., Chung, E., 1996. Localisation of pseudohypoaldosteronism

genes to chromosome 16p12.2–13.11 and 12p13.1-pter by homozygosity mapping. Hum. Mol. Genet. 5, 293–299.

- Studer, R.A., Person, E., Robinson-Rechavi, M., Rossier, B.C., 2011. Evolution of the epithelial sodium channel and the sodium pump as limiting factors of aldosterone action on sodium transport. Physiol. Genomics 43, 844–854. http://dx.doi.org/10.1152/ physiolgenomics.00002.2011.
- Su, Y.R., Menon, A.G., 2001. Epithelial sodium channels and hypertension. Drug Metab. Dispos. 29, 553–556.
- Sugiyama, T., Kato, N., Ishinaga, Y., Yamori, Y., Yazaki, Y., 2001. Evaluation of selected polymorphisms of the Mendelian hypertensive disease genes in the Japanese population. Hypertens. Res. 24, 515–521.
- Szabo, R., Bugge, T.H., 2011. Membrane-anchored serine proteases in vertebrate cell and developmental biology. Annu. Rev. Cell Dev. Biol. 27, 213–235. http://dx.doi.org/10. 1146/annurev-cellbio-092910-154247.
- Takada, T., Ebata, T., Noguchi, H., Keane, T.M., Adams, D.J., Narita, T., Shin-I, T., Fujisawa, H., Toyoda, A., Abe, K., Obata, Y., Sakaki, Y., Moriwaki, K., Fujiyama, A., Kohara, Y., Shiroishi, T., 2013. The ancestor of extant Japanese fancy mice contributed to the mosaic genomes of classical inbred strains. Genome Res. 23, 1329–1338. http://dx.doi. org/10.1101/gr.156497.113.
- Takei, Y., 2000. Comparative physiology of body fluid regulation in vertebrates with special reference to thirst regulation. Jpn. J. Physiol. 50, 171–186.
- Tapolyai, M., Uysal, A., Dossabhoy, N.R., Zsom, L., Szarvas, T., Lengvárszky, Z., Fülöp, T., 2010. High prevalence of liddle syndrome phenotype among hypertensive US Veterans in Northwest Louisiana. J. Clin. Hypertens. 12, 856–860. http://dx.doi.org/10. 1111/j.1751-7176.2010.00359.x.
- Thomas, C.P., Doggett, N.A., Fisher, R., Stokes, J.B., 1996. Genomic organization and the 5' flanking region of the gamma subunit of the human amiloride-sensitive epithelial sodium channel. J. Biol. Chem. 271, 26062–26066.
- Thomas, C.P., Loftus, R.W., Liu, K.Z., Itani, O.A., 2002. Genomic organization of the 5' end of human beta-ENaC and preliminary characterization of its promoter. Am. J. Physiol. Ren. Physiol. 282, F898–F909.
- Tilley, A.E., Walters, M.S., Shaykhiev, R., Crystal, R.G., 2015. Cilia dysfunction in lung disease. Annu. Rev. Physiol. 77, 379–406. http://dx.doi.org/10.1146/annurevphysiol-021014-071931.

Tinker, S.W., 1988. Whales of the World. E. J. Brill.

- Toka, H.R., Koshy, J.M., Hariri, A., 2013. The molecular basis of blood pressure variation. Pediatr. Nephrol. 28, 387–399. http://dx.doi.org/10.1007/s00467-012-2206-9.
- Tolino, L.A., Okumura, S., Kashlan, O.B., Carattino, M.D., 2011. Insights into the mechanism of pore opening of acid-sensing ion channel 1a. J. Biol. Chem. 286, 16297–16307. http://dx.doi.org/10.1074/jbc.M110.202366.
- Tong, Q., Menon, A.G., Stockand, J.D., 2006. Functional polymorphisms in the alphasubunit of the human epithelial Na⁺ channel increase activity. Am. J. Physiol. Ren. Physiol. 290, F821–F827. http://dx.doi.org/10.1152/ajprenal.00312.2005.
- Tousson, A., Alley, C.D., Sorscher, E.J., Brinkley, B.R., Benos, D.J., 1989. Immunochemical localization of amiloride-sensitive sodium channels in sodium-transporting epithelia. J. Cell Sci. 93 (Pt 2), 349–362.
- Uchiyama, M., Maejima, S., Yoshie, S., Kubo, Y., Konno, N., Joss, J.M.P., 2012. The epithelial sodium channel in the Australian lungfish, *Neoceratodus forsteri* (Osteichthyes: Dipnoi). Proc. Biol. Sci. 279, 4795–4802. http://dx.doi.org/10.1098/ rspb.2012.1945.
- Uchiyama, M., Konno, N., Shibuya, S., Nogami, S., 2014. Cloning and expression of the epithelial sodium channel and its role in osmoregulation of aquatic and estivating African lungfish *Protopterus annectens*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 183C, 1–8. http://dx.doi.org/10.1016/j.cbpa.2014.12.028.
- Uniprot, 2014. UniProt: a hub for protein information. Nucleic Acids Res. 43, D204–D212. http://dx.doi.org/10.1093/nar/gku989.
- Vallet, V., Chraibi, A., Gaeggeler, H.P., Horisberger, J.D., Rossier, B.C., 1997. An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature 389, 607–610. http://dx.doi.org/10.1038/39329.
- Venkatesh, B., Kirkness, E.F., Loh, Y.-H., Halpern, A.L., Lee, A.P., Johnson, J., Dandona, N., Viswanathan, L.D., Tay, A., Venter, J.C., Strausberg, R.L., Brenner, S., 2007. Survey sequencing and comparative analysis of the elephant shark (*Callorhinchus milii*) genome. PLoS Biol. 5, e101. http://dx.doi.org/10.1371/journal.pbio.0050101.
- Viel, M., Leroy, C., Hubert, D., Fajac, I., Bienvenu, T., 2008. ENaCbeta and gamma genes as modifier genes in cystic fibrosis. J. Cyst. Fibros. 7, 23–29.
- Vilella, A.J., Severin, J., Ureta-Vidal, A., Heng, L., Durbin, R., Birney, E., 2009. EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. Genome Res. 19, 327–335. http://dx.doi.org/10.1101/gr.073585.107.
- Voilley, N., Lingueglia, E., Champigny, G., Mattéi, M.G., Waldmann, R., Lazdunski, M., Barbry, P., 1994. The lung amiloride-sensitive Na⁺ channel: biophysical properties, pharmacology, ontogenesis, and molecular cloning. Proc. Natl. Acad. Sci. U. S. A. 91, 247–251.
- Voilley, N., Bassilana, F., Mignon, C., Merscher, S., Mattéi, M.G., Carle, G.F., Lazdunski, M., 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, 560–565. http://dx.doi.org/10. 1006/geno.1995.1188.
- Volk, K.A., Husted, R.F., Snyder, P.M., Stokes, J.B., 2000. Kinase regulation of hENaC mediated through a region in the COOH-terminal portion of the alpha-subunit. Am. J. Physiol. Cell Physiol. 278, C1047–C1054.
- von Heijne, G., 1992. Membrane protein structure prediction. Hydrophobicity analysis and the positive-inside rule. J. Mol. Biol. 225, 487–494.
- Vuagniaux, G., Vallet, V., Jaeger, N.F., Hummler, E., Rossier, B.C., 2002. Synergistic activation of ENaC by three membrane-bound channel-activating serine proteases (mCAP1, mCAP2, and mCAP3) and serum- and glucocorticoid-regulated kinase (Sgk1) in *Xenopus* oocytes. J. Gen. Physiol. 120, 191–201.

Wade, C.M., Giulotto, E., Sigurdsson, S., Zoli, M., Gnerre, S., Imsland, F., Lear, T.L., Adelson, D.L., Bailey, E., Bellone, R.R., Blöcker, H., Distl, O., Edgar, R.C., Garber, M., Leeb, T., Mauceli, E., MacLeod, J.N., Penedo, M.C.T., Raison, J.M., Sharpe, T., Vogel, J., Andersson, L., Antczak, D.F., Biagi, T., Binns, M.M., Chowdhary, B.P., Coleman, S.J., Della Valle, G., Fryc, S., Guérin, G., Hasegawa, T., Hill, E.W., Jurka, J., Kiialainen, A., Lindgren, G., Liu, J., Magnani, E., Mickelson, J.R., Murray, J., Nergadze, S.G., Onofrio, R., Pedroni, S., Piras, M.F., Raudsepp, T., Rocchi, M., Røed, K.H., Ryder, O.A., Searle, S., Skow, L., Swinburne, J.E., Syvänen, A.C., Tozaki, T., Valberg, S.J., Vaudin, M., White, J.R., Zody, M.C., Lander, E.S., Lindblad-Toh, K., 2009. Genome sequence, comparative analysis, and population genetics of the domestic horse. Science 326 (80), 865–867. http://dx.doi.org/10.1126/science.1178158.

Waldmann, R., Lazdunski, M., 1998. H⁺-gated cation channels: neuronal acid sensors in the NaC/DEG family of ion channels. Curr. Opin. Neurobiol. 8, 418–424.

- Waldmann, R., Champigny, G., Bassilana, F., Voilley, N., Lazdunski, M., 1995. Molecular cloning and functional expression of a novel amiloride-sensitive Na⁺ channel. J. Biol. Chem. 270, 27411–27414.
- Wang, Q., Schultz, B.D., 2014. Cholera toxin enhances Na⁺ absorption across MCF10A human mammary epithelia. Am. J. Physiol. Cell Physiol. 306, C471–C484. http://dx. doi.org/10.1152/ajpcell.00181.2013.
- Wang, S., He, G., Yang, Y., Liu, Y., Diao, R., Sheng, K., Liu, X., Xu, W., 2013a. Reduced expression of Enac in placenta tissues of patients with severe preeclampsia is related to compromised trophoblastic cell migration and invasion during pregnancy. PLoS One 8, e72153. http://dx.doi.org/10.1371/journal.pone.0072153.
- Wang, Z., Pascual-Anaya, J., Zadissa, A., Li, W., Niimura, Y., Huang, Z., Li, C., White, S., Xiong, Z., Fang, D., Wang, B., Ming, Y., Chen, Y., Zheng, Y., Kuraku, S., Pignatelli, M., Herrero, J., Beal, K., Nozawa, M., Li, Q., Wang, J., Zhang, H., Yu, L., Shigenobu, S., Wang, J., Liu, J., Flicek, P., Searle, S., Wang, J., Kuratani, S., Yin, Y., Aken, B., Zhang, G., Irie, N., 2013b. The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. Nat. Genet. 45, 701–706. http://dx.doi.org/10.1038/ng.2615.
- Wang, J., Yu, T., Yin, L., Li, J., Yu, L., Shen, Y., Yu, Y., Shen, Y., Fu, Q., 2013c. Novel mutations in the SCNN1A gene causing Pseudohypoaldosteronism type 1. PLoS One 8, e65676. http://dx.doi.org/10.1371/journal.pone.0065676.
- Wang, L.-P., Yang, K.-Q., Jiang, X.-J., Wu, H.-Y., Zhang, H.-M., Zou, Y.-B., Song, L., Bian, J., Hui, R.-T., Liu, Y.-X., Zhou, X.-L., 2015. Prevalence of liddle syndrome among young hypertension patients of undetermined cause in a Chinese population. J. Clin. Hypertens. http://dx.doi.org/10.1111/jch.12598.
- Warren, W.C., Hillier, L.W., Marshall Graves, J.A., Birney, E., Ponting, C.P., Grützner, F., Belov, K., Miller, W., Clarke, L., Chinwalla, A.T., Yang, S.-P., Heger, A., Locke, D.P., Miethke, P., Waters, P.D., Veyrunes, F., Fulton, L., Fulton, B., Graves, T., Wallis, J., Puente, X.S., López-Otín, C., Ordóñez, G.R., Eichler, E.E., Chen, L., Cheng, Z., Deakin, J.E., Alsop, A., Thompson, K., Kirby, P., Papenfuss, A.T., Wakefield, M.J., Olender, T., Lancet, D., Huttley, G.A., Smit, A.F.A., Pask, A., Temple-Smith, P., Batzer, M.A., Walker, J.A., Konkel, M.K., Harris, R.S., Whittington, C.M., Wong, E.S.W., Gemmell, N.J., Buschiazzo, E., Vargas Jentzsch, I.M., Merkel, A., Schmitz, J., Zemann, A., Churakov, G., Kriegs, J.O., Brosius, J., Murchison, E.P., Sachidanandam, R., Smith, C., Hannon, G.J., Tsend-Ayush, E., McMillan, D., Attenborough, R., Rens, W., Ferguson-Smith, M., Lefèvre, C.M., Sharp, J.A., Nicholas, K.R., Ray, D.A., Kube, M., Reinhardt, R., Pringle, T.H., Taylor, J., Jones, R.C., Nixon, B., Dacheux, J.-L., Niwa, H., Sekita, Y., Huang, X., Stark, A., Kheradpour, P., Kellis, M., Flicek, P., Chen, Y., Webber, C., Hardison, R., Nelson, J., Hallsworth-Pepin, K., Delehaunty, K., Markovic, C., Minx, P., Feng, Y., Kremitzki, C., Mitreva, M., Glasscock, J., Wylie, T., Wohldmann, P., Thiru, P., Nhan, M.N., Pohl, C.S., Smith, S.M., Hou, S., Nefedov, M., de Jong, P.J., Renfree, M.B., Mardis, E.R., Wilson, R.K., 2008. Genome analysis of the platypus reveals unique signatures of evolution. Nature 453, 175-183. http://dx.doi.org/10.1038/nature06936.
- Warren, W.C., Clayton, D.F., Ellegren, H., Arnold, A.P., Hillier, L.W., Künstner, A., Searle, S., White, S., Vilella, A.J., Fairley, S., Heger, A., Kong, L., Ponting, C.P., Jarvis, E.D., Mello, C.V., Minx, P., Lovell, P., Velho, T.A.F., Ferris, M., Balakrishnan, C.N., Sinha, S., Blatti, C., London, S.E., Li, Y., Lin, Y.-C., George, J., Sweedler, J., Southey, B., Gunaratne, P., Watson, M., Nam, K., Backström, N., Smeds, L., Nabholz, B., Itoh, Y., Whitney, O., Pfenning, A.R., Howard, J., Völker, M., Skinner, B.M., Griffin, D.K., Ye, L., McLaren, W.M., Flicek, P., Quesada, V., Velasco, G., Lopez-Otin, C., Puente, X.S., Olender, T., Lancet, D., Smit, A.F.A., Hubley, R., Konkel, M.K., Walker, J.A., Batzer, M.A., Gu, W., Pollock, D.D., Chen, L., Cheng, Z., Eichler, E.E., Stapley, J., Slate, J., Ekblom, R., Birkhead, T., Burke, T., Burke, S.V., Yang, S.-P., Li, X., Graves, T., Fulton, L., Nelson, J., Chinwalla, A., Hou, S., Mardis, E.R., Wilson, R.K., 2010. The genome of a songbird. Nature 464, 757–762. http://dx. doi.org/10.1038/nature08819.
- Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191. http://dx.doi.org/10.1093/bioinformatics/btp033.
- Welzel, M., Akin, L., Büscher, A., Güran, T., Hauffa, B.P., Högler, W., Leonards, J., Karges, B., Kentrup, H., Kirel, B., Senses, E.E.Y., Tekin, N., Holterhus, P.-M., Riepe, F.G., 2013. Five novel mutations in the SCNN1A gene causing autosomal recessive pseudohypoaldosteronism type 1. Eur. J. Endocrinol. 168, 707–715. http://dx.doi. org/10.1530/EJE-12-1000.
- Winarski, K.L., Sheng, N., Chen, J., Kleyman, T.R., Sheng, S., 2010. Extracellular allosteric regulatory subdomain within the gamma subunit of the epithelial Na⁺ channel. J. Biol. Chem. 285, 26088–26096.

- Worley, K.C., Warren, W.C., Rogers, J., Locke, D., Muzny, D.M., Mardis, E.R., Weinstock, G.M., Tardif, S.D., Aagaard, K.M., Archidiacono, N., Rayan, N.A., Batzer, M.A., Beal, K., Breiova, B., Capozzi, O., Capuano, S.B., Casola, C., Chandrabose, M.M., Cree, A., Dao, M.D., de Jong, P.J., del Rosario, R.C.-H., Delehaunty, K.D., Dinh, H.H., Eichler, E.E., Fitzgerald, S., Flicek, P., Fontenot, C.C., Fowler, R.G., Fronick, C., Fulton, L.A., Fulton, R.S., Gabisi, R.A., Gerlach, D., Graves, T.A., Gunaratne, P.H., Hahn, M.W., Haig, D., Han, Y., Harris, R.A., Herrero, J., Hillier, L.W., Hubley, R., Hughes, J.F., Hume, J., Jhangiani, S.N., Jorde, L.B., Joshi, V., Karakor, E., Konkel, M.K., Kosiol, C., Kovar, C.L., Kriventseva, E.V., Lee, S.L., Lewis, L.R., Liu, Y., Lopez, J., Lopez-Otin, C., Lorente-Galdos, B., Mansfield, K.G., Marques-Bonet, T., Minx, P., Misceo, D., Moncrieff, J.S., Morgan, M.B., Nazareth, L.V., Newsham, I., Nguyen, N.B., Okwuonu, G.O., Prabhakar, S., Perales, L., Pu, L.-L., Puente, X.S., Quesada, V., Ranck, M.C., Raney, B.J., Raveendran, M., Deiros, D.R., Rocchi, M., Rodriguez, D., Ross, C., Ruffier, M., Ruiz, S.J., Sajjadian, S., Santibanez, J., Schrider, D.R., Searle, S., Skaletsky, H., Soibam, B., Smit, A.F.A., Tennakoon, J.B., Tomaska, L., Ullmer, B., Vejnar, C.E., Ventura, M., Vilella, A.J., Vinar, T., Vogel, J.-H., Walker, J.A., Wang, Q., Warner, C.M., Wildman, D.E., Witherspoon, D.J., Wright, R.A., Wu, Y., Xiao, W., Xing, J., Zdobnov, E.M., Zhu, B., Gibbs, R.A., Wilson, R.K., 2014. The common marmoset genome provides insight into primate biology and evolution. Nat. Genet. 46, 850-857. http://dx.doi.org/10. 1038/ng.3042.
- Yang, L.-M., Rinke, R., Korbmacher, C., 2006. Stimulation of the epithelial sodium channel (ENaC) by cAMP involves putative ERK phosphorylation sites in the C termini of the channel's beta- and gamma-subunit. J. Biol. Chem. 281, 9859–9868. http://dx.doi.org/ 10.1074/jbc.M512046200.
- Yang, H., Yu, Y., Li, W.-G., Yu, F., Cao, H., Xu, T.-L., Jiang, H., 2009. Inherent dynamics of the acid-sensing ion channel 1 correlates with the gating mechanism. PLoS Biol. 7, e1000151. http://dx.doi.org/10.1371/journal.pbio.1000151.
- Yue, G.G., Malik, B., Eaton, D.C.D.C., Yue, G.G., Eaton, D.C.D.C., 2002. Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates epithelial sodium channel activity in A6 cells. J. Biol. Chem. 277, 11965–11969. http://dx.doi.org/10.1074/jbc.M108951200.
- Zelle, K.M., Lu, B., Pyfrom, S.C., Ben-Shahar, Y., 2013. The genetic architecture of degenerin/epithelial sodium channels in *Drosophila*. G3 (Bethesda) 3, 441–450. http://dx.doi.org/10.1534/g3.112.005272.
- Zha, X., 2013. Acid-sensing ion channels: trafficking and synaptic function. Mol. Brain 6, 1. http://dx.doi.org/10.1186/1756-6606-6-1.
- Zhang, G., Fang, X., Guo, X., Li, L., Luo, R., Xu, F., Yang, P., Zhang, L., Wang, X., Qi, H., Xiong, Z., Que, H., Xie, Y., Holland, P.W.H., Paps, J., Zhu, Y., Wu, F., Chen, Y., Wang, J., Peng, C., Meng, J., Yang, L., Liu, J., Wen, B., Zhang, N., Huang, Z., Zhu, Q., Feng, Y., Mount, A., Hedgecock, D., Xu, Z., Liu, Y., Domazet-Lošo, T., Du, Y., Sun, X., Zhang, S., Liu, B., Cheng, P., Jiang, X., Li, J., Fan, D., Wang, W., Fu, W., Wang, T., Wang, B., Zhang, J., Peng, Z., Li, Y., Li, N., Wang, J., Chen, M., He, Y., Tan, F., Song, X., Zheng, Q., Huang, R., Yang, H., Du, X., Chen, L., Yang, M., Gaffney, P.M., Wang, S., Luo, L., She, Z., Ming, Y., Huang, W., Zhang, S., Huang, B., Zhang, Y., Qu, T., Ni, P., Miao, G., Wang, J., Wang, Q., Steinberg, C.E.W., Wang, H., Li, N., Qian, L., Zhang, G., Li, Y., Yang, H., Liu, X., Wang, J., 2012. The oyster genome reveals stress adaptation and complexity of shell formation. Nature 490, 49–54. http://dx.doi.org/10.1038/nature11413.
- Zhang, G., Li, B., Li, C., Gilbert, M.T.P., Jarvis, E.D., Wang, J., 2014. Comparative genomic data of the Avian Phylogenomics Project. Gigascience 3, 26. http://dx.doi.org/10.1186/ 2047-217X-3-26.
- Zheng, H., Zhang, W., Zhang, L., Zhang, Z., Li, J., Lu, G., Zhu, Y., Wang, Y., Huang, Y., Liu, J., Kang, H., Chen, J., Wang, L., Chen, A., Yu, S., Gao, Z., Jin, L., Gu, W., Wang, Z., Zhao, L., Shi, B., Wen, H., Lin, R., Jones, M.K., Brejova, B., Vinar, T., Zhao, G., McManus, D.P., Chen, Z., Zhou, Y., Wang, S., 2013. The genome of the hydatid tapeworm *Echinococcus* granulosus. Nat. Genet. 45, 1168–1175. http://dx.doi.org/10.1038/ng.2757.
- Zhu, L, Zhang, Y., Zhang, W., Yang, S., Chen, J.-Q., Tian, D., 2009. Patterns of exon-intron architecture variation of genes in eukaryotic genomes. BMC Genomics 10, 47. http://dx.doi.org/10.1186/1471-2164-10-47.
- Zhu, K., Zhou, X., Xu, S., Sun, D., Zhou, K., Yang, G., 2014. The loss of taste genes in cetaceans. BMC Evol. Biol. 14, 218. http://dx.doi.org/10.1186/s12862-014-0218-8.
- Zhu, X.-Q., Korhonen, P.K., Cai, H., Young, N.D., Nejsum, P., von Samson-Himmelstjerna, G., Boag, P.R., Tan, P., Li, Q., Min, J., Yang, Y., Wang, X., Fang, X., Hall, R.S., Hofmann, A., Sternberg, P.W., Jex, A.R., Gasser, R.B., 2015. Genetic blueprint of the zoonotic pathogen *Toxocara canis*. Nat. Commun. 6, 6145. http://dx.doi.org/10.1038/ncomms7145.
- Ziemann, A.E., Schnizler, M.K., Albert, G.W., Severson, M.A., Howard, M.A., Welsh, M.J., Wemmie, J.A., 2008. Seizure termination by acidosis depends on ASIC1a. Nat. Neurosci. 11, 816–822. http://dx.doi.org/10.1038/nn.2132.
- Ziemann, A.E., Allen, J.E., Dahdaleh, N.S., Drebot, I.I., Coryell, M.W., Wunsch, A.M., Lynch, C.M., Faraci, F.M., Howard, M.A., Welsh, M.J., Wemmie, J.A., 2009. The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. Cell 139, 1012–1021. http://dx.doi.org/10.1016/j.cell.2009.10.029.
- Zimin, A.V., Cornish, A.S., Maudhoo, M.D., Gibbs, R.M., Zhang, X., Pandey, S., Meehan, D.T., Wipfler, K., Bosinger, S.E., Johnson, Z.P., Tharp, G.K., Marçais, G., Roberts, M., Ferguson, B., Fox, H.S., Treangen, T., Salzberg, S.L., Yorke, J.A., Norgren, R.B., 2014. A new rhesus macaque assembly and annotation for next-generation sequencing analyses. Biol. Direct 9, 20. http://dx.doi.org/10.1186/1745-6150-9-20.